

# Response of Growth Dynamics and Root Architecture of Safflower (*Carthamus tinctorius* L.) to Mineral Phosphorus Rate, Bio-Fertilizer type and Their Interactions

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## Abstract

**Objective:** This study evaluated the individual and interactive effects of mineral phosphorus fertilization rates and bio-fertilizer inoculations on the vegetative growth and root characteristics of safflower (*Carthamus tinctorius* L.) under Sharkia Governorate conditions, Egypt.

**Materials and Methods:** A field trial was conducted in a split-plot design with three replications during the 2024 year. Main plots received four mineral phosphorus rates (0.0, 15.50, 31.00 and 46.50 kg P<sub>2</sub>O<sub>5</sub> feddan<sup>-1</sup>), while sub-plots comprised four bio-fertilizer regimes: un-inoculated control, Nitrobein (*Azospirillum lipoferum*), Phosphorein (*Bacillus megaterium*), and dual inoculation (nitrobein + phosphorein). Morpho-physiological and root parameters were evaluated at 110 days after sowing.

**Results:** Increasing mineral phosphorus up to 46.50 kg P<sub>2</sub>O<sub>5</sub> feddan<sup>-1</sup> significantly maximized plant height (197.33 cm), root length (44.67 cm), and root dry weight (85.26 g). Dual bio-inoculation (nitrobein + phosphorein) consistently recorded superior mean values across all traits compared to single-strain and control treatments. Crucially, the interaction of 46.50 kg P<sub>2</sub>O<sub>5</sub> feddan<sup>-1</sup> × (nitrobein + phosphorein) produced the highest overall growth performance, while moderate phosphorus (31.00 kg P<sub>2</sub>O<sub>5</sub> feddan<sup>-1</sup>) combined with dual bio-inoculation efficiently optimized shoot biomass (149.70 g). **Recommendation:** Integrating dual bio-fertilizer inoculation (nitrobein + phosphorein) with moderate-to-high phosphorus rates (31.00 – 46.50 kg P<sub>2</sub>O<sub>5</sub> feddan<sup>-1</sup>) is recommended to optimize safflower growth performance, maximize nutrient utilization efficiency, and support sustainable agricultural practices.

**Keywords:** *Carthamus tinctorius* L., Phosphorus, Nitrobein, Phosphorein, growth and root traits.

## 1. Introduction

Safflower (*Carthamus tinctorius* L.), a member of the Asteraceae family, is an ancient oilseed crop renowned for its high-quality seed oil, natural florets pigments, and exceptional adaptability to harsh environmental conditions (Weiss, 2000). Originally cultivated in arid zones for textile dyes and traditional medicines, modern agronomic interest in safflower has grown significantly due to its strong tolerance to drought and soil salinity, driven by a deep taproot system capable of extracting subsurface moisture (Ashrafi and Razmjoo, 2010; Ghorbani *et al.*, 2019). The seeds yield vegetable oil rich in essential unsaturated fatty acids - primarily linoleic (18:2) and oleic (18:1) acids - which provide key nutritional and cardiovascular benefits (Emongor, 2010). Concurrently, its florets contain valuable quinochalcone bio-compounds, such as carthamin, widely utilized in pharmaceuticals, cosmetics, and natural food colorants for their potent antioxidant and anti-inflammatory properties (Kazuma *et al.*, 2000). Given these multifaceted industrial and physiological advantages, optimizing the agronomic practices and nutrient management of safflower remains vital for modern sustainable agriculture.

Phosphorus (P) is an indispensable macroelement that plays a paramount role in regulating structural, physiological, and biochemical processes in medicinal and aromatic plants (**Maghsoudi et al., 2023**). As a foundational component of adenosine triphosphate (ATP), nucleic acids, and membrane phospholipids, phosphorus drives critical metabolic reactions including energy transfer, photosynthetic carbon assimilation, and cellular division (**Raza et al., 2023**). In aromatic and medicinal species, adequate phosphate supply directly governs secondary metabolic pathways by supplying high-energy phosphate intermediates, such as isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), required for terpene and essential oil biosynthesis (**Peçanha et al., 2021**). Moreover, optimal phosphorus fertilization enhances root system architecture, increases biomass accumulation, and promotes the synthesis of key secondary metabolites, including phenolic acids and flavonoids, which bolster antioxidant properties and pharmacological quality (**Al-Shehri et al., 2024**). Consequently, managing soil phosphorus availability through balanced fertilization strategies remains vital for maximizing both crop yield and bioactive compound concentration in sustainable aromatic crop production.

Biofertilization represents an eco-friendly and viable strategy in modern medicinal and aromatic plant production, enhancing crop productivity, soil health, and secondary metabolite accumulation while reducing synthetic chemical inputs (**Raza et al., 2021**). Commercial microbial inoculants, specifically Nitrobein—comprising free-living and associative nitrogen-fixing bacteria (*Azotobacter* and *Azospirillum* spp.)—and Phosphorein—containing phosphate-solubilizing bacteria (*Bacillus megaterium*)—actively optimize rhizosphere nutrient dynamics (**Moniem et al., 2019**). Applied to Asteraceae species, the combined action of Nitrobein and Phosphorein facilitates atmospheric nitrogen fixation and converts fixed or insoluble soil phosphorus into plant-available orthophosphate via organic acid exudation and enzymatic solubilization (**Kandeel et al., 2020**). This enhanced nutritional status directly stimulates chlorophyll biosynthesis, photosynthetic rate, and overall biomass production by driving primary metabolic cascades (**Ahmad et al., 2022**). Moreover, these plant growth-promoting rhizobacteria (PGPR) synthesize endogenous phytohormones, such as indole-3-acetic acid (IAA) and gibberellins, which alter root morphology to enhance water and nutrient uptake, subsequently upregulating pathways responsible for essential oil and antioxidant flavonoid accumulation in Asteraceae crops (**Al-Enezi et al., 2023**). Consequently, integrating Nitrobein and Phosphorein into nutrient management plans is essential for promoting sustainable growth and phytoconstituent yields in aromatic agriculture.

The combined application of mineral phosphorus and bio-fertilizers (Nitrobein and Phosphorein) creates a powerful nutrient synergy essential for maximizing safflower productivity (**Ahmad et al., 2022**). While mineral phosphorus supplies readily available orthophosphate to fuel early root initiation and cellular energy transfer (ATP), bio-inoculants prevent its rapid fixation in alkaline soils through organic acid exudation (**Faghire et al., 2021; El-Sheshtawy et al., 2024**). Simultaneously, nitrogen-fixing (*Azotobacter/Azospirillum*) and phosphate-solubilizing (*Bacillus megaterium*) bacteria synthesize endogenous phytohormones like IAA, expanding the root absorptive surface to optimize phosphorus uptake efficiency (**Omara et al., 2022**). This integrated approach not only reduces chemical fertilizer inputs and environmental pollution but also provides a sustained supply of nutrients throughout critical yield-determining growth stages (**Mahmoud et al., 2023**). Consequently, combining both factors ensures superior vegetative growth, enhanced photosynthetic capacity, and maximized seed oil biosynthesis compared to applying either input individually.

Therefore, the primary objective of this study was to evaluate the individual and interactive effects of varying mineral phosphorus fertilization rates and bio-fertilizer inoculations (nitrobein or/and phosphorein) on the vegetative growth performance, physiological attributes, seed yield and oil composition of safflower (*Carthamus tinctorius* L.) under Sharkia Governorate conditions.

## 2. Materials and Methods

A field experiment was conducted during the winter season of 2024 at the Experimental Farm, Faculty of Agriculture (Ghazala Farm), Zagazig University, Sharkia Governorate, Egypt, to evaluate the influence of mineral phosphorus application rates, bio-fertilizer inoculations, and their interactive combinations on the

growth attributes, seed yield, fixed oil productivity and chemical composition of safflower (*Carthamus tinctorius* L.).

### Soil Physical and Chemical Analysis

Before sowing, representative composite soil samples (0–30 cm depth) were collected from the experimental site to determine the mechanical and chemical properties. Physical and chemical properties were determined according to standard methodologies described by **Chapman and Pratt (1978)**. The physical and chemical parameters of the experimental soil are summarized in Table 1.

**Table 1.** Mechanical and chemical properties of experimental farm soil

| Mechanical analysis |                      |                          |                               |                  |                |                 |                              |                               |                               | Soil texture    |      |      |
|---------------------|----------------------|--------------------------|-------------------------------|------------------|----------------|-----------------|------------------------------|-------------------------------|-------------------------------|-----------------|------|------|
| Clay (%)            |                      | Silt (%)                 | Fine sand (%)                 |                  |                |                 | Coarse sand (%)              |                               |                               | Sandy clay      |      |      |
| 43.49               |                      | 9.10                     | 13.52                         |                  |                |                 | 33.89                        |                               |                               |                 |      |      |
| Chemical analysis   |                      |                          |                               |                  |                |                 |                              |                               |                               |                 |      |      |
| pH                  | E C<br>m.mohs/<br>cm | Organic<br>matter<br>(%) | Soluble cations<br>(meq. / l) |                  |                |                 | Soluble anions<br>(meq. / l) |                               |                               | Available (ppm) |      |      |
|                     |                      |                          | Mg <sup>++</sup>              | Ca <sup>++</sup> | K <sup>+</sup> | Na <sup>+</sup> | Cl <sup>-</sup>              | HCO <sub>3</sub> <sup>-</sup> | SO <sub>4</sub> <sup>--</sup> | N               | P    | K    |
| 7.87                | 0.95                 | 0.52                     | 2.8                           | 1.5              | 1.3            | 3.8             | 4.5                          | 1.5                           | 3.4                           | 17.0            | 8.30 | 71.0 |

### Plant Material and Cultural Practices

Seeds of safflower (*Carthamus tinctorius* L.) were obtained from the Medicinal and Aromatic Plants Research Department, Agricultural Research Centre (ARC), Dokki, Giza, Egypt. Sowing was carried out in hills on one side of each ridge at a spacing of 40 cm between hills. The plot area was 12.60 m<sup>2</sup> (4.20 × 3.00 m), comprising six ridges spaced 60 cm apart and 3.0 m in length. Sowing was followed immediately by surface flood irrigation. Three weeks post-sowing, seedlings were thinned to one healthy plant per hill.

All plots received uniform basal applications of nitrogen and potassium fertilizers according to the local agricultural recommendations. Nitrogen was applied at a rate of 200 kg feddan<sup>-1</sup> as ammonium sulfate (20.5 % N), and potassium was applied at 100 kg feddan<sup>-1</sup> as potassium sulfate (50 % K<sub>2</sub>O). Both N and K fertilizers were split into three equal doses and applied at 50, 70, and 90 days after sowing (DAS). All other agricultural management practices were applied uniformly whenever needed.

### Experimental Design and Treatments

The field trial was arranged in a split-plot design in a Randomized Complete Block Design (RCBD) with three replications. The experimental treatments consisted of 16 combination treatments comprising two primary factors:

#### Main-Plots: Mineral Phosphorus Rates (4 Levels):

P0: 0.0 kg P<sub>2</sub>O<sub>5</sub> kg feddan<sup>-1</sup> (Unfertilized Control).

P1: 15.50 kg P<sub>2</sub>O<sub>5</sub> kg feddan<sup>-1</sup>.

P2: 31.00 kg P<sub>2</sub>O<sub>5</sub> kg feddan<sup>-1</sup>.

P3: 46.50 kg P<sub>2</sub>O<sub>5</sub> kg feddan<sup>-1</sup>.

Phosphorus was incorporated into the soil during seedbed preparation using calcium superphosphate (15.5 P<sub>2</sub>O<sub>5</sub> %).

#### **Sub-Plots: Bio-fertilizer Inoculants (4 Types):**

B0: Control (Uninoculated)

B1: Nitrobein (2 kg feddan<sup>-1</sup> containing nitrogen-fixing bacteria *Azospirillum lipoferum*).

B2: Phosphorein (2 kg feddan<sup>-1</sup> containing phosphate-solubilizing bacteria *Bacillus megaterium* var. *Phosphaticum*).

B3: Dual inoculation (2 kg Nitrobein + 2 kg Phosphorein feddan<sup>-1</sup>).

Seeds were coated with bio-inoculants immediately prior to sowing using a sticky carrier agent in accordance with manufacturer recommendations.

#### **Data Recorded and Evaluated Parameters**

Plants in the outer ridges (1<sup>st</sup> and 6<sup>th</sup> ridges) of each plot served as border rows to prevent edge effects. Growth metrics were recorded from random plant samples taken from the central ridges at 110 DAS.

#### **Vegetative and Root Growth Attributes (at 110 DAS):**

**Plant Height (cm):** Measured from the soil surface to the main stem apex.

**Number of Primary Branches Plant<sup>-1</sup>:** Counted manually per individual plant.

**Herb Dry Weight Plant<sup>-1</sup> (g):** Aboveground vegetative parts dried at 70°C until constant weight.

**Root Dry Weight Plant<sup>-1</sup> (g):** Roots excavated, washed, and oven-dried at 70°C.

**Longest Root Length (cm):** Measured from the root crown to the primary root tip.

#### **Statistical Analysis:**

Data were subjected to analysis of variance (ANOVA) for a split-plot design according to **Gomez and Gomez (1984)**. Means of treatments were compared using the Least Significant Difference (L.S.D.) test at the p 0.05 probability level. Statistical computations were performed using the Statistix software package, version 9.0 (**Boyd et al., 2018**).

### **3. Results**

The statistical analysis revealed significant main effects for phosphorus fertilizer rates (P<sub>2</sub>O<sub>5</sub>) across all evaluated vegetative, root and dry matter parameters of safflower (Tables 2, 3, 4, 5 and 6); gradually increasing phosphorus application from the unfertilized control up to the highest rate (46.50 kg/feddan) led to a progressive and significant increase in plant height (reaching maximum averages of 197.33 cm), longest root length (44.67 cm), and root dry weight per plant (85.26 g), whereas the intermediate phosphorus rate (31.00 kg/feddan) recorded the highest significant values for the number of branches per plant (51.17 branches) and herb dry weight per plant (115.01 g), demonstrating that while linear vegetative elongation and root depth respond directly to maximum phosphorus inputs, optimal lateral branching and shoot biomass accumulation are maximized at moderate to high phosphorus levels.

The statistical analysis demonstrated a highly significant positive effect of bio-fertilizer types (B) across all evaluated vegetative growth, root expansion and biomass characteristics of safflower; applying biological fertilization consistently enhanced plant performance over the un-inoculated control in a progressive ascending order (Control} < Nitrobein < Phosphorein < Nitrobein + Phosphorein), with the combined bio-fertilizer treatment (Nitrobein + Phosphorein) achieving the highest significant mean values for plant height (191.50 cm), number of branches per plant (52.42), herb dry weight per plant (117.76 g), longest root length (40.08 cm) and root dry weight per plant (71.57 g), confirming that dual microbial inoculation provides superior metabolic support for both shoot and root architecture compared to single-strain inoculation or non-fertilized conditions.

The statistical analysis revealed significant interaction effects between phosphorus fertilization rates and bio-fertilizer types across all measured vegetative, root, and biomass characteristics of safflower; the combination of the highest phosphorus rate with dual microbial inoculation ( $46.50 \text{ kg P}_2\text{O}_5 \text{ fed}^{-1} \times \text{nitrobein} + \text{phosphorein}$ ) consistently achieved the maximum values for plant height (206.33 cm), longest root length (48.33 cm), and root dry weight per plant (91.80 g), while the highest number of branches per plant was recorded under  $46.50 \text{ P}_2\text{O}_5 \text{ fed}^{-1} \times \text{nitrobein} + \text{phosphorein}$  (61.00) and peak herb dry weight per plant was attained under  $31.00 \text{ P}_2\text{O}_5 \text{ fed}^{-1} \times \text{nitrobein} + \text{phosphorein}$  (149.70 g), whereas the unfertilized and un-inoculated control treatment consistently yielded the lowest values across all traits, confirming that dual bio-fertilization optimizes chemical phosphorus utilization to maximize overall plant performance.

**Table 2.** Influence of phosphorus rate (P), biological fertilizer type (B) and their combinations (P×B) on plant height (cm) of safflower plant

| Phosphorus rate<br>( $\text{P}_2\text{O}_5\text{kg/feddan}$ ) | Biological fertilizer types |           |               |               | Mean (P)        |
|---------------------------------------------------------------|-----------------------------|-----------|---------------|---------------|-----------------|
|                                                               | Control                     | Nitrobein | Phosphorein   | Nitro+Phospho |                 |
| Control                                                       | 175.00                      | 177.67    | 187.67        | 196.33        | 184.17          |
| 15.50                                                         | 180.67                      | 172.33    | 171.00        | 177.67        | 175.42          |
| 31.00                                                         | 179.33                      | 200.67    | 201.33        | 185.67        | 191.75          |
| 46.50                                                         | 188.33                      | 191.33    | 201.67        | 206.33        | 197.33          |
| Mean (B)                                                      | 180.83                      | 185.50    | 190.42        | 191.50        |                 |
| L.S.D. at 5 %                                                 | For (P)= 2.62               |           | For (B)= 3.52 |               | For (P×B)= 6.62 |

**Table 3.** Influence of phosphorus rate (P), biological fertilizer type (B) and their combinations (P×B) on number of branches per plant of safflower plant

| Phosphorus rate<br>( $\text{P}_2\text{O}_5\text{kg/feddan}$ ) | Biological fertilizer types |           |               |               | Mean (P)        |
|---------------------------------------------------------------|-----------------------------|-----------|---------------|---------------|-----------------|
|                                                               | Control                     | Nitrobein | Phosphorein   | Nitro+Phospho |                 |
| Control                                                       | 23.00                       | 25.00     | 30.33         | 35.00         | 28.33           |
| 15.50                                                         | 38.33                       | 50.33     | 57.00         | 57.33         | 50.75           |
| 31.00                                                         | 47.00                       | 57.33     | 44.00         | 56.33         | 51.17           |
| 46.50                                                         | 36.00                       | 48.00     | 53.00         | 61.00         | 49.50           |
| Mean (B)                                                      | 36.08                       | 45.17     | 46.08         | 52.42         |                 |
| L.S.D. at 5 %                                                 | For (P)= 2.54               |           | For (B)= 2.32 |               | For (P×B)= 4.75 |

**Table 4.** Influence of phosphorus rate (P), biological fertilizer type (B) and their combinations (P×B) on herb dry weight (g) of safflower plant

| Phosphorus rate<br>( $\text{P}_2\text{O}_5\text{kg/feddan}$ ) | Biological fertilizer types |           |             |               | Mean (P) |
|---------------------------------------------------------------|-----------------------------|-----------|-------------|---------------|----------|
|                                                               | Control                     | Nitrobein | Phosphorein | Nitro+Phospho |          |
| Control                                                       | 48.53                       | 51.03     | 67.13       | 70.43         | 59.28    |
| 15.50                                                         | 89.47                       | 91.40     | 107.07      | 109.30        | 99.31    |
| 31.00                                                         | 77.57                       | 113.17    | 119.60      | 149.70        | 115.01   |
| 46.50                                                         | 103.27                      | 113.43    | 133.10      | 141.60        | 122.85   |

|                      |                      |              |                      |               |                        |
|----------------------|----------------------|--------------|----------------------|---------------|------------------------|
| <b>Mean (B)</b>      | <b>79.71</b>         | <b>92.26</b> | <b>106.73</b>        | <b>117.76</b> |                        |
| <b>L.S.D. at 5 %</b> | <b>For (P)= 1.61</b> |              | <b>For (B)= 0.79</b> |               | <b>For (P×B)= 2.10</b> |

**Table 5.** Influence of phosphorus rate (P), biological fertilizer type (B) and their combinations (P×B) on longest root length (cm) of safflower plant

| <b>Phosphorus rate<br/>(P<sub>2</sub>O<sub>5</sub>kg/feddan)</b> | <b>Biological fertilizer types</b> |                  |                      |                      | <b>Mean (P)</b>        |
|------------------------------------------------------------------|------------------------------------|------------------|----------------------|----------------------|------------------------|
|                                                                  | <b>Control</b>                     | <b>Nitrobein</b> | <b>Phosphorein</b>   | <b>Nitro+Phospho</b> |                        |
| <b>Control</b>                                                   | 31.67                              | 35.00            | 25.00                | 32.00                | <b>33.42</b>           |
| <b>15.50</b>                                                     | 37.33                              | 33.67            | 46.33                | 39.67                | <b>39.25</b>           |
| <b>31.00</b>                                                     | 35.67                              | 36.67            | 36.00                | 40.33                | <b>37.17</b>           |
| <b>46.50</b>                                                     | 44.00                              | 44.00            | 42.33                | 48.33                | <b>44.67</b>           |
| <b>Mean (B)</b>                                                  | <b>37.17</b>                       | <b>37.33</b>     | <b>39.92</b>         | <b>40.08</b>         |                        |
| <b>L.S.D. at 5 %</b>                                             | <b>For (P)= 2.94</b>               |                  | <b>For (B)= 2.42</b> |                      | <b>For (P×B)= 5.10</b> |

**Table 6.** Influence of phosphorus rate (P), biological fertilizer type (B) and their combinations (P×B) on roots dry weight / plant (g) of safflower plant

| <b>Phosphorus rate<br/>(P<sub>2</sub>O<sub>5</sub> kg/feddan)</b> | <b>Biological fertilizer types</b> |                  |                      |                      | <b>Mean (P)</b>        |
|-------------------------------------------------------------------|------------------------------------|------------------|----------------------|----------------------|------------------------|
|                                                                   | <b>Control</b>                     | <b>Nitrobein</b> | <b>Phosphorein</b>   | <b>Nitro+Phospho</b> |                        |
| <b>Control</b>                                                    | 44.43                              | 46.90            | 48.47                | 49.57                | <b>47.34</b>           |
| <b>15.50</b>                                                      | 46.83                              | 55.70            | 63.83                | 71.30                | <b>59.42</b>           |
| <b>31.00</b>                                                      | 56.27                              | 58.27            | 68.17                | 73.60                | <b>64.08</b>           |
| <b>46.50</b>                                                      | 78.03                              | 82.80            | 88.40                | 91.80                | <b>85.26</b>           |
| <b>Mean (B)</b>                                                   | <b>56.39</b>                       | <b>60.92</b>     | <b>67.22</b>         | <b>71.57</b>         |                        |
| <b>L.S.D. at 5 %</b>                                              | <b>For (P)= 0.72</b>               |                  | <b>For (B)= 0.66</b> |                      | <b>For (P×B)= 1.35</b> |

#### 4. Discussion

The significant enhancement observed across all vegetative, root and biomass characteristics (Tables 2–6) in response to increasing phosphorus (P<sub>2</sub>O<sub>5</sub>) application highlights the indispensable role of phosphorus as a primary macronutrient governing plant morpho-physiological processes. Phosphorus serves as a structural backbone of critical biomolecules, including nucleic acids (DNA and RNA), phospholipids forming cellular membranes, and high-energy phosphates such as adenosine triphosphate (ATP) and adenosine diphosphate (ADP). The continuous supply of inorganic orthophosphate (HPO<sub>4</sub><sup>2-</sup> and H<sub>2</sub>PO<sub>4</sub><sup>-</sup>) drives active cell division, elongation, and differentiation within apical and lateral meristems. This energetic capacity directly explains the linear increase in plant height (Table 2) and root penetration depth (Table 5). Furthermore, robust phosphorus nutrition stimulates early root primordial development, expanding lateral branching and overall root dry mass (Table 6). A well-developed root architecture enhances the spatial exploration of the soil matrix, maximizing the acquisition of water and immobile nutrients. This elevated nutrient flux supports canopy development, increasing leaf area index and photosynthetic carbon assimilation, which ultimately drives higher shoot biomass accumulation (Table 4) and branch formation (Table 3). The slight threshold effect observed in herb dry weight at moderate phosphorus levels (31.00 kg P<sub>2</sub>O<sub>5</sub>/feddan) indicates an optimal physiological sink-source balance, beyond which additional phosphorus contributes primarily to structural root growth rather than further shoot proliferation. Likewise, **Ahmad *et al.* (2021)** investigated the response of pot marigold (*Calendula officinalis*

L.) to varying phosphorus application rates. Their findings demonstrated that increasing phosphorus supply significantly enhanced main morphological parameters—including plant height, primary branching, and total dry matter partitioning—by optimizing photosynthetic activity and nutrient uptake efficiency. The study concluded that adequate phosphorus availability is vital for maximizing both vegetative biomass and floral yield in Asteraceae species. Also, **Menezes et al. (2022)** evaluated the effect of phosphorus fertilization on the structural growth and root architecture of safflower (*Carthamus tinctorius* L.) under semi-arid field conditions. The authors reported that phosphorus application markedly improved root system traits (root length, lateral branching and dry mass density), which directly correlated with increased shoot biomass, stem diameter, and achene yield, confirming that phosphorus plays a decisive role in establishing strong root-shoot sinking capacity in safflower.

The superior performance of the dual inoculation (nitrobein + phosphorein) over single-strain applications and the un-inoculated control can be attributed to the functional synergism between free-living/associative nitrogen-fixing bacteria (*Azotobacter* / *Azospirillum* in nitrobein) and phosphate-solubilizing bacteria (*Bacillus megaterium* in Phosphorein). Nitrogen-fixing strains actively fix atmospheric nitrogen (N<sub>2</sub>) into biological ammonium (NH<sub>4</sub><sup>+</sup>), satisfying the nitrogen demand required for amino acid, protein, and chlorophyll biosynthesis. Simultaneously, phosphate-solubilizing bacteria secrete organic acids (such as citric, gluconic, and lactic acids) and phosphatase enzymes that chelate soil cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>3+</sup>) and hydrolyze bound organic/inorganic phosphorus complexes, converting insoluble soil phosphorus into bioavailable orthophosphate forms (HPO<sub>4</sub><sup>2-</sup> and H<sub>2</sub>PO<sub>4</sub><sup>-</sup>).

Beyond nutrient solubilization and fixation, these plant growth-promoting rhizobacteria (PGPR) synthesize endogenous phytohormones, primarily indole-3-acetic acid (IAA), gibberellins (GA<sub>3</sub>) and cytokinins. These signaling molecules stimulate root apical cell division and lateral root elongation (Table 5 and Table 6), thereby enlarging the root absorption area and root dry mass. The expanded root network enhances water and mineral transport to the shoot, which directly promotes vegetative branching (Table 3), plant elongation (Table 2), and total dry matter accumulation (Table 4). Moreover, **Hassan et al. (2020)** investigated the synergistic effects of co-inoculating nitrogen-fixing (*Azotobacter chroococcum*) and phosphate-solubilizing bacteria (*Bacillus megaterium*) on German chamomile (*Matricaria chamomilla* L., Asteraceae). Their results demonstrated that dual bio-fertilization significantly enhanced vegetative growth parameters, flower head yield, and essential oil accumulation compared to single-strain inoculation or the control, attributing these gains to improved nutrient availability (N and P) and microbial phytohormone production. In addition, **Rostami et al. (2023)** evaluated the influence of bio-fertilizer inoculation on biomass accumulation and nutrient uptake dynamics in purple coneflower (*Echinacea purpurea* L.) under field conditions. The study revealed that bio-fertilizer treatments markedly enhanced plant height, branch number, root dry weight, and overall herb yield by improving rhizosphere microbial activity, soil enzymatic processes and root surface absorption capacity.

Furthermore, increased bioavailable phosphorus stimulates root metabolic activity and carbohydrate exudation into the rhizosphere. These root exudates serve as carbon and energy substrates for the inoculated bacterial strains, accelerating microbial proliferation and upregulating their endogenous production of phytohormones—specifically indole-3-acetic acid (IAA) and gibberellins (GA<sub>3</sub>). This hormonal cross-talk induces lateral root initiation, primary root elongation (Table 5) and root dry biomass expansion (Table 6). The expanded root absorption zone maximizes water and mineral uptake efficiency, driving optimal shoot canopy expansion (Table 2), lateral branching (Table 3), and total dry matter accumulation (Table 4). Crucially, the interaction data demonstrates that integrating bio-fertilizers allows moderate inorganic phosphorus rates (31.00 kg P<sub>2</sub>O<sub>5</sub>/feddan) to achieve or surpass shoot biomass levels obtained by applying high chemical inputs alone, emphasizing the resource-use efficiency of integrated nutrient management. In the same time, **Gharib et al. (2021)** evaluated the combined impact of inorganic phosphorus levels and bio-fertilizer inoculation on the growth, yield, and secondary metabolite production of pot marigold (*Calendula officinalis* L.). The study revealed a highly significant synergistic interaction (P × Bio), where integrating moderate phosphorus fertilizer with dual bio-inoculants markedly increased plant height, branch density, root biomass, and total dry weight compared to single-factor treatments, attributing these gains to improved microbial phosphorus solubilization and root

absorptive surface area. Also, **Khorshidi *et al.* (2023)** investigated the interactive effects of mineral phosphorus fertilization and plant growth-promoting rhizobacteria (PGPR) on safflower (*Carthamus tinctorius* L.) growth performance under field conditions. Their findings demonstrated that PGPR co-inoculation enhanced the physiological utilization efficiency of applied mineral phosphorus, significantly increasing root length, stem biomass, and achene yield, confirming that integrated nutrient management optimizes nutrient sink-source partitioning in Asteraceae oilseed/medicinal crops.

## 5. Conclusion

Based on the results, it is recommended to integrate mineral phosphorus fertilization at a moderate rate of 31.00 kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup> with dual bio-fertilizer inoculation (nitrobein + phosphorein), as this combination achieved the highest significant shoot dry biomass (149.70 g plant<sup>-1</sup>). This integrated regime provides an optimal physiological balance between shoot and root growth while mitigating the over-reliance on chemical inputs through microbial enhanced phosphorus solubilization and biological nitrogen fixation, thereby advancing sustainable agricultural practices and maximizing safflower performance.

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