Effect of mycorrhizal inoculation on plant growth and medicinal properties of fruits in *Opuntia ficus-indica*

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ABSTRACT

Opuntia ficus-indica (prickly pear) is a drought-tolerant cactus species valued for its nutritional fruits rich in antioxidants and medicinal compounds. This study explores how arbuscular mycorrhizal fungi (AMF) influence the growth and phytochemical characteristics of Opuntia ficus-indica. A greenhouse experiment was conducted using two treatments: non-inoculated controls and plants inoculated with a consortium of Glomus intraradices and Rhizophagus irregularis. Key growth indicators, including plant height, number of cladodes, and biomass, were assessed, along with evaluations of fruit yield and quality. Phytochemical assays were performed to quantify total phenolics, flavonoids, betalains, and antioxidant activity using standardized colorimetric methods. Results indicated that AMF-treated plants showed significant improvements in growth metrics, fruit yield, and bioactive compound concentrations compared to controls. Specifically, inoculated plants exhibited up to 60% higher flavonoid levels and 40% greater antioxidant activity. These findings suggest that AMF symbiosis not only enhances nutrient uptake and plant development but also stimulates the biosynthesis of health-promoting metabolites in prickly pear fruits. This research highlights the potential of mycorrhizal biotechnology as a sustainable practice to boost both the agronomic and medicinal value of cactus crops, particularly under conditions of environmental stress.

Keywords: Antioxidants, biofertilizer, phytochemicals, Sustainable agriculture,Symbiosis.

INTRODUCTION

Opuntia ficus-indica (L.) Mill., commonly known as prickly pear cactus, is a member of the Cactaceae family and is widely cultivated in arid and semi-arid regions due to its exceptional drought resistance and minimal agronomic requirements (Jorge *et al*., 2023). Beyond its ecological adaptability, the plant is valued for its fruits and cladodes, which are rich in bioactive compounds including phenolics, flavonoids, betalains, and ascorbic acid, all of which contribute to its antioxidant, anti-inflammatory, and metabolic health benefits (Stintzing and Carle, 2005). Increasing consumer demand for nutritionally enhanced and medicinally valuable crops has led researchers to explore ecologically sustainable cultivation methods that enhance both productivity and phytochemical composition. Among these methods, the use of arbuscular mycorrhizal fungi (AMF) has received considerable attention. Arbuscular mycorrhizal fungi (AMF) associate with the roots of most terrestrial plants, aiding in the absorption of key nutrients like phosphorus and micronutrients, while also improving plant tolerance to environmental stresses such as salinity and drought (Smith and

Read, 2008). AMF are also known to influence root architecture and soil structure, further contributing to plant resilience and growth efficiency (Miransari, 2010). The positive effects of AMF on medicinal and aromatic plants have been well documented, with multiple studies showing increased production of secondary metabolites following inoculation (Kapoor *et al*., 2004; Selvakumar *et al.*, 2020). Despite extensive work on other horticultural crops, limited data exist regarding the mycorrhizal responsiveness of *O. ficus-indica*, particularly in relation to its medicinal fruit properties. Given that this cactus is often grown in nutrient-poor soils, the introduction of AMF could play a crucial role in improving its agronomic performance and functional compound synthesis. Inoculation with species such as *Rhizophagus irregularis* and *Glomus intraradices* has been shown to improve fruit yield and quality in related fruit-bearing species (Berta *et al*., 2005), suggesting potential benefits for prickly pear as well. Emerging evidence suggests that mycorrhizal colonization not only affects mineral nutrition but also modulates the metabolic pathways responsible for the biosynthesis of flavonoids, betalains, and phenolic acids (Schweiger and Müller, 2015). These changes can enhance the nutritional and pharmacological properties of fruits, making AMF a valuable biofertilizer for functional food crops. Additionally, the ecological advantages of mycorrhizal symbiosis—including reduced fertilizer dependency and improved soil health align with the principles of sustainable agriculture and agroecology (Jeffries *et al*., 2003). This study evaluates how AMF inoculation influences the growth traits, fruit production, and phytochemical composition of *Opuntia ficus-indica*. By evaluating both agronomic and biochemical outcomes, this research seeks to contribute to the development of environmentally friendly strategies for improving the nutritional and medicinal quality of cactus fruits under low-input conditions.

MATERIALS AND METHODS

The greenhouse experiment was conducted over a 24-week period, from 15 March 2025 to 29 August 2025, encompassing both vegetative and reproductive phases of *Opuntia ficus-indica* under controlled conditions at the Landscaping Plants and Nursery Research Unit of the Italian Council for Agricultural Research and Economics (CREA) in Pescia (PT), Italy. The greenhouse conditions were regulated with daytime temperatures of $28 \pm 2°C$ and nighttime temperatures of $20 \pm 2°C$, while relative humidity ranged from 60% to 70%.

Healthy, uniform cladodes of *Opuntia ficus-indica* were obtained from a certified nursery. Each cladode was 25–30 cm long and 2–3 cm thick. After harvesting, cladodes were air-dried in shade for 5 days to induce suberization and prevent rotting. Cladodes were then transplanted into 10-liter size polyethylene pots filled with sterilized growing medium. At the time of planting, the cladodes were considered 0 days old for tracking developmental milestones.

A soil mixture composed of sandy loam, composted manure, and perlite in a ratio of 2:1:1 (v/v/v) was used. The mixture was autoclaved at 121°C for 1 hour on two consecutive days to eliminate indigenous microorganisms, including native mycorrhizal fungi and soil pathogens. The final soil pH was adjusted to 6.8 ± 0.2 using dolomitic lime prior to planting.

The experiment was structured using a completely randomized design (CRD) consisting of three treatment groups:

- T1 – Absolute Control (No AMF): Plants grown in sterilized substrate without any arbuscular mycorrhizal fungi (AMF) inoculants or commercial biostimulants.
- T2 – Commercial Control: Plants treated with a commercially available organic biofertilizer (non-mycorrhizal), commonly used in local prickly pear cultivation. The biofertilizer included humic acids, seaweed extract, and beneficial rhizobacteria (*Bacillus spp.*, *Azospirillum spp.*).

- T3 – Mycorrhizal Treatment (AMF): A mixture of two arbuscular mycorrhizal fungi species *Rhizophagus irregularis* and *Glomus intraradices* was used to inoculate the plants.

Each treatment was replicated 10 times, resulting in a total of 30 experimental units. All other agronomic practices (soil type, pot size, watering regime, and environment) were kept constant across treatments to isolate treatment effects. All plants were inoculated on 0-day *i.e.*, the same day of planting. The mycorrhizal inoculum was obtained from a certified microbial supplier and contained a viable propagule density of >1000 spores/g. For T3- plants, 10 grams of inoculum were applied directly into the rhizosphere at the time of planting. To ensure minimal microbial contamination, control plants received an equivalent volume of autoclaved inoculum. All pots were irrigated with sterile distilled water to maintain uniform moisture content.

No synthetic phosphorus fertilizers were applied during the experimental period to avoid interference with mycorrhizal colonization. A balanced nutrient solution containing nitrogen, potassium, magnesium, and trace elements (excluding phosphorus) was applied fortnightly. Irrigation was conducted twice weekly using deionized water to maintain consistent soil moisture without waterlogging.

Monthly measurements were taken for plant height, cladode count, and thickness. Plant height was determined using a ruler, measuring from the base to the topmost point. Cladode thickness was determined using a digital Vernier caliper at the midpoint of each new cladode. At harvest, plants were uprooted, washed, and weighed to determine fresh biomass. Samples were oven-dried at 65°C until constant weight to assess dry biomass.

Time to flowering and fruiting was recorded for each plant by tracking the number of days from planting (considered as 0-day) to the appearance of visible flower buds, which was defined as days to first flowering, and continuing until the fruits reached full maturity, characterized by noticeable color change and tissue softening, which was recorded as days to full fruiting from 0-day. These parameters were used to evaluate the influence of treatments on the reproductive timing of *Opuntia ficus-indica* under controlled conditions. This parameter was subjected to ANOVA and Tukey's HSD test, showing significant differences among treatments ($p < 0.05$), with AMF-treated plants fruiting earlier than others.

Fruits were harvested at full maturity (identified by color change and softening). The experiment was terminated 168 days after inoculation, so all plants were 168 days old at harvest, regardless of treatment. Data were collected on fruit count per plant, average fruit weight, and overall yield. Pulp from harvested fruits was homogenized and stored at –20°C prior to biochemical analysis.

Phytochemical and antioxidant analysis of fruit pulp

At harvest, fully mature fruits of *Opuntia ficus-indica* were collected from each treatment and immediately transported to the laboratory. Fruits were washed with distilled water, manually peeled, and the pulp was separated from seeds. The pulp samples were homogenized using a chilled stainless-steel blender to obtain a uniform slurry. For phytochemical extraction, 5 g of fresh fruit pulp were mixed with 25 mL of 80% (v/v) methanol in amber centrifuge tubes. The mixture was vortexed for 2 min and then subjected to extraction under continuous shaking at 150 rpm for 24 h at 4 **°C** in the dark to minimize oxidative degradation of bioactive compounds. Following extraction, samples were centrifuged at 10,000 × g for 15 min at 4 °C**,** and the supernatant was carefully collected. The obtained extracts were filtered through Whatman No. 1 filter paper**,** transferred to airtight amber vials, and stored at −20 °C until analysis. All phytochemical and antioxidant assays were performed within 48 h of extraction to ensure compound stability.

Total phenolics were estimated using the Folin-Ciocalteu assay. Extracts from the fruit pulp were treated with the reagent and sodium carbonate, and absorbance was measured at 765 nm. Values were expressed in mg of gallic acid equivalents per gram of fresh weight.

Flavonoid levels were determined by the aluminum chloride colorimetric assay. Extracts were mixed with aluminum chloride and potassium acetate, and absorbance was measured at 415 nm. Quantification was based on a quercetin standard curve and reported as mg quercetin equivalents (QE)/g FW.

Betacyanin and betaxanthin concentrations were determined spectrophotometrically. Absorbance was measured at 538 nm and 480 nm, respectively, and concentrations were calculated using published molar extinction coefficients. Total betalains were reported in mg/100 g of fruit pulp.

Antioxidant capacity was determined by combining the fruit extract with a DPPH methanolic solution and measuring the absorbance drop at 517 nm after 30 minutes. The outcome was reported as the percentage of radical inhibition relative to the control.

Mycorrhizal colonization assessment

Root colonization was evaluated 60 days after inoculation, corresponding to the early reproductive phase when root–fungus interactions are typically well established. Fine lateral roots were carefully excavated from each treatment group and thoroughly washed to remove soil particles. Root samples were cleared in 10% potassium hydroxide (KOH) at 90 °C for 30 minutes and subsequently stained with 0.05% trypan blue in lactoglycerol. Stained roots were mounted on microscope slides and examined under a compound microscope at 200× magnification. The percentage of mycorrhizal colonization was calculated using the grid-line intersect method, based on the proportion of root segments exhibiting fungal structures (hyphae, vesicles, and arbuscules) (Giovannetti & Mosse, 1980).

Statistical analysis

All data were statistically analyzed using SPSS version 26 or R software. Analysis of variance (ANOVA) was used to evaluate differences among treatment means, with significance set at $p < 0.05$. Tukey's HSD test was conducted for post hoc comparisons.

RESULTS AND DISCUSSION

Growth performance of *Opuntia ficus-indica*

Mycorrhizal fungi application (T3) led to a significant improvement in the vegetative growth of *Opuntia ficus-indica* compared to the untreated control (T1) and the commercial biofertilizer treatment (T2). Mycorrhizal inoculation (T3)led to the highest plant height (56.3 ± 2.4 cm), number of cladodes (5.9 ± 0.5), and biomass accumulation (298.7 ± 17.3 g) (Table 1). These results reflect the role of AMF in improving nutrient uptake—particularly phosphorus and micronutrients—which in turn supports more robust vegetative development (Smith and Read, 2008; Miransari, 2010).

The commercial treatment also improved plant growth metrics compared to the control, though to a lesser extent than T3. These effects can likely be attributed to humic substances and rhizobacteria in the commercial formula, which have been shown to stimulate root development and nutrient solubilization (Vessey, 2003; Selvakumar *et al.*, 2020).

Root colonization and symbiotic efficiency

Root staining and microscopic assessment revealed clear differences in arbuscular mycorrhizal colonization among treatments (Figure 1). Plants inoculated with arbuscular mycorrhizal fungi (T3) exhibited a high level of root colonization (63.4 ± 4.2%), characterized by abundant intraradical hyphae, vesicles, and well-developed arbuscules, indicating an active and functional symbiosis.

In contrast, the absolute control (T1) and the commercial biofertilizer treatment (T2) showed very low colonization levels (6.2 ± 1.1% and 12.7 ± 1.4%, respectively). These values were significantly lower than those observed in T3 ($p < 0.05$) and are considered biologically negligible. The low background

colonization detected in non-inoculated treatments may be attributed to the survival of heat-tolerant fungal propagules during substrate sterilization or minor airborne or handling-related contamination during the experimental period. However, these minimal colonization levels did not translate into measurable improvements in growth or yield parameters.

The substantial colonization observed in T3 strongly correlates with enhanced vegetative growth, earlier reproductive development, and increased fruit yield and phytochemical accumulation. This confirms the high symbiotic efficiency of the applied *Rhizophagus irregularis* and *Glomus intraradices* consortium in *Opuntia ficus-indica*, supporting the role of AMF in improving nutrient uptake, particularly phosphorus, and stimulating physiological and metabolic processes.

Flowering and fruiting timeline

Mycorrhizal inoculation significantly influenced the reproductive phenology of *Opuntia ficus-indica* (Table 2). Plants inoculated with arbuscular mycorrhizal fungi (T3) initiated flowering significantly earlier, with first flower emergence recorded at 92.3 ± 2.4 days after planting, compared to 105.1 ± 2.7 days in the commercial biofertilizer treatment (T2) and 112.4 ± 3.2 days in the absolute control (T1). One-way ANOVA revealed a significant treatment effect on flowering time ($p < 0.05$), and Tukey's HSD test confirmed that all treatments differed significantly from each other.

Similarly, the time required to reach full fruit maturity was significantly reduced by AMF inoculation. Fruits from T3 plants reached physiological maturity at 150.2 ± 2.6 days, whereas T2 and T1 plants required 160.3 ± 3.1 days and 167.1 ± 2.8 days, respectively. Statistical analysis confirmed significant differences among treatments (ANOVA, $p < 0.05$), with Tukey's HSD test grouping T3, T2, and T1 into distinct statistical classes (Table 2).

The earlier onset of flowering and accelerated fruit development observed in AMF-treated plants indicate that mycorrhizal symbiosis not only improves nutrient acquisition but also modulates internal phytohormonal signaling, likely involving auxin and gibberellin pathways, which are known to regulate floral induction and fruit set (Kapoor *et al*., 2004). The reduction in time to reproductive maturity represents a critical agronomic advantage for commercial cactus production, particularly in short growing seasons or stress-prone environments.

Fruit yield and reproductive output

Fruit yield data also reflected significant treatment effects. Plants under mycorrhizal inoculation produced the highest average fruit yield (342.6 ± 18.2 g/plant), surpassing both commercial (276.8 ± 14.7 g) and control (210.3 ± 12.4 g) groups (Figure 2). These results support earlier findings that AMF can enhance reproductive output by optimizing water and nutrient availability during critical developmental stages (Kapoor *et al*., 2004; Giovannetti *et al.*, 2006; Barman *et al.*, 2016; Kumar *et al*., 2023). The improved yield in T3 can be attributed to better nutrient assimilation and water regulation, particularly under high-radiation greenhouse conditions, where AMF symbiosis enhances drought resilience and carbon partitioning toward reproductive structures (Giovannetti *et al.*, 2006).

Phytochemical composition and medicinal quality

Biochemical analysis of the fruit pulp showed that the AMF treatment significantly elevated the concentration of health-related metabolites (Table 3). Total phenolics increased from 1.85 ± 0.09 mg GAE/g FW in the control to 2.69 ± 0.10 mg GAE/g FW in T3. Similarly, flavonoid levels and betalain pigments were highest in the mycorrhizal group, indicating that AMF may stimulate secondary metabolism pathways linked to plant defense and antioxidant function (Schweiger and Müller, 2015; Zhang *et al*., 2013).

Antioxidant activity, measured via DPPH radical scavenging, was also enhanced under T3,

reaching 59.4 ± 2.1%, compared to 52.1 ± 1.8% in the commercial and 41.8 ± 1.5% in the control groups. This reinforces the growing body of evidence suggesting that mycorrhizal symbiosis can influence the biosynthesis of polyphenols and improve the nutraceutical value of fruits (Stintzing and Carle, 2005; Kapoor *et al*., 2004).

CONCLUSION

This study demonstrates that arbuscular mycorrhizal fungi (AMF) significantly enhance both the agronomic performance and phytochemical quality of *Opuntia ficus-indica*. Mycorrhizal inoculation improved plant height, biomass, and root colonization while also boosting fruit yield and the accumulation of health-promoting compounds such as phenolics, flavonoids, and betalains. Compared to both untreated and commercially fertilized plants, AMF-treated individuals consistently exhibited superior growth and higher antioxidant activity, highlighting the symbiotic fungi's capacity to optimize nutrient acquisition and stimulate secondary metabolism. The inclusion of a commercial control allowed for direct comparison with current organic inputs, reinforcing the potential of AMF as a more sustainable and effective alternative. These findings support the integration of mycorrhizal biotechnology in cactus cultivation, particularly in regions facing environmental stress and resource limitations. Broader adoption of AMF in prickly pear production could contribute to environmentally responsible agriculture while enhancing the nutritional and medicinal value of cactus-based food products.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Growth parameters of *Opuntia ficus-indica* under different treatments

Treatment	Plant Height (cm)	Cladode Number	Cladode Thickness (mm)	Biomass (g)
Control (T1)	42.5±2.1 c	3.1±0.3 c	12.5±0.8 b	215.4±2.3 c
Commercial (T2)	49.8±2.3 b	4.4±0.4 b	14.3±0.9 a	262.3±2.8 b
Mycorrhizae (T3)	56.3±2.4 a	5.9±0.5 a	16.1±1.0 a	298.7±3.6 a

Table shows average values and standard deviations for growth parameters, including plant height, number and thickness of cladodes, and total biomass.

Table 2: Effect of different treatments on flowering and fruiting time of *Opuntia ficus-indica*

Treatment	Days to First Flowering	Days to Full Fruiting
Control (T1)	112.4±3.2 a	167.1±2.8 a
Commercial (T2)	105.1±2.7 b	160.3±3.1 b
Mycorrhizae (T3)	92.3±2.4 c	150.2±2.6 c

Values represent mean ± SD. Different letters within each column indicate significant differences among treatments according to Tukey's HSD test at $p < 0.05$.

Table 3: Phytochemical properties of *Opuntia ficus-indica* fruits under different treatments

Treatment	Total Phenolics (mg GAE/g FW)	Flavonoids (mg QE/g FW)	Betalains (mg/100g FW)	Antioxidant Activity (%)
Control (T1)	1.85±0.09 c	0.76±0.04 c	31.2±2.4 c	41.8±1.3 c
Commercial (T2)	2.36±0.11 b	1.02±0.05 b	42.6±2.7 b	52.1±0.8 b
Mycorrhizae (T3)	2.69±0.10 a	1.25±0.06 a	48.9±2.9 a	59.4±0.6 a

Table summarizes key biochemical compounds and antioxidant activity in the fruits, along with associated standard deviations

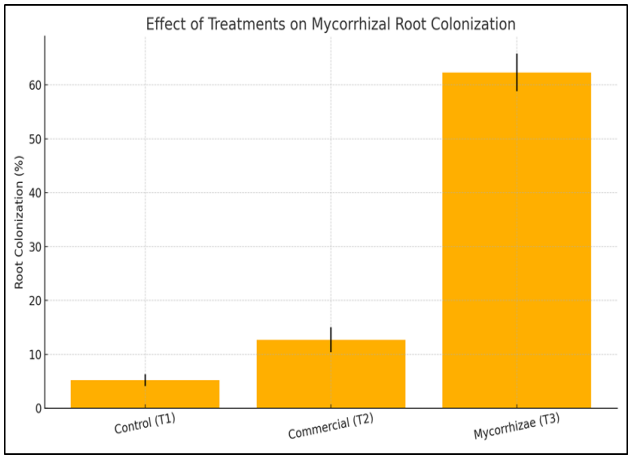

Figure 1: Root colonization (%) in *Opuntia ficus-indica* observed 60 days after inoculation

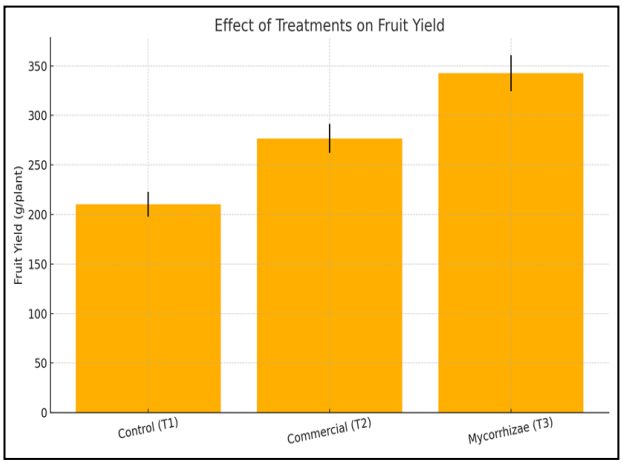

Figure 2: Fruit yield (g/plant) under different treatments