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# Phosphate Solubilizing Bacteria as Drought Stress Alleviators for *Achillea santolina* L.

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**ABSTRACT**

Drought stress mitigation may involve multiple strategies that engage plants physiologically, one of which is the application of beneficial rhizobacteria that enhance plant tolerance. This study aimed to assess the ability of phosphate-solubilizing bacteria (PSB) to improve the growth and physiology of *Achillea santolina* (Santalin yarrow) under drought stress. The interaction effects of PSB (*Pseudomonas fluorescens* isolates FRPF4, FRPF6, and FRPF12) (at 107 CFU mL-1) and drought stress (30, 50, and 80% field capacity) appeared in a factorial test based on a completely randomized design located in a greenhouse (n=3; $P<0.05$). Several vegetative and physiological parameters came under study at full flowering. Measurable growth factors were plant shoot height, canopy diameter, root length, and fresh and dry weights. The results revealed a significant decrease in all growth parameters under drought stress. However, PSB isolates, especially FRPF4, significantly mitigated the adverse effects of stress on vegetative factors. Drought stress significantly affected the leaf samples by increasing their total soluble sugars (TSS), free proline, total flavonoids (TF), and DPPH contents. Drought-stressed plants inoculated with the PSB isolates showed a significant decrease in free proline and DPPH concentrations. However, the TSS and TF contents increased in the stressed plants treated with PSB isolates. Carotenoid, chlorophyll a, b, and total chlorophyll contents also decreased in the stressed plants. However, these factors increased in plants treated with PSB isolates in response to water-deficit stress. Thus, PSB isolates may mitigate the adverse effects of drought stress on Santalin yarrow plants through several direct and indirect mechanisms.

**Abbreviations:** Analysis of variance (ANOVA), Dry weight (DW), Essential oil (EO), Field capacity (FC), Fresh weight (FW), Optical density (OD), Plant growth-promoting rhizobacteria (PGPR), Phosphate-solubilizing bacteria (PSB), Reactive oxygen species (ROS), Total flavonoid content (TFC), Total soluble sugars (TSS)

## Introduction

Santalin yarrow (*Achillea santolina* L.) is a valuable perennial medicinal plant that belongs to the Asteraceae family (Compositae, Asteraceae). This herbaceous species is naturally found on the slopes of Alborz, Tabriz, and around

Damavand, Iran (Omidbeigi, 2000). Its EO is extracted mainly from flowering branches and has a bitter taste and a pungent smell. Its EO has several antiviral and antibacterial properties that stand out in the cosmetic, health, and pharmaceutical industries. Its applications include the alleviation of stomachache and intestinal disorders, bile and liver diseases, and skin and wound inflammation (Benedek et al., 2008). The major components in its EO include polyphenolic compounds, flavones, sesquiterpenes, lactones, betaines, tannins, acetylene compounds, resin, achillines, nitrates, potassium salts, phosphates, and organic acids (Emami et al., 2006).

Several genetic and environmental factors may affect medicinal plant growth and yield. Drought stress significantly limits plant growth and physiology while disregulating various biochemical and physiological processes, such as hormonal signaling, respiration, transpiration, enzymatic activities, and photosynthesis in plants (Gao et al., 2019). Moreover, hyperaccumulation of ROS occurs in drought-stressed plants. Plants deal with abiotic stresses by producing and storing osmotic regulatory substances, such as amino acids, organic acids, soluble sugars, ions, hormones, and proteins (Parida and Das, 2005). Additionally, the adverse effects of ROS on plant membranes and chlorophyll structures can be mitigated by the production of plant antioxidants (Begum et al., 2020). Plant defense mechanisms can moderate the adverse effects of drought stress, and the application of PGPR may successfully increase plant tolerance to stress conditions (Lalay et al., 2022). PGPR agents can directly improve plant growth and productivity by supplying nutrients such as nitrogen and phosphorus or through the secretion of siderophores, hormones, and plant vitamins. Several biocontrol mechanisms, such as the production of various siderophores and antibiotics or the induction of systemic resistance, can limit pathogen growth on plant hosts, suppress pathogenic activity, and stimulate plant growth (Samavat, 2009). Among the PGPR agents, fluorescent pseudomonads have received particular attention (Bossis et al., 2000).

Fluorescent pseudomonads are gram-negative, rod-shaped, motile, spore-producing bacteria with high ecological diversity (Palleroni, 1993). These bacteria can produce fluorescent pigments under iron deficiency conditions. They all have high rRNA homology (Bossis et al., 2000). This group includes *Pseudomonas fluorescens*, *P. putida*, *P. chlororaphis*, *P.* syringae, and *P. aeruginosa.* Isolates of the fluorescent pseudomonads can promote plant growth like other PGPR and increase plant tolerance as drought-tolerant isolates (Yasmin et al., 2022). The subsequent application of these PGPR agents enhances productivity in medicinal plants that grow in water-deficit soils.

Considering the prevalence of drylands in the world and the progressive nature of drought stress on the quantitative and qualitative yields of medicinal plants, this research considered the effects of *Pseudomonas fluorescens* isolates, as PSB, on the physiology and growth of Santalin yarrow plants under drought stress.

## Materials and Methods

### *Plant materials*

Santalin yarrow seeds were gathered from their natural habitats in Abadeh County, Fars Province, at 2100 m above sea level, at a longitude of 52o 28' 06'' and latitude of 31o 21' 40'' in August 2021.

### *Bacterial isolates*

*P. fluorescens* isolates FRPF4, FRPF6, and FRPF12 in this study were obtained from the Beneficial Microorganisms Collection of the Research Institute of Forests and Rangelands, Tehran, Iran. According to previous studies (Samavat and Rahimifard, 2021), these isolates were superior at solubilizing inorganic phosphate and producing siderophores. The bacterial suspensions (107 CFU mL-1) were prepared from the 48 h cultures of the isolates in King's B media.

### *Greenhouse trials*

This study was conducted in a research greenhouse located at the Research Institute of Forests and Rangelands, Tehran, Iran (35°41'39.80'' N, 51°25'17.44'' E, 1189 m from sea level) in September 2021. The seeds were cultivated in seedling trays containing peat moss and perlite (1:1) until the 6- to 8-leaf stage. The plants were grown in a greenhouse under controlled conditions of 25/15 °C day/night temperature, 14/10 h day/night photoperiod, and 60% relative humidity. The Santalin yarrow seedlings were subsequently transplanted into 3 Kg pots containing equal portions of sand, soil, and leaf compost.

### *Preparation of the treatments*

The first set of treatments included drought stress (30% (severe), 50% (moderate), and 80% (control) FC). The second set of treatments comprised bacterial isolates (*P. fluorescens* isolates FRPF4, FRPF6, and FRPF12) and the non-inoculated control.

Before planting, the seeds were immersed in 50 mL of the corresponding bacterial suspension (107 CFU $mL^{-1}$). Seeds of the non-inoculated control were immersed in 50 mL of sterile distilled water. Two months after the seedlings were transplanted, the pots were inoculated with 1 mL of the corresponding bacterial suspension, after which the drought stress treatments were applied (Abideen et al., 2022). Drought stress was imposed by maintaining soil moisture in the aforementioned FC (%) via daily weight measurements (Paymaneh et al., 2019).

To determine field capacity, some pots were randomly saturated with excess water, after which the extra water was allowed to drain freely from the bottom of the pots (after approximately 24 h). The surface of the pots was covered by plastic to avoid evaporation. The plants in each pot were weighed regularly until they reached a stable weight. The soil samples were then removed to record their DW and FW after they were placed in an oven at 105 °C for 24 h. The FC was calculated using the following formula (Ogbaga et al., 2014):

$$FC = \frac{(FW - DW)}{(DW)} \times 100$$

Sampling was carried out at full flowering, which almost coincided with eight months after planting the seeds. Subsequently, several vegetative and physiological factors were investigated and compared with the control. Plant growth parameters, such as shoot height, root length, and dry and fresh weight, were measured using a ruler, a Vernier caliper, and an electronic scale.

## *Total soluble sugars (TSS)*

The TSS content was measured according to the anthrone method (Irigoyen et al., 1992). For this purpose, a volume of 0.2 mL of concentrated plant extract was incorporated with 3 mL of anthrone reagent, consisting of 150 mg of anthrone in 100 mL of 13 M sulfuric acid, and then placed in a hot water bath at 100 °C for 20 min. After cooling, the absorbance of each sample was measured at 620 nm. The amount of total soluble sugars was determined based on a standard glucose curve.

## *Free proline content*

A rapid colorimetric method (Bates et al., 1973) determined the free proline content. In brief, 10 mL of 3% sulfosalicylic acid entered into 0.5 g of freshly crushed plant material. The mixture was centrifuged at 6000 rpm for 10 min at 4 °C. The supernatant (2 mL) was mixed well with glacial acetic acid (2 mL) and ninhydric acid (2 mL) in the test tubes. The sample was boiled at 100 °C for an hour. After stopping the reaction in an ice bath, 4 mL of toluene was added to each tube, and the absorbance was recorded at 520 nm. To determine the proline concentration, a standard curve was used. Free proline concentrations were calculated based on DW (μM $g^{-1}$).

## *Total flavonoid content (TFC)*

A colorimetric method based on the detection of aluminum chloride assisted in measuring the TFC. Briefly, the plant extract (0.5 mL) was mixed with 0.1 mL of 10% aluminum chloride, 1.5 mL of 95% ethanol, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The sample absorbance was recorded at 415 nm. The curve was generated using a quercetin standard (Chlopicka et al., 2012). The data were statistically analyzed using a completely randomized design (n=3).

## *Total antioxidant activity (DPPH)*

Total antioxidant activity was measured using a method described by Mahdavian et al. (2022). The absorbance at 515 nm was expressed as percentages according to the following formula:

$$DPPH\ (\%) = \frac{(Absorbance\ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control} \times 100$$

## *Determination of carotenoid, chlorophyll a, b, and total chlorophyll*

These parameters were measured based on Turner (1981). For this purpose, 0.05 g of fresh plant leaf tissue was homogenized with 80% acetone (2 mL) and kept in a refrigerator for 24 h. The extract was centrifuged for 10 min at 3500 rpm and then filtered and mixed with 80% acetone (2 mL). The OD of the centrifuged extracts was measured at 490, 638, 645, and 663 nm using a spectrophotometer. These parameters were measured using the following formula:

$$Chlorophyll\ a\ (g\ L^{-1}) = (0.0127 \times OD_{663}) - (0.00269 \times OD_{645})$$

$$Chlorophyll\ b\ (g\ L^{-1}) = (0.0229 \times OD_{645}) - (0.00468 \times OD_{663})$$

$$Total\ chlorophyll\ (g\ L^{-1}) = (0.0202 \times OD_{645}) + (0.00802 \times OD_{663})$$

$$Carotenoid = (OD_{490}) - (0.114 \times OD_{663}) - (0.638 \times OD_{645})$$

### *Statistical analysis*

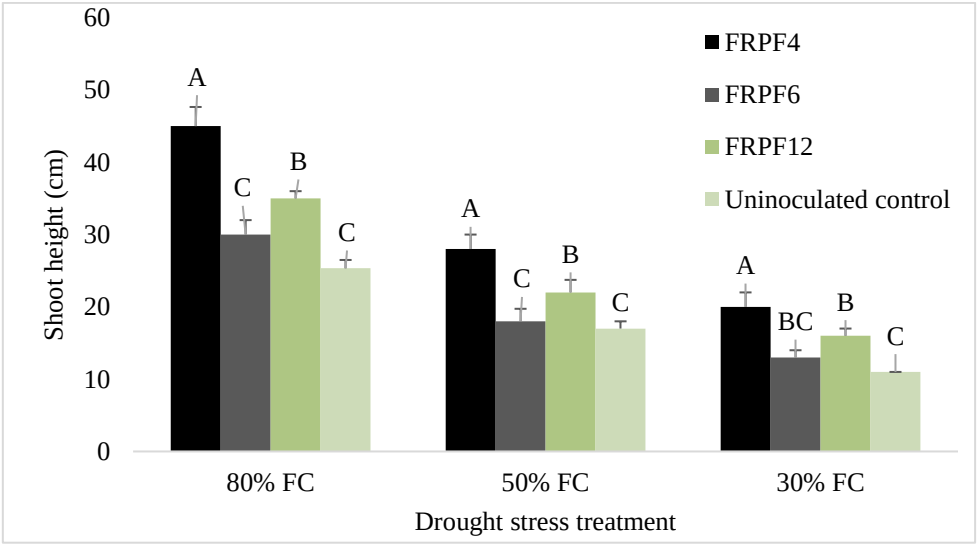
*In vitro* data were statistically analyzed using a completely randomized design (n=3). Greenhouse trials were conducted via a completely randomized design with three replications as a factorial test. Three out of ten replications were randomly selected for each set of treatments. Accordingly, the population and sample sizes were 120 and 92, respectively. ANOVA was performed using the IBM SPSS 26 Statistics Program (Chicago, USA). The comparison of mean values was performed using Duncan's multiple-range test.

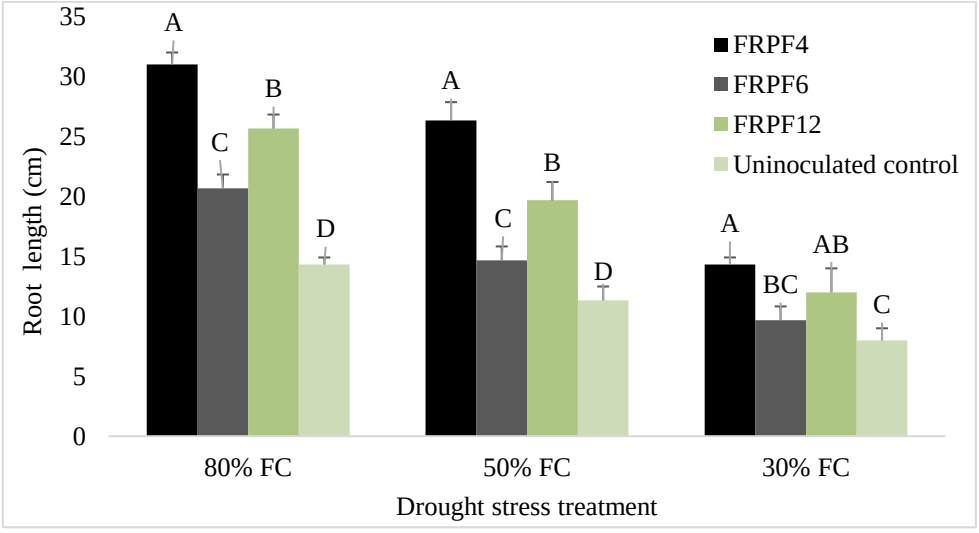
## Results

### *Growth factors*

*P. fluorescens isolates* (FRPF4, FRPF6, and FRPF12) affected the shoot height, root length, and dry and fresh weight of drought-stressed Santalin yarrow plants (Fig. 1). According to the results, all plant growth factors decreased significantly under drought stress compared to those under the irrigated control. Drought stress from 80% to 30% FC led to a logical decrease in all the above growth parameters. The highest values of the growth factors were recorded in response to 80% FC+FRPF4, whereas the lowest amounts occurred in the non-inoculated control at 30% FC. For all growth parameters, the FRPF6 treatment had no significant statistical difference from the control at 30% FC ($P<0.05$). Even though drought stress adversely affected all growth parameters, the FRPF4 and FRPF12 treatments significantly mitigated the effects of drought stress on the plants ($P<0.05$).

ANOVA results for shoot height, root length, and dry weight demonstrated that the interaction effects between drought stress and PSB application were significant ($P<0.01$). However, the interaction effect was only significant for the fresh weight ($P<0.05$) (Table 1).



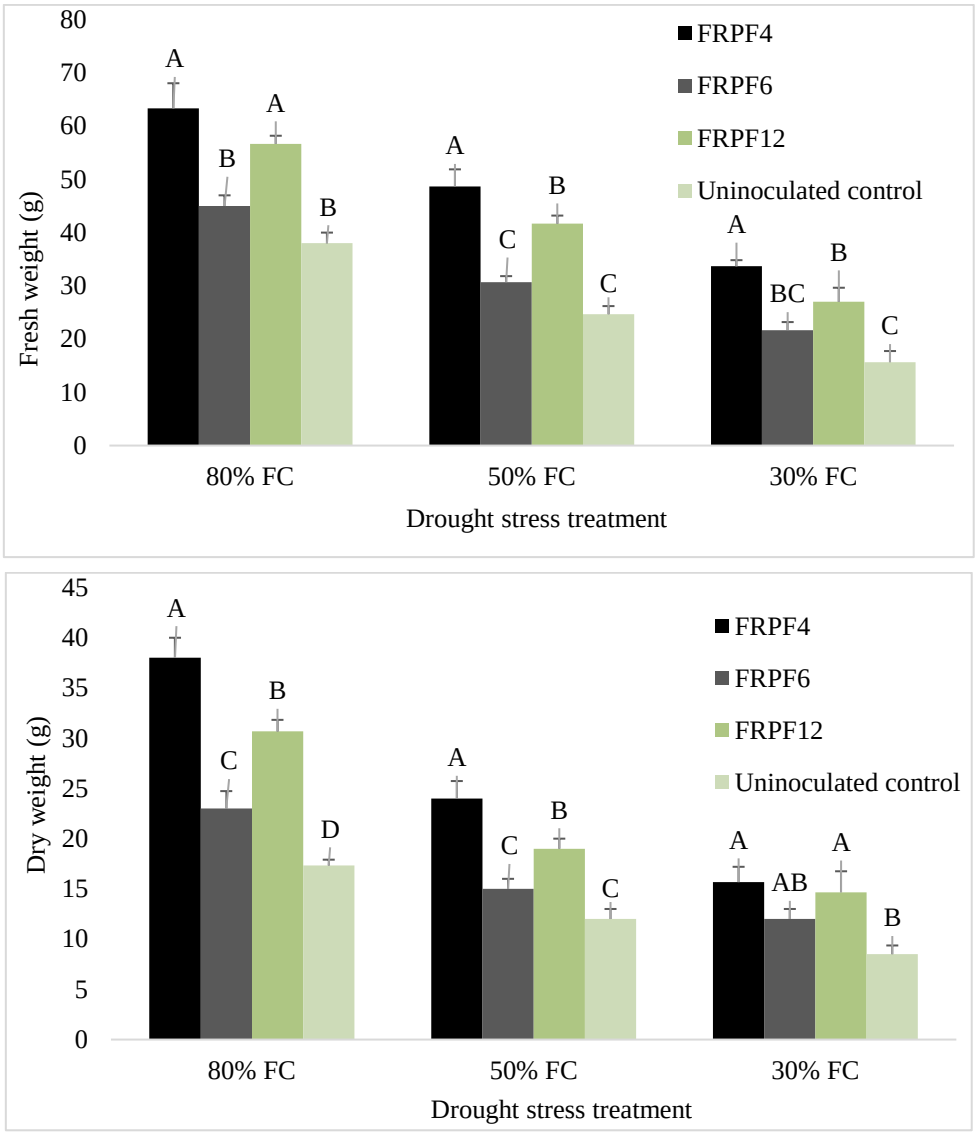



**Fig. 1.** Effects of *P. fluorescens* isolates (FRPF4, FRPF6, FRPF12) on the shoot height, root length, fresh weight, and dry weight of Santalin yarrow plants under drought stress (80%, 50%, and 30%) (n=3) (P<0.05).

**Table 1.** ANOVA (mean squares) of the fresh weight (FW), dry weight (DW), shoot height (SH), and root length (RL) of Santalin yarrow plants treated with PSB isolates under drought stress (n=3) (P<0.05).

| | | Mean squares | | | |
|---|---|---|---|---|---|
| **S.O.V** | **DF** | **FW** | **DW** | **SH** | **RL** |
| **Drought** | 2 | 2073.028** | 658.965** | 1104.194** | 430.361** |
| **PSB** | 3 | 886.444** | 298.803** | 298.917** | 266.769** |
| **Drought×PSB** | 6 | 13.806* | 28.317** | 17.417** | 17.769** |
| **Error** | 24 | 5.333 | 1.924** | 2.528 | 1.500 |
| **CV (%)** | | 15.72 | 20.17 | 18.29 | 14.44 |

**Significance (*P*<0.01); *Significance (*P*<0.05).

### *TSS*

According to Table 2, drought stress caused an increase in the amount of TSS in the Santalin yarrow plants compared to that in the irrigated control plants. Moreover, the content of TSS in the plants directly correlated with the level of stress.

The bacterial isolates also significantly increased the quantity of TSS in the drought-stressed plants compared to their respective stress treatments ($P<0.05$). Among the PSB treatments, the highest (268 mg $g^{-1}$) and lowest (128 mg $g^{-1}$) levels of TSS occurred in response to the FRPF4+30% FC and FRPF6+80% FC treatments, respectively. The ANOVA revealed no significant correlations between drought stress and PSB application for the TSS content (Table 3).

**Table 2.** Effects of *P. fluorescens* isolates (FRPF4, FRPF6, FRPF12) on the total soluble sugars (TSS), free proline, total flavonoid content (TFC), DPPH scavenging of Santalin yarrow plants under drought stress (n=3) ($P<0.05$).

| FC (%) | Bacterial isolate | TSS (mg $g^{-1}$ DW) | Free proline (µmol $g^{-1}$ DW) | TFC (mg QE $g^{-1}$DW) | DPPH (%) |
|---|---|---|---|---|---|
| 80 | NC | 120.33$^{c}$ | 1.26$^{a}$ | 0.63$^{b}$ | 75.33$^{a}$ |
| | FRPF4 | 162.67$^{a}$ | 0.77$^{c}$ | 1.47$^{a}$ | 70.33$^{b}$ |
| | FRPF6 | 128.00$^{c}$ | 1.03$^{b}$ | 0.90$^{b}$ | 72.00$^{ab}$ |
| | FRPF12 | 145.00$^{b}$ | 0.95$^{b}$ | 1.07$^{ab}$ | 73.00$^{ab}$ |
| 50 | NC | 162.00$^{d}$ | 1.87$^{a}$ | 0.83$^{c}$ | 86.67$^{a}$ |
| | FRPF4 | 210.67$^{a}$ | 1.02$^{c}$ | 1.90$^{a}$ | 78.00$^{b}$ |
| | FRPF6 | 180.67$^{c}$ | 1.47$^{b}$ | 1.03$^{bc}$ | 82.33$^{ab}$ |
| | FRPF12 | 195.33$^{b}$ | 1.29$^{bc}$ | 1.33$^{b}$ | 83.00$^{ab}$ |
| 30 | NC | 227.67$^{c}$ | 2.57$^{a}$ | 1.03$^{c}$ | 96.67$^{a}$ |
| | FRPF4 | 268.00$^{a}$ | 1.73$^{c}$ | 2.33$^{a}$ | 82.34$^{c}$ |
| | FRPF6 | 243.33$^{b}$ | 2.23$^{ab}$ | 1.40$^{bc}$ | 91.67$^{ab}$ |
| | FRPF12 | 261.00$^{a}$ | 2.02$^{bc}$ | 1.81$^{ab}$ | 90.00$^{b}$ |

NC: Non-inoculated (control). Values marked by different letters are significantly different ($P<0.05$).

**Table 3.** ANOVA (mean squares) of the total soluble sugars (TSS), free proline, total flavonoid content (TFC), and DPPH of Santalin yarrow plants treated with PSB isolates under drought stress (n=3) ($P<0.05$).

| | | Mean squares | | | |
|---|---|---|---|---|---|
| S.O.V | DF | TSS | Free proline | TFC | DPPH |
| Drought | 2 | 37178.111** | 3.973** | 1.197** | 923.444** |
| PSB | 3 | 3280.704** | 0.834** | 1.871** | 131.259** |
| Drought×PSB | 6 | 33.815$^{ns}$ | 0.023$^{ns}$ | 0.039$^{ns}$ | 12.815* |
| Error | 24 | 18.778 | 0.011 | 0.024 | 3.528 |
| CV (%) | | 15.63 | 13.72 | 25.25 | 10.13 |

**Significance ($P<0.01$); *Significance ($P<0.05$). ns: not significant.

### *Free proline content*

Drought stress significantly increased the proline content of the Santalin yarrow leaves ($P<0.05$) (Table 2). A positive correlation occurred between the free proline content and the stress level. However, compared with those in the corresponding stress treatment, the proline content in the drought-stressed plants treated with the PSB isolates was significantly lower ($P<0.05$). Among the PSB treatments, the highest (2.23 µmol $g^{-1}$) and lowest (0.77 µmol $g^{-1}$) amounts of proline were associated with the FRPF6+30% FC and FRPF4+80% FC treatments, respectively. Based on the ANOVA results, no significant associations occurred between drought stress and PSB application for free proline content (Table 3).

### *TFC*

The effects of the FRPF4, FRPF6, and FRPF12 isolates on the TFC of Santalin yarrow plants under drought stress appear in Table 2. A significant increase in the TFC of the plants occurred under drought stress compared to the

irrigated control (P<0.05). Only the FRPF4 treatment significantly increased the plant TFC at 80% FC. Additionally, the TFC of the plants treated with FRPF6 did not differ significantly from that of the corresponding control at 50% and 30% FC. The ANOVA demonstrated no significant correlations between drought stress and PSB application for the TFC (Table 3).

### *DPPH*

Although the amount of DPPH increased under drought stress conditions, it was not significantly different from that in the corresponding control (Table 2). Furthermore, the DPPH activity of the drought-stressed plants treated with the PSB isolates was lower than that of the corresponding stress-treated plants (P<0.05). At 30% FC, the FRPF4 and FRPF12 treatments significantly decreased the DPPH activity in the plants. Among the PSB treatments, the highest (91.67%) and lowest (70.33%) DPPH radical scavenging occurred in response to the FRPF6+30% FC and FRPF4+80% FC treatments, respectively. According to the ANOVA on DPPH, the interaction effects between drought stress and PSB application were significant (P<0.05 (Table 3).

### *Carotenoid, chlorophyll a, b, and total chlorophyll*

Drought stress decreased the carotenoid, chlorophyll a, b, and total chlorophyll contents of the Santalin yarrow plants (Table 4). Moreover, the levels of these factors significantly increased in the plants treated with the FRPF4 isolate under both irrigated and stress conditions (P<0.05). These parameters increased in plants treated with FRPF6 and FRPF12 but were not significantly different from those in the corresponding control plants. Based on the ANOVA results, no significant associations occurred between drought stress and PSB application for these parameters (Table 5).

**Table 4.** Effects of *P. fluorescens* isolates (FRPF4, FRPF6, FRPF12) on the total colorophyll, chlorophyll a, chlorophyll b, and carotenoid of Santalin yarrow plants under drought stress (n=3) (P<0.05).

| FC (%) | Bacterial isolate | Carotenoid (mg $g^{-1}$ FW) | Chlorophyll a (mg $g^{-1}$ FW) | Chlorophyll b (mg $g^{-1}$ FW) | Total chlorophyll (mg $g^{-1}$ FW) |
|---|---|---|---|---|---|
| 80 | NC | $1.37^{ab}$ | $1.13^{b}$ | $0.73^{b}$ | $1.87^{b}$ |
| | FRPF4 | $1.67^{a}$ | $1.83^{a}$ | $1.30^{a}$ | $3.13^{a}$ |
| | FRPF6 | $1.23^{b}$ | $1.23^{b}$ | $0.87^{ab}$ | $2.10^{b}$ |
| | FRPF12 | $1.20^{b}$ | $1.27^{b}$ | $0.83^{ab}$ | $2.10^{b}$ |
| 50 | NC | $0.40^{b}$ | $0.53^{b}$ | $0.30^{b}$ | $0.83^{b}$ |
| | FRPF4 | $1.03^{a}$ | $1.20^{a}$ | $0.90^{a}$ | $2.10^{a}$ |
| | FRPF6 | $0.67^{b}$ | $0.63^{ab}$ | $0.33^{b}$ | $1.03^{b}$ |
| | FRPF12 | $0.50^{b}$ | $0.90^{ab}$ | $0.33^{b}$ | $1.23^{b}$ |
| 30 | NC | $0.23^{b}$ | $0.23^{b}$ | $0.13^{b}$ | $0.37^{b}$ |
| | FRPF4 | $0.87^{a}$ | $0.90^{a}$ | $0.60^{a}$ | $1.50^{a}$ |
| | FRPF6 | $0.23^{b}$ | $0.30^{b}$ | $0.17^{b}$ | $0.50^{b}$ |
| | FRPF12 | $0.27^{b}$ | $0.33^{b}$ | $0.23^{b}$ | $0.57^{b}$ |

NC: Non-inoculated (control). Values marked by different letters are significantly different ($P$<0.05).

**Table 5.** ANOVA (mean squares) of the carotenoid, chlorophyll a, b, and total chlorophyll of Santalin yarrow plants treated with PSB isolates under drought stress (n=3) (P<0.05).

| S.O.V | DF | Mean squares | | | |
|---|---|---|---|---|---|
| | | Carotenoid | Chlorophyll a | Chlorophyll b | Total chlorophyll |
| **Drought** | 2 | 3.021** | 2.597** | 1.348** | 7.551** |
| **PSB** | 3 | 0.593** | 0.821** | 0.565** | 2.681** |
| **Drought×PSB** | 6 | $0.033^{ns}$ | $0.016^{ns}$ | $0.007^{ns}$ | $0.010^{ns}$ |
| **Error** | 24 | 0.013 | 0.024 | 0.016 | 0.053 |
| **CV (%)** | | 16.32 | 18.57 | 16.03 | 14.17 |

**Significance ($P$<0.01); *Significance ($P$<0.05). ns: not significant.

## Discussion

Drought stress adversely affects plant growth and development. Due to reduced water availability for plants, the surface area of the leaves decreases, and plant stomata close in part or entirely. Therefore, the evaporation rate and CO2 absorption decrease, thus affecting the photosynthesis rate and plant growth (Sharifi Ashoorabadi et al., 2009; Neumann, 2008). An initial symptom of drought stress in plants is a rapid inhibition of branch and root growth (Neumann, 2008). The greater the drought stress, the less the plant absorbs enough water and nutrients. Therefore, the energy required for plant growth and development, cell division, and reproduction will go to root system development, the absorption of water, and nutrient uptake. Accordingly, stressed plants exhibit slower and lower growth rates than those unaffected by stress (Neumann, 2008). The present study revealed a logical decrease in all Santalin yarrow growth parameters, including shoot height, root length, and dry and fresh weight, when 80% to 30% FC was applied during drought stress. In this regard, Ramezan and Abbaszadeh (2016) investigated the effects of drought stress (30, 60, and 90% FC) on the quantitative and qualitative yield of *Nepeta pogonosperma* under field conditions. Based on their findings, drought stress (30 and 60% FC) adversely affected leaf length and width, inflorescence length, yield per hectare, EO percentage and yield, and the number of flowering and inflorescence stems. In contrast, the unstressed control (90% FC) exhibited improved growth and development. Therefore, our results confirm previous research in a similar context.

Plant susceptibility to drought stress reflects several factors, including plant species and growth stage, drought level, and plant-associated microorganisms (Sharifi Ashoorabadi et al., 2009; Abideen et al., 2022). PGPR are beneficial plant-associated microorganisms that can enhance plant growth and protect plants against several types of abiotic stress by producing siderophores, volatile compounds, and phytohormones while reducing plant ethylene synthesis (Abideen et al., 2022). Additionally, Lotfi et al. (2022) reported that drought stress increased the production of siderophores, phytohormones, HCN, and solubilization of phosphate by *Bacillus velezensis* strain ZM39 and *Bacillus amyloliquefaciens* strain Cha43 as PGPR agents. Here, the effects of *Pseudomonas fluorescens* isolates on the abovementioned growth parameters of Santalin yarrow plants under drought stress received focus. According to the results, drought stress had weaker adverse effects on the abovementioned growth parameters in plants treated with the FRPF4 and FRPF12 isolates. Khan et al. (2019) also demonstrated that a consortium of PGPR (*Bacillus subtilis* P1, *B. thuringiensis* P2, and *B. megaterium* P3 strains) and plant growth regulators (salicylic acid and putrescine) assisted in increasing shoot and root dry weight of drought-stressed chickpea (*Cicer arietinum* L.) plants.

Drought stress may significantly affect several plant physiological processes, including proline metabolism, photosynthesis, and the antioxidant defense system (Witt et al., 2012). Organic acids, sugars, polyols, and amino acids play crucial roles in plant tolerance to drought stress (Farooq et al., 2012). On the other hand, water potential gradients from soil to plants function regularly by organic acids and amino acids, while osmotic balance remains regulated by sugars (Jouve et al., 2004). In this regard, Abid et al. (2017) reported that the soluble sugars, proline, and protein contents in the leaves of faba bean plants significantly increased under water-deficit stress ($P<0.05$). Nakabayashi et al. (2014) demonstrated that drought stress caused accumulations of antioxidants, including flavonoids and anthocyanins. This accumulation enables plants to act as free radical scavengers, which helps to reduce the impact of both oxidative stress and dehydration. Based on our findings, drought stress also significantly increased the TSS, free proline, total flavonoid, and DPPH contents of Santalin yarrow leaves. Accordingly, our findings confirm previous findings in other research cases.

The drought-stressed plants treated with the PSB isolates exhibited a significant reduction in free proline and DPPH contents compared to those in the corresponding stress treatments. However, the TSS content and total flavonoids increased in Santalin yarrow plants inoculated with the PSB isolates under drought stress. A similar finding reportedly occurred in chickpea plant treatments with *P. putida* (Tiwari et al., 2016). The authors revealed that inoculation with *P. putida* caused a significant decrease in the chickpea proline content at all drought stress levels. According to Vardharajula et al. (2011), inoculating maize seedlings with strains of *Bacillus* spp. as PGPR agents decreased plant antioxidant enzyme activities under drought stress. Ghosh et al. (2018) also revealed that inoculating with *P. putida* GAP-P45 decreased the activities of all antioxidant enzymes in *Arabidopsis thaliana* seedlings under

water-deficit conditions. Indeed, PGPR strains could alleviate the adverse effects of drought stress on the activity of antioxidant enzymes (Han and Lee, 2005).

Similarly, Vardharajula et al. (2011) showed that strains of *Bacillus* species increased the accumulation of soluble sugars in drought-stressed maize seedlings. According to Mirzaie et al. (2020), the highest TPC in lemongrass (*Cymbopogon citratus*) plants occurred while having interactions between PGPRs (*Pseudomonas* sp. and *Azospirillum* sp.) and their application at 50% FC. Drought stress also decreased the carotenoid, chlorophyll a, b, and total chlorophyll contents of the Santalin yarrow plants. Similarly, Soroori and Danaee (2023) reported that the lowest carotenoid and total chlorophyll contents in *Calendula officinalis* occurred in response to 25% FC. Additionally, our results indicated that inoculation with PSB isolates increased the levels of these factors in Santalin yarrow plants in response to water-deficit stress. Abideen et al. (2022) also detected a significant increase (approximately 18%) in drought-stressed *Hordeum vulgar* plants treated with *Pseudomonas* sp. or *Pantoea* sp. as well as in sufficiently watered plants compared to the corresponding stress-affected plants in the control group.

## Conclusion

Applying *P. fluorescens* isolates FRPF4 improved growth and physiological factors in drought-stressed Santalin yarrow plants. Inoculation with the isolate FRPF4 significantly increased the TSS, TFC, carotenoid, chlorophyll a, b, and total chlorophyll contents in Santalin yarrow plants under water-deficit stress. However, the DPPH and free proline contents decreased. Moreover, other indirect moderating mechanisms might be involved in coping with the unpleasant effects of drought stress on the plants. As drought stress might limit plant access to nutrients such as nitrogen, phosphorous, and iron, PGPR agents can compensate while providing such nutrients to increase plant tolerance to these stressful conditions. Since the FRPF4 isolate is an efficient phosphate-solubilizing and siderophore-producing PGPR, PSB isolates may mitigate the adverse effects of drought stress on Santalin yarrow plants through several direct and indirect mechanisms.

### Conflict of Interest

The authors indicate no conflict of interest in this work.

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