



Allelopathic Potential of Aqueous Extracts and Essential Oils of *Rosmarinus officinalis* L. and *Thymus vulgaris* L

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Abstract

Allelopathic effects of essential oils and aqueous extracts from *Rosmarinus officinalis* (rosemary) and *Thymus vulgaris* (thyme) have been investigated as a source of new natural herbicidal compounds to suppress two weeds: *Chenopodium album* and *Echinochloa crus-galli* associated with two important economic crops, *Triticum aestivum* (wheat) and *Oryza sativa* (rice), respectively. A petri-dish experiment was performed, and 10, 20, 50, and 100% aqueous solutions, and doses of 0, 2, 4, 8, and 16 µL of essential oil per petri dish were applied. The number of germinated seeds in each Petri dish was counted daily. After 15 days, germination percentage, the coefficient of velocity of germination, speed of germination, germination energy, germination index, mean germination time, seedling shoot length, root length, shoot–root ratio, and seedling vigour index were recorded. The chemical profiles of both essential oils were identified with GC–MS analysis. Eucalyptol (1,8-cineole), α-pinene, and camphor were identified as the dominant constituents of the rosemary essential oil. The major components detected in thyme essential oil were thymol, o-cymene, caryophyllene, and gamma-terpinene. The results showed that thyme essential oils had the most negative effect by inhibiting the germination of weeds. Both rosemary and thyme essential oils had a generally reducing effect on the growth parameters of weeds. The essential oils of rosemary and thyme have allelopathic potential and should be assessed as allelopathic agents to be used for weed control.

Keywords *Chenopodium album* · *Echinochloa crus-galli* · Essential oils · Natural herbicides · Organic farming · *Oryza sativa* · *Triticum aestivum*

1 Introduction

Wheat is considered one of the most important crops in the winter season in Egypt. While rice is dominant among the summer crops, the local production of wheat is about

8.8 million tons obtained from 3.13 million feddan (FAO 2018) and it requests about 30% of caloric intake of the Egyptians dietary. Nearly half of the world's population relies on rice as a staple food (Lu 2004). Undoubtedly, weeds are representing severe biotic stress toward these important economic crops. More specifically, weeds are responsible for reduction of 23% of wheat yields worldwide (Oerke 2006) also, caused a significant decline in rice output. Some weeds are also shown for their allelopathic or toxic effect on the wheat germination and seedling growth (Fragasso et al. 2013). *Chenopodium album* one of the most predominant weed in the wheat fields (Siddiqui and Bajwa 2001) which lead to significant reduction in grain yield up to 65% (Siddiqui 2005). *Echinochloa crus-galli* (L.) Beauv. is the most prevalent rice weed. One of the worst rice weeds is thought to be this one. *Echinochloa* spp. infestations in rice can result in extremely severe and variable yield losses depending on the cultivar and the length of the competition (Fischer et al. 1997). Accurate weed control is needed for wheat- and rice-based food

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security for every country of the world. The expense of cultivation is increased by using conventional weed management techniques that involve human weeding (Singh et al. 2016).

Synthetic pesticides are used in agriculture all over the world, but their widespread usage has led to pest resistance, and they can also have a negative impact on both the environment and human health. This has caused alternative development tactics for novel environmentally friendly biodegradable compounds, particularly in organic farming, to be given more thought (Jabran et al. 2015). Therefore, replacement of them with natural compounds would significantly advance environmental protection. Plant-derived natural compounds have advantages over synthetic chemicals such as fast biodegradability, low risk of subsequent pest/weed resistance, and relatively weak toxicity to non-target organisms (Chandler et al. 2011; Isman 2015; Pavela and Benelli 2016). A biological approach to weed control provides a fantastic alternative in organic systems. Allelochemicals form the basis of the biological process. The majority of allelochemicals, including O, alkaloids, tannins, and glycosides, are categorized as secondary metabolites in plants and are used as substitute weed-control methods (Kruse et al. 2000). Allelochemicals can simulate or inhibit the germination and growth of plants. Proverbially, plant-derived allelochemicals do not exert residual or toxic effects. Therefore, they are considered as the perfect alternates for synthetic herbicides (Cheng and Cheng 2015). According to Bonanomi et al (2006) and Macías et al. (2007), plant essential oils (EOs) and water extracts can be employed as a medium for the production of allelochemicals activity to inhibit the growth of other species. Allelochemicals have been recommended by a number of studies for usage in field applications as well as laboratory settings for the reduction of weeds (Farooq et al. 2011). According to Jamil et al (2009), using allelopathic water extract to control weeds is a significant and practical technique to take advantage of the allelopathic potential of agricultural plants. Due to their volatility, essential oils make effective fumigants. EOs are less hazardous to humans, animals, and the environment than synthetic products. Due to their unique composition and quick degradation in soil, EOs are regarded as environmentally safe (Grosso et al. 2010). EOs have been shown to produce allelopathic effects (Verdeguer et al. 2020). Terpenoids and allelochemicals in EOs can be utilized to stop the germination and growth of weeds (Nikolova and Berkov 2018).

The aims of this study were to characterize the chemical constituents of essential oils in two therapeutic plants, rosemary and thyme using GC–MS, and evaluate their water extracts and essential oils as allelopathic agents on seed germination and seedling growth of wheat and rice, as well as their associated weeds *Chenopodium album* and *Echinochloa crus-galli*.

2 Materials and Methods

2.1 Preparation of Plant Materials

The plant samples of rosemary and thyme were supplied from a local farm in Burg El-Arab (45 km west of Alexandria), Egypt. The leaves of the two species were carefully selected, rinsed under running water to remove dust and impurities from the surface, and then dried at room temperature for 5 days before being ground into powder using a Wiley mill (Model 4-GMI). Seeds of wheat and rice were purchased from the Agriculture Research Centre, El-Dokki, Giza, Egypt. While during the spring of 2022, seeds of *Chenopodium album* and *Echinochloa crus-galli* were gathered from some agricultural fields near Alexandria. The seeds were lightly washed on surface and kept in paper bags in the refrigerator until needed.

2.2 Preparation of Aqueous Extracts

By soaking 100 g of the air-dried donor plant materials (rosemary and thyme) in 1 L of distilled water at $(20 \pm 2^\circ\text{C})$ for 24 h with shaking, stock aqueous extract was made. To remove the fibre debris, the mixture was filtered through four layers of cheesecloth. Then, Whatman No. 1 filter paper was used, and the purified extract was then adjusted to pH 6.8 with NaOH 10%. From the stock solution, various concentrations (10, 20, 50, and 100%) as well as the control with distilled water were prepared.

2.3 Extraction of Essential Oils

The essential oils were extracted from rosemary and thyme by hydro-distillation through passing steam produced in one round-bottomed flask, into one more 3-L flask containing 1 kg of the pure grinded leaves of rosemary and thyme along with 1 L distilled water. The steam crosses the plant, charged with essential oil so the vapour mixture of water–oil passes to the condenser, where it is condensed. The process was carried out for 2 h to obtain maximum oil and was repeated until no more oil was left. After condensation, the condensate (water–oil) was collected in a round-bottomed flask. Subsequently, the oil was separated from water by decantation after every fraction. Finally, the essential oil was dried on anhydrous sodium sulphate and stored at 4°C before, GC–MS analysis.

2.4 GC–MS analysis of Essential Oil

Shimadzu-QP-2010 ULTRA gas chromatography/mass spectrometry was used to evaluate both essential oil samples.

The oven temperature was programmed at 60°C for 3 min and then raised to 260°C with 5 rate and hold time 10.00 min. In the TRB-WAX column (30 m×0.25 mm internal diameter and film thickness 0.25 m) at a flow rate of 1.9 mL/min, helium carrier gas was used, injection mode was split, and injection temperature was 200°C. The oven temperature was programmed at 60°C for (3 min), then raised to 260°C with 5 rate, and hold time 10.00 min, and the ionization mode was electronic impact mode (SEI). By comparing their mass spectra with the National Institute of Standards and Technology (NIST11s) database library and other available references, the components of the oil were identified.

2.5 Germination Bioassay

The research was carried out in the lab of Faculty of Science, Botany and Microbiology Department in 2022. The experiment was run as a factorial with three replications using a completely randomized statistical design. Sodium hypochlorite solution was used to sterilize seeds of wheat and rice, as well as *Chenopodium album* and *Echinochloa crus-galli* for about 2 min. The seeds were then repeatedly rinsed in distilled water. Under standard laboratory settings with day temperature range of 25–30°C and night temperature range of 20–25°C, a total of seeds from each plant were distributed in 9-cm diameter Petri-dishes on 2 discs of Whatman No.1 filter paper wetted with the solutions previously prepared of water extracts with varying concentrations (10, 20, 50, and 100%) and essential oil with various doses of 2, 4, 8, and 16 µL per petri dishes in three replicates to test the inhibitory effect of the two plants: rosemary and thyme. Control was prepared by adding distilled water. Each Petri dish's number of germinations was counted every day.

2.6 Specific Analyses

Germination parameters such as germination percentage (GP%), the coefficient of velocity of germination (CVG), speed of germination (SG), germination energy (GE), germination index (GI), mean germination time (MGT), seedling shoot length (SL), seedling root length (RL), shoot–root ratio (SL/RL ratio), and seedling vigour index (SVI) were recorded by the following equations:

Germination percentage (GP%) = number of germinated seeds/total number of seeds × 100.

The Coefficient of Velocity of Germination (CVG) = $N / (1 + N_2 + \dots + N_i / 100 \times N_1 T_1 + \dots + N_i T_i)$ where N is the total number of seeds germinated every day and T is the number of days corresponding to N and i is the last day of germination (Jones and Sanders 1987).

Speed of germination (SG) = N_i / D_i number/day (Khan and Ungar 1998).

As N_i is the number of seeds germinated on day i , D_i is the number of days after sowing,

Germination energy (GE) = Number of germinated seeds at 4 days/Total number of seeds tested × 100.

Germination index (GI) = $\sum (E_t / D_t)$ where E_t is the number of emergence seedlings in t days and D_t is the number of corresponding germination days.

Mean germination time (MGT) = $\sum f_x / (\sum f_1 \times 1 + \dots)$.

Seedling shoot length (SL), seedling root length (RL), shoot–root ratio (SL/RL ratio): Five plant individuals per treatment were used for determination of shoot and root lengths using measuring tape. The shoot/root ratio was calculated for each treatment.

Seedling vigour index (SVI) = Seedling total length (cm) × germination percentage as described by Hossain et al. (2006)

2.7 Statistical Analysis

Results were given as mean ± SE of the mean. Using the CoHort Software Company's COSTAT 2.00 statistical analysis programme, the data from the current investigation were subjected to the standard one-way analysis of variance (ANOVA) (Zar 1984). Using Duncan's multiple range test, the differences between treatment means were separated at a 5% level of significance.

3 Results

3.1 Essential Oil Composition

The GC–MS analysis of the pale-yellow oil isolated from Egyptian rosemary leaves has been shown 50 identified components representing 100% of the total oil (Table 1 and Fig. 1). The dominant constituents were eucalyptol (1,8-cineole) with 48.01% of the relative area, α-pinene (11.50%) and camphor (8.65%). Concerning thyme, GC–MS analysis of the extracted reddish-brown oil has been shown 65 identified components representing 100% of the total oil (Table 2 and Fig. 2). The dominant components were thymol with 14.79% of the relative area, o-cymene (8.97%), caryophyllene (8.91%), and gamma-terpinene (4.66%).

3.2 Allelopathic Effect

The potential allelopathic effects of rosemary and thyme of both aqueous extracts and essential oils in germination of wheat and its associated weed, *Chenopodium album*, were illustrated in Fig. 3 and Plate 1. The inhibitory activity was observed significantly with the increase of concentration of both aqueous extracts and essential oils of both plants

Table 1 Composition of *Rosmarinus officinalis* L. essential oil (the dominant constituents illustrated in bold)

Peak no	Compound	Retention time (min)	% of relative area
1	2-Methylbutane	1.091	11.19
2	6-Chloro-2-(O-methoxyphenyl)-4-phenylquinoline	1.125	0.10
3	Stannic bromide	1.259	0.19
4	Allene	1.291	0.24
5	Tricyclene	2.086	0.11
6	α-Pinene	2.197	11.50
7	Toluene	2.373	0.13
8	α-Fenchene	2.518	0.15
9	Camphene	2.605	2.86
10	2-methyl-2-pentanol	2.971	0.11
11	L-β-Pinene	3.105	1.19
12	2,4(10)-Thujadiene	3.364	0.07
13	β-Myrcene	4.043	1.29
14	(+)-4-Carene	4.298	0.34
15	D-Limonene	4.665	2.22
16	Eucalyptol	4.855	48.01
17	γ-Terpinene	5.670	0.15
18	o-Cymene	6.255	1.98
19	2-Carene	6.523	0.08
20	1-Octen-3-ol	10.969	0.13
21	Copaene	11.708	0.20
22	Camphor	12.243	8.65
23	β-Linalool	13.489	0.79
24	Pinocarvone	13.609	0.11
25	Bornyl acetate	14.027	0.15
26	2-Norbornanol	14.221	0.08
27	Caryophyllene	14.308	1.41
28	L-terpinen-4-ol	14.650	0.50
29	Humulene	16.075	0.12
30	α-Terpineol	16.403	0.34
31	γ-Murolene	16.611	0.11
32	Isoborneol	17.021	3.27
33	β-Cadine	18.265	0.21
34	3-phenyldecane	18.917	0.06
35	6-phenylundecane	20.188	0.06
36	2-phenyldecane	20.289	0.10
37	5-phenylundecane	20.346	0.13
38	4-phenylundecane	20.625	0.14
39	3-phenylundecane	21.258	0.16
40	6-phenyldodecane	22.382	0.10
41	5-phenyldodecane	22.547	0.10
42	2-phenylundecane	22.606	0.25
43	4-phenyldodecane	22.917	0.09
44	3-Phenyldodecane	23.517	0.12
45	6-phenyltridecane	24.487	0.11
46	5-phenyltridecane	24.699	0.07

Table 1 (continued)

Peak no	Compound	Retention time (min)	% of relative area
47	2-Phenyldodecane	24.838	0.20
48	4-phenyltridecane	25.082	0.07
49	3-phenyltridecane	25.691	0.10
50	2-Phenyltridecane	26.982	0.16
Total:			100.00

specially thyme essential oil which prevent the germination of weed totally (Plate 1d). Data showed that there is notable increase in germination percentage of wheat for both aqueous extracts of the two plants in low concentrations (10 and 20%) compared to respective control, while it reduced at high concentrations. In general, the inhibition in germination percentage was higher for the essential oil of thyme than rosemary, and it was more pronounced at increasing dose of the respective oil.

The potential allelopathic effects of different doses for both aqueous and essential oils of rosemary and thyme in different germination parameters of *Triticum aestivum* (wheat) and its associated weeds *Chenopodium album* were illustrated in Table 3 and 4, respectively. Results showed that doses of essential oils for rosemary were affected less compared to thyme which has negative effect on the coefficient of velocity of germination (CVG), speed of germination (SG), germination index (GI), and seedling vigour index (SVI).

The germination energy (GE) of wheat was reported to increase as the dose of aqueous extract for both thyme and rosemary increase; however, it decreased as the dose of the two essential oils increased. In comparison to rosemary essential oil, thyme essential oil had a stronger allelopathic effect on the tested weed. The inhibitory effects of the two different essential oil types and doses against *Chenopodium album* were also found to differ significantly, according to the results.

Mean germination time (MGT) was increased as the concentration of aqueous and essential oil increased; the highest increase of wheat (5.28%) was attained at 10% aqueous extract of rosemary treatment when compared with control. While the highest increase (78%) was recorded at 8 and 16 µL essential oil of rosemary, results showed that there was notable increase for MGT of *Chenopodium album* in response to the effect of the aqueous and essential oil of rosemary, 20% attained with the highest increase (4%) and 16 µL essential oil attained with the highest value (3.36%) compared with control.

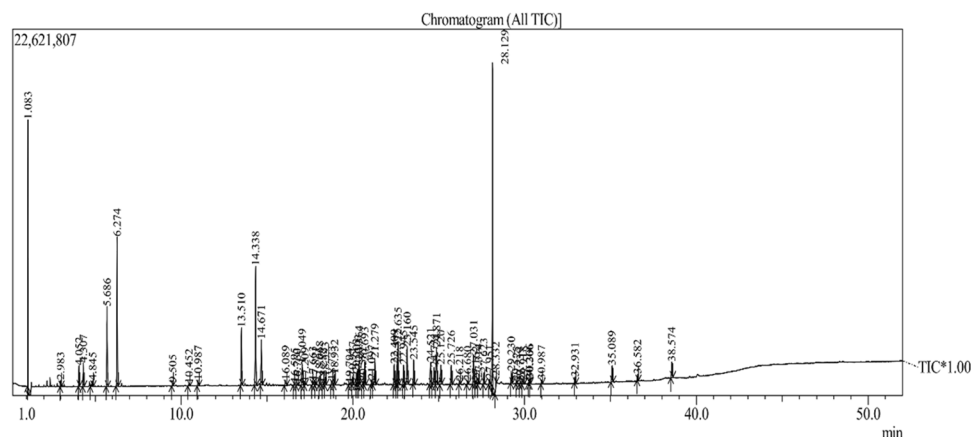
Wheat seedling shoot length (SL) increased significantly when aqueous extract and essential oils of thyme and rosemary were used at lower dosages. The highest increase

Table 2 Composition of *Thymus vulgaris* L. essential oil (the dominant constituents illustrated in bold)

Peak no	Compound	Retention time (min)	% of relative area
1	2-Methylbutane	1.083	7.01
2	2-methyl-2-pentanol	2.983	0.34
3	β -Myrcene	4.052	1.10
4	(+)-4-Carene	4.307	1.21
5	trans-Sabinene hydrate	4.845	0.45
6	γ-Terpinene	5.686	4.66
7	α-Cymene	6.274	8.97
8	3-Octanol	9.505	0.18
9	2,6-Dimethylstyrene	10.452	0.11
10	1-Octen-3-ol	10.987	0.35
11	β -Linalool	13.510	3.71
12	Caryophyllene	14.338	8.91
13	L-terpinen-4-ol	14.671	3.14
14	Humulene	16.089	0.39
15	β -Citral	16.595	0.19
16	(+)-Ledene	16.760	0.17
17	Isoborneol	17.049	1.80
18	Carvenone	17.209	0.21
19	β -Bisabolene	17.663	0.29
20	α -Citral	17.821	0.20
21	5-phenyldecane	18.068	0.62
22	β -Cadine	18.282	0.17
23	4-phenyldecane	18.393	0.55
24	α -Bergamotene	18.773	0.31
25	3-phenyldecane	18.932	0.69
26	β -Damascenone	19.794	0.13
27	Anethole	19.977	0.56
28	6-phenylundecane	20.203	0.76
29	2-phenyldecane	20.303	1.19
30	5-phenylundecane	20.364	1.62
31	p-Cymen-8-ol	20.582	0.30
32	4-phenylundecane	20.693	1.62
33	Carvacryl acetate	21.097	0.37
34	3-phenylundecane	21.279	1.97
35	6-phenyldodecane	22.409	1.49
36	5-phenyldodecane	22.575	1.62
37	2-phenylundecane	22.635	3.05
38	4-phenyldodecane	22.945	1.42
39	Caryophyllene oxide	23.160	2.82
40	3-Phenyldodecane	23.545	1.77
41	6-phenyltridecane	24.521	1.87
42	5-phenyltridecane	24.731	1.42
43	2-phenyldodecane	24.871	2.83
44	4-phenyltridecane	25.120	1.58
45	3-phenyltridecane	25.726	1.40
46	(-)-Spathulenol	26.218	0.21
47	Methyl 9,10-bis[(trifluoroacetyl)oxy]octadecenoate	26.680	0.25
48	2-phenyltridecane	27.031	2.33
49	Chavibetol	27.162	0.24

Table 2 (continued)

Peak no	Compound	Retention time (min)	% of relative area
50	Isothymol	27.314	0.40
51	Thymol	27.623	0.78
52	Isothymol	27.951	0.32
53	Thymol	28.129	14.01
54	3-Butyn-1-ol	28.332	0.46
55	3-Benzylsulfonyl-2,6,6-trimethylbicyclo(3.1.1)heptane	29.230	0.81
56	Alloaromadendrene oxide-(1)	29.525	0.15
57	3-Benzylsulfonyl-2,6,6-trimethylbicyclo(3.1.1)heptane	29.739	0.09
58	5,15-Rosadiene	29.918	0.29
59	Caryophyllene oxide	30.266	0.27
60	Verticillol	30.306	0.32
61	Viridiflorol	30.987	0.26
62	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	32.931	0.39
63	Nerolidol isobutyrate	35.089	1.25
64	2,2-Dimethyl-3-[(3E,7E,11E,15E)-3,7,12,16,20-pentamethyl-3,7,11,15,19-henic-osapentaenyl]oxirane	36.582	0.42
65	Nerolidol isobutyrate	38.574	1.21
	Total:		100.00

Fig. 2 Gas chromatography–mass spectrometry (GC–MS) Chromatogram of *Thymus vulgaris* L. essential oil

8-cineole (eucalyptol) (14.01%), and terpinen-4-ol (13.8%), respectively. Our data recorded the dominant components of thyme were thymol, o-cymene, caryophyllene, and gamma-terpinene. Many researchers reported the chemical composition of the thyme essential oils, which were found to have abundant thymol, carvacrol, p-cymene, γ -terpinene, and caryophyllene oxide (Zeynep et al. 2018; Behnaz et al. 2020; Zhou et al. 2021). Although there are variations quantitatively, the composition of rosemary and thyme leaf extracts was qualitatively identical to that obtained by previous authors.

The internal regulation of seed germination is influenced by a variety of external variables. According to Kucera et al. (2005) and Finkelstein (2010), one of these elements is the existence of specific chemical substances (phytohormones or

organic acids) that have a significant impact on this process. Data from the current experiment revealed that each two studied aromatic plants had a different allelopathic effect on seed germination; a wide negative effect was shown on weed seeds rather than in crops. According to Fischer et al. (1988), cineol was extremely poisonous to *Schizachyrium scoparium* but not to *Leptochloa dubia* in this regard. As a result, a compound's inhibitory action on one species of plant may not always persist on another. Also, a compound's inhibitory action on one species of plant may not always persist on another. According to Dudai et al. (2004), monoterpenes like carvacrol have highly potent inhibitors of wheat seed germination.

Our data recorded wide negative effect on weed seeds rather than in crops. Allelochemicals can inhibit the growth

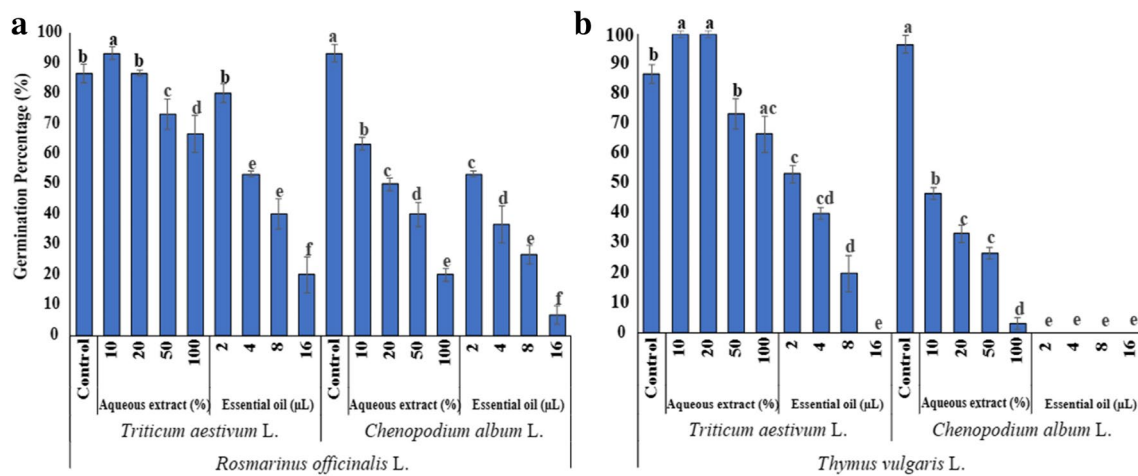


Fig. 3 Effect of different concentrations of aqueous extract and essential oils of *Rosmarinus officinalis* L. (**a**) and *Thymus vulgaris* L. (**b**) on germination percentage (%) of *Triticum aestivum* L. and *Chenopodium album* L.

Columns with same letters did not differ between treatments using a Tukey's test at 5% of probability, figures expressed as mean $\pm$ standard error

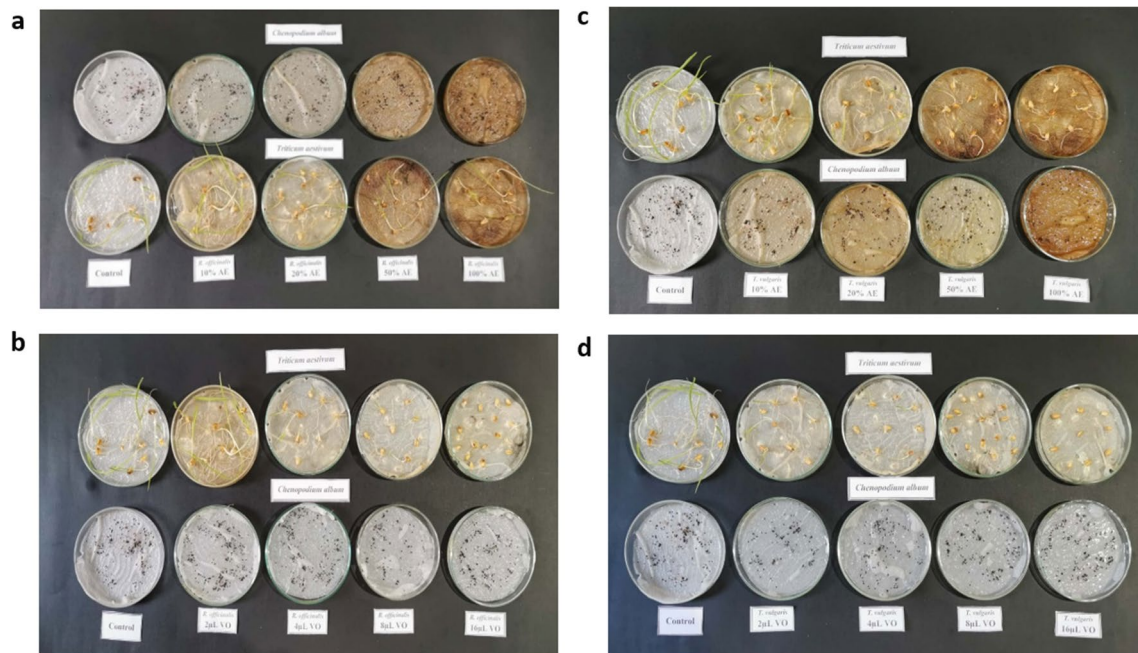


Plate 1 Effect of different concentrations of aqueous extract and essential oils of *Rosmarinus officinalis* L. (**a**, **b**) and *Thymus vulgaris* L. (**c**, **d**) on germination of *Triticum aestivum* L. and *Chenopodium album* L.

of weeds through inhibition of photosynthesis, decline in chlorophyll content, disruption of the cell membrane, and inhibition of enzymatic activity (Ghanizadeh et al. 2014). Aqueous leaf extract of *Rosmarinus officinalis* was reported with potential phytotoxic activity (Appiah et al. 2018 and Rahimi et al. 2015).

This might be because the essential oils in the two aromatic plants have different composition. According to Kotan et al. (2010), a high concentration of oxygenated

monoterpenes is linked to strong phytotoxic action of plant essential oils, and it may be the responsible for the inhibitory effects on weeds and crops (Gitsopoulos et al. 2013; Uremis et al. 2009). These reports agreed completely with our findings. Our data also showed that thyme essential oils had the strongest allelopathic impacts on the plants and related weeds that were under study. The inhibitory effect of thyme oil was greater than that of rosemary oil

Table 3 Effect of different concentrations of aqueous extract and essential oils of *Rosmarinus officinalis* L. on different germination parameters of *Triticum aestivum* L. and *Chenopodium album* L.

			CVG (%)	SG (%)	GE(%)	GI (% day ⁻¹)	MGT (days)	SL (cm)	RL (cm)	SL/RL ratio	SVI
<i>Rosmarinus officinalis</i> L.	<i>Triticum aestivum</i> L.	Control	8.78 ^a ± 0.09	7.93 ^a ± 0.01	6.67 ^a ± 0.01	59.20 ^a ± 0.05	0.22 ^a ± 0.01	5.20 ^a ± 0.20	9.02 ^a ± 0.03	0.58 ^a ± 0.01	12.32 ^a ± 0.04
		Aqueous extract (%)	8.75 ^a ± 0.07	7.40 ^a ± 0.02	6.67 ^a ± 0.01	57.93 ^a ± 0.06	0.24 ^a ± 0.01	5.88 ^a ± 0.09	7.12 ^b ± 0.11	0.83 ^b ± 0.04	12.13 ^a ± 0.06
		20	9.35 ^{ab} ± 0.04	10.80 ^a ± 0.01	20.00 ^b ± 0.02	69.87 ^b ± 0.04	0.19 ^b ± 0.03	5.64 ^a ± 0.06	5.76 ^c ± 0.08	0.98 ^c ± 0.03	9.88 ^b ± 0.01
	Essential oil (µL)	50	9.98 ^b ± 0.12	14.82 ^b ± 0.02	26.67 ^b ± 0.05	81.53 ^c ± 1.02	0.13 ^c ± 0.01	4.04 ^b ± 0.31	4.34 ^d ± 0.07	0.93 ^c ± 0.05	6.15 ^c ± 0.03
		100	9.54 ^b ± 0.11	10.21 ^b ± 0.03	20.00 ^b ± 0.01	64.27 ^b ± 1.03	0.16 ^{bc} ± 0.03	3.00 ^c ± 0.11	2.66 ^e ± 0.12	1.13 ^d ± 0.05	3.77 ^d ± 0.01
		2	9.62 ^b ± 0.08	10.15 ^a ± 0.01	13.33 ^c ± 0.01	61.00 ^b ± 0.09	0.20 ^b ± 0.05	5.34 ^a ± 0.12	5.48 ^c ± 0.13	0.97 ^c ± 0.05	8.66 ^a ± 0.01
		4	8.08 ^c ± 0.05	1.82 ^c ± 0.02	0	17.33 ^d ± 0.05	0.46 ^d ± 0.05	4.64 ^b ± 0.05	2.94 ^e ± 0.11	1.58 ^e ± 0.03	4.04 ^b ± 0.03
	<i>Chenopodium album</i> L.	8	6.67 ^d ± 0.09	0.40 ^c ± 0.03	0	6.00 ^e ± 0.04	1.00 ^e ± 0.07	4.60 ^b ± 0.06	2.42 ^e ± 0.07	1.90 ^e ± 0.03	2.81 ^c ± 0.01
		16	6.67 ^d ± 0.09	0.20 ^d ± 0.01	0	3.00 ^e ± 0.06	1.00 ^e ± 0.09	3.90 ^b ± 0.14	1.16 ^f ± 0.08	3.36 ^f ± 0.03	1.01 ^c ± 0.01
		Control	9.95 ^a ± 0.05	30.60 ^a ± 0.01	40.00 ^a ± 0.01	86.10 ^a ± 0.08	0.16 ^a ± 0.01	2.90 ^a ± 0.05	0	0	2.7 ^a ± 0.00
	<i>Triticum aestivum</i> L.	Aqueous extract (%)	9.70 ^{ab} ± 0.06	19.12 ^b ± 0.02	20.00 ^b ± 0.02	58.43 ^b ± 1.09	0.16 ^a ± 0.02	1.14 ^b ± 0.07	0	0	0.72 ^b ± 0.00
		20	9.21 ^b ± 0.09	11.11 ^c ± 0.02	10.00 ^c ± 0.05	38.00 ^c ± 1.03	0.20 ^b ± 0.06	0.74 ^c ± 0.02	0	0	0.37 ^c ± 0.00
		50	9.08 ^b ± 0.05	8.69 ^d ± 0.01	6.67 ^{cd} ± 0.02	30.83 ^c ± 0.08	0.19 ^b ± 0.04	0.52 ^d ± 0.51	0	0	0.21 ^c ± 0.00
	Essential oil (µL)	100	9.49 ^b ± 0.04	5.66 ^e ± 0.03	3.33 ^d ± 0.04	18.27 ^d ± 0.07	0.16 ^a ± 0.01	0.28 ^e ± 0.22	0	0	0.07 ^d ± 0.00
		2	10.02 ^a ± 0.04	18.41 ^b ± 0.01	26.67 ^b ± 0.03	50.57 ^b ± 0.06	0.16 ^a ± 0.05	0.72 ^c ± 0.33	0	0	0.38 ^a ± 0.00
		4	9.76 ^{ab} ± 0.08	11.71 ^c ± 0.01	13.33 ^c ± 0.02	34.83 ^c ± 1.05	0.16 ^a ± 0.09	0.54 ^d ± 0.12	0	0	0.20 ^a ± 0.00
		8	9.34 ^b ± 0.05	6.95 ^{cd} ± 0.02	6.67 ^{cd} ± 0.01	23.20 ^d ± 0.04	0.17 ^b ± 0.02	0.32 ^e ± 0.04	0	0	0.08 ^b ± 0.00
		16	8.51 ^c ± 0.07	1.08 ^f ± 0.03	0	4.70 ^e ± 0.06	0.21 ^b ± 0.01	0.18 ^f ± 0.13	0	0	0.01 ^b ± 0.00
		Control	9.95 ^a ± 0.05	30.60 ^a ± 0.01	40.00 ^a ± 0.01	86.10 ^a ± 0.08	0.16 ^a ± 0.01	2.90 ^a ± 0.05	0	0	2.7 ^a ± 0.00
		Aqueous extract (%)	9.70 ^{ab} ± 0.06	19.12 ^b ± 0.02	20.00 ^b ± 0.02	58.43 ^b ± 1.09	0.16 ^a ± 0.02	1.14 ^b ± 0.07	0	0	0.72 ^b ± 0.00
		20	9.21 ^b ± 0.09	11.11 ^c ± 0.02	10.00 ^c ± 0.05	38.00 ^c ± 1.03	0.20 ^b ± 0.06	0.74 ^c ± 0.02	0	0	0.37 ^c ± 0.00

Mean with same letter(s) on the same column are not significantly different at 5% probability using Duncan's multiple range test

CVG coefficient of velocity of germination, SG speed of germination, GE germination energy, GI germination index, MGT mean germination time, SL seedling shoot length, RL seedling root length, SL/RL ratio shoot–root ratio, and SVI seedling vigour index

Table 4 Effect of different concentrations of aqueous extract and essential oils of *Thymus vulgaris* L. on different germination parameters of *Triticum aestivum* L. and *Chenopodium album* L.

<i>Thymus vulgaris</i> L	<i>Triticum aesti- vum</i> L	Control	CVG (%)	SG (%)	GE(%)	GI (% day ⁻¹)	MGT (days)	SL (cm)	RL (cm)	SL/RL ratio	SVI
	Aqueous extract (%)	10	9.74 ^a ±0.12	11.87 ^a ±0.02	26.67 ^a ±0.41	66.40 ^a ±0.85	0.20 ^a ±0.02	11.8 ^a ±0.52	10.8 ^a ±0.01	1.09 ^a ±0.05	19.85 ^a ±0.05
		20	10.04 ^b ±0.07	17.08 ^b ±0.01	40.00 ^b ±0.82	89.67 ^b ±1.55	0.17 ^b ±0.01	14.2 ^b ±0.22	10.3 ^a ±0.02	1.38 ^b ±0.02	24.50 ^a ±0.04
		50	10.16 ^b ±0.08	18.29 ^b ±0.01	46.67 ^b ±1.02	93.87 ^b ±1.02	0.16 ^b ±0.03	14.8 ^b ±0.14	9.8 ^b ±0.05	1.51 ^c ±0.06	24.60 ^a ±0.03
		100	9.44 ^a ±0.07	9.21 ^a ±0.02	13.33 ^c ±0.09	55.07 ^a ±0.65	0.20 ^a ±0.01	11.2 ^a ±0.32	10.8 ^a ±0.09	1.04 ^a ±0.02	16.13 ^b ±0.01
	Essential oil (µL)	2	9.24 ^a ±0.04	6.84 ^a ±0.02	13.33 ^c ±0.08	44.00 ^c ±0.32	0.23 ^a ±0.01	10.6 ^a ±0.48	10.9 ^a ±0.22	0.97 ^a ±0.04	14.33 ^b ±0.01
		4	6.80 ^c ±0.04	0.68 ^c ±0.01	0	9.80 ^d ±0.54	0.82 ^c ±0.02	10.0 ^a ±0.95	11.0 ^a ±0.62	0.91 ^a ±0.03	11.21 ^b ±0.02
		8	6.67 ^c ±0.03	0.40 ^c ±0.00	0	6.00 ^{de} ±0.22	1.00 ^c ±0.02	8.20 ^c ±0.25	7.74 ^c ±0.85	0	6.36 ^c ±0.00
		16	6.67 ^c ±0.04	0.20 ^c ±0.00	0	3.00 ^e ±0.41	1.00 ^c ±0.01	5.00 ^c ±0.11	3.90 ^d ±0.32	0	1.78 ^c ±0.00
	<i>Chenopodium album</i> L	Control	9.01 ^a ±0.07	21.57 ^a ±0.02	13.33 ^a ±0.05	80.30 ^a ±1.25	0.18 ^a ±0.02	2.90 ^a ±0.01	0	0	2.80 ^a ±0.00
		Aqueous extract (%)	10	9.48 ^b ±0.06	12.34 ^b ±0.01	16.67 ^b ±0.06	39.03 ^b ±0.85	0.18 ^a ±0.01	1.14 ^b ±0.02	0	0.53 ^b ±0.00
		20	9.65 ^b ±0.05	9.20 ^{bc} ±0.02	10.00 ^a ±0.05	27.97 ^{bc} ±1.11	0.18 ^a ±0.01	0.74 ^c ±0.05	0	0	0.25 ^c ±0.00
		50	9.36 ^b ±0.05	6.56 ^c ±0.01	6.67 ^c ±0.04	21.73 ^c ±0.46	0.18 ^a ±0.01	0.52 ^c ±0.02	0	0	0.14 ^c ±0.00
	Essential Oil (µL)	100	10.53 ^c ±0.03	1.48 ^d ±0.01	3.33 ^d ±0.01	3.80 ^d ±0.75	0.13 ^b ±0.00	0.40 ^c ±0.04	0	0	0.01 ^d ±0.00
		2	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0
		8	0	0	0	0	0	0	0	0	0
		16	0	0	0	0	0	0	0	0	0

Mean with same letter(s) on the same column are not significantly different at 5% probability using Duncan's multiple range test

CVG coefficient of velocity of germination, SG speed of germination, GE germination energy, GI germination index, MGT mean germination time, SL seedling shoot length, RL seedling root length, SL/RL ratio shoot–root ratio, and SVI seedling vigour index

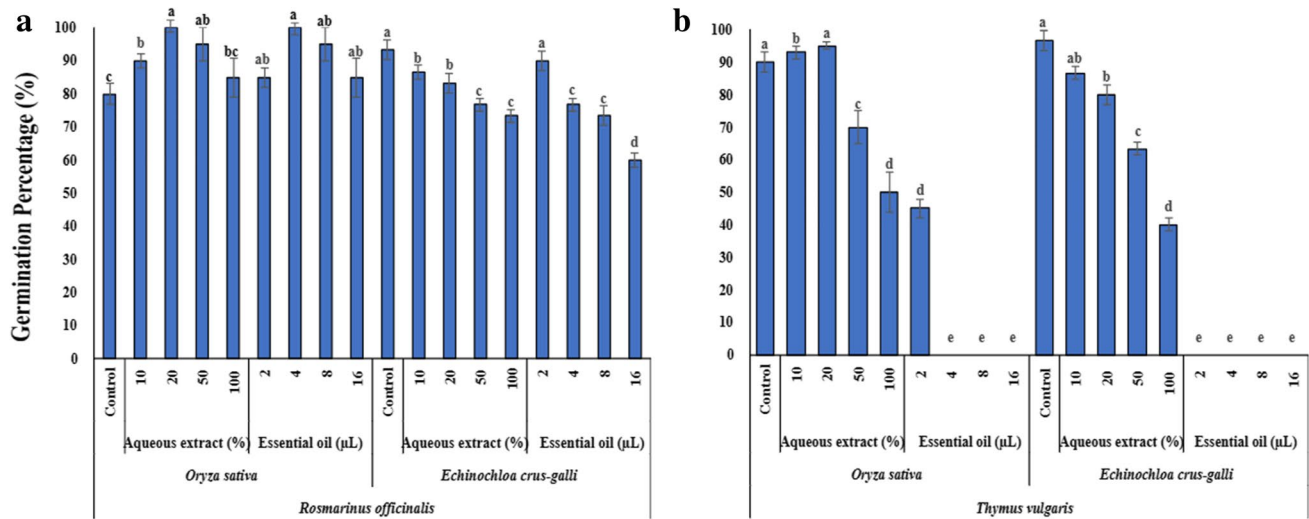


Fig. 4 Effect of different concentrations of aqueous extract and essential oils of *Rosmarinus officinalis* L. (**a**) and *Thymus vulgaris* L. (**b**) on germination percentage (%) of *Oryza sativa* L. and *Echinochloa*

crus-galli L. Columns with same letters did not differ between treatments using a Tukey's test at 5% of probability, figures expressed as mean $\pm$ standard error

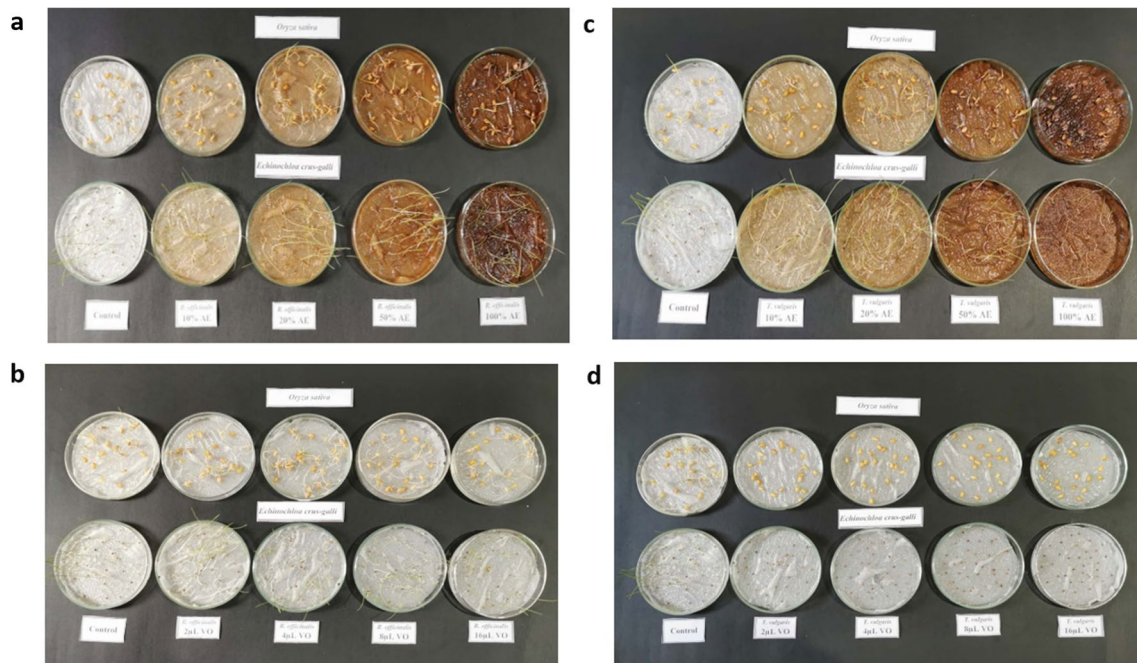


Plate 2 Effect of different concentrations of aqueous extract and essential oils of *Rosmarinus officinalis* L. (**a, b**) and *Thymus vulgaris* L. (**c, d**) on germination of *Oryza sativa* L. and *Echinochloa crus-galli* L.

because the monoterpenes in thyme oil are more diverse with larger percentages.

The seeds that germinate on the first day are given the most weight in the GI, while seeds that germinate later are given less weight. The seeds that germinated on the tenth day would have the lowest weight. As a result, the GI places emphasis on both the rate of germination and its percentage.

A higher GI rating indicates a higher germination percentage and rate (Bench et al. (1991)). Our findings indicated that dosages of rosemary's essential oils were less impacted by weed germination index (GI) than were those of thyme.

In comparison to the corresponding control treatments, rosemary essential oil and aqueous extract did not significantly reduce the seedling shoot length (SL) of wheat

Table 5 Effect of different concentrations of aqueous extract and essential oils of *Rosmarinus officinalis* L. on different germination parameters of *Oryza sativa* L. and *Echinochloa crus-galli* L.

<i>Rosmarinus officinalis</i> L.	<i>Oryza sativa</i> L.	Control	CVG (%)	SG (%)	GE(%)	GI (% day ⁻¹)	MGT (days)	SL (cm)	RL (cm)	SL/RL ratio	SVI
	Aqueous extract (%)	10	9.73 ^a ± 0.15	16.19 ^a ± 0.13	30.00 ^a ± 0.13	73.45 ^a ± 0.16	0.18 ^a ± 0.05	3.90 ^a ± 0.06	8.22 ^a ± 0.07	0.47 ^a ± 0.01	0.10 ^a ± 0.00
		20	9.99 ^b ± 0.49	19.85 ^b ± 0.23	45.00 ^{bc} ± 0.06	83.55 ^b ± 0.23	0.17 ^b ± 0.04	3.96 ^a ± 0.04	6.18 ^b ± 0.06	0.64 ^b ± 0.01	0.09 ^a ± 0.01
		50	10.05 ^b ± 0.12	22.94 ^c ± 0.09	50.00 ^c ± 0.09	96.55 ^c ± 0.28	0.17 ^b ± 0.07	4.04 ^b ± 0.01	2.74 ^c ± 0.09	1.47 ^c ± 0.02	0.07 ^b ± 0.00
		100	9.89 ^{ab} ± 0.39	20.44 ^c ± 0.17	40.00 ^b ± 0.03	89.45 ^{bc} ± 0.35	0.17 ^b ± 0.03	3.74 ^a ± 0.03	1.30 ^d ± 0.05	2.88 ^d ± 0.03	0.05 ^c ± 0.01
		100	9.93 ^b ± 0.67	18.95 ^{ab} ± 0.13	40.00 ^b ± 0.08	81.55 ^b ± 0.50	0.17 ^b ± 0.02	2.96 ^c ± 0.06	0.40 ^e ± 0.03	7.40 ^e ± 0.01	0.03 ^d ± 0.00
	Essential Oil (µL)	2	9.81 ^{ab} ± 0.12	17.96 ^{ab} ± 0.15	25.00 ^d ± 0.15	80.50 ^d ± 0.62	0.17 ^b ± 0.01	3.48 ^a ± 0.07	2.74 ^c ± 0.03	1.27 ^c ± 0.03	0.05 ^c ± 0.01
		4	10.10 ^b ± 0.19	23.98 ^c ± 0.18	55.00 ^e ± 0.13	100.5 ^d ± 0.25	0.16 ^c ± 0.01	3.12 ^{ac} ± 0.06	1.30 ^d ± 0.08	2.40 ^d ± 0.04	0.04 ^c ± 0.00
		8	9.55 ^c ± 0.29	18.28 ^b ± 0.28	20.00 ^d ± 0.18	87.95 ^{bc} ± 0.75	0.18 ^a ± 0.04	2.64 ^c ± 0.05	0.40 ^e ± 0.02	6.60 ^e ± 0.06	0.03 ^d ± 0.00
		16	9.78 ^{ab} ± 0.23	17.41 ^{ab} ± 0.22	30.00 ^a ± 0.14	78.25 ^{ab} ± 0.22	0.18 ^a ± 0.02	1.34 ^d ± 0.03	0.40 ^e ± 0.03	3.35 ^d ± 0.04	0.01 ^e ± 0.01
		10	10.52 ^a ± 0.23	40.71 ^a ± 0.14	70.00 ^a ± 0.11	101.67 ^a ± 0.16	0.14 ^a ± 0.03	6.38 ^a ± 0.07	2.22 ^a ± 0.08	2.87 ^a ± 0.07	0.08 ^a ± 0.02
	Aqueous extract (%)	20	10.35 ^{ab} ± 0.18	34.16 ^b ± 0.14	53.33 ^b ± 0.07	88.23 ^b ± 0.34	0.16 ^b ± 0.02	5.34 ^b ± 0.08	5.26 ^b ± 0.07	1.02 ^b ± 0.02	0.09 ^a ± 0.01
		50	10.31 ^b ± 0.12	32.16 ^b ± 0.19	53.33 ^b ± 0.15	83.43 ^b ± 0.65	0.16 ^b ± 0.03	5.38 ^b ± 0.08	1.28 ^c ± 0.09	4.20 ^c ± 0.07	0.06 ^b ± 0.01
		100	10.12 ^c ± 0.11	26.82 ^c ± 0.32	36.67 ^c ± 0.09	72.77 ^c ± 0.19	0.17 ^c ± 0.01	4.24 ^c ± 0.03	0.92 ^d ± 0.02	4.61 ^c ± 0.09	0.04 ^c ± 0.00
		100	10.08 ^c ± 0.29	24.60 ^c ± 0.17	36.67 ^c ± 0.13	67.10 ^c ± 0.19	0.18 ^d ± 0.04	2.58 ^d ± 0.03	0.92 ^d ± 0.03	2.80 ^a ± 0.05	0.03 ^{cd} ± 0.01
		2	10.22 ^b ± 0.17	33.25 ^b ± 0.09	53.33 ^b ± 0.16	89.07 ^b ± 0.24	0.16 ^b ± 0.03	6.24 ^a ± 0.08	5.26 ^b ± 0.08	1.19 ^b ± 0.03	0.10 ^a ± 0.02
	Essential Oil (µL)	4	10.23 ^c ± 0.09	28.68 ^c ± 0.11	40.00 ^{bc} ± 0.18	76.23 ^c ± 0.37	0.16 ^b ± 0.05	5.26 ^b ± 0.06	1.28 ^c ± 0.07	4.11 ^c ± 0.03	0.05 ^{bc} ± 0.01
		8	9.77 ^c ± 0.05	21.08 ^c ± 0.15	26.67 ^d ± 0.16	61.73 ^{cd} ± 0.29	0.20 ^e ± 0.06	3.56 ^d ± 0.09	0.92 ^d ± 0.06	3.87 ^{ac} ± 0.09	0.03 ^{cd} ± 0.00
		16	9.59 ^d ± 0.22	15.51 ^d ± 0.13	20.00 ^d ± 0.13	47.97 ^d ± 0.14	0.21 ^e ± 0.02	2.38 ^d ± 0.03	0.92 ^d ± 0.04	2.59 ^a ± 0.03	0.02 ^d ± 0.00

Mean with same letter(s) on the same column are not significantly different at 5% probability using Duncan's multiple range test

CVG coefficient of velocity of germination, SG speed of germination, GE germination energy, GI germination index, MGT mean germination time, SL seedling shoot length, RL seedling root length, SL/RL ratio shoot–root ratio, SVI seedling vigour index

Table 6 Effect of different concentrations of aqueous extract and essential oils of *Thymus vulgaris* L. on different germination parameters of *Oryza sativa* L. and *Echinochloa crus-galli* L.

		CVG (%)	SG (%)	GE(%)	GI (% day ⁻¹)	MGT (days)	SL (cm)	RL (cm)	SL/RL ratio	SVI	
<i>Thymus vulgaris</i> L	Control		9.52 ^a ± 0.06	14.60 ^a ± 0.23	20.00 ^a ± 0.88	69.85 ^a ± 0.05	0.22 ^a ± 0.01	4.36 ^a ± 0.06	8.22 ^a ± 0.08	0.53 ^a ± 0.01	0.11 ^a ± 0.01
		10%	9.06 ^a ± 0.15	11.33 ^b ± 0.28	15.00 ^b ± 0.22	60.70 ^a ± 1.22	0.22 ^a ± 0.01	4.68 ^a ± 0.06	6.18 ^b ± 0.09	0.76 ^b ± 0.01	0.09 ^a ± 0.01
		20%	10.06 ^b ± 0.15	22.38 ^c ± 0.33	45.00 ^c ± 0.36	93.95 ^b ± 1.12	0.16 ^b ± 0.03	2.32 ^b ± 0.05	2.74 ^c ± 0.01	0.85 ^b ± 0.02	0.05 ^b ± 0.00
		50%	10.06 ^b ± 0.11	15.43 ^{bc} ± 0.11	40.00 ^c ± 0.11	63.15 ^a ± 0.25	0.18 ^c ± 0.03	1.26 ^c ± 0.02	1.30 ^d ± 0.02	0.97 ^b ± 0.03	0.02 ^c ± 0.00
		100%	9.17 ^a ± 0.10	7.31 ^d ± 0.09	10.00 ^b ± 0.09	38.70 ^c ± 0.44	0.22 ^a ± 0.02	1.00 ^c ± 0.01	0.40 ^e ± 0.00	2.50 ^c ± 0.02	0.01 ^c ± 0.00
	Essential Oil (μL)	2μL	8.60 ^c ± 0.13	5.21 ^d ± 0.02	0	33.15 ^c ± 1.23	0.23 ^a ± 0.01	0.72 ^d ± 0.02	0.10 ^e ± 0.00	7.20 ^d ± 0.01	0
		4μL	0	0	0	0	0	0	0	0	0
		8μL	0	0	0	0	0	0	0	0	0
		16μL	0	0	0	0	0	0	0	0	0
<i>Echinochloa crus-galli</i> L	Control		10.32 ^a ± 0.15	37.67 ^a ± 0.00	60.00 ^a ± 0.54	97.53 ^a ± 1.02	0.16 ^a ± 0.02	6.14 ^a ± 0.07	2.22 ^a ± 0.01	2.77 ^a ± 0.09	0.08 ^a ± 0.01
		10%	10.32 ^a ± 0.24	34.05 ^{ab} ± 0.01	56.67 ^b ± 0.01	89.43 ^b ± 1.55	0.15 ^b ± 0.03	5.94 ^b ± 0.06	5.26 ^b ± 0.02	1.13 ^b ± 0.12	0.10 ^a ± 0.01
		20%	10.30 ^a ± 0.12	31.55 ^b ± 0.02	43.33 ^c ± 0.36	83.20 ^b ± 0.92	0.15 ^b ± 0.01	5.88 ^b ± 0.03	1.28 ^c ± 0.02	4.59 ^c ± 0.01	0.06 ^b ± 0.00
		50%	9.91 ^b ± 0.15	20.12 ^c ± 0.02	33.33 ^d ± 0.85	57.17 ^c ± 1.02	0.18 ^c ± 0.02	4.24 ^c ± 0.90	0.92 ^d ± 0.03	4.61 ^c ± 0.06	0.03 ^c ± 0.00
		100%	10.04 ^b ± 0.09	13.20 ^d ± 0.01	20.00 ^e ± 0.13	36.20 ^d ± 2.01	0.18 ^c ± 0.01	2.88 ^d ± 0.50	0.92 ^d ± 0.01	3.13 ^d ± 0.05	0.02 ^c ± 0.00
	Essential Oil (μL)	2μL	0	0	0	0	0	0	0	0	0
		4μL	0	0	0	0	0	0	0	0	0
		8μL	0	0	0	0	0	0	0	0	0
		16μL	0	0	0	0	0	0	0	0	0

Mean with same letter(s) on the same column are not significantly different at 5% probability using Duncan's multiple range test. CVG coefficient of velocity of germination, SG speed of germination, GE germination energy, GI germination index, MGT mean germination time, SL seedling shoot length, RL seedling root length, SL/RL ratio shoot-root ratio, SVI seedling vigour index

or rice. However, the rate of SL reduction in weeds was somewhat higher, particularly with the increasing dose of both types of essential oils. Compared to the essential oil of rosemary, the essential oil of thyme had a stronger allelopathic impact. Elghobashy et al. (2023) recorded that aqueous extract of natural medicinal plants have allelochemical potentials and showed a remarkable reduction in the organs length in weed *Chenopodium album*. Previous studies reported that essential oil effect on various cereal crops, including bread wheat germination inhibition (Dudai et al. 1999; 2004), and bread wheat germination inhibition (Atak et al. 2016a, b).

As the dose of thyme essential oil was increased, mean germination time (MGT) delayed. However, in the case of rosemary, the MGT increased as the aqueous and essential oil concentrations did as well. Previous research by Atak et al. (2016a, b) demonstrated that different essential oil concentrations and cultivars have different effects on MGT in durum wheat cultivars.

Our experiment reveals that thyme and rosemary can be used as an environmentally friendly technique to control weeds and pests in field crops, but they must be applied selectively so as not to harm crops that are intended to grow (Azirak and Karaman 2008). Numerous plant extract chemicals have specialized weed growth inhibition properties but do not adversely affect crop health (El-Darier et al. 2014). The different sensitivity of the target enzymes or particular weed receptors that recognize and respond to the chemicals may help to explain this (Hosni et al. 2013). The herbicidal effect of the essential oil should be assessed for this purpose both at the field level using different cultivars and relevant hazardous weed species. Our earlier research revealed that rice and wheat were less impacted compared to weed species, indicating that a suitable amount of these aqueous and essential oils could be utilized as a bio-herbicide to manage weeds. Both Dudai et al. (1999) and Gitsopoulos et al. (2013) found that cereal crops respond differently to essential oils. In the current investigation, thyme essential oil showed a stronger phytotoxic effect on germination and seedling characteristics for both two crops and associated weeds than rosemary oil.

5 Conclusion

Generally, the essential oils of the two studied species rosemary and thyme indicated different allelopathic activity on seeds of *Chenopodium album* and *Echinochloa crus-galli*. These essential oils were promising as alternatives to conventional synthetic herbicides and could be used in the development of bioherbicide products. Further, pot experiments and field studies could provide more useful

information concerning the wheat and rice crops, weed species, and applied dose to achieve more reliable weed control.

Abbreviations

GP%: Germination percentage; **CVG:** The coefficient of velocity of germination; **SG:** Speed of germination; **GE:** Germination energy

GI germination index

MGT: Mean germination time; **SVI:** Seed vigour index; **SL:** Seedling shoot length; **RL:** Seedling root length; **SE:** Standard error; **ND:** Not detected

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Author Contribution SME conceived of the presented idea. RME developed the theory and performed the computations. RME and MZ verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

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Declarations

Conflict of Interest The authors declare no competing interests.

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