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# Inoculation with *Piriformospora indica* Enhances Yield and Oil Quality in Sunflower: A Genotype-Dependent Response

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**ABSTRACT**

Sunflower (*Helianthus annuus* L.) is a globally significant oilseed crop, yet its actual yield in Iran remains substantially below its potential due to environmental and nutritional constraints. This study evaluated the application of the growth-promoting fungus *Piriformospora indica* as a biological strategy to enhance the yield and quality of three sunflower hybrids (Barzegar, Farrokh, and Ghasem). A factorial experiment was conducted based on a randomized complete block design with four replications during the 2022-2023 growing season at the research farm of Agricultural Sciences and Natural Resources University of Khuzestan, located in Mollasani. Treatments included two inoculation levels (non-inoculated control and inoculated with *P. indica*) and three hybrids. Results demonstrated that fungal inoculation significantly influenced all measured morphological and phenological traits except leaf area and leaf weight. Inoculation significantly increased plant height, head diameter, number of seeds per head, 1000-seed weight, seed yield, oil percentage, oil yield, and the number of filled seeds. Furthermore, inoculated plants exhibited a reduced vegetative period and fewer days to maturity. The interaction between hybrid and inoculation was significant for most traits, indicating a genotype-specific response. Correlation analysis revealed strong positive relationships between seed yield and both the number of seeds per head (r = 0.858) and oil yield (r = 0.884). Among hybrids, Barzegar proved superior for most traits and showed the most pronounced response to fungal inoculation, achieving a 42% increase in seed yield and a 37% increase in oil yield compared to its control. In conclusion, inoculation with *P. indica* represents an effective biological approach for enhancing sunflower seed yield and quality through improved growth and physiological performance, contributing to sustainable agriculture by reducing the reliance on chemical inputs.

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## 1. Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops globally, ranking fourth in total vegetable oil production after palm, soybean, and canola (FAO, 2024). According to FAO statistics, the global sunflower cultivation area is about 28 million hectares, with a seed production of nearly 57 million tons (FAO, 2024). In Iran, with a cultivation area of approximately 50 to 60 thousand hectares, this crop produces about 120 thousand tons of seeds annually, with its average yield being lower than the global average and the genetic potential of improved cultivars (Soleymani, 2023). It is valued for its high-quality oil, rich in polyunsaturated fatty acids, and its adaptability to diverse agro-ecological conditions. Despite its economic significance, the average yield of sunflower in Iran remains considerably lower than both the global average and the genetic potential of improved cultivars (Soleymani, 2023). This yield gap is primarily attributed to abiotic stress factors such as drought, salinity, high temperatures, and low soil fertility, which are prevalent in many of Iran's agricultural regions, including Khuzestan (Ghorbani *et al.*, 2018). Conventional agriculture has historically relied on high

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inputs of chemical fertilizers and water to maximize yields. However, this approach has led to environmental degradation, soil salinization, and groundwater pollution, undermining the sustainability of agricultural systems (Alom *et al*., 2025; Li *et al*., 2023). In response, there is a growing interest in harnessing beneficial soil microorganisms as a biological and sustainable strategy to enhance plant growth, nutrient uptake, and stress tolerance.

Among these microorganisms, the root-colonizing endophytic fungus *Piriformospora indica* (now also classified under the genus *Serendipita*) has garnered significant attention since its discovery in the Indian Thar Desert (Varma *et al*., 1999). *P. indica* exhibits a remarkable ability to form symbiotic relationships with a wide range of host plants, including important crops like wheat, barley, maize, and sunflower. A key agronomic advantage of *P. indica* over arbuscular mycorrhizal fungi is its ability to be cultured axenically (without a host plant) on synthetic media, facilitating its mass production and practical application (Varma *et al*., 2001). Recent studies on woody plant cuttings have further elucidated that *P. indica* inoculation can significantly enhance root system architecture by upregulating the expression of key root development genes, such as members of the WOX transcription factor family (e.g., WOX1, WOX4, WOX13), leading to increased root length, biomass, and activity (Wang *et al*., 2025).

The symbiotic relationship between *P. indica* and its host plant leads to a multitude of benefits. The fungus extensively colonizes the root cortex, forming pear-shaped chlamydospores, effectively extending the root system's absorptive surface area. This enhances the plant's uptake of water and essential nutrients, particularly phosphorus, zinc, and other less-mobile soil elements (Johnson *et al*., 2023). Beyond improved nutrient acquisition, *P. indica* is known to produce or modulate plant growth regulators, including auxins and cytokinins, which directly stimulate root and shoot development (Varma *et al*., 2001).

Perhaps one of its most valuable attributes is its role as a bioprotectant against abiotic stresses. Inoculation with *P. indica* has been shown to enhance plant tolerance to drought, salinity, and heavy metal toxicity. The underlying mechanisms include the upregulation of antioxidant defense systems (e.g., increased activity of catalase, peroxidase, and superoxide dismutase), maintenance of leaf relative water content, improved photosynthetic efficiency, and stabilization of cell membranes under stress conditions (Xu *et al*., 2017; Boorboori and Zhang, 2022). Specifically, under drought and heat stress, *P. indica* colonization has been demonstrated to reduce oxidative damage by lowering malondialdehyde (MDA) content and enhancing the activities of superoxide dismutase (SOD) and peroxidase (POD), while also protecting photosynthetic apparatus by maintaining higher chlorophyll fluorescence (Fv/Fm) values (Ji *et al*., 2024). For instance, under salt stress, the fungus aids in maintaining ionic homeostasis by sequestering sodium ions in the roots and limiting their translocation to the shoots, thereby protecting photosynthetic tissues (Ghorbani *et al*., 2018).

While Khuzestan province offers great potential for sunflower cultivation, environmental stresses often hinder production. We launched this study to find sustainable ways to boost both yield and oil quality. Although the growth-promoting benefits of *P. indica* are well-known in many plants, we still don't fully understand how it interacts with specific commercial Iranian hybrids. Our goal was to see how *P. indica* affects the growth and yield of three popular hybrids and to identify which one benefits most from this symbiotic partnership.

## 2. Materials and methods

### *2.1. Site description and experimental period*

This research was conducted at the research farm of the Agricultural Sciences and Natural Resources University of Khuzestan, located in Mollasani. The experimental site is located at 31°34' N, 48°52' E, with an altitude of approximately 20 meters above sea level. According to data from the regional meteorological station, the mean annual rainfall is 269 mm, the mean annual temperature is 25.8°C, with absolute mean maximum and mean minimum temperatures of 34°C and 17.5°C, respectively. Based on the Köppen-Geiger climatic classification system, the region falls within an arid to semi-arid climate category. The total rainfall during the growing season was 145 mm, and the average temperature was 27.2°C.

### *2.2. Soil physicochemical properties*

Prior to establishing the experiment, soil samples were collected from depths of 0-30 cm and 30-60 cm using a randomized sampling method across the experimental field. Various physicochemical properties were analyzed in the university's soil laboratory. The results of the soil analysis are presented in Table 1. As shown, the soil texture at both depths is sandy loam. The soil pH ranges from neutral to slightly alkaline (7.3-7.5), and its electrical conductivity (EC) ranges from 7.50 to 8.80 dS $m^{-1}$. According to soil salinity classification criteria (e.g., McGeorge, 1954), these values indicate moderate to relatively high salinity in this field, which can pose nutritional constraints for sunflower cultivation. The organic carbon content was low (0.45%), and available phosphorus and potassium were 12.5 and 240 mg $kg^{-1}$, respectively.

**Table 1. Physicochemical properties of the soil at the experimental site**

| Depth (cm) | Soil Texture | pH (1:2.5) | EC (dS $m^{-1}$) | Organic carbon (%) | Available P (mg $kg^{-1}$) | Available K |
|---|---|---|---|---|---|---|
| 0-30 | Sandy loam | 7.5 | 7.50 | 0.45 | 12.5 | 240 |
| 30-60 | Sandy loam | 7.3 | 8.80 | 0.38 | 9.8 | 215 |

*2.3. Land preparation and experimental design*

The field had previously been cultivated with corn and chamomile. Land preparation included initial plowing with a moldboard plow to a depth of 25-30 cm, followed by disc harrowing for clod breaking and leveling. The field was then marked into furrows and ridges with a distance of 70 cm between the ridges. The experimental design was a factorial arrangement based on a randomized complete block design (RCBD) with four replications. The first factor consisted of three sunflower hybrids (Barzegar, Farrokh, and Ghasem), and the second factor comprised two inoculation levels (non-inoculated control and inoculated with *P. indica*). Each experimental plot consisted of four 5-meter-long rows, with a planting spacing of 25 cm between plants within rows. The two central rows were used for data collection to avoid border effects.

*2.4. Characteristics of the studied hybrids*

The three sunflower hybrids selected for this study-Barzegar, Farrokh, and Ghasem- are all medium-maturing, high-yielding cultivars developed by the Karaj Seed and Plant Improvement Research Center. Each hybrid possesses distinct agronomic strengths: Barzegar offers high yield potential coupled with a desirable oil content of 45–48%, relatively early maturity, and good resistance to downy mildew. Farrokh is characterized by its early maturity, resistance to downy mildew, and documented tolerance to water-deficit stress. Ghasem, another early-maturing hybrid, provides satisfactory yield performance and is particularly suited for both first and second cropping seasons in the target region of Khuzestan.

*2.5. Fungal inoculum preparation and inoculation method*

The pure culture of *P. indica* (strain DSM 11827) was obtained from the Fungal Biotechnology Collection at the University of Tehran. The fungus was initially propagated on solid Complete Medium (CM) and subsequently in liquid Modified Murashige and Skoog (MS) medium for large-scale biomass production. The cultures were incubated at 28 ± 2°C in the dark for 21 days. To prepare the soil inoculum, sterilized barley grains (*Hordeum vulgare*) were inoculated with the fungal culture and incubated for four weeks until fully colonized. For seed treatment, sunflower seeds were surface-sterilized with 2% sodium hypochlorite for 3 minutes and rinsed thoroughly with sterile distilled water. The seeds were then divided into two groups: (1) Control seeds: soaked in sterile distilled water for 72 hours. (2) Treated seeds: soaked in a fungal spore suspension (containing $10^4$ spores per milliliter, as determined by a hemocytometer) for 72 hours.

*2.6. Cultivation and crop management*

Two planting methods were employed simultaneously: (a) For the control plots, 3-4 pre-soaked (water) seeds were sown per hill at a depth of 7 cm. (b) For the inoculated plots, one pre-soaked (spore suspension) seed was sown per hill alongside 10 *P. indica*-colonized barley grains (Fig. 1). Thinning was performed at the 4-5 leaf stage to maintain one uniform plant per hill. Irrigation was applied uniformly to all plots using a furrow irrigation system. Weeds were controlled manually. Nitrogen (150 kg N $ha^{-1}$ as urea 46%), phosphorus (100 kg $P_2O_5$ $ha^{-1}$ as superphosphate), and potassium (100 kg $K_2O$ $ha^{-1}$ as potassium sulfate) were applied based on soil test recommendations, with 1/3 of N and all P and K applied at planting, and the remaining N applied at the 8-leaf stage. Successful root colonization by the fungus was confirmed prior to flowering (50 days after sowing) through microscopic examination of washed and cleared roots, which revealed the presence of intracellular hyphae and characteristic pear-shaped chlamydospores (Fig. 2).

a

b

**Figure 1. Sunflower seed planting method: (a) sowing normal seeds, (b) sowing fungus-treated seeds with inoculated barley.**

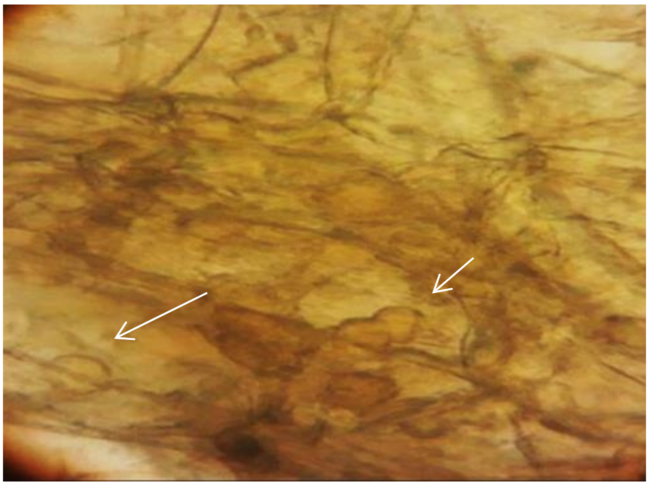

**Figure 2. Microscopic view (x40 magnification) of a sunflower root colonized by *Piriformospora indica*, showing intracellular hyphae and chlamydospores.**

## *2.7. Measured traits*

Phenological traits were recorded based on standardized growth stages. The duration of the vegetative period was measured as the number of days from planting until the complete flowering stage (R5). Days to flowering was defined as the time from planting until 50% of plants in a plot had open flowers (R5.1). Days to maturity was recorded as the number of days from planting until 50% of the heads reached physiological maturity, indicated by a yellowing of the back of the head (R9). Morphological and yield traits were measured at physiological maturity. Five representative plants from each plot were randomly selected and harvested for this purpose. The assessed traits included: plant height (cm), measured from the soil surface to the base of the head; head diameter (cm) at its widest point; and the area ($cm^2$) of the largest fully expanded leaf, measured with a portable leaf area meter (LI-3000, LI-COR, USA). We also recorded the fresh leaf weight (g) per plant.

Yield components were evaluated by analyzing the harvested heads. The total number of seeds per head was counted. Seeds were then separated by flotation in water; sunken seeds were classified as filled, while floating seeds were considered empty. The weight of 1,000 randomly selected filled seeds was determined by counting and weighing five 200-seed samples of filled seeds from each plot and calculating their average. After threshing, we recorded the head weight without seeds (receptacle weight) and the total weight of filled seeds per plant as the seed yield (g $plant^{-1}$). To determine oil percentage (%), a Soxhlet extraction method was employed using n-hexane as a solvent on a dry-weight basis, following the standard AOCS procedure (6-hour extraction on samples ground to pass a 1-mm sieve). Oil yield per plant (g $plant^{-1}$) was subsequently calculated from the seed yield and oil percentage. To minimize pre-harvest seed loss, all heads were covered with paper bags at the R7 stage (Fig. 3) as a protective measure against birds.

**Figure 3. Covering sunflower heads with paper bags at the R7 stage to prevent bird damage before harvest.**

### *2.8. Statistical analysis*

All data were tested for assumptions of ANOVA. After confirming normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test), a two-way analysis of variance (ANOVA) was performed using the GLM procedure in SAS software version 9.4 (SAS Institute, Cary, NC). Treatment means were separated using Duncan's multiple range test at α = 0.05. Additionally, Bayesian correlation analysis was conducted using the bayes.cor function in the psych package in R software (version 4.3.0, R Foundation for Statistical Computing, Vienna, Austria) to explore relationships between all measured traits, with results visualized as a correlation heatmap."

## 3. Results and discussion

### *3.1. Analysis of variance (ANOVA)*

The analysis of variance for phenological and morphological traits is presented in Table 2. Hybrid (H) had a highly significant effect (P≤0.001) on vegetative period, days to flowering, days to maturity, plant height, and head diameter, but not on leaf weight. Fungal inoculation (F) with *P. indica* also exhibited highly significant effects on all these traits except leaf weight. The hybrid × fungus (H × F) interaction was significant for vegetative period (P≤0.01), plant height (P≤0.05), head diameter (P≤0.01), and leaf weight (P≤0.01), indicating genotype-dependent responses to inoculation. The ANOVA for yield, yield components, and quality traits (Table 3) showed that hybrid significantly affected all traits except oil content. *P. indica* inoculation had highly significant positive effects on seeds head$^{-1}$, 1000-seed weight, seed yield, oil yield, filled seeds, empty head weight, and oil content, but did not affect empty seed number. The H × F interaction was significant for every trait in Table 3, confirming strong genotypic variation in responsiveness to the fungus.

**Table 2. Analysis of variance (mean squares) for phenological and morphological traits**

| Source | df | Vegetative period | Days to flowering | Days to maturity | Plant height | Head diameter | Leaf weight |
|---|---|---|---|---|---|---|---|
| Replication | 3 | 2.1$^{ns}$ | 0.4$^{ns}$ | 10.9$^{ns}$ | 28.6$^{*}$ | 1.1$^{ns}$ | 309.0$^{ns}$ |
| Hybrid (H) | 2 | 19.5$^{**}$ | 285.8$^{**}$ | 246.2$^{***}$ | 1283.8$^{**}$ | 83.6$^{**}$ | 27022$^{ns}$ |
| Fungus (F) | 1 | 13.5$^{**}$ | 315.4$^{**}$ | 384.0$^{***}$ | 1434.1$^{**}$ | 173.8$^{**}$ | 5408$^{ns}$ |
| H × F | 2 | 8.0$^{**}$ | 7.6$^{ns}$ | 10.5$^{ns}$ | 28.5$^{*}$ | 14.0$^{**}$ | 56221$^{**}$ |
| Error | 15 | 0.7 | 4.3 | 10.2 | 7.7 | 1.4 | 8493 |
| CV (%) | | 3.0 | 4.3 | 3.4 | 4.6 | 8.1 | 15.5 |

ns, *, **, ***: non-significant or significant at P≤0.05, 0.01, and 0.001, respectively.

**Table 3. Analysis of variance (mean squares) for yield, yield components and quality traits**

| Source | df | Seeds per head | 1000-seed weight | Seed yield | Oil yield | Empty seeds | Filled seeds | Empty head weight | Oil content |
|---|---|---|---|---|---|---|---|---|---|
| Replication | 3 | 992.1$^{ns}$ | 30.0$^{ns}$ | 170.8$^{*}$ | 18.3$^{ns}$ | 6177.5$^{ns}$ | 28318.8$^{ns}$ | 136.6$^{ns}$ | 59.3$^{ns}$ |
| Hybrid (H) | 2 | 17808.7$^{*}$ | 178.6$^{**}$ | 333.1$^{**}$ | 80.1$^{*}$ | 79068.9$^{**}$ | 140652.4$^{**}$ | 17419.4$^{***}$ | 161.8$^{**}$ |
| Fungus (F) | 1 | 1019209.9$^{***}$ | 228.2$^{**}$ | 4866.9$^{***}$ | 1959.1$^{***}$ | 24832.7$^{ns}$ | 976470.0$^{***}$ | 9600.8$^{***}$ | 693.8$^{***}$ |
| H × F | 2 | 67523.1$^{***}$ | 396.4$^{**}$ | 212.3$^{*}$ | 90.0$^{**}$ | 52276.3$^{**}$ | 251693.8$^{***}$ | 425.8$^{*}$ | 86.5$^{*}$ |
| Error | 15 | 3841.3 | 23.6 | 39.0 | 13.5 | 6150.2 | 15613.6 | 87.5 | 19.5 |
| CV (%) | | 15.3 | 10.2 | 19.3 | 31.9 | 29.5 | 25.0 | 8.1 | 15.5 |

ns, *, **, **: non-significant or significant at P≤0.05, 0.01, 0.001 respectively.

### *3.2. Mean comparison of traits (Mean performance of hybrids and inoculation treatments)*

Inoculation with *P. indica* accelerated phenological development and improved morphological traits (Table 4). Across hybrids, inoculation shortened days to flowering by 6–9 days and days to maturity by 5–10 days. The tallest plants (183.5 cm) and largest head diameter (35.8 cm) were recorded in inoculated Barzegar, significantly superior to all other treatments. The mean comparison for yield and yield components is presented in Table 5. The results clearly indicated the positive impact of *P. indica* inoculation on traits. The number of seeds per head was highest in inoculated Barzegar (1364.6), which was 62% higher than its non-inoculated control.

Similarly, 1000-seed weight, a critical determinant of yield, was significantly greater in inoculated plants of Barzegar and Farrokh hybrids. Consequently, seed yield was dramatically increased by inoculation. The inoculated Barzegar hybrid achieved the highest seed yield of 80.6 g plant$^{-1}$, a 42% increase over its control. This trend was mirrored in oil yield, where inoculated

Barzegar produced 35.9 g plant$^{-1}$, a 37% increase. Furthermore, inoculation significantly improved seed quality by increasing the number of filled seeds and reducing the number of empty seeds across all hybrids, with the most notable improvement observed in Barzegar.

**Table 4. Mean comparison of phenological and morphological traits**

| Hybrid | Fungus | Vegetative period (days) | Days to flowering (days) | Days to maturity (days) | Plant height (cm) | Head diameter (cm) | Leaf weight (g) |
|---|---|---|---|---|---|---|---|
| Barzegar | With | $56.5^{a}$ | $66.8^{bc}$ | $92.8^{b}$ | $183.5^{a}$ | $35.8^{a}$ | $827.3^{ab}$ |
| | Without | $56.0^{ab}$ | $73.0^{cd}$ | $102.8^{cde}$ | $165.0^{b}$ | $27.4^{b}$ | $668.2^{c}$ |
| Farrokh | With | $53.3^{b}$ | $57.8^{a}$ | $85.8^{a}$ | $165.3^{b}$ | $26.9^{b}$ | $785.3^{bc}$ |
| | Without | $54.8^{ab}$ | $63.8^{b}$ | $94.3^{bc}$ | $154.0^{c}$ | $23.7^{c}$ | $873.7^{ab}$ |
| Ghasem | With | $51.5^{c}$ | $67.3^{c}$ | $98.0^{cd}$ | $157.3^{bc}$ | $31.9^{a}$ | $779.9^{bc}$ |
| | Without | $55.0^{ab}$ | $76.8^{d}$ | $103.5^{e}$ | $140.7^{d}$ | $27.4^{b}$ | $940.6^{a}$ |

Means in each column followed by the same letter are not significantly different (Duncan's Multiple Range Test, P≤0.05).

**Table 5. Mean comparison of yield, yield components and other traits**

| Hybrid | Fungus | Seeds head$^{-1}$ | 1000-seed weight (g) | Seed yield (g plant$^{-1}$) | Oil yield (g plant$^{-1}$) | Empty seeds (count) | Filled seeds (count) | Empty head weight (g) | Oil content (%) |
|---|---|---|---|---|---|---|---|---|---|
| Barzegar | With | $1364.6^{a}$ | $85.0^{a}$ | $80.6^{a}$ | $35.9^{a}$ | $90.3^{a}$ | $1296.8^{a}$ | $183.5^{a}$ | $47.4^{a}$ |
| | Without | $844.8^{b}$ | $68.2^{b}$ | $46.6^{b}$ | $10.3^{b}$ | $211.0^{b}$ | $573.3^{b}$ | $127.1^{b}$ | $29.5^{b}$ |
| Farrokh | With | $1161.0^{c}$ | $65.6^{b}$ | $59.0^{b}$ | $24.9^{b}$ | $437.0^{c}$ | $724.0^{b}$ | $77.5^{c}$ | $35.5^{b}$ |
| | Without | $961.0^{d}$ | $75.4^{a}$ | $42.4^{b}$ | $12.1^{b}$ | $259.0^{d}$ | $702.0^{b}$ | $48.9^{d}$ | $30.5^{b}$ |
| Ghasem | With | $1268.8^{e}$ | $73.0^{ab}$ | $74.4^{a}$ | $25.0^{b}$ | $338.0^{e}$ | $930.8^{c}$ | $113.9^{b}$ | $34.2^{b}$ |
| | Without | $752.1^{b}$ | $61.5^{b}$ | $39.6^{b}$ | $9.1^{b}$ | $202.3^{b}$ | $466.0^{d}$ | $78.9^{c}$ | $24.9^{c}$ |

Means in each column followed by the same letter are not significantly different (Duncan's Multiple Range Test, P≤0.05).

### *3.3. Correlation analysis*

The Bayesian correlation analysis revealed a network of significant relationships among the 15 measured traits, visualized in the correlation matrix heatmap (Fig. 4). The intensity and hue (red for positive, blue for negative) within the heatmap correspond to the strength and direction of each pairwise correlation. Several strong, biologically meaningful positive correlations (0.7 ≤ |r|) were identified. Not surprisingly, seed yield and oil yield were tightly linked (r = 0.884). Yield components also showed strong interconnections: the number of seeds per head was highly correlated with both the number of filled seeds (r = 0.873) and the final seed yield (r = 0.858). As expected, the phenological traits days to flowering and days to maturity were closely associated (r = 0.860). Furthermore, plant height was positively correlated with head diameter (r = 0.577), seed yield (r = 0.631), and oil percentage (r = 0.747).

Key negative correlations (|r|≤-0.5) were also observed. Notably, the duration of the vegetative period showed a moderate negative relationship with the number of empty seeds (r = -0.602). More importantly, days to flowering was negatively correlated with the number of seeds per head (r = -0.548), suggesting that under the conditions of this experiment, earlier flowering was associated with a greater seed set.

The results of this study unequivocally demonstrated that seed inoculation with the endophytic fungus *P. indica* can serve as a powerful bio-effector to enhance growth, accelerate development, and significantly boost the yield and quality of sunflower hybrids. The significant increases in plant height, head diameter, 1000-seed weight, and the number of filled seeds in inoculated plants align with the well-documented plant growth-promoting functions of *P. indica* (Varma *et al*., 1999; Johnson *et al*., 2023). The primary mechanism is likely the fungus's ability to develop an extensive mycelial network within the root cortex, effectively acting as an extension of the root system (Johnson *et al*., 2023). This significantly increases the surface area for the absorption of water and essential nutrients, particularly phosphorus, which is often a limiting factor in alkaline and calcareous soils (Pang *et al*., 2024). Additionally, by enhancing root activity and potentially upregulating genes involved in root development, *P. indica* fosters a more vigorous and efficient root system (Wang *et al*., 2025). The improved phosphorus nutrition, confirmed in other studies (Li *et al*., 2023), directly supports energy metabolism, cell division, and seed filling processes, leading to heavier seeds and a greater proportion of filled seeds, as observed in our study. Beyond nutrient uptake, the fungus also fortifies the plant's antioxidant defense system. The observed increase in yield components and seed filling could be partly attributed to reduced oxidative stress, as *P. indica* is known to elevate the activity of antioxidant enzymes like SOD and POD, thereby minimizing cellular damage under suboptimal soil conditions (Ji *et al.*, 2024).

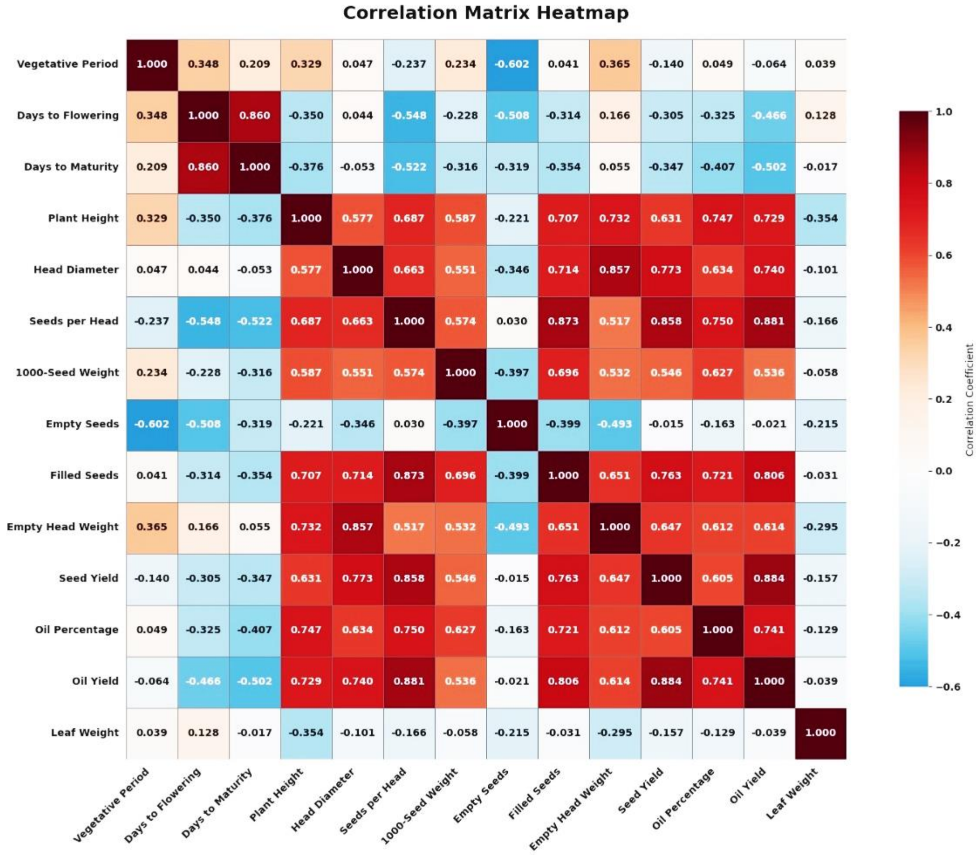


**Figure 4. Heat map of Bayesian correlation coefficients between different morphological, phenological, and yield-related traits in sunflower.**

The correlation analysis provides robust statistical support for the biological interpretations of our ANOVA and mean comparison results. The strong positive correlation between seed yield and the number of seeds per head (r = 0.858) is a classic relationship in sunflower and other grain crops (Soleymani, 2023). This explains why the Barzegar hybrid, which exhibited the highest number of seeds per head under inoculation, also achieved the highest seed yield. This finding underscores the critical importance of this yield component and suggests that *P. indica* positively influences the processes of floret initiation, fertilization, and/or seed set. The strong negative correlation between days to flowering and seeds per head (r = -0.548) suggests that under the conditions of this experiment, a slightly shorter vegetative period was advantageous for seed set, possibly by aligning the critical seed-filling period with more favorable environmental conditions, thus avoiding terminal heat or drought stress. The significant reduction in the vegetative period and days to maturity in inoculated plants, particularly in the Farrokh and Ghasem hybrids, represents a notable agronomic advantage. This phenomenon, often referred to as "physiological earliness," can be a crucial escape mechanism from late-season abiotic stresses such as terminal heat and drought, which are common in Khuzestan (Boorboori and Zhang, 2022). It appears that by providing resources more efficiently during the early growth stages, the fungus promotes a more vigorous and rapid development, allowing the plant to transition earlier to the reproductive phase and complete seed filling before the onset of stressful conditions. This accelerated development might also be linked to improved physiological performance under stress. Studies have shown that *P. indica* helps maintain photosynthetic efficiency under high temperatures by protecting photosystem II, as indicated by stable chlorophyll fluorescence (Fv/Fm) parameters (Ji *et al*., 2024), allowing inoculated plants to sustain assimilate production during the critical grain-filling stage even under mild thermal stress.

The dramatic improvement in oil percentage and oil yield in inoculated plants, especially in the Barzegar hybrid, is of paramount economic importance. The strong correlation between oil yield and both seed yield (r = 0.884) and oil percentage (r = 0.741) indicates that improvements in oil yield can be achieved through both increased biomass production and enhanced oil concentration. The superior performance of Barzegar in both aspects under fungal inoculation highlights the potential of this symbiotic relationship for simultaneous quantitative and qualitative improvement. The increase in oil percentage can be attributed to the fungus's role in enhancing photosynthetic efficiency and the translocation of photoassimilates to the developing seeds (Johnson *et al*., 2023). Furthermore, improved nutrient uptake, particularly of elements like sulfur and magnesium which are involved in oil biosynthesis, may have contributed to this effect (Rahimi Tanha *et al*., 2014). Moreover, *P. indica* can modulate plant hormone homeostasis, including increasing auxin (IAA) levels (Kundu and Vadassery, 2022), which may influence source-sink relationships and promote the partitioning of assimilates towards oil synthesis in the seeds.

The significant genotype × fungus interaction for most traits underscores a critical point: the success of microbial inoculation is not universal but is dependent on the genetic background of the host plant. The superior performance and responsiveness of the Barzegar hybrid suggest it possesses genetic traits such as a more favorable root architecture, specific signaling pathways, or hormonal cross-talk that make it more amenable to a beneficial symbiosis with *P. indica*. This aligns with recent studies emphasizing the importance of host genotype in determining the outcome of plant-microbe interactions (Li *et al*., 2023). The differential response could stem from variations in the efficiency of root colonization, the activation of specific molecular pathways (such as the OXI1 kinase pathway or putrescine-mediated hormone elevation), or the degree to which the fungus upregulates host genes involved in growth and stress adaptation, like the WOX genes crucial for root development (Kundu and Vadassery, 2022; Wang *et al*., 2025). This significant interaction highlights the importance of host genotype-microbe compatibility for achieving maximum symbiotic benefits. Differences in hybrid responses could be due to factors such as root colonization efficiency, differences in initial signaling, or differences in hormonal and metabolic transduction pathways modulated by the fungus (Li *et al*., 2023). Therefore, future breeding programs could aim to select for "microbe-responsive" genotypes to maximize the benefits of bio-inoculants.

In conclusion, this study provides compelling evidence that *P. indica* inoculation is a highly effective biological strategy for improving sunflower productivity in sub-optimal soil conditions. By enhancing nutrient uptake, modulating phenology, and improving yield components and oil quality, this fungus can help bridge the yield gap in a sustainable manner. The application of *P. indica* aligns perfectly with the principles of sustainable agriculture by reducing dependence on chemical fertilizers and enhancing crop resilience.

## 4. Conclusion

This study demonstrates that applying *P. indica* as a biological stimulant offers an effective and sustainable strategy to enhance both the quantitative and qualitative yield of sunflower. The fungus acts through multiple, concurrent mechanisms: it promotes key morphological traits such as plant height, head diameter, 1000-seed weight, and the number of filled seeds; improves final yield and quality by significantly increasing seed yield, oil percentage, and consequently oil yield; and modulates crop phenology by inducing physiological earliness, which shortens the growth cycle and may help the plant escape terminal stresses.

These combined effects can translate into direct economic benefits for farmers through higher output and reduced reliance on chemical inputs. Among the tested hybrids, Barzegar exhibited the most pronounced and comprehensive positive response to inoculation. Therefore, integrating *P. indica* with high-yielding, responsive cultivars like Barzegar is strongly recommended for sustainable sunflower production, particularly in regions challenged by soil salinity and low fertility. To further optimize this promising symbiosis, future research should focus on elucidating the molecular dialogue between the fungus and different sunflower genotypes.

## Conflict of interests

All authors declare no conflict of interest.

## Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

## Consent for publications

All authors read and approved the final manuscript for publication.

## Availability of data and material

All the data are embedded in the manuscript.

## Authors' contributions

Conceptualization, P.P.M. and M.G.; methodology, A.A. and P.P.M.; formal analysis, A.A. and M.F.; investigation, A.A.; resources, P.P.M. and M.G.; data curation, A.A.; writing original draft preparation, A.A.; writing review and editing, P.P.M., M.F., and M.G.; supervision, P.P.M. and M.F.; project administration, P.P.M.; funding acquisition, P.P.M.

## Informed consent

The authors declare not to use any patients in this research.

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