Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 22 | No. 3 | pp. 1343-1351 | 2023

Original Research Paper

https://doi.org/10.46488/NEPT.2023.v22i03.021

Open Access Journal

Plant Growth Promoting Efficacy of Endophytic Fungi Isolated from the Terrestrial Plants of North India

Urvasha Patyal*, Vikas Kumar*†, Manoj Singh* and Kulbir Singh**

*Department of Bio-Sciences and Technology, MMEC, Maharishi Markandeshwar (Deemed to be University), Mullana (Ambala), 133207, Haryana, India

**Department of Civil Engineering, MMEC, Maharishi Markandeshwar (Deemed to be University), Mullana (Ambala), 133207, Haryana, India

†Corresponding author: Vikas Kumar; vmeashi@gmail.com

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 02-12-2022
Revised: 08-01-2023
Accepted: 19-01-2023

Key Words:
Endophytes
Antimicrobial
Phylogenetics
Sustainable agriculture
Plant pathogens

ABSTRACT

Enhanced crop health, which is crucial for sustainable agriculture, is facilitated by a unique endophyte or endophytic community that is frequently linked to a variety of crops. Plant growth-promoting (PGP) characteristics of endophytes can directly or indirectly boost crop growth. Endophytic fungi have been proven to create a high percentage of new compounds, making them a particularly potential source of physiologically active chemicals. In this study, we have isolated two endophytic isolates, *i.e., Paecilomyces* sp. (Isolate AT1) and *Aspergillus flavus* (Isolate AT3), from different host plants, namely *Melaleuca citrine* and *Carica papaya*. These endophytes have shown significant plant growth-promoting potential toward different assays such as IAA production, phosphate solubilization, amylase production, cellulose-degrading assay, and ammonia production. These endophytic fungi also exhibit visible antimicrobial action towards selected crop pathogenic fungi (*Aspergillus* sp. and *Penicillium* sp.). Additionally, these fungal strains are reported for the first time from these plants, as we have found no reports in the literature. The research aims to explore the growth-promoting efficacy of endophytic fungi to boost plant growth.

INTRODUCTION

Endophytes that promote plant growth are symbiotic organisms (fungi and bacteria) that the host plant uses to protect itself from herbivores in a secretive manner. These are common microsporic ascomycetes that live inside the healthy tissues of living plants for the duration of their lives and are found below the layer of epidermal cells in those tissues (Mengistu 2020). It offers the plant several advantages, including the ability to absorb vital nutrients and defense against predatory insects, birds, and animals. Endophytes are ubiquitous and distinct from all categories of plant species in an environment, from huge trees to seagrasses. An estimated 300,000 host plant species were naturally distributed among the endophytic fungi in the temperate region and tropical rainforest (Strobel & Daisy 2003). The endophytic fungus can create a large range of novel bioactive secondary metabolites, which are employed in industries to manufacture a variety of natural goods (Sharma et al. 2016). Endophytes are a good source of bioactive substances that improve the nutritional value of the host plant and increase resistance to pests, diseases, and physical stress (Gouda et al. 2016). These substances (alkaloids, flavonoids, terpenoids, steroids, phenols, phenolic acids, tannins, and peptides), which act as enzymes and exhibit antimicrobial and anti-malarial activities, can be used in the food, agricultural, and pharmaceutical industries (Tungmunnithum et al. 2018). *Taxomyces andrenae*, an endophytic fungus that belongs to the family of hyphomycetes, produces the anticancer drug taxol (Stierle et al. 1993). Plant tissues get inhabited by PGPE (plant growth-promoting endophytes) and exhibit an intimate connection within plant tissues, which improves the activities of enzymes and the flow of nutrients, but this much-appropriate hormone distribution encourages plant development (Hassan 2017). Endophytes have the dynamic ability to activate insoluble phosphate and also feed their host plant with the right amount of nitrogen (Mehta et al. 2019).

In this work, we have selected nine terrestrial plants of the north Indian regions for the isolation of endophytic fungi. We have isolated several different fungal strains from

ORCID details of the authors:

Urvasha Patyal: https://orcid.org/0000-0001-5249-0601
Vikas Kumar: https://orcid.org/0000-0002-6044-3239

these plants. These strains were restricted to the host plants' healthy tissues and did not produce any disease symptoms. When checked for their plant growth-promoting potential, two out of the nine strains exhibited positive results in different assays. The fungal morphology and molecular identification were determined. For molecular characterization, the DNA of these strains was isolated using the CTAB method and then subjected to 18S rRNA sequencing by the Sanger dideoxy method. The obtained sequence was analyzed, and a consensus sequence was prepared, which was then subjected to BLAST analysis and phylogenetic studies (Kumar et al. 2014, Kumar et al. 2017). The consensus sequence was submitted to the National Center for Biotechnology Information (NCBI). The selected isolates were tested against plant pathogens. In this assay, some of them showed a positive antimicrobial response. Also, these two isolates were further checked for their potential to promote plant growth in two different beans, i.e., *Vigna radiata* and *Vigna mungo*. Various growth parameters were checked for these selected beans using the seed germination assay. It is evident from the results that fungal isolates have promoted plant growth in both selected beans.

MATERIALS AND METHODS

To evaluate the plant growth promotion potential of the endophytic fungal isolates, various assays were performed to select the potent isolates. The selected fungal isolates were further identified by microscopic characterization as well as molecular techniques.

Collection of plant parts and isolation technique of endophytic fungi: The fresh, healthy, or disease-free plant parts were collected from *Melaleuca citrina, Phyllanthus emblica, Terminalia arjuna, Eucalyptus globulus, Psidium guajava, Azadirachta indica, Acacia nilotica, Tagetes erecta,* and *Carica papaya*. These plant parts were carefully excised with a sterile scalpel, kept inside sterile poly bags, and brought to the laboratory for storage at 4°C. All plant parts were cut into small pieces (0.5–1.0 cm) and thoroughly washed before processing under running tap water. After surface sterilization, potato dextrose agar medium supplemented with streptomycin was used to culture the fungal isolate and incubated properly at 28±2°C to complete their growth cycle (Kumar et al. 2016).

***In-vitro* testing for plant growth promotion by endophytic isolates:** The isolated endophytes were subjected to different plant growth promoting parameters.

Phosphate solubilizing assay: To check the phosphate solubilizing capacity, each isolated fungal culture was inoculated on the Pikovskayas agar medium plates separately and incubated at 25-28°C for 48-72 hours (Talukdar &Tayung 2019).

Indole-3-acetic acid (IAA) synthesis test: Fungal cultures were dipped into LB broth conical flasks and incubated at 25˚C in a BOD shaker for 48 hours. Add 1-2 mL of Salkowski's reagent to the supernatant of the fungal culture and keep it in the dark at room temperature for 30 minutes (Bric et al. 1991). A change in the color of the media indicates positive results.

Amylase activity: Fungal cultures were cut into small discs utilizing a sterilized cork borer and placed over the medium plates (Glucose- 0.5g, Yeast extract- 0.05g, peptone- 0.25g, agar- 8g, and pH-6) supplemented with 1% soluble starch, and incubated at 25–28˚C for 5 days. After incubation, cultured plates were flooded with 1% iodine and 2% potassium iodide (Hankin & Anagnostakis 1975). A change in the color of the medium from yellow to pink indicates positive results.

Cellulose degrading assay: To select a fungus for cellulolytic activity, fungal isolates were grown in a carboxymethylcellulose (CMC) agar medium. A disc of 8 mm fungal culture was used to inoculate the plates. The test plates were incubated at 25°C for 5–7 days. To visualize the hydrolysis zones, the plates were flooded with an aqueous solution of 0.1% Congo-red (1 mg/mL) for 15 min and washed with 1 M NaCl. Cellulolytic organisms produced a clear zone around the colonies because of the digestion of carboxymethylcellulose (CMC) (Dar et al. 2013).

Ammonia production activity: Peptone medium (broth) was inoculated with different fungal culture discs separately and incubated at 27°C for 48–72 hours. After incubation, 0.5 mL of Nessler's reagent was added individually over the fungal growth in each conical flask (Szilagyi-Zecchinet al. 2014). The change from brown to yellow colour was a positive test for ammonia production.

Identification of Endophytic Fungi

Morphological Identification of Fungi: Morphological identification of all isolated fungal cultures was done by culturing each culture on PDA medium plates (without streptomycin) for seven days and continuously observing the growth appearance on both sides of the culture plates (top and bottom). Based on the standard taxonomic key, we were able to identify each culture according to its color, the diameter of the fungal colony, texture, morphology, and dimensions of conidia and hypha (Barnett & Hunter 1998).

Microscopic identification: By using the tease-mount method, microscopic slides of each isolated fungal culture were prepared using lactophenol cotton blue reagent for tentative recognition, and each slide was observed under a compound microscope. According to the characteristics of culture, the formation of mycelium and spores helps us

This publication is licensed under a Creative Commons Attribution 4.0 International License

identify all unknown isolated endophytic fungi (Aggarwal & Hasija 1986).

Molecular Identification: Using the 18S rRNA gene sequencing method, endophytic fungi were molecularly identified. The DNA extraction was carried out by employing the cetyl trimethyl ammonium bromide (CTAB) method (O'Donnell et al. 1997). The pure DNA extracted was amplified by PCR using universal and degenerate primers. The PCR product was then subjected to gel electrophoresis to check the purity of the DNA, followed by analysis using Sanger's dideoxy method to obtain a DNA sequence (Cubero 1999). The forward and reverse DNA sequences of every sample were subjected to the National Centre for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST) algorithm for identification, and the phylogenetic tree was also constructed using NCBI BLAST online (https://blast.ncbi.nlm.nih.gov/Blast.cgi). All the identified 18S rRNA sequences were submitted to GenBank in fast-all format, and accession numbers for the same were obtained. The identification was confirmed by observing the morphological characteristics through microscopic studies of the spores, hyphae, and colony.

Evaluation for Antagonistic Activity of Isolated Endophytic Strains Against Crop Diseases Causing Fungi

To check the antimicrobial activity of each fungal isolate, test organisms (*Penicillium* sp. and *Aspergillus* sp.) were taken from the fresh plate and strewn around the fungal culture discs in PDA plates and incubated at 25°C for 24-48 hours (Huang et al. 2000). Antagonism between both the isolated endophytic fungi and the fungal pathogen was assessed on an individual basis. The testing was carried out in Petri dishes using PDA medium. To remove excess water from the agar surface, 20 mL of melted medium (40–45°C) was placed into sterile Petri plates, cooled, and the plates were left inverted for 24 hours. A mycelial disc of 8 mm in diameter from the actively growing edges of a 4-5 day old culture of a plant pathogen was brought adjacent to the media of the Petri plate to test resistance. An identical 8 mm-diameter mycelial disc from an actively developing culture of isolated strains (to be evaluated for antibacterial activity) was placed next to the pathogenic fungus after a 24-hour interval. At 25°C, the cultures were incubated. Every 24 hours, observations were taken to investigate the antimicrobial activity.

Preparation of the Formulation from Potent Strains and Evaluation of Plant Growth Promoting Efficacy

To confirm the efficacy of seed coating with endophytic fungi to boost the emergence and plant growth of two pulse crops, *Vigna radiata* and *Vigna mungo*, the experiment was first carried out in a growth chamber. Healthy, disease-free seeds were selected and sterilized (Khatun et al. 2008). By covering the culture with sterile saline containing 0.01% (v/v) Tween (BDH) and spreading the spores with a sterile glass spreader, it is possible to collect spores from lawn cultures of the organism on potato dextrose agar media. The spore solution was then filtered through sterile absorbent cotton wool plugs in a row to get rid of any hyphal fragments that might have been present (Jaber & Enkerli 2016). The spore suspension was then transferred into spray bottles. The sterile filter paper was transferred to autoclaved Petri plates, and seeds of two different samples of crop plants were put in the plates in triplicate. The seeds were sprayed with the fungus suspension, and growth conditions of a 12-hour light cycle with corresponding 22˚C light/ 18˚C darkness and 65% moisture content were provided. The seeds were watered daily, and root and shoot growth were routinely analyzed.

RESULTS

Isolation and Identification of Endophytic Fungi

Various parts were used to isolate fungus strains (stem, root, leaf, bud) of various terrestrial plants (*Melaleuca citrina, Phyllanthus emblica, Terminalia arjuna, Eucalyptus globulus, Psidium guajava, Azadirachta indica, Acacia nilotica, Tagetes erecta, Carica Papaya)* (Fig. 1). Nine endophytic strains were isolated from all the selected plants.

Identification of Endophytic Fungi

Morphological and microscopic identification: According to the standard protocol of (Barnett & Hunter 1998), fungal strains were characterized based on their microscopical and cultural properties (Fig. 2). The fungal strains which were tested positive for different plant growth-promoting activities were subjected to molecular identification.

Paecilomyces sp. Isolate AT1 was characterized as endophyte *Paecilomyces* sp. isolated from the host plant *Melaleuca citrina*. The cultural characteristics include fast growth, powdery, gold, green-gold, yellow-brown, tan colored growth. Phialides are bulbous at their bottoms and have penicillate crowns. They eventually taper into a long, slender neck. In basipetal continuation from the phialides, long, dry chains of single-celled, hyaline to black, smooth or rough, round to fusid conidia are formed.

Aspergillus flavus Isolate AT3 was characterized as endophyte *Aspergillus flavus* isolated from the host plant *Carica papaya*. The macroscopic characteristics include the white colony changing to greenish, and the central region showing dark greenish growth. The microscopic

This publication is licensed under a Creative Commons Attribution 4.0 International License

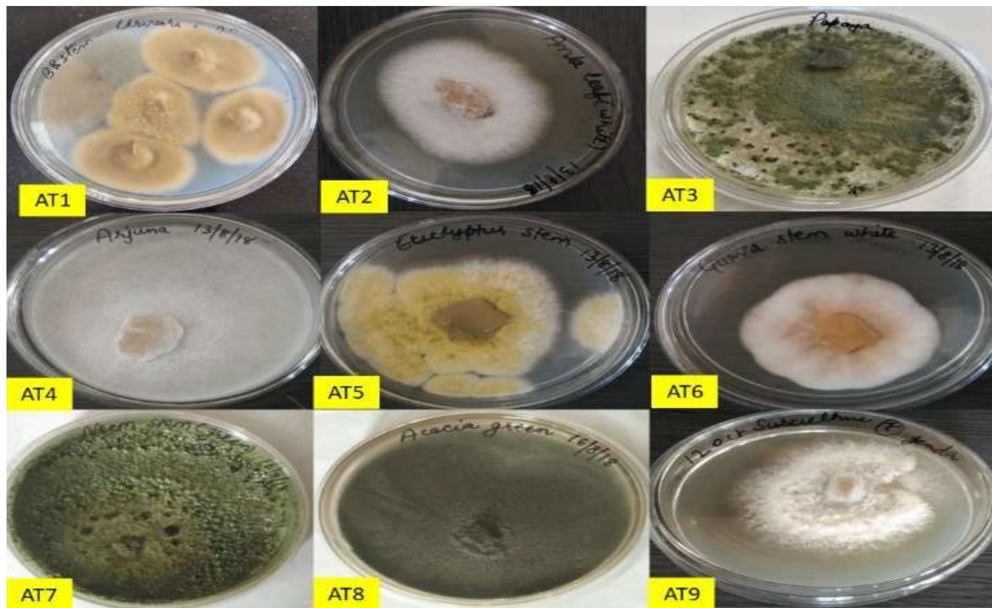

Fig. 1: Endophytic strains isolated from different terrestrial plants.

Fig. 2: Morphology (B, E) (colony appearance) and Microcopy (C, F) (conidia, hypha) view of isolated endophytic strains from different host plants (A- *Melaleuca citrina*, D- *Carica papaya*).

characteristics include a short and columnar conidial head, smooth and brown-colored stripes, biseriatephialides, and globose, smooth conidia.

Molecular Identification

Using the 18S rRNA gene sequencing approach, endophytic fungi were molecularly identified. *Paecilomyces* sp. (Isolate AT1) and *Aspergillus flavus* (Isolate AT3), two endophytic fungi, were amplified and sequenced using universal primers for the 18S rRNA gene. Using BLAST to analyze the sequence similarity of the resulting sequencing products, it was determined that the isolates were *Paecilomyces* sp. (513 bp) and *Aspergillus flavus* (549 bp). The following accession numbers represent the fungal pathogens' gene sequences that have been submitted to NCBI (Table 1). The phylogenetic trees were constructed to represent the evolutionary relationship of isolated organisms with different biological organisms (Fig. 3).

Screening of Endophytic Fungi for Plant Growth Promoting Activities (PGPA)

Isolated endophytic fungal cultures were screened to

This publication is licensed under a Creative Commons Attribution 4.0 International License

Table 1: Detail about the fungal isolates with their accession number and GenBank submission name.

S. No.	Fungal Fugus	Host plant	Plant Part	Accession number	GenBank submission name
1.	*Paecilomyces* sp. (Isolate UP1)	*Melaleuca citrina*	Stem	OP453360	*Paecilomyces* sp. Isolate AT1
2.	*Aspergillus flavus* (Isolate UP3)	*Carica papaya*	Leaf	OP435719	*Aspergillus flavus* Isolate AT3

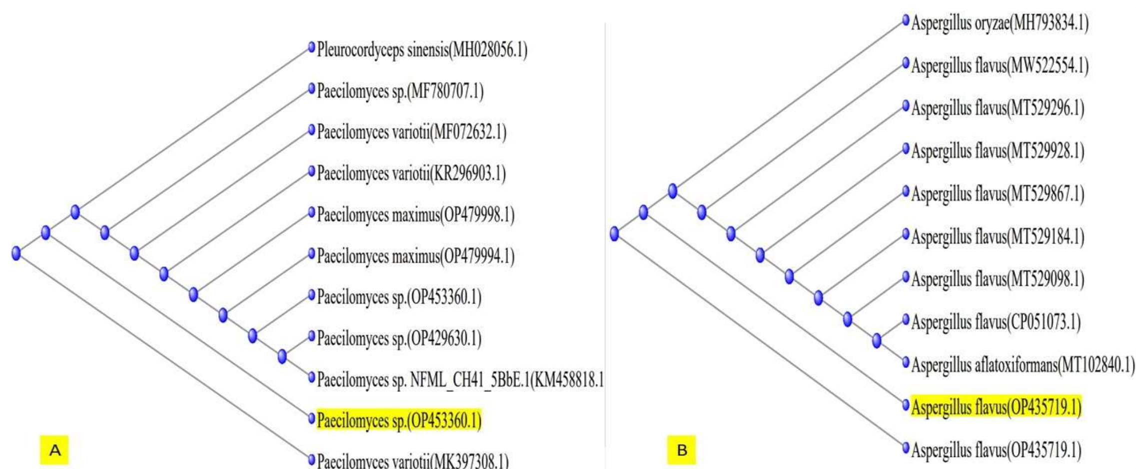

Fig. 3: Phylogenetic analysis of endophytic isolates (A) *Melaleuca citrine* (B) *Carica papaya* with related genera.

determine the various plant growth-promoting activities (Phosphate solubilizing activity, IAA synthesis test, amylase production activity, cellulolytic activity, HCN production activity, ammonia production activity) and the results are depicted in Table 2. *Melaleuca citrine* (Isolate AT1) and *Carica papaya* (AT3) showed highly positive results in different plant growth-promoting assays (Fig. 4).

Screening of Isolated Fungal Cultures for Antagonistic Activity

Two fungal endophytes (Isolates AT1 and AT3) were selected which showed highly positive plant growth-promoting activities and were then subjected to antimicrobial assessment against various test organisms (fungal plant pathogens) (Fig. 5). As a result, isolates AT1 and AT3 exhibited positive antagonistic response against *Aspergillus* sp. and regarding the other test organism, i.e. *Penicillium* sp, isolate AT3 was somewhere seen inhibiting its growth (Fig. 6).

Preparation of the Formulation from Potent Strains and Evaluation of Plant Growth Promoting Efficacy

The selected endophytes were screened for their ability to colonize two preferred host plant species, namely Mung bean (*Vigna radiata*) and Urad bean (*Vigna Mungo*) through seed inoculation. The fungal elicitors namely Isolate AT1 and AT3 showed a positive growth induction in plants in terms of the radicle length and plumule length elongation along with an increased biomass rate in both cases when compared to the control culture. The growth rates were compared with the

Table 2: Comparison of PGP assay of different endophytic strains.

Isolate	Phosphate solubilizing activity	IAA production	Amylase production activity	Cellulolytic activity	Ammonia production
AT1	positive	positive	positive	positive	negative
AT2	negative	positive	negative	negative	negative
AT3	positive	positive	positive	positive	positive
AT4	negative	positive	negative	negative	positive
AT5	negative	positive	negative	positive	positive
AT6	negative	negative	negative	positive	negative
AT7	positive	positive	negative	negative	negative
AT8	positive	negative	negative	negative	positive
AT9	positive	negative	negative	negative	positive

This publication is licensed under a Creative Commons Attribution 4.0 International License

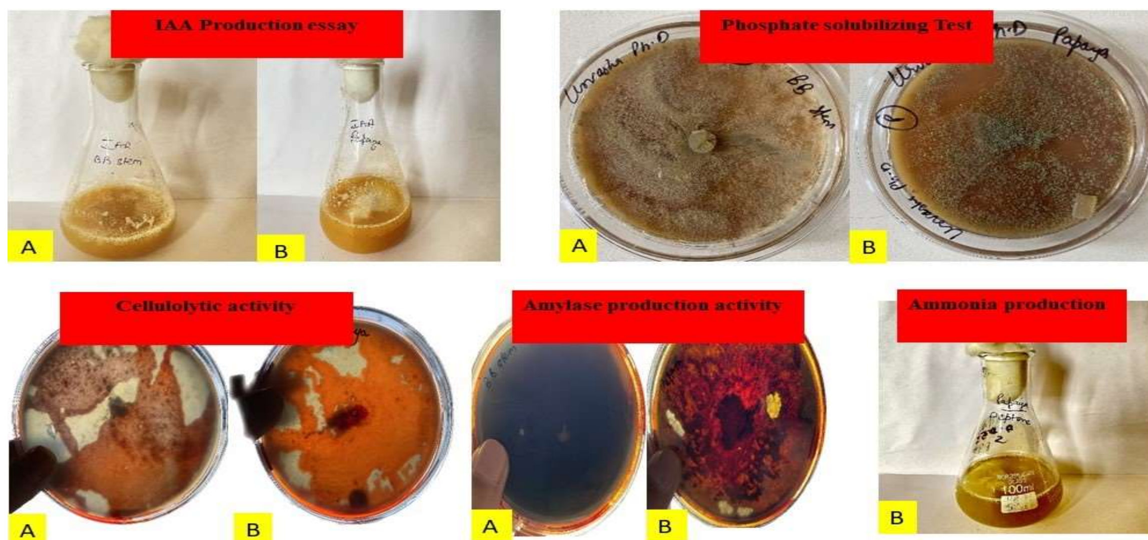

Fig. 4: Positive Plant Growth promoting activities by Endophytes isolated from (A) *Melaleuca citrine;* (B) *Carica papaya*.

Fig. 5: Plant pathogens (A- *Aspergillus* sp., B-*Penicillium* sp.).

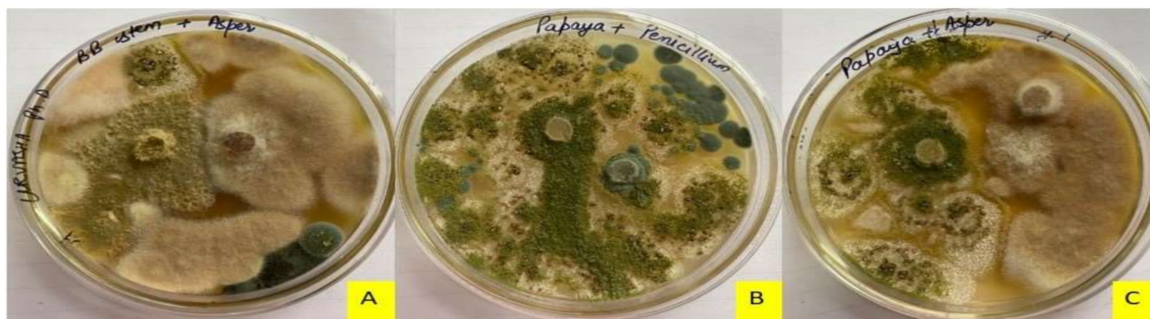

Fig. 6: Endophytes showing antimicrobial activity against test organisms.

help of different graphs. Another component to determine the seed quality was testified by calculating the seed vigor index.

Effect of Culture Supernatant on Mung Bean and Urad Bean Germination

A seed germination essay was carried out and seeds germinated each day were recorded for up to 5 days. The experiment was performed in triplicates. The seeds of *Vigna Radiata* and *Vigna mungo* exhibited a high germination rate when treated with endophytic isolates AT1 and AT3 as compared to the control sample which was treated with water (Fig. 7).

Effect of Culture Supernatant on Growth of Mung Bean (*Vigna radiata*) Plants

Growth promotion was studied using a 15-day plant growth

This publication is licensed under a Creative Commons Attribution 4.0 International License

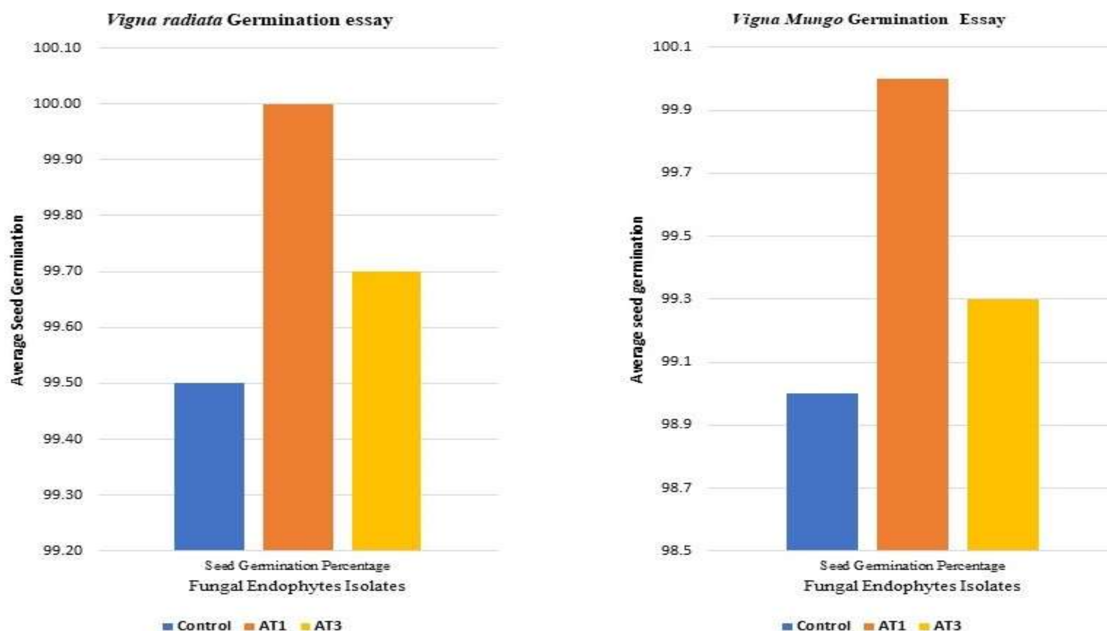

Fig. 7: Seed Germination percentage essay of *Vigna radiata* and *Vigna mungo*.

assay. The average fresh weight of the seedlings was calculated as 3.612 g for Control C, 5.213 g for Isolate AT1, and 4.055 g for Isolate AT3. The average dry weight of the seedlings was calculated as 0.198 g for Control C, 0.213 g for Isolate AT1, and 0.215 g for Isolate AT3. The seed vigor index was calculated to be 1582.05 for Control C, 1840 for isolate AT1, and 2811.54 for isolate AT3. Root and shoot lengths, along with the average seedling length of treated plants, were recorded and are depicted in Fig. 8. The inoculation of *Vigna radiate* seeds with endophytes showed a variable change in the lengths of the plumule and radicle as compared to the control sample C.

Effect of Culture Supernatant on Growth of Urad Bean (*Vigna mungo*) Plants

Growth promotion was studied using a 15-day plant growth assay. The average fresh weight of the seedlings was calculated as 3.883 g for Control C, 7.210 g for Isolate AT1, and 6.148 g for Isolate AT3. The average dry weight of the seedlings was calculated as 0.197 g for Control C,

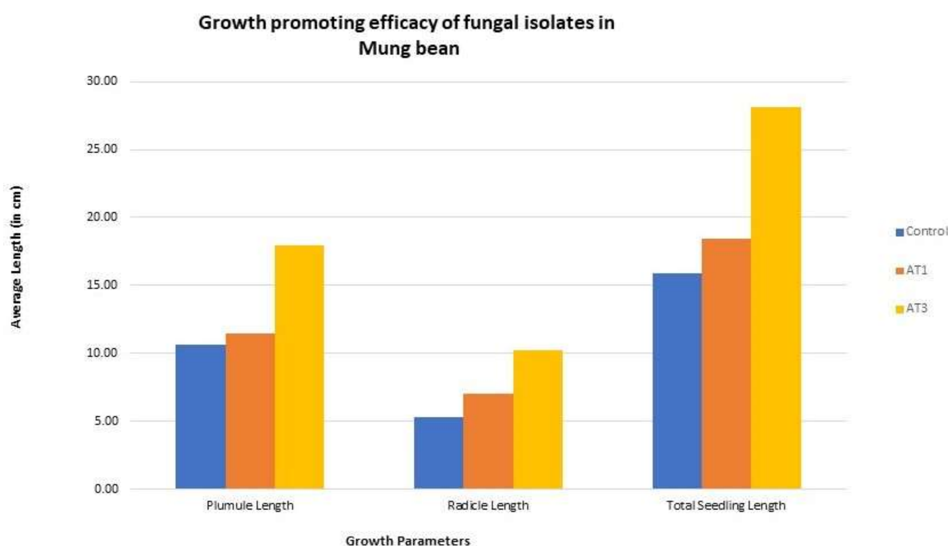

Fig. 8: Effect of Supernatent on Root and shoot lengths of *Vigna Radiata*.

This publication is licensed under a Creative Commons Attribution 4.0 International License

0.208 g for Isolate AT1, and 0.291 g for Isolate AT3. The seed vigor index was calculated to be 1732.5 for Control C, 2340 for isolate AT1, and 2392.13 for Isolate AT3. Root and shoot lengths, along with the average seedling length of treated plants, were recorded and are depicted in Fig. 9. The inoculation of *Vigna mungo* seeds with endophytes showed a variable change in the lengths of the plumule and radicle as compared to control sample C.

Upon seed inoculation with fungal elicitors, in comparison to control it was found that isolates AT1 and AT3 boosted plant growth by initiating root and shoot length and can be noticed visibly (Fig. 10).

DISCUSSION

In this study, according to our research, the isolated fungal isolates *Paecilomyces* sp. and *Aspergillus flavus* have not previously been reported in the host plants *Melaleuca citrina* and *Carica papaya*. The plant growth-promoting assays of fungal endophytes isolated from host plants *Melaleuca citrina* and *Carica papaya* were analyzed to compare

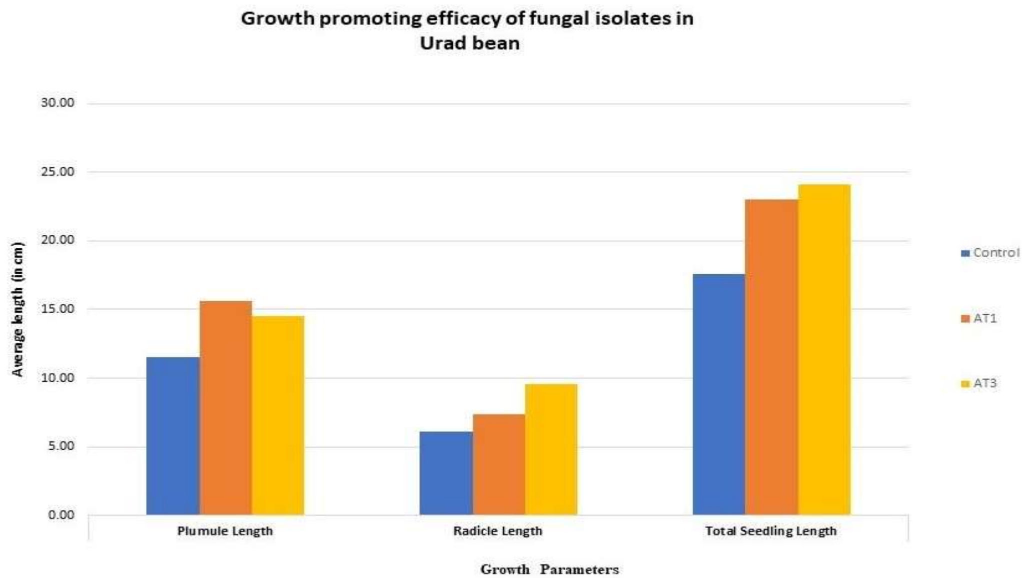

Fig. 9: Effect of the supernatant on root and shoot lengths of *Vigna mungo*.

Fig. 10: Comparison of growth shown by crop beans A (*Vigna radiata*), B (*Vigna mungo*) when inoculated with Control (C) and endophytic fungi (isolates AT1 and AT3).

This publication is licensed under a Creative Commons Attribution 4.0 International License

and evaluate their effects on root and shoot growth. The isolates' indirect plant-promoting actions, such as phosphate mobilization, were also investigated. Out of the nine strains, it is important to emphasize that two strains were found to show positive results in other plant growth-promoting assays, *i.e.,* the Indole-3-Acetic Acid production assay, the cellulose degradation essay, and the ammonia and amylase production essays. The strains were found in various plant parts, such as stems (Isolate AT1) and leaves (Isolate AT3). To further confirm the efficiency of these isolates, they were subjected to an antimicrobial essay against various test organisms (*Aspergillus* sp. and *Penicillium* sp.) and surprisingly showed positive results by suppressing the growth. Also, in preparing the formulation of these endophytes, seeds of *Vigna radiata* and *Vigna mungo* were inoculated with the preparation, and it was confirmed by the results that these endophytes have an important role in visibly enhancing the growth of plants. Isolates AT1 and AT3 boosted plant growth remarkably as compared to controlled culture conditions.

Our research added new knowledge to the area and helped researchers better grasp how fungal endophytes influence plant growth. However, because of how complicated these parameters' effects are, it is necessary to further examine them using a variety of methods, particularly if field applications are to be taken into account.

CONCLUSION

According to the current study, various putative fungal endophytes can find an ecological niche in bottle brush and papaya plants. These microorganisms' activities that encourage plant growth help the host plant adapt to stressful situations. The results of this study encourage us to carry out more research on the chosen fungal endophytes.

REFERENCES

Aggarwal, G.P. and Hasija, S.K. 1986. Microorganisms in the laboratory: a laboratory guide of Mycology, Microbiology and Plant Pathology. Print House, Lucknow, Uttar Pradesh, India.

Barnett, H.L. and Hunter, B.B. 1998. Illustrated Genera of Imperfect Fungi. APS press. St. Paul Minnesota, USA.

Bric, J.M., Bostock, R.M. and Silverstone, S.E. 1991. RapidIn Situassay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl. Environ. Microbiol., 57(2): 535-538.

Cubero, O.F., Crespo, A., Fatehi, J. and Bridge, P.D. 1999. DNA extraction and PCR amplification methods suitable for fresh, herbarium-stored, lichenized and other fungi. Pl. Syst. Evol., 216: 243-249. https://doi.org/10.1007/BF01084401

Dar, R.A., Shahnawaz, M., Sangale, M.K., Ade, A.B., Rather, S.A. and Qazi, P.H. 2013. Isolation, purification and characterization of carboxymethyl cellulase (CMCase) from endophytic *Fusarium oxysporum* producing podophyllotoxin. Adv. Enzym. Res., 1(4): 91-96.

Gouda, S., Das, G., Sen, S.K., Shin, H.S. and Patra, J.K. 2016. Endophytes: a treasure house of bioactive compounds of medicinal importance. Front. Microbiol., 7: 1538. doi: 10.3389/fmicb.2016.01538

Hankin, L. and Anagnostakis, S.L. 1975. The use of solid media for detection of enzyme production by fungi. Mycologia, 67(3): 597-607.

Hassan, S.E.D. 2017. Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of *Teucrium polium* L. J. Advanced Res., 8(6): 687-695.

Huang, X., Xie, W. and Gong, Z. 2000. Characteristics and antifungal activity of a chitin binding protein from Ginkgo biloba. FEBS Letters, 478(1-2): 123-126.

Jaber, L. R. and Enkerli, J. 2016. Effect of seed treatment duration on growth and colonization of *Vicia faba* by endophytic *Beauveria bassiana* and *Metarhizium brunneum*. Biological Control, 103: 187-195.

Khatun, M.K., Haque, M.S., Islam, S. and Nasiruddin, K.M. 2008. In vitro regeneration of mungbean (*Vigna radiata* L.) from different explants. Progressive Agriculture, 19(2): 13-19.

Kumar, V., Aneja, K.R., Aggarwal, N. and Kaur, M. 2014. First report of *Cochliobolus spicifer* causing leaf spot disease of *Trianthema portulacastrum*. J. Pl. Pathol., 96(4, Supplement), S4: 122.

Kumar, V., Kumar, N. and Aneja, K.R. 2017. Three fungal pathogens associated with horse purslane (*Trianthema portulacastrum*) in North India. Ind. J. Weed Sci., 49(4): 411-413.

Kumar, V., Kumar, N., Aneja, K.R. and Kaur, M. 2016. *Gibbago trianthemae*, phaeodictyoconidial genus, causes leaf spot disease of *Trianthema portulacastrum*. Archives of Phytopathology and Plant Protection, 49(1-4): 48-58.

Mehta, P., Sharma, R., Putatunda, C. and Walia, A. 2019. Endophytic fungi: role in phosphate solubilization. In: Singh, B. (eds) Advances in Endophytic Fungal Research. Fungal Biology. Springer, Cham. pp. 183-209. https://doi.org/10.1007/978-3-030-03589-1_9.

Mengistu, A.A. 2020. Endophytes: colonization, behaviour, and their role in defense mechanism. Int. J. Microbiol., 2020: 1-8.Article ID: 6927219 https://doi.org/10.1155/2020/6927219

O'Donnell, K., Cigelnik, E., Weber, N.S. and Trappe, J.M. 1997. Phylogenetic relationships among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. Mycologia, 89(1): 48-65.

Sharma, D., Pramanik, A. and Agrawal, P.K. 2016. Evaluation of bioactive secondary metabolites from endophytic fungus *Pestalotiopsis neglecta* BAB-5510 isolated from leaves of *Cupressus torulosa* D. 3 Biotech, 6(2): 210.

Stierle, A., Strobel, G. and Stierle, D. 1993. Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungi of Pacific Yew. Sci., 260(5105): 214-216.

Strobel, G. and Daisy, B. 2003. Bioprospecting for microbial endophytes and their natural products. Microbiol. Molecul. Biol. Reviews, 67(4): 491-502.

Szilagyi-Zecchin, V.J., Ikeda, A.C., Hungria, M., Adamoski, D., Kava-Cordeiro, V., Glienke, C. and Galli-Terasawa, L.V. 2014. Identification and characterization of endophytic bacteria from corn (*Zea mays* L.) roots with biotechnological potential in agriculture. AMB Express. 7(4):26. doi: 10.1186/s13568-014-0026-y. PMID: 24949261; PMCID: PMC4052694.

Talukdar, R. and Tayung, K. 2019. Antimicrobial activity of endophytic fungi isolated from *Eryngium foetidum*, an ethnomedicinal plant of Assam. In. J. Pharmaceut. Sci. Drug Res., 11(6): 370-375.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A. and Yangsabai, A. 2018. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicines (Basel). 5(3):93. doi: 10.3390/medicines5030093. PMID: 30149600; PMCID: PMC6165118.

This publication is licensed under a Creative Commons Attribution 4.0 International License