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Plant Growth Promotion and *In-vitro* Bio-activities Evaluation of Endophytic Fungal Communities Associated with *Oryza sativa* L.

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ABSTRACT

Utilization of plant-associated beneficial microorganisms, particularly endophytes, is growing, as they occupy a relatively privileged niche within various plant tissues, where they face less competition for food and shelter than in the rhizo-sphere. The plant and these beneficent endophytes form a harmonious interaction that has profound effects on the host plant's physiological and developmental process in its native habitat. The purpose of this study aims to emphasize the endophytic fungi found on flourishing rice seedlings as well as to investigate the preordained factors affecting the growth of the plant. 56 endophytic fungi were isolated from various rice plant tissues and their percent of dominance was determined. The fungal strains were assessed for *in-vitro* mechanisms that boost plant growth-promoting activities studies.Out of 14 isolates, 92.85% were able to produce Gibberellic acid, 7.14% ammonia production, 71.42% for phosphate solubilization activity, 92.85% for zinc solubilization activity, 21.42% for potassium solubilization activity, 7.14% for HCN production and 64.28% for siderophore development, indicated the potency to promote crop growth enhancement. Therefore, endophytic fungi isolated from rice plants have the potential to be a fortuitous bio-stimulant; further research into this area is required to aid in bridging the void in agricultural and biotechnological prospects.

Key words *: Rice seedlings, Endophytic fungi,GA_3,Phosphate solubilization, HCN, Siderophore production*

Introduction

There is an inherent connection between plants and the various kinds of microbes that thrive in their phyllosphere (epiphytic bacteria), rhizosphere (rhizobacteria), and plant tissue (endophytes) Qin *et al.* (2011). An endophytic fungus establishes itself within and between plant cells without causing infection (Abedinzadeh *et al.*, 2019). The variety and structure of plant communities are influenced by myco-endophytes, which are decisive and perceptible components of fungal life and ecology in plants. Forestalling to their host plants from both predators and pathogens, endophytes perform a very imperative character in ecosystems (Scannerini *et al.*, 2001). Pretty fascinating crop traits mostly in agricultural production, this unique critter has an array of mechanisms for encouraging crop production, including phyto-stimulation, bio-fertilization, and persuading plants to withstand simultaneously biotic and abiotic challenges (Hassan *et al.*, 2017); Plant growth-promoting fungi (PGPF), for example,

precisely foster growth of the plant by producing phyto-hormones such as indole-3-acetic acid (IAA) and gibberellic acid (GA) Hashim *et al.* (2020). Fungal GA reacts synergistically with endogenous plant GA, stimulating plant growth through cell expansion. Furthermore, PGPF stimulates plant development by producing ammonia (Fouda *et al*., 2015) and multiple enzymes (including amylase, cellulase, and protease) Hassan *et al.* (2017) in synchrony with fixing nitrogen and solubilizing phosphate (Zamin *et al.*, 2020).

One important contribution of fungal endophytes to agriculture is their role as a biological control agent towards several pests and diseases carried by insects, nematodes, and other microbial pathogens (Murali *et al.*, 2017).

Conjointly, enhancing the host's defense, every endophytic microbe contributes through an as-yet-unknown mechanism, and there are several unresolved queries in endophytic research. As a consequence, the purpose of our research is to utilize morphological techniques to assess the growth-promoting activities of fungal endophytic strains cognate with various tissue portions of wholesome, rice crops.

Materials and Methods

Sampling, Isolation and Identification of Endophytes

Sample Assemblage and Surface Sterilization

Sample processing was done during seedling stage of rice plant. Rice seedlings of four different kinds (*Oryza sativa* L. var. Khandagiri/ Gitanjali/ Hiranyamayee/ Lalat) were collected from the OUAT field in Bhubaneswar, Odisha, India (Lat. 200 16'N, Long. 85047'E, Elevation 25.9 m). Immediately after collection, sterile containers were employed to transport the materials to the lab for processing within 24-48 hours.

Samples were carefully sliced and placed on Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Malt Extract Agar (MEA), Water Agar WA and Czapek Dox Agar (CDA). Moreover, the plates were maintained in an incubator at 28°C± 2°C for 2 weeks unless fungal growth was perceived (Schulz *et al*., 1998). Triplicate media plates spread out with 50 µl aliquots of the last rinse water demonstrated the cogency of the surface sterilization process because no microbial growth cropped up.

Both the frequency of isolation (IF) and the pervasiveness of the most abundant fungus was determined following the methods described elsewhere (Fisher and Petrini, 1991; Goveas *et al.*, 2011):

IF= (Total number of isolates yielded in a given trial/ Total number of sample segments in the same trial) × 100

% Dominance = (Number of isolates collected from the samples/ Total number of leaf/stem samples) × 100

Comparative Morphological Analysis of a Variety of Culture Media

Different media were utilized to determine the mycelium type, colony color, and fungal growth rate. The isolates described in this manuscript were stained with lacto-phenol cotton blue (*via* scotch tape method) and identified using the standard identification manuals based upon colonial morphology and microscopic observation (Gilman, 1971).

Investigation of Plant Growth-Promoting Traits

Screening for Gibberellic Acid (GA) Production

The Follin-Wu technique was used to demonstrate the qualitative and quantitative production of GA_3 by the isolates (Henderson and Grahm 1961, Tripathy *et al*., 2020). The concentration of GA_3 was measured by comparing it to a standard curve created with GA_3 obtained from Sigma-Aldrich, Pvt. Ltd.

Ammonia Production

According to Nessler's reagent method, all of the fungal isolates were checked for their potential to produce ammonia. The non-inoculated peptone water medium serves as the control (Marques *et al.*, 2010).

Phosphate Solubilization

According to Jasim *et al*. (2013), the ability of fungal isolates to dissolve inorganic phosphate using tricalcium phosphate contained in the medium was tested *in vitro*. On Pikovskaya's medium plates, the fungus plugs were inoculated and maintained for 5-7 days at 28 °C ± 2 °C. The phosphate solubilizing capacity was qualitatively assessed by measuring the diameter (mm) of the clear halo zones formed encircling the colony. The formula for determining the Phosphate Solubilization Index (PSI) has been provided below:

PSI= Total diameter of the halo/Diameter of the colony

Zinc Solubilization

Isolates' abilities to solubilize zinc were tested in plates comprising insoluble ZnO or ZnS (0.1%) in a Basal media by using the halo zone formation procedure (Venkatakrishnan *et al*. 2003). The solubilization index was calculated according to the established formula as presented below:

ZSI = Total diameter of the halo/Diameter of the colony

Solubilization of Potassium

Aleksandrov agar medium, which contains 0.2% of the insoluble potassium-containing material like mica, was used to test the isolates for potassium solubilizing activity (Hu *et al*., 2006). Incubating the plates at 28°C ± 2°C for 5 days yielded positive results for potassium solubilization activity, as indicated by the presence of clear halo zones around the colonies. The following formula was used to get the index of potassium solubilization as shown below:

KSI = Total diameter of the halo/Diameter of the colony

Disease Suppressive Mechanisms

Siderophore Evaluation

Isolates were screened for their capacity to produce siderophores by using the modified technique of (Schwyn and Neilands, 1987). The fungal isolates were inoculated onto Chromazurol S (CAS) agar media plate and incubated at 28 °C ± 2 °C for 7 days. Siderophores were thought to be produced by colonies that turned yellow-orange/ pink, on growth color change from blue to yellow-orange, pink, purple, or deep purple-red was used to determine the kinetics of CAS.

Test for HCN Production

Miller and Higgins (1970) modified protocol was used for hydrogen cyanide formation studies. A solution of 0.3% picric acid and 1.5% sodium carbonate was combined to produce HCN medium. The solution was applied to a sterile strip of Whatman No.1 filter paper (China) and dried under sterile conditions. After inoculating a PDA plate with fungus cultures, the processed filter paper sheets were laid on top of the plate and the plate was wrapped with Parafilm to prevent contamination. The duration of incubation was spanning from 7 days to 10 days. Filter paper strips were monitored for their color shift from yellow to brown or reddish brown as a measure of HCN production rate. The levels of intensity were categorized as mild (yellow to light red), moderate (brown), and intense (reddish brown).

Statistical analysis

SPSS (Version 22.0) was used to perform Analysis of Variance (one-way ANOVA) on the data to determine standard deviation and error. The means were compared using Duncan's multiple range test (DMRT) to find significant differences at the $P \leq 0.05$ levels. To further examine the differences between means, a t-test was also employed.

Results

Isolation and identification of endophytic fungi

In toto 56 endophytic fungi were isolated by culturable methods from 290 sterile stem and leaf fragments of four rice plant varieties (Gitanjali, Hiranyamayee, Khandagiri, and Lalat), and 14 isolates were selected for further characterization based on morphological and functional characteristics. Endophytic fungi were isolated from leaf segments more frequently than stem segments (Table 1). Using lactophenol cotton blue and sticky tape techniques, these endophytic fungi were identified morphologically.

All fungal endophytes were kept at 4 °C in PDA slants and -80 °C in glycerol stock as future use. *Aspergillus*, *Talaromyces*, *Fusarium*, and *Sterile mycelia* were isolated from at least two distinct plant parts and exhibited ubiquitous colonization, with *Aspergillus* being the most dominant endophytic fun-

Table 1. Frequency of isolation (%) of endophytic fungi of four different Rice plant (Gitanjali-V_1, Hiranyamayee-V_2, Khandagiri-V_3, Lalat-V_4)

Characters studied	Variety -1		Variety-2		Variety-3		Variety-4	
Plant parts usesd	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Total no. of samples	42	32	40	32	40	32	40	32
Number of isolated endophytic fungi	11	7	7	5	6	3	12	5
% frequency of isolation	26.18	21.72	17.31	15.44	15.08	9.37	30.08	15.59

gus (Table 2). The presence of *Aspergillus*, *Talaromyces*, *Fusarium*, and *Sterile mycelia* in both tissue sections of four distinct rice plants indicates the obtained endophytic isolates had no preference for any particular tissue.

Table 2. Dominanat Endophytic fungi % and their colonized plant part of four different Rice plant (Gitanjali-V_1, Hiranyamayee-V_2, Khandagiri-V_3, Lalat-V_4)

Isolates	Plant parts colonized (Stem/Leaf)	% Dominance
Aspergillus sp. 1	Stem, Leaf	6.89
Aspergillus sp.2	Stem, Leaf	3.44
Talaromyces sp.	Leaf, Stem	2.06
Colletotrichum sp.	Leaf	1.72
Penicillium sp.1	Stem	1.37
Fusarium sp.	Stem, Leaf	1.03
Aspergillus niger	Leaf	0.68
Sterile mycelia (SM) 2	Leaf, Stem	0.68
Unidentified genus 1	Leaf	0.34
Sterile mycelia (SM) 1	Stem	0.34
Rhizopus sp.	Stem	0.34
Unidentified genus 2	Stem	0.34
Aspergillus sp.3	Leaf	0.34
Penicillium sp.2	Leaf	0.34

Growth Promotion and Disease Suppression

GA Production

The potency of selected fungal endophytes for promoting plant growth, biochemical enzyme activity, and mineral solubilization was evaluated. Using qualitative and quantitative GA production assays, GA-producing isolates were screened and quantified. The ability to produce GA was identified in 13 (92%) of the isolates, most notably in the Ascomycota group. The isolate with the highest reported GA production was LS13 (67.78 ± 0.082 µg/ml), followed by LL12 (65.92±0.082 µg/ml) and the lowest production was reported in isolate HL07 (38.75±0.082 µg/ml) (Table 3).

Ammonia Production

Test results pertaining to ammonia production are depicted in the Table 3. According to the findings of the present study, only one endophytic fungus, KL09, produced ammonia.

Phosphate Solubilization

Nine isolates (64%) utilized the incorporated phosphate on Pikovskaya's agar (PVKA) medium, producing a clear zone around the fungal colony, validating the possible phosphate solubilizing activity. The phosphate solubilizing index (2.94 ± 0.005) for isolate LL14 was the highest of all the tested isolates, indicating that it exhibited the most phosphatase activity, whereas LL11 had the lowest phosphate solubilizing activity (1.13 ± 0.003) reported in Table 3.

Zinc Solubilization

When all of the isolates were subjected to the zinc solubilization test, thirteen isolates (92.8%) were found to be positive for zinc solubilization. LS13 had the highest zinc solubilizing index (2.65 ± 0.005), while GS03 had the lowest zinc solubilizing index (1.01 ± 0.008, Table 3).

Potassium Solubilization

All of the isolates were subjected to a test for postassium solubilization. Three isolates (21.42%) (GS01, GL02, and HS06) were determined to be positive for the potassium solubilization test. The isolate HS06 had the highest solubilizing index (1.41 ± 0.003), while the isolate GL02 observed to have lowest solubilizing index (1.17 ± 0.003, Table 3).

Siderophore Production

After 72 hours of incubation on CAS agar, the results of inoculation with all fungal isolates were observed. Nine (GS01, GL02, GS03, GS04, HS08, KL09, KL10, LL14, and LS13) isolates (64.28%) observed to have positive siderophore production ability. The color changed from blue to orange/pink, indicating siderophore production, suggesting that these isolates have the capability to generate siderophore, which chelates iron from the medium. Isolate LS13 exhibited the highest siderophore producing index (SI) (2.96 ± 0.005), whereas, isolate GS03 exhibited the lowest (SI) (1.28 ± 0.008, Table 3).

Production of HCN

Only one of fourteen isolates, KL10, was positive for HCN production (Table 3). All fourteen endophytic fungal isolates were evaluated for their ability to produce HCNas illustrated by the yellow-to-reddish brown transformation of filter paper.

Discussion

With the advancement of technology, the world has risen, but it is disheartening to consider food pro-

Table 3. Plant growth promoting (PGP) traits and antifungal assessment of fungal endophytes

Isolats code	Plant growth stimulating traits of fungal endophytic isolates of four different Rice plants								Antifungal mechanism			
	Stem/leaf endophytic fungi	GAP	GAP (µg/ml)	AMP	PS	PSI	ZnS	ZSI	KS	KSI	HCN	SP	SI
GS01	*Aspergillus* sp.1	+	39.89±0.082^{cd}	-	+	1.25±0.005^{a}	+	1.55±0.005^{b}	+	1.23±0.010^{abc}	–	+	2.05±0.018^{ab}
GL02	*Aspergillus niger*	+	45.76±0.082^{d}	-	+	1.44±0.005^{b}	+	2.12±0.003^{c}	+	1.17±0.003^{cd}	–	+	1.55±0.006^{bc}
GS03	*Rhizopus* sp.	+	63.06±0.082^{c}	-	-	-	+	1.01±0.008^{d}	-	-	–	+	1.31±0.008^{abc}
GS04	*Fusarium* sp.	+	48.62±0.082^{e}	-	+	1.18±0.003^{c}	+	1.76±0.005^{e}	-	-	–	+	1.48±0.003^{de}
GS05	*Unidentified* sp.	+	42.80±0.265^{ab}	-	+	1.36±0.003^{d}	+	1.56±0.003^{b}	-	-	–	–	–
HS06	*Penicillium* sp.1	+	57.05±0.082^{a}	-	+	1.62±0.005^{e}	+	2.14±0.020^{c}	+	1.41±0.003^{efg}	–	–	–
HL07	*Penicillium* sp.2	+	38.75±0.082^{b}	-	+	1.51±0.008^{f}	-	-	-	-	–	–	–
HS08	*Sterile mycelia* sp.1	-	-	-	-	-	+	1.12±0.00^{h}	-	-	–	+	1.98±0.003^{efg}
KL09	*Aspergillus* p.2	+	58.77±0.082^{c}	+	+	1.81±0.003^{g}	+	1.43±0.005^{p}	-	-	–	+	4.41±0.012^{h}
KL10	Sterile mycelia sp.2	+	54.19±0.082^{bc}	-	-	-	+	1.09±0.017^{h}	-	-	+	+	1.70±0.031^{i}
LL11	Unidentified sp.	+	43.32±0.082^{f}	-	+	1.13±0.003^{h}	+	1.03±0.008^{d}	-	-	–	–	–
LL12	*Colletotrichum* sp.	+	65.92±0.082^{g}	-	-	-	+	1.17±0.014^{l}	-	-	–	–	–
LS13	*Aspergillus* sp. 3	+	67.78±0.082^{h}	-	+	2.00±0.028^{i}	+	2.65±0.005^{m}	-	-	–	+	2.96±0.005^{k}
LL14	*Talaromyces* sp.	+	41.61±0.082^{k}	-	+	2.94±0.005^{j}	+	2.11±0.003^{e}	-	-	–	+	2.48±0.008^{j}

(GS- Gitanjali Stem, GL- Gitanjali Leaf, HS- Hiranyamayee Stem, HL- Hiranyamayee Leaf, KS-Khandagiri Stem, KL- Khandagiri Leaf, LS- Lalat Stem, LL- Lalat Leaf)
(GAP, Gibberellic acid production, AMP, Ammonia production; PS, Phosphate Solubilization; PSI, Phosphate Solubilization Index, ZnS, Zinc Solubilization; ZSI, Zinc Solubilization Index, KS, Potassium Solubilization; KSI, Potassium Solubilization Index, HCN, Hydrogen cyanide; SP, Siderophore production; SI, Siderophore production Index)
[Data presented here are Mean ± SE from three replicates (n= 3).When examined with the Duncan Multiple Range Test (DMRT), the mean values denoted by different superscripts along a column show a significant difference at the 5% level(P<0.05)]

ductivity in light of rising demand and the recurrence of food crises around the globe. In crop production, environmental pollution issues caused directly or indirectly by the use of chemical fertilizers, pesticides, herbicides, insecticides, etc. are a major concern. Recent climate change has huge adverse effects on survival and yields of crops as well as quality of derived products for food requirements. In the present past more research is being conducted on novel microorganisms as a means of improving plant health and crop yield, besides their uses as bio-fertilizers, bio-pesticides and bio-insecticide. Therefore, researchers are focused to seek an alternative route based on natural origins for the sustainable growth of plants in agriculture. Myco-endophytes are less studied but of great interest, since they possess numerous traits that protect plants from biotic and abiotic challenges and phyto-pathogens, allowing them to thrive and flourish.

Consequently, during this investigation 56 endophytic fungi belonging to various genera were isolated from 290 leaf and stem fragments of four varieties of healthy rice. Based on *in-vitro* plant growth-promoting assays and bio-control activity results, 56 isolates were narrowed down for further research. The present research indicated that the endophytes belonging to Ascomycota genera were substantially more predominant than other categories, as they are members of frequently detected genera of soil fungi, such as *Fusarium*, *Penicillium*, *Aspergillus*, *Colletotrichum* and *Talaromyces*, with *Mucoromycota* being the least dominant, consistent with the findings of (Atugala and Deshappriya, 2015).

Unidentified fungi and form groups were distributed uniformly throughout the rice plants. *Aspergillus, Penicillium*, *Fusarium*, and *Talaromyces* were identified as stem and leaf endophytes from rice tissue sections. Species of *Colletotrichum* and *Rhizopus* were restricted to the leaf and stem, respectively. *Fusarium*, *Aspergillus* and *Penicillium* were among the most prevalent endophytes isolated from healthy paddy plants in China, according to a study on fungal endophytes (Tian *et al.*, 2004). Intriguingly, a new species of the fungus

Talaromyces was isolated from the leaves of rice plants, corroborating to (Miyake *et al*., 2012) findings that *Talaromyces* sp. KNB422, isolated from a rice seedling, was highly effective against several rice seedling diseases in Japan. According to our knowledge, this is the first report from this region on the bio-prospecting of rice endophytic fungi for agricultural applications.

Through the production of phytohormones such as GA_3, nitrogen fixation, siderophore formation, and phosphate solubilization, endophyte-induced direct promotion contributes to plant growth and development. It has also been demonstrated endophytes can boost crop development through the production of phytohormones such as GA, which not only improve stem elongation but also stimulate seed germination. In this investigation, 13 isolates observed to have the potential to produce gibberellic acid (GA). Our findings that numerous spp. of *Aspergillus* and other fungi can produce GA_3 are corroborated by the findings of Waqas *et al*., (2014), who found that *Aspergillus fumigatus* and other fungi isolated from roots of (*Glycine max* L.) produced diverse subtypes of phytohormones (GA_1, GA_3, GA_4, GA_7, and GA_9).

In addition, 7.14% of the isolates in the present investigation synthesized ammonia by hydrolyzing urea. By hydrolyzing urea into ammonia and carbon dioxide, microorganisms produce ammonia as a secondary metabolite, which meets the nitrogen requirements of the host plant and reduces pathogen colonization (Mbai *et al.,* 2013). Another aspect of PGPF that influences plant growth is the formation of ammonia (Ngoma *et al*., 2014). Our results showing that *Aspergillus sp*. isolate KL09 produced ammonia are in consistent with those of an earlier study (Chadha *et al*., 2015) showing that the fungus *Aspergillus* sp. can produce ammonia.

Generation of organic acids and protons is the primary process for mineral solubilization, including phosphate and other insoluble mineral salts, as this lowers the pH of the medium (Nath *et al*., 2015). Phosphorus is a vital macronutrient for plant development. According to Mengel and Kirkby (1978), most (95–99%) of the phosphorus in soil is present as insoluble organic and inorganic phosphates that slowly leach out of the surface soils and are not readily accessible to plants. In phosphorus-deficient conditions, plant development is hindered. Plant productivity can be enhanced by improving phosphate allocation and assimilation through fungi by solubilizing phosphates. Compared to bacteria, phosphate solubilization by fungi appears to be more promising.

Results showed that when cultured on Pikovskaya agar medium supplemented with tricalcium phosphate, 10 fungal isolates (71.42%) exhibited phosphate solubilization activity, as indicated by the existence of a clear zone all around the fungal colony; 92.85% of fungal isolates were able to solubilize zinc; 21.42% of isolates were positive for potassium (Table 3).

According to reports, *Penicillum* sp. and *Aspergillus* sp. are the most prominent genera employed for phosphate solubilization (Seshadri *et al.,* 2004; Wakelin *et al.,* 2004). *Aspergillus niger*, *Penicillium simplicissimum*, *Penicillium expansum*, and *Scopulariopsis brevicaulis* have been documented to dissolve undissolved potassium salts, which including potassium aluminosilicates (Sterflinger, 2000). Several species of fungus, notably ericoid mycorrhizal fungi (*Woollsia pungens* mycorrhizal endophytes; *Epacridaceae*; *Hymenos cyphus ericae*; *Oidiodendron maius*), have been observed effectively dissolve hydroxyapatite, Zn oxide, and other related minerals (Martino *et al*., 2003). These outcomes align with the conclusions of the existing investigation (Table 3).

Significant studies, such as the production of protease, siderophore, and HCN in enhancing plant defense and survival have been recorded by (Dellagi *et al*., 2009). HCN has a crucial role in disease prevention. Here, in this study, only 7.14 % of samples tested positive for HCN. There is very little known about fungi that produce HCN. Therefore, these PGP-exhibiting isolated strains are incredibly interesting.

Siderophore synthesis is an additional trait that stimulates plant growth by attaching to the accessible iron form (Fe^{3+}) in the rhizosphere, rendering iron unavailable to the phyto-pathogen. Siderophores exhibit a high degree of structural polymorphism and iron affinity, which determines a microbe's growth under competitive situations when iron supply is the limiting factor (Cao *et al.,* 2005). Tan *et al.* (2006) proposed that the formation of siderophore is a key component for phyto-pathogen antagonism and plant development. In our investigation, siderophore synthesis was identified in 64.2% of isolates (Table 3). *Aspergillus* sp. is well-known for producing siderophores, which restrict phyto-pathogen growth by competing for iron in

rhizosphere soils (Haas, 2014).

It was seen that the maximum intensity of siderophore production was shown by *C. globosum*, *A. vericolor*, and *T. peudokoningii*, followed by *F. oxysporum*, *Mucor* sp, *A. niger*, and *M. hiemalis*. However, *F. semitectum*, *F. solani*, *F. fusarioides*, and *F. moniliforme* showed siderophore production (Chadha *et al*., 2015). The results of the current investigation are aligned with the aforementioned findings, which could be attributable to plant growth-boosting activities.

Conclusion

The results of this study imply that endophytic fungi might be used not only as an efficient antifungal agent in the management of diverse crop diseases, but also as valuable agricultural assets in the search for effective antifungal metabolites. The production of phyto-hormones (GA_3), solubilization of inorganic phosphorous, zinc, and potassium, production of siderophores, hydrogen cyanide, ammonia, and protection of plants from phyto-pathogens are just some of the ways in which these species promote plant growth. The findings of the current research encourage to do empirical investigation on the preferred fungal endophytes in order to formulate a persuasive bio-stimulant with wide-ranging applicability to multiple fields, which would ultimately pave the way to organic food crops and help bring in a brighter tomorrow by eliminating our reliance on harmful synthetic chemicals.

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Conflict of interest

All the authors declare that there is no conflict of interest.

References

Abedinzadeh, M., Etesami, H. and Alikhani, H.A. 2019. Characterization of rhizosphere and endophytic bacteria from roots of maize (*Zea mays* L.) plant irrigated with waste water with biotechnological potential in agriculture. *Biotechnology Reports.* 21: e00305.https://doi.org/10.1016/j.btre.2019.e00305.

Atugala, D. M. and Deshappriya, N. 2015. Effect of endophytic fungi on plant growth and blast disease incidence of two traditional rice varieties. *Journal of National Science Foundation Sri Lanka.* 43(2): 173–187. DOI: 10.4038/jnsfsr.v43i2.7945.

Bacon, C.W. and White, Jr. J.F. 2000. Physiological adaptations in the evolution of endophytism in the Clavicipitaceae. In: *Microbial Endophytes*, Eds., pp. 237–261, Marcel Dekker Inc, New York, NY, USA.

Cao, L.X., Qiu, Z.Q., You, J. L., Tan, H.M. and Zhou, S. 2005. Isolation and characterization of endophyticstreptomycete antagonists of *Fusarium* wilt pathogen from surface-sterilized banana roots. *FEMS Microbiology Letters*. 247(2): 147–152. https://doi.org/10.1016/j.femsle.2005.05.006.

Chadha, N., Prasad, R. and Varma, A. 2015. Plant promoting activities of fungal endophytes associated with Tomato roots from Central Himalaya, India and their interaction with *Piriformospora indica. International Journal of Pharma and Bio-Sciences.* 6(1): (b) 333-343.

Dellagi, A., Segond, D., Rigault, M., Fagard, M., Simon, C. and Saindrenan, P. 2009. Microbial siderophores exert a subtle role in *Arabidopsis* during infection by manipulating the immune response and the iron status. *Plant Physiology*. 150(4): 1687–1696. https://doi.org/10.1104/pp.109.138636.

Fisher, P. J. and Petrini, O. 1991. Fungal saprobes and pathogens as endophytes of rice (*Oryza sativa* L.). *New Phytology.* 120 (1): 137–143.

Fouda, A. H., Hassan, S. E.-D., Eid, A.M. and Ewais, E.E.-D. 2015. Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepiassinaica* (Bioss.). *Annals of Agricultural Science.* 60(1): 95–104. https://doi.org/10.1016/j.aoas.2015.04.001

Gilman, J. C. 1971. *A manual of Soil Fungi*. Iowa State College Press. Ames, Iowa: P.450.

Goveas, S. W., Madtha, R., Nivas, S. K. and D'Souza, L. 2011. Isolation of endophytic fungi from *Coscinium fenestratum*- a red listed endangered medicinal plant. *Eurasian Journal of Bio Sciences*. 17 (6): 767-772.DOI: 10.5053/ejobios.2011.5.0.6.

Haas, H. 2014. Fungal siderophore metabolism with a focus on *Aspergillus fumigatus*. *Natural Product Reports. 31*(10): 1266–1276. DOI https://doi.org/10.1039/C4NP00071D.

Hashim, A. M., Alharbi, B. M., Abdulmajeed, A. M., Elkelish, A., Hozzein, W.N. and Hassan, H.M. 2020. Oxidative stress responses of some endemic plants to high altitudes by intensifying antioxidants and secondary metabolites content. *Plants*. 9(7): 869. https://doi.org/10.3390/plants9070869.

Hassan, S.E. D. 2017. Plant growth-promoting activities for

bacterial and fungal endophytes isolated from medicinal plant of *Teucrium polium* L. *Journal of Advanced Research*. 8(6): 687–695.doi: 10.1016/j.jare.2017.09.001.

Henderson, J.H.M. and Graham, H.D. 1961. Reaction of gibberllic acid and giberllins with Folin-Wu Phospho molybdic acid Reagent and it's use for quantitative assay. *Plant Physiology*. The Carver Foundation, Tuskegee Institute, Alabam. Pages: 405-408.

Hu, X. F., Chen, J. and Guo, J. F. 2006. Two phosphate and potassium solubilizing bacteria isolated from Tiannumountain, Zhejiang, China. *World Journal of Microbiology and Biotechnology*. 22: 983-990. https://doi.org/10.1007/s11274-006-9144-2.

Jasim, B., Jimtha, C.J., Jyothis, M. and Radhakrishnan, E. 2013. Plant growth promoting potential of endophytic bacteria isolated from *Piper nigrum*. *Plant Growth Regulation*. 71: 1–11. https://doi.org/10.1007/s10725-013-9802-y.

Marques, A. P., Pires, C., Moreira, H., Rangel, A.O. and Castro, P.M. 2010. Assessmentof the plant growth promotion abilities of six bacterial isolates using *Zea mays* as indicator plant. *Soil Biology and Biochemistry*. 42(8): 1229–1235.https://doi.org/10.1016/j.soilbio.2010.04.014.

Martino, E., Perotto, S., Parsons, R. and Gadd, G. M. 2003. Solubilization of insoluble inorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. *Soil Biology and Biochemistry*. 35(1): 133–141.https://doi.org/10.1016/S0038-0717(02)00247-X.

Mbai, F.N., Magiri, E.N., Matiru, V.N., Nganga, J. and Nyambati, V.C.S. 2013. Isolation and characterization of bacterial root endophytes with potential to enhance plant growth from Kenyan Basmati rice. *American International Journal of Contemporary Research*. 3(4): 25-40.

Mengel, K. and Kirltby, E.A. 1978. *Principles of Plant Nutrition*. International potash institute, Switzerland.

Miller, R.L. and Higgins, V.J. 1970. Association of cyanide with infection of Birdfoot Trefoil by *Stemphylium loti*. *Phytopathology*. 60: 104–110.

Miyake, T., Kato, A., Tateishi, H. and Teraoka, T. 2012. Mode of action of *Talaromyces* sp. KNB422, a biocontrol agent against rice seedling diseases. *Journal of Pesticide Science*. 37(1): 56-61. DOI: 10.1584/jpestics.D11-002.

Murali, M., Mahendra, C., Hema, P., Rajashekar, N., Nataraju, A., Sudarshana, M. and Amruthesh, K. 2017. Molecular profiling and bioactive potential of an endophytic fungus *Aspergillus sulphureus* isolated from *Sida acuta*: A medicinal plant *Pharmaceutical Biology*. 55(1): 1623–1630. DOI: 10.1080/13880209.2017.1315435.

Nath, R., Sharma, G.D. and Barooah, M. 2015. Plant growth promoting endophytic fungi isolated from Tea (*Camellia sinensis*) shrubs of Assam, India, *Applied Ecology and Environmental Research*. 13(3) : 877-891. DOI: 10.15666/aeer/1303_877891.

Ngoma, L., Mogatlanyan, K. and Babalola, O.O. 2014. Screening of endophytic bacteria towards the development of cottage industry: An *in vitro* study. *Journal of Human Ecology*. 47(1): 45-36. DOI: 10.1080/09709274.2014.11906738.

Petrini, O., Stone, J. and Carroll, F. E. 1982. Endophytic fungi in evergreen shrubs in western Oregon: a preliminary study. *Canadian Journal of Botany*. 60: 789–796.https://doi.org/10.1139/b82-102.

Qin, S., Xing, K., Jiang, J.H., Xu, L., H. and Li, W. J. 2011. Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actino bacteria. *Applied Microbiology and Biotechnology*. 89 (3): 457–473.Doi: 10.1007/s00253-010-2923-6.

Scannerini, S., Fusconi, A. and Mucciarelli, M. 2001. The effect of endophytic fungi on host plant morphogenesis. In: Seckbach, J. (eds) *Symbiosis. Cellular Origin, Life in Extreme Habitats and Astrobiology*. 4: 427-447. Springer, Dordrecht. https://doi.org/10.1007/0-306-48173-1_27.

Schulz, B., Guske, S., Dammann, U. and Boyle, C. 1998. Endophyte-host interactions II.Defining symbiosis of the endophyte-host interaction. *Symbiosis*. 25: 213–227.

Schwyn, B. and Neilands, J.B. 1987. Universal chemical assay for the detection and determination of Siderophores. *Analytical. Biochemistry*. 160(1): 47–56.https://doi.org/10.1016/0003-2697(87)90612-9.

Seshadri, S., Ignaimuthu, S. and Lakshminarasimhan, C. 2004. Effect of nitrogen and carbon sources on the inorganic phosphate solubilization by different *Aspergillus niger* strains. *Chemical Engineering Communications*. 191 (8): 1043-1052. https://doi.org/10.1080/00986440490276308.

Siddiqui, Z. A. 2005. PGPR: Prospective biocontrol agents of plant pathogens. In: Siddiqui Z.A, (Ed.).*PGPR: Biocontrol and Biofertilization*. Netherlands, Springer. P: 111–142.

Sterflinger, K. 2000. Fungi as geologic agents. *Geomicrobiolog Journal. 1*. 17(2): 97-124. https://doi.org/10.1080/01490450050023791.

Tan, H. M., Cao, L. X., He, Z. F., Su, G.J., Lin, B. and Zhou, S. N. 2006. Isolation of endophytic actinomycetes from different cultivars of tomato and their activities against *Ralstonia solanacearum In vitro*. *World Journal of Microbiology and Biotechnology*. 22. 1275–1280 https://doi.org/10.1007/s11274-006-9172-y.

Tian, X.L., Cao, L.X., Tan, H.M., Zaeng, Q.G., Jia, Y.Y., Han, W.Q. and Zhou, S.N. 2004. Study on the communities of endophytic fungi and endophytic actinomycetes from rice and their antipathogenic activities

in vitro. *World Journal of Microbiology and Biotechnology*. 20: 303–309.https://doi.org/10.1023/B:WIBI.0000023843.83692.3f.

Tripathy, A. and Rath, C.C. 2020. Endophytic Fungi from *Oryza sativa* L.: Isolation, Characterization, and Production of GA_3 in Submerged Fermentation. *International Journal of Current Microbiology and Applied Science*. 9(11): 1007-1020. 10.20546/ijcmas.2020.911.119.

Venkatakrishnan, S.S., Subramoniam, S.R. and Savariappan, A.R. 2003. Assessing *in vitro* solubilization potential of different zinc solubilizing bacterial (zsb) isolates. *Brazilian Journal of Microbiology*. 34: 121-125. https://doi.org/10.1590/S1517-83822004000100020.

Wakelin, S.A., Warren, R.A., Harvey, P.R. and Ryder, M.H. 2004. Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. *Biology and Fertility of Soils*. 40 : 36-43. https://doi.org/10.1007/s00374-004-0750-6.

Waqas, M., Khan, A. L. and Lee, I.J. 2014. Bioactive chemical constituents produced by endophytes and effects on rice plant growth. *Journal of Plant Interaction*. 9(1): 478–487. https://doi.org/10.1080/17429145.2013.860562.

Wei, G., Kloepper, J. W. and Sadik, T. 1991. Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth promoting rhizo bacteria. *Phytopathology*. 81: 1508–1512.

Zamin, M., Fahad, S., Khattak, A.M., Adnan, M., Wahid, F., Raza, A., Wang, D., Saud, S., Noor, M. and Bakhat, H.F. 2020. Developing the First Halophytic Turf grasses for the Urban Landscape from Native Arabian Desert Grass. *Environmental Science and Pollution Research*. 27: 39702–39716.