

## Isolation of Mycorrhiza from Roots of *Cymbidium borneense* J.J. Wood.

Ah Nge Htwe<sup>1</sup>

### Abstract

*Cymbidium borneense* J.J. Wood. was collected from Goktwin area, between Naungcho Township and Kyaukme Township of Northern Shan State in Myanmar. Root of *Cymbidium borneense* J.J. Wood. was cultured for the isolation of mycorrhiza and to get pure strain. This research work was carried out in the Microbiology Laboratory, Department of Botany, University of Mandalay from May 2016 to June 2017. In this study, the character of ANH was found the septate hyphae after 7 day, mycelium structure after 14 day and right angle branching of mycelia after 28 day. The isolation and identification of mycorrhiza fungi was referred to Sneh *et al.* (1996) and Sharma (2009).

**Keywords:** Mycorrhiza, *Cymbidium borneense*, fungi

### Introduction

Orchidaceae is a very large family of over 725 genera and 17,500 species widely distributed in the temperate and warmer part of the world (Soon 1980). Terrestrial orchids require the presence of suitable fungi in the living cells of the plant embryo and development of multicellular structures in order to develop and mature successfully (Currah *et al.* 1990).

*Cymbidium borneense* J.J. Wood. is a species of orchid. It is widespread across much of Asia, including China, Japan, India, Thailand, Indonesia, Myanmar. etc. *Cymbidium borneense* J.J. Wood. is a 5.0 to 6.0 cm tall plant with creamy white flower. It does not creep. Mycorrhizal fungi are associated with the root systems of more than 90% of terrestrial plant in a mutual symbiosis. Fungi form a mutually beneficial association with plant root forming mycorrhiza ("fungal root"). Fungal hyphae infect cortical cells, forming masses of tightly — interwoven coil called pelotons. Pelotons are considered to be the most distinctive characteristic of orchid mycorrhiza (Currah *et al.* 1990). The pelotons are digested by the host orchid in a controlled manner, this enables carbohydrates tied up within the hyphal cells to be released and absorbed. Most peloton forming fungi have been classified as members of anamorphic form genus *Rhizoctonia*, on the basis of overall morphological characteristics. Mycorrhiza isolates were strictly found in the roots of *Cymbidium borneense* J.J. Wood. (Athipunyakom *et al.* 2004).

Mycorrhizal associations are found throughout the orchid family. Orchid mycorrhizas formed between an orchid and a fungus. Most putative orchid mycorrhizal symbionts belong to the genus *Rhizoctonia*. Orchid mycorrhizal fungi have been studied the isolation and establishment of pure cultures from roots of *Cymbidium borneense* J.J. Wood.

The aims of this study was to find out specific fungus from roots of *Cymbidium borneense* J.J. Wood., to study the suitable media for orchid mycorrhiza

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<sup>1</sup> Dr., Lecturer, Department of Botany, Myingyan Degree College

and to isolate orchid mycorrhiza and pure strain. Therefore, in the present research, the orchid mycorrhiza *Rhizoctonia* species were isolated from roots of *Cymbidium borneense* J.J. Wood.

## Materials and Methods

### Collection of Plant Samples

*Cymbidium borneense* J.J. Wood. samples were collected from Goktwin area, between Naungcho Township and Kyaukme Township of Northern Shan State. The roots samples are used as source of screening and isolation of mycorrhiza fungi. Identification of orchid is carried out by referring to Flora of British India (1894), Flora of Ceylon (1918) and the orchids of Indochina (1992).

### Selection of Media and their Composition

In the present study Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) are used as basal media for isolation. In order to avoid the growth of bacteria contamination, the addition of chloramphenicol (0.025 g/l) is added in the preparation of media (Sneh *et al.*, 1996). These media were sterilized by autoclave before pouring into petridishes of 90 mm in diameter.

### Composition of media

Potato Dextrose Agar Medium (PDA) (Sharma 2009)

Agar	20.0 g
Glucose	20.0 g
Potato infusion	20.0 ml
Distilled water	100.0 ml
pH 5.6 ± 0.2 at 25°C	

Sabouraud Dextrose Agar (SDA) (Sharma 2009)

Dextrose	20.0 g
Peptone	10.0 g
Agar	20.0 g
Distilled Water	100.0 ml
pH 6.9 ± 0.2 at 25°C	

### Isolation of Mycorrhiza Fungi from Root Samples

In the present study, the isolation of mycorrhiza from roots samples of *Cymbidium borneense* J. J. Wood. are done by the use of culture method. This research work was carried out in the Microbiology Laboratory, Department of Botany, University of Mandalay. Roots were washed by water to remove the soil. The roots are cut about 0.1 cm for each segments. All the segments are dipped in 10% Clorox solution about 3 minutes for surface sterilization. After dipping about 15 min, they were rinsed 5 times with distilled water. All the sterilized pieces of roots were carried out in laminar air flow under aseptic conditions. The medium was sterilized by autoclaving at 121°C for 15 minutes. After sterilizing, an appropriate amount (20 ml)

of medium was separately poured into petridishes of 90 mm in diameter. The sterilized medium in petridishes was cool down about 28°C. When the agar was solidified, one segment of root was inoculated into each petridishes. After 7 days, fungal colonies developed on the surface of PDA and SDA agar plates. After that fungi were separately taken for inoculating into other petridishes. These petridishes were contained the same sterilized medium to make subculture for obtaining pure culture of mycorrhiza. Fungal colony were observed on PDA and SDA medium after 7, 14, 28 days.

### Macroscopical and Microscopical Characters

In the present study, all the pure culture of the isolated mycorrhiza are subjected to identification procedures. It consist of direct morphological examination after staining with lactophenol cotton blue. The culture characteristic of isolated mycorrhiza on different media are also recorded for identification.

### Microscopic Examination of Isolated Mycorrhiza

Lactophenol cotton blue is used as both a mounting fluid and a stain. Lactic acid acts as a cleaning agent and aids in preserving the fungal structures (Sharma, 2009).

### Composition of Lactophenol Cotton Blue

Lactic acid	20 ml
Phenol concentrated	20 ml
Glycerine	40 ml
Distilled water	20 ml
Cotton blue	0.05g

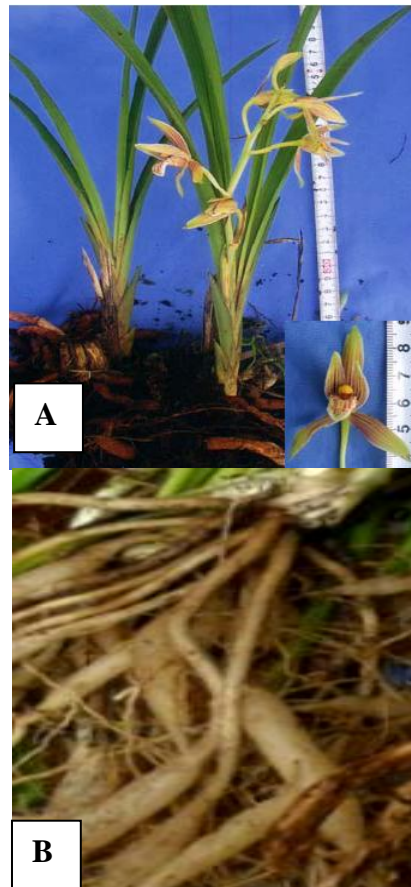
The morphological characteristics of isolated mycorrhiza are done with the help of lactophenol cotton blue staining procedure. This stain gives better view than other because lactic acid acts as a clearing agents and aids in preserving the mycelium. Phenol acts as a clearing agent and glycerine prevent drying. Cotton blue makes a colour to the mycelial structure.

On a clean slide, a loopful amount of fungal culture is smeared with the help of water drop. This smear is attained with one or two drops of lactophenol cotton blue and examine under the microscope. The morphological characteristic of isolated mycorrhiza are recorded by photomicrographic methods.

## Results

### *Cymbidium borneense* J.J. Wood.

Family	: Orchidaceae
Scientific Name	: <i>Cymbidium borneense</i> J.J. Wood.
English Name	: Unknown (Fig 1 A,B)
Local Name	: Unknown
Flowering period	: June to August



**Figure 1.** *Cymbidium borneense* J.J. Wood.  
**A.** Habit of *Cymbidium borneense* J.J. Wood.  
**B.** Roots of *Cymbidium borneense* J.J. Wood.

#### **Description of Mycorrhiza Fungi ANH 01**

On PDA, colony reaching after 7 days incubation at 28°C, white, submerged. Vegetative hyphae, septate, binucleate, containing numerous oil globules. Cluster of monilioid cells loosely arranged, submerged and developed to minute sclerotia.

On PDA, colony grew moderately, reaching after 14 days incubation at 28°C, white to cream, submerged with dense aerial hyphae, forming granular aggregations in the centre. Vegetative hyphae, septate, branching at upright angles.

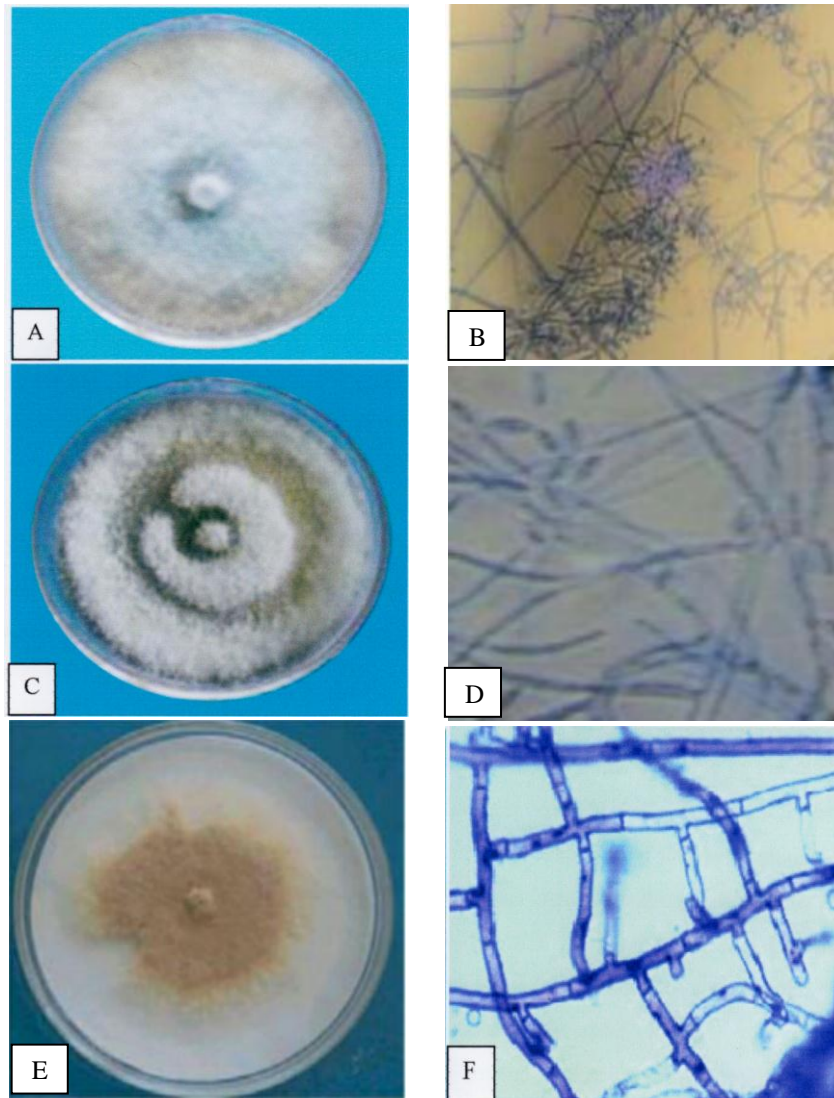
On PDA, colony reaching after 28 days incubation at 28°C, initially flat, yellowish brown, aerial hyphae. Vegetative hyphae, septate, bearing abundant lateral conidia. Conidia smooth walled, globose to ellipsoidal.

#### **Characteristics and Identification of Mycorrhiza**

Fungal hyphae infect cortical cells, forming masses of tightly-interwoven coil called pelotons. Pelotons are considered to be the most distinctive characteristic of orchid mycorrhiza, this enables carbohydrates tied up within the hyphal cell to be released and absorbed. Most peloton forming fungi have been classified as members of the anamorphic form of genus *Rhizoctonia*, on the basis of overall morphological characteristics.

*Rhizoctonia* species are characterized by right-angle branching, a constriction at the branch point, and a septum in the branch hyphae near its point of origin.

Frequently, they have chains of inflated hyphae, known as monilioid cells. Sexual stages are rarely encountered in the laboratory. One morphological feature that has helped in the classification of *Rhizoctonia* is the number of nuclei present in the young cells. Nevertheless, the most common group of orchid mycorrhizal fungi is binucleate *Rhizoctonia*. Based on the macroscopical and microscopical characters, according to Sneh (1996), the mycorrhiza ANH 01 was classified as *Rhizoctonia* species (Figure. 2 A – F)



**Figure 2 Morphological characteristic of *Rhizoctonia* sp.**

- A. Fungal Colony character (7 day old culture)
- B. Photomicrograph of septate hypha
- C. Fungal Colony character (14 day old culture)
- D. Photomicrography of mycelium structure
- E. Fungal Colony character (28 days old culture)
- F. Photomicrograph of right angle branching of mycelia

## Discussion and Conclusion

In the present investigation, *Rhizoctonia* species of orchids mycorrhiza were isolated from the root of *Cymbidium borneense* J.J. Wood. from Goktwin area, between Naungcho Township and Kyaukme Township of Northern Shan State in Myanmar, 2016 to study the mycorrhiza fungi.

In this study, the two culture media of Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) were utilized for the isolation of mycorrhiza. After reviewing the development of fungal colony, PDA are selected for further experiments because fungal growth are better in PDA media than SDA media.

The macroscopical characters of *Rhizoctonia* species ANH 01 after 7 days were white colonies at 28°C in PDA medium. Vegetative hyphae was septate. After 14 days, it was white to creamy colonies at 28°C in PDA medium. Vegetative hyphae was septate, mycelium structure. After 28 days, aerial hyphae was initially flat, yellowish brown, septate, right angle branching of mycelia bearing abundant lateral conidia.

Most orchid mycorrhizal fungi are found in the genus *Rhizoctonia* species. However the classification of *Rhizoctonia* species is complicated due to the lack of spore production (Sneh *et al.* 1996). The study on orchid mycorrhizae would be most successful in term of the specificity between mycorrhizal fungi and the orchid hosts. *Rhizoctonia* species of mycorrhizal fungi were isolated from the peloton in the root cortical cells of *Cymbidium borneense* J.J. Wood., the terrestrial orchids.

The mycorrhiza layers originating from the meristem of the root, except the limiting layers of epidermis and endodermis can be separated into a fungal host cell layer, a fungal digestion cell layer, and a storage layer. This differentiation is apparent even in the meristem. It can be recognized by the development of cells. The infection occurs through hairs, hair papillae, epidermal cells, cell of the velamen, or the passage cells of the exodermis. After that, cells of the most peripheral layer of the fungal host cells, and immediately after that almost simultaneously the adjacent digestion layer are infected by the continuously growing hyphae. These observation serve to examine and give proof for the hypothesis that orchids gained materials from the digestion of the fungus, but still emphasized that the plant might still need other acquiring organic substances (Shan *et al.* 2002).

The prominent results, isolated mycorrhiza strain from the *Cymbidium borneense* J.J. Wood., belong to the genus *Rhizoctonia* species. This result is in agreement with Sneh *et al.* (1996).

The isolation and identification of orchid mycorrhizal fungi are important to understand the orchid-fungus relationship. This research work showed that mycorrhiza *Rhizoctonia* species, isolated from *Cymbidium borneense* J.J. Wood.

In the present research, orchid mycorrhiza strain was screened from root of orchid *Cymbidium borneense* J.J. Wood. The fungus *Rhizoctonia* species isolated from the *Cymbidium borneense* J.J. Wood. enhance the growth of orchid seeds (Smith *et al.* 1997). The specificity of the species was confirmed by the present investigation. It is agreed with Batty *et al.* 2007 and Shan *et al.* 2002 who reported that mycorrhiza isolated from the same species from more or less different localities. The presence of an orchid mycorrhizal fungal symbiont could be considerable

important. It is also needed investment and encouraged capable scientists to become actively involved in biotechnology researches and developments. However, the relationship between fungus mycorrhiza and orchid plants still need to pursue further investigation.

According to the macroscopical and microscopical characters was based on the literatures of Chen *et al.* (2012), Sharma (2009), Sneh *et al.* (1996), Smith & Read (2008), Athipunyakom *et al.* (2004), Gezgin & Eltem (2009) and Masuhara & Ratsuya (1994), these isolated fungus have been previously reported as benefit effect on plant pathogens.

In these studies, to investigate the specificity and distribution of fungal endophytes associated with orchid *Cymbidium borneense* J.J. Wood. The present research work will be very helpful to know the suitable media. To get a knowledge to isolate the pure strains, and to become more understanding on the structure of orchid mycorrhiza *Rhizoctonia* species. It will be more applicable for the further research works of orchid mycorrhiza in Myanmar.

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