

Evaluating the Allelopathic Effects of *Salvia rosmarinus* on Selected Weeds and *Triticum aestivum* L.

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Abstract. Allelopathic effects of *Rosmarinus officinalis* (rosemary) have been investigated on 3 weeds: *Vicia sativa*, *Vicia ervilia*, *Hordeum spontaneum* and one important cereal crop *Triticum aestivum* L. (bread wheat). A Laboratory experiment was laid out and 0.00, 1.00, 1.50 and 2.00 mg/ ml solutions of the ethanolic leaf extract were applied each with 5 replications. After 2 weeks, several germination indices and seedling growth characters were recorded. The chemical constituent of the extract was identified with GC–MS analysis. Eucalyptol, camphor, terpinene, linalool, ferruginol were identified as a dominant allelochemicals. A significant decrease was recorded in the germination % (G %) and germination velocity (GV) of *Vicia ervilia*, *Vicia sativa* and *Hordeum spontaneum*. While, seedling vigor index (SVI) was decreased significantly in *Hordeum spontaneum* and *Triticum aestivum* L. Although, a significant increase observed only in the root and shoot length of *Triticum aestivum* L., but it decreased significantly in the other parameters. A significant reduction in the seedling biomass of *Vicia ervilia* and *Hordeum spontaneum* was recorded. A remarkable increase was recorded in the seedling moisture content of *Hordeum spontaneum* and *Triticum aestivum* L. Generally, it can be concluded that rosemary exhibited a negative allelopathic effects on the weeds that can be used as a herbicide, but a positive promoter effect on *Triticum aestivum* L.

Keywords. Rosemary, Seedling vigor, Allelochemicals, Eucalyptol, *Vicia sativa*, *Vicia ervilia*, *Hordeum spontaneum*, *Triticum aestivum* L.

1. Introduction

Allelopathy is the process where allelochemicals of a plant has an effect on other plants in the environment [1]. Based on the allelochemicals impact on the targeted plant, it can be negative or positive interaction between 2 plants [2]. Allelochemicals are mostly secondary metabolites such as; alkaloids, tannins, and glycosides are used as weed-bio control methods [3]. *Salvia rosmarinus* (rosemary) is a perennial herbal plant natively habituates of the western Mediterranean region [4]. It has an allelopathic behavior upon seed germination and growth of neighbor plants [5]. [6] found that an acetone extract of *Salvia moorcraftiana* can inhibit the growth of *Lemna aequinoctialis*. Similarly, [7] recorded that rosemary aqueous extract caused reduction in the root growth of *Solanum nigrum* due to its ability for lipid peroxidation. [8] observed that an aqueous extract from *Salvia macrosiphon* impacts on different growth stages of *Zea mays* in reducing its fresh and dry biomass, and a reduction in the germination rate. Methanolic extract of *Salvia macrochlamys* used by [9] found to inhibit *Portulaca oleracea* germination due its influence on enzymes activity and hormonal balance. [10] announced that *Salvia officinalis* extracts inhibited the germination of *Hordeum vulgare* and *Portulaca*

oleracea. [11] reported that various *Salvia* species produce exudates from their aerial parts that inhibit the germination and growth of *Papaver rhoeas* and *Avena sativa*. Common and major allelochemicals of rosemary are terpenes and phenolic; their effect differs from one plant species to another [12]. The major allelochemicals of *R. officinalis* include α -pinene, 1,8-cineole, and piperitone, that proved to inhibit germination of weed seed species, e.g., *Eleusine indica* L., *Cynodon dactylon* L., *Digitaria sanguinalis* L., *Amaranthus retroflexus* L., and *Lolium perenne* [13]. [14] found the anti-cyanobacterial activity of *R. officinalis* essential oil on *Microcystis aeruginosa* to be more sensitive compared to *Chroococcus minor*. Due to the remarkable effects of these allelochemicals in altering the growth of a targeted plant, they should be studied more accurately. For instance, the allelopathic influence of rosemary extract to inhibit the growth of different crop species, highlighting its potential use as a weed bio control agent [15]. The frequent use of pesticide caused resistance of most of the pests and diseases, beside of their impact on polluting the environment. Therefore, seeking for the eco-friendly alternative strategies via using natural compounds is crucial nowadays [16]. Fast biodegradation, low risk of resistance subsequently, and relatively their low toxicity makes them more preferable to be studied and used as alternatives for herbicides [17]. The allelopathic effect of *Salvia rosmarinus* is crucial point to be studied and needs further investigations on different weeds and crops, like *Vicia sativa*, *Vicia ervila*, *Hordeum spontaneum* L., and *Triticum aestivum* L. through identifying its allelochemicals using Gas Chromatography-Mass Spectrometry (GC-MS) to distinguish its potential inhibitor or stimulator effects on germination and early growth stage to conclude its biochemical interactions (allelopathy) and its potential agricultural use.

2. Materials and Methods

2.1. Preparation of *Salvia Rosmarinus* Leaf Extract

Collected leaf samples were washed with tap water to remove the debris and then dried at 40 °C for 7 days. Grind into a fine powder using an electronic grinder, and stored at room temperature. Pure ethanol used as an extraction solvent. A total of 30 g dried powdered leaves kept in 1 L of ethanol shaken for 2 h in a dark condition. The whole samples refrigerated and were wrapped for 24 h. The precipitant decanted after centrifugation (Avanti J-26 XPI centrifuge, Beckman Coulter, USA) at 6000 rpm for 10 min. The supernatant was filtered through Whatman filter paper number 1 (ALBERTR). The extract evaporated in a rotary evaporator (Hahn Shin Scientific Co., Taiwan) till drying. Preserved in a tightly sealed sterile glass bottle and kept at 4 °C.

2.2. Qualitative and Quantitative Analyses of Extracts by GC/MS

The analyzing process for the bioactive compounds was detected by (GC/MS) using a (Shimadzu GCQP 2010 Ultra GC/MS) system. The temperature of (GC) oven was at the start (40 °C) and then gradually increased to (220 °C) at a rate of (10 °C) min⁻¹. For carrier, helium was used; and with (49.7 kPa) inlet pressure, and (36.7 cm sec⁻¹) of linear velocity. The column flow rate was set at 1.00 mL min⁻¹, with an injector temperature of 220 °C, using less-splitting injection mode. The MS conditions included a source temperature of 200 °C, an interface temperature of 220 °C, and a detector gain of 0.90 kV. The mass range was set between m/z 40-600. The different extracts constituents were identified through the retention matching indices accompanied by those of quality constituents packed in the (NIST) library 2005 or from the literature. The GC peak areas were used for calculating the components peak area concentrations [18].

2.3. Seed Material and Germination Test

Surface sterilization of *Vicia sativa*, *Vicia ervilia* L., *Hordeum spontaneum* L., and *Triticum aestivum* L. seeds were done with NaClO (8.00%) for 8 min. Then, washed 7 times using sterilized distilled water. The dried rosemary leaves weighed then dissolved in DMSO for preparing 3 concentrations; 1.00 (C1), 1.50 (C2) and 2.00 (C3) mg/ ml distilled water compared to control (C0). The seed germination was conducted in Petri dishes lined with 2 layers of Whatman No. 1 filter paper. For each crop species, 20 seeds were placed in each Petri dish, and 9 ml of the respective rosemary extract concentrations was added to each dish. Only distilled water and DMSO mixture was used for treating

the control group. The Petri dishes were then sealed with parafilm to avoid moisture loss and incubated in a growth chamber at a constant temperature of 19 °C and dark condition.

2.4. Experimental Measurements

2.4.1. Germination Indices

(G %): After emerging 1 mm long radicle the seeds regarded as germinated case. (G %) = germinated seed number/ Total planted seeds number × 100

(GV %) = $\sum G \% / t$. where, G %; is the germination percent and t; is total germination time.

(SVI): was calculated as: $SVI = G \% \times (\text{Root length} + \text{Shoot Length})$ [19]

2.4.2. Seedling Growth Characters

- Radicle length and plumule height (cm).
- Seedling fresh weight (g).
- Biomass (g): the seedlings dried at 72 °C for 24- 48 h till the constant weight obtained [20].

2.4.3. Moisture Content (MC %)

It was calculated due to obtaining the fresh and dry weight (FW and DW) of the shoot system and applied in the following equation used previously by [21]:

$$MC \% = (FW - \text{shoot system} - DW - \text{shoot system} / FW - \text{shoot system}) * 100$$

2.5. Study Design and Data Analysis

The study evaluates the allelopathic activity of *Salvia rosmarinus* leaf extract using 3 concentrations C1:1.00, C2: 1.5 and C3: 2 mg/ml compared to control treatment (C0). Four targeted crop plants were used; *Vicia sativa*, *Vicia ervilia*, *Hordium spontaneum*, and *Triticum aestivum* L. The experiment lay out followed a completely randomized design (CRD). Each treatment replicated 4 times. ANOVA test was calculated to examine the significant effect of the extract on germination indices, seedling growth characters and seedling moisture content using Statistical Package for the Social Sciences (SPSS) model- 25. The Duncan's multiple range test (DMRT) was employed to examine the pairwise comparison among the mean values under the significant probability (0.01).

3. Results

The GC–MS analysis of the extract from the rosemary leaves has been shown 83 identified chemical compounds (Table 1). The mostly common components (Figure 1) were; Eucalyptol with a relative area of 9.712 %, (+)-2-Bornanone (camphor) with 11.786 %, Endo-Borneol (Norborneol) with 12.17 %, Terpinen-4-ol (4-terpineol) had 12.343% relative area, and Linalool (18.73%), Ferruginol (25.171%).

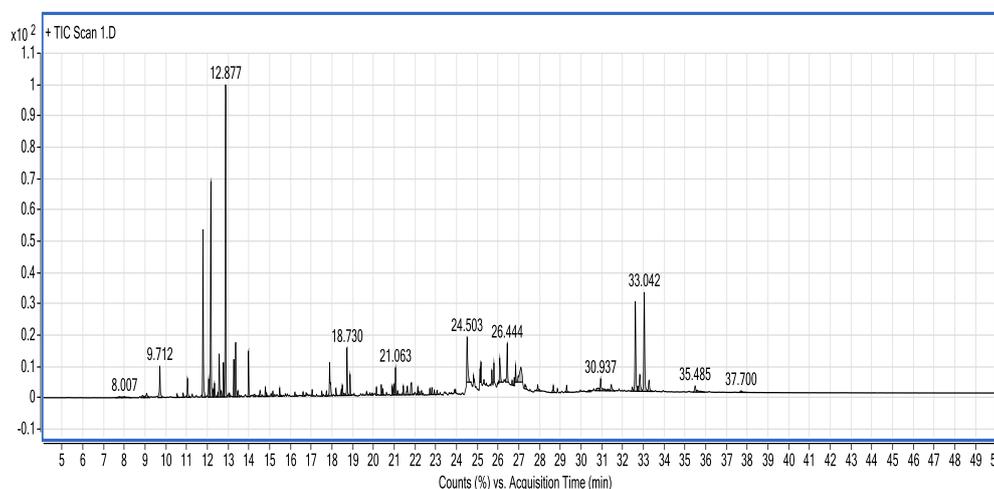


Figure 1. Dominant phytocompounds of *Salvia rosmarinus* leaf extract detected by GC-MS.

Table 1. Phytochemicals of *Salvia rosmarinus* leaf extract detected by GC-MS.

Peak	Retention time	Relative area (%)	Compound
1	7.748	0.1524	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-
2	8.007	0.2	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-
3	8.871	0.1974	Heptane, 3,4-dimethyl-
4	9.068	0.3495	3-Heptanol, 5-methyl-
5	9.712	1.5956	Eucalyptol
6	10.544	0.1793	2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-, cis-
7	10.835	0.2177	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate
8	11.039	0.9147	(4E,8E,13E)-1-(2-Hydroxyethyl)-1,5,9-trimethylcyclotetradecatriene
9	11.786	6.9121	(+)-2-Bornanone (camphor)
10	12.06	0.8285	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1.alpha.,2.alpha.,5.alpha.)-endo-Borneol (Borneol)
11	12.17	9.0657	Terpinen-4-ol (4-terpineol)
12	12.343	0.8522	.alpha.-Terpineol (α -terpineol)
13	12.571	1.8996	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-
14	12.673	0.2773	Isoborneol
15	12.775	1.1605	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-exo-2-Hydroxycineole
16	12.877	14.3222	Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-
17	13.066	0.2073	Tricyclo[4.2.1.1(2,5)]decane
18	13.27	1.3447	Bicyclo[3.1.1]heptan-3-one, 2-hydroxy-2,6,6-trimethyl-
19	13.364	2.0045	1,6-Octadiene, 2,5-dimethyl-, (E)-
20	13.467	0.4323	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-
21	13.828	0.1441	Cyclohexene, 2-ethenyl-1,3,3-trimethyl-
22	13.985	1.6656	1,3-Cyclopentadiene, 5,5-dimethyl-1-ethyl-
23	14.276	0.2326	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)-
24	14.535	0.3402	Bicyclo[2.2.1]heptane, 7,7-dimethyl-2-methylene-
25	14.794	0.5801	Tetradecane
26	15.155	0.3462	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-
27	15.485	0.3444	(1S,2S,4S)-Trihydroxy-p-menthane
28	16.216	0.3019	1,Z-5,E-7-Dodecatriene
29	16.609	0.1862	Caryophyllene oxide
30	17.049	0.2879	Caryophyllenyl alcohol
31	17.512	0.2724	Caryophyllene oxide
32	17.732	0.2151	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene
33	17.889	1.8531	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5.beta.-ol
34	18.195	0.2996	Linalool
35	18.494	0.6769	Caryophyllene oxide
36	18.73	1.7384	2,12-Dimethylidene cyclododecan-1-one
37	18.871	0.7565	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-
38	19.389	0.2001	Octadecane
39	19.664	0.1465	cis-Z-.alpha.-Bisabolene epoxide
40	20.144	0.3767	7-Hexadecene, (Z)-
41	20.379	0.7596	(3S,3aS,6R,7R,9aS)-1,1,7-Trimethyldecahydro-3a,7-methanocyclopenta[8]annulene-3,6-diol
42	20.976	0.8612	Nonadecane
43	21.063	1.0301	Isopimara-9(11),15-diene
44	21.157	0.2228	4-Indancarboxylic acid, 7-methyl-, methyl ester
45	21.44	0.5055	Dibutyl phthalate
46	21.636	0.5453	Eicosane
47	21.825	0.8243	3,7-Nonadienamide, 4,8-dimethyl-N-phenyl-, (E)-
48	22.131	0.3432	
49	22.312	0.2442	

Peak	Retention time	Relative area (%)	Compound
50	22.712	0.2526	1,15-Hexadecadiene
51	22.814	0.2903	Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)-
52	22.924	0.1929	5-Octadecene, (E)-
53	23.058	0.2465	Heneicosane
54	23.207	0.1557	Phytol
55	23.435	0.3763	9-Octadecenoic acid
56	23.953	0.3722	Nonadecane, 9-methyl-
57	24.503	6.7231	4,6-Bis(1,1'-dimethylethyl)-2',5'-dimethoxy-1,1'-biphenyl-2-ol
58	24.817	1.5597	1,1,4a-Trimethyl-5,6-dimethylenedecahydronaphthalene
59	25.171	2.1992	Ferruginol
60	25.312	0.4522	7-Bromo-2-chloroquinoline-3-carbaldehyde
61	25.697	0.5636	Acetic acid, 1-ethyl-9a,11a-dimethylhexadecahydrocyclopenta[a]phenanthren-7-yl ester
62	25.799	1.1163	Acetic acid, 1-ethyl-9a,11a-dimethylhexadecahydrocyclopenta[a]phenanthren-7-yl ester
63	26.082	2.6478	1,2,3-Trimethoxy-5-[2-(4-methoxyphenyl)ethynyl]benzene
64	26.444	2.0455	1-N,4-N-bis(Pyridin-4-ylmethylidene)benzene-1,4-diamine
65	26.671	0.2411	Propenone, 1-adamantan-1-yl-3-(1,3-dimethyl-1H-pyrazol-4-yl)-
66	26.844	0.8041	Bis(2-ethylhexyl) phthalate
67	27.103	3.8326	3-Hydroxy-6H-benzo[c]chromen-6-one, O-TMS
68	27.3	0.6861	3,6-Dipentyl-2,5-dimethylpyrazine
69	27.912	0.5259	Octadecane
70	28.659	0.4497	12-O-Methylcarnosol
71	28.855	0.1646	Squalene
72	29.303	0.3338	Eicosane
73	30.819	0.3886	Tris(tert-butyldimethylsilyloxy)arsane
74	30.937	0.5019	Vitamin E
75	31.455	0.5394	1-N,4-N-bis(Pyridin-4-ylmethylidene)benzene-1,4-diamine
76	32.461	0.2108	4-Methyl-2-trimethylsilyloxy-acetophenone
77	32.61	4.6884	.beta.-Amyrone
78	32.728	0.2269	Tris(tert-butyldimethylsilyloxy)arsane
79	32.822	0.9945	.beta.-Amyrin
80	33.042	5.2949	.beta.-Amyrone
81	33.27	0.7899	.alpha.-Amyrin
82	35.485	0.5642	Ethoxy(phenyl)silane diol, 2TMS
83	37.7	0.1542	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene

Figure (2) provided is a line graph showing the effect of different concentrations of rosemary leaf extract; C0, C1, C2, and C3 on (G %) of four plant species: *Vicia sativa*, *Vicia ervilia*, *Hordeum spontaneum*, and *Triticum aestivum* L. Likely increasing concentrations of an allelopathic agent (rosemary extract) applied led to decrease G % for most species. *Vicia ervilia* showed a significant decrease in the G% as the concentration increased. The lowest value was 30% due to 2 mg/ml (C2) which is significantly differ from the others except C1. *Vicia sativa* shows a mild tolerance to 1.5 mg/ml (C1). However, 2 mg/ml had a significant effect in declining the rate of germinated seeds and led to be the lowest value (71.33) recorded. *Vicia ervilia* exhibit a steep decline as the concentration increased. While, *Vicia sativa* is more stable except at the higher concentrations (2.5 and 2mg/ L). *Hordeum spontaneum* germination percent decreased sharply, with a significant decline at C3. The lowest value was 34.40 % due to C2 which is significantly differ from the G % due to other concentrations. The rosemary leaf extract not impacted significantly on the ability of the *Triticum aestivum* L. grains to germinate. *Triticum aestivum* L. exhibited more resilience, followed by *Vicia sativa*. But, *Hordeum spontaneum* and *Vicia ervilia* germination percent remarkably declined, particularly due to high concentrations of rosemary leaf extract.

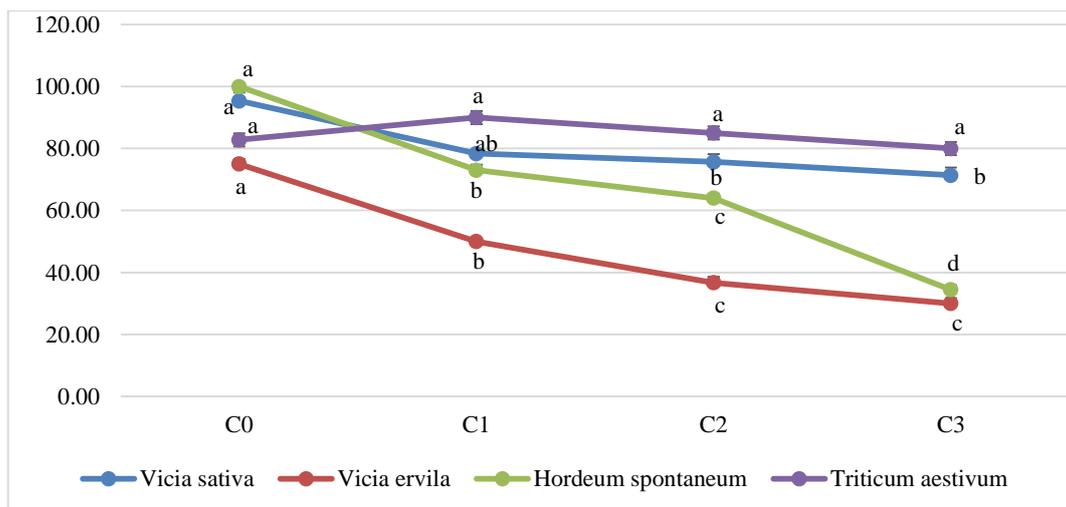


Figure 2. Effects of *Salvia rosmarinus* extract on germination percent (G %) of *Vicia sativa*, *Vicia ervila*, *Hordeum spontaneum* and *Triticum aestivum* L. (C0, C1, C2 and C3: control, 1, 1.5 and 2 mg/ml).

Figure (3) illustrates the impact of rosemary leaf extract concentrations on GV (GV). At C0 (control), *Vicia sativa* had a GV of approximately 13.80. The lowest concentration of the rosemary leaf extract not affected significantly on the GV of *Vicia sativa*. While at C2 and C3 a further decrease observed (10.89 and 10.11), with a significant difference compared to C0. *Vicia ervila* showed a significant difference in their GV due to the applied concentrations 1, 1.5 and 2 mg/ml of rosemary extract. A significant and gradual decline of GV observed 7.56, 4.74, and 4.14 in order, as the concentration increased compared to the control. Thus, *Vicia ervila* seeds vigor could be regarded most sensitive to all concentrations of the extract used. A sharp decline observed in the velocity of *Hordeum spontaneum* seed germination. As the concentration increased the velocity decreased, and the lowest velocity recorded was 5.12 due to treating the seeds with 2mg/ml. In contrast, *Triticum aestivum* L. GV not affected significantly due to the concentrations that used and remained stable.

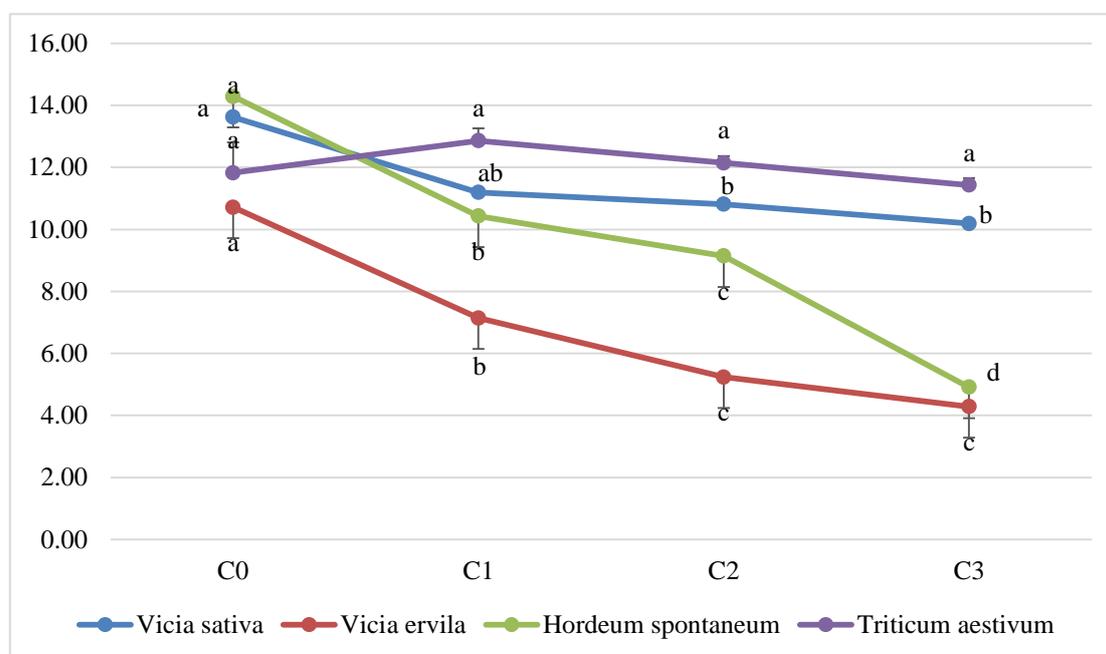


Figure 3. Effect of *Salvia rosmarinus* extract on GV of *Vicia sativa*, *Vicia ervila*, *Hordeum spontaneum* and *Triticum aestivum* L. (C0, C1, C2 and C3: control, 1, 1.5 and 2 mg/ml).

Figure (4) shows the impact of rosemary extract levels on the (SVI) of the 4 species. The *Vicia sativa* seedlings not affected by the rosemary extract and no significant difference observed in the seedling vigor. This suggests that its vigor remains unaffected with increasing concentration, and suggested species specify allelopathically effect of rosemary leaf extract. Similarly, the SVI of *Vicia ervilia* seedlings were not affected significantly. While, *Hordeum spontaneum* exhibited a gradual significant decreased of their SVI as the concentration of the extract increased. The lowest value of the vigor index 2.63 was recorded due to treatment of the seeds with C2 compared to control (2.13). It means that vigor of the *Hordeum spontaneum* had negative allelopathic response to rosemary extract. *Triticum aestivum*'s vigor decreased significantly due to the applied concentrations, but not sharp decline as compared to *Hordeum vulgare*. The lowest values were recorded due to treating the seeds with C2 and C3.

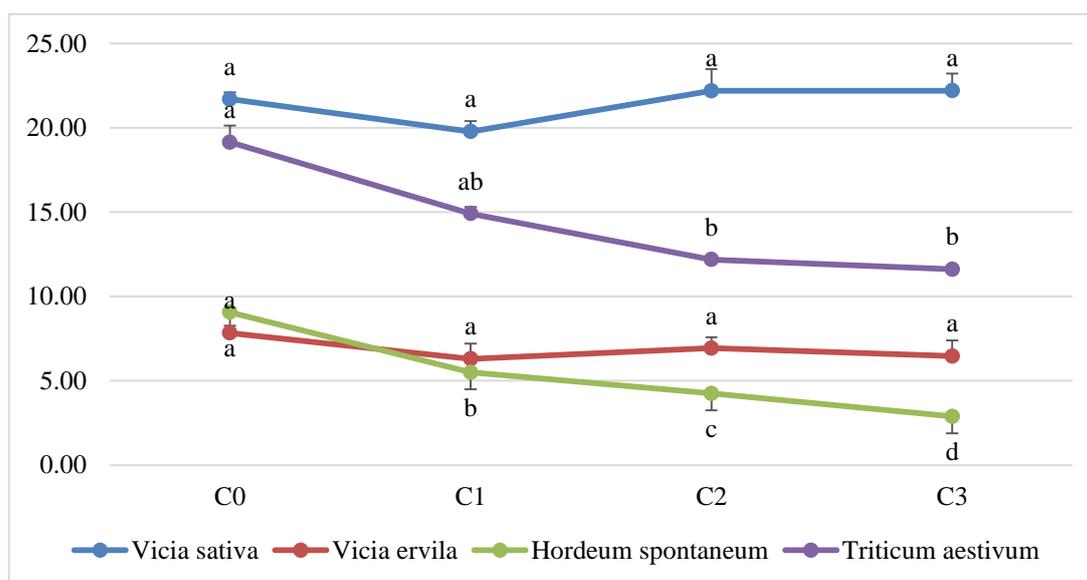


Figure 4. Effect of *Salvia rosmarinus* extract on SVI of *Vicia sativa*, *Vicia ervilia*, *Hordeum spontaneum* and *Triticum aestivum* L. (C0, C1, C2 and C3: control, 1, 1.5 and 2 mg/ ml).

Figure (5) shows the effect of rosemary leaf extract on shoot length of *Vicia sativa*, *Vicia ervilia*, *Hordeum spontaneum*, and *Triticum aestivum* L. The shoot length of *Vicia sativa* not affected by the low concentration (C1). But, the high concentrations (C2 and C3) were impacted significantly on the shoot length. It means that rosemary extract at higher concentrations had a negative allelopathic effect that led to a reduction on the shoot length and the lowest value was 2.91 cm was recorded due to C3. All used concentrations caused a gradual decrease of *Vicia ervilia* shoot length. It means as the concentration of the extract increased, the shoot length decreased significantly; thus, the lowest value was recorded 0.90 cm at C2. *Hordeum spontaneum* remained stable due to applying the low and moderate concentrations (C1 and C2). But the highest concentration (C3) caused a significant decrease in the seedling height which was 3.20 cm. In contrast to other studied species, *Triticum aestivum* L. shoot length increased significantly by treating the seeds with all concentrations of the rosemary leaf extract. A gradual increase 6.00, 7.10, and 6.98 cm, respectively was observed due to effects of C1, C2, and C3 compared to control group (4.15 cm).

Figure (6) shows the effect of rosemary extracts on the root length of the studied species. *Vicia sativa* root length decreased significantly at C2 and C3 (3.80 and 3.78 cm) as compared to the control treatment. While C1 led to a slight decrease but was not significantly different from C0, C2, and C3. *Vicia ervilia* root length reduced due to C1, C2, and C3 as compared to C0. The shortest radicle was 1.51 cm was recorded due to applying C3. The same trend of root length declines due to rosemary extract observed in *Hordeum spontaneum*. The lowest value was 3.80 cm recorded by C3. This suggests the inhibitory role of the allelopathic agent used (rosemary leaf extract) on the root length of *Vicia sativa*, *Vicia ervilia*, and *Hordeum spontaneum*. The opposite effect was observed in the root

length of *Triticum aestivum* seedlings. C1, C2, and C3 were regarded as promoters for increasing its radicle as compared to control (C0). The longest root was 8.80 cm due to the act of adding 1.5 mg/ml of rosemary leaf extract.

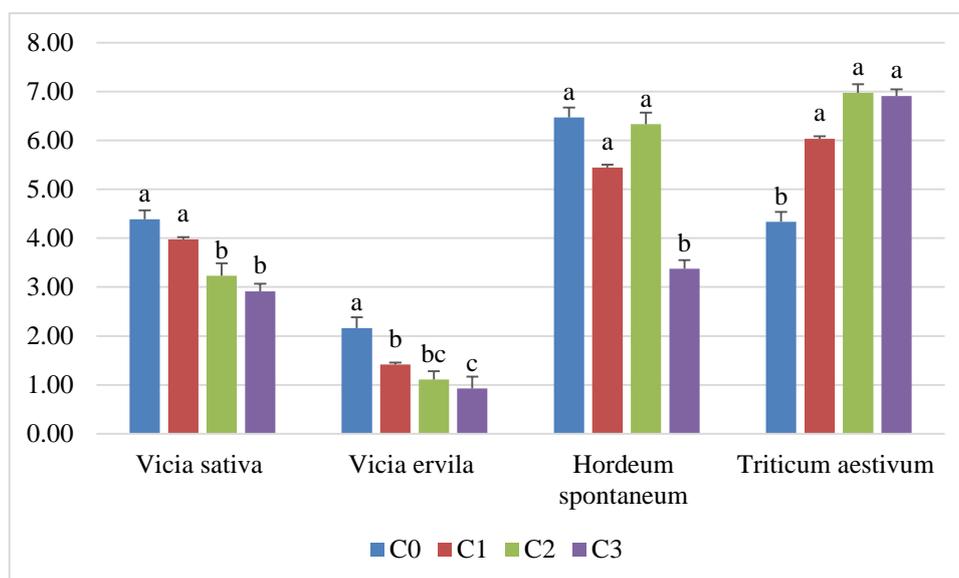


Figure 5. Effect of *Salvia rosmarinus* extract on shoot length (cm) of *Vicia sativa*, *Vicia ervila*, *Hordeum spontaneum* and *Triticum aestivum* L. seedlings. (C0, C1, C2 and C3: control, 1, 1.5 and 2 mg/ml).

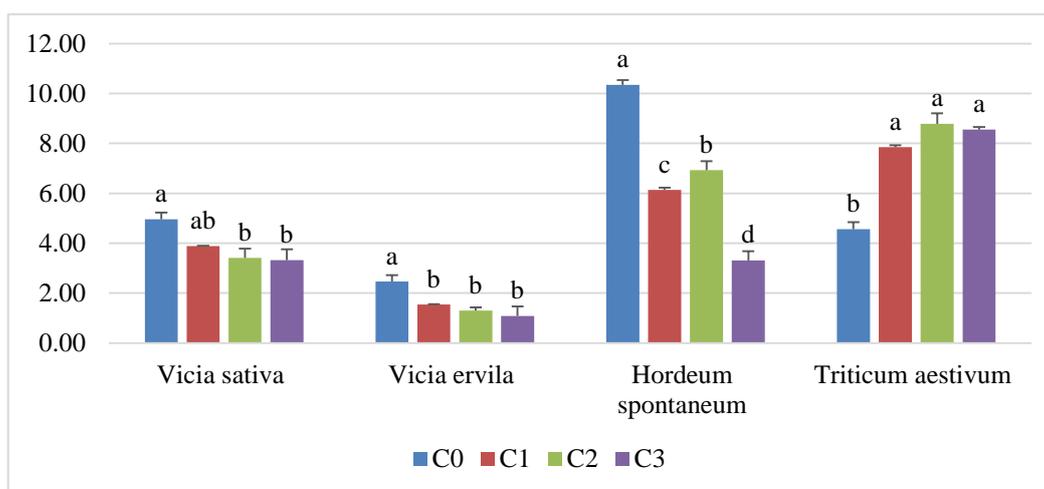


Figure 6. Effect of *Salvia rosmarinus* leaf extract on root length (cm) of *Vicia sativa*, *Vicia ervila*, *Hordeum spontaneum* and *Triticum aestivum* L. seedlings. C0, C1, C2 and C3: control, 1, 1.5 and 2 mg/ml.

Table 2 shows the effect of rosemary leaf extract on seedling biomass (g) and moisture content (%) of the 4 species. The seedling dry matter content and moisture content of *Vicia sativa* were not significantly affected by the applied concentrations of rosemary leaf extract. *Vicia sativa* biomass is relatively stable across all the treatments. The differences between treatments are not statistically significant. Despite a slight decrease but not significant in C1 and C3, the values are comparable to the control (C0). As well, the moisture content remains steady across all treatments, with no significant variations. The seedling biomass of *Vicia ervila* decreased significantly due to the allelopathic effects of C1, C2, and C3. But the remarkable and significant decrease in dry matter accumulation of the seedlings was 0.24 g due to C3, and being different significantly from the control (0.44 g), which negatively affected the biomass accumulation. The seedling moisture content varied slightly but not significantly. So, it might be suggested that, while biomass is affected due to the inhibition effect of

the extract on the metabolic function, water content remained stable across all the treatments. A clear, sharp pattern decrease was observed in biomass accumulation with an increase in rosemary leaf extract concentrations in *Hordeum spontaneum* seedlings, where the lowest value was 0.12 g at C3 significantly differ compared to other treatments. The seedling's moisture content increased with increased concentration progressively. The highest value was 94.67% recorded due to the effect of the 3rd concentration (2 mg/ml). Which means the retention of water due to not assimilating it in the metabolic process to store the biomass, which is declined due to the negative allelopathic effect of the extract. The seedling biomass of *Triticum aestivum* L. increased significantly (0.48 and 0.52 g) by treating them with C1 and C2, but a slight drop was recorded (0.40 g) due to the highest concentration used (C3) as compared to the lowest biomass (0.34 g) recorded by the control. But the moisture content did not differ significantly among the seedlings due to the different concentrations applied. Although *Triticum aestivum* L. seedlings' moisture content and biomass changed slightly, the variations were not as noticeable as those found in *Hordeum spontaneum*.

Table 2. Effect of *Salvia rosmarinus* extract on seedling biomass (g) and moisture content (%) of *Vicia sativa*, *Vicia ervila*, *Hordeum spontaneum* and *Triticum aestivum*.

	Seedling biomass (g)	Moisture content (%)
<i>Vicia sativa</i>		
C0	0.43 ± 0.01 a	82.82 ± 2.55 a
C1	0.35 ± 0.11 a	84.27 ± 0.93 a
C2	0.42 ± 0.001 a	81.97 ± 3.26 a
C3	0.37 ± 0.005 a	82.59 ± 4.18 a
<i>Vicia ervila</i>		
C0	0.44 ± 0.008 a	70.34 ± 1.98 a
C1	0.32 ± 0.003 ab	73.44 ± 2.39 a
C2	0.31 ± 0.002 ab	69.89 ± 3.95 a
C3	0.24 ± 0.003 b	71.41 ± 1.82 a
<i>Hordeum spontaneum</i>		
C0	0.45 ± 0.009 a	89.02 ± 3.89 c
C1	0.25 ± 0.007 c	92.24 ± 2.21 b
C2	0.33 ± 0.006 b	92.08 ± 2.11 b
C3	0.12 ± 0.02 d	94.67 ± 1.08 a
<i>Triticum aestivum</i> L. (V4)		
C0	0.34 ± 0.007 c	80.48 ± 2.79 ab
C1	0.48 ± 0.008 a	78.11 ± 1.03 b
C2	0.52 ± 0.010 a	82.05 ± 1.46 ab
C3	0.40 ± 0.001 b	81.42 ± 2.65 a

4. Discussion

The data related to GC-MS analysis showed that the dominant constituents of the rosemary leaf extract were in parallel with the results found in other studies. However, there are usually considerable variations in the % of the major components of rosemary cultivated in different geographical origins [22]. [23] identified α -pinene as being 19.01%, followed by the eucalyptol (5.49%) and camphor (5.71%). [24] recognized α -pinene (23.98%), camphor (22.62%), and eucalyptol (18.76%) respectively. [25] showed that the major components of Brazilian rosemary were α -pinene (40.55–45.10%), 1,8-cineole (17.40–19.35%), and camphene (4.73–6.06%). Alternatively, [26] defined 1,8-cineole (23.04%), eucalyptol (14.01%), and terpinen-4-ol (13.8%) as a dominant component of rosemary. [27] identified essential oil derived from rosemary leaves and defined camphor as a significant component (12.4%). As well as highlighted eucalyptol (40.1%) and α -pinene (12.9%) as two other major components of the rosemary essential oil. Similarly [28] reported the dominant constituents as eucalyptol (1,8-cineole) with 48.01% of the relative area, α -pinene (11.50%) and camphor (8.65%).

Seed germination is regulated internally and affected by various external factors. Phytohormones or organic acids have a significant effect on the process [29] and [30]. The data obtained in the study revealed the allelopathic effect of rosemary leaf extract on seed germination; the influence was highly negative on *Vicia sativa*, *Vicia ervila*, and *Hordeum spontaneum* rather than *Triticum aestivum* L.

Allelochemicals can negatively affect the growth of weeds, due to its impact on the chlorophyll content and decline in photosynthesis rate, stability of cell membrane, enzymatic activity decline [31]. Thus, the inhibitory action of a compound may vary plant to plant, as well as form one species of plant to another. The potential phytotoxicity of the leaf extract of *Rosmarinus officinalis* was reported by [32]. [33] reported that cineol had a toxic effect on *Schizachyrium scoparium* but not on *Leptochloa dubia*. The allelopathic property of eucalyptol as an inhibitor for seed germination was observed in various plant species. In addition, their use even at low concentrations can significantly reduce the germination rate of weeds, which encouraged its potential use as a natural herbicide [4]. Linalool had an inhibitory effect on certain weed species, because it can disrupt the physiological nature of seed germination, leading to decreased germination %s and slow growth of the seedling [34]. Camphor had been found to negatively impact the seed germination, and its use as a natural herbicide supported [35]. The volatile ability of camphor makes it to diffuse fast in its environment and interact. It can diffuse in to the seed and reach the embryo. Impacts negatively on embryo respiration and disrupt the enzymatic activity for braking down the stored food for embryonic growth [36]. This results in a reduction in germination % and seedling growth, particularly at higher concentrations. Terpinen-4-ol as well has been studied for its significant inhibitory influence on the germination of specific seed weeds thought to interfere with seed metabolism and hormonal balance [37]. Eucalyptol causes oxidative stress in the cell and driven of reactive oxygen species (ROS). They could damage the cellular structures and interfere the radicle emergence and elongation [38].

Conclusions

The allelopathic effects of *Salvia rosmarinus* ethanolic leaf extract demonstrate its potential as a natural bioherbicide due to it enrich content with linalool, ferruginol, camphor, α -terpineol, and eucalyptol. The response could be concluded species-specific, *Triticum aestivum* demonstrated an increase in germination indices and growth characters even at lower concentrations, while *Vicia sativa*, *Vicia ervilia* and *Hordeum spontaneum* were sensitive, demonstrating its effectiveness in weed control.

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