

Enhancing Lavender Germination and Growth: A Assessment of Biofertilizer Seed Priming on Physiological and Biochemical Attributes under Water Stress

Somayeh Sarfaraz¹, Ahmad Asgharzadeh^{1*}, and Hamidreza Zabihi²

¹Department of Agriculture, Shirvan Branch, Islamic Azad University, North Khorasan, Iran

²Assistant Professor, Khorasan Razavi Agricultural and Natural Resources Research and Education Center, Iran

*Corresponding author: Email: ahmad.asgharzadeh@iau.ac.ir

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ABSTRACT

Enhancing herbal plant germination through biological priming is crucial under water stress conditions. This study investigated the effects of different biopriming techniques on the physiological and biochemical attributes of two lavender genotypes (English and French) under water stress induced by PEG-6000 (-1, -0.5, -0.25, and 0 MPa) during 2022. Results indicated that the English genotype outperformed the French genotype regarding germination parameters and photosynthetic pigment content, while the French genotype exhibited higher activity levels of antioxidant enzymes. Under well-watered conditions, hydropriming (HP) and Azetobacter + Pseudomonas (AzPs) treatments led to the most excellent germination percentages and tallest seedlings in the English genotype. Hydropriming and AzPs treatments resulted in the most elevated levels of chlorophyll a, b, and total. Additionally, when subjected to water stress, the AzPs treatment showed the highest chlorophyll b and total. Proline content was found to vary between genotypes and treatments, with the highest mean associated with the English genotype and seed priming with AzPs + Arbuscular mycorrhizal fungi (AMF) under the highest level of water stress. The study suggests that bio-priming with biofertilizers and hydropriming can improve germination parameters and physiological characteristics, with individual biofertilizers being more effective than their combined application. Moreover, the English genotype exhibited superior features to the French genotype, and using of AMF and AzPs alone can enhance seed germination and improve the biochemical characteristics of lavender under water stress conditions.

Keyword: Antioxidant enzymes, Arbuscular mycorrhizal fungi, Hydropriming; PEG-6000, Photosynthetic pigment content, Proline content.

INTRODUCTION

Lavender (*Lavandula* spp.), belonging to the Lamiaceae family, is a renowned genus of flowering plants. It is often grouped alongside fragrant herbs like mint, basil, and thyme. This perennial plant is indigenous to the Mediterranean region but has gained popularity worldwide due to its varied uses in horticulture, medicine, and gastronomy [1, 2]. There are several species of lavender, but two of the most commonly cultivated and commercially important species are *Lavandula angustifolia* (English lavender) and *Lavandula stoechas* (French lavender) [3]. English lavender is native to the Mediterranean region and is widely cultivated for its fragrant flowers and essential oil. French lavender is found in some areas of Southern Europe and North Africa. It is also known as Spanish lavender or butterfly lavender. The name "French lavender" may be due to its popularity in French gardens and its use in French perfumes and soaps. However, it is important to note that French lavender is different from the species of lavender native to France, which is *Lavandula angustifolia* [3, 4, 5].

Lavender, known for its versatility and pleasant fragrance, is widely used in aromatherapy, perfumes, cosmetics, and Mediterranean cuisine. Its medicinal properties have been historically utilized in herbal medicine for treating conditions such as anxiety, insomnia, and headaches [6, 7]. Due to its numerous benefits, lavender has recently become a popular research subject. Scientists have investigated its chemical makeup, biological activities, and potential therapeutic uses. On the other hand, a low percentage and slow germination rate are some of the problems in cultivating lavender [8]. It seems that studying the improvement of germination indices using various techniques could be a fascinating area for research. Methods such as seed priming with biofertilizers and exposure to salinity, and drought stresses have been studied [9, 10, 11]. Under drought-stress conditions in plants, seed priming with biofertilizers, growth hormones, and micronutrients is beneficial for inducing drought stress [12].

Seed priming, a method in which seeds are hydrated to activate metabolic processes without actual germination and then dried, enhances germination, stand establishment, and stress tolerance in various crops. Biopriming, which involves applying plant growth-promoting rhizobacteria to seeds, accelerates and evens out germination, ensuring rapid, uniform crop establishment and ultimately enhancing harvest quality and yield. This technique enables bacteria to adhere to seeds and adapt to prevailing conditions [13]. Seed priming with biofertilizers is an important technique studied in recent years for its potential to enhance the germination and growth of various medicinal plants, including *Calendula officinalis* L. [14] and *Melissa officinalis* L. [15]. Biofertilizers are natural fertilizers that contain living microorganisms, such as bacteria, fungi, and algae. These microorganisms interact with the plant's root system and soil, improving nutrient uptake and promoting plant growth [16]. Seed priming with biofertilizers involves soaking the seeds in a solution containing beneficial microorganisms before planting. This process enhances the seed's ability to absorb water and nutrients and stimulates the growth of beneficial microorganisms in the soil. As a result, seed priming with biofertilizers can lead to faster germination, higher germination rates, and better plant growth [11]. Seed priming with biofertilizers has improved seed germination, seedling emergence, and alteration of physiological and biochemical traits [17, 18].

Biochar, a carbon-rich organic material produced through the pyrolysis of biomass, has garnered attention for its unique structure and characteristics. It has demonstrated potential in enhancing soil fertility, boosting crop productivity, and promoting carbon sequestration [19, 20]. Some studies have reported positive effects on seed germination and seedling growth [21], while others have reported adverse or no significant impact [22]. Although the biochar derived from high-temperature biochar hindered rice seed germination, they all positively influenced rice seedlings' growth, leading to a notable increase in both root and shoot length [23]. Given the importance of the herbal plant lavender, its industrial utilization, and the growing global water stress, conducting the present study becomes imperative. Considering the factors mentioned above, we experimented to explore the impact of seed priming with biofertilizers and biochar on the germination, growth, physiological, and biochemical responses of two Lavender species (English and French) under water stress conditions.

MATERIALS AND METHODS

Experimental Design and Plant Material

This experiment was conducted three factorial experiments using a completely randomized design (CRD) with three replications to evaluate the impact of different seed priming techniques (including unprimed seed as control, hydropriming (HP), Azetobacter and Pseudomonas (AzPs), Biochar (Bi), AzPs + Arbuscular mycorrhizal fungi (AMF), AzPs + Bi, AMF + Bi, and AzPs + AMF + Bi) on the germination and growth parameters, as well as physiological and biochemical characteristics of seedlings from two lavender genotypes (English and French) under water stress (induced by polyethylene glycol 6000: PEG-6000) at different osmotic potentials -1, -0.5, -0.25, and 0 MPa. This setup achieved the desired osmotic potentials by irrigating the respective Petri dishes.

In June 2018, mature seeds of English lavender were gathered from Zargiyah Farms in Firuzabad County, Fars Province, Iran, located at 34° 37' _N, 54° 45' _E, at an altitude of 1790 m. The seeds were freshly collected for production purposes. Additionally, French lavender seeds were procured from Pakan Bazar Company in Isfahan, Iran. The average dry weight of 1000 seeds was 1 ± 0.15 g, and the seed moisture ranged around 11.2%.

Germination Test and Measurement of Germination Traits

Biopriming was conducted utilizing *Azetobacter chroococcum* strain 5 and *Pseudomonas fluorescens* strain 186. These native soil bacteria were obtained from the Soil and Water Research Institute in Karaj, Iran. In the process of seed inoculation, a blend comprising 7 g of bacterial inoculant was applied, with each gram housing 10^7 viable and dynamic bacterial cells. Furthermore, Arabic gum was integrated at a weight ratio of 10% to enhance the adherence of the inoculant to the seeds. All inoculation operations were conducted in a shaded environment, away from direct sunlight, and after the seeds had dried, they were sown [24]. The mycorrhizal fungal species used was *Funneliformis mosseae*, which is native to the country and was sourced from the Biological Research Division of the Soil and Water Research Institute in Karaj, Iran. For inoculating arbuscular mycorrhizal fungi, a quantity of 30 g of inoculant mixture was prepared, containing spores (10 to 12 spores per gram), hyphae, and crushed colonized vesicles (75 to 85%) along with uncolonized root fragments, all mixed with seeds in a

container. This mixture was maintained under ambient conditions for 24 h to ensure complete inoculation [25]. The biochar was sourced from Fasje Pajam Company, Iran. Seed priming was carried out using a 10% solution (as recommended by the manufacturer) of this mixture, and seed priming was conducted for 24 hours.

After applying all priming treatments, the seeds were kept at room temperature for 24 hours for storage and drying. Before performing the seed experiment, the seeds were subjected to a disinfection process. This involved using 70% ethanol for one minute, followed by a 5% sodium hypochlorite solution for 30 seconds. Finally, the seeds were washed thrice with sterile distilled water to ensure cleanliness [9].

The experiment involved placing 50 seeds on Whatman paper No.1 in each Petri dish (10 × 1.5 cm), then adding 7 mL of PEG-6000 solution with the required concentration for each dry treatment. To prevent water evaporation, the dishes were covered with paraffin and moved to a germinator, where they were subjected to a temperature of 23 ± 2 °C, relative humidity of $75 \pm 5\%$, 16 h of light, and 8 h of darkness. The final germination count (normal seedlings) was conducted on the 10th day of the experiment, and after three consecutive days, no further increase in germination was observed. The Michel and Kaufmann [26] equation was used with PEG-6000 to replicate the water stress conditions in the growth chamber. In this equation, Ψ_s represents the osmotic potential in MPa, C represents the concentration of PEG-6000 in g per liter of water, and T represents the temperature of the experiment in °C (which was 23 °C in this experiment). The equation is as follows:

$$\Psi_s = - (1.18 \times 10^{-2}) \times C - (1.18 \times 10^{-4}) \times C^2 + (2.67 \times 10^{-4}) \times CT + (8.39 \times 10^{-7}) \times C^2 T.$$

After the 10-day germination period, several parameters were measured, including seed germination percentage (GP), germination rate (GR), mean germination time (MGT), seedling length (SL), seedling vigor index (SVI), coefficient of variation of the germination time (CVT), coefficient of uniformity of germination (CUG), peak value (PV), and germination value (GV). These parameters were calculated based on previous studies such as Maguire [27], Liopa-Tsakalidi et al. [28], Aghighi Shahverdi et al. [9], Gorzi et al. [29]; Aravind et al. [30].

Measurement of Physiological and Biochemical Characteristics

Following two weeks of growth, the seedlings from each replication were gathered and rapidly frozen in liquid nitrogen. The frozen samples were then stored in an ultra-low freezer at -80 °C for future physiological and biochemical characteristics. It is worth mentioning that biochemical traits were measured using the seedling (root + shoot) as the sample.

To measure the absorbance spectrum of chlorophyll a (Chl-a), chlorophyll b (Chl-b), and carotenoids (Car) in the seedlings of both lavender genotypes, the Lichtenthaler and Wellburn [31] method was employed. In this method, 1.0 g of plant material was ground in a mortar, and liquid nitrogen was added to freeze and crush it. Then, 5 mL of 80% acetone was added to the sample and centrifuged at 5000 rpm for 10 min. The resulting extract was transferred to a glass vial. Three mL of this solution was poured into a cuvette, and the absorbance was measured at wavelengths of 663.2 nm and 646.8 nm for Chl-a and Chl-b, respectively, and at a wavelength of 470 nm for Car, using a spectrophotometer (Perkin Elmer Lambda 25, USA). The concentration of the photosynthetic pigments was determined using the following formulas, and the results were presented as $\mu\text{g}\cdot\text{g}^{-1}$ FW:

$$\text{Chl-a} = (12.25 \times A_{663.2}) - (2.79 \times A_{646.8})$$

$$\text{Chl-b} = (21.51 \times A_{646.8}) - (5.1 \times A_{663.2})$$

$$\text{Total-Chl} = \text{Chl-a} + \text{Chl-b}$$

$$\text{Car} = (1000 \times A_{470} - 1.82 \times \text{Chl-a} - 85.02 \times \text{Chl-b})/198$$

Bates et al. [32] described the method used to measure the free proline content in lavender seedlings. In this method, 0.5 g of seedling material from each petri dish was placed in 10 mL of 3% sulfosalicylic acid, and the resulting mixture was homogenized in a mortar. The homogenate was then centrifuged at 4000 rpm for 10 min. The resulting extract was filtered through Whatman filter paper (no. 2). Two mL of the purified extract were mixed with 2 mL of ninhydrin reagent and 2 mL of glacial acetic acid in a test tube. The test tubes were then placed in a boiling water bath for one hour at 100 °C, and immediately after removal from the bath, the samples were placed in an ice bath for a few min. In the next step, 4 mL of toluene was added to each test tube. The proline concentration was determined using a spectrophotometer at a wavelength of 520 nm, based on the standard proline curve. This method is commonly used to measure the proline content in seedlings.

The Chance and Maehly [33] method was used to measure the activity of catalase enzyme (CAT: EC.1.11.1.6). For this purpose, a reaction mixture containing 0.75 mL of 100 mM potassium buffer with pH 7.0, 20 µL of protein solution, and 1500 µL of distilled water was added to a quartz cuvette. 750 µL of 70 mM hydrogen peroxide (H₂O₂) was added to the reaction mixture to measure the enzyme activity. The activity of the CAT was measured by determining the amount of hydrogen peroxide consumed over 60 seconds at 240 nm and 25 °C using a spectrophotometer. The enzyme activity was calculated using the extinction coefficient of 39.4 per mM per cm. The MacAdam et al. [34] method with slight modifications was used to measure the quantitative activity of the peroxidase enzyme (POD: EC 1.11.1.7). The measurement was based on this enzyme's level of guaiacol oxidation, which converts guaiacol to tetraguaiacol. This method mixed 50 µL of enzyme extract with 3 mL of 1.0 mM potassium phosphate buffer solution (pH 6) and 50 µL of pure guaiacol liquid (as an electron donor). Then, 50 µL of 3% hydrogen peroxide solution (as an electron acceptor) was added to the mixture, and changes in light absorption at a wavelength of 436 nm were immediately recorded for 3 min at 15-second intervals using a spectrophotometer. After adding oxygenated water and guaiacol solution, the mixture turned reddish-brown. To zero the instrument (blank), a mixture of 3 mL of 1.0 mM potassium phosphate buffer solution, 50 µL of pure guaiacol, and 50 µL of 3% oxygenated water was used.

The superoxide dismutase activity (SOD) was measured using the method developed by Beauchamp and Fridovich [35]. To summarize, a reaction mixture consisting of 3 mL containing 0.1 mL of 200 mM methionine, 0.01 mL of 2.25 mM nitro blue tetrazolium, 0.1 mL of 3 mM EDTA, 1.5 mL of 100 mM potassium phosphate buffer, 1 mL of distilled water, and 0.05 mL of the extracted enzyme was prepared in duplicate from each enzyme sample. Two tubes without enzyme extract were also included as controls. The reaction was initiated by adding 0.1 mL of riboflavin (60 µM) and exposing the tubes to a light source of two 15 W fluorescent lamps for 15 min. The reaction was stopped by switching off the light and covering the tubes with black cloth, and the absorbance was measured at 560 nm. This method is commonly used to measure SOD activity with accuracy and reliability.

Statistical Analysis

The Statistical Analysis System software (SAS Institute, Cary, NC, USA, Version 9.2) was used to conduct three-way ANOVA analysis of the studied traits statistically after checking their normality distribution assumption using the Kolmogorov-Smirnov and Shapiro-Wilk test. For significant characteristics identified in the analysis of variance, the least significant difference test (LSD) at a 5% significance level was employed. Additionally, correlation analysis between the traits was conducted using SAS software. Furthermore, the stepwise regression was performed using Minitab statistical software version 18. In this analysis, GP was considered the dependent variable, while physiological and biochemical characteristics were considered independent variables. Principal component analysis (PCA) was conducted using the mean of all data for genotype, drought stress, and priming treatments, utilizing Minitab software version 19.

RESULTS

Seed Germination Characteristics

The significant impact of genotype, water stress, seed priming, and their interaction on germination and growth indices was revealed based on the results obtained from data analysis. The affected indices include the GP, GR, MGT, SL, SVI, CVT, CUG, PV, and GV. The English lavender genotype exhibited a significant advantage over the French genotype regarding germination and growth characteristics (excluding MGT and CUG). Most of the germination and growth indices were affected in opposite ways by water stress and seed priming, causing a decrease and an increase, respectively (Table 1).

In the well-watered conditions, the English genotype seeds exhibited the highest GP through various priming treatments, including HP, Bi as well as the integrated application of AzPs, AMF, and Bi, achieving GP of 92.7%, 91.3%, 7 and 86%, respectively. Furthermore, utilizing AzPs under -0.25 MPa water stress conditions resulted in the highest GP in the English genotype (90.7%). Under water stress conditions (-0.5 and -1 Mpa) in both genotypes, seed priming decreased the mean GP. The French genotype exhibited the lowest GP when subjected

to combined priming treatments of AMF + Bi and AzPs + AMF + Bi under -1 MPa conditions, registering an average of 14.67% (Table 2).

Under well-watered conditions, the English genotype showed the highest GR in both the HP and Bi treatments, with mean values of 13.14 and 12.97 seeds per day, respectively, according to the results of the mean effect comparison. In addition, priming seeds, compared to unprimed seeds, increased the GR at all water stress levels and in both genotypes. The lowest mean value of this trait belonged to non-primed seeds of the French genotype at the highest water stress level (-1 MPa), with an average of 1.18 seeds per day (Table 2).

Overall, water stress induced by PEG-6000 resulted in a significant increase in the MGT in both genotypes. The highest MGT was observed at the highest water stress level (-1 MPa) and in the unprimed and priming with Bi-treated French genotype, with 6.6 and 6.6 days, respectively. At this water stress level, HP, AzPs, the combination of AzPs + AMF, and AzPs + Bi treatments in the French genotype had the highest mean value for this trait. The lowest mean value for this trait was observed in the well-watered treatment and priming with a combination of AzPs + AMF in the English genotype, with a mean of 3.58 days (Table 2).

The mean SL ranged from 3 to 15 cm. The tallest seedlings were observed in treating the English genotype seeds with AMF under well-watered conditions. Additionally, HP and AzPs treatments in the same genotype and under the same water stress level had the highest means. The unprimed seeds of the French genotype under water stress at -1 MPa showed the shortest SL (Table 2).

The results showed that HP and AMF treatments of lavender had the highest mean SVI at water stress levels 0 and -0.25 MPa, respectively (1232 and 1219.3, respectively). The findings indicated that the French genotype had a weaker SVI than the English genotype. The French unprimed seeds under severe water stress conditions (-1 MPa) had the lowest mean for this attribute, averaging 58 (Table 2).

The results showed that HP, AzPs, AMFs, Bi treatments, as well as the combination of AzPs + AMF, had the highest CVT under well-watered conditions in the English genotype. On the other hand, the lowest CVT belonged to the non-priming and Bi treatments under water stress conditions in the French genotype, with means of 15.16 and 15.16, respectively (Table 2).

When using Bi treatment, the French genotype had the highest mean for CUG under well-watered conditions, averaging 0.0516. The lowest CUG was attained by priming seeds of the English genotype using HP, AzPs, AMF, Bi, and the combined AzPs + AMFs treatment, all conducted under well-watered conditions (Table 2).

Peak value or emergence energy is the number of seeds that have started germinating by the point on the germination curve where the GR begins to slow down. This value is calculated by determining the highest quotient obtained by dividing successive cumulative GV by the corresponding incubation time. According to the findings, when treated with HP and Bi, the English genotype had the highest mean for PV (6.89 and 6.94 % .time⁻¹) and GV (58.08 and 57.67) under well-watered conditions. In contrast, the French genotype had the lowest mean PV (0.41 % .time⁻¹) and GV (0.56) when subjected to severe water stress conditions and non-priming treatment (Table 2).

Table 1 Effect of water stress (0, -0.25, -0.5, and -1 MPa induced by PEG-6000) and seed priming with biofertilizers on seed germination and growth parameters of lavender genotypes (English and French)

Treatments	GP (%)	GR (seed per day)	MGT (day)	SL (cm)	SVI	CVT	CUG	PV (% time ⁻¹)	GV
Genotypes									
English	63.4±16.5a	7.88±2.8 a	4.73±0.6 b	8.84±3.4 a	578.8±312.7a	21.5±3.1 a	0.026±0.004 b	3.49±1.6 a	22.14±15.2 a
French	21.2±5.0 b	2.29±0.76 b	5.19±0.5 a	6.33±1.69 b	135.7±54.9 b	19.5±2.1 b	0.030±0.007 a	0.73±0.24 b	1.49±0.8 b
LSD = 0.05	1.41	0.19	0.07	0.48	31.3	0.29	0.0008	0.12	1.17
Water stress (MPa)									
0	47.7±31.4 a	6.25±4.7 a	4.68±1.0 d	8.93±3.6 a	506.4±442.5 a	22.3±4.5 a	0.027±0.01 c	3.08±2.7 a	20.8±12.2 a
-0.25	47.5±25.0 a	6.01±3.4 a	4.81±0.3 c	7.46±2.6 b	388.5±312.7 b	20.9±1.4 b	0.026±0.002 d	2.18±1.4 b	12.53±6.9 b
-0.5	40.1±19.4 b	4.68±2.5 b	5.04±0.4 b	7.28±2.0 bc	305.3±188.6 c	19.9±1.5 c	0.028±0.004 b	1.81±1.1 c	8.58±5.8 c
-1	33.9±16.4 c	3.38±1.7 c	5.3±0.3 a	6.68±3.1 c	228.7±176.6 d	18.9±1.1 d	0.031±0.003 a	1.35±0.8 d	5.35±2.8 d
LSD = 0.05	2.0	0.27	0.10	0.67	44.26	0.41	0.0011	0.17	1.65
Priming									
Control	32.9±17.8 c	3.67±2.5 f	5.3±0.7 a	6.66±2.6 c	242.1±224.9 d	19.2±2.8 c	0.032±0.009 a	1.5±1.0 d	6.15±5.7 e
Hydropriming (HP)	45.4±28.3 a	5.58±4.0 ab	4.84±0.5 c	7.51±3.7 abc	392.6±315.6 ab	20.9±2.8 a	0.026±0.004 de	2.38±2.1 a	14.99±9.7 a
Azetobacter and Pseudomonas (AzPs)	45.7±26.5 a	5.57±3.8 ab	4.94±0.6 bc	8.19±3.6 a	455.3±343.7 a	20.5±2.9 ab	0.027±0.005 b-e	2.31±1.9 ab	13.71±6.5 ab
Arbuscular mycorrhizal fungi (AMF)	43.4±22.9 a	5.25±3.2 bc	4.88±0.5 c	7.66±3.5 abc	378.6±350.8 bc	20.8±2.8 a	0.026±0.004 de	2.15±1.8 ab	11.77±4.0 bc
Biochar (Bi)	46.3±26.9 a	5.71±3.8 a	4.94±0.8 bc	6.76±2.3 bc	320.0±246.2 c	20.7±3.4 a	0.028±0.009 b	2.25±2.0 ab	13.97±8.4 ab
AzPs + AMF	38.6±19.8 b	4.76±3.0 de	4.87±0.6 c	8.34±2.2 a	339.8±245.8 bc	20.9±3.2 a	0.027±0.006 b-e	2.1±1.9 bc	10.45±4.1 cd
AzPs + Bi	39.7±22.2 b	4.62±3.0 e	4.97±0.6 bc	7.6±2.2 abc	323.0±227.5 c	20.4±2.8 ab	0.028±0.005 bcd	1.86±1.3 c	9.26±7.8 d
AMF + Bi	44.4±27.1 a	5.1±3.6 cd	5.03±0.5 b	7.82±2.9 a	377.5±294.4 bc	20.0±2.8 b	0.028±0.004 bc	2.12±1.7 abc	12.57±4.8 abc
AzPs + AMF + Bi	44.4±26.3 a	5.47±3.7 abc	4.83±0.4 c	7.75±3.2 ab	386.1±312.0 bc	20.9±2.4 a	0.026±0.003 e	2.29±1.9 ab	13.46±5.7 ab
LSD = 0.05	3.0	0.41	0.15	1.01	66.4	0.62	0.0016	0.26	2.48

Means ± SD followed by the same letter in each column are not significantly different according to LSD test at 5% level. GP: Germination percentage; GR: Germination rate; MGT: Mean germination time; SL: Seedling length; SVI: Seedling vigor index; CVT: Coefficient of variation of the germination time; CUG: Coefficient of uniformity of germination; PV: Peak value; GV: Germination value.

Table 2 Lavender seed germination indices (English and French genotypes) under different water stress levels (0, -0.25, -0.5, and -1 MPa induced by PEG-6000) and priming treatments

Genotype	Water stress (MPa)	Priming	GP (%)	GR (seed per day)	MGT (day)	SL (cm)	SVI	CVT	CUG	PV (% time ⁻¹)	GV		
0	0	Unprimed seed	54.7±6.4ij	7.1±0.7lmn	4.02±0.07f-l	11.7±4b-e	652.7±301.4f-k	24.9±0.43bc	0.021±0.0004x-A	3.11±0.19f-j	15.5±2.83j-n		
		HP	92.7±3.1a	13.1±0.5a	3.63±0.02kl	13.3±4.2abc	1232±372.1a	27.6±0.14a	0.018±0.0001A	6.89±0.51a	58.08±5.42a		
		AzPs	76.7±4.6cd	10.9±0.4bcd	3.66±0.09jkl	13.3±2.3abc	1029.3±244.8bc	27.4±0.64a	0.019±0.0004A	6±0.33bc	41.74±1.2bc		
		AMF	76±5.3cd	10.7±0.6b-f	3.68±0.13jkl	15±2.6a	1135.3±167.4ab	27.2±0.94a	0.019±0.0006A	6.22±0.38abc	42.95±3.18bc		
		Bi	91.3±2.3a	13±0.3a	3.62±0.02kl	8.3±3.5f-l	756.7±306.6d-h	27.6±0.17a	0.018±0.0001A	6.94±0.42a	57.67±3.89a		
		AzPs + AMF	74.7±4.6cde	10.8±0.5b-e	3.58±0.14l	11.7±2.1b-e	866.7±122.5cde	28±1.12a	0.018±0.0007A	6.56±0.69ab	44.44±4.58b		
		AzPs + Bi	70±6d-g	9.2±0.6hij	3.97±0.11h-l	10.3±2.1d-h	715.3±93e-i	25.2±0.7bc	0.02±0.0006yzA	4.25±0.43de	27.18±5efg		
		AMF + Bi	77.3±11.7cd	10.1±1.7d-h	4±0.12g-l	10.7±4c-g	794±180.4def	25±0.78bc	0.02±0.0007x-A	4.67±0.8d	33.38±10.19de		
		AzPs+AMF+Bi	86±5.3ab	11.8±0.6b	3.79±0.05i-l	11±1b-f	943.3±56.7cd	26.4±0.32ab	0.019±0.0002zA	5.78±0.51c	45.17±4.71b		
		Unprimed seed	62±5.3ghi	8.2±0.6jkl	4.74±0.12c-l	8±2.6g-l	486.7±128.6k-n	21.1±0.52e-j	0.026±0.0009r-w	3.17±0.29f-i	17.94±3.21j-m		
		HP	74±14c-f	9.8±2.3d-h	4.84±0.19b-k	10.3±2.1d-h	780±285.3d-g	20.7±0.84f-n	0.026±0.0016m-v	3.83±1.44ef	27±15.6e-h		
		-0.25	-0.25	AzPs	90.7±11.4a	11.5±2.1bc	5.02±0.37b-i	13.3±1.5abc	1219.3±282a	20±1.54h-q	0.028±0.0034g-u	4.33±1.44de	36.42±15.9cd
AMF	67.3±4.2e-h			8.4±0.3ijk	5.06±0.15b-h	7.3±2.5i-n	491.3±155.4k-n	19.8±0.56i-r	0.028±0.0014f-u	2.83±0.29g-l	17.36±2.32j-m		
Bi	74.7±3.1cde			9.7±0.1e-h	4.8±0.13b-l	5.7±2.11-r	426.7±173.9l-p	20.8±0.56e-l	0.026±0.0011o-w	3.17±0.29f-i	21.45±1.37g-j		
AzPs + AMF	51.3±4.2jk			6.5±0.8no	5.04±0.45b-i	10.3±2.5d-h	524±90.4j-n	19.9±1.83h-q	0.028±0.0042f-t	2.39±0.67j-n	11.21±3.44m-q		
AzPs + Bi	72.7±2.3c-f			9.4±0.5ghi	4.82±0.27b-l	8±1g-l	580±57h-l	20.8±1.15e-l	0.026±0.0024n-v	3.33±0.29fgh	22±1.7g-j		
AMF + Bi	78.7±13.3bc			10.4±2.2c-g	4.82±0.24b-l	7±2.6i-o	573.3±319h-l	20.8±1e-l	0.026±0.0021n-v	4.33±1.44de	32.15±16.56def		
AzPs+ AMF +Bi	63.3±4.6gh			8.4±0.6ijk	4.87±0.14b-k	8.3±0.6f-l	526±3.5j-m	20.5±0.59f-n	0.027±0.0013k-v	3.5±0.87fg	20.27±5.93g-k		
Unprimed seed	39.3±3.1mn			3.8±0.2s-w	5.33±0.14b-e	5.6±0.7i-r	221.3±30.6q-u	18.8±0.51o-t	0.031±0.0015d-k	1.93±0.31m-q	6.88±0.99o-t		
HP	44.7±9.5klm			5.9±1.4opq	4.85±0.29b-k	7.8±1.3g-m	354.8±120.5m-q	20.7±1.25f-m	0.027±0.0025l-v	2.33±0.76k-o	9.85±4.56n-r		
AzPs	62±5.3ghi			8±0.9klm	4.89±0.16b-j	10.3±1.8d-h	634.7±59.1f-k	20.5±0.65f-o	0.027±0.0014k-v	3±0.5g-k	17.06±4.27j-m		
AMF	66±5.3fgh			8.2±0.6i-l	5.1±0.03b-h	9.5±1.5d-i	623±68.5f-k	19.6±0.11j-s	0.029±0.0003f-t	3.33±0.29fgh	20.09±3.24h-k		
-0.5	-0.5			Bi	54±2ij	6.9±0.5mno	4.89±0.29b-j	9.5±0.5d-i	513.7±46j-n	20.5±1.26f-o	0.027±0.0025j-v	2.5±0i-n	12.27±0.45m-p
		AzPs + AMF	54±6ij	6.8±0.7no	5.1±0.37b-h	6.8±1.5i-o	364.2±67.7m-q	19.7±1.41j-r	0.029±0.0036f-s	2.83±0.29g-l	13.91±2.16k-o		
		AzPs + Bi	52±7.2jk	5.2±0.8pqr	5.13±0.06b-h	6.6±1.5j-o	337±44.1n-r	19.5±0.22j-s	0.029±0.0006f-s	2.67±0.7h-m	12.8±4.42l-p		
		AMF + Bi	69.3±12.9d-h	7±1.5mno	5.13±0.13b-h	8±0.3g-l	555.1±107i-l	19.5±0.47j-s	0.029±0.0013f-s	3.2±0.53f-i	20.58±7.39g-k		
		AzPs+ AMF +Bi	72±9.2c-f	9.6±1.5fgh	4.77±0.14b-l	6.5±0.5j-o	471±94.4k-o	21±0.62e-k	0.026±0.0012q-w	3.83±1.15ef	25.7±10.13f-i		
		Unprimed seed	38±3.5mn	3.7±0.5t-x	5.36±0.2a-e	4.3±0.7o-r	162.9±36.9r-u	18.7±0.69p-t	0.032±0.0023d-i	1.48±0.28p-s	5.18±1.35q-t		
		HP	67.3±9.9e-h	6.9±1mno	5.02±0.15b-i	3.3±0.9pqr	225.6±86.7q-u	19.9±0.6h-q	0.028±0.0014h-u	3.13±0.42f-i	19.37±4.99i-l		
		AzPs	41.3±4.2lm	4±0.4r-v	5.29±0.05b-e	4.3±1o-r	176.7±28q-u	18.9±0.19n-t	0.031±0.0006d-o	1.8±0.2n-q	6.75±0.87p-t		
		-1	-1	Unprimed seed	38±3.5mn	3.7±0.5t-x	5.36±0.2a-e	4.3±0.7o-r	162.9±36.9r-u	18.7±0.69p-t	0.032±0.0023d-i	1.48±0.28p-s	5.18±1.35q-t
				HP	67.3±9.9e-h	6.9±1mno	5.02±0.15b-i	3.3±0.9pqr	225.6±86.7q-u	19.9±0.6h-q	0.028±0.0014h-u	3.13±0.42f-i	19.37±4.99i-l
				AzPs	41.3±4.2lm	4±0.4r-v	5.29±0.05b-e	4.3±1o-r	176.7±28q-u	18.9±0.19n-t	0.031±0.0006d-o	1.8±0.2n-q	6.75±0.87p-t

	AMF	48.7±3.1jkl	4.6±0.3rst	5.45±0.12a-d	4.8±0.4n-r	231.2±8.3q-u	18.3±0.39q-u	0.033±0.0014d-h	1.6±0.17o-r	7.11±1.22o-t
	Bi	61.3±4.2hi	6.3±0.5nop	5.04±0.1b-i	4.3±0.4o-r	265.7±29.7p-t	19.9±0.38h-q	0.028±0.0009g-u	2.48±0.28i-n	13.91±2.51k-o
	AzPs + AMF	39.3±8.1mn	4.1±1r-v	5.01±0.27b-i	6.5±0.5j-o	254±41.3p-t	20±1.06h-q	0.028±0.0026h-u	2.05±0.68m-p	7.66±4.02o-s
	AzPs + Bi	39.3±2.3mn	3.8±0.3t-w	5.44±0.1a-d	10.9±2.2b-f	425.8±64.7l-p	18.4±0.34q-u	0.032±0.0012d-h	1.42±0.18p-t	5.09±0.92q-t
	AMF + Bi	50±3.5jk	5.1±0.2qr	5.14±0.25b-h	12±3bcd	600±149.9g-l	19.5±0.93j-s	0.029±0.0025f-s	2.13±0.34l-p	9.63±0.96n-r
	AzPs+ AMF +Bi	50.7±5jk	4.9±0.5qrs	5.26±0.06b-f	13.7±2.1ab	690±102.2e-j	19±0.2m-s	0.03±0.0006d-q	2.47±0.5i-n	11.28±1.91m-q
	Unprimed seed	14.7±2.3v	1.2±0.2E	6.6±0.09a	6.1±0.8l-p	89±15.3tu	15.2±0.2v	0.052±0.002a	0.41±0.08v	0.56±0.18t
	HP	18.7±1.2r-v	1.8±0.1B-E	5.45±0.41a-d	5.9±1l-q	111.6±25.7stu	18.4±1.43q-u	0.033±0.0047c-f	0.53±0.06uv	0.9±0.08st
	AzPs	18±2s-v	1.8±0.3B-E	5.46±0.27a-d	5.7±0.4l-r	101.4±4.7stu	18.4±0.89q-u	0.033±0.0032d-g	0.57±0.06uv	0.93±0.19st
	AMF	21.3±1.2q-v	2.4±0.3z-D	5.04±0.44b-i	6.1±0.8l-p	129.9±15stu	19.9±1.83h-q	0.028±0.004f-t	0.74±0.07tuv	1.43±0.09st
0	Bi	14.7±2.3v	1.2±0.2E	6.6±0.09a	6.3±0.6k-o	91.9±16.7tu	15.2±0.2v	0.052±0.002a	0.41±0.08v	0.56±0.18t
	AzPs + AMF	18.7±1.2r-v	1.8±0.1B-E	5.45±0.41a-d	6.9±1.7i-o	128.6±26.5stu	18.4±1.43q-u	0.033±0.0047c-f	0.53±0.06uv	0.9±0.08st
	AzPs + Bi	18±2s-v	1.8±0.3B-E	5.46±0.27a-d	6.3±0.6k-o	113.4±19.8stu	18.4±0.89q-u	0.033±0.0032d-g	0.57±0.06uv	0.93±0.19st
	AMF + Bi	21.3±1.2q-v	2.4±0.3z-D	5.04±0.44b-i	6.3±0.9k-o	134.1±23.5stu	19.9±1.83h-q	0.028±0.004f-t	0.74±0.07tuv	1.43±0.09st
	AzPs+ AMF +Bi	15.3±2.3uv	1.6±0.3CDE	5.18±0.22b-h	5.8±0.8l-r	90.3±24.1tu	19.3±0.82k-s	0.03±0.0023e-r	0.5±0uv	0.7±0.1st
	Unprimed seed	20±2r-v	2.1±0.1z-E	5.08±0.27b-h	5.6±0.4l-r	111.5±18.5stu	19.7±1.07j-r	0.029±0.0025f-t	0.67±0.08uv	1.23±0.24st
	HP	22±3.5q-v	2.4±0.3y-D	4.8±0.26b-l	7±0.3i-o	153.3±17.6r-u	20.9±1.18e-l	0.026±0.0022o-w	0.81±0.17s-v	1.65±0.63st
	AzPs	29.3±4.6opq	3.6±0.4t-y	4.58±0.14c-l	6.6±0.8j-o	195±46.1q-u	21.9±0.69efg	0.024±0.0011t-y	1.22±0.38q-u	3.31±1.38rst
	AMF	23.3±3.1p-u	2.8±0.3w-B	4.65±0.23c-l	6.4±1.1k-o	150.5±43.2r-u	21.5±1.04e-h	0.025±0.0019s-x	0.93±0.35r-v	2.04±1st
-0.25	Bi	26±3.5o-s	3.2±0.5u-z	4.52±0.13d-l	7.8±3.7h-m	210.6±129.4q-u	22.2±0.64ef	0.024±0.001u-z	1.03±0.29r-v	2.48±1.03st
	AzPs + AMF	23.3±6.1p-u	2.9±1v-B	4.45±0.18d-l	6.5±1.4j-o	152.2±49r-u	22.5±0.93de	0.023±0.0013v-z	0.92±0.14r-v	1.98±0.75st
	AzPs + Bi	31.3±2.3nop	4.2±0r-u	4.17±0.37e-l	6.1±0.4l-p	191.4±20.1q-u	24.1±2.08cd	0.022±0.0024w-A	1.42±0.22p-t	4.02±0.47rst
	AMF + Bi	20.7±2.3r-v	2.1±0.2z-E	5.3±0.42b-e	6.8±0.8i-o	142.3±30.2stu	19±1.5m-s	0.031±0.0046d-l	0.64±0.04uv	1.21±0.12st
	AzPs+ AMF +Bi	25.3±2.3o-s	2.8±0.1w-B	4.94±0.12b-i	3.2±0.1qr	80±9.3tu	20.2±0.49g-p	0.027±0.0011i-v	0.75±0.08s-v	1.74±0.34st
	Unprimed seed	16±3.5tuv	1.4±0.4DE	6±0.26ab	6±2l-q	100±53.8stu	16.7±0.73uv	0.04±0.0039b	0.53±0.06uv	0.79±0.26st
	HP	24±2o-t	2.7±0.3w-C	4.86±0.34b-k	9.3±2.1d-j	226±62.4q-u	20.6±1.39f-n	0.027±0.0031k-v	0.89±0.19r-v	1.94±0.46st
	AzPs	26.7±2.3o-r	2.9±0.2v-B	5.12±0.19b-h	6±1l-q	160±31.2r-u	19.6±0.72j-s	0.029±0.0018f-s	0.87±0.12r-v	2.11±0.4st
	AMF	24.7±3.1o-s	2.9±0.5v-B	4.67±0.37c-l	5.7±3.1l-r	134±58.3stu	21.5±1.66e-i	0.025±0.003r-w	0.92±0.14r-v	2.06±0.46st
-0.5	Bi	26.7±1.2o-r	3.2±0.1u-A	4.74±0.27b-l	5.7±2.1l-r	150±51r-u	21.1±1.21e-j	0.026±0.0022r-w	0.87±0.12r-v	2.1±0.24st
	AzPs + AMF	32±2no	3.7±0.3t-w	4.81±0.33b-l	9±1e-k	286.7±14o-s	20.9±1.47e-l	0.026±0.0027n-v	0.87±0.12r-v	2.52±0.37st
	AzPs + Bi	19.3±3.1r-v	2.1±0.2z-E	4.96±0.4b-i	7±1i-o	134±17.8stu	20.3±1.71g-p	0.028±0.0034i-v	0.72±0.1tuv	1.26±0.29st
	AMF + Bi	20±2r-v	1.9±0.2B-E	5.6±0.26a-d	5±1m-r	100.7±25.3stu	17.9±0.86stu	0.034±0.0032cd	0.61±0.1uv	1.12±0.27st
	AzPs+ AMF +Bi	19.3±2.3r-v	2.2±0.2z-E	4.76±0.53b-l	6.7±2.3i-o	130.7±53.3stu	21.2±2.37e-j	0.026±0.0044p-w	0.77±0.03s-v	1.35±0.14st

	Unprimed seed	18.7±1.2r-v	1.9±0.2B-E	5.25±0.5b-f	6.1±0.5l-p	113.2±13.8stu	19.2±1.8ll-s	0.031±0.0055d-n	0.67±0.12uv	1.13±0.17st
	HP	20±3.5r-v	2±0.2A-E	5.29±0.39b-e	3±1r	58±12.2u	19±1.4lm-s	0.031±0.0041d-m	0.62±0.13uv	1.14±0.39st
	AzPs	21.3±3.1q-v	2±0.3A-E	5.55±0.31a-d	5.9±0.8l-q	126.6±25.5stu	18±0.98r-u	0.034±0.0041cde	0.69±0.1tuv	1.35±0.38st
	AMF	20±2r-v	2±0.1A-E	5.35±0.33a-e	6.6±1.9j-o	133.6±51.7stu	18.8±1.13o-t	0.032±0.0039d-j	0.59±0.08uv	1.08±0.26st
-1	Bi	22±4q-v	2.3±0.7z-E	5.29±0.44b-e	6.6±0.5j-o	145.4±38stu	19±1.53m-s	0.031±0.005d-l	0.64±0.13uv	1.3±0.42st
	AzPs + AMF	16±4tuv	1.6±0.6CDE	5.52±0.7a-d	9±1e-k	142.7±30stu	18.3±2.18q-u	0.035±0.0099cd	0.63±0.2uv	0.96±0.48st
	AzPs + Bi	15.3±4.6uv	1.4±0.5DE	5.82±0.17abc	5.6±0.8l-r	87.5±35.9tu	17.2±0.49tu	0.037±0.0024bc	0.53±0.12uv	0.78±0.36st
	AMF + Bi	18±2s-v	1.8±0.2B-E	5.25±0.36b-g	6.8±2.6i-o	121±45.8stu	19.1±1.3l-s	0.03±0.0038d-p	0.67±0.12uv	1.09±0.22st
	AzPs+ AMF +Bi	23.3±5p-u	2.6±0.6x-D	5.09±0.32b-h	6.8±1i-o	158.1±36.7r-u	19.7±1.23j-r	0.029±0.0031f-t	0.71±0.08tuv	1.49±0.2st

Means ± SD followed by the same letter in each column are not significantly different according to LSD test at 5% level. Capital letters were used in sequence following the completion of mean grouping with small letters. GP: Germination percentage; GR: Germination rate; MGT: Mean germination time; SL: Seedling length; SVI: Seedling vigor index; CVT: Coefficient of variation of the germination time; CUG: Coefficient of uniformity of germination; PV: Peak value; GV: Germination value.

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Table 3 Effect of water stress (0, -0.25, -0.5, and -1 MPa induced by PEG-6000) and seed priming with biofertilizers on physiological and biochemical attributes of lavender seedling (English and French genotypes)

Treatments	Chl-a ($\mu\text{g/g FW}$)	Chl-b ($\mu\text{g/g FW}$)	Total-Chl ($\mu\text{g/g FW}$)	Car ($\mu\text{g/g FW}$)	Proline ($\mu\text{mol/g FW}$)	CAT (U/mg protein.min)	POD (U/mg protein.min)	SOD (U/mg protein)
Genotype								
English	8.78 \pm 3.3 a	4.4 \pm 1.8 a	13.18 \pm 4.5 a	1.49 \pm 0.8 a	245.4 \pm 163.9a	1.85 \pm 1.4 b	4.54 \pm 2.3 b	3.03 \pm 1.6 b
French	8.07 \pm 6.0 b	3.39 \pm 3.2 b	11.45 \pm 9.0 b	1.47 \pm 0.9 a	103.7 \pm 51.8 b	3.01 \pm 2.4 a	6.22 \pm 3.7 a	5.26 \pm 3.7 a
LSD = 0.05	0.37	0.23	0.43	0.12 (NS)	7.66	0.35	0.55	0.43
Water stress (MPa)								
0	13.32 \pm 5.3 a	5.76 \pm 3.4 a	19.08 \pm 8.3 a	2.1 \pm 1.0 a	78.5 \pm 41.9 c	1.34 \pm 0.7 d	3.68 \pm 2.3 c	2.97 \pm 1.9 c
-0.25	7.89 \pm 4.0 b	3.38 \pm 2.1 b	11.26 \pm 5.5 b	1.45 \pm 1.0 b	149.8 \pm 88.6 b	1.9 \pm 1.0 c	5.26 \pm 3.2 b	3.23 \pm 2.8 c
-0.5	6.95 \pm 3.2 c	3.18 \pm 2.1 b	10.13 \pm 5.1 c	1.42 \pm 0.5 b	157.7 \pm 65.5 b	2.41 \pm 1.2 b	5.39 \pm 2.3 b	4.1 \pm 3.3 b
-1	5.54 \pm 2.2 d	3.26 \pm 1.9 b	8.79 \pm 4.0 d	0.97 \pm 0.4 c	312.3 \pm 191.3 a	4.08 \pm 2.3 a	7.193.6 a	6.28 \pm 2.8 a
LSD = 0.05	0.53	0.33	0.60	0.18	10.83	0.50	0.78	0.61
Priming								
Unprimed seed	7.36 \pm 3.8 d	3.05 \pm 1.8 c	10.41 \pm 5.5 e	1.49 \pm 0.7 bc	135.1 \pm 101.6 d	2.87 \pm 1.4 a	6.47 \pm 2.6 a	4.61 \pm 2.8 ab
Hydropriming (HP)	9.01 \pm 7.3 ab	4.52 \pm 3.8 b	13.53 \pm 10.7 ab	1.04 \pm 0.4 e	161.1 \pm 142.4 c	2.7 \pm 2.4 a	5.77 \pm 3.5ab	3.92 \pm 2.7 abc
Azotobacter and Pseudomonas (AzPs)	9.39 \pm 7.1 a	5.04 \pm 4.1 a	14.42 \pm 11.1 a	1.21 \pm 0.7 de	167.1 \pm 126.3 bc	2.41 \pm 2.2 a	5.29 \pm 3.7 bc	3.9 \pm 2.9 abc
Arbuscular mycorrhizal fungi (AMF)	7.75 \pm 3.6 cd	3.4 \pm 2.0 c	11.14 \pm 5.5 de	1.5 \pm 0.6 bc	188.7 \pm 158.0 a	2.77 \pm 2.3 a	5.8 \pm 4.0 ab	4.73 \pm 4.0 a
Biochar (Bi)	9.04 \pm 5.2 ab	5.15 \pm 3.4 a	14.18 \pm 8.3 a	1.35 \pm 0.8 cd	178.2 \pm 131.4 ab	2.78 \pm 2.2 a	5.7 \pm 3.2 ab	4.45 \pm 3.4 abc
AzPs + AMF	7.68 \pm 3.3 d	3.51 \pm 1.4 c	11.19 \pm 4.2 de	1.52 \pm 0.9 bc	189.7 \pm 165.1 a	2.39 \pm 1.5 a	5.77 \pm 3.1 ab	3.76 \pm 1.8 bc
AzPs + Bi	7.56 \pm 3.0 d	3.45 \pm 1.6 c	11.01 \pm 4.2 e	1.45 \pm 0.6 bcd	180.4 \pm 150.8 ab	2.26 \pm 2.2 a	5.45 \pm 3.7 abc	3.98 \pm 3.1 abc
AMF + Bi	8.53 \pm 4.3 bc	3.52 \pm 2.0 c	12.05 \pm 5.7 cd	1.66 \pm 1.1 b	188.7 \pm 155.3 a	2.39 \pm 2.1 a	4.46 \pm 1.6 cd	4.3 \pm 3.0 abc
AzPs + AMF + Bi	9.5 \pm 3.8 a	3.42 \pm 1.5 c	12.92 \pm 4.6 bc	2.12 \pm 1.1 a	182.3 \pm 138.2 ab	1.32 \pm 1.0 b	3.7 \pm 1.7 d	3.65 \pm 3.5 c
LSD = 0.05	0.79	0.50	0.91	0.27	16.25	0.75	1.17	0.92

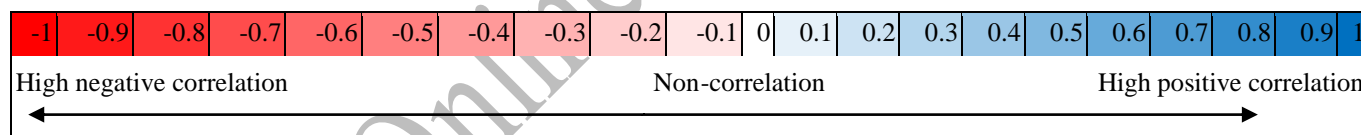
Means \pm SD followed by the same letter in each column are not significantly different according to LSD test at 5% level. Chl: chlorophyll; Car: carotenoid; CAT: catalase activity; POD: peroxidase activity; SOD: superoxide dismutase.

	AzPs +								
	AMF	11.6±2.93f-j	2.82±0.44n-t	14.42±2.89g-k	3.09±1.06b	75.6±17.6w-E	1.64±1.09k-t	5.22±3.5i-t	1.46±0.89w-C
	AzPs + Bi	6.05±0.87r-B	4.85±1.45f-l	10.9±2.19l-p	0.45±0.18uv	107.4±8.8s-z	2.28±1.46i-s	6.32±3.33f-o	1.32±0.77x-C
	AMFs + Bi	5.74±1.21t-D	3.11±0.66m-s	8.85±1.85o-s	0.99±0.12j-v	114.5±12r-w	3.29±1.47e-m	5.77±1.4g-r	1.87±1.12s-C
	AzPs+ AMF								
	+Bi	13.37±0.99def	2.09±0.54r-w	15.46±1.02f-i	4.01±0.37a	111.3±14.7r-x	0.03±0.01t	4.72±3.46j-x	0.76±0.24BC
	Unprimed								
	seed	4.37±0.44z-H	1.03±0.43w	5.39±0.87v-A	1.49±0.14g-o	61.6±15.1z-F	2.52±0.53h-q	4.6±1.28j-y	2.68±0.49n-C
	HP	4.39±0.86z-H	1.62±0.3t-w	6±1.09t-z	1.34±0.25g-r	138±12.6o-t	1.33±0.34l-t	5.24±0.6i-t	1.58±0.46v-C
	AzPs	2.2±0.88H	1.37±0.32uvw	3.57±1.1zA	0.69±0.24p-v	155.5±9.1m-r	1.42±0.24l-t	1.53±0.41w-z	2.16±0.3q-C
	AMF	3.83±1.09B-H	1.28±0.54uvw	5.11±1.61v-A	1.4±0.31g-q	166±10.5m-q	7.15±0.49a	10.49±1.22abc	13.67±1.29a
-0.5	Bi	4.91±1.97x-G	1.34±0.52uvw	6.26±1.47t-y	1.37±0.91g-r	165.5±4.6m-q	7±1.78ab	9.57±2.9a-f	12.25±2.12ab
	AzPs +								
	AMF	5.33±0.38v-E	2.27±0.99q-w	7.6±1.37q-v	1.28±0.37g-t	177.4±7.2k-o	2.13±0.51i-t	2.93±0.78p-z	3.57±1.01l-A
	AzPs + Bi	2.96±0.76FGH	0.95±0.23w	3.91±0.94yzA	1.15±0.2i-u	178.5±18.6k-o	2.24±0.11i-s	3.82±0.68l-z	4.5±0.33i-r
	AMF + Bi	4.43±1.58z-H	1.03±0.38w	5.46±1.25u-A	1.48±0.49g-o	184.5±14.8j-n	2.53±0.52h-q	5.15±1.19i-u	4.42±0.83i-t
	AzPs+ AMF								
	+Bi	4.77±1.14y-G	1.51±0.28t-w	6.28±1.37s-y	1.53±0.29g-n	121.6±16.3q-v	1.84±0.3i-t	3.5±0.67m-z	3.28±0.36m-B
	Unprimed								
	seed	2.42±1.06H	0.89±0.25w	3.31±1.31A	0.61±0.28r-v	110.3±20.6r-y	4.67±0.57c-g	7.53±1.98c-j	7.96±3.36def
	HP	3.32±0.37E-H	1.44±0.25t-w	4.76±0.26x-A	0.68±0.12q-v	47.6±19.2B-F	3.86±0.57e-j	4.8±1.39j-x	4.27±0.6i-u
	AzPs	3.54±1.73D-H	1.34±0.66uvw	4.88±2.4w-A	0.68±0.4p-v	130.9±17.5p-u	4.65±3.17c-h	7.6±4.45c-j	5.99±3.43f-l
	AMF	3.19±1.26E-H	1.3±0.39uvw	4.49±1.62yzA	0.69±0.3p-v	140.4±5.7n-t	5.23±1.99a-e	12.56±4.12a	6.28±2.02e-k
-1	Bi	2.89±1.21GH	1.01±0.25w	3.9±1.46yzA	0.69±0.28p-v	142±9.4n-t	2.79±0.55g-p	7.2±2.45c-k	4.17±0.85i-v
	AzPs +								
	AMF	4.32±1.12z-H	2.07±0.42r-w	6.38±1.21r-y	0.77±0.36n-v	141.5±6.5n-t	5±2.44b-f	12.49±1.45a	6.59±1.41e-i
	AzPs + Bi	5.17±0.27w-F	2.05±1r-w	7.22±1.27q-x	1.06±0.32i-v	165.2±5.8m-q	6.86±3.78ab	11.16±6.53ab	9.5±5.89cd
	AMF + Bi	2.37±0.59H	0.86±0.08w	3.24±0.66A	0.56±0.17s-v	163.9±6.7m-q	6.1±5.56a-d	5.32±3.4h-t	9.92±4.23bcd
	AzPs+ AMF								
	+Bi	6.33±2.21q-A	2.6±0.54o-u	8.93±2.56o-r	0.91±0.84l-v	161±16.3m-q	3.3±1.15e-m	1.47±0.91xyz	11.86±2.46abc

Means ± SD followed by the same letter in each column are not significantly different according to LSD test at 5% level. Capital letters were used in sequence following the completion of mean grouping with small letters. Chl: chlorophyll; Car: carotenoid; CAT: catalase activity; POD: peroxidase activity; SOD: superoxide dismutase.

Table 5 Simple correlation coefficients (Pearson) between seed germination indices and seedling growth, physiological, and biochemical attributes of lavender genotypes under the influence of water stress and priming treatments

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2	0.98															
3	-0.61	-0.69														
4	0.57	0.61	-0.52													
5	0.89	0.91	-0.65	0.85												
6	0.62	0.7	-0.98	0.56	0.69											
7	-0.57	-0.63	0.96	-0.44	-0.57	-0.89										
8	0.94	0.97	-0.73	0.65	0.92	0.77	-0.63									
9	0.92	0.95	-0.7	0.63	0.91	0.76	-0.6	0.99								
10	0.07	0.09	-0.07	0.1	0.12	0.11	-0.01	0.12	0.12							
11	0.1	0.08	0.07	0.07	0.09	-0.02	0.13	0.11	0.09	0.84						
12	0.08	0.09	-0.02	0.1	0.11	0.07	0.04	0.12	0.11	0.98	0.93					
13	0.05	0.08	-0.19	0.11	0.12	0.22	-0.13	0.11	0.1	0.56	0.16	0.43				
14	0.24	0.13	0.13	0.03	0.09	-0.18	0.04	0.09	0.03	-0.16	0.09	-0.07	-0.22			
15	-0.38	-0.41	0.19	-0.38	-0.43	-0.21	0.14	-0.38	-0.38	-0.43	-0.34	-0.41	-0.29	0.17		
16	-0.45	-0.49	0.38	-0.44	-0.52	-0.4	0.33	-0.48	-0.49	-0.14	-0.06	-0.12	-0.21	0.1	0.73	
17	-0.46	-0.48	0.32	-0.38	-0.48	-0.35	0.24	-0.49	-0.47	-0.3	-0.23	-0.28	-0.29	0.07	0.79	0.58



1. Germination percentage; 2. Germination rate; 3. Mean germination time; 4. Seedling length; 5. Seedling vigor index; 6. CVT; 7. CUG; 8. PV; 9. GV; 10. Chlorophyll a; 11. Chlorophyll b; 12. Total chlorophyll; 13. Carotenoids; 14. Proline; 15. CAT activity; 16. POD activity; 17. SOD activity

Seedling Physiological and Biochemical Attributes

The physiological and biochemical data analysis indicated that the content of Chl-a, -b, and total, proline and the activity of antioxidant enzymes CAT, POD, and SOD were significantly affected by the genotype, water stress, and various priming treatments (Table 3). Furthermore, all measured physiological and biochemical traits demonstrated significant interactive effects of genotype, water stress, and priming (Table 4). The results showed that photosynthetic pigments and proline content were higher in the English genotype than in the French genotype. On the other hand, the activity of antioxidant enzymes was higher in the French genotype (Table 3).

The data revealed that HP seeds from the English genotype under non-water stress conditions led to the highest average levels of Chl-a, -b, and total (25.83, 12.75, and 38.57 $\mu\text{g}\cdot\text{g}^{-1}$ FW, respectively). Priming these seeds with AzPs also produced the highest average levels of Chl-b and total (13.19 and 36.17 $\mu\text{g}\cdot\text{g}^{-1}$ FW, respectively) under non-water stress conditions. Conversely, the French genotype demonstrated the lowest mean levels of photosynthetic pigments, including Chl-a, -b, and total, under severe water stress conditions when the seeds were not primed (2.42, 0.89, and 3.31 $\mu\text{g}\cdot\text{g}^{-1}$ FW, respectively) or when they were primed with a combination of AMF + Bi (2.37, 0.86, and 3.24 $\mu\text{g}\cdot\text{g}^{-1}$ FW, respectively) (Table 4).

The Car content in the seedlings ranged from 0.37 to 4 $\mu\text{g}\cdot\text{g}^{-1}$ FW and varied across different treatments. The French genotype exhibited the highest mean Car content under well-watered conditions when the seeds were primed with fungi and Bi (AMF + Bi). On the other hand, the French genotype showed the lowest mean Car content under the highest level of water stress without any seed priming treatment (Table 4).

The mean comparison revealed that the proline content demonstrated variability, ranging from 29.3 to 588.7 $\mu\text{mol}\cdot\text{g}^{-1}$ FW. Water stress induced by PEG-6000 increased proline content, with the highest mean of this trait associated with the highest level of water stress (-1 MPa) and seed priming of the English genotype with a combination of AzPs + AMF. Moreover, priming these same seeds with fungi alone or combined with Bi (AMF + Bi) resulted in the highest mean proline content. The lowest proline content was observed in the priming of French seeds with AzPs + Bi under well-watered conditions (Table 4).

The results showed a significant increase in the activity of antioxidant enzymes with the increasing severity of water stress. The highest CAT activity (7.15 $\text{U}\cdot\text{mg protein}^{-1}\cdot\text{min}$) was associated with the water stress level of -1 MPa and non-priming treatment of seeds from the French genotype. Moreover, priming these same seeds at the same level of water stress with HP, AMF, a combination of AzPs + Bi, and AMF + Bi also resulted in the highest mean activity of this enzyme. On the other hand, the lowest mean activity was observed in the priming of seeds from the French genotype with a combination of AzPs+AMF+Bi under a water stress level of -0.25 MPa, with a mean of 0.03 $\text{U}\cdot\text{mg protein}^{-1}\cdot\text{min}$.

According to the results, the highest activity of the POD was observed under the most severe water stress conditions when the seeds of the French genotype were primed with fungi alone (AMF) or in combination with bacteria (AzPs + AMF) with average values of 12.56 and 12.49 $\text{U}\cdot\text{mg protein}^{-1}\cdot\text{min}$, respectively. Conversely, the lowest activity of this enzyme was associated with seed priming of the English genotype with Bi under non-stressed treatment, with an average of 0.75 $\text{U}\cdot\text{mg protein}^{-1}\cdot\text{min}$ (Table 4).

Like the other two enzymes, the activity of the SOD significantly increased under water stress conditions. The highest activity was found in the French genotype with non-priming treatment under the highest level of water stress, with an average of 13.67 $\text{U}\cdot\text{mg protein}^{-1}$. At the same stress level, HP of seeds also resulted in the most increased activity of the SOD enzyme. In contrast, the priming of seeds from the English genotype under non-stress conditions had the lowest activity of this enzyme, with an average of 0.65 $\text{U}\cdot\text{mg protein}^{-1}$ (Table 4).

Correlation, Regression, and PCA Analysis

Table 5 presents the results of a simple correlation analysis, which examines the relationships between germination traits, seedling growth, and physiological and biochemical traits. The study revealed significant and non-significant negative and positive correlations between these traits. The GP positively and significantly correlated with GR, SL, SVI, CVT, PV, and GV traits. However, it had a negative and significant correlation with average MGT, CUG, and the activity of antioxidant enzymes such as CAT, POD, and SOD.

A stepwise regression analysis was performed to determine the most influential physiological and biochemical traits on GP, and the outcomes are illustrated in Table 6. The results indicated that three characteristics, namely

proline content and the activity levels of SOD and POD enzymes, were included in the regression model. These three traits explained 34.8% of the variation in seed GP.

PCA analysis for the mean data obtained from studying 17 germination, growth, and biochemical traits is presented in Fig. 1. According to the results, the first and second components justified 46.8% and 19.6% of the variations, respectively. In the first component, the most influential traits included GP, GR, SVI, PV, and GV. In contrast, in the second component, the content of photosynthetic pigments, including chlorophyll a, b, and total, had a significant impact.

Table 6 Stepwise regression for lavender seed germination percentage as the dependent variable and other physiological and biochemical characteristics as the independent variable

Term	Coef	SE Coef	T-Value	P-Value
Constant	58.70	5.85	10.03	0.000
Proline (X1)	0.0512	0.0170	3.01	0.004
POD activity (X2)	-2.67	1.06	-2.51	0.014
SOD activity (X3)	-2.46	1.04	-2.55	0.013

R-sq= 34.89%

$$Y = 58.70 + 0.0512 (X1) - 2.67 (X2) - 2.64 (X3)$$

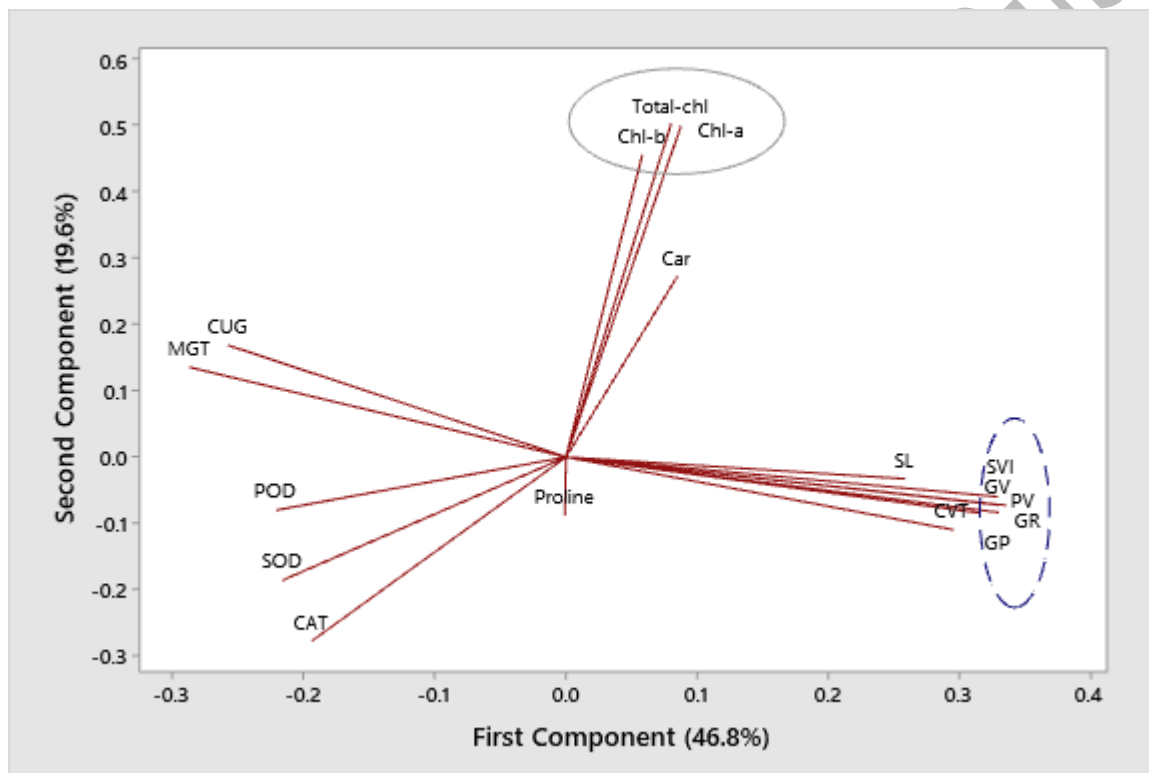


Fig. 1 Principal component analysis (PCA) of germination, growth, physiological, and biochemical attributes of lavender seedling (English and French genotypes) under water stress and priming treatments

DISCUSSION

The present study evaluated the germination, growth, physiological, and biochemical responses of two lavender genotypes (English and French) to different priming treatments with biofertilizers under water stress conditions. Additionally, the study aimed to evaluate biofertilizers efficacy in separate or combined applications. According to the results, the presence of water stress caused a significant reduction in the seed germination parameters and seedling growth (GP, GR, SL, SVI, CVT, PV, and GV), especially at the levels of -0.5 and -1 MPa of water stress (Table 1). Gorzi et al. [29] on *Stevia rebaudiana*, Afshari et al. [36] on *Stevia rebaudiana*, and Saha et al. [37] reported that the main reasons for the decrease in seed germination indices under water stress induced by PEG-6000 were the reduction in water potential, accumulation of reactive oxygen species due to oxidative stress, and

disruption of metabolic processes and energy supply to the germinating seeds. The researchers reported similar results showing the adverse effects of water stress on the germination indices of the medicinal plant.

When seeds experience higher levels of water stress, their ability to take up water decreases, reducing their hydraulic conductivity. This reduction impacts the physiological and metabolic processes involved in seed germination, causing a decrease in their rate or intensity. Suppose water absorption by the seed is disrupted or slow. In that case, the metabolic activities of germination will also occur at a slower pace, resulting in a longer time required for radical emergence and a slower GP [36, 38]. It has been reported that PEG has caused a decrease in the GR. The molecules of PEG restrict the passage of oxygen through the cell walls and disrupt the oxygen supply to the roots [39].

As water stress increases, the SVI decreases in inoculated and control seeds, with a more significant reduction observed in control seeds. Water stress leads to decreased GP, resulting in a reduced number of germinated seeds. This decrease in the number of normally germinated seeds also leads to a reduction in the SVI. The SVI is generally considered one of the crucial parameters for determining seed germination, as it directly relates to seed quality and viability. In other words, better seed quality results in a higher GP and more germinated seeds, leading to a higher SVI [9]. Other researchers in this field have also reported similar findings on *Stevia rebaudiana* seeds [29].

The mean comparison of data revealed that in the majority of seed germination and seedling growth characteristics, the use of biofertilizer included AzPs, AMF, and Bi in combination with HP (either separately or together) resulted in an increase in the average traits compared to the control treatment. Furthermore, the results indicated that the HP had a significant advantage over other treatments regarding seed germination traits and plant growth parameters. Seed priming or pre-treatment is a method by which injecting moisture and nutrients into the seed improves germination and seedling growth, reduces the time required for seedling development, increases tolerance to stress conditions, boosts yield, and reduces production costs. Additionally, seed priming increases rooting and nutrient absorption, reduces the risk of diseases, and increases resistance to unfavorable environmental conditions and diseases. Seed priming methods include controlled water supply, chemical and physical priming, growth-stimulating bacteria, and plant extracts, which are used based on plant requirements [9, 36]. Hydropriming with water is one of the seed priming methods in which seeds are soaked in water to improve germination and seedling growth. The benefits of HP include improving germination speed, reducing the time required for seedling growth, increasing tolerance to stress conditions, boosting yield, reducing production costs, and reducing seed loss [40]. Although using biofertilizers improved certain traits, as the difference was insignificant compared to HP, farmers tend to choose the easiest and most cost-effective method. Similarly, other researchers have highlighted seed hydropriming's advantages in improving seed germination and seedling growth parameters of bitter melon (*Momordica charantia*) [41]. Reduced seed germination under water stress may be associated with decreased water uptake by seeds. In this regard, Salleh et al. [42] reported an improvement in *Oryza sativa* L. seed germination of HP seeds under water stress.

The present study's findings demonstrated that the application of AMFs had positive and beneficial impacts on seed germination traits and physiological and biochemical parameters. This could be attributed to the effect of beneficial bacteria interacting with spores or mycelium, which subsequently contribute to the formation of the mycorrhizosphere in the soil. This interaction promotes the growth and development of both AMFs and the host plant. Mycorrhizal fungi are among the most significant microorganisms that establish a symbiotic relationship with a diverse range of plants in three forms, including ectomycorrhizae, endomycorrhizae, and ectendomycorrhizae, thereby enhancing plant nutrient uptake. According to the researchers, mycorrhizal fungi directly improve plant nutrition by increasing nutrient and water uptake while indirectly mitigating the adverse effects of biotic and abiotic stresses (such as drought and salinity) [43].

Using bacterial fertilizers (AzPs) for seed inoculation can lead to improved plant growth, accelerated seed germination, and enhanced seedling development in response to external stressors. Saadaoui et al. [44] conducted a study investigating the effects of various priming methods (biopriming, bio-osmo priming, osmo-priming, and non-primed) on wheat seed germination and SVI. The researchers reported that biopriming significantly increased seed germination and SVI compared to non-primed seeds.

The current study's findings revealed that the mean content of photosynthetic pigments, such as Chl-a, -b, and total, was significantly higher under the AzPs treatment (Table 3). Growth-promoting bacteria, such as *Pseudomonas* and *Azotobacter*, can significantly improve the content of photosynthetic pigments under water stress conditions. Studies have shown that these bacteria can considerably enhance the content of Chl-a, -b, and total for seedlings under water stress [36]. As photosynthetic pigments play a significant role in photosynthesis, increasing their content can improve plant performance and resilience to environmental stressors. Therefore, using growth-promoting bacteria can be an effective strategy to mitigate the negative impacts of water stress on plant growth and development. Moreover, water stress led to a significant reduction in the content of photosynthetic pigments. The damage to mesophyll chloroplasts resulting in a lower rate of photosynthesis under stress has been associated with a loss of Chl in previous research [9, 29]. This reduction in Chl content is a typical symptom of oxidative stress and can be attributed to pigment photo-oxidation and Chl degradation. Moreover, studies have shown that water stress can decrease photosynthetic pigments such as Chl-a, -b, and total, as well as carotenoids in safflower [45].

Previous studies have demonstrated that water stress increases proline content in seedlings of *Stevia rebaudiana* [36]. Proline aids in improving plant resilience to water stress by facilitating osmotic adjustment, preventing enzyme damage, and scavenging hydroxyl radicals. Other researchers have also reported increased proline content in stressed plants following seed priming [29, 45]. Furthermore, biofertilizers have been shown to enhance proline content and modulate phytohormone levels, nutrient absorption, redox potential, ion balance, photosynthetic efficiency, and the expression of stress-related genes, positively correlating with heightened water shortage resistance [46]. Proline also plays a crucial role in safeguarding enzymes, preventing macromolecule degradation, and upholding cell wall strength during environmental stress. Hence, applying AMF and AzPs, which boost proline levels, seems to amplify this effect, thereby enhancing the plant's resilience to water stress [47].

Lavender seedlings increase the activity of antioxidant enzymes, including CAT, POD, and SOD, in response to water stress to mitigate oxidative stress effects. Based on the data analysis and stepwise regression results, the high activity level of these enzymes significantly influenced the percentage and rate of germination. The findings imply that the increased enzyme activity in the seedlings likely reduced cellular damage in the plant by eliminating excess free radical oxygen produced under drought stress conditions. Indeed, the elevation in antioxidant enzyme activity under water stress conditions could be attributed to the synergistic presence of AMFs or bacterial fertilizers application, combined or individually, in contrast to their absence. The utilization of biofertilizers enhances nutrient uptake, consequently boosting antioxidant enzyme performance. Prior research has highlighted the significant impact of AMFs and biological fertilizer treatments on enzymatic antioxidant responses during environmental stresses [48].

CONCLUSION

Results indicated that biopriming with biofertilizers and HP improved germination parameters and physiological characteristics. Individual application of biofertilizers was more effective than the combined application of fungus and bacteria (AzPs + AMF). Additionally, the English genotype exhibited superior characteristics compared to the French genotype. These findings suggest that seed HP and using AMF and AzPs alone can enhance seed germination attributes and improve the biochemical characteristics of lavender under water stress conditions.

Conflict of Interest

The authors have not declared any conflict of interests.

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