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Fungal Endophytes as Growth Promoters and Inducers of Resistance in Tomato (*Lycopersicon esculentum* Mill.) against *Alternaria solani*

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Abstract: Bio control strategies are gaining importance presently due to adverse effects caused by chemical pesticides on the environment. During our study, a total of 78 fungal endophytes were isolated from five medicinally important plants and evaluated for their efficacy in inducing resistance against the early blight pathogen caused by *Alternaria solani*. Among the isolates evaluated, only five endophytes were able to antagonize *A. solani* with a maximum inhibition of 58.53% offered by ENSM-08. All the antagonistic endophytes were molecularly identified based on ITS1 and ITS4 regions and sequence analysis was submitted to GenBank, NCBI to acquire accession numbers. In addition, seed treatment with the conidial suspension of the antagonistic fungal endophytes were able to enhance seed, vegetative and reproductive growth parameters in tomatoes with the highest enhancement observed upon the application of *Pestalotiopsis microspora*- ENSM-08. Also, seed treatments with select endophytes were also able to induce resistance in tomato plants against the early blight pathogen with a maximum protection of 70.25% observed in *P. microspora*- ENSM-08 treated plants. The results of the study validate the application of fungal endophytes as inoculants for sustainable agriculture.

Keywords: Endophytic fungi; early blight disease; *Lycopersicon esculentum*; *Alternaria solani; Pestalotiopsis* sp.

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1. INTRODUCTION

The ever-growing population has instigated a question to the researchers on how to supply food with changing environmental conditions that have led to leakage in crop yield production.[1, 2] Currently, better management strategies for both biotic and abiotic stress inferred to plants apart from using non-environmental friendly chemicals are the talk of the hour. To date, microorganisms are considered to be best suited for the role of biocontrol agent's viz., plant growth-promoting rhizobacteria, fungi, arbuscular mycorrhizae, etc. as they have shown a positive impact on plants with respect to growth as well as induction of resistance against biotic and abiotic stress. [3,4] Among the plant stress, fungal pathogens are the principal source affecting crop yield in several plants globally.[5-7] Endophytes (both fungi and bacteria) are the microorganisms that inhabit inter/ intra cellularly within the host forming systemic or local associations showing mutuality to that of a symbiotic relationship without causing apparent disease symptoms.[8-11] These endophytes are often known to promote plants' growth, recycle nutrients, fitness and improve their ability to cope with stress.[12-15] Among the different forms of endophytes, myco-endophytes have been least explored compared to that of bacterial forms irrespective of their application in the scientific field. Also, fungal endophytes possess potential biocontrol properties and are important to isolate and screen potential endophytes under *in-vitro* conditions followed by *in-vivo* studies under different agro-climatic conditions.[16-18] Apart from these, endophytes have also been noted to provide defense in plants against the invading herbivory [19]. Biocontrol in plants through fungal endophytes is through recycling of nutrients, antibiosis, production of lytic enzymes, volatile substances, antagonism, mycoparasitism or just competing with the pathogen for nutrients and ecological niches.[20] These actions from the fungal endophytes reduce the infection in plants through disruption in the invading pathogens' life cycle through reduced sporulation and colonization in host tissues, thereby affecting survivability.[21] To date, several reports on the control of phytopathogens, nematodes, and insects through the application of fungal endophytes apart from the early emergence of seedlings and plant growth have been reported.[22,23,16, 17,24-26,15]omato (*Lycopersicon esculentum* Mill.) is a known, nutritionally valued, important vegetable crop globally as it possesses a rich source of vitamins, minerals and shows antioxidant properties.[28] It shows an important role in metabolic activities and maintains good health.[29] It is well known that every plant is prone to diseases and tomato is not a part. The plant is prone to many bacterial, viral and fungal infections, which lead to a reduction in crop yield. One of the major diseases of tomato is the early blight, caused by *Alternaria solani* (Ellis & Martin), as it affects leaves, stems and fruits, resulting in the reduction of the fruit's quality and quantity.[30] The use of chemical fertilizers to control the disease is expensive. Its repeated usage develops the resistance in pathogens against the chemicals apart from deteriorating the environment and soil fertility.[31] Hence to reduce the risk, biological control method using the application of endophytic fungi isolated from some medicinal plants are used to evaluate its efficacy in inducing resistance against the early blight pathogen and plant growth in tomato.

2. MATERIALS AND METHODS

2.1 Collection of the seed sample

Tomato seed samples susceptible (PKM1) to the early blight pathogen were collected from the Local Sri Venkateshwara ago traders. The collected seed sample was washed thoroughly under tap water and surface sterilized using sodium hypochlorite solution (2 min) followed by 2-3 rinses with sterile distilled water (SH_2O) and used throughout the study.

2.2 Isolation and identification of Alternaria solani

The early blight disease-infected tomato plants (leaves and stem) were collected from agricultural fields and subjected to pathogen isolation. The collected samples were washed with tap water and subjected to surface sterilization using sodium hypochlorite solution (1%) followed by 2-3 washes with sterile distilled water (SH_2O). The sterilized samples were incised into small pieces (1-2 cm) and placed on Petri dishes containing three layers of moistened blotter discs (previously sterilized) and incubated for seven days at 25 ± 2 °C. The incubated samples were visualized under a stereomicroscope. The fungal colonies showing typical sporulating structures of *A. solani* were subcultured on Petri plates with potato dextrose agar (PDA) medium aseptically supplemented with chloramphenicol (200 µg L^{-1}) and incubated for seven days. The isolated pathogen was further identified based on morphological, cultural and conidial characters). Besides, molecular identification of the pathogen was carried out by extracting genomic DNA by CTAB method. [32]and subjecting to amplification using universal primer sets (ITS-1 and ITS-4) in an Eppendorf's Thermal Cycler (Germany) following the conditions as mentioned in previous studies.[33] The PCR amplified products were electrophoretically detected on agarose gel [1.5% (w/v)] containing 0.5 µg mL^{-1} of ethidium bromide and were subjected to sequencing. The obtained sequence analysis was deposited to GenBank, NCBI and Accession number was acquired).

2.3 Pathogenicity of A. solani

The susceptible (PKM-1) tomato plants (30-day old) grown under greenhouse conditions were challenged inoculated with the conidial suspension of *A. solani* ($5x10^4$ conidia ml^{-1}) until runoff. They were monitored daily for the development of typical symptoms of early blight viz., black or brown lesions that enlarge with time surrounded by yellow halo up to 15 days post-inoculation. Also, detached leaf assay was performed on leaves collected from health plants as mentioned above. The collected leaves were placed on moistened sterile blotter discs in Petri dishes and were pricked with a sterile needle. To the pricked region, 100 µL of conidial suspension of the pathogen (100 µL) was injected and subjected to incubation for seven days 25 ± 2 °C. The development of sporulation on the injected leaves confirmed the pathogenicity. Plants (mock-inoculated) and leaves (injected) with SH_2O served as control.

2.4 Selection of medicinal plants

A total of five locally available medicinal plants *viz*., *Solanum macranthum*, *Dillenia indica*, *Simarouba glauca*, *Cissus quadrangularis* and *Crescentia alata* were selected based on their ethnopharmacology for the isolation of endophytes from their leaf tissues. All the selected plants were identified with the help of a Taxonomist, Department of Studies in Botany, University of Mysore, Mysuru and validated with Flora of Presidency of Madras. [34]The healthy leaves of each

plant were collected and immediately processed for isolation of endophytic fungi.

2.5 *Isolation and identification of endophytic fungi*

The collected healthy leaves were washed thoroughly, followed by sterilization with ethanol (70% for 2 min), NaOCl (4% for 2 min) and final washes with Sterilized H_2O to remove sterilants' traces. The sterilized leaf samples were cut into small pieces (1×1 cm) and placed on Petri plates containing PDA medium and incubated for 2 to 3 weeks at 25 ± 2 °C. The effectiveness of surface sterilization was authenticated by the imprint method, according to Sculz et al[35]A total of 200 segments from each plant were screened to isolate endophytic fungi. The incubated Petri plates were visualized to develop any fungal colonies and colonization frequency (CF) of developed colonies were calculated.[36] The emerging fungal colonies were subcultured onto Petri plates containing PDA to obtain pure cultures and identified on their morphological and cultural characters.

$$Colonization\ Frequency\ (\%) = \frac{Number\ of\ Segments\ Colonized\ by\ Fungus}{Total\ number\ of\ segments}\ X\ 100$$

2.6 *In vitro* antagonism of endophytic fungi

The dual culture technique was used to determine all the isolated endophytic fungi' antagonistic activity against *A. solani*. In brief, about 6 mm discs of each of the isolated endophytic fungi and the pathogen were placed individually against each other 1 cm apart from the periphery of the 90 cm Petri plates containing PDA medium.[38] The Petri plates on PDA medium containing 6 mm discs of pathogen and the agar plug served as control. Each of the inoculated plates was subjected to incubation at 25 ± 2 °C for seven days and the percent inhibition was calculated.

$$Inhibition\ Percentage\ (I) = \frac{R1 - R2}{R1}\ X\ 100$$

Where R1 is the colony radius of *A. solani* in control and R2 is the colony radius of *A. solani* towards endophytic fungus.

2.7 *Evaluation of antagonistic endophytic fungi for pathogenicity and its molecular identification*

Pathogenicity of the antagonistic endophytic fungi was determined according to Koch's postulates (https://phytopath.ca/wp-content/uploads/2014/09/What-are-Koch.pdf). The genomic DNA isolated (CTAB method) from each antagonistic and non-pathogenic endophytic fungi were further identified based on molecular characterization. The isolated genomic DNA was amplified using universal primer sets (ITS-1 and ITS-4) as mentioned above and the obtained sequences were deposited at GenBank, NCBI to acquire accession numbers.

2.8 *Preparation of inducer and seed treatment*

Each of the antagonistic and non-pathogenic endophytic fungi grown on Petri plates containing PDA medium (10 day-olds) was amended with 10 mL of Sterile H_2O. The conidia produced were dislodged aseptically from the culture medium using a sterile scalpel and brush and the concentration was maintained at $1x10^8$ CFU mL^{-1} using a Haemocytometer.[1] The surface-sterilized seeds (PKM-1) were treated with conidial suspension ($1x10^8$ CFU mL^{-1}) of each of the selected endophytic fungi by keeping it in a rotary shaker (100 rpm) for 3 and 6 h, respectively at 25 ± 2 °C. After treatment, the seeds were air-dried aseptically and used for further studies. The susceptible seeds treated with SH_2O served as control.

2.9 *Effect of seed treatment with inducers in tomato*

2.9.1 *Seed germination and seedling vigor*

The inducer treated and untreated tomato seeds were equidistantly placed on Petri dishes containing three layers of moistened sterile blotter discs to evaluate seed germination. A set of seeds from all the treatments was subjected to between paper methods to evaluate seedling vigor.[38] The treatments were incubated for 14-days at 25 ± 2 °C and percent seed germination and seedling vigor were calculated accordingly. Each experiment consisted of four replicates of 400 seeds each.

$$Percent\ Seed\ Germination = \frac{Total\ number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ plated} \times\ 100$$

Vigor index = [Mean Root Length (cm) + Mean Shoot Length (cm)] × Per cent seed

2.9.2 *Evaluation of plant growth parameters*

The efficacy of seed treatment on vegetative and reproductive growth parameters was evaluated under greenhouse conditions. The treated and control seeds were sown in earthen pots containing sterilized potting medium (2:1:1 of soil: sand: farmyard manure) and maintained under greenhouse conditions with regular watering (with 85% relative humidity and 25 ± 2 °C). Each of the treatments consisted of four replicates of 10 plants. At the end of 45 days of sowing, the plants were uprooted carefully without damage to assess the vegetative growth parameters (plant height, shoot fresh and dry weight). In another set of plants, the days for flowering were recorded and the fruit weight was taken for the first harvest.

2.9.3 *Disease protection*

The three-week-old tomato plants (both treated and untreated) grown under greenhouse conditions were

challenge inoculated with the conidial suspension of *A. solani* (5×10^4 CFU mL^{-1}) by hand spray till runoff.[39] Each of the treatments consisted of four replicates of 10 plants. The challenge inoculated plants were maintained under greenhouse conditions and were observed daily for the development of early blight disease symptoms. Each plant was observed daily for the development of early blight disease symptoms. At the end of 21-days of post-inoculation, percent disease protection was calculated.

$$Disease\ Protection\ (\%) = \frac{Number\ of\ infected\ plants}{Total\ number\ of\ plants\ evaluated} \times 100$$

3. STATISTICAL ANALYSIS

The data obtained from different experiments were statistically analyzed separately and subjected to analysis of variance (ANOVA) using the SPSS version. The effect of treatment was determined by F value ($p \leq 0.05$). Mean values were separated by Tukey's honest significant difference (HSD) test.

4. RESULTS

4.1 Isolation and identification of endophytic fungi

A total of one thousand leaf segments (200 × 5 plants) of medicinal plants were screened for isolation of endophytic fungi. The results of the screening revealed a total of 78 endophytic fungi were associated with the plant tissue.) with an isolation rate ranging between 5 to 11% (Table1). The isolated endophytic fungi were classified into thirteen genera of *Chaetomium* sp., *Penicillium* sp., *Helminthosporium* sp., *Fusarium* sp., *Curvularia* sp., *Xylaria* sp., *Corynascus* sp., *Alternaria* sp., *Colletotrichum* sp., *Pestalotiopsis* sp., *Acremonium* sp., *Aspergillus* sp., *Trichoderma* sp., apart from *Mycelia* sterilia. The highest isolation rate (11%) of fungal endophytes was noticed in *Solanum macranthum* followed by 8.5% observed in *Crescentia alata*. It was also observed that, among the isolated endophytic fungi, *Aspergillus* sp., was found to be the dominant fungi (89.74%) while *Colletotrichum* sp. was the least (6.41%) associated among the plants evaluated (Table1).

4.2 In vitro antagonism of endophytic fungi

All the isolated endophytes were screened for their antagonistic nature towards the growth of *A. solani* by the dual culture method. From the results of the study, it was observed that five endophytic fungi were able to antagonize the test pathogen, while others did not (Fig. 1). The maximum (58.53%) and minimum (41.46%) percent inhibition was observed against *Pestalotiopsis* sp. ENSM-08 and *C. sependonium* ENSG-45, respectively (Table 2). Further, only the antagonistic endophytic fungi were carried forward for subsequent studies.

4.3 Evaluation of antagonistic endophytic fungi for pathogenicity and its molecular identification

The pathogenicity test for the antagonistic endophytic fungi in tomato plants revealed that all the endophytes were non-pathogenic in nature (Table 2). Further, PCR amplification with a universal set of primers (ITS-1 and ITS-4) of the selected fungi showed bands ranging from 500 to 600 base pairs. The amplified product was subjected for sequencing and the obtained data were analyzed with GenBank database for identifying the similarity using nBLAST and results confirmed the isolates ENSM-08, ENSM-15, ENDI-36, ENSG-42 and ENSG-45 as *Pestalotiopsis microspora*, *Penicillium griseofulvum*, *Colletotrichum* sp., *Fusarium* sp. and *Corynascus sependonium*, respectively. Also, the obtained sequence was submitted to GenBank, NCBI, and accession numbers were acquired ().

4.4 Effect of seed treatment with inducers in tomato

4.4.1 Seed germination and seedling vigor

The seed treatment of selected endophytes significantly enhanced the seed growth parameters compared to untreated seeds at both the time points evaluated except for ENSG-42 and ENSG-45 treatments (Table 3). Among the five endophytes evaluated for seed treatment, *Pestalotiopsis* sp. treated seeds for 3 h offered the highest seed germination 85.50%, and seedling vigor 1072.43 compared to all other treatments followed by treatment with *Penicillium griseofulvum* and *Colletotrichum* sp., respectively (Fig. 2). Likewise, no significant enhancement in seed germination and vigor was noticed between the treatments but in some treatments, the seed growth parameters decreased with an increase with the duration of the treatment. The untreated control plants showed 75.0% of seed germination and 752.32 seedling vigor. From the results, it was noted that, as the seed treatment with inducers for 3 h was effective compared to 6 h treatments, further studies were carried upon 3 h seed treatment.

4.4.2 Evaluation of plant growth parameters

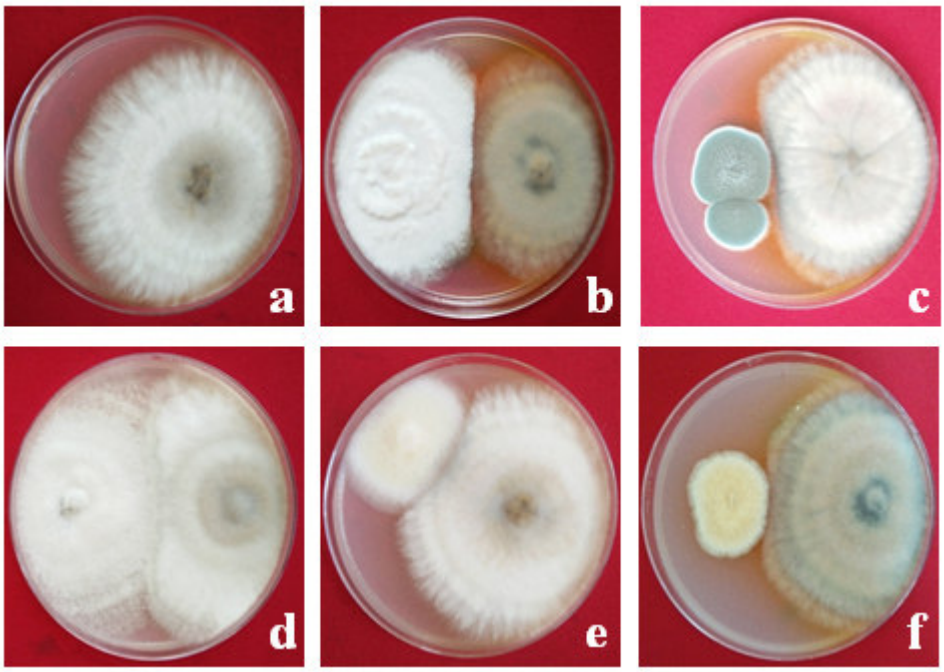
The vegetative and reproductive growth parameters evaluated in tomato plants maintained under greenhouse conditions, raised upon inducer treatment showed significant ($p \leq 0.05$) enhancement in all the test parameters compared to control plants. Among the endophytic inducers evaluated, *Pestalotiopsis* sp. treated plants showed the highest record in plants' height (70.40 cm), fresh (54.13 g) and dry weight (4.12 g) of shoot followed by plants treated with *P. griseofulvum*. In addition, *Pestalotiopsis* sp. treated plants also showed early flowering (earlier by 10 days) and a maximum number of fruits and its weight compared to all other endophytic treatments (Table 4).

4.5 Disease protection

The effect of endophytic fungal treatment in tomato plants upon challenge inoculation with the pathogen *A. solni* was evaluated after 21-days of post-inoculation. The results of the study revealed that all the treatments were able to induce disease resistance significantly upon their treatment. During the examination of plants, it was observed that *P. microspora* seed treatment effectively induced disease resistance with 70.25% protection followed by the treatment of *P. griseofulvum* which offered 65.5% protection against the invading pathogen. The SH_2O treated plants showed a disease incidence of 98.75% (Table 5).

Table 1. Fungal endophytes associated with selected medicinal plants

Endophytes	*S. macranthum*	*D. indica*	*S. glauca*	*C. quadrangularis*	*C. alata*	Colonization Rate (%)	Dominant Fungi (%)
Chaetomium sp.	2	1	-	-	-	1.5	19.23
Penicillium sp.	3	1	2	3	1	5	64.10
Helminthosporium sp.	-	2	-	1	-	1.5	19.23
Fusarium sp.	4	1	1	3	2	5.5	70.51
Curvularia sp.	2	1	-	1	-	2	25.64
Xylaria sp.	1	-	-	1	2	2	25.64
Corynascus sp.	-	2	1	1	1	2.5	32.05
Alternaria sp.	-	1	2	-	3	3	38.61
Colletotrichum sp.	-	-	-	-	1	0.5	6.41
Pestalotiopsis sp.	2	1	-	-	-	1.5	19.23
Acremonium sp.	1	-	-	1	1	1.5	19.23
Aspergillus sp.	4	4	1	2	3	7	89.74
Trichoderma sp.	-	-	2	1	-	1.5	19.23
Sterile mycelia	3	-	1	1	3	4	51.28
Total No. of isolates	22	14	10	15	17	7.8	
Isolation Rate (%)	**11**	**7**	**5**	**7.5**	**8.5**		



a-Alternaria solani **b-** ***Pestalotiopsis microspora*****; c-** ***Penicillium griseofulvum*****;**
d- ***Colletotrichum sp.*** **; e-** ***Fusarium*** **sp.; f-** ***Corynascus sependonium***

Fig 1. Antagonistic nature of fungal endophytes against *A. solani*

Table 2. In vitro antagonism and pathogenicity of fungal endophytes

Endophytic Fungi	Code	*Antagonism (%)	#Pathogenicity
ENSM-08	*P. microspora* ENSM-08	58.33 ± 0.53^{a}	-
ENSM-15	*P. griseofulvin* ENSM-15	48.78 ± 0.74^{b}	-
ENDI-36	*Colletotrichum* sp. ENDI-36	46.34 ± 0.62^{bc}	-
ENSG-42	*Fusarium* sp. ENSG-42	43.90 ± 0.53^{c}	-
ENSG-45	*C. sependonium* ENSG-45	41.46 ± 1.2^{cd}	-

Values are means of four independent replicates (n=4) and ± indicate standard errors. Mean values followed by the same letter(s) within the same column are not significantly ($p \leq 0.05$) different according to Tukey's HSD. *Antagonistic to A. solani; #Pathogenic to susceptible tomato plants. '+' indicates positive and '–' indicates negative for the experiments.

Table 3. Effect of seed treatment with fungal endophytes on seed germination and seedling vigor

Endophytes	Seed Treatment in hours	Seed Germination (%)	Seedling Vigour
ENSM-08	3	85.50±0.64^{a}	1072.43 ±0.25^{a}
	6	84.0±0.40^{ab}	1031.03 ±9.23^{b}
ENSM-15	3	83.0±0.57^{bc}	951.41± 2.21^{c}
	6	82.0±0.70b^{cd}	916.32± 2.08^{d}
ENDI-36	3	81.0±0.40^{cd}	892.92 ±3.82^{e}
	6	80.0±0.70^{de}	885.20±5.07^{e}
ENSG-42	3	78.25±0.47^{ef}	817.07± 1.78^{f}
	6	77.0±0.40^{fg}	802.07 ± 1.10^{g}
ENSG-45	3	76.75±0.47^{fg}	769.32 ± 3.94^{h}
	6	76.0±0.40^{fg}	761.07 ± 1.72^{hi}
Control		75.0±0.40^{g}	752.32±2.4^{i}

Values are means of four independent replicates (n=4) and ± indicate standard errors. Mean values followed by the same letter(s) within the same column are not significantly ($p \leq 0.05$) different according to Tukey's HSD.

a - Control; b – ENDI-36; c-ENSM-08; d- ENSG-42; e- ENSG-45; f- ENSM-15

Fig 2: Effect of seed treatment with fungal endophytes on seedling vigour of tomato under *in vitro* conditions.

Table 4. Effect of seed treatment with fungal endophytes on vegetative and reproductive growth parameters in tomato

Treatments	Shoot Length (cm)	Shoot Fresh Weight (gm)	Shoot Dry Weight (gm)	Flowering (in days)	Fruit/ plant	Mean fruit weight (gm)
ENSM-08	70.40±0.77^{a}	54.13±0.83^{a}	4.12±0.56^{a}	52±0.40^{e}	32±0.40^{a}	49.15±0.63^{a}
ENSM-15	59.15±0.82^{b}	48.29±0.53^{b}	3.77±0.17^{ab}	54±0.40d^{e}	28±0.81^{b}	47.21±0.36^{b}
ENDI-36	52.05±0.72^{c}	45.50±0.89^{bc}	3.54±0.17^{cd}	56±0.40^{cd}	26±0.40^{c}	45.20±1.04^{b}
ENSG-42	50.77±0.56^{c}	43.13±1.27^{cd}	3.30±0.12^{bcd}	58±0.81^{bc}	24±0.40^{d}	44.14±0.65^{bc}
ENSG-45	45.12±0.72^{d}	41.60±0.65^{d}	3.00±0.96^{cd}	59±0.70^{a}	23±0.40^{d}	41.20±0.99^{c}
Control	32.80±0.82^{e}	28.12±0.64^{e}	2.94±0.14^{d}	62.0±0.71^{a}	19.0±0.40^{e}	38.03±0.90^{d}

Values are means of four independent replicates (n=4) and ± indicate standard errors. Mean values followed by the same letter(s) within the same column are not significantly ($p \leq 0.05$) different according to Tukey's HSD.

Table 5. Efficacy of seed treatment with fungal endophytes on induction of resistance in tomato plants against early blight disease

Endophytes	Disease Protection (%)	Disease Incidence (%)
ENSM-08	70.25±0.47^{a}	29.75±0.47e
ENSM-15	65.50±0.64^{b}	34.50±0.64^{d}
ENDI-36	62.00±0.40^{c}	38.0±0.40^{c}
ENSG-42	60.25±0.47^{cd}	39.75±0.85^{bc}
ENSG-45	58.75±0.47^{d}	41.25±0.47^{b}
Control	1.25±0.25^{e}	98.75±0.47^{a}

Values are means of four independent replicates (n=4) and ± indicate standard errors. Mean values followed by the same letter(s) within the same column are not significantly ($p \leq 0.05$) different according to Tukey's HSD

5. DISCUSSION

Biocontrol strategies through the application of microorganisms are gaining importance from the present-day usage of chemical pesticides as they are causing severe damage to the soil. Among the microorganisms of importance, endophytic fungi are gradually gaining importance in biocontrol strategies as they are previously known to produce many secondary metabolites of importance to human welfare in various fields.[40,13,11,15] Hence, in the study endophytic fungi isolated from some of the medicinally important plants and screened for their effectiveness against the induction of resistance against the early blight disease and plant growth promotion in tomato. A total of five medicinal plants namely *S. macranthum*, *D. indica*, *S. glauca*, *C. quadrangularis* and *C. alata* were collected from the Mysore region and 78 endophytic fungi were isolated following the imprint method. All the isolated endophytes were identified up to their generic level based on morphological, cultural, and conidial characters. Similarly, many researchers have used the same method in order to isolate endophytic fungi from many medicinally important plants.[41-44,17,7] Further, all the isolates were evaluated for their antagonistic nature against the early blight pathogen *A. solani* by the dual culture method. From the results, it was noted that only five endophytic fungi revealed against the test pathogen with a maximum inhibition of 58.33% offered by *P. microspora*. It has been observed from the literature that many endophytic fungi have offered antagonistic nature towards phytopathogens viz., *Alternaria solani, A. alternata, Colletotrichum capsici, Fusarium solani* and *Pythium aphanidermatum*, *Phytophthora infestans*, etc. with a varied percentage of inhibition which was evaluated by dual culture method[44-45],[14] The antagonistic nature towards the pathogen offered by endophytes is attributed towards the production of antibiotics/ biologically active metabolites or through secretion of hydrolytic enzymes at the cell wall.[41,42,15] It has been observed that even though the endophytes are known for their mutualism to that of a symbiotic relationship with the host plants. There are reports that, eventhough they are endophytes some of them are known to cause pathogenicity upon application to other hosts.[46] and hence pathogenicity of the isolated endophytes should be conducted before their application to any other plants. From the study it was noted that all the antagonistic endophytes were non-pathogenic to tomato plants. Parallel studies have been conducted by [47] wherein, the isolated endophytic fungi were checked for their pathogenicity and also before application as biocontrol agents.[48] After confirmation of both antagonistic and pathogenicity of the selected endophytes, they were identified based on ITS regions (ITS1 and ITS4) which are in employed as a regular tool for the identification of fungi studies of .[43] The antagonistic and non-pathogenic fungal endophytes were further evaluated for their effectiveness against plant growth and induction of resistance against early blight disease in tomato upon seed treatment. From the results of seed, vegetative and reproductive plant growth parameters studies it was observed that all the endophytes were able to significantly enhance the test parameters except ENSG-42 and ENSG-45. In corroboration with the findings of the study, seed treatment with conidial suspension of endophytic fungi isolated from medicinal plants was able to enhance seed germination, seedling vigor, vegetative and reproductive plant growth parameters in sorghum and turmeric plants. [14,7] The beneficial nature towards the enhancement in seed, vegetative and reproductive plant growth parameters from microbial endophytes is mainly correlated to the production of plant hormones and antimicrobial compounds, therefore, helping the plants in nutritional improvement which positively impacts plant growth.[49,50,17,15] In addition to plant growth-promoting studies, the application of fungal endophytic seed treatment was also able to induce disease resistance against the early blight pathogen in tomato. It was observed that a maximum of 70.25% disease protection was observed in tomato plants upon application of *P. microspora* compared to other isolates evaluated. In agreement with the results of the present study [16,14] have reported the potential disease protection against *Rhizoctonia solani* and *Pythium aphanidermatum* in potato and turmeric plants upon the application of endophytes. In addition, endophytes isolated from a different host (tomato, mangrove, star arise, and agarwood) apart from their application plant (cucumber) have also helped in the induction of resistance against the invading pathogen *Fusarium oxysporum* f.sp. *cucumerinum* the causal agent of wilt disease.[26] The mechanism of induced resistance offered by these endophytic fungi involves antagonism, mycoparasitism, competition apart from the production of hormones/ bioactive metabolites that induce increased immunity of the host eventually resulting in protection against the invading pathothogens.[17,15,7]

6. CONCLUSION

The present work reveals the efficacy of the fungal endophytes isolated from medicinal plants as plant growth promoters and also as biocontrol agents against the early blight pathogen in tomatoes. In the study, a total of 78 fungal endophytes were isolated from five different medicinal plants out of which five isolates were found antagonistic against *A. solani* , the causal agent of early blight disease in tomatoes. In addition, all the endophytic fungi were able to enhance vegetative and reproductive plant growth parameters compared to control plants apart from inducing resistance against the early blight pathogen under greenhouse conditions. The results of the study validate the application of fungal endophytes as inoculants for sustainable agriculture.

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8. AUTHORS CONTRIBUTION STATEMENT

Ms. Sujatha H.S. conducted experiments and gathered data. Dr. Murali M analysed these data and design this manuscript. Prof. K.N. Amruthesh discussed the methodology and results, suggested for necessary inputs to finalise the manuscript

9. CONFLICT OF INTEREST

Conflicts of interest declared none.

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