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Isolation, Identification and Plant Growth-Promoting Potential of Endophytic Fungi Associated with *Solanum melongena* L. under Abiotic Stress

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Abstract

Endophytic fungi can improve plant nutrition and help crops tolerate environmental stress. This study isolated culturable endophytic fungi from healthy roots, stems and leaves of brinjal (*Solanum melongena* L.) and evaluated their plant growth-promoting potential under salinity and polyethylene glycol-induced drought stress. Surface-sterilized tissue segments yielded six fungal isolates, designated EF-1 to EF-6. Morphological and microscopic examination provisionally assigned the isolates to *Aspergillus*, *Fusarium*, *Trichoderma*, *Phomopsis*, *Penicillium* and *Alternaria*. The isolates were screened for indole-3-acetic acid production, phosphate solubilization and siderophore production. EF-3 (*Trichoderma* sp.) and EF-5 (*Penicillium* sp.) showed the strongest combined plant growth-promoting traits. Relative to the stressed uninoculated control, EF-3 treatment increased germination by 26%, root length by 32%, shoot length by 28% and biomass by 30%; EF-5 produced corresponding increases of 22%, 28%, 24% and 27%. Treated seedlings also showed higher chlorophyll status, lower apparent oxidative stress and greater antioxidant activity. These findings identify EF-3 and EF-5 as promising candidates for further molecular identification, dose optimization and field validation before use as brinjal bioinoculants.

Keywords: abiotic stress; bioinoculant; brinjal; endophytic fungi; plant growth promotion; *Solanum melongena*

1. Introduction

Abiotic stresses such as drought and salinity constrain plant establishment, biomass production and crop yield. Abiotic stress arises by climate changes has reduced global agricultural production by 1 to 5% during the last few decades elevating global food demand from 70 to 100% by 2050 (Newbery et al., 2016; Pais et al.,2020). Their effects are increasingly important under climate variability, creating a need for biological approaches that can complement conventional crop management (Kumar et al., 2025; Zhang et al., 2025). Agriculture is one of the sectors most vulnerable to climate change, particularly in tropical regions. Various abiotic stress conditions, such as high carbon dioxide, drought, high temperature, salinity, etc., impede food production. The production of reactive oxygen species (ROS) during stress conditions such as singlet oxygen (1O2), hydrogen peroxide (H2O2), super oxide radicals(O2), hydroxyl radicals (OH), etc., hampers the crop yield (Keswani et al.,2019a). Increasing temperatures, changing precipitation patterns, and water scarcity threaten crop productivity, especially in South Asian wheat-growing regions (Chakrabarti et al., 2025; Fiaz et al., 2025). Historical temperature records also indicate a warming trend in India (Pant & Rupa Kumar, 1997).

Endophytic fungi inhabit internal plant tissues without producing visible disease symptoms. Depending on the host, fungal strain and environmental conditions, these organisms can influence nutrient acquisition, phytohormone balance, antioxidant defence and tolerance to osmotic or ionic stress. Several endophytes

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produce indole-3-acetic acid, mobilize sparingly available phosphorus or release siderophores that improve iron acquisition. They may also reduce stress-related cellular damage by supporting osmotic adjustment and antioxidant activity (Khan et al., 2012; Lata et al., 2018; Poveda et al., 2022). Endophytic fungi are an ecological, non-pathogenic, polyphylectic group of highly diverse fungi of ascomycetes, basidiomycetes and anamorphic fungi (Gupta et al., 2020).

Brinjal (*Solanum melongena* L.) is an economically important solanaceous vegetable, but early growth and productivity are sensitive to water deficit and salinity. The culturable endophytic community of brinjal may therefore contain strains useful for stress-resilient crop production. The present study aimed to (i) isolate culturable endophytic fungi from healthy brinjal tissues, (ii) provisionally identify the isolates using cultural and microscopic characteristics, (iii) screen them for selected plant growth-promoting traits, and (iv) assess their effects on brinjal germination and early seedling growth under simulated salinity and drought stress.

2. Materials and Methods

2.1. Collection of plant material

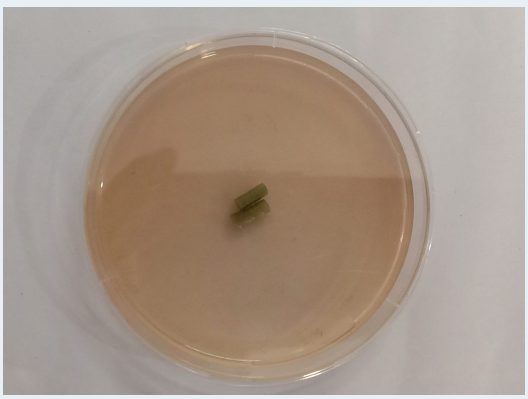
Healthy brinjal plants were collected from agricultural fields in Kushinagar district, Uttar Pradesh, India. Roots, stems and leaves showing no visible disease symptoms were selected and transported for laboratory processing. Representative field plants are shown in Figure 1A.

2.2. Surface sterilization and isolation

Plant material was washed under running tap water. Tissue pieces were treated with 70% ethanol for 1–3 min and 1% sodium hypochlorite for 3–5 min, then rinsed repeatedly with sterile distilled water. Surface-sterilized tissues were cut into approximately 0.5–1.0 cm segments and placed on potato dextrose agar supplemented with streptomycin. Plates were incubated at 25 ± 2 °C for 5–7 days. Hyphae emerging from tissue margins were transferred to fresh medium until visually pure cultures were obtained (Figure 1B).



A



B

Figure 1. Field and isolation stages. (A) Representative brinjal plants at the sampling site. (B) Surface-sterilized brinjal tissue segment placed on agar for endophyte isolation.

2.3. Morphological identification and trait screening

Isolates were designated EF-1 to EF-6 and provisionally identified from colony colour, texture, growth pattern, sporulation and microscopic structures observed after lactophenol cotton blue staining. Because DNA sequence data were not available, identification was restricted to genus level. Isolates were screened qualitatively for indole-3-acetic acid production, phosphate solubilization on Pikovskaya's agar and siderophore production. Relative responses were recorded as absent (–), low (+), moderate (++) or strong (+++). Representative culture morphologies are shown in Figure 2.



A



B

Figure 2. Representative colony morphologies of culturable endophytic fungi recovered from brinjal tissues. (A) Dark-pigmented sporulating colony. (B) Rapidly spreading green sporulating colony. Species-level identity cannot be assigned from photographs alone.

2.4. Stress and seedling assays

Selected isolates were evaluated under salinity imposed with sodium chloride and drought stress simulated with polyethylene glycol 6000. Brinjal seeds were treated with fungal inoculum and maintained under controlled laboratory conditions. Germination percentage, root length, shoot length and biomass were recorded after 10–14 days. Chlorophyll status, oxidative stress and antioxidant activity were assessed comparatively. Treatment effects are reported as percentage change relative to the corresponding stressed, uninoculated control.

3. Results

3.1. Isolation and provisional identification

Six culturable endophytic fungal isolates were recovered from roots, stems and leaves. Root tissues yielded the highest apparent colonization frequency, followed by stems and leaves. Based on cultural and microscopic features, the isolates were provisionally assigned to *Aspergillus* (EF-1), *Fusarium* (EF-2), *Trichoderma* (EF-3), *Phomopsis* (EF-4), *Penicillium* (EF-5) and *Alternaria* (EF-6).

Table 1. Tissue origin and plant growth-promoting traits of the six endophytic fungal isolates.

Isolate Code	Identified Genus	Tissue Source	IAA Production	Phosphate Solubilization	Siderophore Production	Overall Potential
EF-1	*Aspergillus* sp.	Root	+	+	–	Moderate
EF-2	*Fusarium* sp.	Stem	+	–	+	Moderate
EF-3	*Trichoderma* sp.	Root	+++	+++	++	High
EF-4	*Phomopsis* sp.	Leaf	+	–	–	Low
EF-5	*Penicillium* sp.	Stem	++	++	+	High
EF-6	*Alternaria* sp.	Leaf	+	+	–	Moderate

3.2. Plant growth-promoting traits

Five isolates produced a positive IAA response, four solubilized phosphate and three produced siderophores. EF-3 showed the strongest combined response, including strong IAA production and phosphate solubilization, while EF-5 ranked second. All remaining isolates showed moderate or low combined activity (Table 1).

3.3. Seed germination and seedling growth under stress

Salinity and simulated drought reduced germination and seedling growth in the uninoculated control. Inoculation improved all measured growth variables, although the response differed among isolates. EF-3 produced the largest increases: 26% in germination, 32% in root length, 28% in shoot length and 30% in biomass. EF-5 was the next most effective treatment, with increases of 22%, 28%, 24% and 27%, respectively (Table 2).

Table 2. Percentage improvement in brinjal growth variables relative to the stressed uninoculated control.

Treatment	Germination Increase (%)	Root Length Increase (%)	Shoot Length Increase (%)	Biomass Increase (%)
Control (Stress)	0	0	0	0
EF-1	10	15	12	14
EF-2	12	18	15	16
EF-3	26	32	28	30
EF-4	8	12	10	11
EF-5	22	28	24	27
EF-6	14	20	18	19

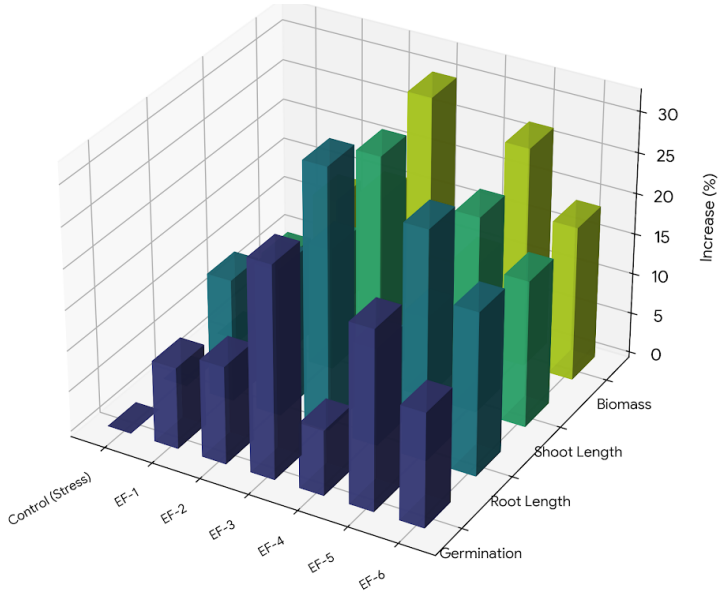


Figure 3:Comparative efficacy of endophytic fungal treatments (EF-1 to EF-6) on plant growth parameters (Germination, Root Length, Shoot Length, and Biomass Increase %) under stress conditions relative to the Control baseline. Isolate EF-3 exhibited maximum growth promotion across all tested attributes.

3.4. Biochemical responses

The stressed control showed low chlorophyll status, high oxidative stress and low antioxidant activity. EF-3 treatment reversed this pattern most strongly, whereas EF-5 produced an intermediate response (Table 3). These qualitative changes were consistent with the superior seedling growth recorded for the two isolates.

Table 3. Comparative biochemical responses of brinjal seedlings under stress.

Treatment	Chlorophyll Content	Oxidative Stress (ROS)	Antioxidant Activity
Control	Low	High	Low

Treatment	Chlorophyll Content	Oxidative Stress (ROS)	Antioxidant Activity
EF-3	High ↑	Low ↓	High ↑
EF-5	Moderate–High ↑	Moderate ↓	Moderate ↑

4. Discussion

The recovery of six culturable fungi from symptomless brinjal tissues supports the view that roots, stems and leaves provide distinct niches for endophytic colonization. The greater recovery from roots is biologically plausible because roots remain in close contact with soil microorganisms and root exudates can support fungal establishment. Nevertheless, culture-based isolation detects only the fraction able to grow under the selected medium and incubation conditions.

The strong responses of EF-3 and EF-5 across IAA production, phosphate solubilization and siderophore assays suggest complementary mechanisms of plant growth promotion. IAA-producing endophytes may influence root development, while phosphate solubilization and siderophore production can improve nutrient availability. Similar mechanisms have been reported for plant-associated fungi under stressful conditions (Khan et al., 2012; Lata et al., 2018).

Improved germination, root and shoot growth, biomass and chlorophyll status in inoculated seedlings indicate that selected isolates reduced the impact of osmotic and salt stress during early development. EF-3 showed the most consistent response, followed by EF-5. The reduction in apparent oxidative stress together with greater antioxidant activity provides a plausible physiological explanation, although the responsible enzymes and metabolites were not individually quantified in the present dataset.

The findings should be interpreted as an initial laboratory evaluation. Morphological identification is provisional, the assay concentrations and inoculum dose require explicit standardization, and performance under controlled conditions may not predict field efficacy. Molecular identification, replicated dose-response experiments, quantitative biochemical measurements and multi-location field trials are therefore required before agronomic application.

5. Conclusion

Brinjal tissues harboured six culturable endophytic fungal isolates with differing plant growth-promoting traits. EF-3 (provisionally *Trichoderma* sp.) and EF-5 (provisionally *Penicillium* sp.) produced the strongest responses and improved germination, seedling growth and biochemical performance under simulated salinity and drought stress. These isolates are promising candidates for further molecular characterization and field validation as components of stress-resilient brinjal production systems.

6. Author Contribution: Each author contributes equally.

7. Conflict of Interest: Authors declare no conflict of interest.

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