Co-application of arbuscular mycorrhizae fungi and putrescine improves essential oil production and drought tolerance cumin (Cuminum cyminum L.)

Hamid Reza Anjam
Islamic Azad University
Moslem Abdipour
m.abdipour@areeo.ac.ir
Agricultural Research, Education and Extension Organization (AREEO)
Mehdi Hosseinifarahi
Islamic Azad University

Article

Keywords: Chemical compounds, Cumin, Drought stress, Mycorrhizae, Putrescine

Posted Date: July 19th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4627617/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on April 27th, 2026. See the published version at https://doi.org/10.1038/s41598-026-43180-w.

Abstract

The effects of arbuscular mycorrhizae fungi (AMF), putrescine (Put), and their combination on essential oil (EO) content, chlorophyll (Chl), carotenoid and EO chemical compounds of cumin (*Cuminum cyminum* L.) were studied under optimal and drought stress conditions. The foliar application of Put or inoculation with AMF significantly ($P \leq 0.05$) increased seed yield, EO content, EO yield, Chl a, Chl b, total Chl, and carotenoid. Moreover, AMF or Put application increased cuminaldehyde, limone, and β-Pinene and decreased γ-Terpinene, o-Cymene, and linalool concentration in cumin plants compared to non-treated plants in both optimal and drought stress conditions. However, the highest EO content, EO yield, Chl a, Chl b, total Chl, carotenoid, and major EO chemical components were obtained when AMF and Put were used together under optimal and drought stress conditions. This study showed that Put and AMF can be co-applied to the cumin plant to significantly improve drought tolerance in field conditions greatly.

1. Introduction

Cumin (*Cuminum cyminum* L.), is an essential spice and medicinal plant from the Apiaceae family with a wide range of uses in various industries [1]. This plant is widely cultivated in arid and semi-arid regions due to its short growing season and low water requirement [2]. However, cumin plants face a variety of abiotic stresses during growth in these regions, such as drought stress, which can greatly affect their morphological and physiological characteristics and the concentration of their secondary metabolites and essential oil [3]. Limited water resources, besides progressive demand from food, pharmaceutical, and cosmetic industries, have encouraged researchers to improve water use in medicinal and aromatic plants with an emphasis on sustainable agriculture.

It has been documented that defense systems in plants such as antioxidant may be insufficient to reduce the negative consequence of radical oxygen species (ROS) in stressful conditions such as drought stress [4]. Most recently, researchers have been drawn towards the application of biofertilizers to cope with drought stress. Biofertilizers are an emerging alternative considered a safer, cost-effective, and eco-friendly source of sustainable crop production with reduced chemical input [5].

Arbuscular mycorrhizal fungi (AMF) are known as the most important biofertilizers to improve the quantity and quality of plants by increasing the availability and maintaining the ecological balance of soil nutrients [6]. AMF can also efficiently increase plant tolerance to different stressful conditions, e.g., drought stress [7,8], salinity [9] and heavy metals [10], by set up a common symbiotic connection with roots [11]. Increased tolerance to drought stress in plants inoculated with AMF can be due to regulation of leaf stomatal conductivity, increase hydraulic conductivity of roots, reinstate ionic homeostasis, and balance biological process [12,13].

AMF alters the biosynthesis of essential oils by inducing the synthesis of secondary metabolites through the so-called induced systemic resistance (ISR) mechanism [14].

Polyamines (PAs) are a class of biogenic amines with multiple vital roles in the growth of plants. Three ubiquitous amines, viz., spermidine, spermine, and putrescine, are the most frequent PAs in plants and are widely used as plant growth regulators (PGRs) [15]. These osmotically active substances have been determined as potential candidates to ameliorate the adverse effects of environmental stresses [16].

The accumulation of PAs in plants exposed to environmental stresses and its vital role in stress tolerance have motivated researchers to apply PAs exogenously to cope with the adverse effects of various environmental stresses, such as drought [17]. Several reports have confirmed the role of PAs as a regulatory factor in improving the drought tolerance in medicinal plants, such as chamomile, thyme, fennel, and flame lily [17–20].

So far, there is no report on the effects of co-application of PAs and AMF on EO content and EO components in cumin plants under drought stress. Therefore, the main object of this research was to confirm whether AMF and Put as PBs can increase seed yield, EO content, and EO yield in *Cuminum cyminum* L. under normal irrigation and drought stress conditions. Furthermore, changing the EO profiles of *Cuminum cyminum* L. with the use of AMF and Put was investigated.

2. Materials and Methods

2.1. Study site and experimental layout

A two-year field experiment was conducted at the Chamkhani Research Station, Yasouj, Iran (51°31′06″ N, 30°41′59″ E, Elevation 1740 m), with an average temperature of 16°C and 11.5°C and humidity of 47.6% and 49% during the growing season in 2018 and 2019, respectively.

The soil at the experimental site was characterized as a clay loam with a hygroscopicity index of 7.3, a C:N of < 17, organic matter content of 0.96%, N content of 1%, available P content of 24 ppm, available K content of 337 ppm, and pH of 7.1.

Planting was done by hand in individual plots (4m × 3m) with an inter-row spacing of 20 cm and a plant distance of 5 cm. Cumin plants were sown on 10 March 2018 and 13 March 2019 and harvested on 10 June 2018 and 15 June 2019.

This research was performed in a factorial experiment according to a randomized complete block design (RCBD) with three replications. The factors were (1) irrigation treatment, including irrigation after 20% (normal irrigation) and 75% (drought stress) of the available soil water was depleted, (2) AMF inoculum (*Glomus intraradices*), including control (non-inoculation) and inoculation with the fungi, and (3) the foliar application of Put at three levels, i.e., control (0 mg L^{-1}), 1000, and 2000 mg L^{-1}.

All treatments were irrigated with a drip irrigation system every three days for five weeks to facilitate seedling establishment. Irrigation treatments were applied from April 16 and 19 in the first and second year, respectively, and continued throughout the growing season (end of June in both years).

The water requirement (m^3) for each treatment was determined based on the soil water content (θi) using the TDR method (Time Domain Reflectometry, Model 4593) according to the following Eq. 2[1]:

Vw: $(\theta F.C - \theta i) \times Bd \times D \times A$

where *Vw* is the irrigation water volume, *θF.C* is the volume of soil moisture at field capacity (FC), *θi* is the soil water content, *Bd* is the soil bulk deity (kg/m^3), *D* is the rooting depth (0.3 m), and *A* is the main plot area (m^2).

The AMF inoculation was down according to Khalediyan, et al. [22] with an inoculum of 35 g containing approximately 1450 spores. The AM fungi (*G. intraradices*) were obtained from the Soil and Water Research Institute, Iran.

The foliar application of Put was performed twice. The first application was performed one week before drought treatment (April 9 and 12), and the second one was performed two weeks after the first application. Put was sprayed by an atomizer as the leaves were completely covered.

2.3. Plant sampling and data collecting

At the flowering stage (May 10 and 13 in 2018 and 2019, respectively), 10–12 plants were randomly selected and the contents of chlorophyll (a, b, and total) and carotenoid pigments were measured.

Chlorophyll *a*, *b*, and a + b (total) was measured based on [23] procedure. To measure the amount of chlorophyll, 1 g of leaf sample of each plot was beaten with 5 mL acetone 80% in a porcelain mortar. After purifying the resulting extract, the remaining leftovers were completely washed with 10 mL of acetone and passed through filter paper. Finally, the samples were as completely homogenized liquid to 10 mL volume. Optical absorbance was read by a spectrophotometer device at 663 and 645 (for chlorophyll *a* and *b*, respectively). Carotenoids content was determined using the method of Gu, et al. [24].

The seed yield was measured on plants harvested from 2 m^2 of each plot.

2.4. EO extraction and Gas chromatography/mass spectrometry (GC-MS) analysis

EO was extracted using distillation with a glass Clevenger-type apparatus as recommended by the British Pharmacopoeia [25].

EO components analyses were down according to de Lira, et al. [26] with a Hewlett-Packard 6890 gas chromatograph. More details can be found in Sharafabad, et al. [27].

The retention indices (RI) were calculated using the standard solution of C8–C20 n-alkane (Fluka® Analytical, Munich, Germany) in hexane according to Adams [28] method. The bioactive constituents of cumin were confirmed by comparison of their RI with those reported in similar studies [29,30].

2.5. Statistical analysis

The data after testing for normality and homogeneity of variance, were subjected to a combined analysis of variance (ANOVA). The differences among the means were tested by Duncan's multiple range test. Due to the insignificant ($P \le 0.05$) effect of year on the traits, the average of the two-year data was used for further analyses. Statistical Analysis System (SAS) package version 9.4 [31] was used for statistical analysis.

3. Results and Discussion

3.1. Seed yield

The combined ANOVA indicated that the effects of water deficit treatments, putrescine (Put), arbuscular mycorrhizal fungi (AMF), and their interactions were significant on the seed yield (Table 1). However, the effect of year and it’s interaction with water deficit treatments, Put, and AMF were not significant ($p \leq 0.05$) on this trait. Duncan’s test indicated that the drought stress (25% FC) drastically reduced the seed yield of the cumin plants by 79.1% compared to the control (Table 2). The results are in line with the studies of Alipour, et al. [32] on fennel and the research of Jahani, et al. [33] on peppermint. Water deficit conditions, especially in the reproductive stage, result in the closure of stomatal apparatuses, which reduces chlorophyll florescence and then photosynthesis duration [34]. Such reduction in the photosynthesis period leads to a decrease in material mobilization to the seeds and the share of remobilization of material stored in the stem to seeds and a decrease in the seed yield [35,36].

Table 1
Combined analysis of variance related to the studied traits in two years

SOV	df	Seed yield	Essential oil (%)	Essential oil yield	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids
Year	1	20480	2.731	45.43	0.119	0.112	0.255	0.155
Block (Year)	4	2876	0.421	11.59	0.070	0.034	0.038	0.207
Water deficit	1	49603**	2.221**	59.47**	0.881**	0.527**	1.427**	2.124**
Year × Water deficit	1	2435	0.292	10.25	0.077	0.070	0.399	0.284
Block (Year × Water deficit)	4	493.2	0.084	2.487	0.032	0.017	0.058	0.053
Putrescin	2	7360**	1.212**	9.660**	0.454**	0.059**	0.408**	0.418**
Mycorrhizal fungi	1	11139**	2.057**	13.92**	0.392**	0.103**	0.731**	0.399**
Year × Putrescin	2	3202	0.354	4.131	0.139	0.027	0.080	0.098
Year × Mycorrhizal fungi	1	4116	0.511	2.820	0.087	0.049*	0.163	0.170
Water deficit × Putrescin	2	10152**	1.893**	15.86**	0.309**	0.098**	0.489**	0.513**
Water deficit × Mycorrhizal fungi	1	17919**	2.416**	16.51**	0.508**	0.177**	0.574**	0.646**
Putrescin × Mycorrhizal fungi	2	129159**	1.415**	17.16**	0.491**	0.057*	0.605**	0.558*
Year × Water deficit × Putrescin	2	1239	0.456	5.101	0.162	0.021	0.161	0.138
Year × Water deficit × Mycorrhizal fungi	1	1134	0.197	2.487	0.053	0.038	0.257	0.164
Year × Putrescin × Mycorrhizal fungi	2	1722	0.260	7.125*	0.101	0.032	0.089	0.115
Water deficit × Putrescin × Mycorrhizal fungi	2	14297**	0.944**	12.80**	0.591**	0.135**	0.396**	0.453**
Year × Water deficit × Putrescin × Mycorrhizal fungi	2	3202	0.396	3.230	0.169	0.031	0.203	0.140
Error	40	1050	0.158	1.673	0.053	0.011	0.065	0.046
CV† (%)		10.61	15.95	16.07	12.72	17.21	10.64	4.89

*Significant at the level of 0.05; **Significant at the level of 0.01; †CV = Coefficient of variance

Table 2
Effects of polyamine and arbuscular mycorrhiza fungi (AMF) on traits in cumin plant under water deficit.

Water deficit	Putrescine	Mycorrhizal fungi	Seed yield (kg. ha^{-1})	Essential oil (%)	Essential oil yield (kg. ha^{-1})	Chlorophyll a (mg g^{-1}.FW)	Chlorophyll b (mg g^{-1}.FW)	Total chlorophyll (mg g^{-1}.FW)	Carotenoids (mg g^{-1}.FW)
80% available soil water	0 mg L^{-1}	Non-inoculation	308.89^{de}	1.91^{h}	5.89^{f}	1.44^{e}	0.41^{c}	1.85^{gh}	4.25^{f}
		AMF-inoculation	402.68^{b}	2.43^{e}	9.78^{b}	2.01^{c}	0.54^{b}	2.55^{cd}	4.8^{d}
	1000 mg L^{-1}	Non-inoculation	322.06^{d}	2.01^{g}	6.47^{e}	1.52^{e}	0.45^{bc}	1.97^{g}	4.31^{f}
		AMF-inoculation	417.03^{a}	2.79^{c}	11.63^{a}	2.36^{b}	0.66^{ab}	3.02^{b}	5.79^{a}
	2000 mg L^{-1}	Non-inoculation	374.37^{c}	2.17^{f}	8.12^{c}	1.91^{c}	0.47^{bc}	2.38^{e}	4.7^{d}
		AMF-inoculation	423.68^{a}	3.05^{b}	12.92^{a}	2.77^{a}	0.75^{a}	3.52^{a}	5.57^{ab}
30% available soil water	0 mg L^{-1}	Non-inoculation	172.44^{i}	2.13^{f}	3.67^{i}	0.96^{fg}	0.31^{d}	1.27^{k}	3.24^{ij}
		AMF-inoculation	267.61^{g}	2.61^{d}	6.98^{d}	1.87^{d}	0.39^{c}	2.26^{f}	3.68^{h}
	1000 mg L^{-1}	Non-inoculation	178.35^{i}	2.16^{f}	3.85^{h}	1.12^{f}	0.36^{cd}	1.48^{j}	3.38^{i}
		AMF-inoculation	285.55^{f}	2.94^{b}	8.39^{c}	2.06^{c}	0.43^{c}	2.49^{d}	4.58^{e}
	2000 mg L^{-1}	Non-inoculation	220.34^{h}	2.38^{e}	5.24^{g}	1.26^{f}	0.38^{cd}	1.64^{i}	3.94^{g}
		AMF-inoculation	291.1^{f}	3.31^{a}	9.63^{b}	2.15^{bc}	0.56^{b}	2.71^{c}	5.08^{c}

Values marked with the same alphabets are not significantly different (LSD, $p \leq 0.05$).

Under non-stress conditions, the application of Put (1000 and 2000 mgL^{-1}) or AMF significantly increased the seed yield by 4.26, 21.19, and 30.36% compared to the non-treated plants, respectively (Table 2). A 3.43, 27.78 and 55.19% increase was recorded in the cumin plants treated with 1000 and 2000 mgL^{-1} or inoculated with AMF over the non-treated plants under water deficit conditions, respectively. These results are consistent with the researchers who found a significant increase in seed yield when plants were inoculated with AMF under non-water stress conditions [37–39] and water deficit stress conditions [40,41].

The higher resistance of mycorrhizal plants to environmental stress can be related to the fact that AMF inoculation improves root architecture and the change of endogenous phytohormone contents in plants [42]. The improvement of root architecture that leads to the absorption of more water and nutrients from the soil is mainly attributed to the increase in the lateral root number, total root length, root surface area, and root volume in AMF-inoculated plants [43].

Compared to non-stress conditions, the cumin plants treated with Put and/or AMF under water deficit stress produced a higher seed yield. For example, Put application to the cumin plants in water stress conditions led to a 15.6% increase in this trait, while it led to a 12.73% increase in the seed yield in non-stress conditions (Table 2). Similarly, AMF under water stress conditions led to a significant increase compared to the non-stress conditions (55.19▯30.36%). Although foliar application of Put and AMF inoculation increased seed yield, it seems that cumin plants inoculated with AMF compared those treated with Put showed a greater extent of seed yield (on average, 42.77% compared to 14.16%). Interestingly, the Put and AMF treatments had a synergistic effect, where the highest seed yield was recorded in the plants treated with 2000 mgL^{-1} Put and AMF.

Numerous studies have been reported that the co-application of the two PBs can increase seed yield more than their individual application [44,45].

3.2. Percentage of essential oil

According to the results of combined ANOVA, the effects of water deficit stress, Put, AMF, and their interactions were significant on essential oil percentage (EO) (*p*0.01, Table 1). However, the effect of year and its interactions with other treatments was not significant on this trait (*p*0.05, Table 1).

Interestingly, a significant increase (about 12%) in EO percentage with water deficit stress was observed compared with the plants under non-stress conditions (Table 2). The accumulation of secondary metabolites due to adverse environmental conditions, like drought, is a defense response to keep up cell balance and reduce the damaging results of stress [46]. In line with the results of the present study, in their research on the effect of drought stress on the EO percentage of *Pelargonium graveolens*, *Mentha piperita* L., and *Melissa officinalis* L., respectively, Amiri, et al. [47], Jahani, et al. [33], and Eshaghi Gorgi, et al. [7] reported that the EO percentage significantly increased with enhancing the intensity of drought stress.

In addition, the results of this study showed that the application of Put increased the percentage of EO by 9.42 and 6.57% under non-stress and stress conditions, respectively. These results are consistent with those of Abd Elbar, et al. [48], according to which the *Thymus vulgaris* L. plants treated with Put showed higher EO content under both non-drought and drought stress than the plants without Put application.

Our findings showed a significant difference in response to Put concentrations (1000 and 2000 mgL^{-1}). The cumin plants treated with 2000 mgL^{-1} Put had a higher percentage of EO than those treated with 1000 mgL^{-1} of Put. Our results support the results of Mohammadi, et al. [18], who obtained the highest increase in the percentage of EO under both non-drought stress and stress when the *Thymus vulgaris* L. plants were treated with the highest concentration of Put.

Furthermore, the results showed that AMF colonization under non-stress and water deficit stress conditions caused a statistically significant increase of 27.23% and 20.83% in the EO percentage compared with the control treatment (Table 2). It has been documented that colonization with mycorrhizae can improve the EO percentage by improving soil microbial activity, increasing P availability, and producing plant growth regulators [22,49]. Under both non-stress and water deficit stress conditions, the cumin plants inoculated with AMF had a higher EO percentage than those treated with Put. However, the maximum rise in the EO percentage was exhibited by the co-application of AMF and Put compared to their individual use.

3.3. Essential oil yield

The EO yield was remarkably (*p*0.01) impressed by the trial factors and their interactions (except year, Table 1). Duncan's multiple range test showed that water deficit stress significantly reduced the EO yield by 60.99% in the cumin plants compared to the control. Basically, the EO yield in medicinal plants depends on the percentage of EO and the seed yield/flower yield, so changes in either factor can affect the EO yield [50]. According to the results, although the oil content increased in drought stress conditions, the seed yield decreased drastically (79.1%), leading to a decrease in the EO yield under drought stress conditions. Our results agree with those formerly reported by Mehrabi, et al. [51] on cumin and Alipour, et al. [32] on fennel, who reported that the EO yield was significantly influenced by drought stress.

A pronounced increase was observed in the cumin plants inoculated with AMF under non-stress and water deficit stress conditions (66.04% and 90.19%, respectively, Table 2). The symbiosis between AMF and plants enhances phosphorous, iron, calcium, sulfur, copper, and zinc uptake and can elevate resistance to biotic and abiotic stresses [52]. The application of AMF, especially under drought stress, as an approach to increasing the EO yield has been reported in medicinal plants, such as *Pelargonium graveolens* L. [47], *Ocimum basilicum*, and *Satureja hortensis* [22], *Foeniculum vulgare* Mill [32], and *Melissa officinalis* L. [7]. The cumin plants treated with Put also exhibited a significant increase compared to control under both non-stress and water deficit stress conditions.

Owing to its tissue and outer layer stabilizing properties, its acid-neutralizing and antioxidant functions, Put can maintain a high water status, especially in dry environments, and can alleviate the impact on water status [53]. The pivotal role of Put in maintaining higher water status under drought stress and enhancing seed yield and EO yield have been reported by Mohammadi, et al. [18], Zeynali, et al. [54] on *Salvia officinalis* L., and Nazarli and Naderi Arefi [55] on *Matricaria chamomilla* L. However, the maximum increase in EO yield was manifested by the cumin plants treated with both Put and AMF (Table 2).

3.4. Leaf chlorophyll content and carotenoid

The results of combined ANOVA showed that the effects of water deficit stress, Put, AMF, and their interactions were significant on chlorophyll *a*, *b*, total chlorophyll, and carotenoid (Table 1). The cumin plants subjected to drought stress exhibited a notable reduction in above traits (Table 2). Chlorophyll a, b, total chlorophyll, and carotenoid of the cumin plants dropped significantly by 50%, 32.25%, 45.67%, and 31.17%, respectively, under induced water stress than under the non-stressed control plants (Table 2). Like our results, Mohammadi, et al. [18] found that the thyme plants exhibited 38.82, 51.11, and 41.61% lower chlorophyll a, chlorophyll b, and total chlorophyll contents under drought stress over the control, respectively.

Chlorophyll, a key pigment to regulate the strength of the photosynthesis operation and crop production, is very sensitive to drought stress [56]. Drought stress can reduce chlorophyll content through destruction of chlorophyll substrate, chloroplast peroxidation, chlorophyll photo-oxidation, imbalance of protein complexes, inhibition of chlorophyll biosynthesis, and the increase in chlorophyllase activity Taïbi, et al. [57]. In this work, chlorophyll a was found to be more sensitive to drought stress than chlorophyll b (Table 2). Our results are in agreement with Kamrava, et al. [58] and Alipour, et al. [32], who reported that chlorophyll a was more sensitive to water deficit and drastically decreased compared to chlorophyll b in rapeseed and fennel.

The decline in carotenoid content due to drought stress has been reported in *Melissa officinalis* L. and *Nigella sativa* L. [7,59]. The malfunction of beta-carotene and the shaping of xanthine in the xanthophyll cycle has been reported as one of the reasons for carotenoid content reduction under drought stress conditions [59].

However, the cumin plants significantly benefitted from AMF inoculation, leading to increased chlorophyll a, b, total chlorophyll, and carotenoid compared to the control plants without AMF inoculation. For example, the inoculation of AMF led to an increase of 39.58%, 31.71%, 37.83%, and 12.94% under non-stress conditions and led to an increase of 94.79%, 25.81%, 77.95%, and 13.58% under water deficit stress conditions in chlorophyll a, b, total chlorophyll, and carotenoid, respectively (Table 2). Our results are in agreement with those of Zhu, et al. [60] and Hashem, et al. [61], who reported that AMF inoculation increased chlorophyll content. Improving chlorophyll content by AMF inoculation, especially under drought stress, can be related to enhancing magnesium uptake and, consequently, improving plant growth [62]. The role of AMF in restoring the contents of pigments under drought stress, especially carotenoids, can be associated with the reduction of oxidative stress and ROS levels, thereby improving plant adaptation to drought stress [62].

Likewise, the cumin plants treated with Put showed an increase in chlorophyll a, b, total chlorophyll, and carotenoid by 19.09%, 12.19%, 17.56%, and 6% under non-stress conditions and an increase of 23.95%, 19.35%, 22.83%, and 12.96% under water deficit stress conditions, respectively.

Similar to our results, Talaat, et al. [63] and Mohammadi, et al. [18] reported that the application of Put on *Catharanthus roseus* and *Thymus vulgaris* L. increased chlorophyll a, b, and carotenoid contents. Serafini-Fracassini, et al. [64] attributed the reduction of the destructive effects of drought stress due to spermine treatment in *Lactuca sativa* L. to an increase in TGase activity. The interactive effects of AMF and Put in both conditions were significant on chlorophyll a, b, total chlorophyll, and carotenoid (*p*0.05, Table 1). The highest chlorophyll a, b, total chlorophyll, and carotenoid contents were obtained when AMF and Put were applied together (Table 2).

3.5. Correlation coefficient

The results of Pearson correlation between traits are shown as a heatmap in Fig. 1. A strong positive correlation was found between the seed yield and EO yield ($r_{0.01}$=0.883), chlorophyll a ($r_{0.01=}$0.834), chlorophyll b ($r_{0.01}$=0.851), total chlorophyll ($r_{0.01=}$0.850), and carotenoids ($r_{0.01=}$0.886). Therefore, an increase in seed yield will be associated with an increase in EO yield and vice versa. Furthermore, the positive and significant correlation between the seed yield and pigments showed the key role of these pigments, especially under drought stress, in gaining an acceptable seed yield. The importance of chlorophyll contents (a,b, and total chlorophyll) and carotenoids in resistance to environmental stress through eliminating free oxygen radicals [65] is consistent with the results of this study.

3.6. EO components

The cumin EO samples in different treatments were subjected to a comparative chemical analysis through GC-MS. Cuminaldehyde was identified as the major compound, followed by limonene, γ- Terpinene, o-Cymene, linalool, and β-Pinene accounting for more than 45% of the total components (Table 3). In line with the results of the present study, Ebrahimabadi, et al. [29] and Ouryemchi, et al. [66] found that cuminaldehyde was the predominant component of cumin EO. However, the content of cuminaldehyde significantly varied under different experimental conditions.

Table 3
Effects of polyamine and arbuscular mycorrhiza fungi (AMF) on traits in cumin plant under water deficit.

Water deficit	Putrescine	Mycorrhizal fungi	cuminaldehyde	limonene	γ-Terpinene	o-Cymene	linalool	β-Pinene
80% available soil water	0 mg L^{-1}	Non-inoculation	14.25^{e}	12.08^{f}	11.1^{f}	6.74^{e}	5.84^{d}	4.41^{g}
		AMF-inoculation	16.33^{c}	13.82^{b}	12.25^{d}	7.94^{ab}	6.47^{c}	5.57^{b}
	1000 mg L^{-1}	Non-inoculation	15.74^{cd}	13.37^{c}	10.87^{fg}	7.26^{c}	5.52^{e}	5.24^{c}
		AMF-inoculation	18.01^{a}	$14.1a^{b}$	13.9^{a}	7.72^{b}	7.29^{a}	5.94^{a}
	2000 mg L^{-1}	Non-inoculation	17.78^{a}	13.59^{bc}	13.01^{b}	6.92^{d}	6.77^{b}	4.97^{d}
		AMF-inoculation	17.59^{ab}	14.41^{a}	13.72^{a}	8.21^{a}	6.92^{b}	5.73^{a}
30% available soil water	0 mg L^{-1}	Non-inoculation	14.7^{de}	12.75^{e}	9.07^{h}	5.87^{f}	4.68^{h}	4.64^{ef}
		AMF-inoculation	16.06^{c}	13.45^{c}	11.36^{e}	7.21^{c}	5.84^{d}	4.96^{d}
	1000 mg L^{-1}	Non-inoculation	15.31^{d}	13.05^{d}	10.41^{g}	7.01^{cd}	5.01^{fg}	4.81^{e}
		AMF-inoculation	16.88^{bc}	13.6^{bc}	12.43^{c}	7.52^{bc}	5.92^{d}	5.12^{c}
	2000 mg L^{-1}	Non-inoculation	15.21^{d}	13.53^{bc}	11.32^{e}	7.61^{b}	5.21^{f}	5.06^{cd}
		AMF-inoculation	17.24^{b}	13.69^{b}	11.29^{e}	7.2^{c}	6.11^{cd}	5.22^{c}

Values marked with the same alphabets are not significantly different (LSD, $p \le 0.05$).

A significant variation in response to water deficit stress was observed among the major constituents of cumin EO. Cuminaldehyde, limonene, and β-Pinene increased by 3.15%, 5.54%, and 5.22% under water deficit stress conditions compared to non-stress conditions, while γ- Terpinene, o-Cymene, and linalool decreased by 22.38%, 14.82%, and 24.78%, respectively (Table 3).

These results are consistent with Rebey, et al. [67], who found a notable increase in cuminaldehyde, limonene, and β-Pinene under water deficit stress in cumin plants. Mohammadi, et al. [18] reported similar results for *Thymus vulgaris* L. under drought stress, according to which the plants exhibited lower rates of γ- Terpinene, *p*-Cymene, and linalool compared with well-watered plants.

Importantly, cuminaldehyde, limonene, β-Pinene, γ- Terpinene, o-Cymene, and linalool in cumin plants treated with AMF increased by 14.59%, 14.40%, 10.36%, 17.80%, 10.78%, and 26.30% under non-stress conditions and by 9.25%, 5.49%, 25.24%, 22.83%, 24.78% and 6.89% under water deficit stress conditions compared to the control. In agreement with our results, Ebrahimabadi, et al. [29] showed that AMF application significantly increased cuminaldehyde, limonene, β-Pinene, γ- Terpinene, o-Cymene, and linalool in cumin plants. Changes in the ratio of EO compounds with AMF inoculation under normal and drought stress conditions have been reported for black cumin [68], lemon balm [7], and Ajowan [40].

Likewise, the foliar application of Put under both non-stress and stressful conditions led to an average increase of 7.31%, 6.52%, 8.42%, 13.58%, 0.78%, and 11.24% in cuminaldehyde, limonene, β-Pinene, γ- Terpinene, o-Cymene, and linalool, respectively (Table 3). Similar observations were reported by Mohammadi, et al. [18], who showed a higher accumulation of some major EO constituents in thyme under non-drought stress and drought stress conditions due to the treatment with Put compared to the control. Changes in the proportion of EO compounds with the use of different concentrations of Put have been reported for lavender [27], marigold [69], and sage [54]. However, the maximum increase in cuminaldehyde, limonene, β-Pinene, γ- Terpinene, o-Cymene, and linalool was exhibited by the co-application of AMF and Put (2000 mgL^{-1}).

4. Conclusion

Our results provide insights into understanding the effects of AMF and Put on seed yield, EO yield, EO content, and composition in cumin under drought stress. Based on the results, AMF and Put can be considered advantageous for enhancing cumin production in arid and semi-arid regions because they improve photosynthetic pigments, seed yield, and EO yield accumulation. Conclusively, due to the synergistic effects of AMF and Put, the co-application of AMF and Put in cumin plants is suggested to improve plant tolerance to drought stress and enhance commercial value.

Abbreviations

AMF, Arbuscular mycorrhizae fungi; Put, putrescine; EO, Essential oil; Chl, chlorophyll, ROS, Radical oxygen species; ISR, Induced systemic resistance; PGR's, Plant growth regulators, PAs, Polyamines; PBs, Plantbiostimulants; ANOVA, Analysis of variance; RCBD, Randomized complete block design; TDR, Time Domain Reflectometry; F.C, Field capacity; GC-MS, Gas chromatography-mass spectrometry; RI, Retention index; SAS, Statistical analysis system.

Declarations

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Author Contribution

Hamid Reza Anjam: Investigation, data collection, and original draft preparation. Moslem Abdipour & Mehdi Hosseinifarahi: Conceptualization, Methodology, Validation, Investigation, Supervision, Software, Formal analysis, Writing - review & editing.

Data Availability

We confirm that we provide same data availability statement in the manuscript and in submission system. The datasets generated and/or analysed during the current study are available in the [data] Supplementary material, [https://submission.springernature.com/submission/54fab868-4b2f-4982-930a-2fdc2d92e63c/file/121cc2f9-feca-465c-95c4-760ed58d87ea]

References

1. Lodha, S. & Mawar, R. Cumin wilt management: a review. J. Spice. Aromat. Crop 23, 145–155 (2014).
2. Kumar, S. *et al.* Understanding Cuminum cyminum: An important seed spice crop of arid and semi-arid regions. Int. J. Seed Spices 5, 1–19 (2015).
3. Hajmohammadnia Ghalibaf, K. *et al.* Effects of Planting Date and Deficit Irrigation on Water Use Efficiency of Cumin (Cuminum cyminum L.) at two different Densities in Mashhad Conditions. Iranian Journal of Field Crops Research 18, 213–224 (2020).
4. Mishra, N. *et al.* Achieving abiotic stress tolerance in plants through antioxidative defense mechanisms. Frontiers in Plant Science 14, 1110622 (2023).
5. Nosheen, S., Ajmal, I. & Song, Y. Microbes as biofertilizers, a potential approach for sustainable crop production. Sustainability 13, 1868 (2021).
6. Kuila, D. & Ghosh, S. Aspects, problems and utilization of Arbuscular Mycorrhizal (AM) application as bio-fertilizer in sustainable agriculture. Current Research in Microbial Sciences 3, 100107 (2022).
7. Eshaghi Gorgi, O., Fallah, H., Niknejad, Y. & Barari Tari, D. Effect of Plant growth promoting rhizobacteria (PGPR) and mycorrhizal fungi inoculations on essential oil in Melissa officinalis L. under drought stress. Biologia 77, 11–20 (2022).
8. Arpanahi, A. A., Feizian, M., Mehdipourian, G. & Khojasteh, D. N. Arbuscular mycorrhizal fungi inoculation improve essential oil and physiological parameters and nutritional values of Thymus daenensis Celak and Thymus vulgaris L. under normal and drought stress conditions. European Journal of Soil Biology 100, 103217 (2020).

9. Duc, N. H., Vo, A. T., Haddidi, I., Daood, H. & Posta, K. Arbuscular mycorrhizal fungi improve tolerance of the medicinal plant Eclipta prostrata (L.) and induce major changes in polyphenol profiles under salt stresses. Frontiers in plant science 11, 612299 (2021).
10. Riaz, M. *et al.* Arbuscular mycorrhizal fungi-induced mitigation of heavy metal phytotoxicity in metal contaminated soils: A critical review. Journal of Hazardous Materials 402, 123919 (2021).
11. Wu, C. *et al.* Quo vadis: signaling molecules and small secreted proteins from mycorrhizal fungi at the early stage of mycorrhiza formation. Symbiosis 85, 123–143 (2021).
12. Aroca, R., Porcel, R. & Ruiz-Lozano, J. M. How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in Phaseolus vulgaris under drought, cold or salinity stresses? New Phytologist 173, 808–816 (2007).
13. Bitterlich, M., Sandmann, M. & Graefe, J. Arbuscular mycorrhiza alleviates restrictions to substrate water flow and delays transpiration limitation to stronger drought in tomato. Frontiers in Plant Science 9, 154 (2018).
14. van Loon, L. C. & Glick, B. R. in *Molecular ecotoxicology of plants* 177–205 (Springer, 2004).
15. Kaur, Y. & Das, N. Roles of polyamines in growth and development of the Solanaceous crops under normal and stressful conditions. Journal of Plant Growth Regulation 42, 4989–5010 (2023).
16. Vocciante, M., Grifoni, M., Fusini, D., Petruzzelli, G. & Franchi, E. The role of plant growth-promoting rhizobacteria (PGPR) in mitigating plant’s environmental stresses. Applied Sciences 12, 1231 (2022).
17. Mustafavi, S., Shekari, F. & Abbasi, A. Putrescine improve low temperature tolerance of fennel (Foeniculum vulgare Mill.) seeds. Cercetari Agronomice in Moldova 48, 69–76 (2015).
18. Mohammadi, H., Ghorbanpour, M. & Brestic, M. Exogenous putrescine changes redox regulations and essential oil constituents in field-grown Thymus vulgaris L. under well-watered and drought stress conditions. Industrial Crops and Products 122, 119–132 (2018).
19. Hassan, F., Ali, E. & Alamer, K. Exogenous application of polyamines alleviates water stress-induced oxidative stress of Rosa damascena Miller var. trigintipetala Dieck. South African Journal of Botany 116, 96–102 (2018).
20. Sivakumar, S. *et al.* Influence of exogenous polyamines and plant growth regulators on high frequency in vitro mass propagation of Gloriosa superba L. and its colchicine content. Biocatalysis and Agricultural Biotechnology 18, 101030 (2019).
21. Mokhtassi-Bidgoli, A. *et al.* Agronomic performance, seed quality and nitrogen uptake of Descurainia sophia in response to different nitrogen rates and water regimes. Industrial Crops and Products 44, 583–592 (2013).
22. Khalediyan, N., Weisany, W. & Schenk, P. M. Arbuscular mycorrhizae and rhizobacteria improve growth, nutritional status and essential oil production in Ocimum basilicum and Satureja hortensis. Industrial Crops and Products 160, 113163 (2021).
23. Arnon, A. Method of extraction of chlorophyll in the plants. Agronomy journal 23, 112–121 (1967).
24. Gu, C., Hornberger, G. M., Mills, A. L., Herman, J. S. & Flewelling, S. A. Nitrate reduction in streambed sediments: Effects of flow and biogeochemical kinetics. Water Resources Research 43 (2007).
25. Pharmacopoeia, B. HM Stationery Office, London, 1988. *Search PubMed*, 153 (1988).
26. de Lira, C. S. *et al.* Evaluation of the toxicity of essential oil from Alpinia purpurata inflorescences to Sitophilus zeamais (maize weevil). Crop Protection 71, 95–100 (2015).
27. Sharafabad, Z. H., Abdipour, M., Hosseinifarahi, M., Kelidari, A. & Rashidi, L. Integrated humic acid and vermicomposting changes essential oil quantity, and quality in field-grown Lavandula angustifolia L. intercropped with Brassica nigra L. Industrial crops and products 178, 114635 (2022).
28. Adams, R. P. *Identification of essential oil components by gas chromatography/mass spectrometry*. Vol. 456 (Allured publishing corporation Carol Stream, IL, 2007).
29. Ebrahimabadi, A. H., Hatami, M., Karimzadeh, A. & Ghorbanpour, M. Effect of mycorrhizal fungi and biophosphor fertilizer on growth features, yield and yield components, and essential oil constituents in Cuminum cyminum L. Journal of Medicinal Plants 17 (2018).
30. Wanner, J. *et al.* Chemical composition and antimicrobial activity of cumin oil (Cuminum cyminum, Apiaceae). Natural product communications 5, 1934578X1000500904 (2010).
31. SAS Institute, C., NC. Base SAS 9.4 Procedures Guide: Statistical Procedures. SAS Institute. (2017).
32. Alipour, A., Rahimi, M. M., Hosseini, S. M. A. & Bahrani, A. Mycorrhizal fungi and growth-promoting bacteria improves fennel essential oil yield under water stress. Industrial Crops and Products 170, 113792 (2021).
33. Jahani, F., Tohidi-Moghadam, H. R., Larijani, H. R., Ghooshchi, F. & Oveysi, M. Influence of zinc and salicylic acid foliar application on total chlorophyll, phenolic components, yield and essential oil composition of peppermint (Mentha piperita L.) under drought stress condition. Arabian Journal of Geosciences 14, 1–12 (2021).

34. Sun, H., Shi, Q., Liu, N.-Y., Zhang, S.-B. & Huang, W. Drought stress delays photosynthetic induction and accelerates photoinhibition under short-term fluctuating light in tomato. Plant Physiology and Biochemistry 196, 152–161 (2023).
35. Tafresh, R. S. *et al.* Role of barley stem reserves in the maintenance of grain yield under terminal drought. Crop Science 63, 1408–1420 (2023).
36. Nyaupane, S. *et al.* Drought stress effect, tolerance, and management in wheat–a review. Cogent Food & Agriculture 10, 2296094 (2024).
37. El-Sawah, A. M. *et al.* Arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria enhance soil key enzymes, plant growth, seed yield, and qualitative attributes of guar. Agriculture 11, 194 (2021).
38. Rafiee, H. *et al.* Application of plant biostimulants as new approach to improve the biological responses of medicinal plants-A critical review. Journal of Medicinal Plants 3, 6–39 (2016).
39. Rafiee, S., Yadavi, A., Dehnavi, M. M. & Balouchi, H. Arbuscular mycorrhizal fungi, phosphate-solubilizing bacteria, and zinc foliar application improve physiological responses in black cumin (Nigella sativa) under drought stress. (2023).
40. Rezaei-Chiyaneh, E. *et al.* Co-inoculation of phosphate-solubilizing bacteria and mycorrhizal fungi: Effect on seed yield, physiological variables, and fixed oil and essential oil productivity of ajowan (Carum copticum L.) under water deficit. Journal of Soil Science and Plant Nutrition 21, 3159–3179 (2021).
41. Paymaneh, Z., Sarcheshmehpour, M., Mohammadi, H. & Hesni, M. A. Vermicompost and/or compost and arbuscular mycorrhizal fungi are conducive to improving the growth of pistachio seedlings to drought stress. Appl. Soil Ecol. 182, 104717 (2023).
42. Sheteiwy, M. S. *et al.* Inoculation with Bacillus amyloliquefaciens and mycorrhiza confers tolerance to drought stress and improve seed yield and quality of soybean plant. Physiologia Plantarum 172, 2153–2169 (2021).
43. Huang, G.-M., Zou, Y.-N., Wu, Q.-S., Xu, Y.-J. & Kuča, K. Mycorrhizal roles in plant growth, gas exchange, root morphology, and nutrient uptake of walnuts. Plant, Soil & Environment 66 (2020).
44. Dehsheikh, A. B., Sourestani, M. M., Zolfaghari, M. & Enayatizamir, N. Changes in soil microbial activity, essential oil quantity, and quality of Thai basil as response to biofertilizers and humic acid. J. Clean. Prod. 256, 120439 (2020).
45. Gholami, H., Saharkhiz, M. J., Fard, F. R., Ghani, A. & Nadaf, F. Humic acid and vermicompost increased bioactive components, antioxidant activity and herb yield of Chicory (Cichorium intybus L.). Biocatalysis and Agricultural Biotechnology 14, 286–292 (2018).
46. Farouk, S. & Al-Huqail, A. A. Sodium nitroprusside application regulates antioxidant capacity, improves phytopharmaceutical production and essential oil yield of marjoram herb under drought. Industrial Crops and Products 158, 113034 (2020).
47. Amiri, R., Nikbakht, A., Rahimmalek, M. & Hosseini, H. Variation in the essential oil composition, antioxidant capacity, and physiological characteristics of Pelargonium graveolens L. inoculated with two species of mycorrhizal fungi under water deficit conditions. Journal of Plant Growth Regulation 36, 502–515 (2017).
48. Abd Elbar, O. H., Farag, R. E. & Shehata, S. A. Effect of putrescine application on some growth, biochemical and anatomical characteristics of Thymus vulgaris L. under drought stress. Annals of Agricultural Sciences 64, 129–137 (2019).
49. Weisany, W., Raei, Y. & Pertot, I. Changes in the essential oil yield and composition of dill (Anethum graveolens L.) as response to arbuscular mycorrhiza colonization and cropping system. Industrial Crops and Products 77, 295–306 (2015).
50. Machiani, M. A., Javanmard, A., Morshedloo, M. R. & Maggi, F. Evaluation of yield, essential oil content and compositions of peppermint (Mentha piperita L.) intercropped with faba bean (Vicia faba L.). Journal of cleaner production 171, 529–537 (2018).
51. Mehrabi, Y., Movahhedi Dehnavi, M., Salehi, A., Mohatashami, R. & Hamidian, M. Improving physiological traits, yield and essential oil of cumin (Cuminum cyminum L.) via application of phosphorus bio-fertilizers and supplementary irrigation under dryland condition. Iranian Journal of Medicinal and Aromatic Plants Research 35, 1044–1057 (2020).
52. Kara, Z., Arslan, D., Güler, M. & Güler, Ş. Inoculation of arbuscular mycorrhizal fungi and application of micronized calcite to olive plant: Effects on some biochemical constituents of olive fruit and oil. Scientia Horticulturae 185, 219–227 (2015).
53. Gill, S. S. & Tuteja, N. Polyamines and abiotic stress tolerance in plants. Plant signaling & behavior 5, 26–33 (2010).
54. Zeynali, R., Najafian, S. & Hosseinifarahi, M. Exogenous putrescine changes biochemical (antioxidant activity, polyphenol, flavonoid, and total phenol compounds) and essential oil constituents of Salvia officinalis L. Chemistry & Biodiversity 20, e202301043 (2023).
55. Nazarli, H. & Naderi Arefi, A. Effect of putrescine on dried flower yield and essential oil components changes of Chamomile Plants (Matricaria chamomilla L.) under different moisture regimes. Journal of Crops Improvement 25, 669–684 (2023).
56. Rezai, S., Etemadi, N., Nikbakht, A., Yousefi, M. & Majidi, M. M. Effect of light intensity on leaf morphology, photosynthetic capacity, and chlorophyll content inSage (Salvia officinalis l.). Horticultural Science and Technology 36, 46–57 (2018).
57. Taïbi, K. *et al.* Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in Phaseolus vulgaris L. South African Journal of Botany 105, 306–312 (2016).

58. Kamrava, S., Babaeian Jolodar, N. & Bagheri, N. Evaluation of drought stress on chlorophyll and proline traits in soybean genotypes. Journal of Crop Breeding 9, 95–104 (2017).
59. Kabiri, R., Farahbakhsh, H. & Nasibi, F. Effect of drought stress on physiological and biochemical characteristics of Nigella sativa L. Iranian Journal of Medicinal and Aromatic Plants Research 30, 600–610 (2014).
60. Zhu, X. Q., Tang, M. & Zhang, H. Arbuscular mycorrhizal fungi enhanced the growth, photosynthesis, and calorific value of black locust under salt stress. Photosynthetica 55, 378–385 (2017).
61. Hashem, A. *et al.* The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of Acacia gerrardii under salt stress. Frontiers in microbiology 7, 1089 (2016).
62. Al-Arjani, A.-B. F., Hashem, A. & Abd_Allah, E. F. Arbuscular mycorrhizal fungi modulates dynamics tolerance expression to mitigate drought stress in Ephedra foliata Boiss. Saudi Journal of Biological Sciences 27, 380–394 (2020).
63. Talaat, I. M., Bekheta, M. & Mahgoub, M. H. Physiological response of periwinkle plants (Catharanthus roseus L.) to tryptophan and putrescine. International Journal of Agriculture and Biology 7, 210–213 (2005).
64. Serafini-Fracassini, D., Di Sandro, A. & Del Duca, S. Spermine delays leaf senescence in Lactuca sativa and prevents the decay of chloroplast photosystems. Plant Physiology and Biochemistry 48, 602–611 (2010).
65. Gill, S. S. & Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant physiology and biochemistry 48, 909–930 (2010).
66. Ouryemchi, I. *et al.* GC-MS Characterization, Antioxidant, Antimicrobial and Insecticidal Potential of Moroccan Cuminum Cyminum L. Essential Oil. Tropical Journal of Natural Product Research 8 (2024).
67. Rebey, I. B. *et al.* Effect of drought on the biochemical composition and antioxidant activities of cumin (Cuminum cyminum L.) seeds. Industrial Crops and Products 36, 238–245 (2012).
68. Darakeh, S. A. S. S., Weisany, W., Tahir, N. A.-R. & Schenk, P. M. Physiological and biochemical responses of black cumin to vermicompost and plant biostimulants: Arbuscular mycorrhizal and plant growth-promoting rhizobacteria. Industrial Crops and Products 188, 115557 (2022).
69. Beheshti, F., Abdipour, M., Hosseinifarahi, M. & Kelidari, A. Characterization of Lavender Essential Oil and Its Chemical Composition Intercropped with Marigold as Affected by Polyamines and Arbuscular Mycorrhizal Fungi Inoculation. Communications in Soil Science and Plant Analysis 55, 343–364 (2024).

Figures

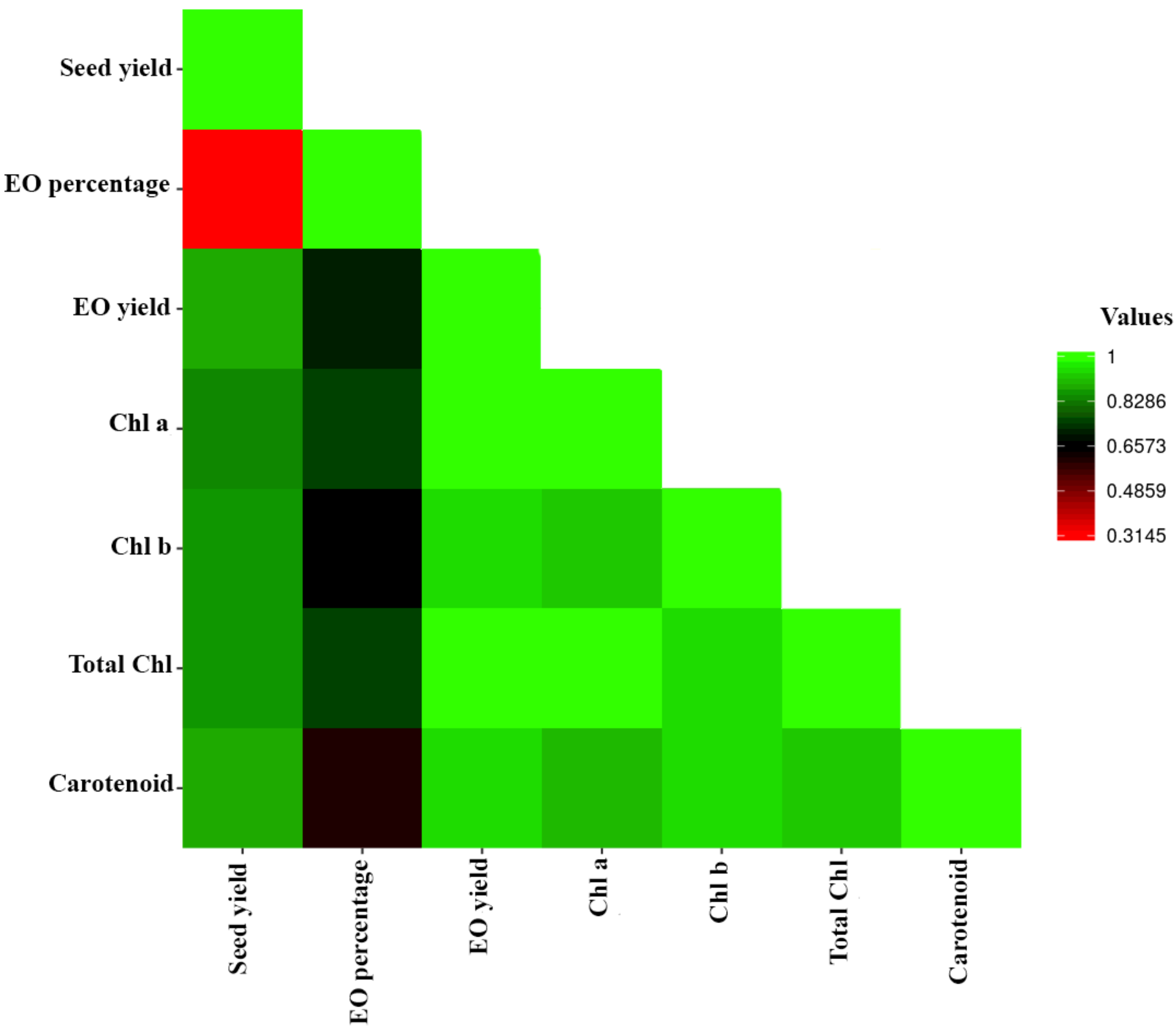

Figure 1

Heat-map matrix of the correlation between traits of cumin from different treatments. Each square indicates *r* (Pearson's correlation coefficient of a pair of traits).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Data.xlsx