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***Research***

# Identification and Characterization of Endophytic Fungi from *Garcinia atroviridis* for Potential Antagonistic Against Phytopathogenic, *Colletotrichum gloeosporioides*

**Nur Afeeqah Mohamed Zanudin[1,2], Nor'aishah Hasan[2,3]* and Patayah Mansor[4]**

1. Faculty of Applied Sciences, Universiti Teknologi MARA, 404500 Shah Alam, Selangor
2. Faculty of Applied Sciences, Universiti Teknologi MARA, Cawangan Negeri Sembilan, Kampus Kuala Pilah, 72000 Kuala Pilah, Negeri Sembilan, Malaysia
3. Biotechnology, Microbiology and Environmental Collaborative Science, Universiti Teknologi MARA, Cawangan Negeri Sembilan Kampus Kuala Pilah, 72000, Negeri Sembilan, Malaysia
4. Laboratory Mycology and Pathology, Forest Biodiversity Division, Forest Research Institute Malaysia, 52109 Kuala Lumpur, Malaysia
*Corresponding author: aishahnh@uitm.edu.my

**ABSTRACT**

Biological control is referred to as the "use of natural or modified organisms, genes" to minimize the effects of undesirable pests, pathogenic microorganisms, and diseases on plant crops. This measure has become a suitable and safe alternative for chemical fungicides in plant disease management. Endophytic fungi have received much attention as biological control agents against many plant pathogens through antibiosis, parasitism, invading spores, mycelium, and cells of the pathogen, and secreting bioactive metabolites. While the therapeutic properties of *Garcinia atroviridis* have been studied, the existence of microbial endophytes and their properties is still less documented. In this research, *G. atroviridis* endophytic fungi were isolated and identified by fungal colony morphology observation combined with the PCR-amplified fungal internal transcribed spacer (ITS) sequence analyses. Fungal endophytes were assessed for their biocontrol potential against *Colletotrichum gloeosporioides*. In total, 111 endophytic fungal isolates harboring inside the leaf, branch, and fruit of *G. atroviridis* belonged to 5 different species with 3 different genera and two unidentified genera. All the endophytic fungal species isolated were evaluated using an in vitro dual culture assay against *C. gloeosporioides*, a common pathogen that causes anthracnose disease. The results of the present study clearly showed that seven species of isolated fungal endophytes were capable of inhibiting the mycelial colony growth of *C. gloeosporioides* with an inhibition percentage between 54.67% to 87.94%. Among these species, *Nigrospora sphaerica* recorded the highest PIRG with 87.94%. Our work indicates that endophytic fungi isolated from *G. atroviridis* have a biocontrol effect on *C. gloeosporioides* and are expected to be a potential source of bioactive metabolites.

**Key words:** Antagonistic activity, biocontrol agent, *Colletotrichum gloeosporioides*, endophytic fungi, *Garcinia atroviridis*

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## INTRODUCTION

There are an estimated 500, 000 species of terrestrial plant in the world, and many species are still unknown (Corlett, 2016). Plants protect themselves from herbivores and pathogens by optimizing the distribution of chemical defenses (Gershenzon & Ullah, 2022). De Bary (1866) provided the first definition of an endophyte, as "any organism that grows within plant tissues are termed as endophytes," (Patra *et al*., 2016). Endophytes, often bacterial or fungal microorganisms that colonize healthy plant tissues without causing any visible symptom of disease or adverse effects on the host and have been found in all plants studied to date (Chowdhary & Kaushik, 2015; Saad *et al*., 2019). The complex endophyte-plant relationship causes the microorganisms to produce diverse potent bioactive compounds that confer considerable benefits to the host such as stimulating host plant growth, and inducing host plant resistance to biotic and abiotic stress (Kandel *et al*., 2017). The criteria taken into account when considering a microbial antagonist as a biocontrol agent (BCA) against phytopathogen are generally based on their

capacity to compete with the pathogen for the available nutrient and space, hyperparasitism, secretion volatile and/or non-volatile secondary metabolites (Naidu *et al*., 2016). According to Kaul *et al*. (2012), fungal endophytes produced a vast amount of secondary metabolites compared to any other group of endophytic microorganisms. Also, some studies show that isolated endophytic fungi from medicinal plants are capable of replicating their host novel compounds (Venieraki *et al*., 2017). Therefore, fungal endophyte bioprospecting research has been increased for decades as these microbes have been proven to produce a wide range of valuable metabolites for agricultural, pharmaceutical, and industrial purposes (Manganyi & Ateba, 2020).

Medicinal plants have long been utilized in traditional medicine due to their highly valuable natural compounds that have slight to no side effects. Due to the rising demand for herbal drugs and pharmaceuticals, the use of medicinal plants is on the rise. However, the commercialization of plant bioactive compounds may be impractical as the plant population reduces significantly from overharvesting without replanting, long period of plant development, and a small number of secondary metabolites accumulated in the plant (Adeleke & Babalola, 2021). Thus, the use of endophytes is necessary as an alternative to reduce the production cost and environmental impact by reducing the reliance on the plant itself. *Garcinia atroviridis*, also known as 'Asam Gelugor' in Malaysia, belongs to the family Guttiferae having its own economical and folk-medicinal applications. The dried slices of fruits called 'asam keeping is used as a sour flavoring in cuisines and the young shoots can be eaten raw as 'ulam'. This plant has been used as a post-partum treatment as well for cough, dandruff, and earache, and improves blood circulation (Sultana *et al*., 2014). The various parts of the plant possess a range of medicinal benefits such as antioxidant, anti-inflammatory, and anti-obesity (Hamıdon *et al*., 2017). A study on the antimicrobial activity of endophytic fungi isolated from this plant has been conducted by Phongpaichit *et al*. (2006). While much is known about the phytochemistry of this plant, there is still limited information available about the endophyte biology. Hence, this study was conducted to evaluate the diversity of endophytic fungi isolated from *G.atroviridis* and further screen them as potential biocontrol agents against *Colletotrichum gloeosporioides*, an anthracnose causal agent. This fungus pathogen infects about 470 different host genera and causes post-harvest problems (Pillai & Jayaraj, 2015). Therefore, research is warranted to identify the potential endophyte fungal with biocontrol activity which will help in plant disease management.

## MATERIALS AND METHODS

### Collection of plant samples

Plant samples (Figure 1) were collected from Nasuha Herbal Farms, Muar, Johor, Malaysia. The symptomless and healthy leaves, branches, and fruits were selected, kept separately in a sealed plastic bag, and transported back to the laboratory by method described by Deepthi *et al*. (2018).


**Fig. 1.** Different plant parts of *G. atroviridis* (leaf, branch, fruit)

### Isolation of Endophytic Fungi

A standard protocol was followed with few modifications (Zheng *et al*., 2017). All the plant samples were thoroughly washed under running tap water, cut into 0.5 $cm^2$ pieces, and subjected to a sequence of submersions in different solutions for surface disinfection purposes as follows: 70% ethanol for 2 min, 2% sodium hypochlorite for 3 min, sterile distilled water for three times and allowed to dry on sterilized filter paper. Then, the small fragments of each plant part were inoculated in a petri dish containing Potato Dextrose Agar (PDA) enriched with chloramphenicol (50 µg/mL) and incubated at 28°C for two weeks. A pure culture of endophytic fungi was obtained by transferring the hyphal tips

onto a new PDA petri dish.

**Morphological Identification of endophytic fungi**

The texture, color, and reproductive properties of each pure culture were first evaluated macroscopically and microscopically. All the fungal isolates were identified using a microscope, and all of the methods were followed as previously reported by Guo *et al*. (2000). The isolated endophytes were sorted into groups based on the appearance of the colonies.

**DNA extraction and PCR amplification**

The isolated endophytes were sorted into groups based on the appearance of the colonies. Based on the groupings, one isolate was selected representing each group for molecular identification. The extraction of genomic DNA was conducted using the CTAB protocol proposed by Umesha *et al*. (2016). The isolates were grown first in potato dextrose broth for 7 days at room temperature. Then, approximately 200 mg mycelia were transferred into a 1.5 mL centrifuge tube containing 500 µL of 2× CTAB buffer. By using a cell homogenizer, the mixture was completely homogenized before being incubated in a water bath at 65 °C for 30 min with intermittent mixing at every 15-min interval. Then, the tubes were centrifuged at 10,000 r.p.m for 10 min. The supernatant formed was transferred to a new tube and an equal volume of chloroform:isoamyl alcohol (24:1) was added. The solution was vortexed until an emulsion formed and centrifuged at 13,000 rpm for 12 min to separate the phases. The aqueous upper phase was transferred to a new tube and this extraction step was repeated until the upper phase was clear. To precipitate the DNA, two volumes of ice-cold isopropanol were added to the tube and incubated overnight at -20 °C. The precipitation was collected by centrifugation at 10,000 r.p.m. The supernatant was decanted without disturbing the pellet and subsequently washed with 1mL of 70% ethanol. The residual ethanol was removed by drying in a speed vac. The pellet was allowed to air dry long enough to remove the alcohol. The dried pellet was dissolved in 50 µL of TE buffer.

The ITS sequences of each sample were amplified with the universal primers, forward: ITS 1 (5'TCCGTAGGTGAACCTGCGG3') and ITS 4 reverse: (5'TCCTCCGCTTATTGATATGC3'). The amplification mixture was set up in 25 µL comprising 12.5 µL of Go Taq ® Green Master Mix, 1.0 µL of each primer, 2.0 µL of each DNA template, and 8.5 µL of sterile distilled water. The PCR amplification method was performed as described by Katoch and Pull (2017). The PCR products were sequenced by Bio Basic Asia Pacific Private Limited using the same primers. The sequences were analyzed against the nucleotide database using the BLASTn tool of the National Centre for Biotechnology Information (NCBI).

***In vitro* inhibition of mycelial growth of *C.gloeosporioides* with endophytic fungi**

The ability of the fungal endophytes to inhibit the mycelial growth of the pathogenic fungus, *C. gloeosporioides*, was determined with the dual culture method. The pathogenic fungus was obtained from the culture collection (FRIM 1319) at the Mycology and Pathology Laboratory, Forest Research Institute, Malaysia. Mycelial plugs (5 mm) of 7-day-old pathogen and endophyte were cultured 3 cm apart. All plates were incubated at 28 °C for two weeks and the radial growth of the pathogen was measured daily. The recorded data was translated into percentage inhibition of radial growth (PIRG) using the following equation:

$$PIRG = \left( \frac{R1 - R2}{R1} \right) \times 100$$

Where, R1 was the radial growth of the pathogen without endophytic fungi and R2 was the radial growth of the pathogen in the presence of endophytic fungi (Katoch & Pull, 2017). The percentage of radial inhibition growth was grouped as follows: 70% - 100% (strong inhibition), 50% - 69% (moderate inhibition), 1 - 49% (weak inhibition) and 0% (no inhibition). Statistical analysis of the PIRG values was performed using ANOVA in SPSS.

**RESULTS**

A total of 111 endophytic fungal strains were recovered from three different tissues (leaf, branch & fruit) of *Garcinia atroviridis*. Of these, the highest colonization frequency was found in branch (86%) followed by leaf (72.2%) and fruit (44.4%) as shown in Figure 2.

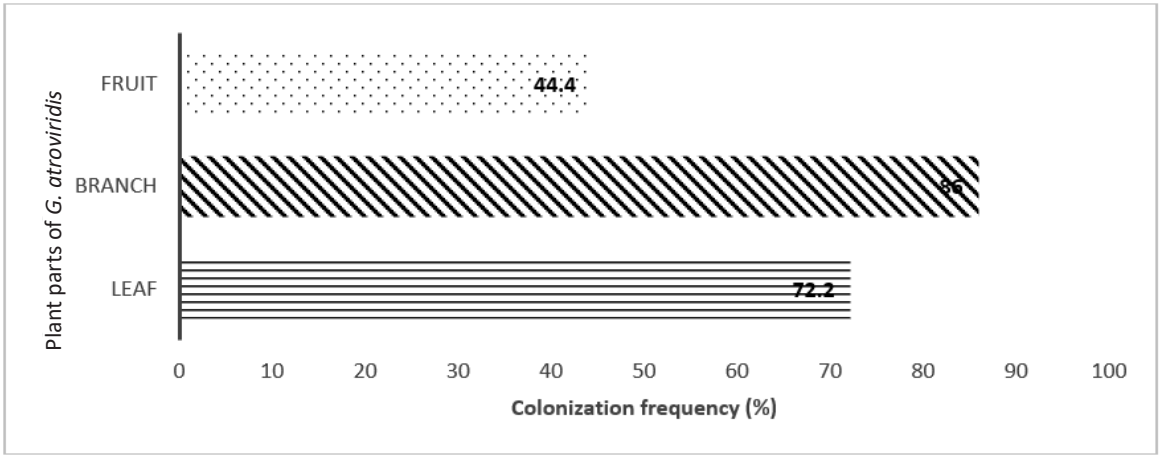


**Fig. 2.** Colonization frequency of endophytic fungi of *G.atroviridis*

The isolated endophytes were then divided into phenotype groups based on their macromorphological attributes such as colony form, mycelium color and reverse media color (Table 1 & Figure 3). Those isolates were divided into 14 groups based on their macromorphological characteristics. During the incubation period, the growth of hyphae of each isolate can be seen as remarkably rapid, intermediate, and slow to fill the petri dish. From the result obtained in Figure 3, isolates Type A, B, F, I, J, K and N express similar white mycelium color. However, they were differentiated through some attributes. Isolate Type A has a flat, wooly mycelium with dark brown pigmentation and brown to blackish brown colony reverse color. While the cottony mycelia of Type B were white to light grey at the center with cream to the brown center colony reverse color. Both isolates Type F and I possessed white mycelia with black and small acervular conidiomata but distinguished by the numbers of conidiomata on mycelia. Their mycelia are also differentiated by the form and texture. Isolate Type J is one of the fast-growing endophytic fungi with white, cottony mycelia both upper and reverse colony color. The mycelia of isolate Type K and N were both in white color but have been contrasted by the grey center of mycelia in Type K. The slowest growing fungus, isolate Type C, had olivaceous grey to black centers with the granular texture of the colony. Next, the colony of isolate Type E was flat with the entire margin, light to olivaceous dark grey mycelia with salmon to orange acervular conidiomata and olivaceous dark grey with black pigment at the reverse colony. Both isolates Type D and Type G secrete chemicals that change the color of PDA to red to maroon and yellowish to light brownish respectively. The most rapid-growing endophytic fungus was isolate Type H. The mycelia color of the isolate initially was white to pale grey and became dark grey to black as it older. Also, the flat, wooly mycelia of isolate Type L was light grey and turned to dark grey as it grew older. Lastly, isolate Type M showed light grey to dark brown upper color with dark brown reverse color with raised, threadlike mycelia in irregular form.

Each of the isolates from these groupings was selected for molecular identification. Endophytic isolates were successfully amplified using primer ITS1 and ITS 4 (Figure 4). Among the identified endophytic fungi, five of them were identified at the species level (*Bjekandera adusta, Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Nigospora sphaerica* and *Pestaliotiopsis neglecta*) and three at the genus level (*Colletotrichum sp*., *Diaporthe sp*. & *Pestalioptiosis sp*.), in which most of the isolates belongs to phylum Ascomycota and one of them was Basidiomycota. However, six of the isolates were unidentified. Isolate type B was eliminated from further antagonistic studies due to having similar identification with pathogenic used in this study.

Direct inhibition antagonism test was conducted to verify the antagonism potential of fungal endophytes against C.gloeosporioides for two weeks. Overall, this test revealed that most of the isolates were capable of inhibiting the mycelial growth of the pathogen with varying degrees of inhibition, from no inhibition (0.00%) to strong inhibition activity (87.94%) as shown in Table 2. As seen in Figure 5, the most common mechanism in antagonism activity was competition between endophyte and pathogen. This mechanism can be seen from the dual culture interaction of isolates E (*Colletotrichum* sp*.),* F (*Pestalotiopsis sp*.), H (*L. theobromae*), K (*N. sphaerica*), L (unidentified fungus), M (unidentified fungus) and N (*Diaporthe sp*.). Both endophytes and pathogens grow towards each other, but the growth stopped before their mycelia came in physical contact with no inhibition zone. The mycoparasitism action of isolates Type J, namely *Bjerkandera adusta*, can be seen on the 7th day as the mycelial of the endophytes started to overgrow and completely overgrow on pathogen on the 10th day with visible creamy exudates at the area of contact. Interestingly, there was a change in pathogen mycelium color from grey to light brown and red when interacting with the isolates from Type A and Type D respectively. Unfortunately, endophyte isolates from Type C (unidentified fungus) and Type G were unable to inhibit the growth of *C.gloeosporioides.* Among all the endophytes tested, 36 isolates showed

strong inhibition towards *C.gloeosporioides*, with the highest inhibition percentage possessed EL 41, namely *N.sphaerica* (87.94%) (Table 2). Meanwhile, 58 isolates moderately inhibited the pathogen, 14 isolates had weak inhibition activity and 2 isolates showed no inhibition.

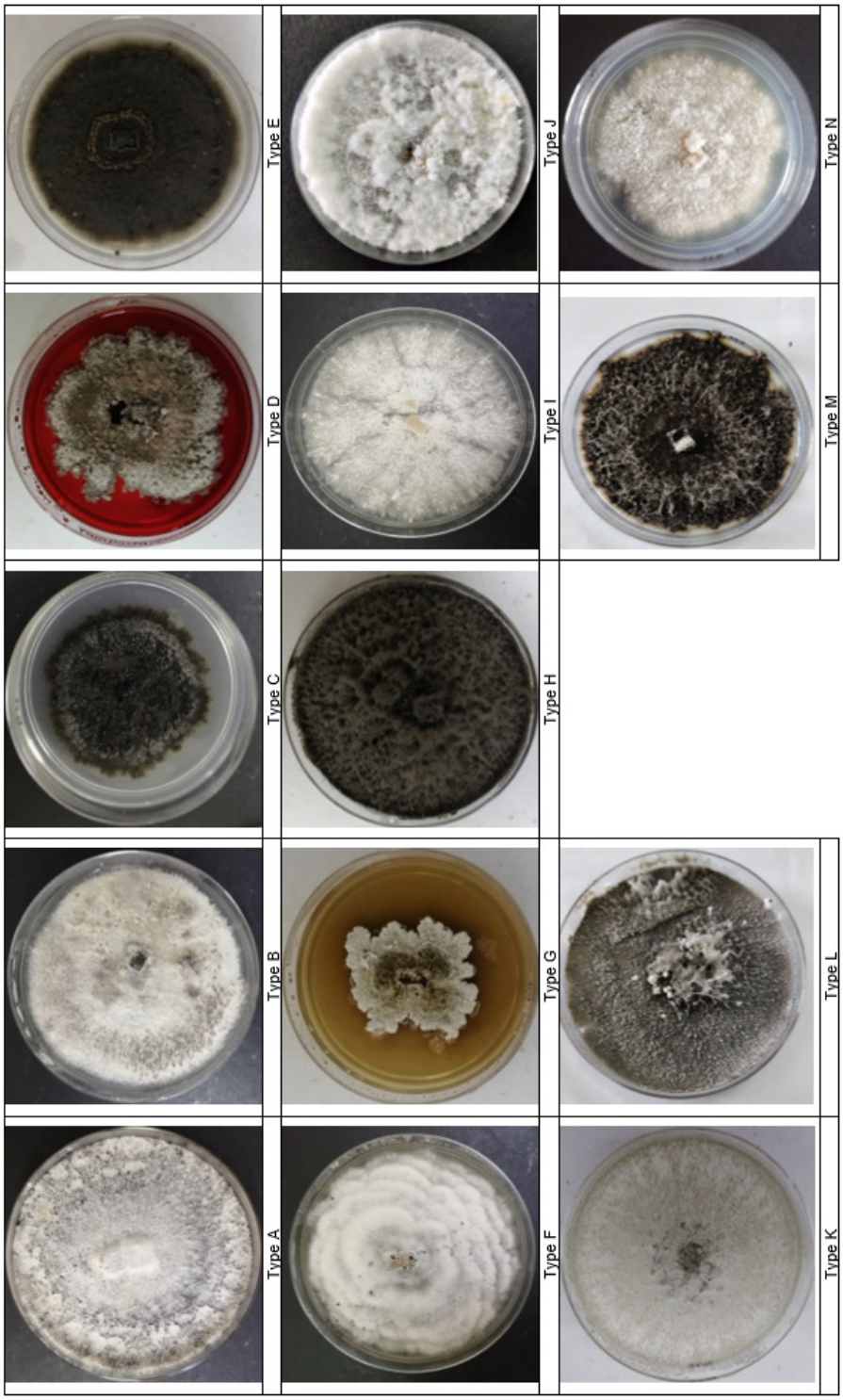


**Fig. 3.** Representative of the isolates from each group that have been differentiated based on their macromorphological characteristic

**Table 1.** Morphological and cultural descriptions of endophytic fungi isolated from *G. atroviridis*

| Isolate type | Scientific name | Culture morphological characteristics of endophytic fungi isolated from *G.atroviridis* | | |
|---|---|---|---|---|
| | | Colony upper colour | Colony reverse colour | Colony texture |
| A | Unidentified | White to cream mycelia | Brown to blackish brown | Flat, dark brown pigment |
| B | *Colletotrichum gloeosporioides* | White to light grey centers | Cream with brown centres | Raised, cottony mycelia |
| C | *Unidentified* | Olivaceous grey to black centers | Olivaceous grey | Granular, irregular |
| D | *Unidentified* | Grey with brown centers | Light grey | Cottony, turns PDA to red to maroon |
| E | *Colletotrichum* sp. | Salmon to orange spores color, light to olivaceous dark grey | Olivaceous dark grey with black pigment at reverse | Flat, cottony |
| F | *Pestalotiopsis* sp. | White mycelia with black and small acervular conidiomata | White to cream | Raised, wooly mycelia with concentric form |
| G | Unidentified | Light grey with light brown centers | Light grey | Turns PDA to yellowish to light brownish |
| H | *Lasiodiplodia theobromae* | initially white to pale grey becoming dark grey to black | initially white to pale grey becoming dark grey to black and black pigment | Raised, wooly mycelia |
| I | *Pestalotiopsis neglecta* | White | White to cream | Flat, Wooly mycelia with filamentous form |
| J | *Bjerkandera adusta* | White to cream | White to cream | Raised, cottony mycelia |
| K | *Nigospora sphaerica* | White to grey center | White to cream | Raised, wooly mycelia |
| L | Unidentified | Light grey to dark grey | Dark grey | Flat, wooly mycelia |
| M | Unidentified | Light grey to dark brown | Dark brown | Raised, threadlike mycelia with irregular form |
| N | *Diaporthe* sp. | White to cream | White to cream centers | Raised, wooly mycelia with undulate margin |

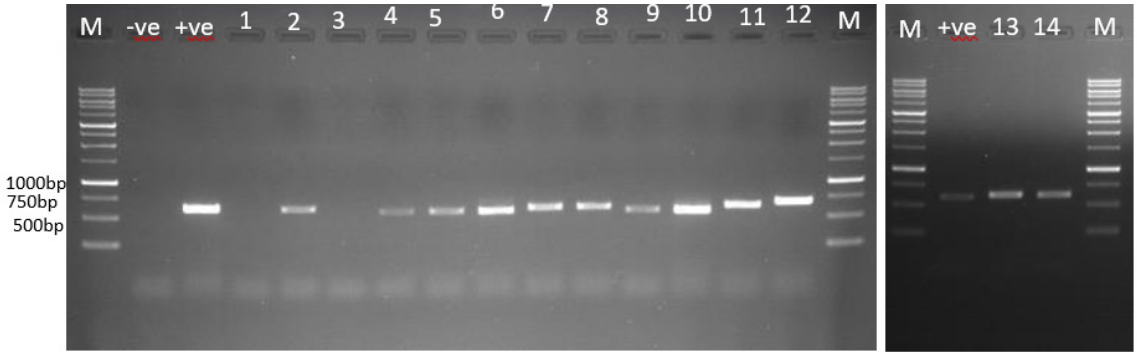


**Fig. 4.** Banding pattern of isolated endophytic fungi *C.gloeosporioides* (1), *Colletotrichum sp.* (2), *Pestalotiopsis sp*. (3), *P. neglecta* (4), *L. theobromae* (5), unidentified fungus, Type A (6), *N. sphaerica* (7), *Bjerkandera adusta* (8), unidentified fungus, Type C (9), unidentified fungus, Type D (10), *Diaporthe sp*. (11) unidentified fungus, Type G (12), unidentified fungus, Type L (13) and unidentified fungus, Type M (14).

**Table 2.** Antifungal activity (percentage of inhibition radial growth %) of endophytic fungi against *C.gloeosporioides*

| Isolate type | Scientific name | PIRG (%) |
|---|---|---|
| A | Unidentified | $73.33^{b} \pm 2.31$ |
| C | Unidentified | $1.33^{e} \pm 2.31$ |
| D | Unidentified | $73.7^{b} \pm 3.94$ |
| E | *Colletotrichum* sp. | $79.82^{a} \pm 0.71$ |
| F | *Pestalotiopsis* sp. | $77.67^{b} \pm 3.01$ |
| G | Unidentified | $0.00^{a} \pm 0.00$ |
| H | *Lasiodiplodia theobromae* | $84.82^{a} \pm 1.20$ |
| I | *Pestalotiopsis neglecta* | $57.33^{d} \pm 9.24$ |
| J | *Bjerkandera adusta* | $80.71^{a} \pm 4.09$ |
| K | *Nigospora sphaerica* | $87.94^{a} \pm 2.37$ |
| L | Unidentified | $77.33^{b} \pm 6.11$ |
| M | Unidentified | $65.33^{c} \pm 2.30$ |
| N | *Diaporthe* sp. | $76.79^{b} \pm 0.55$ |

Means with the same letter are significantly different at $p<0.05$

## DISCUSSION

Over the years, endophytic microorganisms, mainly bacteria and fungi, have been exploited for their bioactive compounds which have enormous prospects in agricultural applications such as biological control of plant pathogens and plant growth improvement as well as in the biotechnological and pharmaceutical industries. Many studies have been displaying successful data on their antagonism capabilities against phytopathogens and phytophagous insects.

In total, 111 fungal endophytes were isolated from leaves, branches, and fruits of *G. atroviridis*. The surface sterilization method used in this study was effective as no microbial growth was observed on the control culture plates. Thus, any isolates obtained from the plant material were considered endophytes, as stated by Yu *et al*. (2018). The colonization frequency of endophytic fungi isolated was varied in different plant parts, with the highest frequency in branch (86%) and the lowest frequency in fruit (44.4%). Leaf recorded 72.2% of the colonization frequency of endophytic fungi. Successful endophytic colonization is dependent on many factors including plant tissue type, plant genotype, microbial taxon, and strain type (Miguel *et al*., 2017). This frequency was measured to contrast the degrees of fungal endophyte infection between various plant tissues (Sun *et al*., 2008). The result was aligned with the study done by Phongpaichit *et al*. (2006) in a similar plant. On the contrary, the colonization frequency of endophytic fungi isolated from *Camellia oleifera* was found to be highest in leaves compared to other plant parts (Yu *et al*., 2018). According to Shankar Naik *et al*. (2014), the variances of microbial endophyte colonization may be attributed to previous microhabitat, environmental stress, host senescence, endophyte virulence, and host defense responses. This particular dispersion has been viewed as a technique for reducing severe rivalry among endosymbionts and protecting the plant host from an overpopulation of microbial endophytes (Gimenez *et al*., 2007).

The isolates were then grouped based on morphological characteristics which allowed the identification of 14 different macromorphological colonies. From that, the isolated endophytic fungi were identified as *Bjekandera adusta, Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Nigospora sphaerica, Pestaliotiopsis neglecta, Colletotrichum sp*., *Diaporthe sp*. and *Pestalioptiosis sp.* Some of

the endophytic fungi isolated in this study (*Colletotrichum sp*., *Diaporthe sp*., *Pestatiolopsis sp*.,) are well-known as a plant pathogen. It is known that there are different ranges of endophyte-plant host relationships such as mutualism, neutralism, and antagonism. Also, as mentioned by Gashgari *et al*. (2016), an endophyte in one plant can be a pathogen in another host. The lifestyle of an endophyte can be shifted to a latent pathogen on account of environmental stress factors, genotype of host and microbial endophytes, also, nutrition exchange disequilibrium between the endophyte and the plant host (Khare *et al*., 2018; Mengistu, 2020). The fungal endophytes recovered in this study have been claimed earlier as endophytes from a diverse host plant such as *Cynometra travancorica* (Pillai & Jayaraj, 2015), *Bruguiera gymnorrhiza* (Liu *et al*., 2010) and *Hydnocarpus anthelminthicus* (Mandavid *et al*., 2015). According to Choudhary *et al*. (2021), *B. adusta* is an endophyte that can produce a novel compound of biocatalytic namely huperzine. Meanwhile, the ethyl acetate extract of *L. theobromae*, an endophyte of *Psidium guajava* has antimicrobial properties (Ujamp *et al*., 2020). Similarly, the extract of *N. sphaerica* shows bacteriocidal activity against MRSA and Klebsiella pneumoniae cells (Ibrahim *et al*., 2015). Six samples did not show any relevant fungal identification by ITS sequencing and were therefore assessed negative.

Potential antagonism capabilities of endophytic fungi to suppress or stop the growth of plant pathogen, *C. gloeosporioides* were tested using a dual culture method for two weeks for consistency purposes. Antagonism in the phytopathology context refers to the action of any organism, normally bacteria or fungi, suppressing or interfering with the normal growth and activity of a phytopathogen. These organisms are referred to as biocontrol agents (BCAs) that can be used in plant disease management. According to Rajani *et al*. (2020), this action was manifested in plate assays through several mechanisms of inhibition such as mycoparasitism, space competition, or antibiosis, which incorporates the production of secondary metabolites and volatile organic compounds (VOC). This statement is also can be supported by Scott (2016) and Hamzah *et al*. (2018). Most of the endophytic fungi isolated in this study were able to negatively affect the growth of the pathogen and showed different levels of antagonistic activity. Most of the endophytic fungi tested in this study showed the most frequent mechanism in antagonistic action against phytopathogen, which was competition. In this strategy, there is no inhibition zone formed as the mycelial growth of both endophyte and pathogen was halted before coming into physical contact. Endophytic fungi isolated in the study of Rosli *et al*. (2020) showed a similar antagonistic strategy in depleting the *F. verticillioides* mycelial growth. Endophyte isolate*, Choiromyces aboriginum* in Cao *et al*. (2009) also shows a similar mechanism, which is mycoparasitism, as isolate Type J (*B. adusta*) in inhibiting the mycelial growth of *R.solani*. Meanwhile, the isolates of Type D (unidentified fungus) and Type I (*P. neglecta*) were said to employ an antibiosis mechanism, in line with the mechanism used by *Penicillium* sp. in a study by Zuhria *et al*. (2016). This method was remarked by the clear zone formation between the mycelial mass of both endophyte and pathogen. Interestingly, there was a change in pathogen mycelium color from grey to light brown and red when interacting with the isolates from Type A (unidentified fungus) and Type D (unidentified fungus) respectively. According to Hamzah *et al*. (2018) and Almeida *et al*. (2020), the changes in color and production of pigment may have been formed as a defensive strategy by the fungus to keep their mycelia from being degraded by the enzyme produced by other microorganisms. Unfortunately, unidentified endophyte isolates from Type C and Type G were unable to inhibit the growth of *C.gloeosporioides.*

## CONCLUSION

A total of 111 fungal endophytes were isolated from *G.atroviridis*, which were grouped based on their morphology and colonies characteristic of 14 different types. These isolates have been identified as *Bjekandera adusta, Colletotrichum sp*., *Colletotrichum gloeosporioides,Diaporthe sp*., *Lasiodiplodia theobromae, Nigospora sphaerica Pestalotiopsis sp*., *Pestaliotiopsis neglecta* and some of the type were unsuccessfully to be identified. Most of the isolated endophytes have the capability of inhibiting the mycelial growth of the *C.gloeosporioides* pathogen, where *Nigospora sphaerica* (EL 41), an isolate of Type K demonstrated the highest PIRG of 87.94%. Meanwhile, various interactions demonstrated between the endophyte and pathogen should be explored more for the production of natural bioactive compounds which will help in plant disease management and the conservation of medicinal plants.

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## ETHICAL STATEMENT

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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