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EFFICACY OF FUNGAL ENDOPHYTES AGAINST PURPLE BLOTCH DISEASE OF ONION [*Alternaria porri* (Ellis) Cifferi]

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ABSTRACT :

Onion belongs to genus *Allium* belongs to family Amaryllidaceae, used for both domestic and commercial purposes. Globally onions are the top most export commodity among vegetables severe to many fungal diseases which leads to heavy yield loss. Out of which Purple Blotch is a major disease caused by *Alternaria porri,* require temprature and humidity for disease development is 24± 2°C; 80-90 per cent, respectively. The present study aimed to check Efficacy of Fungal Endophytes against Purple Blotch disease of Onion {*Alternaria porri* (Ellis) Cifferi} through *in vitro* and *in vivo*, Rabi season, Division of Plant pathology, P.G. Department of Agriculture, Khalsa College Amritsar. Total nineteen fungal endophytes were isolated from healthy and fresh onion plants collected from different locations of Punjab. Four isolates of fungal endophytes among nineteen proved to be most efficient. During *In vitro* studies, maximum and minimum mycelial inhibition was shown by isolates LE1 and RE2 by 77.30 and 45.62 per cent, respectively. While during Pot and Field trials, maximum and minimum disease control by isolates LE1 and RE2 with 75.20 and 74.30 per cent and 54.85 and 58.95 per cent, respectively. In Molecular identification, the isolates LE1, RE1, LE3 and RE2 were identified on the basis of ITS sequencing and named as *Aspergillus niger* strain 7M1, *Fusarium solani* voucher JUF0036, *Aspergillus niger* isolate MEBP0060, and *Fusarium sp.* isolate JP36, respectively while the Phylogenetic tree revealed that isolates LE1 and LE3 are homologous to each other while isolates RE1 and RE2 are present in different clades.

Keywords: Onion, Purple blotch, *Alternaria porri*, Endophytic fungi, Molecular identification.

INTRODUCTION

Monocotyledonous crop Onion (*Allium cepa* L., 2n=16) belongs to the family Amaryllidaceae which is a biennial bulbous crop introduced to India from Palestine. Due to its characteristic flavour, high aroma and its medicinal properties it is also called Queen of Kitchen(Pareek *et al.*, 2017; Marrelli *et al*., 2019). It is used in several ways in the kitchen for culinary applications including frozen, dehydrated bulbs, fresh and green bunching varieties."Allyl-propyl-di-sulfide" is the compound present in onions which is characteristics quality of onion for its flavour and pungency (Kareem *et al*., 2018). It consists of minerals, calcium, iron, sulphur, fiber, ascorbic acid, vitamins, proteins, insulin and various other nutrients (Bektas and Kusek, 2021). Onion bulbs also consists of various anti-inflammatory, anti-cholesterol, anticancer and antioxidant phytochemicals such as 'quercetin' which is also known to reduce the risk of cardiovascular diseases (Slimestad *et al*., 2007). Furthermore, Onion possesses some fungicidal and insecticidal properties (Mishra *et al.*, 2014). Purple blotch disease is highly prevalent in all *Allium* growing countries.The optimum temperature for purple blotch falls to 24± 2°C with relative humidity 80 to 90 per cent (Yadav *et al*., 2017). Primary infection is observed on older leaves with purplish, distinctive, lesions like a concentric ring. Endophytes are microorganisms that live within the tissues of plants without causing any apparent harm to the host. Some endophytes have shown the ability to suppress the growth of pathogens and provide protection against diseases. Endophytic fungi on *Allium* crops have demonstrated their potential to enhance plant growth and suppress pathogenic fungi (Xiong *et al*., 2017).

MATERIALS AND METHODS

The present investigation entitled, **"**Efficacy of Fungal Endophytes against Purple Blotch disease of Onion [*Alternaria Porri* (Ellis) Cifferi]**"** was carried in laboratory and pot experiments were conducted in the Division of Plant Pathology at the P.G. Department of Agriculture and field trials were carried out at Student's Research Farm, Khalsa College, Amritsar. The methods adopted during study are elaborated under following headings.

Isolation of pathogen

Infected leaf samples were collected and washed with sterilized water, cut into small bits of 1cm^2 along with healthy parts using a sterilized blade, then small bits were surface sterilized with 1% sodium hypochlorite solution for 60 seconds further washed three times with sterile distilled water. Bits were

placed on sterilized filter paper to remove the excess moisture and transferred to Petri dishes as well as slants containing PDA medium under aseptic conditions. Then, after 7 inocubation slants were observed regularly for fungal growth. Isolates were stored in a refrigerator at 4°C until further use

Isolation of endophytes

Endophytes were isolated from fresh and healthy onion leaves and roots of plant. The selected plant material was brought to the laboratory in sealed sterile bags, gently rinsed under running water to remove dust and debris. Samples were cut into small $1cm^2$ pieces, surface sterilized by dipping in 70% ethanol for one minute then soaked in 4% sodium hypochlorite for 60 seconds followed by rinsing twice in sterilized distilled water. Remove excess moisture and infected samples were transferred under aseptic conditions to Petri dishes containing PDA medium. Petri dishes were incubated at 25±2°C for 7 days for endophytic fungal growth. Hyphal tip method was used to obtain pure cultures of mixed endophytic fungal isolates. These cultures were maintained in a Refrigerator at 4°C onto petri dishes and further identified on the basis of cultural characteristics.

In vitro Antagonism of fungal endophytes against Alternaria porri

Seven-day-old culture discs, each 4mm in diameter of the fungal antagonists were selected and the pathogen were taken from the margin of the actively growing culture using a cork borer and transferred to solidified PDA in Petri dishes. Fungal antagonists were evaluated by inoculating the pathogen on one side of the plate with respect to antagonist on the opposite side along with control with thrice replication. Petri plates were incubated at 25±2°C in the BOD incubator. Mycelial diameter was recorded after 8 days of incubation and per cent, inhibition over control was worked out according to the equation of Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

- I = Per cent inhibition of the mycelium
- C = Growth of the mycelium in control
- T = Growth of the mycelium in treatments

Evaluation under pot conditions (Root dip treatment and foliar application)

The roots of two-month-old onion seedlings (variety N53) were dipped in a suspension of selective strains of endophytic fungi (2×10^6 cfu/ml) for half an hour prior to transplanting in the pots. The pots of size 4" diameter were filled with 10 kg of autoclaved sterilized soil mixed with 2.5 kg of FYM to prepare the potting mixture of a 3:1 ratio followed by sterilization with 5% formaldehyde solution. Subsequently, the treated seedlings were transplanted into pots and each treatment was replicated thrice. For control, the roots of the seedlings were only dipped in sterile distilled water. After three weeks of transplanting of seedlings, two foliar applications of different endophytic suspension were applied at an interval of 15 days before the addition of pathogen inoculation. Then plants were sprayed with spore suspension of pathogen culture after 15 days of the second application of endophytic fungi and per cent disease incidence and per cent disease control (PDC) were recorded after 15, 30, 45 and 60 days of pathogen inoculation. Further it was also observed that root dip treatment with fungal endophytes resulted in prolonging the incubation period.

Evaluation under field conditions (Root dip treatment and foliar application)

The roots of two-month-old onion seedlings (variety N53) were dipped in a suspension of selective strains of endophytic fungi (2×10^6 cfu/ml) for half an hour before transplanting into the field. No endophytic cultures were used as a control. Each treatment was replicated thrice. After three weeks of transplanting the seedlings, when the seedlings were well established, two foliar applications of endophytic suspension were applied at an interval of 15 days before the addition of pathogen inoculation. The spore suspension was evenly sprayed onto the onion seedlings with a small hand sprayer. The plants were challenged 15 days after the second application of endophytic fungi by spraying spore suspension of the culture of pathogen *Alternaria porri* followed by irrigation. Disease incidence were recorded after 15, 30, 45 and 60 days of pathogen inoculation.

Molecular Identification of most Efficient Fungal Endophytes

The most efficient isolates of endophytic fungi were identified on the basis of molecular characteristics. Based on an *in vitro* screening for various antagonistic traits, the four most promising cultures of selected fungal endophytes were outsourced to Barcode Biosciences under refrigerated conditions using gel packs and a thermocol box for sequencing of ITS region. All sequences generated in this study were aligned to the NCBI database sequences to analyze the variability of selected isolates of endophytic fungi at the sequence level.

Statistical Analysis

The experimental data obtained from laboratory, pot, and field experiments were analyzed statistically using standard statistical procedures and interpretation of the data as described by Gomez and Gomez (1984). The significance of the differences among the examined endophytes was

tested by Analysis of Variance (ANOVA). Critical differences (CD) among the treatments in laboratory experiments was calculated using the Completely Randomized Design (CRD) while in Pot and Field, Randomized Block Design (RBD) was used for analysis of data at a 5 per cent level. While in molecular identification of fungal endophytes, the Phylogenetic tree was aligned through T-coffee multiple sequence alignment software.

RESULTS AND DISCUSSION

The results obtained during the investigation are presented and discussed below.

Isolation and identification of Alternaria porri

The pathogen was identified as *Alternaria porri* on the basis of cultural, microscopic characters and pathogenicity test of fungus. Variation in the colony color ranged from grey to light grey, texture of colony varied from coarse to smooth, surface topography appeared to be fluffy to smooth which was radial towards the margin having raised center with regular and irregular margin with shiny to dull luster. Two types of septation horizontal and vertical are noticed in spores of *Alternaria porri.* With respect to septation a maximum of six horizontal and two vertical septa are noticed. Conidia of pathogen were club shaped with or without beak which were light to deep brown in colour and were borne singly at the apex of conidiophore. During pathogenicity test, after four days of inoculation small and white colored elliptical lesions appeared on older leaves. In later stages, the lesions enlarged and appeared as water-soaked spots which turned brown to purple in colour surrounded by a yellow halo. After 8 DAI, leaf tips started to dry out and fell down, whereas control plants remained healthy.

Isolation of Fungal Endophytes

Healthy onion plants collected from different locations of Amritsar, Gurdaspur and Tarn Taran were used for isolation of endophytes. Maximum colonization frequency of (47.36 %) was recorded from Amritsar district followed by Tarn Taran (31.58 %) and least colonization frequency was registered from Gurdaspur (21.05 %). A total of 19 endophytes were isolated from onion plants. Out of 19 isolates, 12 were leaf endophytes, 7 were root endophytes. The predominant fungal endophytic colonies were selected, purified, and the pure culture of each isolate was maintained at 4°C on PDA media for further studies.

Cultural characterization of fungal endophytes

The data revealed that colony development of different isolates of fungal endophytes (designated as LE1, LE3, RE1 and RE2) exhibited great variation with respect to cultural characters and microscopic examination. The colour of fungal colonies ranged from creamish white to green. The mycelium of all the isolates was hyaline, and septate, however, few variations in colony characters were recorded. Significant variations have also been observed with respect to sporulation in different isolates of fungal endophytes. Isolates LE1and LE3 were highly sporulating, whereas, RE1 had exhibited less sporulation however RE2 exhibited very less sporulation on PDA plates.

In vitro antagonism of fungal endophytes against pathogen

The results (Table 1, Plate 1) indicated that the effective isolates of fungal endophytes for their anti-fungal activity were tested by dual culture assay. Perusal of data indicated that all the tested fungal endophytes inhibited the mycelial growth of *Alternaria porri*, but degree of inhibition varied with the antagonists. The maximum inhibition of 77.30 per cent was shown by LE1 (*Aspergillus niger* strain 7M1) and followed by LE3 (*Aspergillus niger* isolate MEBP0060) inhibited 73.25 per cent; RE1 (*Fusarium solani* voucher JUF0036) inhibited 65.87 per cent, whereas, RE2 (*Fusarium sp.* isolate JP36) isolate was least effective in inhibiting the mycelial growth of the pathogen i.e. 45.62 per cent. The above results are in line with Khandagale *et al.* (2022) evaluated that fungal bioagents such as *T. harzianum* caused significant maximum inhibition, 90.58 per cent followed by *T. viridae* inhibit 85.58 per cent against *A. porri* under *in vitro* conditions.

Biocontrol activity of fungal endophytes under pot conditions

The data (Table 2) indicated the evaluation of fungal endophytes on incidence of purple blotch in pot conditions with root dip treatment of onion seedlings in respective suspension of endophytes. Among all treatments, seedling treatment with LE1 (*Aspergillus niger* strain 7M1) was highly effective which exhibited minimum disease incidence i.e., 22.97 per cent up to 60 days of pathogen inoculation. Disease incidence was recorded 11.48 and 21.10 per cent at 30 and 45 days after pathogen inoculation, respectively followed by LE3 (*Aspergillus niger* isolate MEBP0060) that exhibited 0.50, 12.75, 21.98 and 23.14 per cent of disease incidence at 15, 30, 45 and 60 days after inoculation respectively; RE1 ((*Fusarium solani* voucher JUF0036) showed disease incidence of 5.98, 14.62, 25.15 and 26.91 per cent at 15, 30, 45 and 60 days after inoculation, respectively while RE2 (*Fusarium sp.* isolate JP36) showed disease incidence of 7.50, 20.22, 34.80 and 36.42 per cent at 15, 30, 45 and 60 days after inoculation respectively. Disease appeared after 30 days of inoculation, and further increased at a slower rate up to 60 days in comparison to control. The overall maximum per cent disease control was exhibited by LE1 isolate (75.20%) followed by LE3 (73.78 %); RE1 (66.84%). However, RE2 (54.85%) was least effective in disease control (Fig. 4.3). Our results obtained are in conformity with the findings of Elshahawy *et al*. (2022) revealed that endophytic fungal isolate *Chaetomium* can be used to control Late wilt disease caused by *Cephalosporium maydis*. In pots, Chg- I treatments significantly reduced late wilt disease incidence and increase plant growth

Bio-control activity of fungal endophytes in field conditions

The results presented in Table 3, indicated that all effective fungal endophytes tested as root dip treatment under field conditions during Rabi season 2021-22 and 2022-23, significantly reduced the incidence of purple blotch of onion in comparison to control.

In 2021-22, among all LE1 (*Aspergillus sp.* strain 9232) seedling treatment was highly effective which exhibited minimum disease incidence (i.e) 0.00, 14.67, 25.13 and 30.33 per cent at 15, 30, 45 and 60 days after pathogen inoculation respectively followed by LE3 (*Aspergillus oryzae* strain 9177), RE2 (*Penicillium oxalicum* isolate T26) and RE1 (*Fusarium incartanum* strain EX2019- M11) with 1.95, 15.27, 31.33 and 31.67; 9.37, 19.00, 37.78 and 37.83 and 11.80, 27.56, 39.52 and 42.98 per cent respectively. Maximum disease control after 60 days of pathogen inoculation was achieved with LE1 (74.04%). The treatment LE3 was found next best accounting for 70.03 per cent disease control followed by RE1 (64.26 %); RE2 (56.61%). In 2022-23, among all LE1 (*Aspergillus niger* strain 7M1) seedling treatment was highly effective which exhibited minimum disease incidence (i.e) 0.15, 13.95, 27.90 and 29.12 per cent at 15, 30, 45 and 60 days after pathogen inoculation respectively followed by LE3 (*Aspergillus niger* isolate MEBP0060), RE1 (*Fusarium solani* voucher JUF0036) and RE2 (*Fusarium sp.* isolate JP36) with 1.50, 13.95, 28.10 and 30.72; 7.98, 17.74, 35.00 and 36.87 and 10.30, 24.62, 36.92 and 40.63 per cent respectively. Maximum disease control after 60 days of pathogen inoculation was achieved with LE1 (74.30%). The treatment LE3 was found next best accounting for 72.89 per cent disease control followed by RE1 (66.84 %); RE2 (58.95%). Disease appeared mostly after 30 days of inoculation, and further increased at slower rate up to 60 days in comparison to control. Similarly Elshahawy *et al*. (2022) revealed that in field conditions, the endophytes not only reduced late wilt symptoms but also increased ear yield on both maize cultivars when compared to the Untreated control.

Molecular identification

Four most promising endophytes that exhibited significant antagonistic activity under *in vitro*, pot, and field conditions were selected for their molecular identification. The result (Plate 2, Plate 3, Fig. 1) indicated that the isolate LE1 showed sequence homology of *Aspergillus niger* strain 7M1 while isolate LE3 showed sequence homologous with *Aspergillus niger* isolate MEBP0060. The isolate RE1 were 100 per cent sequence homologous with *Fusarium solani* voucher JUF0036 and RE2 showed homology with *Fusarium sp.* isolate JP36. All the sequences of different isolates were deposited in the NCBI database. Phylogenetic tree analysis indicated that the fungal isolates LE1 (*Aspergillus niger* strain 7M1) and LE3 (*Aspergillus niger* isolate MEBP0060) were branched in a clade and showed homology, whereas, isolates RE1 (*Fusarium solani* voucher JUF0036) and RE2 (*Fusarium sp.* isolate JP36) were in different clades.The result is in agreement with the study conducted by Wang *et al*., (2023). He isolated 31 fungal strains from healthy Welsh onion leaves and assigned them to nine fungal genera using morphological and molecular characterization based on DNA sequencing data obtained from nuclear internal transcribed spacer (nrITS) (fungi).

REFERENCES

1. Bektas I and Kusek M (2021) The isolation and characterization of phosphate solubilizing bacteria from the onion rhizosphere and their effect on onion growth. *KSU Journal of Agriculture and Nature* **24**: 1084-1092.
2. Elshahawy I E and Khattab A E N A (2022) Endophyte *Chaetomium globosum* improves the growth of maize plants and induces their resistance to late wilt disease. *Journal of Plant Diseases and Protection* **129**: 1125-1144.
3. Gomez K A and Gomez A A (1984) Statistical Procedures for Agricultural Research. 2nd ed. John Wiley and Sons New York, USA 680p.
4. Kareem M A, Bhat A S, Kueubetta K D, Mesta R K, Allolli T B, Ajjappalavar P S, Shweta K, Masuthi D and Waseem M A (2018) Evaluation of Silicon and Bio-Formulations for The Management of Purple Blotch Disease Of Onion. *Research Journal of Chemical and Environmental Sciences* **6**: 46-50.
5. Khandagale P, Suryawanshi K, Patil D and Arote B (2022) *In vitro* management of purple blotch of onion (*Alternaria porri*) by new fungicides and bioagents. *Asian Journal of Microbiology Biotechnology & Environmental Sciences* **24**: 262-268.
6. Marmath K K, Giri P, Sharma S, Taj G, and Kumar A (2013) In-silicon interaction studies of *Alternaria brassicae* toxin destruxin B and potential partners of MAPK4 cascade. *International Journal of Agriculture Environment and Biotechnology* **6**: 203-210.
7. Marrelli M, Amodeo V, Statti G and Conforti F (2019) Biological Properties and Bioactive Components of *Allium cepa* L. : Focus on Potential Benefits in the Treatment of Obesity and Related Comorbidities. *Molecules* **24**: 119.
8. Mishra R K, Jaiswal R K, Kumar D, Saabale P R and Singh A (2014) Management of major diseases and insect pests of onion and garlic: A comprehensive review. *Journal of Plant Breeding and Crop Science* **6**:160-170.
9. Pareek S, Sagar N A, Sharma S and Kumar V (2017) Onion (*Allium cepa* L.). *Fruit and Vegetable Phytochemicals: Chemistry and Human Health, 2nd Edition* 1145-1162.
10. Slimestad R, Fossen T and Vagen I M (2007) Onions: A source of unique dietary flavonoids. *Journal of Agriculture Food and Chemistry* **55**: 10067-10080.
11. Vincent J H (1947) Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* **15**: 580.
12. Wang J-Y, Jayasinghe H, Cho Y-T, Tsai Y-C, Chen C-Y, Doan H K and Ariyawansa H A (2023) Diversity and Biocontrol Potential of Endophytic Fungi and Bacteria Associated with Healthy Welsh Onion Leaves in Taiwan. *Microorganisms* **11**: 1801.
13. Xiong C, Li Q, Li S, Chen C, Chen Z and Huang W (2017) *In vitro* antimicrobial activities and mechanism of 1-octen-3-ol against food-related bacteria and pathogenic fungi. *Journal of Oleo Science* **66**: 1041-1049.
14. Yadav R K, Singh A, Jain S and Dhatt A S (2017) Management of purple blotch complex of onion in Punjab. *International Journal of Applied Sciences and Biotechnology* **5**: 454-465.

Table 1. *In vitro* antagonism of Fungal Endophytes against *Alternaria porri*

Isolates	**Mycelial growth of pathogen (mm)**	**Mycelial growth inhibition by Endophytes (%)**
LE1 (*Aspergillus niger* strain 7M1)	18.16	77.3
RE1 (*Fusarium solani* voucher JUF0036)	23.45	65.87
LE3 (*Aspergillus niger* strain GFR13)	21.4	73.25
RE2 (*Fusarium sp.* strain S42)	43.5	45.62
Control	80	–
CD $_{0.05}$	2.636	2.736
SE (m)	0.796	0.826

Plate 1: *In vitro* efficacy of Fungal Endophytes against *Alternaria porri*

A **B** **C** **D** **E**

A. LE1 (*Aspergillus niger* strain 7M1) B. LE3(*Aspergillus niger* strain GFR13)
C. RE1 (*Fusarium solani* voucher JUF0036) D. RE2 (*Fusarium sp*. strain S42)
E. Control

Isolates / Intervals	**Disease incidence (%) after pathogen inoculation in days**					**Percent Disease Control (%)**
	15	**30**	**45**	**60**	**Mean**	
LE1 (*Aspergillus niger* strain 7M1)	0.00	11.48	21.10	22.97	13.89	75.20
RE1 (*Fusarium solani* voucher JUF0036)	5.98	14.62	25.15	26.91	18.17	66.84
LE3 (*Aspergillus niger strain GFR13*)	0.50	12.75	21.98	23.14	14.59	73.78
RE2 (*Fusarium sp*. strain S42)	7.50	20.22	34.80	36.42	24.74	54.85
Control	12.60	28.00	52.10	54.80	–	–
CD $_{0.05}$	2.172	2.251	2.08	1.636	–	–
SE(m)	0.616	0.638	0.59	0.464	–	–

Table 2. Efficacy of Fungal Endophytes against Purple Blotch of Onion under Pot conditions

Table 3. Efficacy of Fungal Endophytes against Purple Blotch of Onion under Field conditions during Rabi Season

Isolates / Intervals	Disease incidence (%) after pathogen inoculation in days										Percent Disease Control (%)	
	15		30		45		60		Mean			
	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
LE1*(Aspergillus niger* strain 7M1)	0.00	0.15	14.67	13.24	25.13	27.90	30.33	29.12	17.53	17.60	75.04	74.30
RE1*(Fusarium solani* voucher JUF0036)	9.37	7.98	19.00	17.74	37.78	35.00	37.83	36.87	25.10	24.4	64.26	68.34
LE3 *(Aspergillus niger strain GFR13)*	1.95	1.50	15.27	13.95	31.33	28.10	31.67	30.72	21.05	18.57	70.33	72.89
RE2 *(Fusarium sp*. strain S42)	11.80	10.30	27.56	24.62	39.52	36.92	42.98	40.63	30.47	28.12	56.61	58.95
Control	17.15	16.00	37.89	35.20	64.10	62.85	70.23	68.50	-	-	-	-
CD $_{0.05}$	1.104	1.522	1.149	2.488	1.468	2.814	1.707	2.459	-	-	-	-
SE(m)	0.313	0.431	0.326	0.705	0.416	0.798	0.484	0.697	-	-	-	-

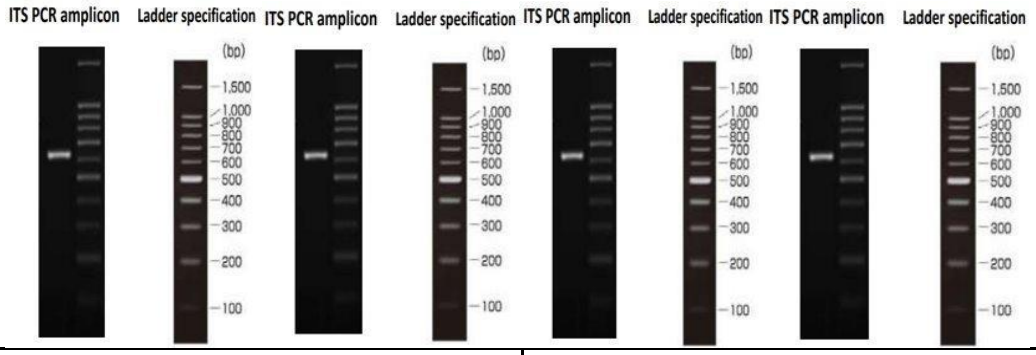
Plate 2: Efficient Fungal Endophytes

A **B** **C** **D**

A. *Aspergillus niger* strain 7M1	C. *Fusarium solani* voucher JUF0036
B. *Aspergillus niger* strain GFR13	D. *Fusarium sp*. strain S42

Plate 3: PCR gel images of Fungal Endophytes

A **B** **C** **D**



A. *Aspergillus niger* strain 7M1(~600bp)	C. *Fusarium solani* voucher JUF0036(~600bp)
B. *Aspergillus niger* strain GFR13(~600bp)	D. *Fusarium sp*. strain S42(~600bp)

Fig 1: Phylogenetic tree of Endophytic Fungal isolates

LE1: *Aspergillus niger* strain 7M1	RE1: *Fusarium solani* voucher JUF0036
LE3: *Aspergillus niger* strain GFR13	RE2: *Fusarium sp*. strain S42