Assiut University Journal of Multidisciplinary Scientific Research (AUNJMSR)
Faculty of Science, Assiut University, Assiut, Egypt.
Printed ISSN 2812-5029
Online ISSN 2812-5037
Vol. 54(2): 229- 253 (2025)
https://aunj.journals.ekb.eg

# Plant growth-promoting endophytic fungi isolated from roots of some wild grasses inhabiting new reclaimed fields

Hala M. Ibrahim [1,*], Mohamed B. H. Mazen [1], T. Ramadan[1], Nivien A. Nafady[1] and Dalia A. Gaber[1,2]

[1]Botany and Microbiology Department, Faculty of Science, Assiut University, Assiut, Egypt
[2] Erfurt Research Centre for Horticultural Crops, University of Applied Sciences Erfurt, Erfurt, 99090, Germany

[*]Corresponding Author: hala.ibrahim@science.aun.edu.eg

## ARTICLE INFO

**Article History:**
Received: 2024-12-01
Accepted: 2025-02-06
Online: 2025-04-27

**Keywords**:
Endophytic fungi, Grasses, Plant growth promoters, *Curvularia spicifera*, *Fusarium pallidiroseum*, Dark sterile mycelia

## ABSTRACT

The objective of the current study was to isolate endophytic fungi and examine their potentiality for promoting plant growth by assaying production of ammonia, indole-3-acetic acid (IAA), and their efficiency for phosphate solubilization. Ninety-eight fungal isolates belonging to 26 genera were obtained from the roots of seven common grasses inhabiting the newly reclaimed fields at Arab El-Awamer, Assiut Governorate. The grasses were *Cynodon dactylon, Dichanthium annulatum, Digitaria sanguinalis, Echinochloa colona, Paspalum dilatatum, Setaria verticillata*, and *Sorghum virgatum*. *Fusarium* was the abundant genus accounting 21.6% of the recorded endophytic fungi, *Curvularia* was next, with a high recovery rate and a total count of 12.5% of all endophytic fungi. *C. dactylon* roots associated with the highest fungal dominance, whereas the lowest dominance was recorded in *P. dilatatum*. The cluster analysis segregated the studied grasses into three distinct groups (A, B and C) of plants concerning the occurrence of fungal communities. Eleven endophytic fungal species (out of 43 spp.) were restricted to the group A (*C. dactylon*, *S. verticillata* and *S. virgatum*), 8 to group B (*D. annulatum* and *D. sanguinalis*) and 6 to group C (*E. colona* and *P. dilatatum*). Eight fungal species were common in the three groups and 10 appeared in two groups. Out of 98 fungal isolates,

only 10 isolates were negative for IAA production; the remaining isolates produced IAA but with varying concentrations. Only two, *F. pallidiroseum* and *F. fujikuroi* were found to solubilize tricalcium phosphate.

## INTRODUCTION

Endophytic fungi are harmlessly present in healthy plant tissues for the entirety or a portion of their life cycle [1]. Endophytic organisms are discovered to be present in nearly every class of vascular plants and grasses that have been studied to date [2]. These fungi inhabit the host plants' organs, including the root, stem, leaf, flower, fruit, and seed, either in intracellular or intercellular spaces [3].

Endophytes are now considered a superb source of natural compounds with antimicrobial properties. Since it was discovered that these microbes may defend against pest and insect diseases, they have drawn a lot of attention [4]. It is documented that endophytic fungi produce beneficial bioactive compounds within the tissues of their host plants. This has led to a great deal of research on the interaction between microbes and plants [1, 5]. Endophytes disrupt the metabolism of their host plants and feed on them, while the plant controls the microbes' metabolic processes to produce and release chemicals that may serve as defenses for the endophytes. The ability of endophytes to associate with other species through chemical signals is essential for the host's survival and ability to adapt to a variety of biological and environmental conditions [6]. However, endophytic fungi can shield the host plant from biotic and abiotic stresses that can pose a serious threat to crop food security and safety. In addition, endophytes may offer new sources of bioactive secondary metabolites which are considered as building blocks of many medications. Therefore, endophytic fungi have greatly influenced industry, agriculture, medicine, and ultimately the economy [7].

The fungal diversity is associated with different tissues of the same host plant and is also dependent on its geographical distribution and climatic conditions [8]. Analyzing the diversity of conglomerated fungal endophytes to find new species that produce biomolecules and their function in the ecosystem is becoming more and more difficult these days. Hence, the diversity of various endophytic microbes has been explored for their metabolic potential [9]. Studies involving endophytes not only shed light on

fascinating aspects of the ecology and the interactions between these microorganisms and plants, but they also aid in understanding the potential advantages of these interactions as well as the variables that need to be investigated to create sustainable human practices, particularly in agriculture.

The characterization of endophytic fungi increases the likelihood of using them as biostimulants, biofertilizers, and biocontrol agents [10, 11]. Endophytes have recently been found to be used in various biotechnological fields, including nanotechnology for creating diverse nanoparticles integrated into various applications and bio-fertilizers to increase crop yield and drastically reduce chemical input into the environment. Immobilizing endophytic microbes in substances that can be applied in organic farming to shield the soil from weeds, drying out, and other problems is another way that microbes can be employed [12, 13].

The discovery of mycelia in healthy seeds of the rye grass *Lolium temulentum* by Guerin and Freeman marked the beginning of research on endophytic fungi that inhabit grasses [14, 15]. Many years later, researches on endophytic fungi concentrated on pasture grasses, particularly after it was discovered that the teleomorph *Epichloe* and endophytic *Neotyphodium* produced animal-toxic alkaloids [16, 17]. Endophytes are widely found in plants other than grasses [18, 19]. Dark septate endophytes are widespread among root-associated fungi, occurring in a variety of plant species. These fungal characteristic melanized hyphae are thought to be crucial for the host's survival under stress because the melanin in their cell walls may absorb and get rid of oxygen radicals produced during abiotic stress [20]. Therefore, the dominant colonization of wild grasses by dark septate endophytes may confer tolerance to a variety of environmental stress factors.

The heterogeneous group of non-pathogenic fungi known as plant growth-promoting fungi can be found inside plant roots, on the surface of roots, or in the rhizosphere. The plant growth-promoting fungi serve to enhance the soil in addition to giving the plant protection from different types of phytopathogens, root extension, and plant growth development (seed germination, seedling vigour, and photosynthetic efficiency). It is unclear exactly how the fungi promote plant growth, but it is believed that they could aid in the high-yield production of plants, agricultural crops, and rare

medicinal and herbal plants. They will also undoubtedly improve human health and the health of our ecosystem [21].

Endophytes can enhance crop development and productivity by producing phytohormones, antimicrobial compounds, and phosphorus, potassium, and zinc solubilization, among other direct or indirect plant growth-promoting characteristics. Growth-promoting fungi in fungal endophytic relationships colonize host plants and show up asymptomatically. They are frequently useful in nutrient uptake, plant growth, stress resistance, suppressing soil-borne pathogenic organisms and the growth of competitors, and disease resistance [22, 23]. Endophytes like *Colletotrichum, Piriformospora indica,* and *Penicillium* have attracted special attention because of their tendency to generate metabolites and enzymes that promote better plant development in stressed environments [24]. According to reports, gibberellic acid, which is released by *Cladosporium sphaerospermum*, promotes the growth of soybean and rice plants [25]. Additionally, pestalotin analogue, a metabolite with gibberellin activity that promotes germination, was discovered to be produced by *Pestalotiopsis microspora* [26]. Endophytes like *Alternaria alternata* and *Fusarium tricinctum* produced derivatives of indole acetic acid that promoted plant growth [27]. Johnson et al. [28] reported that the root endophyte *Piriformospora indica* modulated phytohormones that influenced the host plant growth and development. Additionally, this endophyte improved translocation and nutrient absorption. This is especially important in phosphorus and nitrogen absorption from the soil. In this context, the aim of our study was to explore the fungal endophytic diversity associated with the common grasses in a newly reclaimed field in Arab El-Awamer, Assiut Governorate, and to explore their growth-promoting capabilities.

## MATERIALS AND METHODS

### - Area of study

In this study, the selected grasses were collected from four different sites at Arab El-Awamer, Assiut Governorate. Arab El-Awamer is located at the border between the Nile valley and the Eastern desert, about 20 km southeast of Assiut city. The farmers of Arab El-Awamer have been known to increase their reclamation activities of the desert areas from about 20 years ago, and these activities continue to the present day.

- **Sampling**

Twenty-one samples from seven different plant species were gathered from Arab El Awamer, Assiut Governorate. Seven grass species (family Poaceae) were chosen namely: *Cynodon dactylon* (L.) Pers, *Dichanthium annulatum* (Forssk.) Stapf, *Digitaria sanguinalis* (L.) Scop, *Echinochloa colona* (L.) Link, *Paspalum dilatatum* Poir., *Setaria verticillata* (L.) P. Beauv. and *Sorghum virgatum (*Hack.) Stapf. In order to isolate and identify the fungi, the gathered plants and soil samples were collected during the summers of 2022 and 2023, put into sterile, clean plastic bags, and brought immediately into the lab. After being cleaned of dirt and soil particles with running tap water, the obtained root samples were refrigerated to facilitate the isolation of endophytic fungi.

- **Isolation and purification of endophytic fungi**

Applying Petrini & Fisher's approach [29], fungal endophytes associated with the collected plant roots were isolated. In summary, the roots were cleaned using running tap water to get rid of dust, dirt, and debris. They then underwent surface protocol, which involved immersing the root segments (about 1.5 cm each) in a 2.5% sodium hypochlorite solution for 3.5 minutes. Next, they were treated with 70% ethanol for 2 minutes, and finally they were repeatedly washed with sterilized distilled water.

The sterile filter paper was then used to dry the sterilized root segments. Using sterile forceps, five segments were inserted on an agar plate and cultured for seven days at 25±2 ºC. To stop bacteria from growing, fungal hyphae tips originating from plant tissues were sub-cultured on Potato Dextrose Agar (PDA) treated with 250 mg/L Chloramphenicol. This was done for four days at 26–27 ºC [30]. For each sample, five duplicates were made. The developing fungi were counted, isolated, purified, and identified. For each sample that was obtained, the counts were expressed as colony forming units, or "CFU," per 25 root segments. The process of purifying colonies involved repeatedly sub culturing the fungal colonies in PDA until pure isolates were obtained. The pure fungal isolates were identified morphologically using macroscopic and microscopic features according to the different mycological keys [31].

- **The biodiversity of fungal isolates**

The diversity of the fungal community recovered from the collected plant samples was calculated using the following metrics: fungal taxa, which indicates the number of

isolated fungal species from the plant; dominance, which shows the dominance of fungal taxa in a specific sample; the Simpson index, which indicates the evenness of the fungal community; and the Shannon index, which is the diversity index, which estimates the number of fungal individuals while accounting for the number of fungal taxa. Classification and ordination techniques were used to analyze the endophytic fungal associations. So, a presence/absence data matrix of 7 grasses × 43 fungal species was used and subjected to classification by cluster analysis (Word's method) using Paleontological Statistics "PAST" software v. 4.7.0.0 [32], and a dendrogram was elaborated. Also, ordination procedures were performed by the same software and the Detrended Correspondence Analysis (DCA) was used to check the magnitude of change in fungal composition along the first ordination axis. Canonical Correspondence Analysis (CCA) was elaborated to perform the relationships between grasses and/or the fungal species and the variations in some primary and secondary components that were indicated by CCA biplot. The 7 variables in the CCA biplot (total soluble sugars TSS, total free amino acids TFAA and soluble proteins SP as primary metabolites, total phenolics, flavonoids, alkaloids, and terpenoids as secondary metabolites) were represented by arrows pointing in the direction of maximum variation, with their length proportional to the rate of change.

- **Screening for plant growth-promoting activities by isolated endophytic fungi**
- **Indole-3-acetic acid (IAA) production ability test**

The ability of the fungal isolates to produce indole-3-acetic acid (IAA) was examined according to Stajkovic et al. [33]. Fungal cultures were cultivated in 50 ml of PD broth that had been supplemented with 2 mg/ml of tryptophan for 5-7 days. After centrifuging the cultures, 1 ml of the supernatant was combined with 1 ml of the reagent, which was 10.8 M $H_2SO_4$ with 4.5 g of $FeCl_3$ per litre. The mixture was incubated for 25 minutes at room temperature, and absorbance of the developed pink colour was measured by the spectrophotometer (UV-120 SPECTROPHOTOMETER, MIOSTECH PTY, Egypt) at 530 nm. With pure IAA, a calibration curve was created.

- **Detection of phosphate solubilizing ability**

By employing Pikovskaya phosphate medium (PVK) for the plate assay, the fungal isolates were examined [34]. The medium's contents included 10 g of glucose, 5 g

of $CaHPO_4$, 0.5 g of $(NH_4)_2SO_4$, 0.2 g of NaCl, 0.1 g of $MgSO_4.7H_2O$, 0.2 g of KCL, 0.5 g of yeast extract, 0.002 g of $MnSO_4.H_2O$, and 0.002 g of $FeSO_4.7H_2O$. The mixture was built up to one litre using distilled water and the pH was set to 6.8. The isolates were inoculated on the centre of the plate in three replicates using a sterilised loop. A distinct halo zone surrounded the colony following seven days of incubation at 28 ºC, demonstrating the isolates' capacity to solubilise phosphate.

- **Ammonia production test**

The production of ammonia was detected according to Stajkovic et al [35] by using Nessler's reagent. The test for ammonia production was positive when a yellow-to-brown colour was developed.

**- Statistical analyses**

Data were subjected to statistical analysis using SPSS (version 21). One-way ANOVA was performed followed by the post hoc Duncan's multiple-range test for comparison between means at $P \leq 0.05$.

## RESULTS AND DISCUSSION

### The endophytic fungi associated with different grasses

In this study, 43 fungal endophytic species belonging to 26 genera were isolated from the roots of seven common grasses that were collected from various sites in Arab El-Awamer. As indicated in Table 1, the total fungal count was highest in *P. dilatatum*, while *D. annulatum* had the highest number of fungal genera. Nine species belonging to 8 fungal genera were isolated from *C. dactylon*, 16 species belonging to 13 fungal genera were isolated from *D. annulatum*, 14 species from 10 fungal genera were isolated from *D. sanguinalis*, 15 species belonging to 9 genera were isolated from *E. colona*, 13 species belonging to 7 fungal genera were isolated from *P. dilatatum*, 14 species belonging to 11 fungal genera were isolated from *S. verticillata* and 11 fungal species belonging to 7 genera were isolated from *S. virgatum*. The overall total fungal count in this habitat (newly reclaimed desert) was 366 CFU/ 525 root segment.

**Table 1:** Total fungal count (CFU/525 root segment), number of endophytic fungal genera and species isolated from roots of seven common grasses gathered from Arab El-Awamer site cultivated on PDA media at 25±2ºC for 7-14 days, n = 9.

| Plants | *C. dactylon* | *D. annulatum* | *D. sanguinalis* | *E. colona* | *P. dilatatum* | *S. verticillata* | *S. virgatum* |
|---|---|---|---|---|---|---|---|
| **Total fungal count =366** | 34 | 56 | 72 | 40 | 74 | 48 | 42 |
| **No. of genera=26** | 8 | 13 | 10 | 9 | 7 | 11 | 7 |
| **No. of species=43** | 9 | 16 | 14 | 15 | 13 | 14 | 11 |

In general, the most common genera (found in all plant samples) were *Fusarium* and dark sterile mycelium, which together accounted for 21.6% of all endophytic fungi on PDA medium (Table 2). *Curvularia* sp. was the next most frequent genus, accounting for 12.5% of all endophytic fungi. *Aspergillus* was detected as a moderate occurrence, with a total count accounting for 2.46% of all endophytic fungi. The endophytic fungi: *Candida tropicalis*, *Curvularia lunata,C. spicifera*, *Fusarium fujikuroi*, *F. incarnatum*, *Sarocladium strictum*, and white sterile mycelium were isolated in low frequency with total count at 12.8%, 4.1%, 7.38%, 5.19%, 3.55%, 2.46%, and 4.1%, respectively while the other fungal genera were isolated in rare occurrences.

*Aspergillus* was represented by four species: *A. flavus, A. ochraceus, A. carbonarius,* and *A. terreus,* and they were present in rare occurrences with total count matches 0.55%, 0.27%, 0.82%, and 0.82%, respectively, of total endophytic fungi. The significance of endophytic fungi as biological sources of a variety of valuable compounds, such as metabolites that regulate plant growth, and are antibacterial, antifungal, antiviral, and insecticidal, has been acknowledged in a growing body of literature from a variety of disciplines in recent years. These compounds are used to improve the growth and competitiveness of the host in nature [36, 37, 38, 39, 40, 41]. According to the current findings, endophytic fungal assemblages were found in all of the plant species that were studied. Additionally, several plants had the same fungal genera and species present, suggesting that endophytic fungi can be found in plants that belong to distinct families. Table 2 shows that the root endophytic fungi isolated from the *Cynodon dactylon* varied widely, of which nine fungal species belonging to eight fungal genera were isolated from sites located in Arab El-Awamer. *Aspergillus flavus* recorded

the lowest total fungal count percentage of 5%, while dark sterile mycelia demonstrated the highest total fungal count percentage with TC% reaching 60%. The overall fungal count in this plant was 34 CFU/75 root segment.

Table 2 demonstrates the extensive range of root endophytic fungi that were isolated from the *Dichanthium annulatum*. Of these, 16 fungal species from 13 different genera were isolated from sites in Arab El-Awamer. Dark sterile mycelia showed the highest total count percentage (85.71%), while *Chaetomium elatum, Sarocladium strictum, Verticillium lecanii,* and *F. fujikuroi* showed the lowest count percentage (3.45%). Dark sterile mycelia were the most common genera isolated from this plant. It is worth mentioning that *Periconia macrospinosa* fungus is considered the first recorded endophytic fungus in Egypt and was isolated from Arab El-Awamer site. The overall fungal count in this plant was 56 CFU/75 root segment. Furthermore, only the *Dichanthium annulatum* plant yielded isolates of *Chaetomium elatum, Cladosporium macrocarpum, Periconia macrospinosa, Talaromyces duclauxii, and Verticillium lecanii*, suggesting that these fungi are unique to this plant.

Table 2 shows that the endophytic fungi isolated from the *Sorghum virgatum* roots varied widely whereas 11 fungal species belonging to 7 fungal genera were isolated. *Curvularia spicifera* showed the highest total count percentage, while *Papulaspora equi*, dark sterile mycelia, and *Striaticonidium cinctum* showed the lowest count percentage. The overall fungal count in this plant was 42 CFU/75 root segment. Dark sterile mycelia and *Fusarium incarnatum* were the most common genera isolated from this grass. It is worthy to mention that *Papulaspora equi*, which was isolated from *Sorghum virgatum* roots is regarded as the first endophytic fungus ever discovered in Egypt.

The root endophytic fungi isolated from the *Digitaria sanguinalis* varied greatly, as shown in Table 2. Of them, 14 fungal species from 10 different genera were isolated from sites in Arab El-Awamer. A. *terreus, C. spicifera, C. lunatus*, and *F. solani* had the lowest percentage of total fungal counts, at 3.8%, whereas dark sterile mycelia had the largest percentage (65%). *F. fujikuroi* was the most often isolated genus from this plant.

Table 2 reveals a wide range of root endophytic fungi isolated from the *Echinochloa colona* plant, including 15 fungal species from 9 fungal genera found at sites in Arab El-Awamer. Dark sterile mycelia showed the highest total count percentage

(64%), while *A. terreus, A. alternata, A. carbonarius, A. ochraceus, Fusarium oxysporum,* and white sterile mycelium recorded the lowest total fungal count with a percentage of 6%. *Fusarium oxysporum* and dark sterile mycelia were the most common genera isolated from this plant. It is important to note that the fungus *Periconia macrospinosa*, which was only isolated from *Sorghum virgatum* and *Echinochloa colona* grasses, is thought to be the first endophytic fungus ever discovered in Egypt. The overall fungal count in this plant was 40 CFU/75 root segment.

Table 2 shows that the root endophytic fungi isolated from the *Setaria verticillata* varied widely, of which 14 fungal species belonging to 11 fungal genera were isolated. *Candida tropocalis* showed the highest total count percentage (90.3%), while *Eurotium repenes,* dark sterile mycelia, and *F. incarnatum* recorded the lowest total fungal count percentage, with a percentage of 3.2%. Dark sterile mycelia were the most common genera isolated from this plant. The overall fungal count was 48 CFU/75 root segment.

Table 2 shows that the root endophytic fungi isolated from the *Paspalum dilatatum* plant varied widely, of which 17 fungal species belonging to 13 fungal genera were isolated. Dark sterile mycelia showed the highest total count percentage (70.8%), while *A. carbonarius, C. lunatus*, *F. incarnatum, Sarocladium strictum,* and *F. sporothrichoids* recorded the lowest total fugal count percentage, with a percentage of 4.8%. Dark sterile mycelia and *C. lunatus* were the most common genera isolated from this plant. The overall fungal count was 74 CFU/75 root segment.

**Table 2:** Total fungal count (CFU/75 root segment) and percentage of total counts (TC%), of endophytic fungal genera and species isolated from roots of different studied grasses gathered from Arab El-Awamer area; and cultivated on PDA media at 25±2ºC for 7-14 days, n = 9.

| Plant | | Site 1 | | Site 2 | | Site 3 | | Site 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | **Fungi** | **TC** | **%TC** | **TC** | **%TC** | **TC** | **%TC** | **TC** | **%TC** |
| *Cynodon dactylon* | *Aspergillus flavus* | 1 | 20 | 1 | 5 | 0 | 0 | 0 | 0 |
| | *Beauveria bassiana* | 0 | 0 | 2 | 11 | 0 | 0 | 0 | 0 |
| | *Candida tropicalis* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 10 |
| | *Curvularia spicifera* | 0 | 0 | 7 | 37 | 0 | 0 | 4 | 40 |
| | Dark sterile mycelia | 3 | 60 | 2 | 11 | 0 | 0 | 1 | 10 |
| | *Fusarium incarnatum* | 1 | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *F. proliferatum* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 10 |
| | *Sarocladium strictum* | 0 | 0 | 2 | 11 | 0 | 0 | 3 | 30 |
| | White sterile mycelia | 0 | 0 | 5 | 26 | 0 | 0 | 0 | 0 |

| Plant | | Site 1 | | Site 2 | | Site 3 | | Site 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | **Fungi** | **TC** | **%TC** | **TC** | **%TC** | **TC** | **%TC** | **TC** | **%TC** |
| | **TC = 34** | **5** | | **19** | | **0** | | **10** | |
| *Dichanthium annulatum* | *Aspergillus terreus* | 0 | 0 | 0 | 0 | 1 | 5.0 | 0 | 0 |
| | *Candida tropicalis* | 5 | 17.24 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Chaetomium elatum* | 1 | 3.45 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Cladosporium macrocarpum* | 0 | 0 | 0 | 0 | 2 | 10.0 | 0 | 0 |
| | *Curvularia lunatus* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 14.29 |
| | *C. spicifera* | 2 | 6.90 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Dark sterile mycelia | 14 | 48.28 | 0 | 0 | 2 | 10.0 | 6 | 85.71 |
| | *Fusarium sporothrichoids* | 0 | 0 | 0 | 0 | 2 | 10.0 | 0 | 0 |
| | *F. fujikuroi* | 1 | 3.45 | 0 | 0 | 2 | 10.0 | 0 | 0 |
| | *Periconia macrospinosa* | 0 | 0 | 0 | 0 | 3 | 15.0 | 0 | 0 |
| | *Rhizoctonia solani* | 2 | 6.90 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Sarocladium strictum* | 1 | 3.45 | 0 | 0 | 2 | 10.0 | 0 | 0 |
| | *Talaromyces duclauxii* | 2 | 6.90 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Talaromyces* sp. | 0 | 0 | 0 | 0 | 4 | 20.0 | 0 | 0 |
| | *Torula* sp. | 0 | 0 | 0 | 0 | 2 | 10.0 | 0 | 0 |
| | *Verticillium lecanii* | 1 | 3.45 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **TC = 56** | **29** | | **0** | | **20** | | **7** | |
| *Sorghum virgatum* | *Chaetomium piluliferum* | 0 | 0 | 3 | 9.7 | 0 | 0 | 0 | 0 |
| | *Curvularia spicifera* | 2 | 66.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Dark sterile mycelium | 0 | 0 | 1 | 3.2 | 2 | 25.0 | 0 | 0 |
| | *Exserohilum rostratum* | 0 | 0 | 8 | 25.8 | 0 | 0 | 0 | 0 |
| | *Fusarium proliferatum* | 0 | 0 | 0 | 0 | 2 | 25.0 | 0 | 0 |
| | *F. fujikuroi* | 0 | 0 | 0 | 0 | 1 | 12.5 | 0 | 0 |
| | *F. incarnatum* | 1 | 33.3 | 0 | 0 | 1 | 12.5 | 0 | 0 |
| | *F. Pallidiroseum* | 0 | 0 | 17 | 54.8 | 0 | 0 | 0 | 0 |
| | *Fusarium* sp. | 0 | 0 | 0 | 0 | 2 | 25.0 | 0 | 0 |
| | *Papulaspora equi* | 0 | 0 | 1 | 3.2 | 0 | 0 | 0 | 0 |
| | *Striaticonidium cinctum* | 0 | 0 | 1 | 3.2 | 0 | 0 | 0 | 0 |
| | **TC = 42** | **3** | | **31** | | **8** | | **0** | |
| | **Fungal genera = 7** | **2** | | **6** | | **2** | | **0** | |
| | **Fungal species = 11** | **2** | | **6** | | **5** | | **0** | |
| *Digitaria sanguinalis* | *Aspergillus terreus* | 1 | 3.8 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Stachybotrys elegans* | 5 | 19.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Candida tropicalis* | 0 | 0 | 8 | 30.8 | 0 | 0 | 0 | 0 |
| | *Curvularia lunatus* | 5 | 19.2 | 1 | 3.8 | 0 | 0 | 0 | 0 |
| | *C. spicifera* | 1 | 3.8 | 2 | 7.7 | 0 | 0 | 0 | 0 |
| | Dark sterile mycelia | 0 | 0 | 0 | 0 | 13 | 65 | 0 | 0 |
| | *Fusarium incarnatum* | 8 | 30.8 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *F. oxysporum* | 2 | 7.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *F. solani* | 1 | 3.8 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *F. fujikuroi* | 3 | 11.5 | 7 | 26.9 | 1 | 5 | 0 | 0 |
| | *Papulaspora equi* | 0 | 0 | 2 | 7.7 | 2 | 10 | 0 | 0 |
| | *Periconia macrospinosa* | 0 | 0 | 0 | 0 | 1 | 5 | 0 | 0 |
| | *Torula* sp. | 0 | 0 | 0 | 0 | 3 | 15 | 0 | 0 |
| | White sterile mycelium | 0 | 0 | 6 | 23.1 | 0 | 0 | 0 | 0 |

| Plant | | Site 1 | | Site 2 | | Site 3 | | Site 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | **Fungi** | **TC** | **%TC** | **TC** | **%TC** | **TC** | **%TC** | **TC** | **%TC** |
| | **TC = 72** | **26** | | **26** | | **20** | | **0** | |
| *Echinochloa colona* | *Aspergillus terreus* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 6 |
| | *A. carbonarius* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 6 |
| | *A. ochraceus* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 6 |
| | *Alternaria alternata* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 6 |
| | *Cladosporium cladosporoids* | 1 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *C. herbarum* | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 18 |
| | *Curvularia spicifera* | 3 | 25 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Dark sterile mycelia | 0 | 0 | 7 | 64 | 0 | 0 | 8 | 47 |
| | *Fusarium oxysporum* | 0 | 0 | 3 | 27 | 0 | 0 | 1 | 6 |
| | *F. sporothrichoides* | 2 | 17 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *F. sambucinum* | 2 | 17 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *F. pallidoroseum* | 2 | 17 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Periconia macrospinosa* | 0 | 0 | 1 | 9 | 0 | 0 | 0 | 0 |
| | White sterile mycelia | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 6 |
| | Yellow sterile mycelia | 2 | 17 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **TC = 40** | **12** | | **11** | | **0** | | **17** | |
| | **Fungal genera = 9** | **4** | | **3** | | **0** | | **6** | |
| | **Fungal species = 15** | **6** | | **3** | | **0** | | **8** | |
| *Setaria verticillata* | *A. carbonarius* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 12.5 |
| | *Candida tropicalis* | 0 | 0 | 28 | 90.3 | 0 | 0 | 0 | 0 |
| | *Cladosporium cladosporoids* | 1 | 11.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Curvularia lunatus* | 4 | 44.4 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *C. tuberculata* | 2 | 22.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Dark sterile mycelia | 0 | 0 | 1 | 3.2 | 0 | 0 | 1 | 12.5 |
| | *Eurotium repenes* | 0 | 0 | 1 | 3.2 | 0 | 0 | 0 | 0 |
| | *Exserohilum pedicellatum* | 1 | 11.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *E. rostratum* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 12.5 |
| | *F. incuratum* | 0 | 0 | 1 | 3.2 | 0 | 0 | 0 | 0 |
| | *F. proliferatum* | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 37.5 |
| | *Humicola isolens* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 12.5 |
| | *Macrophomina phasoliana* | 1 | 11.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | white sterile mycelium | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 12.5 |
| | **TC = 48** | **9** | | **31** | | **0** | | **8** | |
| | **Fungal genera = 11** | **4** | | **4** | | **0** | | **6** | |
| | **Fungal species = 14** | **5** | | **4** | | **0** | | **6** | |
| *Paspalum dilatatum* | *A. carbonarius* | 1 | 4.8 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Alternaria alternata* | 2 | 9.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Candida tropicalis* | 5 | 23.8 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Chaetomium piluliferum* | 0 | 0 | 2 | 6.9 | 0 | 0 | 0 | 0 |
| | *Cladosporium cladosporoids* | 2 | 9.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Curvularia lunatus* | 1 | 4.8 | 0 | 0 | 0 | 0 | 3 | 12.5 |
| | *C. spicifera* | 0 | 0 | 6 | 20.7 | 0 | 0 | 0 | 0 |
| | Dark sterile mycelia | 1 | 4.8 | 0 | 0 | 0 | 0 | 17 | 70.8 |
| | *Fusarium incarnatum* | 1 | 4.8 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *F. sporothrichoids* | 1 | 4.8 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *F. fujikuroi* | 4 | 19.0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *F. oxysporum* | 0 | 0 | 6 | 20.7 | 0 | 0 | 0 | 0 |

| Plant | | Site 1 | | Site 2 | | Site 3 | | Site 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | **Fungi** | **TC** | **%TC** | **TC** | **%TC** | **TC** | **%TC** | **TC** | **%TC** |
| | *Rhizoctonia solani* | 0 | 0 | 15 | 51.7 | 0 | 0 | 0 | 0 |
| | *Rhizopus stolonifer* | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 8.3 |
| | *Sarocladium strictum* | 1 | 4.8 | 0 | 0 | 0 | 0 | 0 | 0 |
| | White sterile mycelia | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 8.3 |
| | Yellow sterile mycelia | 2 | 9.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **TC = 74** | **21** | | **29** | | **0** | | **24** | |

**Biodiversity of endophytic fungi in the host grass**

Data in Table 3 indicate that *Cynodon dactylon* has the highest fungal dominance whereas *Paspalum dilatatum* has the lowest dominance. *Setaria verticillata* has the higher Shannon and Simpson diversity indices. The data referred to high variation in the abundances of endophytic fungi associated with *C. dactylon*, compared to other studied grasses, as indicated by the lowest evenness value, while endophytes associated with *Setaria verticillata* have less abundance variation and hence the highest evenness value.

**Table 3:** Biodiversity of endophytic fungal community recovered from collected plant samples.

| **Plants / Fungal diversity** | ***C. dactylon*** | ***D. annulatum*** | ***D. sanguinalis*** | ***E. colona*** | ***P. dilatatum*** | ***S. verticillata*** | ***S. virgatum*** |
|---|---|---|---|---|---|---|---|
| **Taxa (S)** | 9 | 16 | 14 | 15 | 17 | 15 | 10 |
| **Individuals** | 14 | 20 | 19 | 17 | 19 | 16 | 12 |
| **Dominance (D)** | 0.133 | 0.075 | 0.086 | 0.073 | 0.064 | 0.070 | 0.111 |
| **Simpson (1-D)** | 0.867 | 0.925 | 0.914 | 0.927 | 0.936 | 0.920 | 0.889 |
| **Shannon (H)** | 2.107 | 2.692 | 2.552 | 2.670 | 2.799 | 2.686 | 2.254 |
| **Evenness ($e^{H/S}$)** | 0.913 | 0.923 | 0.917 | 0.963 | 0.966 | 0.978 | 0.952 |

The seven studied grasses were separated into 3 groups by the application of cluster analysis to the presence/absence data set of 43 fungal species × 7 grasses (Figure 1). Group "A" included *C. dactylon*, *S. verticillata* and *S. virgatum*. Eleven endophytic fungi were associated only with the roots of one, two, or the three grasses of this group. Group "B" included two grasses, *D. annulatum* and *D. sanguinalis*, and 8 fungal species were associated only with one or both grasses of this group. Also, group "C" included *E. colona* and *P. dilatatum* where 6 fungal species were found to be only isolated from the roots of one or both grasses. It is worth mentioning that 8 endophytic fungi were isolated from at least one plant species from the three groups, while 10 fungal species were isolated from two groups of grasses.

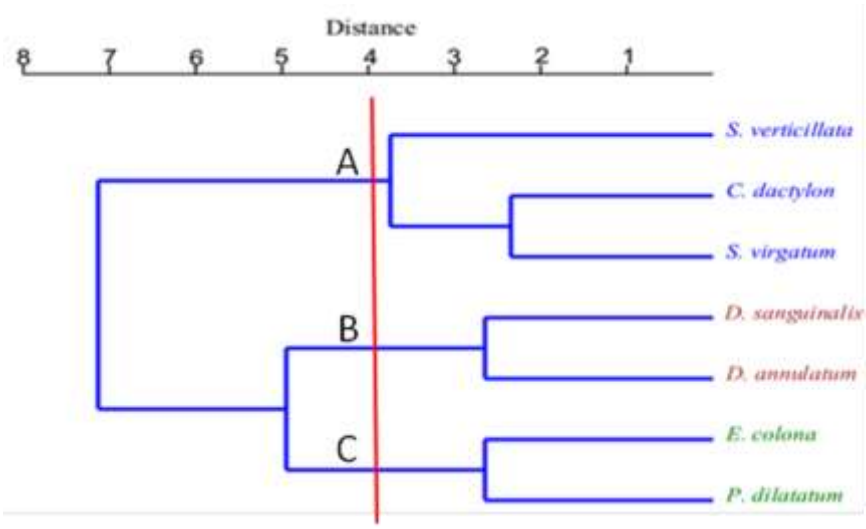


**Figure 1:** Cluster analysis showing the fungal communities recovered from collected plant samples

The ordination analysis (DCA) of the data set separated the three groups along the first and second DCA axes (Figure 2). The first eigenvalue accounts for 53.6% of the total variation and the second eigenvalue accounts for another 32.3% of the variation for a cumulative 85.9%. Thus, the first axis captured the greatest proportion of the variation in the endophytic fungal species composition among grasses. This DCA reflects the differences between these groups especially group "B" that represented far away from axis 1 and group "C" that was close to axis 1, while group "A" represented between both.

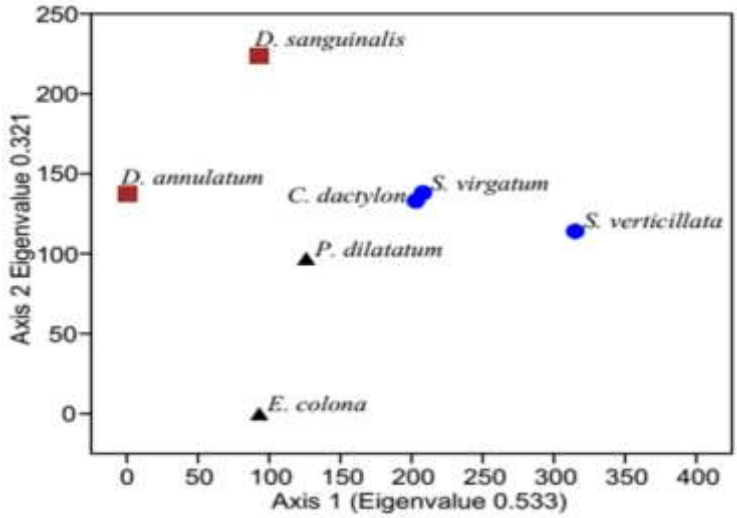


**Figure 2:** Detrended correspondence analysis diagram (DCA) showing ordination of the studied grasses on axes 1 and 2 as classified by cluster analysis into three groups as indicated with different labels

Canonical correspondence analysis (CCA) was used in this study as an attempt to assess the relationship between the endophytic fungal species and some variables in the host grasses (Figure 3). The seven variables, which included some primary and secondary metabolites in grasses, contributed independently to the overall ordination. In the CCA biplot (Figure 3), the first axis was negatively correlated with total terpenoids and flavonoids and positively correlated with other metabolites. The second axis was negatively correlated with total terpenoids and free amino acids and positively correlated with other metabolites. The ordination biplot produced by CCA showed that most fungal species occupied the positive sides of both axes. This led to the suggestion that endophytic fungi prefer to associate with the more nutritive grasses as indicated by the arrow length of soluble sugars and proteins, and also phenolics and alkaloids. On the other hand, the total terpenoids displayed a negative correlation with endophytic fungal association. However, variations in the contents of primary and secondary metabolites showed that there are non-significant differences between the three groups, except in terpenoids (data are not shown here).

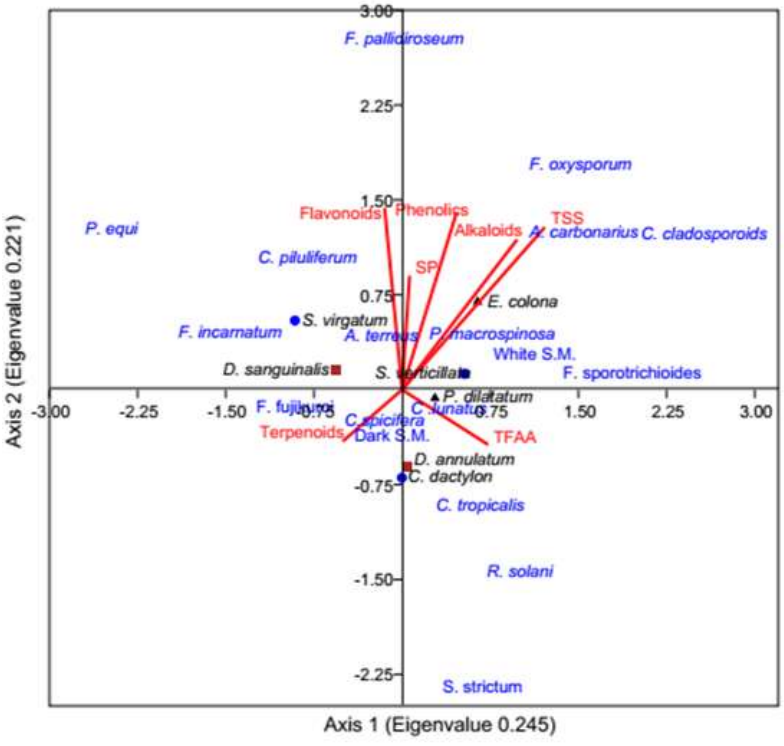


**Figure 3:** Canonical correspondence analysis (CCA) biplot of axes 1 and 2 showing the distribution of studied grasses and associated endophytic fungi as affected by some metabolic variables. The biplot represents the fungal species that associated with two or three groups.

Additionally, the current study found that the plants' fungal endophyte abundance, absence, and presence varied significantly. *Paspalum dilitatum* and *Dichanthium annulatum*, the two plants with the greatest number of fungal species, had 17 and 16 fungal species, respectively, indicating that some plants have more endophytic fungi than others. *Aspergillus* and *Fusarium*, two endophytic fungi that are present in most plants, exhibited varying relative rates in their host plants. The study found that the isolated endophytic fungi had different levels of host specificity. Some species, such as *Alternaria alternata, Aspergillus terreus*, and *A. flavus*, showed a strong affinity for particular plants, whereas others seemed to be able to colonize multiple plant hosts.

Endophytes' host specificity has been the subject of conflicting studies, with some studies suggesting that they can only stimulate the growth of plants that are closely related to their original host [42]. In this study, some endophytic fungi were detected only in a single host grass. For example: *Beauveria bassiana* was isolated only from *C. dactylon*; *Chaetomium elatum, Curvularia. macrocarpum, Verticillium lecanii, Talaromyces duclauxii,* and *Talaromyces* sp. were isolated only from *D. annulatum*; *Cladosporium herbarum* was isolated from *E. colona*; *C. tuberculata, Exserohilum pedicellatum, Macrophomina phasoliana, Exserohilum rostratum, Rhizopus stolonifer* and *Humicola isolens* were isolated from *S. verticillata*; *Eurotium repens* was isolated from *P. dilatatum*; *F. solani and Stachybotrys elegans* were isolated from *D. sanguinalis;* and *Striaticonidium cinctum* was isolated from *S. virgatum.* Bettucci et al. [43] and Arnold et al. [44] had previously reported similar results [45].

On the other hand, a non-specific endophyte like *Periconia macrospinosa* was recently described as a distinct dark septate endophyte that lives in the roots of different plants [46], and this matches with our results where *Periconia* was isolated from three grasses (*D. annulatum, D. sanguinalis,* and *E. colona*). Also, *Fusarium* was the most dominant genus and isolated from the seven studied grasses. This is in agreement with the previous studies, which revealed that *Fusarium* is considered a common endophyte of native and cultivated plants, especially grasses [47, 48]. The wide diversity of grass endophytes includes species from the following *Fusarium* species complexes: *F. chlamydosporum, F. fujikuroi, F. incarnatum, F. equiseti, F. oxysporum* and *F. sambucinum* [49, 50, 51, 52, 53, 54]. *Fusarium verticillioides* and *F. proliferatum* are

found in asymptomatic native grasses in the USA [55]. However, in this study *F. fujikuroi, F. incarnatum, F. oxysporum, F. pallidiroseum, F. proliferatum, F. sambucinum, F. solani* and *F. sporotrichioides* were isolated from the selected grasses.

As an endophytic fungus of wild grass, *Sarocladium strictum* was isolated in this study. These findings are consistent with several earlier investigations, where Sieber et al. [56] reported that *Sarocladium strictum* is a well-known endophyte in Poaceae and has been isolated from wheat tissues in multiple investigations. According to Anjos et al. [57], *Sarocladium* species are widespread endophytes of tropical pasture grass and may help host plants withstand biotic and abiotic stresses.

**Estimation of plant growth promoting activities by isolated endophytic fungi**

Plant growth promoting properties such as indole-3-acetic acid (IAA) production, phosphate solubilizing activity (PSA) and ammonia production of 98 fungal isolates were tested. Ten isolates were negative for IAA production; the remaining isolates were tested positive, albeit at varying concentrations. While the other examined isolates were positive for ammonia production, albeit at varied concentrations, five isolates tested were negative for this as well. The ammonia production capability of the studied endophytic fungal isolates was detected in peptone water media (Table 4). The maximum ammonia production among these isolates was found in *Curvularia spicifera,* followed by *Curvularia lunata*, *Fusarium fujikuroi, Fusarium pallidiroseum*, and *Chaetomium elatum*. Moderate activity was observed in *Sarocladium strictum*, dark sterile mycelia, *Cladosporium cladosporoids*, and *Alternaria alternata*, while the minimum activity of ammonia production was observed in *Exserohilum pedicellatum* (Figure 4).

The ability of endophytic fungus linked to many grasses to synthesize ammonia promotes plant growth and aids in controlling the full metabolism required for plant lifecycles. Host grasses readily absorbed ammonia produced by endophytic fungus. The current study found that a large number of endophytic fungi that were isolated from wild grass roots could produce ammonia in a peptone water broth medium [58].

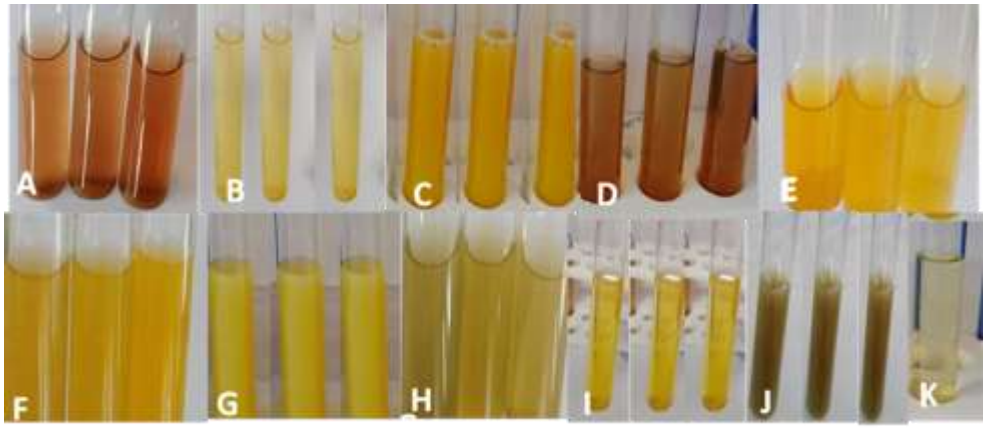


**Figure 4.** Screening for ammonia production by selected fungi were grown on peptone water media for 5-7 days, (n = 3). **(A)** *Curvularia spicifera,* **(B)** *Exserohilum pedicellatum,* **(C)** *Fusarium pallidiroseum,* **(D)** *Curvularia lunatus,* **(E)** *Cladosporium cladosporoids***, (F)** *Sarocladium strictum,* **(G)** Dark sterile mycelia**, (H)** *Fusarium fujikuroi,* **(I)** *Chaetomium elatum***, (J)** *Alternaria alternata,* **(K)** Control**.**

In this study, two isolates only that found to have the activity to solubilize phosphate. This was observed as a distinct halo zone surrounded by an opaque background around the fungal culture spot. Regarding both isolates, *Fusarium pallidiroseum* was found to be more active in tricalcium phosphate solubilization than *Fusarium fujikuroi* as shown in Figure 5. Phosphates are inorganic compounds that can be found in both free and mixed forms in soil, water, and many other plant parts. Phosphates are necessary for the plant's respiration, photosynthesis, and general growth and development. Microbes play a crucial role in the transport and solubilization of organic phosphate in plant roots. In addition, endophytic fungi that were isolated from grass roots shown the ability to break down the complex form of phosphate compounds that were supplied in the medium. These results corroborate the phosphate solubilizing ability of endophytic fungi, which was demonstrated in earlier studies [59, 60].

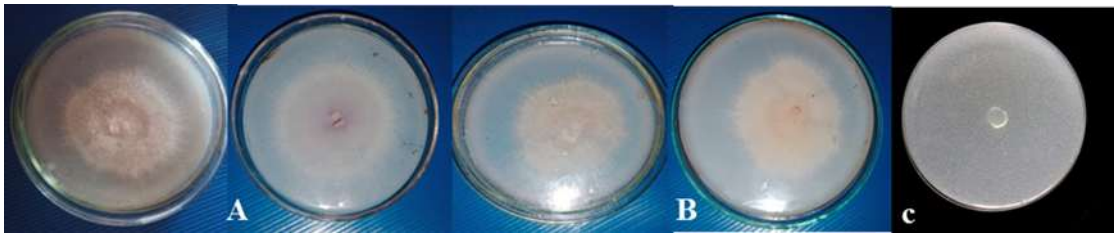


**Figure 5**. Colony features and clear zone formation for TCP solubilization on PVK agar after 7 days (left = above view, right = below view). **(A)** *Fusarium pallidiroseum,* **(B)** *Fusarium fujikuroi***, (C)** Control.

The most potent isolates, from the positive isolates, were chosen to measure the amount of IAA in liquid medium in presence of a fixed concentration of tryptophan. Amongst 10 selected isolates, *Curvularia spicifera* and *Exserohilum pedicellatum* produced the highest quantity of IAA (73.67 and 72.29 $\mu g.ml^{-1}$, respectively) after one week of growth. The isolates of other fungal genera also produced a significant amount of IAA ranging between 19.21 and 63.55 $\mu g.ml^{-1}$ (Figure 6 and Table 4).

Numerous studies and reports have examined the production of IAA by microbes associated with plants [61, 62]. In this study, *Curvularia spicifera*, *Alternaria alternata*, *Fusarium* spp., and *Chaetomium elatum*, isolated from different grasses, produced IAA in liquid medium supplemented with L-Trp. According to earlier research, pure cultures of endophytic fungi (such as *Alternaria alternata*, *Aspergillus fumigatus*, *Chaetomium globosum*, *C. acutatum*, *C. gloeosporioides*, *Fusarium* spp., *M. cinnamomi*, *Paecilomyces* spp., *Penicillium* spp., and *Phoma* spp.) cultivated in liquid medium supplemented with L-Trp could produce IAA [63, 64].

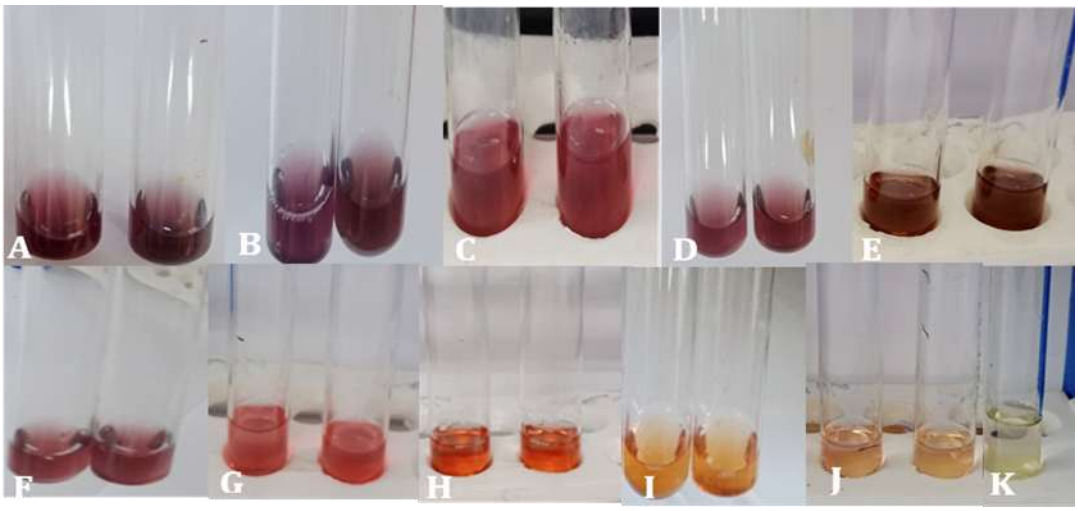


**Figure 6.** Screening for IAA production by selected fungi were grown on media for 5-7 days, (n = 2). **(A)** *Curvularia spicifera*, **(B)** *Exserohilum pedicellatum*, **(C)** *Fusarium pallidiroseum*, **(D)** *Curvularia lunatus*, **(E)** *Cladosporium cladosporoids*, **(F)** *Sarocladium strictum*, **(G)** Dark sterile myclia, **(H)** *Fusarium fujikuroi*, **(I)** *Chaetomium elatum*, **(J)** *Alternaria alternata*, **(K)** Control.

**Table 4:** Screening of plant growth promoting activities of endophytic fungi. Data are means ± SE, means with different letters are significantly different at $P \leq 0.05$ according to Duncan's test.

| Fungal isolate | IAA production (µg/ml) | Phosphate solublization (SI±S.E) | $NH_3$ |
|---|---|---|---|
| *Alternaria alternata* | $19.21 \pm 0.40^{a}$ | - | ++ |
| *Chaetomium elatum* | $23.09 \pm 0.94^{b}$ | - | +++ |
| *Cladosporium cladosporoids* | $55.63 \pm 0.16^{e}$ | - | ++ |
| *Curvularia lunatus* | $57.59 \pm 0.81^{e}$ | - | +++ |
| *Curvularia spicifera* | $73.67 \pm 0.97^{g}$ | - | +++ |
| *Dark sterile mycelia* | $36.72 \pm 0.63^{c}$ | - | ++ |
| *Exserohilum pedicellatum* | $72.29 \pm 0.89^{g}$ | - | + |
| *Fusarium fujikuroi* | $35.37 \pm 1.78^{c}$ | 1.37± 0.06 | +++ |
| *Fusarium pallidiroseum* | $63.55 \pm 1.58^{f}$ | 1.49±0.07 | +++ |
| *Sarocladium strictum* | $41.72 \pm 0.57^{d}$ | - | ++ |

+++ Strong, ++ Moderate, + Weak

## CONCLUSION

Altogether, 98 endophytic fungal isolates belonged to 43 fungal species, and 26 genera were identified from the roots of seven common grass. The diversity of endophytic fungi was found highest in the roots of the *Paspalum dilatatum* plant (17 fungal species, 13 genera). Dark sterile mycelium and *Fusarium* were the most common fungi isolated from all plant samples, followed by *Curvularia,* which was recovered in high occurrence, while *Aspergillus* was recorded in moderate occurrence. After screening of all isolated fungal isolates for IAA production, ammonia production, and phosphate solubilization, data showed that *Curvularia spicifera* produced the maximum amount of IAA among the selected isolates after 1 week of growth. The maximum ammonia production among these isolates was found in *Curvularia spicifera,* followed by *Curvularia lunata*, *Fusarium fujikuroi*, *Fusarium pallidiroseum*, and *Chaetomium elatum*. The maximum tricalcium phosphate (TCP) solubilisation activity among tested fungal isolates was observed in *Fusarium pallidiroseum* with a solubilisation index of followed by *Fusarium fujikuroi.*

## REFERENCES

[1] J. L. Azevedo, Jr. W. Maccheroni, J. O. Pereira and W. L. De Araújo, Endophytic microorganisms: a review on insect control and recent advances on tropical plants, Electronic journal of biotechnology, 3(1) (2000) 15-16.

[2] W. Zang, D. Becker, Q. A. Cheng, Mini review of recent W.O, Resent patents on anti infective drug discovery,1 (2006) 225-230.

[3] G. Strobel, B. Daisy, Bioprospecting for microbial endophytes and their natural products, Microbiology and molecular biology reviews, 67(4) (2003) 491–502.

[4] P. R. Hardoim, V. Overbeek, L. S. G. Berg, P. A. M. S. Compant, A. Campisano et al, The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes, Microbiology and molecular biology reviews, 79(2015) 293–320.

[5] V. K. Kumar, N. Shandilya, C. Mohapatra, S. S. Bhayana, and A. Varma, Revisiting plant–microbe interactions and microbial consortia application for enhancing sustainable agriculture, a review Frontiers in Microbiology, 11(2020) 560406.

[6] M. R. D. Torkamani, M. Jafari, N. Abbaspour, R. Heidary and N. Safaie, Enhanced production of valerenic acid in hairy root culture of Valeriana officinalis by elicitation, Central European Journal of Biology, 9(2014) 853–863.

[7] M. Salehi and N. Safaie, Endophytic fungi: secondary metabolites and plant biotic and abiotic stress management, Frontiers in Microbiology, 15(2024)1345210.

[8] K. Saikkonen, Forest structure and fungal endophytes, Fungal biology reviews, 21(2-3) (2007) 67-74.

[9] J. Dighton, J. F. White, P. Oudemans, The fungal community: its organization and role in the ecosystem, 4th edn. CRC Press, Boca Raton, (2017).

[10] A. Fouda, A. M. Eid, A. Elsaied, E. F. El-Belely, M. G. Barghoth, E. Azab, A. A. Gobouri, S. E . D. Hassan, Plant Growth-Promoting Endophytic Bacterial Community Inhabiting the Leaves of *Pulicaria incisa* (Lam.) DC Inherent to Arid Regions Plants, (2021) 10-76.

[11] N. C. Baron, and E. C. Rigobelo, Endophytic fungi: a tool for plant growth promotion and sustainable agriculture, Mycology, 13(1) (2022) 39-55.

[12] M. d. C. Orozco-Mosqueda, A. Flores, B. Rojas-Sánchez, C. A. Urtis-Flores, L. R. Morales-Cedeño, M. F.Valencia-Marin, S. Chávez-Avila, D. Rojas-Solis, G. Santoyo, Plant Growth-Promoting Bacteria as Bioinoculants: Attributes and Challenges for Sustainable Crop Improvement, Agronomy,11(6) (2021)1167.

[13] A. E. Fadiji, O. O. Babalola, G. Santoyo, M. Perazzolli, The Potential Role of Microbial Biostimulants in the Amelioration of Climate Change-Associated Abiotic Stresses on Crops, Frontiers in Microbiology, 12 (2022) 829099.

[14] D. Guerin, Sur la présence d'un champignon dans l'ivraie (*Lolium temulentum*, L.), Journ. d. Bot, 12 (1898) 230–238.

[15] E. M. Freeman, Philosphical Transactions-The Seed Fungus of *Lolium temulentum*, L., the Darnel, 196 (1902) 1–27.

[16] K. D. Hyde and K. Soytong, The fungal endophyte dilemma, Fungal Divers, 33(2008) 163–173.

[17] S. Marquez, S. Bills, G. F. Herrero and Zabalgogeazcoa, Non-systemic fungal endophytes of grasses, Fungal Ecology, 5(3) (2012) 289–297.

[18] S. P. Richier, Furla, A. Plantivaux, P. L. Merle, and D. Allemand, Symbiosis-induced adaptation to oxidative stress, Journal of Experimental Biology, 208(2) (2005) 277-285.

[19] M.L. Friesen, S. S. Porter, S.C. Stark, E. J. von Wetteberg, Sachs J. L., E. Martinez-Romero, Microbially mediated plant functional traits, Annual Review of Ecology Evolution and Systematics, 42(2011) 23-46.

[20] E. N. Vasilyeva, G. A. Akhtemova, V. A. Zhukov, I. A. Tikhonovich, Endophytic microorganisms in fundamental research and agriculture, Ecological genetics, 17(1) (2019) 19–32.

[21] N. Singh, and P. Dahiya, Plant growth-promoting endophytic fungi from different habitats and their potential applications in agriculture, Recent Trends in Mycological Research, 1(2021) 69-87.

[22] A. N. Yadav, Recent Trends in Mycological Research Agricultural and Medical Perspective, Springer International Publishing, 1(2021).

[23] S. Fráková, M. Šturdíková and M. Múcková, Bioactive secondary metabolites produced by microorganisms associated with plants, Biologia, 62 (2007) 251–257.

[24] R. S. Redman ,O. K .Yong, J. D. A. Claire Woodward, C. Greer, L. Espino, S.L. Doty, Rusty J. Rodriguez , Increased Fitness of Rice Plants to Abiotic Stress Via Habitat Adapted Symbiosis: A Strategy for Mitigating Impacts of Climate Change, 6 (7) (2011) 14823.

[25] M. Hamayun, S. A. Khan, A. L. Rehman, G. Kim, Y. H. Iqbal, I. J. Lee, I. Gibberellin production and plant growth promotion from pure cultures of *Cladosporium* sp. MH-6 isolated from cucumber (*Cucumis sativus* L.), Mycologia, 102(5) (2010) 989–995.

[26] S. J. Li, X. Zhang, X. H. Wang and C. Q. Zhao, Novel natural compounds from endophytic fungi with anticancer activity, European Journal of Medicinal Chemistry, 156 (2018) 316-343.

[27] F. M. Khan, M. Barnathan, M. Montgomery, S. Myers, L. Côté and S. Loftus, "A Wearable Accelerometer System for Unobtrusive Monitoring of Parkinson's Diease Motor Symptoms," 2014 IEEE International Conference on Bioinformatics and Bioengineering, Boca Raton, FL, USA, 2014, pp. 120-125.

[28] J. M. Johnson, T. Alex and R. Oelmüller, *Piriformospora indica*: the versatile and multifunctional root endophytic fungus for enhanced yield and tolerance to biotic and abiotic stress in crop plants, Journal of Tropical Agriculture, 52(2) (2014)103-122.

[29] O. Petrini, P. J. Fisher: Fungal endophytes in *Salicornia perennis*, Transactions of the British Mycological Society, 87(4) (1986) 647-651.

[30] S. K. Gond, Verma, V. C. Kumar, A. Kumar and R. N. Kharwar, Study of endophytic fungal community from different parts of Aegle marmelos Correae (Rutaceae) from Varanasi (India), World Journal of Microbiology and Biotechnology, 23(2007) 1371-1375.

[31] K. H. Domsch, W. Gams and T.-H Anderson. Compendium of Soil Fungi, 2nd Ed, taxonomically revised by W. Gams. IHW- Verlag, Eching, (2007) 672pp.

[32] Ø. Hammer, T. Harper, A. David, and Ryan, P.D. Past: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica, 4(1:4) (2001) 1-9, 178kb.

[33] O. Stajkovic, D. Delic, D. Josic, D. Kuzmanovic, N. Rasulic, and J. Knezevic-Vukcevic, Improvement of common bean growth by co-inoculation with Rhizobium and plant growth-promoting bacteria, Romanian Biotechnological Letters, 16(1) (2011) 5919-5926.

[34] R. I. Pikovskaya, "Mobilisation of phosphorus in soil in connection with vital capacity of source microbial species", Microbiologiya, 17(1948) 362-370.

[35] O. Stajkovic, D. Delic, D. Josic, D. Kuzmanovic, N. Rasulic and J. Knezevic-Vukcevic, Improvement of common bean growth by co-inoculation with Rhizobium and plant growth-promoting bacteria, Romanian Biotechnological Letters, 16(1) (2011) 5919-5926.

[36] F. Anwar, S. Latif, M. Ashraf, A. H. Gilani Moringa oleifera, a food plant with multiplemedicinal uses. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 21(2007) 17–25.

[37] M. Kaur and A. Kalia, *Convolvulus arvensis*: A useful weed. Int J Pharm Pharm Sci, 4(1) (2012) 38-40.

[38] A. K. Khairnar, S. Bhamare, H. Bhamare, *Calotropis procera*: an ethnopharmacological update, Advance Research in Pharmaceuticals and Biologicals, 2(2012) 142–156.

[39] A. Al-Snafi, The chemical contents and pharmacological effects of *Anagallis arvensis*-A review, International Journal of Pharmacy, 5 (2015) 37–41.

[40] S. Syed, N. Fatima, G. L. Kabeer, *Portulaca oleracea*, a mini review on phytochemistry and pharmacology, International journal of Biology and Biotechnology, 13(2016) 637–641.

[41] A. L. Khan, M. Waqas, J. Hussain, A. Al-Harrasi, I. J. Lee, Fungal endophyte *Penicillium janthinellum* LK5 can reduce cadmium toxicity in *Solanum lycopersicum* (Sitiens and Rhe), Biology and fertility of soils, 50 (2014) 75–85.

[42] H. H. Long, D. D. Schmidt and I. T. Baldwin, Native bacterial endophytes promote host growth in a species-specific manner, phytohormone manipulations do not result in common growth responses, PloS one, 3(7) (2008) e2702.

[43] L. Bettucci, S. Simeto, R. Alonso, and S. Lupo, Endophytic fungi of twigs and leave from three native species of Myrtaceae in Uruguay, Sydowia-Horn, 56 (2004) 8-23.

[44] A. E. Arnold, Z. Maynard, and G. S. Gilbert, Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity, Mycological research, 105(12) (2001) 1502-1507.

[45] M. Kvas, W. F. O. Marasas, B. D. Wingfield, M. J. Wingfield, E. T. Steenkamp, Diversity and evolution of *Fusarium* species in the *Gibberella fujikuroi* complex, Fungal Divers 34 (2009) 1–21.

[46] K. Mandyam, Dark septate fungal endophytes from a tall grass prairie and their continuum of interactions with host plants. Ph.D.Thesis, Kansas State University, Manhattan, KS, 2008.

[47] J. F. Leslie, B. A. Summerell, The Fusarium laboratory manual, Blackwell, Sydney, 388p (2006).

[48] M . Kvas, W. F. O. Marasas, B. D. Wingfield, M. J. Wingfield, E. T. Steenkamp, Diversity and evolution of Fusarium species in the *Gibberella fujikuroi* complex, Fungal Divers, 34 (2009)1–21.

[49] A. R. Bentley, T. Petrovic, S. P. Griffiths, L. W. Burgess, B. A. Summerell, Crop pathogens and other Fusarium species associated with *Austrostipa aristiglumis*, Australasian Plant Pathol, 36 (2007) 434–438.

[50] I. N. Nor Azliza, R. Hafizi, M. Nurhazrati, B. Salleh, Production of major mycotoxins by *Fusarium* species isolated from wild grasses in peninsular Malaysia, Sains Malaysiana, 43 (2014) 89–94.

[51] L. Kago, J. Njuguna, D. M. G. Njarui, S. R. Ghimire, Fungal endophyte communities of *Brachiaria grass* (Brachiaria spp.) in Kenya, (2016) 271.

[52] M. H. Laurence, J. L. Walsh, L. A. Shuttleworth, D. M. Robinson, R. M. Johansen, T. Petrovic, Vu. TTH, L. W. Burgess, B. A. Summerell, Liew ECY, Six novel species of *Fusarium* from natural ecosystems in Australia, Fungal Diversity, 77(2016) 349–366.

[53] K. Chehri, S. Hajeb, S. M. Maassoumi, Morphological and molecular identification and PCR amplification to determine the toxigenic potential of *Fusarium graminearum* species complex (FGSC) isolated from wild grasses in Iran, J Agric Sci Technol, 19(2017)1617–1629.

[54] J. F. Leslie, K. A. Zeller, A. Logrieco, G. Mulè, A. Moretti, A. Ritiene. Species diversity of and toxin production by *Gibberella fujikuroi* species complex strains isolated from native prairie grasses in Kansas, Applied and Environmental Microbiology, 70 (2004) 2254–2262.

[55] S. S. Márquez, G. F. Bills, N. Herrero, I. Zabalgogeazcoa, Non-systemic fungal endophytes of grasses, Fungal Ecology, 5(3) (2012) 289-297.

[56] T. Sieber, T. K. Riesen, E. Muller, P.M. Fried, Endophytic fungi in four winter wheat cultivars (*Triticum aestivum* L.) difering in resistance against *Stagonospora nodorum* (Berk.) Cast and Germ. =Septoria nodorum (Berk.) Berk, Journal of Phytopathology, 122(4)(1988) 289–306.

[57] R. M. Anjos, S. I. Moreira, S. S. Costa, L. M. Abreu, E. Alves and P. G. Cardoso, *Sarocladium graminicola*, a new endophytic species from tropical grasses, Mycological progress, 19(6) (2020) 605-614.

[58] M. Waqas, A.L. Khan, M. Kamran, M. Hamayun, S. M. Kang, Y. H. Kim, I. J. Lee, Endophytic fungi produce gibberellins and indole acetic acid and promotes host-plant growth during stress, Molecules, 17(2012 )10754–10773.

[59] A. R. Khan, I. Ullah, M. Waqas, R. Shahzad, S. J. Hong, G. S. Park, B. K. Jung, I. J. Lee, J. H.Shin, Plant growth-promoting potential of endophytic fungi isolated from *Solanum nigrum* leaves, World Journal of Microbiology and Biotechnology,31(2015)1461–1466.

[60] K. R. Chung,T. Shilts, Ü. Ertürk, L. W. Timmer, P. P. Ueng, Indole derivatives produced by the fungus *Colletotrichum acutatum* causing lime anthracnose and postbloom fruit drop of citus. FEMS Microbiology Letters, 226(1) (2003) 23–30.

[61] R. Chutima, S. Lumyong, Production of indole-3-acetic acid by Thai native orchid-associated fungi, Symbiosis, 56 (2012) 35–44.

[62] A. L. Khan, M. Waqas, J. Hussain, A. Al-Harrasi, K. Al-Hosni, M. J. Kim, M. Adnan, I. J. Lee, Endophytes *Aspergillus caespitosus* LK12 and *Phoma* sp. LK13 of *Moringa peregrine* produce gibberellins and improve rice plant growth, Journal of Plant Interactions, 9(2014) 731–737.

[63] A. Kapri, L. Tewari, Phosphate solubilization potential and phosphatase activity of rhizospheric *Trichoderma* spp, Brazilian Journal of Microbiology, 41(2010)787–795.

[64] M. P. Resende, I. C. M. C. Jakoby, L. D. Santos, M. A. Soares, F. D. Pereira, E. L. Souchie and F. G. Silva, Phosphate solubilization and phytohormone production by endophytic and rhizosphere *Trichoderma* isolates of guanandi (*Calophyllum brasiliense Cambess*), African Journal of Microbiol Res, 8 (2014) 2616–2623.