

Growth Effects of Endophytic Fungi Isolated from Flower of *Catharanthus roseus* (L.) G. Don on Different Media

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Abstract

Five types of fungi were carried out from flower of *Catharanthus roseus* (L.) G. Don. The plant specimen of *C. roseus* was collected from Hlaing Township, Yangon Region, January 2018. The direct inoculation method was used to isolate different strains of fungi on Potato glucose agar medium and these were incubated at room temperature. These strains of fungi were designated as F1 from corolla lobe, F2 from corolla lobe, F3 from corolla tube, F4 from anther and F5 from calyx. According to their morphological and microscopic characters, these fungal isolate F2 was also assumed to be genus *Mucor* and F1, F3, F4 and F5 were assumed to be *Aspergillus*. All fungal isolates were cultured on four different medium such as Czapek dox agar (CZA), Potato sucrose agar (PSA), Sucrose yeast agar (SYA) and Sabouraud's dextrose agar (SDA) respectively and their observed color and reverse color were recorded. These isolated fungal strains did not grow well on Sabouraud's dextrose agar medium. This research was conducted in the Microbiology Laboratory of Botany Department, Yangon University.

Keywords : *Catharanthus roseus*, fungi, media, *Mucor*, *Aspergillus*, incubate, isolate

Introduction

Endophytes are microorganisms that inhabit in living tissues of various plants. Endophytes are mainly colonize vegetative parts but are also found in reproductive organs. These endophytes are organisms that colonize internal plant tissues without causing apparent harm to their host. Fungi, bacteria, actinomycetes and mycoplasma are groups of microorganisms and these are reported as endophytes of plants (Arnold and Lutzoni, 2007). Endophytes protect plants against herbivores, insect attacks or tissue invading pathogens and they are mutualistic and commensalis relationship with its host (Marcellano *et al.*, 2017). Filamentous fungi are organisms that can break down organic matter, releasing phosphorus, oxygen, nitrogen and carbon into the atmosphere and the soil (Svahn, 2015).

Catharanthus roseus (L.) G. Don is important to explore endophytic microflora in the medicinal plant. It is belongs to the family Apocynaceae. It is commonly known as the Madagascar periwinkle while it is also known as Thinbawma-hyno-pan. Two common cultivars of *Catharanthus* which is named on the basis of their flower color that is the pink flowered "Rosea" and the white flowers "Alba" Monika (2013). The effective endophytic fungi were screened from flower of *Catharanthus roseus* (L.) G. Don that was grown on Potato glucose agar medium by direct plate method (Atlas, 1993). These isolated fungi were cultured on four media such as Czapek dox agar (CZA), Potato sucrose agar (PSA), Potato glucose agar (PGA), Sucrose yeast agar (SYA), Sabouraud's dextrose agar (SDA), respectively. The aim of this research is to isolate and identify the endophytic fungi from flower of *Catharanthus roseus* (L.) G. Don and to find out their potential on different media.

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Materials and Methods

Collection, Identification and surface sterilization of plant sample (Arnold *et al.*, 2007)

The flower specimens were collected in early January of 2018 from Hlaing Township, Yangon Region. This plant was identified as *Catharanthus roseus* according to Dassanayake and Fosberg (1981). So, this plant was identified into *Catharanthus roseus* (L.) G. Don by using available literature in the Department of Botany. The flower samples were surface sterilized with 70% ethanol for 1 minute and washed with sterilized water for several times. These samples were then sterilized with 2% sodium hypochlorite for 3 minutes and washed again with sterilized water. These were cut into small pieces and dried on filter paper.

Isolation of endophytic fungi from flower of *Catharanthus roseus* (Atlas,1993)

Endophytic fungi were isolated from flower of *Catharanthus roseus* (corolla lobe F1, corolla lobe F2, corolla tube F3, anther F4 and calyx F5). Each 0.5 cm of sterilized segments were directly cultured on Potato glucose agar medium. Single colonies were picked up and subcultured on this medium respectively. Morphological characters of isolated fungi were visually checked and microscopic characters of these fungi were examined under microscope.

Potato Glucose Agar (Atlas, 1993)

Peeled potato	200 g
Glucose	20 g
Agar	20 g
Distilled water	1000 ml
pH	5.6

Effect of isolated fungi F1, F2, F3, F4 and F5 on different medium

The fungal culture were grown in different media namely Czapek dox agar (CZA), Potato sucrose agar (PSA), Potato glucose agar (PGA), Sucrose yeast agar (SYA), Sabouraud's dextrose agar (SDA), respectively. Their observed and reversed colour and sizes of colonies were examined and recorded.

Composition per Liter

Potato Sucrose Agar Medium (Atlas, 1993)

Peeled potato	200.0 g
Sucrose	20.0 g
Agar	20.0 g
pH	5.6

Sucrose Yeast Agar Medium (Strobel and Sullivan, 1999)

Sucrose	10.00 g
Yeast extract	3.00 g
NaCl	0.50 g
CaCO ₃	0.10 g
Agar	20.00 g
pH	6.8

Sabouraud's Dextrose Agar Medium (Larone, 1995)

Agar	15.00 g
Dextrose	40.00 g
pH	5.6

Czapek Dox Agar Medium (Arthur Wayland Dox, 1910)

Sucrose	30.00 g
Sodium Nitrate	2.00 g
Dipotassium phosphate	1.00 g
Magnesium sulfate	0.50 g
Potassium chloride	0.50 g
Ferrous sulfate	0.01 g
Agar	20.00 g
pH	7.3

Results**The outstanding characters of *Catharanthus roseus* (L.) G. Don**

ScientificName	: <i>Catharanthus roseus</i> (L.) G. Don
Family Name	: Apocynaceae
Elglish Name	: Cape periwinkle, Rose periwinkle, Rosy periwinkle and "Old-Maid".
Myanmar Name	: Thinbaw-mahnyo

Perennial shrubs. Leaves opposite and decussate, simple, laminae oblong, margin entire, apex obtuse, base cuneate both surfaces pubescent, petiolate, exstipulate. Inflorescences terminal and axillarycymose. Flowers bracteate, ebrateolate, pedicellate bisexual complete, pink; sepal 5, aposepalous, green; petals 5, sympetalous, corolla lobe (salver shape), pink with a long corolla tube, the throat of the corolla tube hairy. Stamen 5, epipetalous, anther sagittate, ditheous, dorxifixed. Ovary, bicarpellary, stigma capitate, style long, filiform, marginal placentation, superior. Fruits follicle. Seeds small, numerous. The flowering and fruiting period throughout the years in Figure 1.



a. Habit



b. Inflorescence

Figure 1. *Catharanthus roseus* (L.) G. Don

Isolation of endophytic fungi

Endophytic fungi were isolated from flower of *Catharanthus roseus* (corolla lobe F1, corolla lobe F2, corolla tube F3, anther F4 and calyx F5). A total of five strains of fungi were isolated from flowers of *Catharanthus roseus* (L.) G. Don that grown on Potato glucose agar medium (PGA) during 7 days by direct inoculation method. Their culturing periods of fungal isolates were significantly different. These isolates were denoted as F1 (corolla lobe), F2 (corolla lobe), F3(corolla tube), F4 (anther) and F5(calyx), respectively. The colony of fungal isolates F1, F2 and F3 were grown on PGA medium during 3 days but isolate F4 and F5 were well grown during 7 days. The individual colonies of filamentous fungi were picked up and kept on PGA medium to get pure colony.

Morphological characters of isolated fungi

Their morphological and microscopic properties of fungal isolates showed differences in colony observe and reverse color. The observe color of colonies on PGA of isolate F1 and F5 were white margin and center black, F2 was white and F3 was cottony white and F4 was margin pale yellow and center black. The reverse color of fungal isolates had F1, F3 and F5 were white, pale orange in F2 and yellow in F4 respectively. The sizes of colony on PGA medium were all a like (30x30 mm) except F3 (40x40 mm). The colony characters and microscopic characters of isolated fungi from flower of *Catharanthus roseus* (L.) G. Don shown in Figure 2 and Table 1.

Microscopic character of fungal isolates F1, F2, F3, F4 and F5

Fungal isolate F1

Hyphae were septate, hyaline and conidiophore were long, erect, and conidia head round. Conidia were 1- celled and round in Figure 3.

Fungal isolate F2

Hyphae were septate, hyaline. Sporangioophores were hyaline, short and often bunched and bear terminal round spore-filled sporangia, brown. The sporangial walls were dissolved. Spores were round or oblong, numerous spores present in sporangia in Figure 4.

Fungal isolate F3

Hyphae were septate, hyaline and conidiophores were long, erect, globose and dark. Vesicle was globose. Phialide were biserial, covering the entire surface of the vesicle. Conidia were 1- celled, chain, mutulae present, round, black, wall with spine in Figure 5.

Fungal isolate F4

Hyphae were septate and hyaline. Sporangioophores were long, erect and sporangium head globose, round and radiate. The spores were round in Figure 6.

Fungal isolate F5

Hyphae were septate, hyaline and conidiophore were long, erect, and conidia head round. Conidia were 1- celled and round in Figure 7.

Isolated fungi F2 from flower of *Catharanthus roseus* was assumed to be the genus *Mucor* sp. and F3 was also to be the genus *Aspergillus* sp. based on their morphological and microscopic characters.

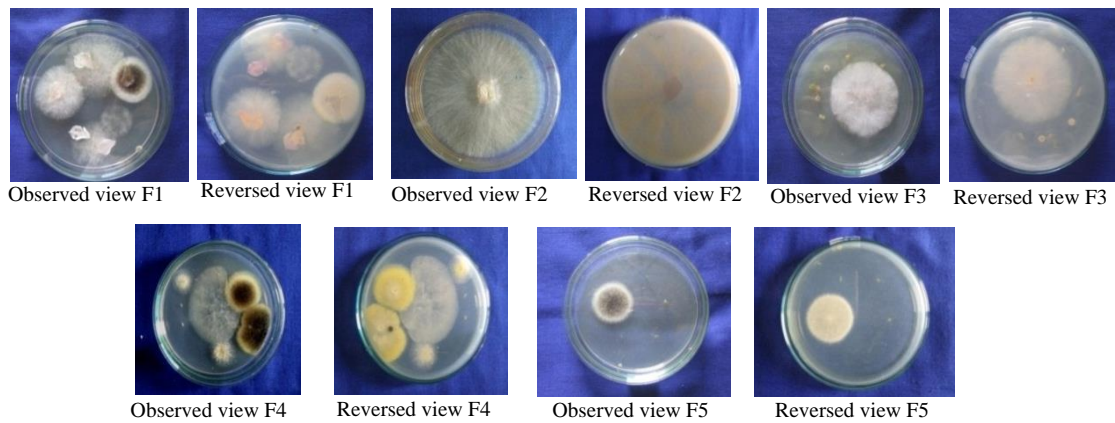


Figure 2. Morphological characters of isolated fungi F1, F2, F3, F4 and F5 from flower of *Catharanthus roseus*(L.) G. Don grown on Potato glucose agar medium

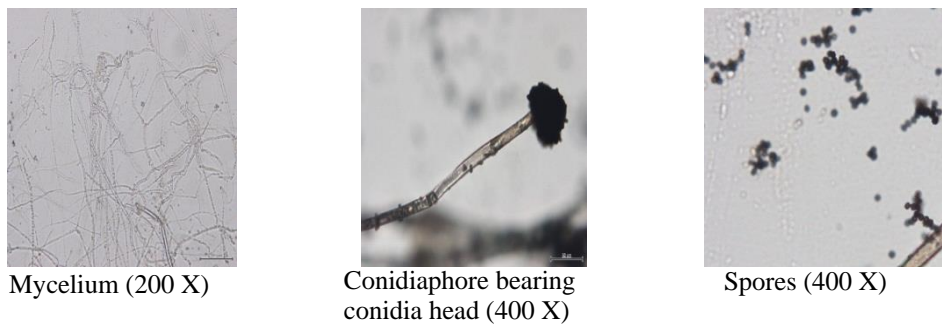


Figure 3. Microscopic character of fungal isolate F1

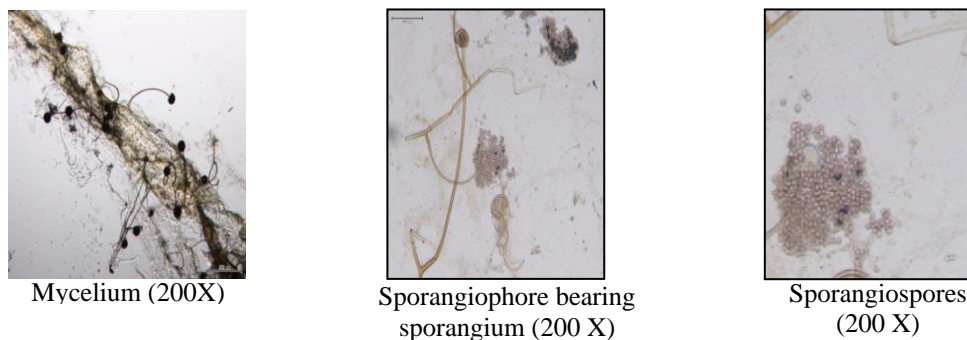


Figure 4. Microscopic character of fungal isolate F2

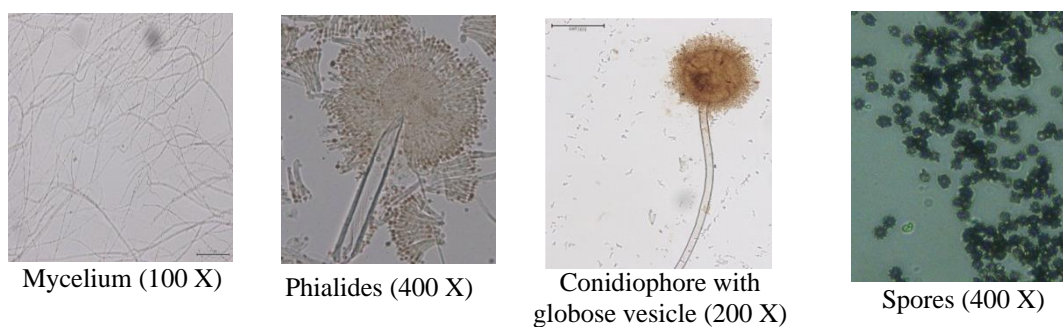


Figure 5. Microscopic character of fungal isolate F3



Figure 6. Microscopic character of fungal isolate F4

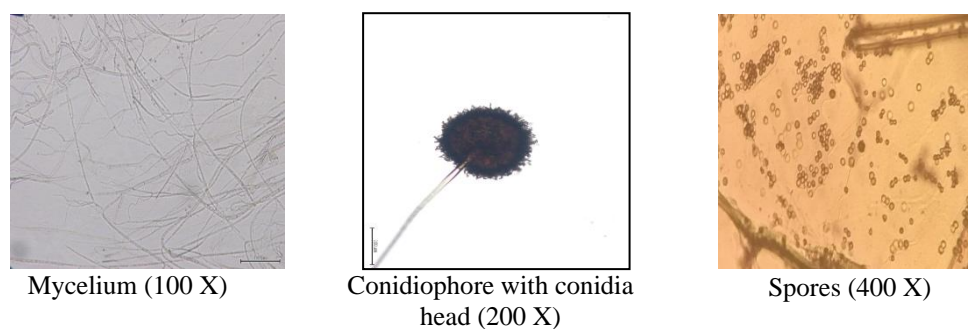


Figure 7. Microscopic character of fungal isolate F5

Table 1. Colony Characters of isolated fungi from flower of *Catharanthus roseus*(L.) G. Don grown on Czapeak Dox Agar, Potato Sucrose Agar, Sucrose Yeast Agar and Saboureaud's Agar media

Morphology	F1	F2	F3	F4	F5
Czapeak Dox Agar Observe view	Brown	White	White margin, Center black	Black	Pale brown, Margin white
Reverse view	Pale yellow	Pale orange	Pale yellow	White	Pale yellow
Colony size (mm)	9.4 × 9.4	30 × 30	13 × 11	12 × 10	40 × 45
Potato Sucrose Agar Observe view	Brown margin and Center black	White	Black	Black	Black
Reverse view	Pale yellow	Pale orange	Pale yellow	Pale yellow	Pale yellow
Colony size (mm)	18 × 18	20 × 20	11 × 12	16 × 20	14 × 12
Sucrose Yeast Agar Observe view	Black	Black	Black margin and Center brown	Black margin and Center brown	Black margin and Center brown
Reverse view	Pale yellow	White	Pale yellow	Pale yellow	Pale yellow
Colony size (mm)	8.0 × 8.0	13 × 12	1.8 × 1.8	7.8 × 6.8	4.2 × 14.2
Saboureaud's Dextrose Agar Observe view	Black	Pale yellow	Margin brown and Center black	Margin brown and Center black	Black
Reverse view	White	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Colony size (mm)	10 × 9.0	19 × 21	12.6 × 13	19 × 18	1.4 × 1.4

Effects of isolated fungi on different media

Fungal cultures isolates had different observed and reversed colony colours. These isolated fungi were cultured on different media such as Potato sucrose agar, Sucrose yeast agar, modified potato sucrose yeast agar and modified potato glucose yeast agar. These were varied in colour and colony sizes. The colony size on CZA medium, the largest size was obtained from F5 isolates (40x45 mm). On Sabouraud's dextrose agar medium, the largest size was observed in F2 (19x21 mm). On Sucrose yeast agar medium, F2 showed the largest size of colony (13x12 mm). As F2 showed the largest size of colony on SY agar medium (19x21 mm) in Figure 8 to 11.

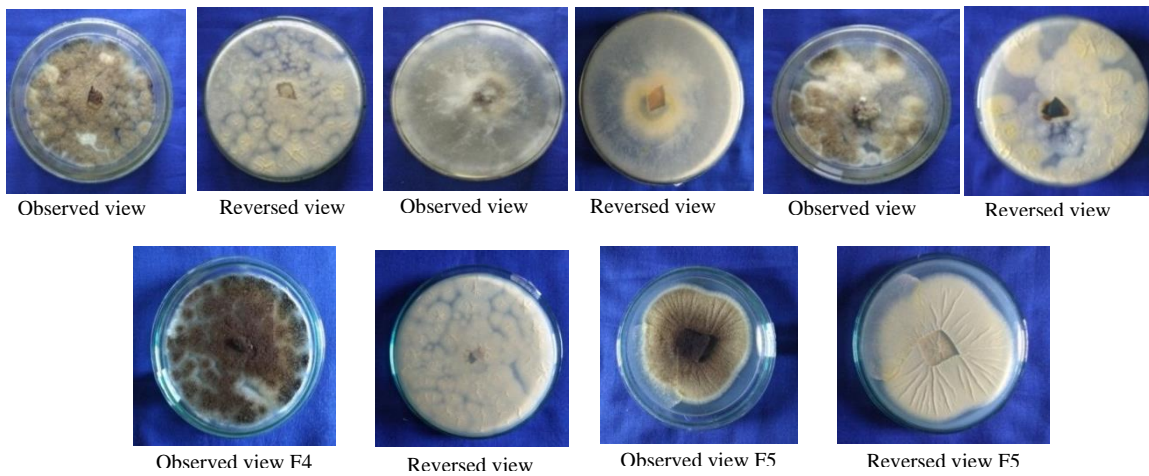


Figure 8. Morphological characters of fungal isolate F1, F2, F3, F4 and F5 from flower of *Catharanthus roseus* (L.) G. Don grown on Czapek dox agar medium

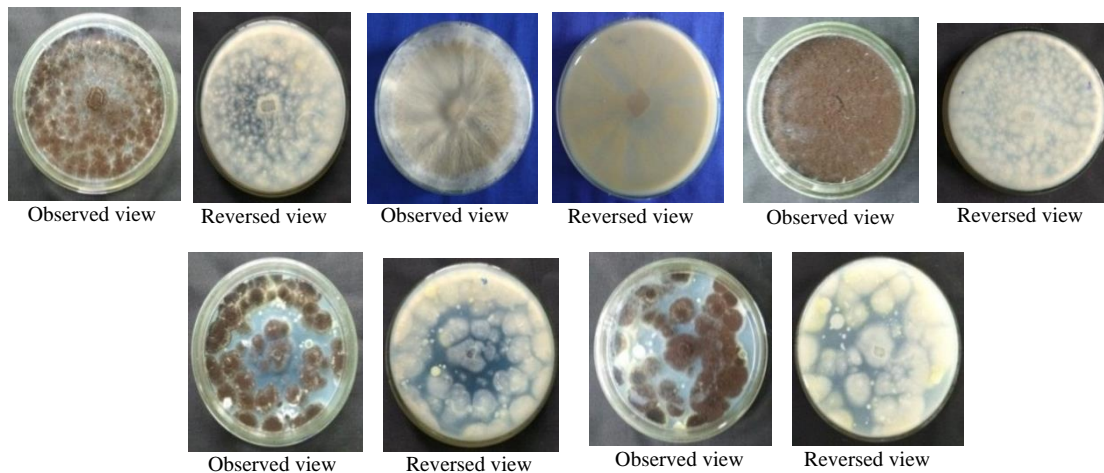


Figure 9. Morphological characters of fungal isolate F1, F2, F3, F4 and F5 from flower of *Catharanthus roseus*(L.) G. Don grown on Potato Sucrose agar medium.

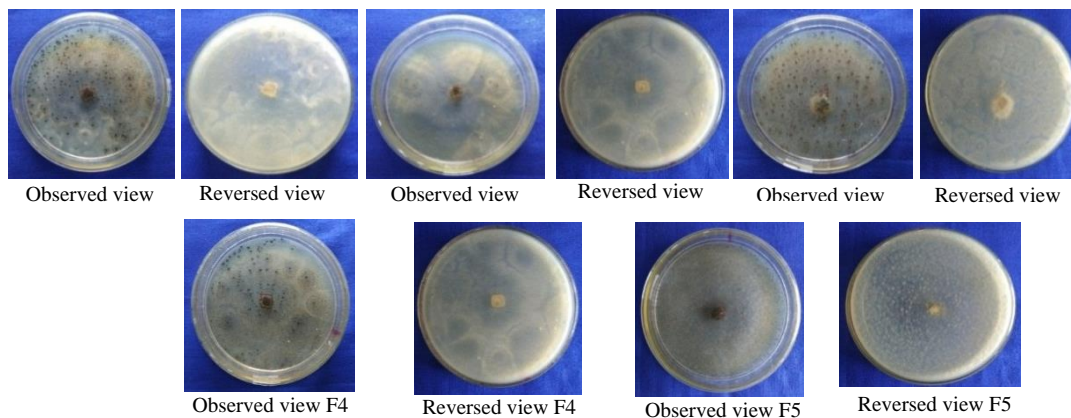


Figure 10. Morphological characters of fungal isolate F1, F2, F3, F4 and F5 from flower of *Catharanthus roseus*(L.) G. Don grown on Sabouraud's dextrose agar medium

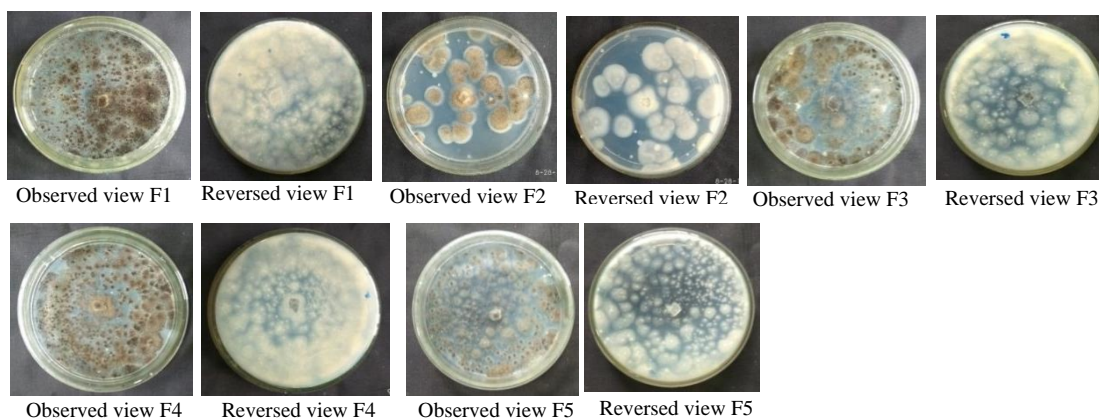


Figure 11. Morphological characters of fungal isolate F1, F2, F3, F4 and F5 from flower of *Catharanthus roseus*(L.) G. Don grown on Sucrose yeast agar medium

Discussion and Conclusion

The flower specimens of *Catharanthus roseus* (L.) G. Don was collected from Hlaing, Township, Yangon Region. It belongs to the family Apyocynaceae. Their morphological character of this plant was confirmed the scientific name. *Catharanthus roseus* is a perennial subshrub. The leaves are opposites and decussate, lamina oblong, entire margin, petioles short, terminal and axillary cymose, flower pink, calyx sepaloid, calyx tube short, corolla tube long, the throat of the corolla tube hairy were outstanding characters of this plant. So, this collected flower sample was agreed Dassanayake and Fosberg (1981) and Soe Myint Aye (2002). The habitat and inflorescence of plant sample were shown in Figure 1.

The five strains of fungi isolated from flower of *Catharanthus roseus* (L.) G. Don that was grown on Potato glucose agar medium. These isolated fungi were denoted as F1(corolla lobe), F2(corolla lobe), F3(corolla tube), F4(anther) and F5(calyx). Based on their morphological and microscopic characters, these isolated fungi F2 and F1, F3, F4 and F5 were identified into genus level.

Ramesha and Srinivas, (2014) discovered the colonies (20.83%) were obtained from flowers of *Plumeria obtusifolia* as (23.52%) was isolated from

Plumeria acuminata. Ayob and Simarani (2016) discovered that two strains of filamentous fungi were found out from violet color of *Catharanthus roseus* (leaf and root). Spore type and hyphae type of these fungi were hyphae septate and rounded shape of spores. Sreekanthet *al.* (2017) found that twenty-five endophytic fungi were isolated from different tissue of *C. roseus*. The 48% from leaves, 44% from stems and 4% from each of the roots and flowers were investigated.

The isolates F2 on PGA medium showed white in colony, the hyphal were septate, hyaline, sporangiophore erect, simple, branched, multispore present in sporangium, spore round and chlamydospore present. Kidd *et al.*, (2016) said that colony of *Mucor* were very fast growing white to yellow with the development of sporangia, sporangiophore hyaline, erect, simple, globose to spherical, multispore present in sporangia. As characters of fungal isolate F2 are in accordance with Kidd *et al.*, (2016).

Isolated fungal F1, F3, F4 and F5 were hyphae septate, hyaline conidiophore long erect, conidia head globose, radiate entire the surface, vesicle round, conidia round 1celled, chain, spine, mutulae present, phalides present. Barnett and Hunter (1979) observed that the conidiophore of *Aspergillus* sp. were cleavate swelling bearing phialides at the apex or radiating from the entire surface and conidia are one celled, globose often various color and mass and dry basipetal chains. Myint Myint Than (2001) stated that *Aspergillus* sp. isolated from soil were showed vesicles globose, conidia in large radiating heads. The conidia were mostly globose, echinulate. Prakash and Jha (2014) proposed vesicles are typical characters of genus *Aspergillus*. These characters of fungal isolate F1, F3, F4 and F5 are similar with previous authors Barnett and Hunter (1979), Myint Myint Than (2001), Prakash and Jha (2014).

Fungal cultures isolates had different observed and reversed colony colours. These isolated fungi were cultured on different media such as Potato sucrose agar, Sucrose yeast agar, modified potato sucrose yeast agar and modified potato glucose yeast agar. These were varied in colour and colony sizes. The colony size on CZA medium, the largest size was obtained from F5 isolates (40x45 mm). On Sabouraud's dextrose agar medium, the largest size was observed in F2 (19x21 mm). On Sucrose yeast agar medium, F2 showed the largest size of colony (13x12 mm). As F2 showed the largest size of colony on SY agar medium (19x21 mm). Carbon sources such as simple hexoses like glucose or complex polysaccharides such as starch and cellulose can be utilized by filamentous fungi for their growth (Helen, 2014).

Pumphrey and Christran (1996) observed that glucose is the most readily metabolized sugar but most fungi could use sucrose and they require ammonia, nitrate and nitrite for nitrogen sources. In conclusion isolated fungal strains F1, F2, F3, F4 and F5 growth affected on different media. Among them the strains F2 and F1, F3, F4 and F5 will be used for biochemical and industrial significance. It should be further studies in fermentation for antimicrobial activities.

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