

Bioherbicidal potential of Aqueous Extracts and Essential Oils of Rosmarinus Officinalis L. and Thymus Vulgaris L.

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Abstract

Purpose

Weed control is a technique attempts to stop or reduce growth of noxious weeds, with the aim of reducing their competition with desired crop and in natural settings preventing nonnative species competing with native ones. The essential oils and aqueous extracts from *Rosmarinus officinalis* (Rosemary) and *Thymus vulgaris* (Thyme), have been investigated as bioherbicides to suppress two weeds; *Chenopodium album* and *Echinochloa crus-galli* associated with two important economic crops; *Triticum aestivum* (wheat) and *Oryza sativa* (rice) respectively.

Methods

Petri-dish experiment was performed and 10, 20, 50, and 100% aqueous solution and doses of 0, 2, 4, 8 and 16 µL of essential oil per petri dishes were applied. Number of germinated seeds in each Petri dish was counted daily. After 15 days, germination percentage, germination velocity, speed of germination, germination energy, germination index, mean germination time as well as seedling shoot length, root length, shoot root ratio and seedling vigour index were recorded. The chemical profile for both essential oils were identified with GC-MS analysis.

Results

Eucalyptol (1,8-cineole), α-pinene and camphor were identified as the dominant constituents of the rosemary essential oil. The detected major components of 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 oil caused a generally reducing effect on growth parameters of weeds.

Conclusions

Essential oil of rosemary and thyme had allelopathic potential and should be assessed as allelopathic agents to be used for weed control.

Introduction

Wheat and Rice are two of the most vital strategic crops in Egypt and worldwide. Almost all people in Egypt depend on them in their food. Wheat is cereals known to have been domesticated and is considered one of the most important crops in the winter season in Egypt. The local production 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. Undoubtedly, weeds represent severe biotic stress toward these important economic crops. More specifically, weeds are responsible for reduction of 23% of wheat yields worldwide (Oerke, 2006). Some weeds are also shown for their allelopathic or toxic effect on the wheat germination and seedling growth (Faragasso *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). Accurate weed control is needed for wheat based food security for every country of the world. To achieve weed control, increased amount of herbicides have been used.

Nearly half of the world's population relies on rice as a staple food. A substantially higher level of rice production is required due to the rapid increase in global population (Bao- Rang, 2004). With an average yield of 10 tons per ha, rice is grown on around 500,000 hectares. This notable increase in productivity is mostly attributable to the development of short-duration varieties with a higher yield potential and biotic and abiotic stress resistance (Khattab, 2019). One type of biotic stress, weeds, caused a significant decline in rice output. *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). The expense of cultivation is increased by using conventional weed management techniques that involve human weeding (Singh *et al.*, 2016).

In long run chemical weed control, some challenges are faced, such as herbicide resistance in weeds and increasing management cost for herbicide. 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. Because they neither stay long in soil nor penetrate underground water, 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).

According to Bonanomi *et al.* (2006) and Macas *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. The majority of the plants are grown for their therapeutic properties. The essential oils from two therapeutic plants, rosemary and thyme, have, however, been investigated in this study for their potential phytotoxicity when used as bioherbicides to suppress weeds. Moreover, their effect on simulation on seed germination of two important crops economic crops wheat and rice was studied.

Materials and Methods

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.

Preparation of aqueous extracts

By soaking 100 g of the air-dried donor plant materials in 1 liter of distilled water at $(20 \pm 2^\circ\text{C})$ for 24 hours with shaking, rosemary and thyme stock aqueous extract was made. To remove the fiber 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.

Extraction of essential oils

The essential oils were extracted by hydro-distillation through passing steam produced in one round-bottomed flask, into one more 3-liter flask containing 1 kg of the pure grinded leaves along with 1 liter distilled water. The steam crosses the plant, charged with essential oil so the vapor 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 sulfate and stored at 4°C before, GC-MS analysis.

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 minutes, then raised to 260°C with 5 rate and hold time 10.00 min. 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.

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 for 20 minutes. The seeds were then repeatedly rinsed in distilled water. Under standard laboratory settings with day temperature range of $25\text{--}30^\circ\text{C}$ and night temperature range of $20\text{--}25^\circ\text{C}$, a total of seed from each plant were distributed in 9 cm diameter Petri-dishes on 2 discs of Whatman No.1 filter paper.

The two medicinal plants (rosemary and thyme) were made up of water extracts with varying concentrations (10, 20, 50, and 100%) and essential oil with various doses of 0, 2, 4, 8 and 16 μL per petri dishes were applied to wheat and rice, as well as to their associated weeds, *Chenopodium album* and *Echinochloa crus-galli*, in three replicates. Each Petri dish's number of germinations was counted every day.

Germination parameters such as germination percentage (GP%), germination velocity (GVe), 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.

Germination velocity (GVe) = $\sum \text{GP} / t$ as GP germination percentage and t is total germination time

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

As T_i is the number of days after sowing, N_i is the number of seeds germinated on day i , S is the total number of seeds planted.

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 seeds in t days; D_t is the number of corresponding germination days.

Mean germination time (MGT) = $\sum f_x / (\sum (f_1 \times x_1) + \dots)$

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) x germination percentage as described by Hossain *et al.* (2006)

Results were given as mean $\pm$ 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.

Results

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, Supplementary **Table (1) and Figure (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, Supplementary **Table (2) and Figure (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%).

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 **Figure 1 and plate 1**. The inhibitory activity was observed significantly with the increase of concentration of both aqueous extracts and essential oils of both plants specially thyme essential oil which prevent the germination of weed totally. Data showed that there is notable increase in germination percentage of wheat for both aqueous extracts of the two plants in low concentrations (10 & 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 1 and 2** respectively. results showed that doses of essential oils for rosemary were affected less compared to thyme which have negative effect on germination velocity (GVe), 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 μ L 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 μ L essential oil attained with the highest value (3.36%) compared with control.

It was significant increase of seedling shoot length (SL) of wheat at lower doses of Aqueous extract and essential oil of rosemary and thyme the highest increase recorded at 20% aqueous extract and 2 μ L essential oil on the other hand seedling root lengths (RL) in all treatment of both two aromatic plants this inhibition is obvious clearly in *Chenopodium album*, Shoot Root ratio (SL/ RL ratio) in wheat increase at lower doses of Aqueous extract of the two aromatic species

Table 1. The effect of different dose of an aqueous extract as well as essential oils of *Rosmarinus officinalis* (rosemary) in different germination parameters of *Triticum aestivum* (wheat) and its associated weeds *Chenopodium album*.

			GP %	GVs	SG	GE%	GI	MGT	S	
<i>Rosmarinus officinalis</i>	<i>Triticum aestivum</i>	Control		86.67 ^a ±0.03	6.19 ^a ±0.06	0.87 ^a ±0.01	6.67 ^a ±0.01	59.20 ^a ±0.05	0.22 ^a ±0.01	5.20 ^a ±0.02
		aqueous extract	10%	93.33 ^b ±0.02	6.67 ^a ±0.04	0.93 ^a ±0.02	6.67 ^a ±0.01	57.93 ^a ±0.06	0.24 ^a ±0.01	5.88 ^a ±0.01
			20%	86.67 ^a ±0.01	6.19 ^a ±0.02	0.87 ^a ±0.01	20.00 ^b ±0.02	69.87 ^b ±0.04	0.19 ^b ±0.03	5.64 ^a ±0.01
			50%	73.33 ^c ±0.05	5.24 ^b ±0.09	0.73 ^b ±0.02	26.67 ^b ±0.05	81.53 ^c ±1.02	0.13 ^c ±0.01	4.04 ^b ±0.03
			100%	66.67 ^d ±0.06	4.76 ^c ±0.08	0.67 ^b ±0.03	20.00 ^b ±0.01	64.27 ^b ±1.03	0.16 ^{bc} ±0.03	3.00 ^c ±0.01
		Essential oil	2μL	80.00 ^a ±0.03	5.71 ^b ±0.06	0.80 ^a ±0.01	13.33 ^c ±0.01	61.00 ^b ±0.09	0.20 ^b ±0.05	5.34 ^a ±0.01
			4μL	53.33 ^e ±0.01	3.81 ^d ±0.02	0.53 ^c ±0.02	0	17.33 ^d ±0.05	0.46 ^d ±0.05	4.64 ^b ±0.01
			8μL	40.00 ^e ±0.05	2.86 ^d ±0.07	0.40 ^c ±0.03	0	6.00 ^e ±0.04	1.00 ^e ±0.07	4.60 ^b ±0.01
			16μL	20.00 ^f ±0.06	1.43 ^e ±0.06	0.20 ^d ±0.01	0	3.00 ^e ±0.06	1.00 ^e ±0.09	3.90 ^b ±0.01
	<i>Chenopodium album</i>	Control		93.33 ^a ±0.03	7.18 ^a ±0.02	0.93 ^a ±0.01	40.00 ^a ±0.01	86.10 ^a ±0.08	0.16 ^a ±0.01	2.90 ^a ±0.01
		aqueous extract	10%	63.33 ^b ±0.02	4.87 ^b ±0.01	0.63 ^b ±0.02	20.00 ^b ±0.02	58.43 ^b ±1.09	0.16 ^a ±0.02	1.14 ^b ±0.01
			20%	50.00 ^c ±0.02	3.85 ^b ±0.05	0.50 ^c ±0.02	10.00 ^c ±0.05	38.00 ^c ±1.03	0.20 ^b ±0.06	0.74 ^c ±0.01
			50%	40.00 ^d ±0.04	3.08 ^b ±0.02	0.40 ^d ±0.01	6.67 ^{cd} ±0.02	30.83 ^c ±0.08	0.19 ^b ±0.04	0.52 ^d ±0.05
			100%	20.00 ^e ±0.02	1.54 ^c ±0.01	0.20 ^e ±0.03	3.33 ^d ±0.04	18.27 ^d ±0.07	0.16 ^a ±0.01	0.28 ^e ±0.02
		Essential oil	2μL	53.33 ^c ±0.01	4.10 ^b ±0.01	0.53 ^c ±0.01	26.67 ^b ±0.03	50.57 ^b ±0.06	0.16 ^a ±0.05	0.72 ^c ±0.03
			4μL	36.67 ^d ±0.06	2.82 ^c ±0.03	0.37 ^d ±0.01	13.33 ^c ±0.02	34.83 ^c ±1.05	0.16 ^a ±0.09	0.54 ^d ±0.01
			8μL	26.67 ^e ±0.03	2.05 ^c ±0.02	0.27 ^{ed} ±0.02	6.67 ^{cd} ±0.01	23.20 ^d ±0.04	0.17 ^b ±0.02	0.32 ^e ±0.01
			16μL	6.67 ^f ±0.03	0.51 ^d ±0.04	0.07 ^f ±0.03	0	4.70 ^e ±0.06	0.21 ^b ±0.01	0.18 ^f ±0.01

Mean with same letter(s) on the same column are not significantly different at 5% probability using Duncan's multiple range test. germination percentage (GP%), germination velocity (GVe), 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).

Table 2. The effect of different dose of an aqueous extract as well as essential oils of *Thymus vulgaris* (thyme) in different germination parameters of *Triticum aestivum* (wheat) and its associated weeds *Chenopodium album*.

			GP %	GVs	SG	GE%	GI	MGT	S	
Thymus vulgaris	Triticum aestivum	Control		86.67 ^a ±0.62	6.19 ^a ±0.09	0.87 ^a ±0.02	26.67 ^a ±0.41	66.40 ^a ±0.85	0.20 ^a ±0.02	11.8 ^a ±0.52
		aqueous extract	10%	100.0 ^b ±0.41	7.14 ^b ±0.05	1.00 ^b ±0.01	40.00 ^b ±0.82	89.67 ^b ±1.55	0.17 ^b ±0.01	14.2 ^b ±0.22
			20%	100.0 ^b ±0.25	7.14 ^b ±0.04	1.00 ^b ±0.01	46.67 ^b ±1.02	93.87 ^b ±1.02	0.16 ^b ±0.03	14.8 ^b ±0.14
			50%	73.33 ^a ±0.36	5.24 ^a ±0.03	0.73 ^a ±0.02	13.33 ^c ±0.09	55.07 ^a ±0.65	0.20 ^a ±0.01	11.2 ^a ±0.32
			100%	66.67 ^a ±0.88	4.76 ^a ±0.01	0.67 ^a ±0.02	13.33 ^c ±0.08	44.00 ^c ±0.32	0.23 ^a ±0.01	10.6 ^a ±0.48
		Essential oil	2μL	53.33 ^c ±0.52	3.81 ^a ±0.01	0.53 ^a ±0.01	0	9.80 ^d ±0.54	0.82 ^c ±0.02	10.0 ^a ±0.95
			4μL	40.00 ^{cd} ±0.11	0.03 ^c ±0.00	0.40 ^c ±0.00	0	6.00 ^{de} ±0.22	1.00 ^c ±0.02	8.20 ^c ±0.25
			8μL	20.00 ^d ±0.12	0.01 ^c ±0.00	0.20 ^d ±0.00	0	3.00 ^e ±0.41	1.00 ^c ±0.01	5.00 ^c ±0.11
			16μL	0	0	0	0	0	0	0
	Chenopodium album	Control		96.67 ^a ±0.55	7.44 ^a ±0.04	0.97 ^a ±0.02	13.33 ^a ±0.05	80.30 ^a ±1.25	0.18 ^a ±0.02	2.90 ^a ±0.01
		aqueous extract	10%	46.67 ^b ±1.10	3.59 ^b ±0.03	0.47 ^b ±0.01	16.67 ^b ±0.06	39.03 ^b ±0.85	0.18 ^a ±0.01	1.14 ^b ±0.02
			20%	33.33 ^c ±0.57	2.56 ^b ±0.02	0.33 ^{bc} ±0.02	10.00 ^a ±0.05	27.97 ^{bc} ±1.11	0.18 ^a ±0.01	0.74 ^c ±0.05
			50%	26.67 ^c ±0.66	2.05 ^b ±0.01	0.27 ^c ±0.01	6.67 ^c ±0.04	21.73 ^c ±0.46	0.18 ^a ±0.01	0.52 ^c ±0.02
			100%	3.33 ^d ±0.32	0.26 ^c ±0.01	0.03 ^d ±0.01	3.33 ^d ±0.01	3.80 ^d ±0.75	0.13 ^b ±0.00	0.40 ^c ±0.04
		Essential Oil	2μL	0	0	0	0	0	0	0
			4μL	0	0	0	0	0	0	0
			8μL	0	0	0	0	0	0	0
			16μL	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. germination percentage (GP%), germination velocity (GVe), 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).

The effects of rosemary and thyme of both aqueous extracts and essential oils in germination of rice and its associated weed *Echinochloa crus-galli* were showed in **Figure 2 and Plate 2**. The study recorded that there was significant increase in germination percentage of rice which treated with both aqueous extract and essential oils of rosemary, the highest germination percentage attained in 20% aqueous extracts and 4 µL essential oils (100% GP). while the percentage of germination reduced significantly for *Echinochloa crus-galli* specially at high concentrations treatments. results showed that essential oil dose of thyme had inhibition effect on seed GP of both rice and its associated weed *Echinochloa crus-galli*, but inhibition was more remarkable in weed.

Data showed that thyme has more negative affect on different germination parameters of rice and its associated weeds *Echinochloa crus-galli* when compared to rosemary (**Table 3 and 4**). Our experiment result also reveals that inhibition was noted in *Echinochloa crus-galli*, On the other hand, low concentrations of Aqueous extract and Essential oils for rosemary were enhance the growth of rice, the highest enhancement recorded at 20% Aqueous extract and 4 µL essential oil in both germination percentage (GP%), germination velocity (GVe), speed of germination (SG), germination energy (GE), germination index (GI) and seedling vigour index (SVI) while aqueous and essential oil not-significantly reduced Mean germination time (MGT) of rice compared to respective control treatments while it was noted that it increase in *Echinochloa crus-galli*.

The inhibition was noted in *Echinochloa crus-galli* in most germination parameters, these results showed that essential oil dose of thyme had inhibition effect on seed GP of both rice and weed, but inhibition effect of *Echinochloa crus-galli* was more remarkable

It was reported that there was significant enhancement of seedling shoot length (SL) of rice at lower doses (20%) of rosemary and (10%) thyme Aqueous extract, while seedling root lengths (RL) fluctuated in both two aromatic plants but data recorded notable reduction in *Echinochloa crus-galli* especially under effect of thyme, shoot root ratio (SL/ RL ratio) in rice increase at all doses of Aqueous extract of the two aromatic species

Table 3. The effect of different dose of an aqueous extract as well as essential oils of *Rosmarinus officinalis* in different germination parameters of *Oryza sativa* L. (rice) and its associated weed *Echinochloa crus-galli*.

				GP %	GVs	SG	GE%	GI	MGT	S	R	SL/ RL ratio
<i>Rosmarinus officinalis</i>	<i>Oryza sativa</i>	Control		80.00 ^a ± 0.03	6.15 ^a ± 0.12	1.07 ^a ± 0.03	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
		aqueous extract	10%	90.00 ^b ± 0.02	6.92 ^b ± 0.42	1.20 ^b ± 0.02	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
			20%	100.0 ^c ± 0.01	7.69 ^c ± 0.09	1.33 ^c ± 0.08	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
			50%	95.00 ^{bc} ± 0.05	7.31 ^{bc} ± 0.33	1.27 ^c ± 0.01	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
			100%	85.00 ^{ab} ± 0.06	6.54 ^{ab} ± 0.60	1.13 ^{ab} ± 0.03	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
		Essential Oil	2µL	85.00 ^{ab} ± 0.03	6.54 ^{ab} ± 0.08	1.13 ^{ab} ± 0.05	25.00 ^d ± 0.15	80.50 ^b ± 0.62	0.17 ^b ± 0.01	3.48 ^a ± 0.07	2.74 ^c ± 0.03	1.27 ^c ± 0.03
			4µL	100.0 ^c ± 0.01	7.69 ^c ± 0.13	1.33 ^c ± 0.10	55.00 ^c ± 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
			8µL	95.00 ^{bc} ± 0.05	7.31 ^{bc} ± 0.23	1.27 ^c ± 0.08	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
			16µL	85.00 ^{ab} ± 0.06	6.54 ^{ab} ± 0.18	1.13 ^{ab} ± 0.09	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
	<i>Echinochloa crus-galli</i>	Control		93.33 ^a ± 0.03	7.18 ^a ± 0.16	0.93 ^a ± 0.04	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
		aqueous extract	10%	86.67 ^b ± 0.02	6.67 ^{ab} ± 0.13	0.83 ^b ± 0.04	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
			20%	83.33 ^b ± 0.03	6.41 ^b ± 0.06	0.83 ^b ± 0.06	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
			50%	76.67 ^c ± 0.02	5.90 ^c ± 0.08	0.73 ^c ± 0.03	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
			100%	73.33 ^c ± 0.02	5.64 ^c ± 0.23	0.67 ^c ± 0.09	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
		Essential Oil	2µL	90.00 ^a ± 0.03	6.92 ^a ± 0.13	0.87 ^b ± 0.07	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
			4µL	76.67 ^c ± 0.02	5.90 ^c ± 0.06	0.77 ^c ± 0.03	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
			8µL	73.33 ^c ± 0.03	5.64 ^c ± 0.03	0.70 ^c ± 0.05	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
			16µL	60.00 ^d ± 0.02	4.62 ^d ± 0.17	0.53 ^d ± 0.03	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

Mean with same letter(s) on the same column are not significantly different at 5% probability using Duncan's multiple range test. germination percentage (GP%), germination velocity (GVe), 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).

Table 4. The effect of different dose of an aqueous extract as well as essential oils of *Thymus vulgaris* in different germination parameters of *Oryza sativa* L. (rice) and its associated weed *Echinochloa crus-galli*.

			GP %	GVs	SG	GE%	GI	MGT	SL	RL	
<i>Thymus vulgaris</i>	<i>Oryza sativa</i>	Control		90.00 ^a ± 0.03	6.92 ^a ± 0.04	1.20 ^a ±0.01	20.00 ^a ±0.88	69.85 ^a ±0.05	0.22 ^a ±0.01	4.36 ^a ±0.06	8.22 ^a ±0.01
		aqueous Extract	10%	80.00 ^b ± 0.02	6.15 ^a ± 0.12	1.07 ^b ±0.01	15.00 ^b ±0.22	60.70 ^a ±1.22	0.22 ^a ±0.01	4.68 ^a ±0.06	6.18 ^b ±0.01
			20%	95.00 ^a ± 0.01	7.31 ^b ± 0.11	1.27 ^b ±0.02	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
			50%	70.00 ^c ± 0.05	5.38 ^c ± 0.08	0.93 ^c ±0.01	40.00 ^c ±0.11	63.15 ^a ±0.25	0.18 ^c ±0.03	1.26 ^c ±0.02	1.30 ^d ±0.01
			100%	50.00 ^d ± 0.06	3.85 ^d ± 0.07	0.67 ^d ±0.01	10.00 ^b ±0.09	38.70 ^c ±0.44	0.22 ^a ±0.02	1.00 ^c ±0.01	0.40 ^e ±0.01
		Essential Oil	2µL	45.00 ^d ± 0.03	3.46 ^d ± 0.09	0.60 ^d ±0.02	0	33.15 ^c ±1.23	0.23 ^a ±0.01	0.72 ^d ±0.02	0.10 ^e ±0.01
			4µL	0	0	0	0	0	0	0	0
			8µL	0	0	0	0	0	0	0	0
			16µL	0	0	0	0	0	0	0	0
	<i>Echinochloa crus-galli</i>	Control		96.67 ^a ± 0.03	7.44 ^a ± 0.11	0.93 ^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
		aqueous Extract	10%	86.67 ^{ab} ± 0.02	6.67 ^b ± 0.20	0.83 ^b ±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 ±0.01
			20%	80.00 ^b ± 0.03	6.15 ^b ± 0.07	0.80 ^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 ±0.01
			50%	63.33 ^c ± 0.02	4.87 ^c ± 0.09	0.60 ^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 ±0.01
			100%	40.00 ^d ± 0.02	3.08 ^c ± 0.05	0.37 ^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
		Essential Oil	2µL	0	0	0	0	0	0	0	0
			4µL	0	0	0	0	0	0	0	0
			8µL	0	0	0	0	0	0	0	0
			16µL	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. germination percentage (GP%), germination velocity (GVe), 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).

Discussion

Data of the GC-MS analysis of rosemary leaves showed that the dominant constituents were eucalyptol (1,8-cineole) with 48.01% of the relative area, α-pinene (11.50%) and camphor (8.65%), in accordance with most results obtained by different authors in several countries (Micić *et al.*, 2021). However, there are usually considerable variations in the percentage of the major components of rosemary EO cultivated in different geographical origins. Saviuc *et al.* (2016) identified α-pinene being 19.01%, followed by the 1,8-cineole (eucalyptol) with 5.49% and camphor (5.71%). Giarratana *et al.* (2016) recognized α-pinene, camphor and eucalyptol with 23.98%, 22.62% and 18.76%, respectively. Atti-Santos *et al.* (2005) obtained α-pinene (40.55 to 45.10%), 1,8-cineole (17.40 to 19.35%) and camphene (4.73 to 6.06%) as the major components of Brazilian rosemary essential oils. Alternatively, the dominant constituents reported by Tawfeeq *et al.* (2018) were camphor (23.04%), 1, 8-cineole (eucalyptol) (14.01%), and terpinen-4-ol (13.8 %), respectively. The dominant components of thyme were thymol with 14.79% of the relative area, o-Cymene (8.97%) and gamma-terpinene (4.66%). Many researchers reported the chemical composition of the thyme essential oils, which were found to have abundant thymol, carvacrol, p-cymene, γ-terpinene, caryophyllene oxide (Zeynep *et al.*, 2018; Behnaz *et al.*, 2020; Zhou *et al.*, 2021).

Data from the current experiment revealed that each two studied aromatic plants had a different allelopathic effect on seed germination, and under various treatments with the essential oil produced from those plants, a wide negative effect was shown on weed seeds rather than in crops. This might be because the essential oils in the two aromatic plants have different composition. Studies have shown that the primary components of thyme oil include thymol, o-cymene, and carvacrol (De Almeida *et al.*, 2010). Additionally, according to Boutekedjiret *et al.* (2003), the primary components of rosemary essential oil are α-pinene, β-pinene, 1,8-cineole and camphor. According to Kotan *et al.* (2010), a high concentration of oxygenated monoterpenes is linked to strong phytotoxic action of plant essential oils. 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 because the monoterpenes in thyme oil are more diverse with larger percentages.

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. According to our findings, weeds showed stronger evidence of the examined essential oils' allelopathic effects on seed germination. 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 reports (Gitsopoulus *et al.*, 2013; Uremis *et al.*, 2009), monoterpenes and sesquiterpenes were the primary constituents of essential oils responsible for the inhibitory effects on weeds and crops. According to Dudai *et al.* (2004), monoterpenes like carvacrol have highly potent inhibitors of wheat seed germination.

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 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.*, 2016).

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.* (2016) 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 & Karaman, 2008). Numerous plant extract chemicals have specialised 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 recognise 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 utilised as a bio-herbicide to manage weeds. Both Dudai *et al.* (1999) and Gitsopoulus *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.

Conclusion

The present study showed that both aquatic and essential oil of rosemary and thyme could be considered as potential allelopathic agents. this feature of the essential oils is very important, particularly for application as bio-herbicides, where specificity of activity is required. Therefore, researches are needed to identify the most drastic compounds for the different target species. Accordingly, compared to the individual use, combining several essential oils may have a greater inhibitory effect on seed germination of weeds. More knowledge about bio-degradable herbicides or pesticides would help to decrease man made environmental damages.

Abbreviations

GP%: germination percentage, **GVe**: germination velocity, **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.

Declarations

Conflict of interest: The authors declare that they have no conflict of interest.

Author contribution: Salama M. El-Darier conceived of the presented idea. Roufaida M. Elghobashy developed the theory and performed the computations. Roufaida M. Elghobashy & Mohamed Zakaria verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

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References

1. Atak, M., Mavi, K., & Üremiş, İ. (2016). Allelopathic effects of oregano and rosemary essential oils on germination and seedling growth of durum wheat. In *Proceedings of the VII International Scientific Agriculture Symposium, "Agrosym"* (pp. 1178-1183).
2. Atak, M., Mavi, K., Uremis, I. (2016). Bio-herbicidal effects of oregano and rosemary essential oils on germination and seedling growth of bread wheat cultivars. *Romanian Biological Letters*, 21, (1), 11149-11159.
3. Atti-Santos, A. C., Rossato, M., Pauletti, G. F., Rota, L. D., Rech, J. C., Panseira, M. R., Agostini, F., Serafini, L.A. & Moyna, P. (2005). Physico-chemical evaluation of *Rosmarinus officinalis* L. essential oils. *Brazilian archives of biology and technology*, 48, 1035-1039.
4. Azirak, S., Karaman, S. (2008). Allelopathic effect of some essential oils and components on germination of weeds species. *Acta Agriculturae Scandinavica. Section-B- Soil and Plant Science*, 58 (1), 88-92.
5. Bao-Rang, Lu. (2004). Gene flow from cultivated rice: Ecological Consequences. ISB. News Report, May. Fundan University. Shanghai, pp. 1-3. (C.F. Computer research).
6. Behnaz, T., Mehdi, R., Ahmad, A., and Mohammad, R. S. (2020). Thymol, carvacrol, and antioxidant accumulation in *Thymus* species in response to different light spectra emitted by light-emitting diodes. *Food Chemistry*. 307:125521.
7. Bench A.R., Fenner, M. and Edwards, P. (1991). Changes in germinability, ABA content and ABA embryonic sensitivity in developing seeds of *Sorghum bicolor* (L.) Moench induced by water stress during grain filling. *New Phytologist*. 118: 339-347.
8. Bonanomi G., M.G. Sicurezza, S. Caporaso, A. Esposito and S. Mazzoleni. (2006). Phytotoxicity dynamics of decaying plant materials. *New Phytologist*, 169: 571-578.
9. Boutekedjiret C, Bentahar F, Belabbes R, Bessiere J. (2003). Extraction of rosemary essential oil by steam distillation and hydrodistillation. *Flavour and Fragrance Journal*, 18: 481-484.
10. Chandler D., Bailey A. S., Tatchell G. M., Davidson G., Greaves J., Grant W. P. (2011). The development, regulation and use of biopesticides for integrated pest management. *Philosophical Transactions of the Royal Society A - Journals*, B 366, 1987–1998.
11. De Almeida LFR, Frei F, Mancini E, De Martino L, De Feo V. (2010). Phytotoxic activities of Mediterranean essential oils. *Molecules*, 15: 4309-4323.
12. Dudai, N., Ben-Ami, M., Chaimovich, R., Chaimovitch, D. (2004). Essential oils as allelopathic agents: Bioconversion of monoterpenes by germinating wheat seeds. *Acta Hort*, 629, 505-508.
13. Dudai, N., Mayer, A.M., Poljakoff-Mayber, A., Putievsky, E., Lerner, H.R. (1999). Essential oil as allelochemicals and their potential use as bio-herbicides. *Journal of Chemical Ecology*, 25, 1079-1089.
14. Elghobashy, R., El-Darier, S., Elnouby, A. & Migahid, M. (2023). Weed management in wheat crop through bio-herbicidal activity of two natural medicinal plants. *Journal of Crop and Weed*, 19 (1): 145-157.
15. FAO (2018) 'Food and Agriculture Organization of United Nations' Vial Delle Terme di Caracalla, 00100. Rome, Italy.
16. Farooq M, Jabran K, Cheema ZA, Wahid A, Siddique KHM (2011) Role of allelopathy in agricultural pest management. *Pest Management Science*, 67:494-506
17. Finkelstein R. 2010. The role of hormones during seed development and germination. – In: Davies P. (eds), *Plant Hormones*, Springer, *Netherlands*, p. 549-573.
18. Fisher, R. A. & Maurer, R. O. (1978) 'Drought resistance in spring wheat cultivars. 1- Grain yield responses', *Australian Journal of Agricultural Research*, 29: pp. 897-912.
19. Fischer NH, Tanrisever N, Bruce Williamson G. (1988). Allelopathy in the Florida scrub community as a model for natural herbicide actions. ACS symposium series: Oxford University Press, pp. 233-249.
20. Fischer, A., H. V. Ramirez and J. Lozano (1997). Suppression of junglerice [*Echinochloa colona* (L.) Link] by irrigated rice cultivars in Latin America. *Agronomy Journal*, 521 (3): 516–521.
21. Fragasso, M., Iannucci, A., Papa, R. (2013). Durum wheat and allelopathy: towards wheat breeding for natural weed management. *Frontiers in Plant Science*, 4, 1-8.
22. Giarratana, F., Muscolino, D., Ragonese, C., Beninati, C., Sciarrone, D., Ziino, G. & Panebianco, A. (2016). Antimicrobial activity of combined thyme and rosemary essential oils against *Listeria monocytogenes* in Italian mortadella packaged in modified atmosphere: Thyme & Rosemary EOs vs L. monocytogenes. *Journal of Essential Oil Research*, 28(6), 467-474.
23. Gitsopoulus, T., Chatzopoulou, P., Georgoulas, I. (2013). Effects of essential oils of *Lavandula x hybrida* Rev, *Foeniculum vulgare* Mill and *Thymus capitatus* L. on the germination and radical length of *Triticum aestivum* L., *Hordeum vulgare* L., *Lolium rigidum* L. and *Phalaris brachystachys* L. *Journal of Essential Oil Bearing Plants*, 16 (6), 817-825.
24. Hossain, M.A.; Arefin, M.K.; Khan, B.M.; Rahman, M.A. (2006) Effects of seed treatments on germination and seedling growth attributes of Horitaki (*Terminalia chebula* Retz.) in the nursery. *Research journal of agriculture and biological sciences*, 1, 135–141.
25. Isman M. B. (2015). A renaissance for botanical insecticides? *Pest Management Science*, 71, 1587–1590.
26. Jabran, K., Mahajan, G., Sardana, V., and Chauhan, B. S. (2015). Allelopathy for weed control in agricultural systems. *Crop Protection*, 72, 57–65.
27. Jamil, M., Z.A. Cheema, M.N. Mushtaq, M. Farooq and M.A. Cheema, (2009). Alternative control of wild oat and canary grass in wheat fields by allelopathic plant water extracts. *Agronomy for Sustainable Development*, 29: 474–482

28. Kader, M. A. (2005). A Comparison of seed germination calculation formulae and the associated interpretation of resulting data. *Journal and proceedings of the Royal Society of New South Wales*, 138, 65–75.
29. Khan MA and Ungar IA. (1998) Germination of salt tolerant shrub *Suaeda fruticosa* from Pakistan: salinity and temperature responses. *Seed Science and Technology*, 26:657-67.
30. Khattab, E. A. (2019). Performance Evaluation of Some Rice Varieties under the System of Planting in Egypt. *Asian Journal of Research in Crop Science*, 3(2): 1-10.
31. Kotan R, Cakir A, Dadasoglu F, Aydin T, Cakmakci R, Ozer H, Kordali S, Mete E, Dikbas N. (2010). Antibacterial activities of essential oils and extracts of Turkish *Achillea*, *Satureja* and *Thymus* species against plant pathogenic bacteria. *Journal of the Science of Food and Agriculture*, 90: 145-160.
32. Kucera B, Cohn MA, Leubner-Metzger G. (2005). Plant hormone interactions during seed dormancy release and germination. *Seed Science Research*, 15: 281-307.
33. Macías F.A., Molinillo J.M., Varela R.M., Galindo J.C. (2007): Allelopathy – A natural alternative for weed control. *Pest Management Science*, 63: 327–348.
34. Micić, D., Đurović, S., Riabov, P., Tomić, A., Šovljanski, O., Filip, S., Tosti, T., Dojčinović, B., Božović, R., Jovanović, D. & Blagojević, S. (2021). Rosemary essential oils as a promising source of bioactive compounds: Chemical composition, thermal properties, biological activity, and gastronomical perspectives. *Foods*, 10(11), 2734.
35. Oerke, E. C. (2006). Crop losses to pests. *Journal of Agricultural Science*, 144, 31–43.
36. Pavela, R., and Benelli, G. (2016). Essential oils as ecofriendly biopesticides? Challenges and constraints. *Trends Plant Science*, 21, 1000–1007.
37. Saviuc, C., Gheorghe, I., Coban, S., Drumea, V., Chifiriuc, M. C., Banu, O. & Lazăr, V. E. R. O. N. I. C. A. (2016). Rosmarinus officinalis essential oil and eucalyptol act as efflux pumps inhibitors and increase ciprofloxacin efficiency against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* MDR strains. *Romanian Biotechnological Letters*, 21(4), 11783.
38. Siddiqui, I. (2005). Fungal pathogens as biological control agents of weeds of wheat. Ph. D. thesis. University of the Punjab, Quaid-e- Azam campus, Lahore, Pakistan.
39. Siddiqui, I., and Bajwa, R. (2001). Variation in weed composition in wheat fields of Lahore and Gujranwala divisions. *Pakistan Journal of Biological Sciences*, 4: 492-504.
40. Singh, V., L. J. Mangi, Z. A. Ganie, B. S. Chauhan and R. K. Gupta (2016). Herbicide options for effective weed management in dry direct seeded rice under scented rice-wheat rotation of western Indo Gangetic Plains. *Crop Protection*, 81:168-176.
41. Tawfeeq, A. A., Mahdi, M. F., Abaas, I. S., & Alwan, A. H. (2018). Isolation, quantification, and identification of rosmarinic acid, gas chromatography-mass spectrometry analysis of essential oil, cytotoxic effect, and antimicrobial investigation of rosmarinus officinalis leaves. *Asian Journal of Pharmaceutical and Clinical Research*, 11(6), 126-32.
42. Uremis, I., Arslan M., Sangun, M.K. (2009). Herbicidal activity of essential oil on the germination of some problem weeds. *Asian Journal of Chemistry*, 21(4), 3199-3210.
43. Vokou D, Douvli P, Blionis GJ, Halley JM. (2003). Effects of monoterpenoids, acting alone or in pairs, on seed germination and subsequent seedling growth. *Journal of Chemical Ecology*, 29: 2281-2301.
44. Zar, J. H. (1984). Statistical Analysis (2nd ed.). Englewood Cliffs, NJ: Prentice-Hall, Inc.
45. Zeynep U., Menderes C., Huseyin, I., and Murat, Y. (2018). Antimicrobial and herbicidal activities of the essential oil from the Mediterranean *Thymus eigii*, *Journal of Essential Oil-Bearing Plants*, 21, 214–222.
46. Zhou, S., Han, C., Zhang, C., Kuchkarova, N., Wei, C., Zhang, C., & Shao, H. (2021). Allelopathic, phytotoxic, and insecticidal effects of *Thymus proximus* Serg. essential oil and its major constituents. *Frontiers in Plant Science*, 12, 689875.
47. El-Darier, S.M., Abdelaziz, H.A., ZeinEl-Dien, M.H. (2014). Effect of soil type on the allelotoxic activity of *Medicago sativa* L. residues in *Vicia faba* L. agroecosystems. *J. Taibah Univ. Sci.*, 8, 84–89.
48. Hosni, K., Hassen, I., Sebei, H., Casabianca, H. (2013). Secondary metabolites from *Chrysanthemum coronarium* (Garland) flowerheads: Chemical composition and biological activities. *Ind. Crop Prod.* 44, 263–271.

Plates

Plates 1 and 2 are available in the Supplementary Files section

Figures

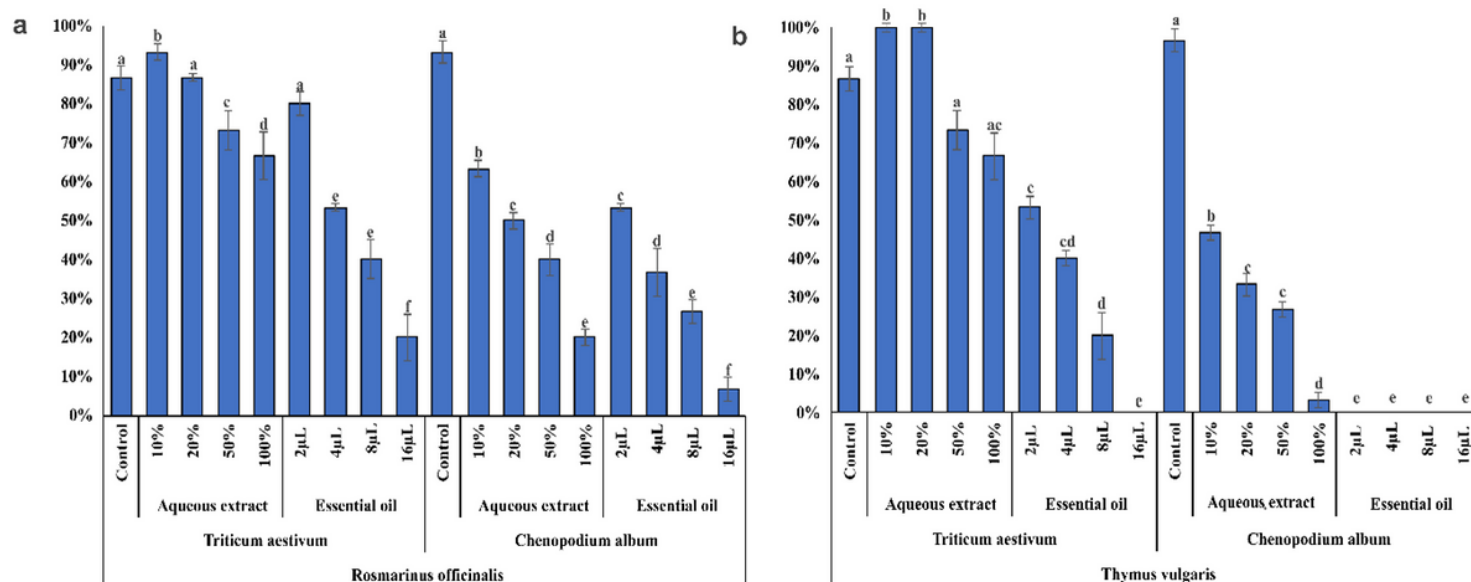


Figure 1

The effect of different dose of an aqueous extract as well as essential oils of **a**: *Rosmarinus officinalis* (rosemary) and **b**: *Thymus vulgaris* (thyme) in germination percentage % of *Triticum aestivum* (wheat) and its associated weed *Chenopodium album*.

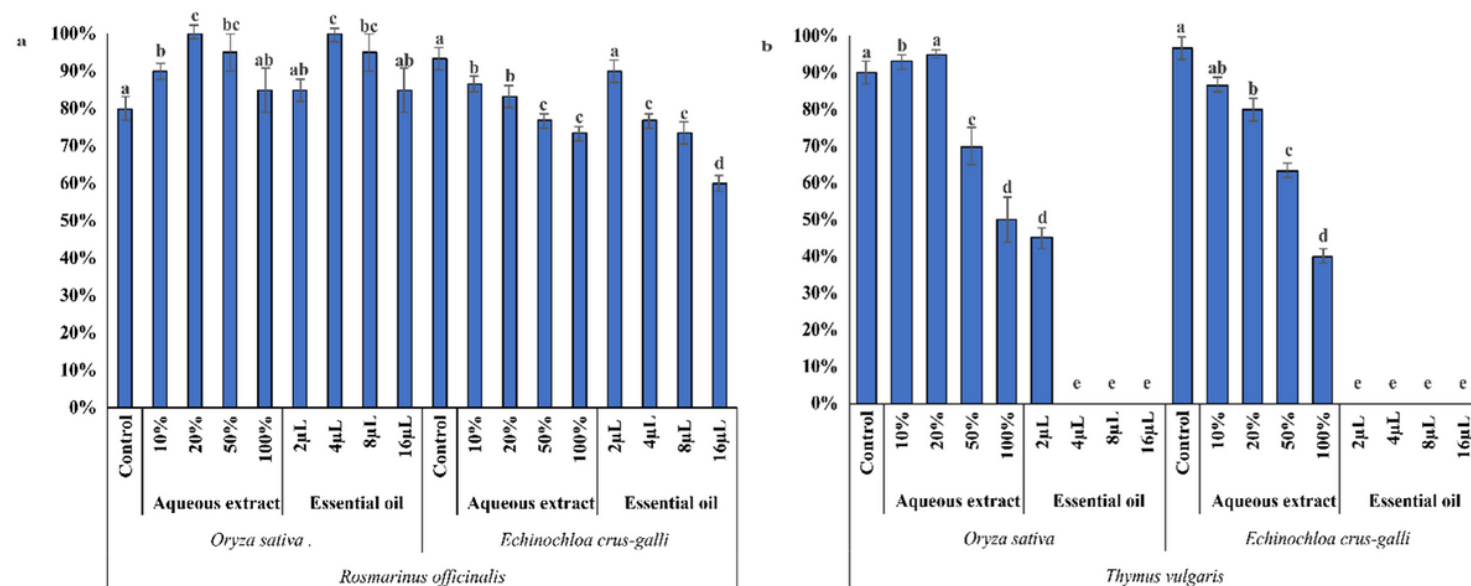


Figure 2

The effect of different dose of an aqueous extract as well as essential oils of **a**: *Rosmarinus officinalis* (rosemary) and **b**: *Thymus vulgaris* (thyme) in germination percentage % of *Oryza sativa* L. (rice) and its associated weed *Echinochloa crus-galli*.

Supplementary Files

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