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Full Length Research Article

NEW RECORDS OF FUNGAL ENDOPHYTES FROM UNILORIN SUGARCANE PLANTATION USING INTERNAL TRANSCRIBED SPACER GENE SEQUENCES

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ABSTRACT
Fungal endophytes are beneficial microbes that confer an added advantage to plants by colonizing their intercellular spaces without causing any harm. Instead, they produce secondary metabolites that play a considerable role in improving the medicinal qualities and protection of the host plants. There are several reports on fungal endophytes isolated from different plants but no report yet on fungal endophytes of *Blighia sapida* and *Euphorbia heterophylla*. These two plants were found growing in the Unilorin Sugarcane plantation as weeds. Leaf samples were collected from the plants, sterilized, and cultured on potato dextrose agar (PDA) for seven days. Emerging fungal mycelia from the leaves were subcultured to obtain pure culture and used for DNA extraction. We used the Internal Transcribed Spacer (ITS) region gene sequence analysis of fungi DNA for the identification of two new records of isolated fungal endophytes of these two plants. The extracted DNA was amplified using Polymerase Chain Reaction (PCR), sequenced and analyzed using the computer softwares; AliView and MEGAX. Molecular phylogeny inferred using maximum likelihood phylogenetic tree based on the ITS DNA sequences showed the relationship between the isolated fungal endophyte and the closest identified relatives from NCBI GenBank. Fungal endophyte isolated from *B. sapida* had the highest sequence similarity to *Curvularia lunata,* and isolate from *E. heterophylla* had the highest similarity to *Chaetomium globosum*. These two results represent the first reports of the fungal isolates as new records of endophytes from their respective host plant. The implications of these new records are fully discussed.

Keywords: Internal Transcribed Spacer (ITS), Polymerase Chain Reaction (PCR), AliView, MEGA X, Maximum likelihood, phylogeny

1.0 INTRODUCTION

The internal tissues of plant species have been inhabited by fungal endophytes that have no overtly negative effect or apparent symptoms on their hosts. The mechanism of their actions alters plants' interaction with their environment (Hamilton *et al*., 2012). The activities of these fungal endophytes greatly influence the physiological activities of the host plants by enhancing resistance against abiotic stress, diseases, insects, and mammalian herbivores (Khiralla *et al*., 2020).
Blighia sapida is a forest food tree in the plant family Sapindaceae native to tropical West Africa (Cameroon, Nigeria, Ghana, Gabon etc.) and reported to grow in the Caribbean and Jamaica (Adeduntan *et al*., 2016; Ouattara *et al*., 2016). *Blighia sapida* has several uses, but it is mainly grown for its edible fruits called arils. The arils obtained from *B. sapida* are consumed fresh, cooked into sauce, fried in oil, or industrially processed into cans for export to the United Kingdom and America (Ouattara *et al*., 2016). *Blighia sapida* has been reported to contain essential elements such as lipids (45%), proteins (11%), ash (4%), and Vitamin C. When employed as a nutritional base, the fruit can provide approximately 50% of the nutritional requirements (Ouattara *et al*., 2010). Besides the consumption of its edible fruits, *B. sapida* leaves are of medicinal importance and have been used to treat different ailments.

Euphorbia heterophylla, a member of the family Euphorbiaceae, is a major weed and medicinal plant usually found in cultivated land and waste places native to tropical and sub-tropical America also widespread in the tropics (Hutchison & Dalziel, 1958). *Euphorbia heterophylla* has been reported as a weed of many crops (Bridges *et al*., 1992; Kissman, 1992; Meschede *et al*., 2002; Olorunmaiye & Ogunfolaji, 2002). It is prevalent because its seeds are capable of germinating in extreme conditions. Germination has been reported in a wide range of temperatures without light requirements (Chachalis, 2015). *Euphorbia heterophylla* competes with crops for water, light, nutrients, and space through phytotoxic natural products leading to reduction in crop yield ranging from 4% to 85% (Tanveer *et al*., 2010).

Fungal endophytes promote the accumulation of bioactive compounds in medicinal plants, thereby influencing their quantity and quality (Chen *et al*., 2016). (Eze *et al*., 2019) in their study on endophytic fungi isolated from healthy leaves of *Carica papaya* identified toxigenic species belonging to genera *Fusarium* and *Epicoccum,* which were confirmed to produce toxic compounds known to have beneficial potentials for pharmaceutical, agricultural and industrial purposes. Phytochemical screening of *B. sapida* by (Dossou *et al*., 2014) revealed the presence of tannins, saponins, and glycosides. Adaptation of weeds to environmental factors and their tolerance to herbicides was described by Tétard-Jones & Edwards (2016) as biotrophic, i.e., weeds are resistant to adverse effects due to their ability to metabolise and detoxify agrochemicals coupled with the actions of free-living and plant-associated fungal endophytes. Márquez *et al*. (2007) reported that *Dichanthelium lenuginosum,* a grass in Yellow stone National Park U.S.A survived a temperature range of 38 – 65 ℃ because of its association with

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fungal endophyte *Curvularia protuberata*. Thus, *E. heterophylla's* evasiveness in a wide variety of crops can be attributed partly to the actions of their fungal endophytes.

Identification of fungal endophytes was previously based on their morphological characters when cultured on artificial medium. Findings of these studies were flawed because fast-growing fungi were isolated quicker than slow-growing ones, as well as the fact that some endophytic fungi are not culturable, and so are difficult to detect (Duong *et al*., 2006). Although there have been advancements in the current cultural techniques for identifying endophytic fungi, the flaws persist to some extent (Kandasamy & Kalra, 2013). In comparison, the molecular techniques of endophytic fungi identification make it possible for accurate classification of their strains at diverse hierarchical taxonomic levels. Several studies have successfully identified and classified endophytic fungi using the molecular phylogenetic method (Arnold, 2007; Kumar & Kaushik, 2013; Singha *et al*., 2016).
Based on our knowledge at the time of writing this paper, there is no previous report yet on fungal endophytes of *B. sapida* and *E. heterophylla*. Therefore the objective of this work was to identify fungal endophytes of *B. sapida* and *E. heterophylla* using the ITS gene sequences and molecular phylogenetic methods.

2.0 MATERIALS AND METHODS

2.1 *Source of plant materials*

Healthy green and fresh leaves of *B. sapida* and *E. heterophylla* were collected at the sugarcane plantation area in the University of Ilorin, Nigeria. The collection was made with a sterile blade and kept in sterile polythene bags at the collection location. Collections were transferred to the laboratory to isolate the fungal endophytes.

2.2 *Fungal isolation and identification*

Leaf samples were prepared for culture using a modified protocol (Stone *et al*., 2004) for endophytic fungi culturing. Using a sterile blade, the leaf sample was cut to sizes of $2cm^2$. Cut samples were surface sterilized by being washed with running water, 70% ethanol for 4 minutes, and then in 10% commercially available hypochlorite solution for 2 minutes. This was followed by further sterilization in 70% ethanol for 1 minute, and finally, the cut pieces were rinsed in 5 changes of distilled water and then brought to dry with sterile filter papers. The surface-sterilized leaf samples were plated aseptically on Potato Dextrose Agar (PDA) using streptomycin sulphate to prevent bacteria contamination. The plated leaf samples setup was observed for fungal activities for five (5) days, and observations were recorded. Pure isolates of the fungi were obtained by inoculation of fragments of mycelia from the initial culture plates. Inoculation on freshly prepared PDA in Petri dishes was done using sterile needles. All sterilization of inoculating instruments was done in flame and 90% ethanol simultaneously. Further isolation of fungal isolate was done in replicates to get isolates free of contamination.

2.3 *DNA extraction*

Mycelia were aseptically scraped and put in the DNA extraction kit to begin the extraction process, following the manufacturer's instructions for extraction. DNA extraction was done using the DNA mini-prep® extraction kit protocol. Ultra-pure DNA was then obtained after the experiment.

2.4 Polymerase Chain Reaction (*PCR) amplification and Sequencing*

PCR amplification and sequencing were done at Inqabba Biotec in South Africa using primers ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990). The PCR mixture contained 12.5 µl of Taq 2X PCR master mix, 1 µl each primer (10 µM) (ITS 1 and ITS 4), 9.5 µl of sterilised distilled water and 1 µl of the DNA. The PCR programme was run for 2 mins at 94 ˚C, followed by 35 cycles at 94 ˚C for 1 min, 55 ˚C for 1 min, 72 ˚C for 1 min and final extension at 72˚C for 10 mins. Sequencing of the PCR products were also done at Inqabba Biotec (South Africa) using the same primers.

2.5 *Phylogenetic analysis*

After the DNA fragment had been amplified, it was sent to a service provider (Inqab Biotech, Pretoria, South Africa) for sequencing.
Raw sequence data were analysed using the following installed computer software: SeqTrace 9.0 (Stucky, 2012) for viewing raw sequence data and consensus generation; AliView (Larsson, 2014) for alignment and MEGAX for alignment and phylogenetic tree construction. Similar nucleotide sequences were carefully selected, aligned and analyzed in MEGA X(Kumar *et al*., 2018) using maximum parsimony.

3.0 RESULTS

Two (2) isolates were successfully amplified and sequenced from the leaves of *Blighia sapida* and *Euphorbia heterophylla*. The ITS sequences of the two isolates showed high percentage of similarities with the GenBank database (*http.//www.ncbi.nhm.gov)* based on BLAST searches. Sequences with high similarities were downloaded (Tables 1 & 2) to identify the two isolates through phylogenetic analyses. The isolates from *B. sapida* was identified as *Curvularia lunata* (Figure 1) while *Chaetomium globosum* was identified from *E. heterophylla*.

The DNA sequence of the ITS region was 566 bp and 485 bp for *Curvularia lunata and Chaetomium globosum* respectively.

Table 1. Sequences used for the phylogenetic tree of *Curvularia lunata* isolated from *Blighia sapida*

Species	Isolate	GenBank accession number
Curvularia lunata	UIL80	LC835946 (from this study)
Botryosphaeria corticola	CBS 112546	AY259090.1 (Outgroup)
Curvularia_geniculata	UPM1192	KP340056.1
Curvularia lunata	PKC1	KX442659.1
Curvularia lunata	KY18.1	MK623264.1
C. lunata	E31	MT524329
Curvularia dactyloctenicola	Cc-M22018	MH282519
Curvularia lunata	CX-3	KR633084.1
Curvularia lunata	MC13	MZ436987.1
Curvularia lunata	E16	MH183194.1
Curvularia geniculata	RSCG-07	MW600265
Curvularia pseudorobusta	SDAU 992347-2	NR 130653
Curvularia muehlenbeckiae	CBS 144	MH858242

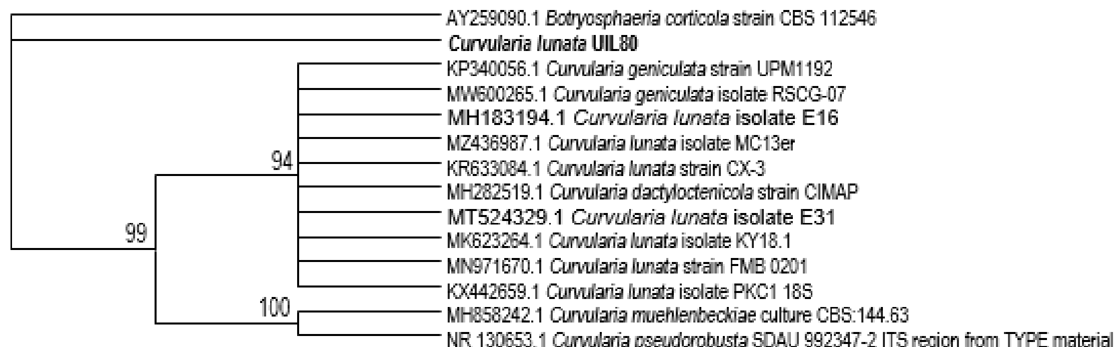

Figure 1. Phylogenetic tree showing the new isolate *C. lunata* UIL80 in bold

The evolutionary history was inferred using the Maximum Parsimony method. The bootstrap consensus tree inferred from 652 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (652 replicates) are shown next to the branches. The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). This analysis involved 14 nucleotide sequences. There were a total of 741 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

Table 2. Sequences used for the phylogenetic tree of *Chaetomium globosum* isolated from *Euphorbia heterophylla*.

Species	Isolate	GenBank accession number
Chaetomium globosum	UIL81	LC835947 (this study)
Botryosphaeria corticola	CBS 112546	AY259090.1 (Outgroup)
Chaetomium longiciliata	HMAS 245782	NG 068525
Chaetomium globosum	TNAU-Cg 108	MK823129.1
Chaetomium_funicola	CBS_158_52	GU563369_1
Chaetomium_funicola		AB746176_1
Chaetomium longiciliata	LC4055	KP336774
Chaetomium_elatum	P10_17a	KT354986_1
Chaetomium_elatum	DTO_333_F8	KX976613_1
Chaetomium_tenue	gao_1	MH619610_1
Chaetomium_pseudoglobosum	CBS_574_71	MH860267_1

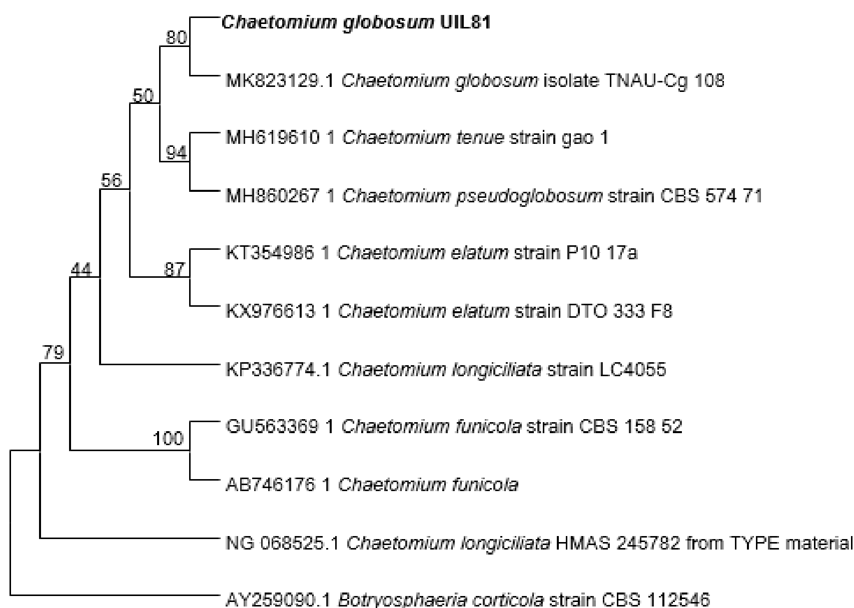

Figure 2. Phylogenetic tree showing the new isolate from *E. heterophylla C. globosum* UIL81 in bold

The evolutionary history was inferred using the Maximum Parsimony method. The most parsimonious tree with length = 525 is shown. The consistency index is (0.716495), the retention index is (0.649682), and the composite index is 0.581620 (0.465493) for all sites and parsimony-informative sites (in parentheses). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). This analysis involved 11 nucleotide sequences. There were a total of 971 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

The sequences of *Curvularia lunata* UIL80 and *Chaetomium globosum* UIL81were deposited in the online sequences repository DNA Data Bank of Japan (DDBJ) with the accession numbers LC835946 and LC835947 respectively

4.0 DISCUSSION

In this study, two endophytic fungi *Curvularia geniculata* and *Chaetomium globosum* were isolated from the leaves of the two plants *B. sapida* and *E. heterophylla* respectively.

Curvularia lunata belongs to the class Dothideomycetes (Kirk *et al*., 2008). The genus *Curvularia* exist in nature as endophytes (Gautam *et al*., 2013), epiphytes (Avila-Díaz & Oyama, 2007), saprophytes (Manamgoda *et al*., 2011) and pathogens (Scott & Carter, 2014). Members of the genus belong to a pathocomplex group, including *Curvularia*, *Cochliobolus,* and *Bipolaris*. In studies conducted by Manamgoda(2015) and Tan *et al*. (2014), phylogenetic analysis using ITS gene regions helped in the reclassification of morphologically defined *Bipolaris* species into the *Curvularia* genus. *Curvularia* species have been reported as endophytes of some plants, such as *Rauwolfia macrophylla* (Kaaniche *et al*., 2019)*, Parthenium hysterophorus* (Priyadharsini & Thangavelu, 2017)*,* and *Cymbopogon caesius* (Avinash *et al*., 2015). *Rauwolfia macrophylla* is a medicinal plant commonly used for the treatment of malaria and other diseases in Cameroon, and was associated with *Curvularia sorghina. Curvularia geniculata* isolated from invasive weed *Parthenium hysterophorus* showed plant growth promoting ability through phosphate solubilization and phytohormones when examined *in-vitro*. *C. lunata* isolated from *Cymbopogon caesius* showed antimicrobial activities when tested against pathogens.

The genus *Chaetomium* are filamentous fungi of different climates associated with plant debris and soil (Sutton *et al*., 1999; Von Arx *et al*., 1986). The genus was established in 1817 (Kunze & Schmidt, 1817) and classified as a non-clavicipitaceous endophyte. Zhao *et al*. (2017) reported the inhibitory activity of *C. globosum* (strain CDW7) against *Sclerotina sclerotinum,* causing rape rot. Zhai *et al*. (2018) described *C. globosum* as a highly beneficial endophytic fungus of *Salvia miltiorrhiza*, promoting the growth of the plant and enhances the accumulation of bioactive compounds. Zhou *et al*. (2016) illustrated the ability of *C. globosum* to increase systemic resistance of wool (*Gossypium hirsutum*) against invertebrate nematodes.

Previous studies have reported the two fungal endophyte isolates from this study to be a rich source of secondary metabolites belonging to diverse structural groups. Some reported secondary metabolites of *Curvularia* species include 4-hydroxylphenylacetic acid, indole-3-acetic acid, and acropyrone, 2'-deoxyribolacetone, hexylitaconic (Kaaniche *et al*., 2019; Nwachukwu *et al*., 2018). Flavipins, chaetoglobins, epipolythiodioxopiperazines, azaphilones, xanthones, anthraquinones, chromones, depsidones, terpenoids, and steroids are reported secondary metabolites of *Chaetomium* species (Zhao *et al*., 2017). Antimicrobial, antioxidant, and acetylcholinesterase inhibitory activities reported to be displayed by these metabolites confirm that they are of valuable pharmaceutical and biocontrol importance, playing a considerable role in improving plants' medicinal qualities and exhibiting potential against plant pathogen.

In addition to identifying the fungal endophytes, the molecular phylogeny method provided an evolutionary relationship between the identified fungal endophytes and other closely related species. The isolate from *B. sapida*, *C. lunata* showed evolutionary relationship with *Curvularia pseudorobusta, Curvularia dactyloctenicola, Curvularia geniculata, Curvularia muehlenbeckiae* and an outgroup *Daldinia raimundi* based on DNA sequence similarity from NCBI GenBank database. *Curvularia globosum* isolated from *E. heterophylla* showed close evolutionary relationship with *Chaetomium longiciliata, Achaetomium strumarium, Chaetomium nigricolor, Chaetomium globisporum, Chaetomium tectifimeti, Curvularia dactyloctenicola, Chaetomium pseudoglobosum, and Chaetomium microthecia*. These species are difficult to distinguish using cultural identification techniques because they belong to a pathocomplex group sharing similar morphological characteristics. Thus, the molecular phylogeny method of identification allowed for an accurate and reliable distinction of the isolates from their close relatives in this study.

Fungal metabolites are of increasing interest in bio-molecular activities studies. Therefore, it will be valuable to investigate the bio-molecular activities of the isolated endophytic fungi from this study.

5.0 Conclusion

With a high degree of clarity, *Curvularia lunata* and *Chaetomium globosum* are the identified endophytic fungi of *B. sapida* and *E. heterophylla,* respectively. Further research on assessing the efficacy of the bioactive constituent of these isolated endophytic fungi of *B. sapida* and *E. heterophylla* is required in order to determine their potential antimicrobial, medicinal, and host plant protection qualities.

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REFERENCES

Adeduntan, S. A., Olawale, A. E., & Oyerinde, O. V. (2016). Utilization of *Blighia sapida* (K.D. Koenig) in Rainforest and Savanna Zones of South-West, Nigeria. *Applied Tropical Agriculture*, *21*(3), 166–173.

Arnold, A. (2007). Understanding the diversity of foliar endophytic fungi: Progress, challenges, and frontiers. *Fungal Biology Reviews*, *21*, 51–66. https://doi.org/10.1016/j.fbr.2007.05.003

Avila-Díaz, I., & Oyama, K. (2007). Conservation genetics of an endemic and endangered epiphytic *Laelia speciosa* (Orchidaceae). *American Journal of Botany*, *94*(2), 184–193. https://doi.org/10.3732/ajb.94.2.184

Avinash, K. S., Ashwini, H. S., Babu, H. N. R., & Krishnamurthy, Y. L. (2015). Antimicrobial Potential of Crude Extract of *Curvularia lunata*, an Endophytic Fungi Isolated from Cymbopogon caesius. *Journal of Mycology*, *2015*, e185821. https://doi.org/10.1155/2015/185821

Bridges, D. C., Brecke, B. J., & Barbour, J. C. (1992). Wild poinsettia (*Euphorbia heterophylla*) interference with peanut (*Arachis hypogaea*). *Weed Science (USA)*. https://agris.fao.org/agris-search/search.do?recordID=US9190456

Chachalis, D. (2015). Wild poinsettia (*Euphorbia heterophylla*): An emerging weed in cotton and processing tomato in Greece. *Hellenic Plant Protection Journal*, *8*. https://doi.org/10.1515/hppj-2015-0005

Chen, L., Zhang, Q.-Y., Jia, M., Ming, Q.-L., Yue, W., Rahman, K., Qin, L.-P., & Han, T. (2016). Endophytic fungi with antitumor activities: Their occurrence and anticancer compounds. *Critical Reviews in Microbiology*, *42*(3), 454–473. https://doi.org/10.3109/1040841X.2014.959892

Dossou, V., Agbenorhevi, J., Combey, S., & Afi-Koryoe, S. (2014). Ackee (Blighia sapida) Fruit Arils: Nutritional, Phytochemicals and Antioxidant Properties. *International Journal of Nutrition and Food Sciences*, *3*, 534. https://doi.org/10.11648/j.ijnfs.20140306.17

Duong, L., Jeewon, R., & Lumyong, S. (2006). DGGE coupled with ribosomal DNA gene phylogenies reveal uncharacterized fungal phylotypes. *Fungal Diversity*, *23*.

Eze, P., Abonyi, D., Abba, C., Proksch, P., Okoye, F., & Esimone, C. O. (2019). Toxic, but beneficial compounds from endophytic fungi of *Carica papaya*. *The EuroBiotech Journal*, *3*, 105–111. https://doi.org/10.2478/ebtj-2019-0012

Gautam, A. K., Kant, M., & Thakur, Y. (2013). Isolation of endophytic fungi from *Cannabis sativa* and study their antifungal potential. *Archives of Phytopathology and Plant Protection*, *46*(6), 627–635. https://doi.org/10.1080/03235408.2012.749696

Hamilton, C., Pedro, G., Helander, M., & Saikkonen, K. (2012). Endophytic mediation of reactive oxygen species and antioxidant activity in plants: A review. *Fungal Diversity*, *54*. https://doi.org/10.1007/s13225-012-0158-9

Hutchison, J., & Dalziel, J. M. (1958). Flora of West Tropical Africa. *Crown Agents for Overseas Governments and Administrations, London, UK*, *1–3*. https://www.feedipedia.org/node/15867

Kaaniche, F., Hamed, A., Abdel-Razek, A. S., Wibberg, D., Abdissa, N., El Euch, I. Z., Allouche, N., Mellouli, L., Shaaban, M., & Sewald, N. (2019). Bioactive secondary metabolites from new endophytic fungus *Curvularia*. Sp isolated from Rauwolfia macrophylla. *PLoS ONE*, *14*(6), e0217627. https://doi.org/10.1371/journal.pone.0217627

Kandasamy, P., & KALRA, A. (2013). Molecular phylogenetic identification of endophytic fungi isolated from resinous and healthy wood of *Aquilaria malaccensis*, a red listed and highly exploited medicinal tree. *Fungal Ecology*, *6*, 205–211. https://doi.org/10.1016/j.funeco.2013.01.005

Khiralla, A., Spina, R., Varbanov, M., Philippot, S., Lemiere, P., Slezack-Deschaumes, S., André, P., Mohamed, I., Yagi, S. M., & Laurain-Mattar, D. (2020). Evaluation of Antiviral, Antibacterial and Antiproliferative Activities of the Endophytic Fungus *Curvularia papendorfii*, and Isolation of a New Polyhydroxyacid. *Microorganisms*, *8*(9), 1353. https://doi.org/10.3390/microorganisms8091353

Kirk, P. M., Canon, P. M., Minter, D. W., & Stalpers, J. A. (2008). *Dictionary of the fungi*. C.A.B International, Wallingford.

Kissman, K. G. (1992). *Plantas infestantes e nocivas*.

Kumar, S., & Kaushik, N. (2013). Kumar, S. & Kaushik, N. Metabolites of endophytic fungi as novel source of biofungicide: A review. Phytochem. Rev. 11, 507-522. *Phytochemistry Reviews*, *11*. https://doi.org/10.1007/s11101-013-9271-y

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Kunze, G., & Schmidt, J. C. (1817). *Chaetomium*. *Myc Heft*, *1*(15).

Larsson, A. (2014). AliView: A fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics (Oxford, England)*, *30*. https://doi.org/10.1093/bioinformatics/btu531

Manamgoda, D. (2015). A taxonomic and phylogenetic re-appraisal of the genus *Curvularia* (Pleosporaceae): Human and plant pathogens. *Phytotaxa*, *212*, 175–198. https://doi.org/10.11646/phytotaxa.212.3.1

Manamgoda, D., Cai, L., Bahkali, A., Chukeatirote, E., & Hyde, K. (2011). *Cochliobolus*: An overview and current status of species. *Fungal Diversity*, *51*, 3–42. https://doi.org/10.1007/s13225-011-0139-4

Márquez, L. M., Redman, R. S., Rodriguez, R. J., & Roossinck, M. J. (2007). A virus in a fungus in a plant: Three-way symbiosis required for thermal tolerance. *Science (New York, N.Y.)*, *315*(5811), 513–515. https://doi.org/10.1126/science.1136237

Meschede, D. K., Oliveira Jr., R. S., Constantin, J., & Scapim, C. A. (2002). Critical period of interference of *Euphorbia heterophylla* in soybean crop under low seeding rate. *Planta Daninha*, *20*, 381–387. https://doi.org/10.1590/S0100-83582002000300008

Nwachukwu, C., Ngwoke, Eze, P., Eboka, C., & Okoye, F. (2018). Secondary Metabolites from Curvularia sp, An Endophytic Fungus Isolated from the Leaves of *Picralima nitida* Durand and Hook (Apocynaceae). *Tropical Journal of Natural Product Research*, *2*(5), 209–213.

Olorunmaiye, K. S., & Ogunfolaji, R. T. (2002). Effect of density and duration of *Euphorbia heterophylla* (L) on the performance of cowpea (*Vigna unguiculata*) (Walp). *NISEB J.*, *21*(1), 217–222.

Ouattara, H., Meite, A., Kouame, P., & Coulibaly, S. (2016). Impact of *Blighia sapida* (k. Koenig) aril powder level in diet on the growth and the well-being of albino Wistar rats. *African Journal of Food Science*, *10*, 164–171. https://doi.org/10.5897/AJFS2016.1441

Ouattara, H., Niamké, B., Dally, T., & Kati Coulibaly, S. (2010). Nutritional composition studies of sun dried *Blighia sapida* (k. Koenig) aril from Côte d'Ivoire. *J Appl Biosci*, *32*, 1989–1994.

Priyadharsini, P., & Thangavelu, M. (2017). The root endophytic fungus *Curvularia geniculata* from *Parthenium hysterophorus* roots improves plant growth through phosphate solubilization and phytohormone production. *Fungal Ecology*, *27*, 69–77. https://doi.org/10.1016/j.funeco.2017.02.007

Scott, E. M., & Carter, R. T. (2014). Canine keratomycosis in 11 dogs: A case series (2000-2011). *Journal of the American Animal Hospital Association*, *50*(2), 112–118. https://doi.org/10.5326/JAAHA-MS-6012

Singha, I. M., Kakoty, Y., Unni, B. G., Das, J., & Kalita, M. C. (2016). Identification and characterization of *Fusarium* sp. Using ITS and RAPD causing fusarium wilt of tomato isolated from Assam, North East India. *Journal of Genetic Engineering & Biotechnology*, *14*(1), 99–105. https://doi.org/10.1016/j.jgeb.2016.07.001

stone, polishook, & White, J. (2004). *Endophytic fungi* (pp. 241–270). https://doi.org/10.13140/RG.2.1.2497.0726

Stucky, B. J. (2012). SeqTrace: A graphical tool for rapidly processing DNA sequencing chromatograms. *Journal of Biomolecular Techniques : JBT*, *23*(3), 90–93. https://doi.org/10.7171/jbt.12-2303-004

Sutton, D., Sanche, S., Revankar, S., Fothergill, A., & Rinaldi, M. (1999). Sutton DA, Sanche SE, Revankar SG, Fothergill AW, Rinaldi MG. In vitro amphotericin B. resistance in clinical isolates of *Aspergillus terreus*, with a head-to-head comparison to voriconazole. J Clin Microbiol. 37: 2343-2345. *Journal of Clinical Microbiology*, *37*, 2343–2345.

Tan, Y. P., Madrid, H., Crous, P., & Shivas, R. (2014). Johnalcornia gen. Et. Comb. Nov., and nine new combinations in *Curvularia* based on molecular phylogenetic analysis. *Australasian Plant Pathology*, *43*, 589–603. https://doi.org/10.1007/s13313-014-0315-6

Tanveer, A., Rehman, A., Javaid, M. M., Abbas, R. N., Sibtain, M., Ahmad, A. U. H., Ibin-I-Zamir, M. S., Chaudhary, K. M., & Aziz, A. (2010). Allelopathic potential of *Euphorbia helioscopia* L. against wheat (*Triticum aestivum* L.), chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medic.). *TURKISH JOURNAL OF AGRICULTURE AND FORESTRY*, *34*(1), 75–81.

Tétard-Jones, C., & Edwards, R. (2016). Potential roles for microbial endophytes in herbicide tolerance in plants. *Pest Management Science*, *72*(2), 203–209. https://doi.org/10.1002/ps.4147

Von Arx, J. A., Guarro, J., & Figueras, M. J. (1986). The ascomycete genus *Chaetomium*. *Beih Nova Hedwig*, *84*, 162.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and Direct Sequencing of fungal Ribosomal RNA genes for Phylogenetics. In *PCR Protocols: A Guide to methods and Applications.* (pp. 315–322). Academic Press.

Zhai, X., Luo, D., Li, X., Han, T., Jia, M., Kong, Z., Ji, J., Rahman, K., Qin, L., & Zheng, C. (2018). Endophyte *Chaetomium globosum* D38 Promotes Bioactive Constituents Accumulation and Root Production in Salvia miltiorrhiza. *Frontiers in Microbiology*, *8*, 2694. https://doi.org/10.3389/fmicb.2017.02694

Zhao, S.-S., Zhang, Y.-Y., Yan, W., Cao, L.-L., Xiao, Y., & Ye, Y.-H. (2017). *Chaetomium globosum* CDW7, a potential biological control strain and its antifungal metabolites. *FEMS Microbiology Letters*, *364*(3). https://doi.org/10.1093/femsle/fnw287

Zhou, W., Starr, J. L., Krumm, J. L., & Sword, G. A. (2016). The fungal endophyte *Chaetomium globosum* negatively affects both above- and belowground herbivores in cotton. *FEMS Microbiology Ecology*, *92*(10). https://doi.org/10.1093/femsec/fiw158