Journal of Applied Horticulture, 28(2): 373-377, 2026
https://doi.org/10.37855/jah.2026.v28i02.59
Journal of Applied Horticulture
ISSN: 0972-1045

Effect of nitrogen fertilization and fungal inoculation on the growth of *Senna surattensis* (*Cassia glauca*) seedlings

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Abstract

This study was conducted in the ornamental plant shade house of the Plant Production Techniques Department at Al-Mussaib Technical College during the period spanning autumn 2024 to spring 2025 to examine the effect of nitrogen addition and fungal inoculation on the growth of *Senna surattensis* (Burm.f.) H.S.Irwin & Barneby seedlings. A factorial experiment was implemented using a completely randomized design with two factors: nitrogen supplied as urea at three levels (N0: 0, N1: 40, and N2: 80 kg N ha^{-1}) and fungal inoculation at four levels (B0: no inoculation, B1: *Trichoderma asperellum* Samuels, Lieckf. & Nirenberg, B2: mycorrhizal fungi (*Glomus* sp.), B3: a mixture of mycorrhizal fungi + *T. asperellum*). The results showed that B3 produced the highest plant height (102.53 cm), stem diameter (9.53 mm), chlorophyll content (39.67 SPAD units), leaflet number (543.00 leaflets plant^{-1}), and shoot dry weight (24.63 g). Nitrogen treatment N2 also improved all measured traits, giving 97.73 cm in height, 8.95 mm stem diameter, 37.58 SPAD units, 517.25 leaflets plant^{-1}, and 22.88 g dry weight. The combined inoculation with mycorrhizal fungi and *T. asperellum* under nitrogen fertilization resulted in the greatest increases overall, with plant height reaching 106.30 cm, stem diameter 10.60 mm, chlorophyll content 41.70 SPAD units, 576.00 leaflets plant^{-1}, and shoot dry weight 27.20 g.

Key words: Nitrogen fertilization, mycorrhiza, *Trichoderma asperellum*, *Senna surattensis*, seedling growth, fungal inoculation

Introduction

Senna surattensis (Burm.f.) H.S.Irwin & Barneby *(syn. Cassia glauca*) is a legume plant (Fabaceae) and widely adopted as an ornamental plant. It produces small flowers in inflorescences and produces fruits that are green (maturing to black) with a pod shape, each containing 3–5 seeds (Hanelt, 2001). *S. surattensis* is becoming more popular in urban landscaping schemes and in nurseries in Iraq, but is often stunted by low soil fertility, especially by nitrogen and phosphorus deficiency in calcareous soils with low organic matter content (Al-Shahbani *et al.,* 2026).

Remarkably, several studies have been undertaken on the associated symbiotic fungi and root-zone bacterial communities in relation to enhancing plant productivity and their nutrient absorptive capacity. Arbuscular mycorrhizal fungi (AMF) form mutualistic associations with the roots of more than 80% of terrestrial plant species and enhance plant acquisition of essential mineral nutrients, particularly phosphorus and nitrogen, in exchange for host-derived carbon (Richardson *et al.*, 2009). The higher leaflet production may be attributed to potential lateral bud outgrowth or cellular expansion influenced by host hormonal dynamics (López-Ráez *et al.*, 2010; Martínez-Medina *et al.*, 2011), although phytohormone levels were not directly quantified in this study.

Furthermore, *Trichoderma* species are frequently abundant in the rhizosphere, where they solubilize essential nutrients to facilitate plant growth and metabolism (Shoresh *et al.,* 2010), while also producing bioactive molecules that suppress phytopathogen proliferation (Manonmani and Ayyandurai, 2025).

Nutrient deficiencies severely disrupt physiological homeostasis, leading to impaired metabolic activity and substantial yield losses. Among essential elements, nitrogen (N) serves as a critical macronutrient required for the synthesis of chlorophyll, amino acids, proteins, nucleic acids, and phytohormones, thereby driving photosynthetic capacity and carbon assimilation (Taiz and Zeiger, 2006; Leghari *et al.*, 2016). Consequently, maintaining optimal nitrogen availability is crucial for supporting vegetative growth, biomass accumulation, and overall plant development (Hawkesford *et al.*, 2012; Al-Freeh *et al.*, 2019).

Although *S. surattensis* is now being used in Iraqi nurseries and landscaping, few data are available regarding the interactive effect of nitrogen fertilization and fungal inoculation on seedling growth. Hence, in the present study, the contributions of mycorrhiza and *Trichoderma* inoculation, nitrogen fertilization, and their interaction were evaluated for enhancing growth of *S. surattensis* seedlings.

Materials and methods

Study site: The experiment was conducted in the shade house nursery of the Plant Production Techniques Department at Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University (32°28′ N, 44°25′ E; altitude 34 m a.s.l.), characterized by a semi-arid climate. During the trial period, daily ambient temperatures ranged between 18°C and 28°C, relative humidity was maintained at 50%–68%, and shadecloth provided 50% photosynthetically active radiation (PAR) attenuation. Irrigation was supplied using municipal tap water. Chemical analysis of the irrigation water yielded: pH 7.31, Electrical Conductivity (EC) 0.85 dS m^{-1}, Total Dissolved Solids (TDS) 544 mg L^{-1}, Na^+ 2.1 meq L^{-1}, Ca^{2+} 3.4 meq L^{-1}, Mg^{2+} 1.8 meq L^{-1}, Cl^- 3.2 meq L^{-1}, and HCO_3^- 2.1 meq L^{-1}.

Plant material: Uniform two-month-old *Senna surattensis* (Burm.f.) H.S.Irwin & Barneby seedlings (mean baseline height 14.80 ± 0.42 cm, stem diameter 2.15 ± 0.08 mm, baseline tissue leaf nitrogen content 1.22% ± 0.05%; values represent Mean ± SE) were transplanted into 10.5 L plastic pots (26 cm depth, 0.0491 m² surface area). Baseline tissue leaf nitrogen content was determined prior to treatment imposition using oven-dried leaf samples (70 °C for 48 h) analyzed via the micro-Kjeldahl digestion and distillation method. Prior to treatment imposition, seedlings were acclimatized in the shade house for 10 days. Pots were filled with river sandy loam soil amended with commercial peat moss (Klasmann-Deilmann GmbH, Geaste, Germany) at a 3:1 (v/v) ratio. The peat moss substrate composition comprised: organic matter >85%, pH 5.8 (in H_2O), EC 0.35 dS m^{-1}, total nitrogen 1.0%–1.5%, P_2O_5 0.1%–0.3%, and K_2O 0.2%–0.4%.

The experiment included two factors, urea fertilizer (N) and biofertilizer inoculation.

Urea fertilizer (N): Nitrogen was applied using commercial urea (46% N) at three elemental nitrogen levels: N0 (0 kg N ha^{-1}, non-fertilized control), N1 (40 kg N ha^{-1}), and N2 (80 kg N ha^{-1}). Terminology throughout the manuscript is consistently expressed as elemental nitrogen (kg N ha^{-1}). Fertilizer dosages per pot were calculated on a pot top-surface area equivalent basis relative to one hectare (10,000 m²). Each pot had a top diameter of 25 cm (r = 0.125 m), yielding a top surface area (A = πr²) of 0.0491 m². The elemental nitrogen rate per pot was calculated as:

Nitrogen dose (g pot^{-1}) = [Field rate (kg N ha^{-1}) × 1,000 g kg^{-1} / 10,000 m²] × 0.0491 m²

Accordingly, the 40 kg N ha^{-1} (N1) and 80 kg N ha^{-1} (N2) treatments supplied 0.196 g and 0.393 g elemental N pot^{-1}, which corresponds to 0.427 g and 0.854 g of commercial urea (46% N) per pot, respectively. Urea was dissolved in distilled water and applied as a soil drench in two equal split applications at 20 and 40 days post-transplanting.

Biofertilizer Inoculation and Application: The biofertilizer treatment factor (B) comprised four distinct inoculation levels using *Trichoderma asperellum* Samuels, Lieckf. & Nirenberg (strain TA-01, obtained from BioWorks, Inc., Victor, NY, USA) and the arbuscular mycorrhizal fungus Glomus sp. (obtained from INVAM, West Virginia University, Morgantown, WV, USA). All inoculants were applied on the day of transplanting (Day 0) by placing and incorporating the carrier-based inoculants directly into the planting hole at the root zone of each pot prior to placing the seedling.

The four biofertilizer levels were defined as follows:

B0 (Non-inoculated Control): Received no biofertilizer inoculants or carrier substrate.

B1 (*Trichoderma asperellum*): Inoculated with *T. asperellum* formulated on a corn waste carrier at a concentration of 7×10^7 CFU g−1, applied at a rate of 10 g pot^{-1}, supplying a total viable dose of 7×10^8 CFU pot^{-1}.

B2 (*Mycorrhizal Fungi – Glomus* sp.): Inoculated with *Glomus* sp. (50 spores g^{-1}) formulated on a peat moss carrier, applied at a rate of 10 g pot^{-1}, supplying 500 spores pot−1.

B3 (Combined Inoculation): Inoculated with a combined mixture consisting of 5 g *T. asperellum* formulation, supplying 3.5×10^8 CFU pot^{-1}, and 5 g *Glomus* sp. formulation, supplying 250 spores pot^{-1}, providing a total application rate of 10 g pot^{-1}.

To maintain a consistent total rate of added organic carrier substrate (10 g pot^{-1}) across all inoculated treatments, treatment B3 was prepared on a substitution basis rather than an additive basis. Consequently, each fungal formulation in B3 was applied at half the mass used in its corresponding single-inoculant treatment (5 g + 5 g instead of 10 g + 10 g). As a result, treatment B3 contained approximately half the individual carrier mass and half the nominal propagule/spore dose of each fungal strain compared with B1 and B2, respectively. Because of these individual dosage differences, B3 was designated as a combined inoculation treatment rather than a dose-equivalent treatment, and performance comparisons between B3 and the single-inoculant treatments were interpreted relative to these proportional inoculant application rates.

Table 1. Initial physicochemical properties of the river soil used in the pots before planting

Property	Value	Unit
pH (1:1 soil: water)	7.6	—
Electrical conductivity (EC)	2.1	dS m^{-1}
Organic matter	0.82	%
Total nitrogen (N)	410	mg kg^{-1}
Available phosphorus (P)	7.4	mg kg^{-1}
Available potassium (K)	128	mg kg^{-1}

Planting and growth conditions: Seedlings were transplanted into plastic pots with a volume capacity of 10.5 L (depth of 26 cm, top diameter of 25 cm, and bottom diameter of 18 cm) containing 8 kg of river sandy loam soil amended with peat moss at a 3:1 (v/v) ratio on November 15, 2024 (late fall). During the trial, the shade-house environmental conditions maintained an average daytime temperature range of 18°C–28°C, nighttime minimum temperatures of 10°C–14°C (under agricultural polyethylene sheet protection during cold winter periods), and relative humidity ranging between 50% and 68%. All pots were irrigated uniformly 2–3 times per week, receiving approximately 600 mL of water per pot per irrigation event to maintain soil moisture around 75% of field capacity. Standard cultural practices were applied as needed. Growth measurements were recorded at 120 days post-transplanting on March 15, 2025 (spring 2025).

Plant height (cm) was measured from the soil surface to the top of the plant using a metric tape, and averaged per treatment. Number of leaflets (leaflets $plant^{-1}$) was counted for each plant and averaged per treatment. Chlorophyll content (SPAD units) was measured on three leaves (lower, middle, upper parts) using a SPAD-225 chlorophyll meter; mean values calculated. Main stem diameter (mm) was measured 2 cm above soil surface using a digital vernier caliper; average per replicate computed. Shoot dry weight (g): To determine the total above-ground biomass, the entire shoot system (including stems and all leaves) was harvested from the seedlings 120 days after transplanting. The samples were thoroughly washed with running water to remove any dust, patted dry with tissue paper, and weighed to obtain the fresh weight. The shoots were then placed in perforated paper bags and dried in an electric oven with forced air ventilation at 70°C until a constant weight was achieved. The final dry weight was recorded using a high-precision sensitive balance.

All growth measurements (plant height, stem diameter, number of leaflets, chlorophyll content, and shoot dry weight) were recorded at 120 days post-transplanting.

Experimental design: The experiment was laid out as a factorial experiment based on a Completely Randomized Design (CRD) with two factors: Fungal Inoculation (4 levels: B0, B1, B2, B3) and Nitrogen Fertilization (3 levels: N0, N1, N2), yielding 12

treatment combinations. Each treatment combination included 3 biological replicates (36 experimental units in total). To strictly avoid pseudo replication, each biological replicate (experimental unit) comprised a physical cluster of 3 adjacent pots (1 seedling per pot) managed as a single experimental unit. Data from the 3 pots within each unit were pooled into a single mean value prior to statistical analysis. To eliminate edge effects and micro-environmental gradients within the shade house, the spatial positions of all 36 experimental unit clusters (rather than individual pots independently) were fully re-randomized on a weekly basis using a random number table.

Statistical analysis: To avoid pseudo replication, data collected from the three individual pots within each experimental unit were averaged to yield a single mean value per replicate prior to statistical analysis. All data were verified for the fundamental assumptions of Analysis of Variance (ANOVA). Normality of residuals was assessed using the Shapiro-Wilk test, and homogeneity of variance was confirmed using Levene's test. All evaluated traits satisfied the underlying ANOVA assumptions ($P > 0.05$); therefore, no data transformations were required.

Data were analyzed via a two-factor factorial ANOVA structured in a Completely Randomized Design (CRD) across 36 experimental units using GenStat software (Version 22, VSN International, Hemel Hempstead, UK). Data were fitted to a two-factor fixed-effects model represented by the equation:

$$Y_{ijk} = \mu + B_i + N_j + (BN)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the observed response variable for the k-th replicate under the i-th biofertilizer level and j-th nitrogen rate; μ is the overall mean; B_i is the fixed effect of fungal inoculation (i = 1, ..., 4); N_j is the fixed effect of nitrogen fertilization rate (j = 1, ..., 3); $(BN)_{ij}$ is the interaction effect between fungal inoculation and nitrogen rate; and ε_{ijk} is the random experimental error term.

Main effects and interaction effects (N × B) were evaluated. Post-hoc mean separations were performed exclusively using Tukey's Honestly Significant Difference (HSD) test at $P \leq 0.05$.

Results and discussion

The two-way analysis of variance (ANOVA) showed statistically significant at $P < 0.05$ main effects of fungal inoculation (B) and nitrogen fertilization (N), as well as significant interaction effects (B × N), for all evaluated growth and physiological traits of *Senna surattensis* seedlings ($P < 0.05$; Table 2).

Seedling height (cm): The results presented in Table 3 show significant differences among treatments for seedling height. The biofertilizer treatment combining *Trichoderma* + *Glomus sp*. produced the highest mean height (102.53 cm), followed by *Glomus* sp. alone (96.7 cm), then *Trichoderma* alone (91.8 cm), while the control treatment (B0) recorded the lowest mean (88.8 cm).

For mineral nitrogen fertilization, treatment N2 (80 kg ha^{-1}) gave the highest mean height (97.73 cm), followed by N1 (40 kg ha^{-1}) at 94.88 cm, with the control (N0) producing the lowest value (92.28 cm). The interaction between the two factors showed that the combined treatment of B3 + N2 (*Trichoderma* + *Glomus* sp. + 80 kg ha^{-1} N) resulted in the highest mean seedling height (106.3 cm), compared with the lowest height observed in the combined control treatment (B0 + N0), which was 86.5 cm.

Table 3. Effect of nitrogen application, fungal inoculation, and their interaction on plant height (cm)

Fungal inoculation	Nitrogen (N kg ha^{-1})			Mean
	0	40	80	
Control (B0)	86.5	88.4	91.5	88.8 d
T. asperellum (B1)	89.2	92.5	93.7	91.8 c
Glomus sp. (B2)	94.6	96.1	99.4	96.7 b
T. asperellum + *Glomus* sp. (B3)	98.8	102.5	106.3	102.53 a
Mean (N)	92.3 c	94.9 b	97.7 a	

Means followed by different lowercase letters differ significantly according to Tukey's HSD test ($P \leq 0.05$). Critical HSD values were 1.11, 0.87, and 2.52 for fungal inoculation, nitrogen fertilization, and their interaction (B × N), respectively.

Seedling stem diameter (mm): Table 4 presents the effect of treatments on seedling stem diameter. The biofertilizer treatment combining *Trichoderma* + *Glomus* sp. recorded the highest mean diameter (9.53 mm), followed by *Glomus sp*. alone (8.57 mm), then *Trichoderma* alone (8.10 mm), while the control (B0) showed the lowest mean (7.57 mm). Treatment N2 (80 kg ha^{-1}) had the largest stem diameter (8.95 mm), followed by N1 (40 kg ha^{-1}) at 8.40 mm, with the control (N0) being the lowest (8.0 mm).

The interaction of fungal inoculation and nitrogen fertilization showed that the combined treatment of B3 + N2 produced the highest stem diameter (10.6 mm), while the combined control (B0 + N0) was the lowest (7.2 mm).

Table 4. Effect of nitrogen application, fungal inoculation, and their interaction on stem diameter (mm)

Fungal inoculation	Nitrogen (N kg ha^{-1})			Mean
	0	40	80	
Control (B0)	7.20 g	7.60 fg	7.90 ef	7.57 d
T. asperellum (B1)	7.80 efg	8.10 def	8.40 cde	8.10 c
Glomus sp. (B2)	8.20 def	8.60 cd	8.90 bc	8.57 b
T. asperellum + *Glomus* sp. (B3)	8.70 bcd	9.30 b	10.60 a	9.53 a
Mean (N)	7.98 c	8.40 b	8.95 a	—

Means followed by different lowercase superscript letters differ significantly according to Tukey's HSD test ($P \leq 0.05$). Critical HSD values were 0.27, 0.21, and 0.62 for fungal inoculation, nitrogen fertilization, and their interaction (B × N), respectively.

Table 5 shows the effect of Fungal inoculation, nitrogen fertilization, and their interaction on the number of leaflets per plant. The combined biofertilizer treatment *Trichoderma* + *Glomus sp*. (B3) produced the highest mean number of leaflets per plant, reaching 543.00 leaflets, followed by *Glomus sp*. alone (B2) with 505.67 leaflets, *Trichoderma* alone (B1) with 484.33

Table 2. Two-way Analysis of Variance (ANOVA) summary for growth and physiological traits of *Senna surattensis* seedlings

Source of variation (SOV)	df	Plant height (cm)	Stem diameter (mm)	Leaflets number	Chlorophyll (spad)	Shoot dry weight (g)
Fungal Inoculation (B)	3	324.94 (445.13***)	6.27 (141.94***)	11851.67 (740.73***)	69.16 (368.84***)	50.73 (317.04***)
Nitrogen Fertilizer (N)	2	89.17 (122.15***)	2.87 (64.92***)	4403.25 (275.20***)	21.71 (115.77***)	20.00 (124.98***)
Interaction (B × N)	6	2.17 (2.97*)	0.32 (7.34***)	261.92 (16.37***)	0.66 (3.54*)	2.18 (13.65***)
Error (MSE)	24	0.73	0.044	16.00	0.188	0.16
Total	35	—	—	—	—	—
Standard Error (SE)	—	0.493	0.121	2.309	0.250	0.231
CV (%)	—	0.90%	2.49%	0.80%	1.20%	1.86%

Values are mean squares (MS), with F-values in parentheses. df = degrees of freedom; MSE = residual mean square error; SE = standard error of the treatment-cell mean; CV = coefficient of variation. *$P < 0.05$; ***$P < 0.001$.

Table 5. Effect of nitrogen application, fungal inoculation, and their interaction on number of leaflets (leaflets plant^{-1})

Fungal inoculation	Nitrogen (N kg ha^{-1})			Mean
	0	40	80	
Control (B0)	442.00 i	456.00 h	473.00 fg	457.00 d
T. asperellum (B1)	468.00 g	482.00 f	503.00 de	484.33 c
Glomus sp. (B2)	495.00 e	505.00 de	517.00 c	505.67 b
B3	511.00 cd	542.00 b	576.00 a	543.00 a
Mean (N)	479.00 c	496.25 b	517.25 a	—

Means followed by different lowercase letters within a row or column differ significantly according to Tukey's HSD test at P ≤ 0.05. ANOVA effect sizes (ηp²): Nitrogen rate (N), ηp² = 0.96, P < 0.001; Biofertilizer inoculation (B), ηp² = 0.99, P < 0.001; Interaction (N × B), ηp² = 0.80, P < 0.001.

leaflets, and the control (B0) with the lowest number, 457 leaflets.

Nitrogen fertilization significantly influenced leaflet number. The highest mean was recorded in N2 (80 kg ha^{-1}) with 517.25 leaflets, followed by N1 (40 kg ha^{-1}) at 496.25 leaflets, and N0 (0 kg ha^{-1}) with 479 leaflets. The significant ($P \leq 0.05$) interaction between fungal inoculation and nitrogen fertilization indicated that the combination B3 + N2 resulted in the maximum number of leaflets per plant (576 leaflets), while the control combination B0 + N0 produced the minimum (442 leaflets).

Shoot dry weight (g): Table 6 presents the effect of Fungal inoculation, nitrogen fertilization, and their interaction on the shoot dry weight of *Senna surattensis* seedlings. The combined biofertilizer treatment *Trichoderma* + *Glomus sp*. (B3) recorded the highest mean shoot dry weight (24.63 g), followed by *Glomus sp*. alone (B2) with 22.03 g, *Trichoderma* alone (B1) with 20.3 g, and the control (B0) with the lowest value, 19.2 g. Nitrogen fertilization also significantly affected shoot dry weight. The highest mean was observed under N2 (80 kg ha^{-1}) with 22.88 g, followed by N1 (40 kg ha^{-1}) at 21.4 g, and N0 (0 kg ha^{-1}) at 20.3 g. The interaction between Fungal inoculation and nitrogen fertilization showed that the combination B3 + N2 produced the maximum shoot dry weight (27.2 g), whereas the control combination B0 + N0 had the lowest value (18.6 g).

Table 6. Effect of nitrogen application, fungal inoculation, and their interaction on shoot dry weight (g)

Fungal inoculation	Nitrogen (N kg ha^{-1})			Mean
	0	40	80	
Control (B0)	18.60 h	19.20 gh	19.80 efg	19.20 d
T. asperellum (B1)	19.50 fgh	20.40 ef	20.90 de	20.27 c
Glomus sp. (B2)	20.70 de	21.80 cd	23.60 b	22.03 b
B3	22.40 c	24.30 b	27.20 a	24.63 a
Mean (N)	20.30 c	21.43 b	22.88 a	—

B3: *T. asperellum* + *Glomus* sp. Means followed by different lowercase superscript letters differ significantly according to Tukey's HSD test ($P \leq 0.05$). Critical HSD values were 0.52, 0.41, and 1.18 for fungal inoculation, nitrogen fertilization, and their interaction (B × N), respectively.

Chlorophyll content in leaves (SPAD units): Table 7 summarizes the effects of fungal inoculation, nitrogen fertilization, and their interaction on leaf chlorophyll content (SPAD units) of *Senna surattensis* seedlings. The combined biofertilizer treatment *Trichoderma* + *Glomus sp*. (B3) achieved the highest mean chlorophyll content (39.67 SPAD), followed by *Glomus sp*. alone (B2) with 36.73 SPAD, *Trichoderma* alone (B1) with 34.9 SPAD, and the control (B0) with the lowest value (33.2 SPAD). Nitrogen fertilization significantly increased chlorophyll content. The highest value was recorded under N2 (80 kg ha^{-1}) at 37.58 SPAD, followed by N1 (40 kg ha^{-1}) at 35.85 SPAD, and N0 (0 kg ha^{-1}) at 34.9 SPAD. The interaction between Fungal inoculation and nitrogen fertilization showed that B3 + N2 produced the maximum chlorophyll content (41.7 SPAD), whereas the control combination B0 + N0 had the lowest value (32.6 SPAD).

Table 7. Effect of nitrogen application, fungal inoculation, and their interaction on leaf chlorophyll content (SPAD units)

Fungal inoculation	Nitrogen (N kg ha^{-1})			Mean
	0	40	80	
Control (B0)	32.60 g	32.90 fg	34.10 ef	33.20 d
T. asperellum (B1)	33.50 fg	34.90 de	36.20 c	34.87 c
Glomus sp. (B2)	35.40 cd	36.50 c	38.30 b	36.73 b
T. asperellum + *Glomus* sp. (B3)	38.20 b	39.10 b	41.70 a	39.67 a
Mean (N)	34.93 c	35.85 b	37.58 a	—

Means followed by different lowercase superscript letters differ significantly according to Tukey's HSD test ($P \leq 0.05$). Critical HSD values were 0.56, 0.44, and 1.28 for fungal inoculation, nitrogen fertilization, and their interaction (B × N), respectively.

S. surattensis seedlings inoculated with *mycorrhizal fungi* (*Glomus* sp.) and *Trichoderma asperellum* exhibited significantly higher vegetative growth across all measured parameters including plant height, stem diameter, number of leaflets, chlorophyll content, and shoot dry weight compared to the non-inoculated controls (Tables 3–7). Enhanced nutrient acquisition and water absorption may explain the observed growth responses; however, these processes were not directly measured (Richardson *et al.*, 2009; Rouphael *et al.,* 2015). Furthermore, *T. asperellum* is known to stimulate root elongation and lateral root branching through the secretion of bioactive secondary metabolites, thereby modifying root system architecture (Contreras-Cornejo *et al.,* 2009).

The observed plant height and stem diameter data are in line with potential nutrient availability and root system development. In seedlings, higher production of leaves was induced by fungal inoculants, consistent with observations in other plant species, including leguminous and ornamental crops (Sohn *et al.*, 2003; Andrzejak *et al.*, 2021). Seedlings inoculated with fungi, particularly the combined Glomus sp. and *T. asperellum* treatment, exhibited significant increases in stem diameter. This improvement may have resulted from overall enhancement in plant biomass accumulation. However, specific tissue nutrient concentrations and stem anatomical modifications (such as vascular cambium activity) were not directly measured in the present study and warrant further investigation.

The significantly higher chlorophyll content observed in seedlings inoculated with mycorrhizal fungi and *T. asperellum* reflects an improved overall physiological status. While fungal inoculation is frequently reported in literature to influence plant nutrition and stress response, reactive oxygen species (ROS) dynamics, antioxidant enzyme activities, tissue mineral concentrations, and actual photosynthetic capacity were not explicitly quantified in the present study. Therefore, mechanisms such as oxidative stress reduction or enhanced photosynthetic rates remain speculative hypotheses rather than demonstrated outcomes.

Fungal inoculation also led to a significant increase in the number of leaflets as well as shoot dry weight. The higher leaflet production may be attributed to potential lateral bud outgrowth or cellular expansion influenced by host hormonal dynamics (Martínez-Medina *et al.*, 2011; Safitri *et al.*, 2023), although phytohormone levels were not directly quantified in this study. Meanwhile, the enhanced shoot biomass accumulation is hypothesized to be supported by optimized metabolic activity induced by the symbiotic fungi (Shoresh *et al.*, 2010; Rouphael

et al., 2015), though specific nutrient uptake rates were not evaluated.

Nitrogen helps plants build chlorophyll and biomass when nothing else is holding growth back (Hawkesford *et al.*, 2012). Too much nitrogen can reduce how well mycorrhizae colonize roots (Treseder, 2004), but combining biofertilizers with nitrogen still gave the best overall performance. We saw a significant positive interaction between nitrogen fertilization and fungal inoculation, and the combined mycorrhizal and T. asperellum treatment under nitrogen (N2) produced better growth across every parameter. This likely reflects general microbial processes in the soil (Richardson *et al.*, 2009): the fungi may expand the roots' capacity to absorb nutrients, while the urea supplies nitrogen that is readily available.

Overall, combining biofertilizers with nitrogen worked better than either alone. Seedlings under the integrated approach consistently showed greater plant height, stem diameter, leaflet number, chlorophyll content (SPAD), and shoot dry weight. This fits with earlier work showing that microbial inoculation plus nitrogen fertilization boosts the growth of ornamental and leguminous plants (Andrzejak *et al.*, 2021).

A few limitations are worth noting. First, we didn't measure root colonization or how well the introduced fungi persisted, so we can't tie the growth gains directly to how well the fungi established. Future studies should quantify AMF colonization under the microscope and track *T. asperellum* with molecular methods over time. Second, the two biofertilizers used different carriers (corn waste for *T. asperellum*, peat moss for *Glomus* sp.), and B0 had no sterile carrier control. So some of the effect may come from the carriers themselves rather than the fungi. Also, the combined treatment (B3) split the dose (5 g + 5 g) instead of adding both in full (10 g + 10 g), meaning each fungus started at a lower density than in the single-inoculation treatments. Going forward, using autoclaved, carrier-matched controls and standardized propagule counts across all treatments would help separate the true symbiotic effects from carrier effects.

The study revealed that the combined application of *T. asperellum* and *mycorrhizal fungi* significantly improves the vegetative growth performance of *S. surattensis* seedlings, particularly when applied alongside nitrogen fertilization (80 kg ha^{-1}). The symbiotic fungi together enhanced shoot dry weight, leaflet number, relative chlorophyll status, and stem diameter under the evaluated experimental conditions. The results indicate that integrating bio-fertilizers with mineral fertilizers represents a promising strategy for producing vigorous seedlings. Future research should evaluate broader biofertilizer combinations, tissue nutrient concentrations, actual photosynthetic rates, and post-transplant survival. The utilization of *S. surattensis* is recommended for landscape applications due to its high ornamental value.

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Received: March, 2026; Revised: April, 2026; Accepted: May, 2026