Seed Germination Ability and Biochemical Features of Two *Teucrium* Taxa (Germander) of Lamiaceae

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Teucrium chamaedrys subsp. *chamaedrys* and *Teucrium polium*, from the Lamiaceae family, have ornamental, medicinal, and aromatic usage potentials. In this study, seed germination and biochemical features of samples of two taxa grown in natural habitat and cultivated were determined. Seed germination tests were carried out in petri dishes. Phenological and biochemical characteristics were performed on the seedlings and grown in viols obtained from two different origins. While germination rate was between 41% and 46% in *T. chamaedrys*, it was 33% in *T. polium*. In *T. chamaedrys* the seedling raising rate was between 16% and 26%, however, it was determined as 33.3% and 26.7% in *T. polium*, natural and cultivated plants, respectively. In terms of macro and micro elements, no statistical difference was observed in seedlings of both origins. Germacrene-D was determined with the highest amount in *T. chamaedrys* as 41.1% in natural plants seedlings and 40.46% in the cultivated ones. Moreover, β-myrcene in *T. polium* was found to be the major component in both seedlings as 20.2% and 23.4%, respectively.

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INTRODUCTION

Climate change as a result of global warming puts great pressure on water resources and causes a decrease in usable water resources. It is necessary to use water in a planned and rational way to ensure its efficient use and its clean transfer to future generations. In addition to agricultural production, the water consumption of plants used in plant design in landscape architecture studies is quite high. The effort to provide an aesthetic appearance by reducing water consumption has revealed some design concepts. One of the conceptual approaches developed for efficient water use in landscape architecture is “Xeriscape”. Xeriscape is described as creative landscape works that use water and energy effectively (Bayramoğlu *et al*. 2013; AlHalim 2020; Zohny and Moaawad 2021). According to the concept of Xeriscape, the main aim is to minimize water use and protect water resources by using plants that need less water. The main task of this design principle, especially in arid regions, is to minimize the need for water using naturally growing plants in designs. In this context, there are many studies related to the cultivation of natural species (Altuntaş 2020; Abd El-Salam 2021; Erken 2022). Because seed germination and seedling formation are the most important and critical stages in plant growth, one of the main problems encountered in the sustainable use of plants that grow naturally in arid environments is the differences between their germination ability in their natural habitat and when cultivated (Gupta 2003; Nejdafi *et al*. 2006).

For these plants to be produced both for industrial use, such as medicinal-aromatic and ornamental plants, their germination capabilities and growth characteristics should be known (Copete *et al*. 2015). It is desired that the structure and amount of essential oils (volatile oils) in medicinal and aromatic plants used in terms of essential oils should be in relation with the plants produced from the seeds taken from the natural habitat. There are several methods that can be evaluated for the extraction and isolation of volatile oils from different tissues of plants (Zancan *et al*. 2002; Cao *et al*. 2006). These methods take a long time, have limited extraction efficiency, always result in some volatile component loss, and leave behind hazardous solvent residues. Therefore, solid phase micro extraction (SPME) is one of the most important methods for determining volatile components of plants. It is a solvent-free approach for analyzing the constituents of volatile components followed by gas chromatography-mass spectrometry (GC-MS). It is an advantage that the extraction and identification of volatile components from medicinal and aromatic plants takes almost 30 min (Hashemi *et al*. 2009; Gholivand *et al*. 2013; Özderin *et al*. 2016).

Teucrium chamaedrys L. and *Teucrium polium* are two natural plant species belonging to the Lamiaceae family that can be evaluated as xeriscape and medicinal-aromatic plants. Most of the plants from Lamiaceae family are thought of as ornamental by taking into consideration their flowers and fragrance, and as medicinal and aromatic in terms of their rich volatile oil content (Dönmez 2019; Wesolowska and Jadczak 2019; Virchea *et al*. 2021). *Teucrium* sp. (germander) having 300 species, can be seen naturally in the temperate climates of Asia, Europe, and North Africa (Bukhari *et al*. 2015), and the Mediterranean is the main center of speciation with 96% of the items (Crespo *et al*. 2018; Sadeghi *et al.* 2022). *Teucrium sp.* grows naturally in arid regions. The plants of arid and semi-arid regions have developed generative trails and physiological mechanisms to survive prolonged dry periods even under the exposition to high temperatures (Levitt 1980; Burghardt *et al.* 2008). *Teucrium chamaedrys* L., which is native to Europe and South West Asia (Nencini *et al*. 2014; Giuliani *et al*. 2021), is found in sparse forests, cliffs, slopes, steppes, and arid meadows. It is a perennial woody plant that can grow 10 cm to 30 cm in length. The leaves are oblong or ovate-oblong, 1.5×2.0 cm^2. The pink flowers are dense, arranged in a row, and there are 4 to 8 flowers in each spiral (Fig. 1). *Teucrium polium* naturally grows in all areas of Türkiye and is widely distributed throughout the Middle East and Mediterranean (Tekin 2007). *Teucrium polium* is a perennial herb with prostrate stems of 10 cm to 35 cm. Grey-green leaves are 0.15×0.75 cm, whitish, flowers are 0.4×0.7 cm (Djabou *et al.* 2012; Elmasri *et al*. 2016). Because its flowers are very small, the leaf and stem structure of the plant is more attractive than its flower (Fig. 2).

Recently, *Teucrium* species has attracted much attention. Thus, studies with these species are mainly focused on the amount of their essential oils, and medicinal and aromatic uses (Bağcı *et al*. 2010; Belmakki *et al*. 2013; Raei *et al*. 2013; Candela *et al*. 2021; Catinella *et al*. 2021; Dönmez 2022; Tafrihi *et al.* 2023). These plants having medicinal and aromatic importance are not commonly cultivated and are usually gathered directly from the wild (Kostas *et al.* 2022). It puts great pressure on the plants grown in the natural environment. Cultivation of these species and determining their seed production capabilities are of great importance for the protection of plants and providing the necessary material for their use in different industries. However, the studies related with the cultivation of these plants are limited.

The objectives in this research were to study the seed germination ability and production possibilities of *T. chamaedrys* and *T. polium*, which have the potential to be used in arid landscape design and as medicinal and aromatic plants, and also the determination of the changes in volatile compounds in newly produced plants.

Fig. 1. *Teucrium chamaedrys* subsp. *chamaedrys*

Fig. 2. *Teucrium polium*

EXPERIMENTAL

The seeds of both species were collected from plants cultivated at the Botanical Garden of Süleyman Demirel University (Trial Area) in Isparta and Aziziye-Burdur, Türkiye, where they are naturally distributed. Seeds of *T. chamaedrys* and *T. polium* were stored at 4 °C until germination experiments. Environmental conditions and coordinates of both areas are given in Table 1.

Table 1. Environmental Conditions and Coordinates of Sampling Areas

		Longitude (E)	Latitude (N)	Alti-tude (m)	Rh (%)	Min. Temp (°C)	Max. Temp (°C)	Annual Temp (°C)	Annual Rainfall (mm)
N.H.	Tc.	30° 15' 25"	37° 25' 30"	1600	55	-15.0	41.0	13.3	429
	Tp.	30° 14' 31"	37° 24' 40	1200					
T.A.		30° 31' 39"	37°50'46"	1025	61	-21.0	38.7	12.3	508.3

N.H.: Natural Habitat (Burdur-Aziziye), T.A.: Trial area (Isparta-SDU Botanical Garden), Tc: *Teucrium chamaedrys* subsp. *chamaedrys*, Tp : *Teucrium polium*, Rh: Relative humidity.

Germination and Seedling Test

Although the seed dormancy of species from Lamiaceae family, which generally spreads in arid regions, diversifies due to temperature and species, it was stated as non-dormant previously by Copete *et al*. (2015) and Panuccio *et al*. (2018). After seeds were soaked for 72 h in distilled water, germination tests were conducted according to Nadjafi *et al*. (2006). Petri dishes of 9 cm in diameter was used for germination tests. About 50 seeds were placed in each dish and germination tests were performed in 3 replicates at 25 °C in the dark. Plants reaching a root length of 1 mm were considered sufficient for germination and the number of germinated seeds was counted daily until the number of germinated seeds fixed. The percentage of the ratio of seeds that germinated to all seeds sowed was used to calculate the "Germination Rate" (GR, %). According to Eq. 1, “Average Germination Speed (AGS, day)” was calculated (Pedersen *et al*. 1993),

$$AGS = (S1.D1 + S2.D2 + \ldots\ldots Sn.Dn) / (S1+S2+\ldots\ldots Sn) \tag{1}$$

where *S* denotes the number of germinated seeds each day, *D* is the day needed for the seed germination*;* and *n* is the days, until the last count

Seedlings to be used for seedling development tests and biochemical analyses were grown in a greenhouse environment (26 °C). Seeds of the plants were sowed in a vial ($4 \times 4 \times 6$ cm^3) that included peat with perlite. It was then kept in a greenhouse with 3 replicates. Each replication of the seedling experiments had 50 seeds. Plants with cotyledon leaves were considered as seedlings and counted every day. The ratio of seedlings to all seeds sowed was used to calculate the seedling raising rate (SRR, %). According to Eq. 2, “Average Seedling Raising Speed (days, ASRS)” was determined (Pedersen *et al*. 1993),

$$ASRS = (S1.D1 + S2.D2 + \ldots\ldots Sn.Dn) / (S1 + S2 + \ldots\ldots Sn) \tag{2}$$

where *S* represents the seeds amount of raising each day, *D* is the amount of day needed for germination, and *n* is the days, until the last count

Phenological Properties and Biochemical Analysis

Seedlings were transferred into flowerpots. Phenological characteristics, such as height, diameter, width of the plant, and the length of leaf, were measured using a ruler and caliper during the vegetation period. On the basis of the measurement of leaf chlorophyll content, the SPAD value was identified using a chlorophyll meter (SPAD-502, Minolta Ltd., Osaka, Japan), where measurements were made between 940 and 650 nm, and the ratio gathered from these transmission values are SPAD values (Monostori *et al*. 2016).

For biochemical analysis of the seedlings in the vegetation period, randomly collected plants, from each plot, were dried in an oven at 65 °C until constant weight was obtained. Total N content was determined as percentage by Kjeldahl method (Trikilidou *et al*. 2022). Atomic absorption spectrometry (AAS) was used to determine the mineral content (K, Ca, Mg, Fe, Cu, Mn, Zn) of plant materials (Mahood 2021). The amount of P was defined with the modifications of spectrophotometric method developed by Olsen (1954).

Identification of Volatile Compounds

Volatile components of *Teucrium* species were determined according to Dönmez and Salman (2017) with some modifications. About 2 g of plant sample separated from branches were transferred into SPME vials (Supelco 27159 15 mL, PTFE/Silicone septa cap), the vials were then placed in the heater set at 60 °C, and it was kept at 60 °C for 15 min for preheating. Injector with suitable fiber tip (Fused silica SPME fiber CAR/PDMS) was dipped in the vial for 30 min and then until absorption. Fiber that absorbed the compounds was injected into the GC-MS injection block for desorption. To detect volatile compounds, GC-MS (Shimadzu QP 2010 Plus) with a Restek Rx-5Sil MS (30 m × 0.25 µm, 0.25 µm film thickness) column was used. Injection port temperature was adjusted to 250 °C, carrier gas used was helium at a rate of 1.61 mL/min constant flow. Temperature was programmed at 40 °C (2 min hold) and was increased at a rate of 4 °C/min to 250 °C (5 min hold). The peaks of the constituents were identified by scanning in Wiley, NIST Tutor, and FFNSC libraries.

RESULTS AND DISCUSSION

Germination Test and Seedling Raising Rate

Although the seed dormancy of species from Lamiaceae family, which generally spreads in arid regions, diversifies due to temperature and species, it was stated as non-dormant previously by Copete *et al*. (2015) and Panuccio *et al*. (2018). *Teucrium chamaedrys* had a 46.7% germination rate in seeds taken from the native environment and a 41.3% germination rate in seeds taken from the trial area. In soil experiments, while the seedling growth percentage was 16.7% in seeds from natural areas, it was observed as 26.7% in the trial area seeds. Average germination speed for *T. chamaedrys* was 18 days. The germination capacity of *Teucrium* species varies (Ferriol *et al*. 2006), and it is known that the germination percentage of *T. polium* (32.7%) is low (Nadjafi *et al*. 2006). The seedling growth percentage (26.7%, 33.3%) and average seedling raising speed (20, 70, 19, and 90 days) values of *T. polium* were higher than germination percentage (Table 2). Species of the Lamiaceae had germination rates ranging between 20% and 50%, and the germination might last up to 30 days (Mandal *et al*. 2008; Mattana *et al*. 2016; Dönmez and Önal 2019).

Table 2. Germination and Seedling Raising Data of Plant

		GR (%)	AGS (day)	SRR (%)	ASRS (day)
T. chamaedrys	Trial	41.33	17.85	16.66	21.08
	Natural	46.66	18.42	26.66	23.08
	sig.	0.23	0.42	0.05	0.54
T. polium	Trial	3.33	19.70	26.66	20.70
	Natural	-	-	33.33	19.90
	sig.			0.14	0.21

In both *Teucrium* taxa, there was no flowering in the vegetation period after germination. Leaf widths of seedlings belonging to *T. chamaedrys* were measured in the samples from both natural and trial area as 1.02 to 1.04 cm., and leaf lengths were 1.59 to 1.71 cm, respectively (Table 3). In a study by Şenkal and Uskutoğlu (2022), the data obtained because of the germination trials of the seeds collected from nature in Yozgat, Türkiye had a similar result with the current study. In *T. polium*, the leaf width was measured as 0.36 to 0.41 and the length as 1.08 to 1.22. Chlorophyll content, which is the most important pigment required for photosynthesis, is one of the main sources reflecting the photosynthetic ability of the leaf and the health condition of plant. Chlorophyll was determined as 52.7 to 53.6 SPAD value in natural and trial area, respectively, in *T. chamaedrys*, and it was 49.9 to 52.5 in *T. polium*. The SPAD value was given previously by Manetas *et al*. (1998) as 49.8 in some *Teucrium* species.

One of the most crucial elements for plant growth is nitrogen. Nitrogen content analyzes were carried out in the studied plants when the plant completed its growth and development at the end of vegetation. The highest nitrogen content was determined in the trial area plants of *T. chamaedrys* (2.62%). While it was found as 0.77% in *T. chamaedrys* by Tunçtürk *et al*. (2019), nitrogen content of *T. polium* was determined as 1.76% in the same study. In medicinal and aromatic plants, quality is as important as yield, even those below a certain quality are not grown even if they are very productive (Şahin 2013). Among the elements that have an important role in plant growth and development, P (0.25%), Ca (2.38%), Mg (0.96%), and Cu (22.41 ppm) were determined at the highest rate in *T. chamaedrys*, and K (4.21%), Fe (157.21 ppm), Mn (42.27 ppm), and Zn (74.58 ppm) in *T. polium*. The amount of P was reported 0.66% (Yücel *et. al*. 2011) and 4.44 g/kg (Tunçtürk *et al*. 2019) in *T. polium*. The differences in biochemical properties is directly related with ecological conditions. Additionally, the nutrient needs in plant growing are also different. When the phenological and biochemical properties of the seedlings obtained from the seeds collected from the trial areas and natural habitat were compared, there was no statistically significant difference according to the "Independent sample t test-P".

Table 3. Phenological and Biochemical Properties

	Teucrium chamaedrys			*Teucrium polium*		
	N	T	sig	N	T	sig
Plant width (cm)	2.60 ± 0.5	2.55 ± 0.4	0.88	2.80 ± 0.4	2.60 ± 0.3	0.40
Plant height (cm)	11.25 ± 1.1	10.35 ± 1.3	0.12	9.55 ± 1.2	9.05 ± 1.3	0.38
Leaf width (cm)	1.02 ± 0.1	1.04 ± 0.1	0.77	0.36 ± 0.1	0.41 ± 0.1	0.40
Leaf length (cm)	1.59 ± 0.4	1.71 ± 0.3	0.49	1.08 ± 0.2	1.22 ± 0.3	0.33
SPAD value	52.68 ± 4.3	53.58 ± 3.3	0.61	49.94 ± 5.0	52.53 ± 2.9	0.17
Nitrogen (N) (%)	2.25 ± 0.1	2.62 ± 0.1	0.05	2.32 ± 0.1	2.14 ± 0.6	0.08
Phosphorus (P) (%)	0.24 ± 0.1	0.25 ± 0.1	0.56	0.21 ± 0.1	0.20 ± 0.1	0.39
Potassium (K)(%)	3.15 ± 0.5	2.94 ± 0.4	0.05	4.15 ± 0.2	4.21 ± 0.1	0.81
Calcium (Ca)(%)	2.21 ± 0.2	2.38 ± 0.4	0.02	1.64 ± 0.2	1.82 ± 0.2	0.06
Magnesium (Mg)(%)	0.91 ± 0.2	0.96 ± 0.2	0.01	0.92 ± 0.1	0.94 ± 0.1	0.80
Iron (Fe)(ppm)	122.01 ± 1.2	123.01 ± 1.4	0.30	157.21 ± 2.5	154.4 ± 1.5	0.09
Copper (Cu) (ppm)	22.14 ± 1.0	22.41 ± 0.8	0.90	18.29 ± 1.9	19.02 ± 0.9	0.43
Manganese(Mn)(ppm)	32.14 ± 1.1	31.72 ± 0.8	0.42	42.27 ± 1.2	41.62 ± 0.9	0.37
Zinc (Zn) (ppm)	62.21 ± 0.4	61.92 ± 1.0	0.67	74.58 ± 1.6	73.84 ± 2.0	0.48

N: The natural habitat seedlings, T: the trial area seedlings

Volatile Compounds

Volatile components determined by SPME in two *Teucrium* species are given in Table 4.

Table 4. Volatile Compounds (%) of *T. chamaedrys* Seedlings

Components	Natural Area	Trial Area
3-Hydroxybutanal	0.09	0.12
1-Penten-3-one	0.08	0.05
3-Hexenal	0.26	0.27
n-Hexanal	0.99	0.92
2-Hexenal	12.81	11.79
Cyclohexanol	0.06	0.03
2,4-Hexadienal	0.34	0.42
α-Pinene	1.51	1.75
Benzaldehyde	0.38	0.39
Furanone	0.04	0.06
Sabinene	0.06	0.05
β-Pinene	0.88	0.92
1-Octen-3-ol	5.98	6.69
β-Myrcene	0.46	0.23
2,4-Heptadienal	0.14	0.08
Hexanol	0.36	0.33
Limonene	2.93	2.81
Benzeneacetaldehyde	0.41	0.35
β-Ocimene	0.14	0.11
Linalool	0.51	0.50
3-Octanyl acetate	0.04	0.05
Me-salicylate	0.55	0.54
Linalyl acetate	0.09	0.08
α-Terpenyl acetate	0.09	0.09
Anethole	0.15	0.12
Bicycloelemene	0.26	0.21
α-Cubebene	0.69	0.62
α-Copaene	0.44	0.49
β-Bourbonene	0.60	0.54
α-Gurjunene	0.11	0.09
β-Cubebene	0.76	0.66
Caryophyllene	14.91	14.81
Germacrene-D	41.06	40.46
Cadina-1,4-diene	0.21	0.36
α-Humulene	2.76	2.95
Aromadendrene	0.53	0.57
Epibicyclosesquiphellandrene	0.64	0.78
Caryophyllene	0.06	0.02
Γ-Cadinene	1.58	1.76
β-Selinene	0.18	0.13
Bicyclogermacrene	2.96	2.93
α-Muurolene	0.44	0.44
α-Panasinsen	3.01	3.17
α-Calacorene	0.09	0.05
1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	0.26	0.20

Table 5. Volatile Compounds of *T. polium* Seedlings (%)

Components	Natural Area	Trial Areas
Acetaldehyde	0.22	0.15
Ethanol	0.25	0.83
2-Butenal	1.26	0.76
2-Ethyl-Furan	0.24	0.13
Valeraldehyde	0.09	0.21
cis-3-Methylcyclohexanol	0.39	0.25
n-Hexanal	1.31	0.83
2-Hexen-1-al	5.84	5.06
3-Hexene-1-ol	0.33	0.62
n-Hexanol	0.25	0.26
2,4-Hexadienal	0.96	0.64
α-Thujene	15.61	11.43
α-Pinene	3.41	2.87
2,6,6-Trimethylbicyclohept-2-ene	0.22	0.31
Bicyclohex-2-ene,	0.09	0.11
Camphene	0.37	0.43
Benzaldehyde	1.06	0.91
Sabinene	7.14	6.24
1-Octen-3-one	0.41	0.39
Bicycloheptane,	1.54	1.06
1-Octen-3-ol	1.01	1.94
β-Myrcene	20.22	23.45
2,4-Heptadienal	1.09	0.76
3-Octanol	0.24	0.29
l-Phellandrene	0.09	0.07
Ethyl-Hexanol	0.06	0.11
2,4-Heptadienal,	0.64	0.73
α-Terpinene	0.82	1.09
p-Cymene	1.98	2.09
Cyclopentanecarboxaldehyde,	1.45	1.69
Cymol	2.46	2.81
Me-cymol	0.06	0.09
Limonene	6.12	7.45
trans-Limonene oxide	0.08	0.08
4-Terpineol	0.71	0.38
Eucalyptol	0.11	0.19
β-Ocimene	2.11	1.87
Cyclopropane	0.09	0.11
1,4-Cyclohexadiene,	0.61	0.51
α-Terpinolene	0.21	0.29
1,6-Octadien-3-ol	0.13	0.11
L-Linalool	0.11	0.09
p-Mentha-1,5,8-triene	0.45	0.32
2,4,6-Octatriene	0.13	0.27
3-Cyclohexen-1-ol	0.31	0.33
α-Cubebene	0.18	0.19
β-Bourbonene	0.31	1.22
Germacrene-B	1.45	1.23

Germacrene-D	4.09	4.86
β-Farnesene	4.56	3.21
Epibicyclosesquiphellandrene	0.24	0.36
Cadina-1,4-diene	0.24	0.16
α-Cubebene	0.61	0.72
Bicyclogermacrene	0.51	1.84
α-Bisabolene	0.55	0.61
α-Muurolene	0.18	0.27
β-Bisabolene	1.09	0.87
γ-Cadinene	2.03	1.61
δ-Cadinene	0.19	0.91
α-Humulene	0.31	0.23
β-Eudesmol	0.21	0.51

Forty-six different volatile components of *T. chamaedrys*, collected from both areas were found. Germacrene-D, the most dominant component, was detected at 41.1% level in the native seedlings and at 40.5% in trial area plants. Caryophyllene was also a major constituent and its content was 14.9% in the native and 14.8% in trial area seedlings. 2-Hexenal (12.8% in natural habitat and 11.8% in trial area plants) and 1-octen-3-ol (5.98% in natural habitat and 6.69% in trial area plants) were also seen as major components in the volatile compounds of *T. chamaedrys*. The results of the study are in agreement with the literature (Katayoun *et al*. 2005; Bağcı *et al*. 2010; Maccioni *et al*. 2021).

Additionally, it was seen that rootstocks where seeds that were collected had the highest concentrations of the same components (Dönmez 2022). *Teucrium polium* had 61 volatile components (Table 5) and β-myrcene, the most abundant component, was found in 20.2% and 23.4% in native and trial seedlings, respectively. α-Thujene (15.6% in the natural habitat and 11.4% in the trial area plants), sabinene (7.14% to 6.24%), limonene (6.12% to 7.45%), and 2-hexen-1-al (5.84% to 5.06%) were the dominant components of *T. polium*. α-Thujene and germacrene-D were previously stated as the dominant component in this species by Saijadi and Ghannadi (2004), Djabou *et al.* (2012), Ciocarlan *et al.* (2022), and Dönmez (2022).

CONCLUSIONS

It is inevitable to apply xeriscape in planting designs to be made in arid areas, especially in our time when water is scarce. The main way to do this is to use natural species. Following are the conclusions derived from the results of the current study:

1. It was revealed that the germination ability of two naturally growing taxa did not change after cultivation.
2. The phenological properties, such as length, height of the plants, and width and length of the leaves as well as elemental contents (N, P, K, Ca, Mg, Fe, Cu, Mn, and Zn) and other biochemical structures were similar to both origin seedlings. In contrast, no increase in germination rates was observed even when the plants were cultivated.
3. Germacrene-D and caryophyllene were the most abundant component in both origin of *T. chamaedrys*. In *T. polium*, β-myrcene and α-thujene had the highest amount. In

addition, 2-hexenal, limonene, and 1-octen-3-ol were seen as dominant components in both plant species.

4. While physiological and biochemical properties of the plants did not change, germination yield remained low. Further studies should be carried out to increase the germination and seedling yield to produce this plant in different industries in terms of both designs and evaluation of its medicinal and aromatic properties.

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