ISOLATION OF ENDOPHYTIC FUNGI FROM LEAVES OF *MURRAYA KOENIGII* (L.) SPRENGEL

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Abstract

The isolation of endophytic fungi from leaves of *Murraya koenigii* (L.) Sprengel has been undertaken. The specimens were collected from University of Mandalay Campus. The research work as conducted at Microbiology Laboratory of Botany Department, University of Mandalay December 2018 to June 2019. Potato Dextrose Agar (PDA) medium was used for the isolation of endophytic fungi. Six endophytic fungi were isolated from leaves, such as TTO 1, TTO 2, TTO 3, TTO 4, TTO 5 and TTO 6. The endophytic fungi were *Aspergillus* sp (TTO 1), *Aspergillus* sp (TTO 2), *Colletotrichum* sp (TTO 3), *Colletotrichum* sp (TTO 4), *Nigrospora* sp (TTO 5), *Paecilomyces* (TTO 6). The morphological, macroscopical and microscopical characters of *Murraya koenigii* (L.) Sprengel were described, recorded and stated with photomicrograph.

Keywords : isolation, endophytic fungi, *Murraya koenigii*

Introduction

Murraya koenigii (L.) Sprengel commonly known as curry leaf is a shrub or a small tree under the family Rutaceae, growing up to 6 m height. The plant is native to India, Pakistan, Sri Lanka, Bangladesh and the Andaman Islands. It is also cultivated widely in South-East Asia and some parts of the United States and Australia. *Murraya koenigii* (L.) Sprengel is the most popular on account of its diverse medicinal properties and its use as a flavouring agent in different curries and foods since ancient times. The phytoconstituents present in *Murraya koenigii* (L.) Sprengel leaves include phenols, steroids, saponins, quinones, alkaloids, flavonoids, tannins, carbohydrates, proteins and volatile oils. (Tripathi *et al*. 2018).

Murraya koenigii (L.) Sprengel has a lot of bioactive principles due to which it has been proven as the medicinally important plant but least or no attention was received by the scientist. *Murraya koenigii* (L.) Sprengel is proven as the natural medicinal plant. There are different forms of *Murraya koenigii* (L.) Sprengel due to which they are found as the useful plant such as extract, essential oil, or directly used due to the presence of active constituent (Manshu *et al.* 2017).

Endophytes are microorganisms that reside the internal tissues of living plants without causing any symptoms and obvious harm to the host plants. Endophytes are ubiquitously found in all plants and are valued for their ability to synthesize various useful bioactive compounds. These bioactive compounds were originally involved in defense mechanisms against phytopathogens. However, in the recent years, endophytic bioactive compounds were gradually integrated in novel drug discoveries due to their wide variety of biological activities as antibiotic, anticancer, antioxidant, and anti-inflammatory agents (Chow & Ting 2014).

The production of bioactive compounds which shows antimicrobial activity against different enzyme like amylase, cellulose, protease, lipase, and lactase (Jingfeng *et al*. 2013). The bioactive substances in plants are produced as secondary metabolites (Selvi & Balagengatharathilagam 2014).

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The aim and objective of this study are to isolate and identify the endophytic fungi from the leaves of *Murraya koenigii* (L.) Spergel and to know the macroscopical and microscopical characters of isolated endophyitc fungi.

Materials and Methods

The specimens were collected from Mandalay University Campus. The plant collections were carried out from December 2018 to June 2019. Fungal strains were isolated from healthy leaves of *Murraya koenigii* (L.) Sprengel. The samples were recorded by photograph. Plant identification was done by description of Kress *et al*. (2003).

The potato dextrose agar (PDA) medium was prepared with PDA (3.9 g), distilled water (100 ml) and pH (6.5). After autoclaving chloramphenicol (0.1) g was added to the medium.

The isolation of endopytic fungi was done by the methods of Hallmann *et al.* (2007). The leaves were rinsed gently in running water to adhere dust and were cut into 1 cm segments. Each sample was surface sterilized with 70 % ethyl alcohol for 1 minute, with sodium hypochlorite for 3 minutes, rinsed with 70% ethyl alcohol for 1 minute and then rinsed with distilled water. Samples were dried on sterile filter paper by drying under laminar airflow chamber. The samples were inoculated into potato dextrose agar (PDA) plates and incubated at room temperature for 3-10 days. Hyphal tips growing out of the plated plant samples were transferred into a PDA medium to get pure colony. Potato Dextrose Agar (PDA) medium was used for examining the macroscopical and microscopical characters.

By using a sterile inoculating needle, small portions of each mycelium colony were aseptically taken and placed on clean microscope slides and loop in a drop of distilled water. The conidia shape and size arrangement of spores on the sporangiophores were noted under a light microscope. The morphological and microscopical characters were observed by the method of Barnett and Hunter (2000) and Dube (1990). The fungal spores were measured according to the method of Kokate (2000).

Results

In the present study, morphological characters of *Murraya koenigii* (L.) Sprengel were described. The six endophytic fungal strains were isolated from leaves of this plant. The macroscopical and microscopical characters were presented as shown in Table.

1. Morphological Characters of *Murraya koenigii* (L.) Sprengel

Murraya koenigii (L.) Sprengel, Syst. veg 2:315.1817

Family : Rutaceae

Myanmar name : Pyin daw thein

Perennial shrubs up to 1.0 m high. Leaves unipinnately compound, impripinnate, alternate, exstipulate, petiolate; leaflets, alternate, ovate, obtuse and oblique at the base, crenulate along the margin, acute at the apex, pellucid-dotted. Inflorescences terminal, paniculate cymes, many-flowered. Flower bisexual, actionomorphic, pentamerous, hypogynous, white, fragrant, pedicelate. Petals 5, free, oblong to oblanceolate, recurved, glabrous. Stamens 10 in two whorls, free; filaments dilate at the base; anthers dithecous-basifixed. Carpels 2, fused; ovary superior, oblongoid, bilocular with one ovule in each locule on the axile placentae; styles stout; stigmas capitate. Fruits baccate, ovoid, bluish black, 1-seeded.

2. Macroscopical and Microscopical Characters isolated from leaf of *Murraya koenigii* (L.) Sprengel

Leaves (Figure 1 A) and pieces of leaves (Figure 1 B) were cultured on PDA medium.

Table 1

Isolated strains	Endophytic fungi	Macroscopical characters		Microscopical characters
		Surface view	Reverse view	
TTO 1	*Aspergillus* sp.	Black in central, white in peripheral (Fig. 2 A and B)	White	Hyphae septate, conidia are emerged, unicellular, spherical, conidiophores upright, simple (Fig. 2 C and G)
TTO 2	*Aspergillus* sp.	White turns to yellowish green (Fig. 2 D and E)	Pale yellow	Hyphae septate, conidia unicellular, spherical, conidiophores upright, smooth (Fig. 2 F and H).
TTO 3	*Colletotrichum* sp.	White (Fig. 3 A and B)	Creamy	Hyphae septate, conidia unicellular, ovoid and smooth wall (Fig. 3 C and G).
TTO 4	*Colletotrichum* sp.	Pink in central, white in peripheral (Fig. 3 D and E)	Pale pink	Hyphae septate, conidia unicellular, hyaline and smooth wall (Fig. 3 F and H)
TTO 5	*Nigrospora* sp.	White turns to black (Fig. 4 A and B)	White, become black	Hyphae septate, conidia unicellular, globose, conidiaphores simple (Fig. 4 C and G)
TTO 6	*Paecilomyces* sp.	White turns to gray (Fig. 4 D and E)	Peripheral in white, dark green in central	Hyphae septate, conidia unicellular, hyaline, conidiophores branches more divergent than *Penipcillum* (Fig. 4 F and H).

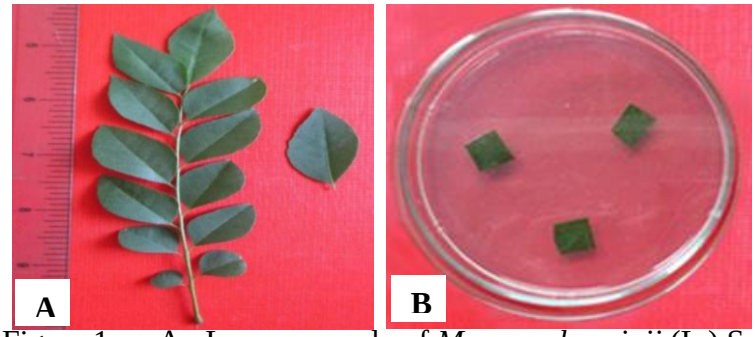

Figure 1 A. Leaves sample of *Murraya koenigii* (L.) Sprengel
B. Pieces of leaves culture on PDA medium

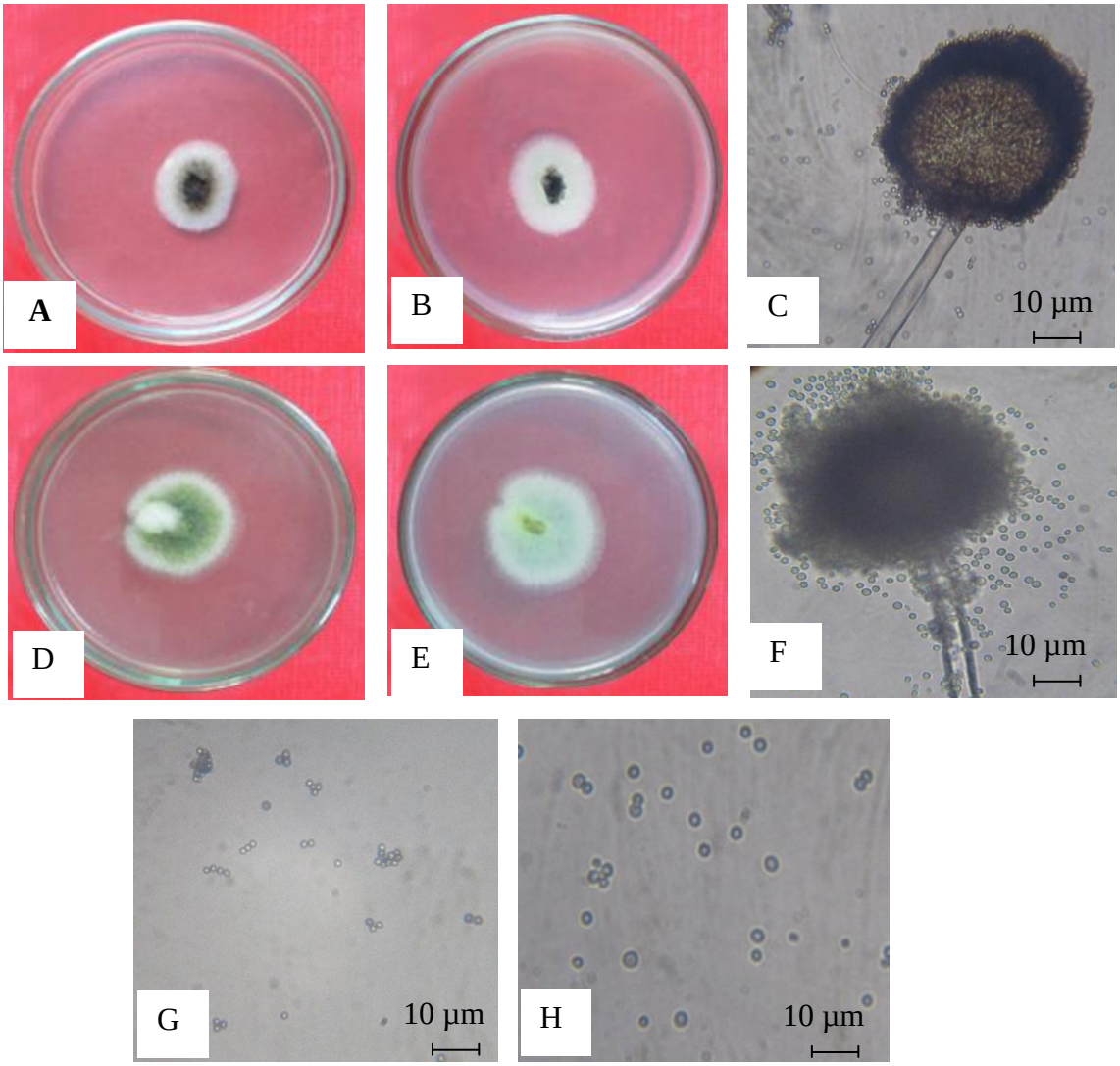

Figure 2 A. Surface colony characters of TTO 1 (2 days)
B. Reverse colony characters of TTO 1 (2 days)
C. Photomicrograph of conidial head of *Aspergillus* sp.(TTO 1)
D. Surface colony characters of TTO2 (2 days)
E. Reverse colony characters of TTO2 (2 days)
F. Photomicrograph of conidial head of *Aspergillus* sp. (TTO 2)
G. Photomicrograph of conidia of *Aspergillus* sp. (TTO 1)
H. Photomicrograph of conidia of *Aspergillus* sp. (TTO 2)

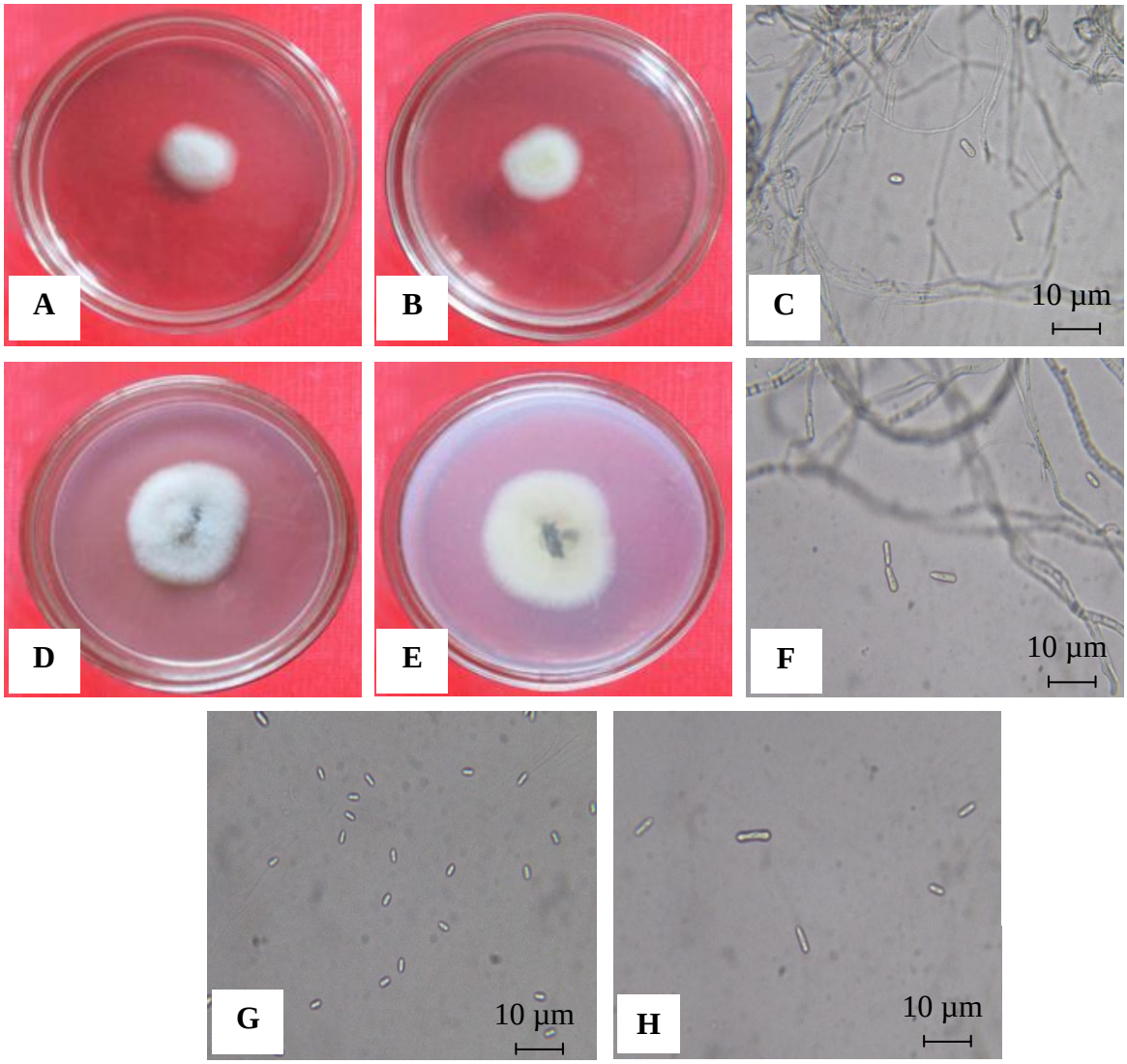

Figure 3 A. Surface colony characters of TTO 3 (3 days)
B. Reverse colony characters of TTO 3 (3 days)
C. Photomicrograph of hyphae of *Colletotrichum* sp. (TTO 3) with conidia
D. Surface colony characters of TTO 4 (3 days)
E. Reverse colony characters of TTO 4 (3 days)
F. Photomicrograph of hyphae of *Colletotrichum* sp. (TTO 4) with conidia
G. Photomicrograph of conidia of *Colletotrichum* sp. (TTO 3)
H. Photomicrograph of conidia of *Colletotrichum* sp. (TTO 4)

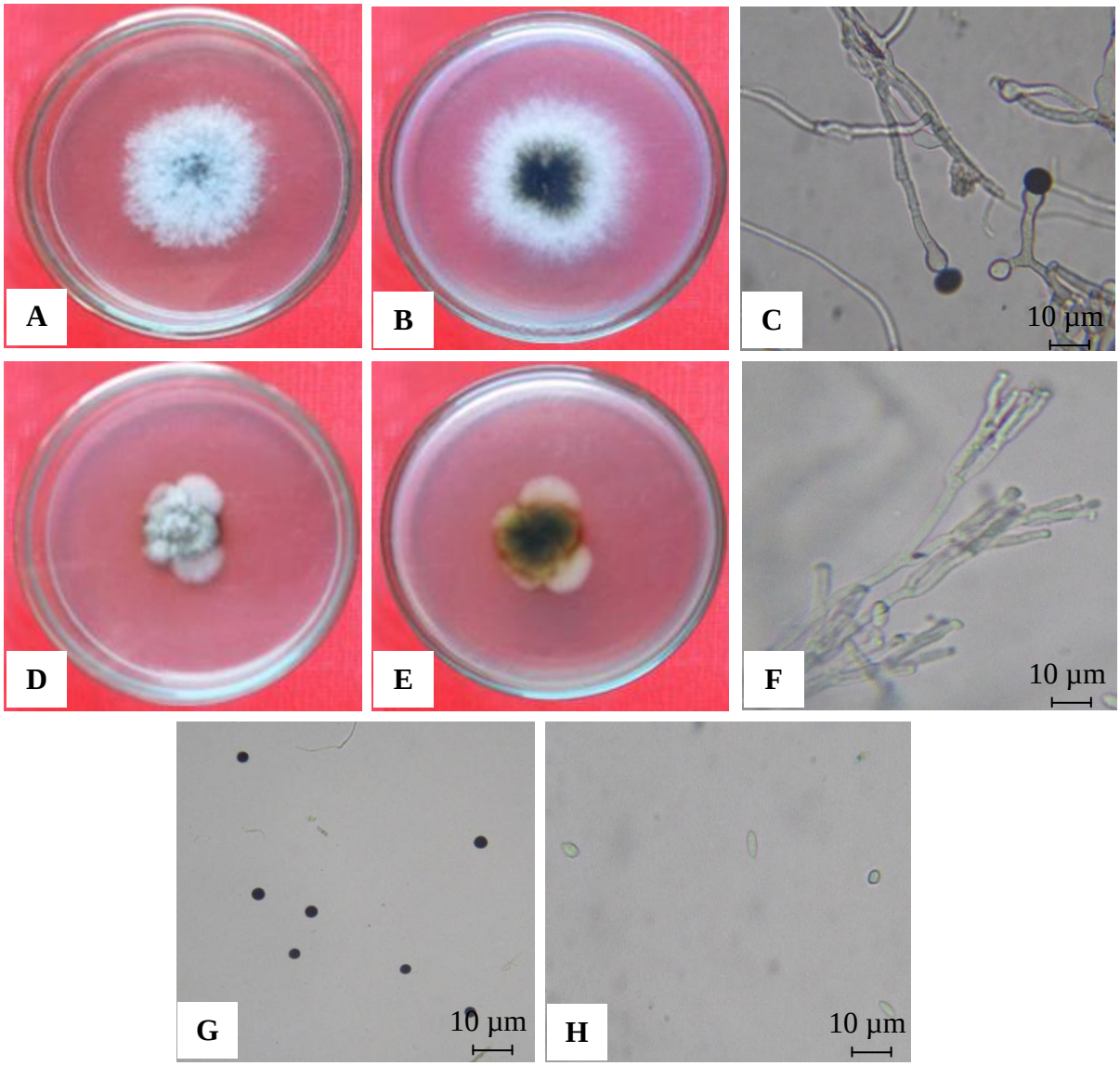

Figure 4 A. Surface colony characters of TTO 5
B. Reverse colony characters of TTO 5
C. Photomicrograph of hyphae of *Nigrospora* sp. (TTO 5) with conidia
D. Surface colony characters of TTO 6
E. Reverse colony characters of TTO 6
F. Photomicrograph of hyphae of *Paecilomyces* sp. (TTO 6) with conidia
G. Photomicrograph of conidia of *Nigrospora* sp. (TTO 5)
H. Photomicrograph of conidia of *Paecilomyces* sp. (TTO 6)

Discussion and Conclusion

The endophytic fungi were isolated from leaves of *Murraya koenigii* (L.) Sprengel. Direct isolation method was used for the isolation of endophytic fungi: the two *Aspergillus* spp., the two *Colletotrichum* spp., *Nigrospora* sp. and *Paecilomyces* sp. were isolated from leaves of *Murraya koenigii* (L.) Sprengel.

The macroscopical characters of TTO 1 fungal colony was black in central, white colour in peripheral. Reverse colony was white colour on potato dextrose agar (PDA) medium. TTO 2 fungal colony was white turns to yellowish green. Reverse colony was pale yellow colour on potato dextrose medium. The microscopical

characters of TTO 1 and TTO 2 fungal strains were septate, conidiophores upright, simple, globose, clavate swelling, bearing phialides at the apex and smooth. These characters were in agreement with those of Barnett & Hunter (2000), who mentioned that the genus of *Aspergillus* spp. were septate. Conidia were unicellular, globose, often variously colour in mass, in dry basipetal chains. Therefore, TTO 1 and TTO 2 fungal strains were confirmed as *Aspergillus* spp.

The macroscopical characters of TTO 3 fungal colony was white in colour. Reverse colony was creamy on potato dextrose agar (PDA) medium. The macroscopical characters of TTO 4 fungal colony were pink in central, white colour in peripheral. Reverse colony was pale pink colour on potato dextrose agar (PDA) medium. The microscopical characters of TTO 3 and TTO 4 fungal strains were septate, conidiophores simple, elongate, conidia hyaline, unicellular and ovoid. These characters were agreement with those of Smith and Spiers (1982) and Barnett and Hunter (2000). Smith and Spiers (1982) stated that the shape of conidia was cylindrical, sides straight with conidia rounded on both ends. Barnett & Hunter (2000), mentioned that the conidia of *Colletotrichum* spp. were unicellular, ovoid and hyaline. Therefore, TTO 3 and TTO 4 fungal strains were confirmed as *Colletotrichum* spp.

The macroscopical characters of TTO 5 fungal colony was white initially and turned to black. Reverse colony was white and become black on potato dextrose agar (PDA) medium. The microscopical characters of TTO 5 fungal strain were septate, conidiophores short, mostly simple, conidia shiny black, unicellular, globose, hyaline vesicle at the end of the conidiophores. These characters were agreement with those of Dube (1990) and Barnett and Hunter (2000). Dube (1990) stated that conidia were flatted or slightly bulged apically. Barnett & Hunter (2000) mentioned that the conidiophores of *Nigrospora* sp. were typically small, forming a single conidium at the tip, the black, unicellular, globular. Therefore, TTO 5 fungal strain was confirmed as *Nigrospora* sp.

The macroscopical characters of TTO 6 fungal colony was white turned to gray. Reverse colony was white in peripheral, dark green in central on potato dextrose agar (PDA) medium. The microscopical characters of TTO 6 fungal strain were septate, conidiophores tall, slender, hyaline, and simple branched. Conidia were unicellular, dark, in dry basipetal chains and lemon-shaped. These characters were agreement with those of Barnett and Hunter (2000) and Nguyen (2006). Barnett & Hunter (2000) mentioned that the conidia of *Paecilomyces* sp. were hyaline, smooth wall, divergent, and basipetal chains. Nguyen (2006) stated that the branched conidiophores were club-shaped, phialide and conidial chain. Therefore, TTO 6 fungal strain was confirmed as *Paecilomyces* sp.

The present studies were in agreement with those of Sharma (2014), Hande and Suradkar (2015) and Rangari *et al*. (2017). Sharma (2014) stated that the endophytic fungal strains of *Aspergillus stelatus* were isolated from *Murraya koenigii* (L.) Sprengel. Hande and Suradkar (2015) also observed *Aspergillus* sp. isolated from different parts of *Murraya koenigii* (L.) Sprengel. Rangari *et al*. (2017) showed that endophytic fungi were isolated from leaves of *Murraya koenigii* (L.) Sprengel and its fungal strains were *Aspergillus flavus*.

It can be concluded that six endophytic fungi are isolated and identified based on the morphological characters, conidiospore structures of the fungal at culture from leaves of *Murraya koenigii* (L.) Sprengel. Endophytes in host plants can stimulate plant growth, increase disease resistance, and improve plant's ability to withstand environmental stresses and recycle nutrient. Hence, this study may facilitate the new

product discovery by using the isolated endophytic fungi from leaves of *Murraya koenigii* (L.) Sprengel.

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