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Isolation and Characterization of Endophytes from *Justicia Secunda*

Inala, E.R.
Department of Biology, Federal University Otuoke, Bayelsa State, Nigeria

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Abstract

Plants are colonized by a diversity of microorganisms including endophytes. Endophytic microorganisms including bacteria and fungi are hidden companions of plants living mutually beneficial life inside the host plant. The study examined the diversity and distribution of endophytic fungal species in *Justicia secunda* using culture-dependent methods. The results revealed the presence of five relatively unharmful endophytic fungi (*Fusarium chlamydosporum*, *Fusarium oxysporum*, *Aspergillus versicolor*, *Acremonium falciform* and *Geotrichum* sp) colonizing *Justicia secunda*. The highest mean values of fungi isolated from the stem were *F. chlamydosporum* (33.3), followed by *Aspergillus versicolor* (8.33), *Fusarium oxysporum* (7.66), *A. falciform* (5.33) and *Geotrichum* (2.33). The mean values of fungi isolated from the leaves were *A. falciform* (15.33), *F. chlamydosporum* (9.00), *F. oxysporum* (3.67), *A.versicolor* (11.67), *Geotrichum* (4.67). Though the fungi genera are not usually pathogenic, effort should be made to thoroughly wash all parts of the plants before consumption as some of the identified fungi such as *Aspergillus versicolor* can cause opportunistic infections in immune-compromised individuals.

***Corresponding Author**: Inala, E.R., inalaer@fuotuoke.edu.ng

Introduction

Plants are colonized by a diversity of microbiota including endophytes (Lindow and Brandl, 2003) and medicinal plants are a huge reservoir of endophytes with pharmacological importance (Strobel, 2004). Endophytic microorganisms are hidden companions of plants inhabiting healthy living plant tissues; as symptomless colonies without causing harm to the host plant (Hallman, *et al.*, 2011). They live mutually beneficial lives inside the host plant, receiving protection and nutrition from the host plant while providing/facilitating nutrient uptake and protection to the plant against biotic and abiotic stress. The presence of endophytes may not only influence plant growth, development, and fitness (quantity and quality) but also population dynamics, plant community diversity and ecosystem functioning (Saikkonen, *et al.*, 1998; Adeleke and Babalola, 2021a).

Justicia is the largest and one of the most important genus of the Acanthaceae family; with about 600 species distributed in pantropical and tropical regions. *Justicia secunda* originates from South Africa and is today also grown in tropical or subtropical African countries. It is a perennial plant that is abundant in the lowland rainforest of the Niger Delta region of Nigeria; it contains several compounds and it is boiled and served as tea by the indigenous people for sickle cell patients (Osioma, *et al.*, 2017). Areal plant parts are used in traditional medicine to treat several health issues including diabetes and diabetic symptoms, anaemia, and hypertension (Mpiana, *et al.*,2010; N'guessan, *et al.*, 2011, Koffi *et al.*, 2013).

Endophytic fungi are one of the important elements in plant micro-ecosystems that influence host plants' growth and development (Jia *et al.*, 2016). Most of the endophytic fungi belong to Ascomycota (Huang et al. 2001, Arnold 2007) and are considered a great reservoir of bioactive compounds, producing secondary metabolites similar to those derived from the host pants (Rashmi et al., 2014, Tiwari, 2015, Adeleke & Babalola, 2021b). Studies have shown that fungal endophytes improve plant vigour, confer tolerance and control plant pathogens (Zabalgogeazcoa, 2008; Bushby, et al., 2016); and have posited possible exploitation of bioactive compounds from microbial endophytes in drug discovery (Yadav & Meena, 2021).

Moreover, plant bioactive characteristics can mirror their activity with associated microorganisms, especially those in the endosphere (Mohotti et al., 2020). Hence, elucidation of plant-endophytic fungi interactions can provide insights into the plant's content which can be applied in drug production (Salehi et al., 2019). Also, information on the microorganisms associated with medicinally important *Justicia secunda* is scarce. Therefore, this study investigated the diversity and distribution of endophytic fungal species in *Justicia secunda*.

Materials and Methods

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Collection of samples

Matured, wound-free and healthy parts (leaves and stems) of *Justicia secunda* from Otuaba, Ogbia L.G.A Bayelsa State. Parts of the plant were excised with a sterile knife placed in plastic bags and transported within one hour after collection to the laboratory for analysis.

Isolation of Fungi

Fresh plant parts were washed thoroughly with distilled water, cut into 1cm segments; submerged in 70% ethanol for 2mins, rinsed with distilled water and followed by treatment in hydrogen peroxide for 1min, then double rinsed with distilled water and blot dried on sterile filter paper.

After sterilization, explants were placed in Petri dishes containing Sabroud Dextrose Agar (SDA) supplemented with 100µg/ml chloramphenicol and gentamycin to inhibit the growth of bacteria. All plates were incubated at 25ºC for 72 hours to promote the growth of mycelia, under controlled conditions followed by pure culture for identification. The growth of the endophytic fungal colonies from the plant tissues was observed daily.

The colonies which developed on the plate were randomly picked and purified by subculturing on Sabroud Dextrose Agar plates and colony morphology (purity) was examined after 7 days of incubation.

Characterization and Identification of the Fungal Isolates

Colony descriptions were based on observations of SDA under ambient daylight conditions. Microscopic observations at X10 and X40 were made from preparations using the lacto phenol cotton blue staining method and the slide culture test (Oyeleke *et al.*, 2008).

Results

Isolation of Fungal Endophytes

Using macroscopic (cultural) and microscopic characteristics; a total of five (5) fungal endophytes belonging to 4 different taxa were isolated and identified from *Justicia secunda* (Table 1). Two isolates belong to *Fusarium*, one belongs to *Aspergillus*, one belongs to *Acremonium* and one is *Geotrichum*. The putative isolates are: *Fusarium chlamydosporum* (brown whole colonies with red pigmentation, frequently growing in blooms), *Fusarium oxysporum* (white colonies tinged with salmon and lavender at maturity), *Aspergillus versicolor* (large velvety colonies with blue-green colour), *Acremonium falciform* (pale grey colonies) and *Geotrichum* sp (white, dry, powdery to cottony colonies, resembling ground glass).

The percentage occurrence of the fungi species in leaves of *Justicia secunda* is presented in Table 2 and Figure 1. The highest occurrence was recorded for *Fusarium chlamydosporum* (34.59%) while the least was recorded for *Geotrichum* (8.27%). Table 3 and Figure 2 show the percentage occurrence of the fungi species isolated from the stem of *Justicia secunda* *Fusarium chlamydosporum* has the highest occurrence (31.65%), while the least was recorded by *Geotrichum* (6.33%).

The mean value of fungal occurrence in parts of *J. ecunda* is presented in Table 4 and Figure 3. The highest mean values of fungi isolated from the stem were *F. chlamydosporum* (33.3), followed by *A. versicolor* (8.33), *F.oxysporum* (7.66), *Acremonium falciforme* (5.33) and *Geotrichum* (2.33)

Table 1: Fungi species isolated from *Justicia secunda*

Isolate	Cultural Characteristics	Morphological Characteristics
Fusarium chlamydosporum	Brown colonies with red pigmentation	Woolly colonies, growing in blooms with production of abundant chlamyconidia
Fusarium oxysporum	White colonies tinged with salmon and lavender at maturity	Presence of non- septate microconidia with a smooth wall appearance.
Acremonium falciform	Pale grey colonies with uncoloured pigmentation	Presence of nonseptate conidiophores which is crescent at times.
Geotrichum sp	White, dry, powdery to cottony colonies	Arthroconidia and coarse true hyphae are observed.
Aspergillus versicolor	Large velvety colonies with blue-green colour	Smooth walled monoverticillate conidiophores

Table 2: Percentage occurrence of fungi isolated from *Justicia secunda* (leaves)

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Fungal Isolate	Number of occurrences	Percentage occurrence (%)
F. oxysporum	27	20.30
F. chlamydosporum	46	34.59
A. falciforme	14	10.52
Geotrichum sp	11	8.27
A. versicolor	35	26.32

Table 3: Percentage occurrence of fungi isolated from *Justicia secunda* (stem)

Isolate	Number of occurrences	Percentage occurrence (%)
Acremonium falciform	10	12.66
Geotrichum sp	5	6.33
Aspergillus versicolor	23	29.11
Fusarium oxysporum	16	20.25
Fusarium chlamydosporum	25	31.65

Table 4: Mean occurrence of Fungi isolated from parts of *Justicia secunda*

Sample	*Fusarium chlamydosporum*	*Geotrichum sp*	*Fusarium oxysporum*	*Acremonium falciform*	*Aspergillus versicolor*
Stem	33.3 ± 1.53	2.33 ± 2.52	7.66 ±2.08	5.33 ± 2.52	8.33 ± 2.08
Leaves	9.00 ± 3.00	4.67 ± 2.52	3.67 ± 1.53	15.33 ± 3.05	11.67 ± 6.66

Each value is mean ± SD of triplicate determination.

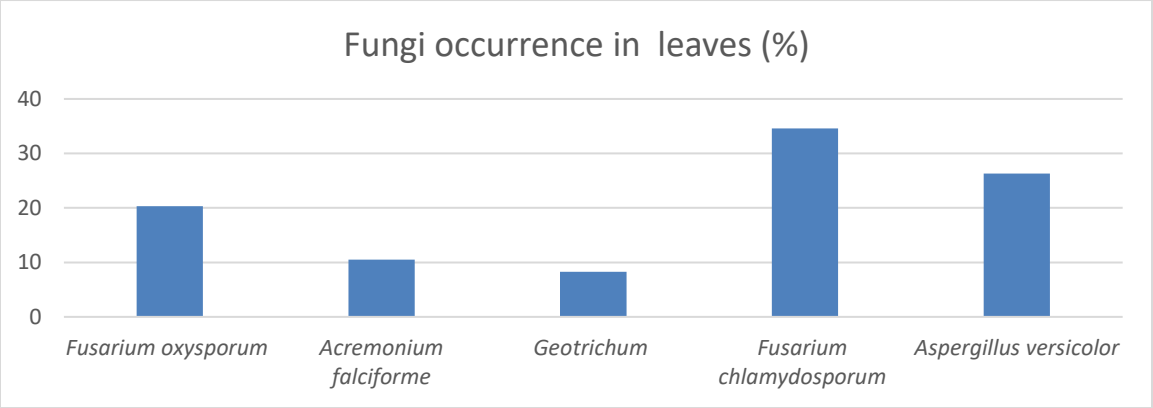

Figure 1: Percentage occurrences of the fungi species in leaves of *Justicia secunda*

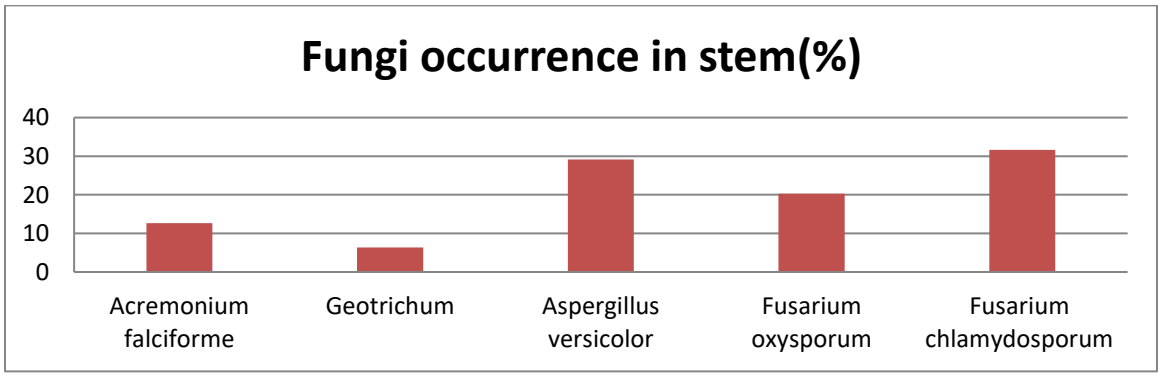

Figure 2: Percentage occurrence of the fungi species in the stem of *Justicia Secunda*

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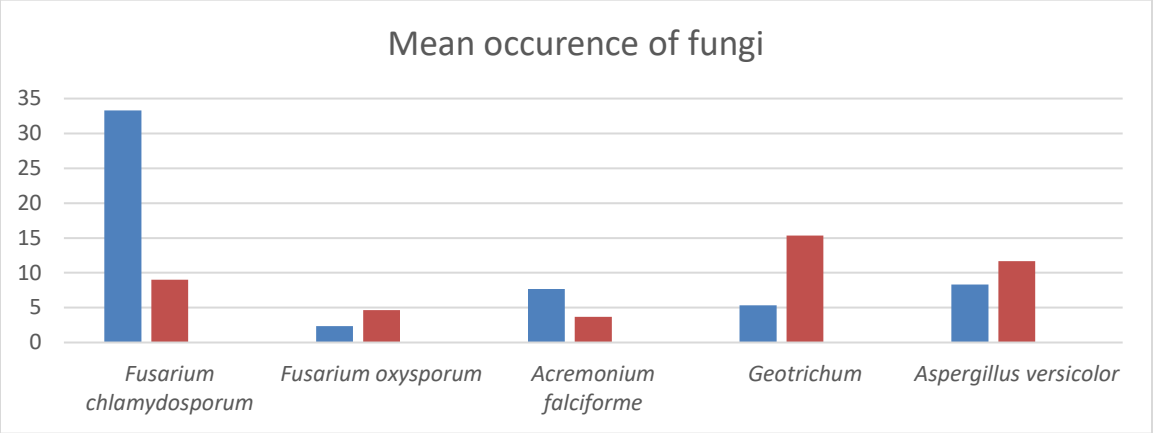

Figure 3: Mean occurrence of fungi in the stem and leaves of *Justicia secunda*

Discussion

The results herein have shown the presence of five fungal species f of Ascmycta: *Fusarium chlamydosporum, Fusarium oxysporum, Aspergillus versicolor, Acremonium falciform* and *Geotrichum* as the putative endophytic fungi occurring in *Justicia secunda*. These fungi isolated are not known to cause diseases of the plant, nor do they cause any major disease on the consumer perhaps except for *Aspergillus versicolor* which is a causative agent of aspergilliosis and can cause opportunistic infections in immune-compromised individuals (Engelhart *et al*., 2002). The fungal genera *Fusarium* and *Aspergillus* are cosmopolitan members of the Ascomycota with a wide host range. Moreso, *Fusarium* is reported to be one of the most dominant endophytic fungi with the ability to grow in diverse substrates (Ahmed *et al*., 2023). Though, *Fusarium spp* are known as mycotoxin producers their occurrence on *Justicia secunda* does not seem to cause any adverse effect on the plant.

The occurrence of the same species of fungi on the stem and leaf of *Justicia secunda* is as a result of nature and not of the health of the plant. *Fusarium chlamydosporum* is an infrequent endophytic fungus, having the lowest incidence of occurrence while the predominant endophytic fungus is *Aspergillus versicolor* in *Justicia secunda*.

Conclusion

This study has shown the presence of relatively unharmful endophytic fungal species (*Fusarium chlamydosporum, Fusarium oxysporum, Acremonium falciform, Geotrichum* and *Aspergillus versicolor*) occurring in *Justicia secunda*. These fungi species have not been implicated in diseases of the plant, their occurrence on *Justicia secunda* may be as a result of natural adaptation, conferring mutualistic benefits to both the plant and fungi.

Although these fungi genera are not usually pathogenic, effort should be made to thoroughly wash all parts of the plants before consumption as some of the identified fungi such as *Aspergillus versicolor*, although not pathogenic can cause opportunistic infections in humans. Further studies are recommended to ascertain the relationship between the fungi and *Justicia secunda* and their input on the growth of the plant.

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