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PCR-BASED IDENTIFICATION, BIODIVERSITY, AND ANTIFUNGAL SCREENING OF ENDOPHYTIC FUNGI FROM DEVIL'S CLAW (*HARPAGOPHYTUM PROCUMBENS*)

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ABSTRACT

Food safety remains a critical global issue, with fungi posing significant challenges due to their ability to produce mycotoxins, contaminate crops, and compromise food quality. These contaminants impact public health, reduce agricultural productivity, and hinder food security, particularly in developing regions. Addressing this problem requires integrated strategies, including biocontrol, fungal-resistant crops, and improved food storage technologies, to ensure global food safety and public health. Recent research has discovered that plants harbour therapeutic valuable endophytes which produce a plethora of unique biocompounds used for medical, pharmacological, and agricultural purposes. The aim of the study is to identify and screen endophytic fungi from Devil's Claw. Thirty (n=30) *Harpagophytum procumbens* (Devil's claw) plants were used to isolate a total of 114 endophytic fungi. Morphological techniques as well as deoxyribonucleic acid (DNA) sequence-based techniques were used to identify the endophytic fungi. The identities of the fungal strains were blasted against the known isolates using the National Center for Biotechnology Information (NCBI) database. Antifungal screening was tested against plant pathogenic fungi using dual technique assay. Colonization rates (CR) in leaves were 50% and isolation rates (IR) were 21%, respectively, whereas stem colonization rates (CR) were 25% and 14%. The lowest CR and IR were found in roots, with CR of 21% and IR of 17%. According to the blast results, isolates were identified to species level based on similarities with existing sequences in GenBank. As a result, predominate species were *Alternaria* sp (16%), followed by *Alternaria alternata* (14%), and *Penicillium* (10%) species only 20 fungal strains were able to demonstrate high bioactivity. *Penicillium* sp. (S4 and S49) had the strongest inhibition at 69% pathogens against *Collectotrichum gleosporioides* (12517), despite this *Alternaria alternata* (L39) exhibiting the lowest activity 24% against the same pathogen. This is the first study of culturable endophytic fungi isolated *from Harpagophytum procumbens* for antifungal activity. Such studies provide leeway to the discovery of new, economical, and effective antifungal agents of natural origin for agricultural use.

Key words: Devil's Claw, Endophytes, *Harpagophytum procumbens*,Microbial community, Plant pathogens, Sustainable

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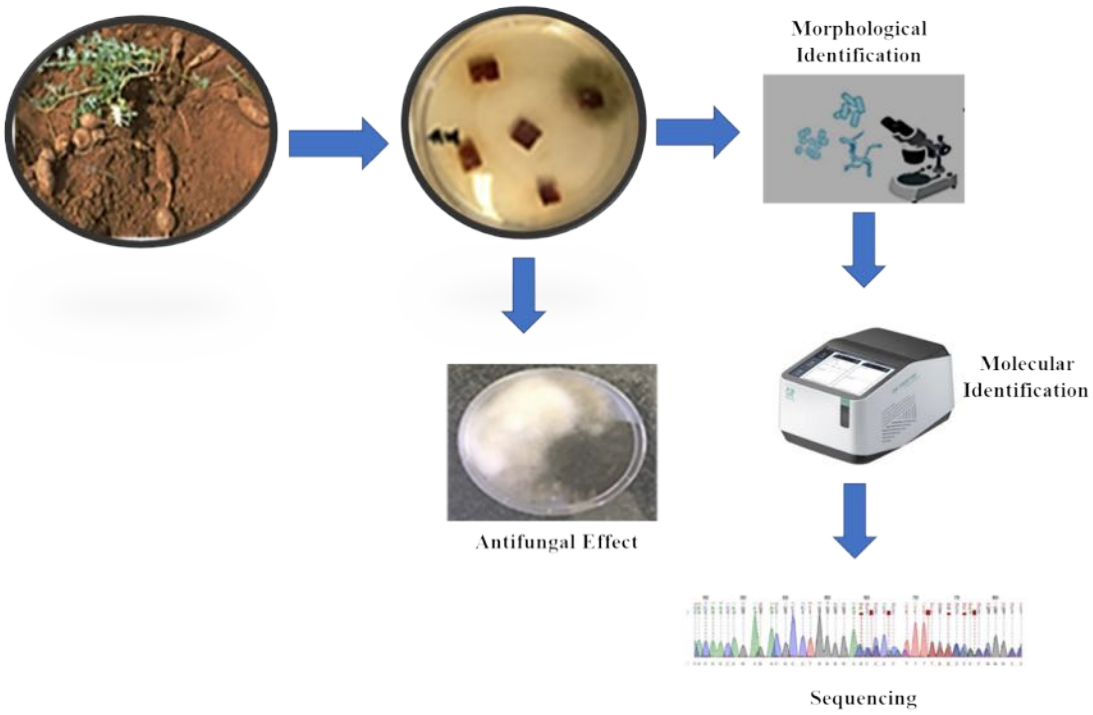

Graphical abstract

INTRODUCTION

A body of evidence supports the fact that endophytes contribute to plant growth, development, fitness, and diversity as well as population dynamics, plant community diversity and the functioning of the ecosystem [1, 2]. Endophytes are bacteria or fungi that colonize internal healthy plant tissue without causing any disease or disease symptoms. Endophytic fungi represent a powerful reservoir of novel bio-compounds with enormous potential for exploitation in agriculture, medicine, and pharmaceutical sectors. They play a crucial ecological role in the succession of plants through the beneficial interaction as a result of long-term evolution of the environment [3]. In addition, endophytes have the capability of producing a variety of novel bioactive metabolites. This has aroused interest in bio-prospecting of endophytic fungi for the exportation of bioactive compounds with potent anticancer, antifungal, antimicrobial, and antioxidant properties [4, 5].

Endophytes help protect the plant from diseases, promote growth, and keep herbivores away [6]. Various studies have found that fungal endophytes produce phytohormones and compounds in order to aid in the fight against abiotic and biotic challenges in the environment, particularly in agricultural industry [1-4]. Hence, maintaining the membrane integrity and stimulate host growth [7]. Fungal pathogens impair agricultural activities and food production, leading to low yield and poor quality and thus are determinants and a threat to both plants and crops, resulting in a major

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economic loss [8]. Iannotti and colleagues [9], estimated that 691-783 million people worldwide face food insecurity, predominantly in Africa and Asia.

Utilization of chemical pesticides comes with its challenges [10]. Some fungicides are typically high-toxic, ineffective and are detrimental to the environment since some plant pathogenic fungi can adapt to fungicide treatments by mutations leading to the development resistance and the loss of fungicide efficacy [11]. The utilization of chemical agents in controlling fungal pathogens remains a plethora of problems for farmers [12]. As the global population has taken on the eco-friendly, more sustainable, safer, and environmentally friendly way of life [13]. Studies in the past have indicated that endophytes linked with medicinal plants are likely to synthesize fungus-host bioactive chemical constituents [14]. Fungal endophytes isolated from medicinal plants produce beneficially potent bioactive compounds for agriculture usage [15]. This study is aimed to isolate endophytic fungi from *Harpagophytum procumbens* plant and screen for antifungal activity.

MATERIAL AND METHODS

Plant samples and collection

A total of 30 fresh, healthy (disease symptom free) *H. procumbens* plants were randomly selected based on availability from Dinkgateng, a tiny town in the North-West province (25°46'10.1"S 24°31'12.0"E). They were chosen with care utilizing random sampling.

Isolation of Endophytic Fungi

Prior to surface disinfection, the plants samples were carefully washed with running water to eliminate dust and dirt. The plants' roots and leaves were sliced into segments (1-3 cm long). After rinsing each sample with 70% ethanol for 1 minute, the surfaces were disinfected with a sodium hypochlorite (NaClO) solution (2%, household jik) for 2 minutes. The plant segments were then rinsed once more in 70% ethanol for 20 seconds before being cleaned again in sterile distilled water. The effectiveness of sterilization was tested by plating out rinse water. The sterilized pieces were placed in five plates, one each on nutrient-poor and nutrient-rich media (Potato carrot agar plus antibiotics (PCA+N), Potato Dextrose Agar (PDA), and Water Agar WA). PDA media is for fast growing fungi while PCA and WA is for slow growing fungi. There were 2-5 pieces of plant stuff on each plate. Each plate had 2-5 pieces of plant material. Finally, the plates were incubated for 7-10 day at 25ºC.

Morphological identification

The morphological features such as hyphal structure, spore shape and size, fruiting body, texture, and coloration were used to make a preliminary identification of the fungal isolates based on the reference book "Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to Species [16]". Fresh 65mm diameter

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PDA (Merck-Biolab, Gauteng, South Africa) plates fortified with kanamycin sulphate (VWR Life Science, England) and chloramphenicol were used to obtain pure isolates (VWR Life Science, England). Special structures were also noted where present [17].

Molecular identification

Deoxyribonucleic acid (DNA) extraction

On PDA plates, the investigating endophytic fungus were spot inoculated. All fungal isolates cultured on PDA, which was supplemented with 50 g.ml^{-1} kanamycin sulfate and 50 g.ml^{-1} chloramphenicol to prevent the growth of unwanted bacteria, were deoxyribonucleic acid extracted using the ZR Fungal/Bacterial DNA KitTM (Zymo Research, Catalogue No. D6005, Inqaba, South Africa), following the manufacturer's instructions [17].

Polymerase chain reaction (PCR)

Internal transcribed spacer (ITS) regions were used in the polymerase chain reaction (PCR) for all fungal strains. The oligonucleotide primer pairs ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) were amplification. The final volume of the PCR mixtures was 20 µL. Each tube contained 10µL of 10X Master-mix, 0.5 µL of each primer (ITS1 and ITS4), 1µL of diluted DNA and 8µL of deionised water. Amplification cycle was set at initial denaturation at 94ºC for 10 mins, 30 cycles of denaturation at 94ºC for 30 seconds, annealing at 50-52ºC, elongation at 72ºC for 45 seconds and a final elongation at 72ºC for 7 mins. To confirm whether the amplification was successful, the PCR products were checked by preparing 1.5% (w/v) of agarose gel at 65V for 1hour. Later, Inqaba Biotec (Pretoria, South Africa) were sequenced using PCR amplicons. A Blast Search with the National Centre for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) database was used to confirm the isolates' identification [17].

Selection of plant pathogenic fungi

The investigated fungal pathogens indicated in Table 1 were purchased from the Agricultural Research Council (ARC), Plant Protection Research Institute (PPRI) in Pretoria, South Africa. In addition, pathogenic fungi were chosen based on their ability to infect a host crop and cause a disease. *Botrytis cinerea*, *Fusarium oxysporum, F. graminearum F. culmorum* and *Collectotrichum gleosporioides* were selected because they are well-known fungal pathogens capable of infecting crops with serious damage.

Bioassay (Dual method) of endophytic fungi against plant pathogenic fungi

The antifungal activity of endophytic fungi was tested against pathogenic fungi using a dual culture approach. Both the endophytes and pathogenic fungi were inoculated

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on the Potato Dextrose Agar media. Afterwards, the inoculated plates were incubated at 28°C for around 5-8 days. The fungal colonies were measured, and growth inhibition was utilized to calculate the percentages of activities using the below formulate.

$$\text{Growth inhibition (GI\%)} = \frac{R1 - R2}{\text{R2}} \text{ X } 100$$

R1 is diameter average of fungal pathogens without endophytic fungus.
R2 is diameter average of fungal pathogens with endophytic fungus on the same plate.

Statistical Analysis

The colonization rate (CR) was calculated as the total number of segments incubated divided by the total number of plant tissue segments. Isolation rate (IR) was the total number of segments incubated divided by the number of endophytic fungi isolated from plant segments. Relative frequency (RF) was calculated by the number of isolates designated type of strain isolated from tissue blocks/number of total tissue blocks) × 100% [18]. Statistical Package for Social Sciences (SPSS) software was used out using analysis of variance (ANOVA) with significant p-values ≤ 0.05.

$$\text{Colonization frequency (CF)} = \frac{\text{Number of segments colonized by the fungi}}{\text{Total number of segments observed}} \text{ X } 100$$

$$\text{Isolation Rate (IR)} = \frac{\text{Number of isolates obtained from tissue segments}}{\text{Total number of segments}} \text{X } 100$$

$$\text{Relative frequency (RF)} = \frac{\text{Number of isolates of a species}}{\text{Total number of isolates}} \text{ X } 100$$

RESULTS AND DISCUSSION

The CR is low, and IR is also low showing a positive correlation. The leaves of the devil's claw have the most endophytes, followed by the stems and roots, which have the least (Figure 1). As a result, colonization rates (CR) in leaves were 50% and isolation rates (IR) are 21%, respectively, whereas stem colonization rates (CR) were 25% and 14%. The lowest CR and IR were found in roots, with CR of 21% and IR of 17%.

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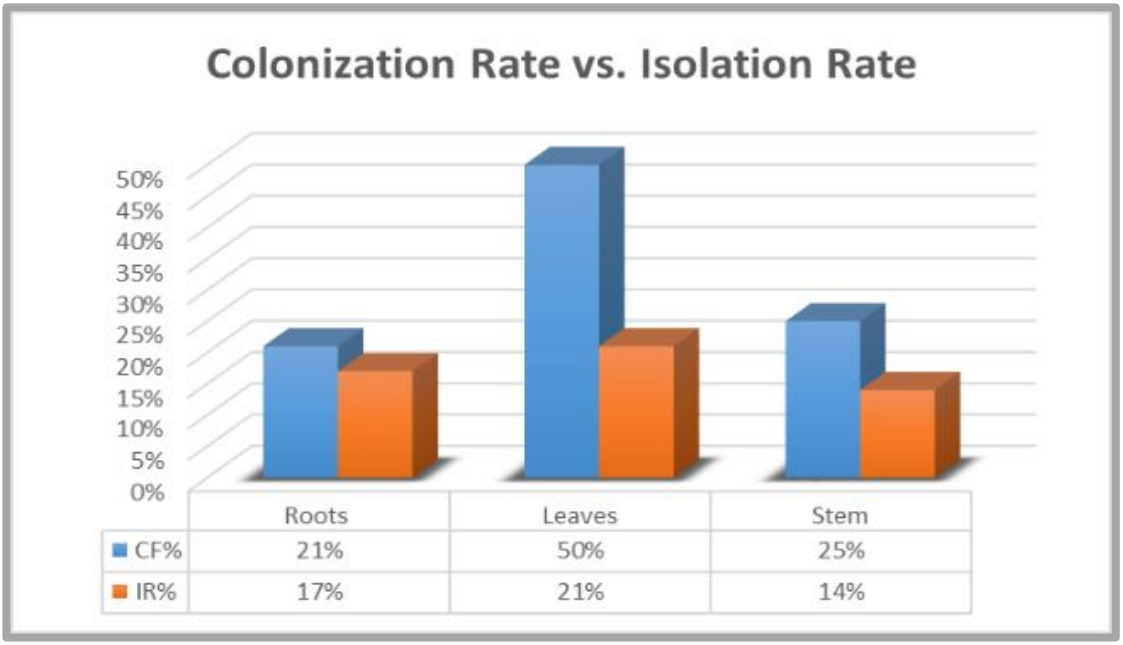

Figure 1: Overall endophytic fungal colonization and isolation rates

From these data set, *Fusaria (n*=13, 11.3%*)*, *Penicillium (n*=33, 28.9%*)*, 11 (9.6%) *Rhizopu*s, 3 (2.6%) *Alternaria*, 4 (3.5%) *Trichoderma*, 13 (11.4%) *Aspergillus* and 40 (35%) where unknown total of 114 endophytic fungi were identified. The BLAST results revealed that endophytic fungi belong to 13 genera, which are divided into four phylum groups (Deuteromycota, Ascomycota, Zygomy-cota, Basidiomycota). According to the genus, the results showed that *Alternaria* sp. (n=68, 16%), *Alternaria alternata* (n=16, 14%), *Penicillium* (n= 12, 10.4%), *Curvularia australiensis* (n=8, 6.9%), *Phoma* sp. (n=7, 6.0%), *Phoma herbarium* (n=7, 6.0%), uncultured Basidomycota (n=7, 6.0%), *Fusarium polyphialidicum* (n=5, 4.3%), *Cytospora* sp. (n=5, 3.4%), *Rhizopus* sp. LG04 (n=5, 3.4%). Tables 3 provide detailed information about the species. The majority of *Alternaria alternata* were most similar to MK972909.1, with similarity percentages ranging from 87 to 99 %, as shown in Table 2. As indicated in Figure 2, the pathogens *Fusarium graminearum, Collectotrichum gleosporioides, Fusarium oxysporum*, and *Botrytis cinerea* were tested.

Antifungal inhibition was observed in all the isolates (100%) against one or more pathogens. Moderate to good activity had the greatest number of activities 81%, followed by the least activity at 14%, and the highest activity at 4%. *Penicillium* sp. (S4 and S49) had the strongest effectiveness and inhibited the most fungal pathogen, particularly pathogens 12517 with 69% inhibition. On the other hand, *Alternaria alternata* (L39) had the lowest antifungal activity of 24% against pathogen *Collectotrichum gleosporioides* (12517), as indicated in Table 3 and Figure 2, followed by *Alternaria alternata* (S48), *Fusarium polyphialidicum* (L42; L73) with 25% inhibition effect against pathogen *Collectotrichum gleosporioides and Fusarium*

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graminearum, respectively. Pathogen *Botrytis cinerea* (13071) showed to be the most resistant pathogen towards endophytic fungi, followed by pathogen *Fusarium graminearum* (10139). Furthermore, these two pathogens displayed similar pathogenic resistance pattern. However, pathogenic *Collectotrichum gleosporioides* (12517), seems to be susceptible to endophytic fungi.

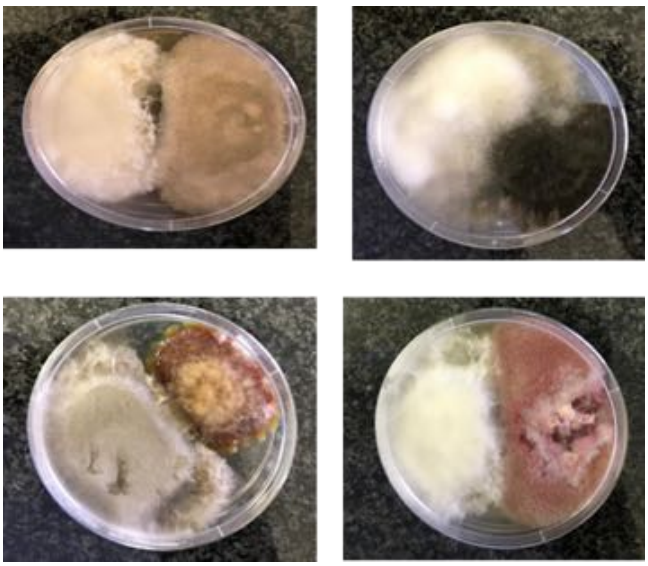

Figure 2: Dual culture assay of endophytic fungi against plant pathogens

From the findings, 77 (66.9%) of the 114 endophytic fungi were identified using morphological features. Shubha and Srinivas [19] found that *Cymbidium aloifolium* were invaded by endophytic fungus. The root (CR = 40.6 %, IR = 0.83) had the highest colonization rate (CR = 40.6 %, IR = 0.83), followed by leaves (CR = 32.12 %, IR = 0.66), and floral parts (CR = 27.27 %, IR = 0.56) [19]. Another study found that the stem section of *Pelargonium graveolens* (geranium) in Middle Egypt has the lowest number of fungal isolates, with CF=39 % and IR=48 % [20]. Species identification can be difficult and subjective [21], and morphology was once the basis of classification [22]. It was reported that that ITS amplicons ranging from 650 to 700 bp [23,24]. Manganyi *et al.* [17] isolated and identified 60 fungal species from *Sceletium tortuosum* into 16 different genera. Furthermore, *Fusarium* species were the most abundant (37%) followed by *Aspergillus* (25%) and *Penicillium* (7%) species [17]. It was estimated that about 5% of the 1.5 million species of fungi are known and catalogued [23]. In all aspects of fungal identification, a wide range of molecular approaches are becoming increasingly valuable instruments [26]. From the results, all of the isolates (100%) were active against one or more pathogens. The highest percentage of moderate activity (81%), followed by 14%, and only 4% exhibited the highest activities. As shown in Table 3, *Alternaria alternata* (L39) had the lowest antifungal activity of 24% against pathogen *Collectotrichum gleosporioides* (12517), followed by *Alternaria alternata* (S48), *Fusarium*

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polyphialidicum (L42; L73), and *Alternaria alternata* (S48) with 25% inhibition effect against pathogen *Collectotrichum gleosporioides* and *Fusarium graminearum. Botrytis cinerea* (13071) was found to be the pathogen most resistant, followed by *Collectotrichum gleosporioides* (10139).

Cosoveanu *et al.* [27] demonstrated that some endophytic organisms have been reported to exhibit *B. cinerea* [27]. *Rhizopus* strain AR17-02 also showed inhibitory activities against plant pathogens [28]. *Ceratobasidium ramicola* IBRLCM127 was also isolated from *Curcuma mangga* and showed significant anti-candidal action [29]. Numerous studies have documented the ability of endophytic *Fusaria* to inhibit pathogenic fungi that cause plant disease and crop losses [30]. Natural products hold a lot of promise as new sources of fungicides for controlling pathogenic fungi [31], particularly bioactive compounds from endophytic fungi. The preliminary, extraction of biocompounds should be the next time and volatile inhibitory testing should hold better results.

The ecological and physiological significance of PCR-based identification and antifungal screening of endophytic fungi from *Harpagophytum procumbens* lies in uncovering the adaptive roles these microorganisms play in supporting their host in arid, nutrient-poor environments [32]. These fungi also represent a unique microbial community adapted to extreme environmental conditions, offering insights into stress tolerance mechanisms and ecological resilience [33]. Furthermore, their metabolic versatility may play a vital role in regulating plant physiology, promoting growth, and maintaining the health of desert ecosystems [34], while also serving as potential sources of novel antifungal compounds for agricultural and pharmaceutical applications.

This study is novel in its comprehensive approach molecular taxonomy, ecological diversity analysis, and functional screening as well as applied to an unexplored medicinal plant. There is no existing research on endophytic fungi associated with *H. procumbens*, a medicinal plant native to arid regions of southern Africa. This adds to global efforts in natural product discovery, microbial biodiversity conservation, and the search for alternative antifungal agents in the face of rising resistance.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

For decades, there has been evidence that many of the synthetic chemicals used in agriculture are detrimental to the environment and human health. Current trends are heading towards a more sustainable, greener, and environmentally friendly lifestyle. Hence, sparking a massive response from consumers, farmers, scientists, and policymakers, by demanding agricultural products to have minimal or no chemical while also encouraging research into new pathogen control measures. This research demonstrates that the endophytic fungi can produce beneficial bioactive compounds

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with significant potential to prevention and/or control fungal disease. The results provide a step in the right direction in the discovery of fungal biocontrol to achieve food safety and security.

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DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

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Table 1: Pathogenic fungi with their origin and accession number

PPRI no.	Fungal Name	Host/substrate	Locality
13071	*Botrytis cinerea*	Chrysanthemum flower	Gauteng, Tarlton
2929	*Fusarium oxysporum*	Wheat	Free state
10139	*Fusarium graminearum*	Maize	Northwest
12517	*Collectotrichum gleosporioides*	Papaya	Mpumalanga, Nelspruit

Table 2: Endophytic fungal identification of blast sequence

Sample ID	Closest related species	GenBank Best BLAST Match		Sample ID	Closest related species	GenBank Best BLAST Match	
		Max identity	Accession No.			Max identity	Accession No.
R3	*Alternaria tenuissima*	99%	MF070759.1	L1	*Bipolaris setariae*	96.97%	JX282411.1
R4	*Penicillium sp.*	98.78%	KJ191440.1	L4	*Fusarium polyphialidicum*	97.45%	HQ607880.1
R5	*Alternaria sp.*	94.96%	MK640595.1	L5	*Alternaria sp.*	95.28%	MK640595.1
R6	*Cytospora sp.*	91.06%	MF495425.1	L6	*Penicillium sp.*	96.26%	MK775949.1
R7	*Alternaria sp.*	95.99%	KY788251.1	L7	*Phoma sp.*	95.00%	MT448656.1
R8	*Alternaria sp.*	96.73%	MK690430.1	L8	*uncultured Ascomycota*	99.07%	EU489900.1
R9	*Cytospora sp.*	91.06%	MF495425.1	L10	*Phoma herbarum*	95.75%	KJ767079.1
R10	*Phoma herbarum*	96.83%	KJ767079.1	L11	*Bipolaris setariae*	96.97%	JX282411.1
R11	*Cytospora sp.*	91.06%	MF495425.1	L12	*Curvularia australiensis*	97.73%	KM999998.1
R12	*Alternaria alternate*	97.38%	MH521173.1	L14	*Alternaria sp.*	98.09%	KM007075.1
R13	*Alternaria alternate*	97.73%	MK972909.1	L15	*dothideomycete sp. 7685*	96.48%	EU680546.1
R15	*Penicillium sp.*	94.06%	KX009141.1	L16	*Rhizopus microsporus*	98.53%	JN943067.1
R16	*Penicillium sp.*	99.15%	MT446131.1	L17	*Penicillium sp.*	99.15%	MT446131.1
R17	*Alternaria brassicae*	83.33%	MG250601.1	L18	*Alternaria sp.*	95.83%	MN115554.1
R18	*uncultured Ascomycota*	99.07%	EU489900.1	L19	*Alternaria alternata*	96.34%	MH521173.1
R19	*Phoma sp.*	95.40%	MT448656.1	L21	*Aspergillus sp.*	98.34%	MT239561.1
R20	*Curvularia aeria*	99.13%	KP131940.1	L22	*Alternaria sp.*	97.90%	MK640595.1

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R21	*Cytospora sp.*	91.06%	MF495425.1	L23	*Rhizopus microsporus*	98.53%	JN943067.1
R22	*Alternaria destruens*	97.10%	MK409129.1	L24	*Alternaria sp.*	97.90%	MK640595.1
R23	*Alternaria alternate*	97.20%	MK972909.1	L25	*Alternaria sp.*	97.20%	MK640595.1
R24	*Penicillium sp.*	98.52%	HQ850350.1	L26	*Alternaria alternata*	87.72%	LN835252.1
R26	*Alternaria sp.*	96.73%	MK690430.1	L27	*Alternaria sp.*	98.08%	MK640595.1
R27	*uncultured Ascomycota*	98.61%	EU490151.1	L28	*Aspergillus sp.*	98.34%	MT239561.1
R28	*Curvularia australiensis*	95.85%	KM999998.1	L30	*Alternaria sp.*	96.37%	MT089982.1
R29	*Alternaria tenuissima*	86.62%	MN893910.1	L31	*Phoma sp.*	95.00%	MT448656.1
R31	*Curvularia australiensis*	95.85%	KM999998.1	L32	*Bipolaris sp.*	98.11%	MH370845.1
R32	*Phoma sp.*	95.40%	MT448656.1	L33	*Curvularia australiensis*	97.73%	KM999998.1
R33	*Ascomycota sp. LM107*	97.96%	EF060476.1	L35	*Alternaria sp.*	97.06%	MK640595.1
R34	*Curvularia australiensis*	97.03%	KM999998.1	L37	*Alternaria sp.*	97.06%	MK640595.1
R35	*Ascomycota sp. LM107*	98.70%	EF060476.1	L36	*Fusarium polyphialidicum*	97.45%	HQ607880.1
R36	*Phoma herbarum*	96.83%	KJ767079.1	L38	*Phoma sp.*	95.00%	MT448656.1
R37	*Rhizopus sp. LG04*	96.28%	HQ876465.1	L39	*Alternaria alternata*	99.30%	MK972909.1
R38	*Aspergillus arcoverdensis*	97.66%	MN431385.1	L40	*Curvularia australiensis*	97.73%	KM999998.1
R39	*Phoma sp.*	95.40%	MT448656.1	L42	*Fusarium polyphialidicum*	97.45%	HQ607880.1
R41	*Alternaria alternate*	94.07%	MK972909.1	L43	*Penicillium sp.*	99.15%	MT446131.1
R42	*Alternaria alternate*	97.04%	MT453271.1	L44	*Alternaria alternata*	96.34%	MH521173.1
R43	*Phoma herbarum*	95.37%	KJ767079.1	L47	*Penicillium sp.*	99.15%	MT446131.1
R44	*Bipolaris setariae*	94.74%	JX282411.1	L50	*Alternaria sp.*	97.55%	MK640595.1
R46	*Aspergillus sp.*	90.98%	MH550493.1	L52	*Alternaria alternata*	97.54%	MW081304.1
S1	*Alternaria alternate*	96.72%	MH521173.1	L53	*Phoma sp.*	95.00%	MT448656.1
S3	*Curvularia australiensis*	95.85%	KM999998.1	L54	*Phoma herbarum*	95.75%	KJ767079.1

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S4	*Penicillium sp.*	98.11%	MH102085.1	L55	*Alternaria alternata*	96.71%	MW081304.1
S5	*Alternaria alternate*	89.49%	HQ674661.1	L56	*Trichoderma viride*	95.72%	MK952215.1
S6	*Phoma herbarum*	95.75%	KJ767079.1	L57	*Fusarium polyphialidicum*	97.45%	HQ607880.1
S7	*Alternaria alternate*	90.09%	MW081304.1	L58	*Bipolaris sp.*	98.11%	MH370845.1
S8	*uncultured Basidiomycota*	97.30%	EU490129.1	L65	*Curvularia australiensis*	97.73%	KM999998.1
S9	*uncultured Basidiomycota*	97.30%	EU490129.1	L69	*Bipolaris sp.*	98.11%	MH370845.1
S10	*Penicillium sp.*	98.52%	HQ850350.1	L73	*Fusarium polyphialidicum*	97.45%	HQ607880.1
S16	*Rhizopus microsporus*	97.29%	MG250464.1	L82	*Alternaria sp.*	97.87%	MK640595.1
S17	*Dothideomycete sp. 7685*	96.48%	EU680546.1	S50	*Phoma herbarum*	95.75%	KJ767079.1
S22	*uncultured Basidiomycota*	97.30%	EU490129.1	S51	*Penicillium sp.*	99.15%	MT446131.1
S26	*Alternaria destruens*	94.10%	MK409132.1	S53	*uncultured Basidiomycota*	97.30%	EU490129.1
S37	*Alternaria sp.*	98.60%	MK640595.1	S54	*uncultured Basidiomycota*	97.30%	EU490129.1
S38	*Rhizopus sp. LG04*	94.16%	HQ876465.1	S55	*Alternaria destruens*	94.10%	MK409132.1
S47	*uncultured Basidiomycota*	98.43%	EU490129.1	S57	*Rhizopus microsporus*	97.29%	MG250464.1
S48	*Alternaria alternate*	90.80%	MK972906.1	S59	*Alternaria alternata*	97.38%	MH521173.1
S49	*Penicillium sp.*	98.11%	MH102085.1	S60	*Alternaria sp.*	88.04%	MK640595.1

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Table 3: Identification of endophytic fungi and activity against pathogenic fungi of plants according to the dual culture technique

Endophytic strains	Specie ID	Growth inhibition %			
		10139	12517	2929	13071
R3	*Alternaria tenuissima*	40 (++)	43 (++)	45 (++)	40 (++)
R4	*Penicillium sp.*	49 (++)	55 (++)	56 (++)	43 (++)
R5	*Alternaria sp.*	39 (++)	43 (++)	40 (++)	45 (++)
R6	*Cytospora sp.*	55 (++)	51 (++)	60 (+++)	61 (+++)
R7	*Alternaria sp.*	49 (++)	45 (++)	63 (+++)	40 (++)
R8	*Alternaria sp.*	55 (++)	51 (++)	37 (++)	45 (++)
R9	*Cytospora sp.*	41 (++)	37 (++)	40 (++)	41 (++)
R10	*Phoma herbarum*	51 (++)	42 (++)	45 (++)	37 (++)
R11	*Cytospora sp.*	40 (++)	41 (++)	40 (++)	40 (++)
R12	*Alternaria alternate*	39 (++)	43 (++)	43 (++)	40 (++)
R13	*Alternaria alternate*	49 (++)	51 (++)	41 (++)	45 (++)
R15	*Penicillium sp.*	43 (++)	45 (++)	40 (++)	43 (++)
R16	*Penicillium sp.*	40 (++)	42 (++)	40 (++)	37 (++)
R17	*Alternaria brassicae*	41 (++)	43 (++)	45 (++)	41 (++)
R18	*uncultured Ascomycota*	59 (++)	61 (+++)	60 (++)	51 (++)
R19	*Phoma sp.*	43 (++)	37 (++)	43 (++)	40 (++)
R20	*Curvularia aeria*	60 (++)	59 (++)	32 (++)	51 (++)
R21	*Cytospora sp.*	39 (++)	45 (++)	40 (++)	45 (++)
R22	*Alternaria destruens*	40 (++)	43 (++)	40 (++)	43 (++)
R23	*Alternaria alternate*	41 (++)	40 (++)	37 (++)	37 (++)
R24	*Penicillium sp.*	43 (++)	45 (++)	45 (++)	41 (++)
R26	*Alternaria sp.*	31 (++)	31 (++)	43 (++)	40 (++)
R27	*uncultured Ascomycota*	40 (++)	37 (++)	40 (++)	45 (++)
R28	*Curvularia australiensis*	39 (++)	43 (++)	37 (++)	55 (++)
R29	*Alternaria tenuissima*	41 (++)	51 (++)	40 (++)	43 (++)
R31	*Curvularia australiensis*	49 (++)	40 (++)	53 (++)	40 (++)
R32	*Phoma sp.*	61 (+++)	53 (++)	45 (++)	37 (++)

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R33	*Ascomycota sp. LM107*	40 (++)	39 (++)	43 (++)	41 (++)
R34	*Curvularia australiensis*	49 (++)	51 (++)	59 (++)	45 (++)
R35	*Ascomycota sp. LM107*	39 (++)	43 (++)	41 (++)	40 (++)
R36	*Phoma herbarum*	40 (++)	37 (++)	37 (++)	41 (++)
R37	*Rhizopus sp. LG04*	43 (++)	37 (++)	56 (++)	40 (++)
R38	*Aspergillus arcoverdensis*	55 (++)	45 (++)	40 (++)	45 (++)
R39	*Phoma sp.*	39 (++)	43 (++)	60 (++)	33 (++)
R41	*Alternaria alternate*	41 (++)	33 (++)	32 (++)	43 (++)
R42	*Alternaria alternate*	61 (+++)	56 (++)	55 (++)	40 (++)
R43	*Phoma herbarum*	40 (++)	36 (++)	45 (++)	37 (++)
R44	*Bipolaris setariae*	43 (++)	37 (++)	40 (++)	41 (++)
R46	*Aspergillus sp.*	49 (++)	51 (++)	40 (++)	45 (++)
S1	*Alternaria alternate*	29 (+)	40 (++)	53 (++)	36 (++)
S3	*Curvularia australiensis*	40 (++)	43 (++)	44 (++)	36 (++)
S4	*Penicillium sp.*	65 (+++)	69 (+++)	66 (+++)	55 (++)
S5	*Alternaria alternate*	39 (++)	51 (++)	40 (++)	51 (++)
S6	*Phoma herbarum*	49 (++)	45 (++)	49 (++)	44 (++)
S7	*Alternaria alternate*	43 (++)	40 (++)	36 (++)	40 (++)
S8	*uncultured Basidiomycota*	39 (++)	48 (++)	44 (++)	36 (++)
S9	*uncultured Basidiomycota*	41 (++)	51 (++)	56 (++)	40 (++)
S10	*Penicillium sp.*	33 (++)	43 (++)	40 (++)	57 (++)
S16	*Rhizopus microspores*	40 (++)	51 (++)	40 (++)	36 (++)
S17	*Dothideomycete sp. 7685*	49 (++)	45 (++)	42 (++)	61 (+++)
S22	*uncultured Basidiomycota*	39 (++)	40 (++)	39 (++)	40 (++)
S26	*Alternaria destruens*	40 (++)	51 (++)	36 (++)	40 (++)
S37	*Alternaria sp.*	39 (++)	45 (++)	42 (++)	43 (++)
S38	*Rhizopus sp. LG04*	41 (++)	43 (++)	40 (++)	32 (++)
S47	*uncultured Basidiomycota*	40 (++)	36 (++)	43 (++)	42 (++)
S48	*Alternaria alternate*	49 (++)	25 (+)	40 (++)	43 (++)
S49	*Penicillium sp.*	65 (+++)	69 (+++)	32 (++)	40 (++)
S50	*Phoma herbarum*	48 (++)	37 (++)	42 (++)	37 (++)

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S51	*Penicillium sp.*	43 (++)	36 (++)	40 (++)	36 (++)
S53	*uncultured Basidiomycota*	39 (++)	51 (++)	60 (++)	40 (++)
S54	*uncultured Basidiomycota*	39 (++)	40 (++)	56 (++)	42 (++)
S55	*Alternaria destruens*	41 (++)	45 (++)	59 (++)	43 (++)
S57	*Rhizopus microspores*	40 (++)	37 (++)	42 (++)	36 (++)
S59	*Alternaria alternate*	40 (++)	43 (++)	40 (++)	40 (++)
S60	*Alternaria sp.*	49 (++)	51 (++)	61 (+++)	40 (++)
L1	*Bipolaris setariae*	43 (++)	35 (++)	41 (++)	43 (++)
L4	*Fusarium polyphialidicum*	39 (++)	37 (++)	55 (++)	40 (++)
L5	*Alternaria sp.*	40 (++)	43 (++)	36 (++)	40 (++)
L6	*Penicillium sp.*	43 (++)	41 (++)	40 (++)	49 (++)
L7	*Phoma sp.*	49 (++)	45 (++)	63 (++)	42 (++)
L8	*uncultured Ascomycota*	43 (++)	40 (++)	40 (++)	43 (++)
L10	*Phoma herbarum*	40 (++)	37 (++)	40 (++)	41 (++)
L11	*Bipolaris setariae*	39 (++)	42 (++)	45 (++)	42 (++)
L12	*Curvularia australiensis*	43 (++)	43 (++)	36 (++)	40 (++)
L14	*Alternaria sp.*	55 (++)	51 (++)	59 (++)	40 (++)
L15	*dothideomycete sp. 7685*	48 (++)	45 (++)	42 (++)	56 (++)
L16	*Rhizopus microspores*	40 (++)	42 (++)	40 (++)	36 (++)
L17	*Penicillium sp.*	31 (++)	32 (++)	40 (++)	61 (++)
L18	*Alternaria sp.*	51 (++)	31 (++)	39 (++)	55 (++)
L19	*Alternaria alternate*	40 (++)	45 (++)	53 (++)	40 (++)
L21	*Aspergillus sp.*	41 (++)	51 (++)	43 (++)	40 (++)
L22	*Alternaria sp.*	39 (++)	37 (++)	40 (++)	42 (++)
L23	*Rhizopus microspores*	40 (++)	43 (++)	40 (++)	40 (++)
L24	*Alternaria sp.*	39 (++)	45 (++)	45 (++)	36 (++)
L25	*Alternaria sp.*	33 (++)	29 (+)	40 (++)	41 (++)
L26	*Alternaria alternate*	49 (++)	68 (+++)	66 (+++)	29 (+)
L27	*Alternaria sp.*	39 (++)	43 (++)	41 (++)	40 (++)
L28	*Aspergillus sp.*	39 (++)	51 (++)	40 (++)	39 (++)
L30	*Alternaria sp.*	40 (++)	37 (++)	43 (++)	42 (++)

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L31	*Phoma sp.*	39 (++)	45 (++)	36 (++)	37 (++)
L32	*Bipolaris sp.*	39 (++)	51 (++)	40 (++)	40 (++)
L33	*Curvularia australiensis*	40 (++)	40 (++)	40 (++)	43 (++)
L35	*Alternaria sp.*	43 (++)	45 (++)	55 (++)	41 (++)
L37	*Alternaria sp.*	40 (++)	39 (++)	40 (++)	43 (++)
L36	*Fusarium polyphialidicum*	49 (++)	45 (++)	57 (++)	40 (++)
L38	*Phoma sp.*	40 (++)	40 (++)	41 (++)	40 (++)
L39	*Alternaria alternate*	65 (+++)	24 (+)	53 (++)	33 (++)
L40	*Curvularia australiensis*	48 (++)	45 (++)	37 (++)	40 (++)
L42	*Fusarium polyphialidicum*	25 (+)	33 (++)	32 (++)	35 (++)
L43	*Penicillium sp.*	60 (++)	57 (++)	40 (++)	32 (++)
L44	*Alternaria alternate*	43 (++)	35 (++)	41 (++)	43 (++)
L47	*Penicillium sp.*	49 (++)	60 (++)	40 (++)	45 (++)
L50	*Alternaria sp.*	39 (++)	37 (++)	36 (++)	40 (++)
L52	*Alternaria alternate*	40 (++)	43 (++)	42 (++)	36 (++)
L53	*Phoma sp.*	32 (++)	42 (++)	40 (++)	40 (++)
L54	*Phoma herbarum*	41 (++)	45 (++)	27 (+)	42 (++)
L55	*Alternaria alternate*	49 (++)	51 (++)	43 (++)	40 (++)
L56	*Trichoderma viride*	39 (++)	37 (++)	40 (++)	36 (++)
L57	*Fusarium polyphialidicum*	29 (+)	43 (++)	29 (+)	36 (++)
L58	*Bipolaris sp.*	40 (++)	40 (++)	41 (++)	40 (++)
L65	*Curvularia australiensis*	65 (+++)	57 (++)	53 (++)	33 (++)
L69	*Bipolaris sp.*	48 (++)	45 (++)	37 (++)	40 (++)
L73	*Fusarium polyphialidicum*	25 (+)	33 (++)	32 (++)	34 (++)
L82	*Alternaria sp.*	60 (++)	57 (++)	40 (++)	32 (++)

% Inhibition activity T= 0 mm = (-) No activity; > **30**% = (+) Slight activity; >**31%** T < **60**% (++) Moderate to Good activity; >**60**% (+++) Excellent activity; 10139 *Fusarium graminearum*; 12517 *Collectotrichum gleosporioides;* 13071 *Botrytis cinerea*; 2929 *Fusarium oxysporum with a significant difference (p < 0.05)*

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