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Research Note:

Common fungal diseases of native butterfly orchids (*Phalaenopsis* sp.)

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ABSTRACT

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Specimens of the most common fungal diseases of native butterfly orchids were collected in Baybay, Leyte and the diseases and their casual organisms were also identified. *Sclerotium rolfsii* Sacc. grew radially showing profuse, white, aerial, straight mycelia and brown sclerotial bodies in potato dextrose agar (PDA). *Colletotrichum* sp. exhibited creeping, whitish, straight mycelia with circular growth and produced abundant spores. Sporulation was observed after two days of growth in PDA. *Phytophthora* sp. showed suppressed growth and did not sporulate in PDA; it displayed creeping, whitish, cottony mycelia with radial growth pattern. However, abundant sporangia were produced in onion agar (OA) and after immersion in sterile water for 2 to 4 days under laboratory condition. Incubation periods of *S. rolfsii, Colletotrichum* sp. and *Phytophthora* sp. were observed 2-3 days, 10-11 days 9-12 days, respectively, after inoculation. Yellowish intact rotted portions characterized the disease caused by *S. rolfsii* while circular to oblong, sunken, necrotic lesions were produced by *Colletotrichum* sp. Watersoaked lesions incited by *Phytophthora* sp. gradually turned blackish and sunken with irregular border and shape. Of the three fungal pathogens, *S. rolfsii* was the most destructive with the highest severity.

Keywords: butterfly orchids. fungal diseases.

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PCARRD (1994) identified orchid diseases and their casual organisms such as black rot caused by *Phytophthora*, the most common species of which is *palmivora*; the Sclerotium rot caused by *Sclerotium rolfsii* Sacc.; anthracnose, whose casual organism is *Gloeosporium*; and bacterial soft caused by *Erwinia carotovora*. Viral diseases are commonly caused by tobacco mosaic virus-orchid strain or TMV-O and cymbidium mosaic virus (CyMV) which induce general or localized chlorosis of various sizes and shapes, streaking, flower spotting or breaking, necrosis and stunting.

Baniqued (1986) listed diseases of ornamental plants including *Phalaenopsis amabilis*, and identified the casual organisms of the diseases. These diseases include anthracnose, Sclerotium rot and soft rot, caused by *Colletotrichum gleoesporioides*, *Sclerotium rolfsii* Sacc. and *Erwinia carotovora* (L.R. Jones) Holland, respectively.

No report has been made on the pathogenesis of orchid diseases. Thus, the study was conducted in order to identify the various fungal diseases affecting native butterfly orchids and their causal organisms, determine the incubation period of the diseases and the disease severity caused by the identified fungal organisms.

The native butterfly orchid was propagated through tissue culture. The plantlets were eventually grown on a piece of wood (2.5 cm L x 3.5-5.0 cm D) placed inside the screenhouse and fertilized regularly with 1 tablespoon of Albatros foliar fertilizer (18-18-18) at the rate of 1 tablespoon per 4 liters of water. Butterfly orchid parts or tissues infected with diseases were collected from the Visayas State College of Agriculture (ViSCA) and its neighboring barangays and brought to the laboratory for pure culture isolation. Plant pathogenic fungi were isolated by tissue planting on potato dextrose agar (PDA) or onion agar (OA).

The collected infected specimens showed different symptoms ranging from rotting to spots. *Phalaenopsis* orchids that were observed to be seriously infected generally exhibited rotting on the base followed by defoliation. Whitish mycelia and brown sclerotial bodies were sometimes visible on the rotted portion of the orchid. The rotted part appeared to be yellowish, watersoaked and remained intact but emitted no foul odor. On the other hand, some diseased leaf specimens had varied

spots on both surfaces. Lesions were found to be black, sunken, some watersoaked with sizes ranging from 0.9 to 12 mm. The spots were circular, elliptical, oblong or irregular in shape. They appeared singly or in coalesced form, showing enlarged lesions or spots.

Butterfly orchid plants were allowed to grow up to the 3-4 leaf stage during which inoculation was carried out. For fungal inoculum preparation, sterile water was added to the pure culture and the spores were dislodged from the fungal growth by scraping. The fungal suspension was filtered so that the final suspension contained only spores and fine mycelial threads. For *Phytophthora*, agar disks with mycelia were immersed in a plate containing sterile water and observed for appearance of sporangia. The disks were later placed in an osterizer to separate the sporangia and break the agar into smaller pieces. The number of spores was calibrated using a hemacytometer.

Inoculation was done by spraying the inoculum to the test plants using an atomizer. For *Sclerotium*, only sclerotial bodies were inoculated on the basal portion of the leaves. Test plants were covered with plastic bags for 48 hours. From the resulting diseased condition, the organisms were reisolated and reinoculated into healthy butterfly orchid seedlings.

Sclerotium rolfsii Sacc. The organism showed a sparse radial growth one day after introduction to PDA. It grew luxuriantly, showing profuse , white, aerial and straight mycelia following 3 days of incubation, with some small visible white mycelial mass. The hyphae were compact, septated and hyaline (Fig.1C). The fungus covered the petri plate within 5 to 6 days after planting . No conidia were produced, however, sclerotial formation was observed 4 to 5 days later. The size of sclerotia ranged from O.8 to 1.5 mm with light to dark brown color and shape from globose to ellipsoid. Usually, sclerotial bodies were situated on the center and majority at the edge of the culture .Yellowish water droplets were very noticeable clinging to the side of the sclerotia after one week of incubation (Fig 1B.). The pathogen possessed the same cultural characteristics as described by Trigo and Palomar (1979)and Montesclaros (1987) on their study of *S. rolfsii* grown on sweetpotato culture medium and the growth of *S. rolfsii* on different indigenous culture media, respectively.

Symptoms appeared on plantlets 2-3 days after inoculation. The disease was characterized by rotting on the affected area, particularly the basal portion of the leaves. The rotted portion was yellowish, but the tissues remained intact (Figure 1A). Moreover, rotting had no foul odor and no oozing was observed, unlike that of diseases caused by *Erwinia*. In advanced stage of the disease, white mycelial bodies were noted spreading on the basal area that became thick with brown sclerotial bodies (Fig. 1A). As the disease became severe, the portion left unaffected exhibited wilting. Later on, the whole leaf manifested rotting which led to defoliation. Defoliation occurred during the early stage of infection but it was only minimal. Furthermore, some of the roots became yellowish to brownish and they shrunk. The diseased plantlets eventually died 8-9 days after inoculation.

Colletotrichum sp. The fungus grew on plated PDA 2 days after incubation. The colony exhibited whitish, straight, hyphae which grew radially, creeping from the center and forming circular colonies on the surface of the culture medium. The rapid increase of mycelial growth was observed at the early stage of development but became slower after several days of incubation. Sporulation started after 2 days of growth. Aggregates of orange droplets or pigments containing the fungal spores were visible on the agar surface 3-4 days after inoculation (Fig.1E). Later, the orange droplets were dominantly found on the PDA surface with the whitish mycelium barely seen. *Colletotrichum* sp. had simple and elongate conidiophores with hyaline, one celled, ovoid or oblong conidia (Fig.1F).

Infection usually started at the leaf margin, visible 10-11 days after inoculation. The lesion appeared necrotic, small at first but increasing in size as the infection progressed. Lesions were brown to black and sunken or thin. The shape was circular to oblong with a diameter of 3 to 5 mm (Fig. 1D). Sometimes dead areas were surrounded with thin yellowish color as the lesion enlarged. Lesions were evident on both surfaces of the leaf . In addition, disease development was found to be slow. Similarly, Singh (1975) as cited by Edurise (1992) stated that some plants have the capability to produce toxic substances which stop the establishment or inhibit the activity of microorganisms that may enter the plant.

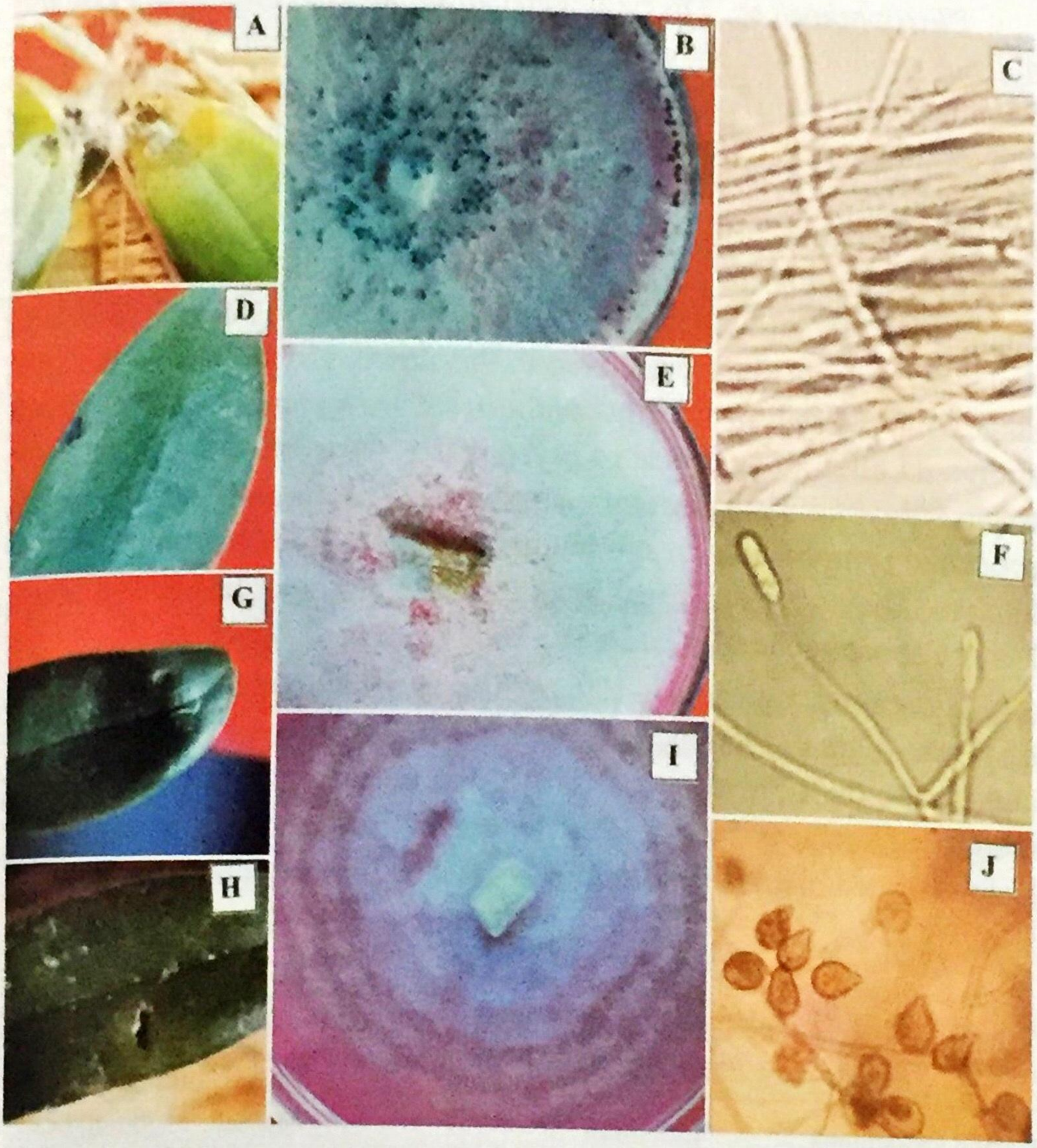

Figure 1. Disease symptoms and their causal organisms. (**A**) Butterfly orchid plantlet infected w/ *S. rolfsii* showing yellowish, intact but rotted portion w/ brown sclerotial bodies 4 days after inoculation (X=1); (**B**) Sclerotial bodies found after 1 wk growth of *S. rolfsii* in PDA (X=0.5); (**C**) Compact, septated and hyaline hyphae of *Sclerotium rolfsii* (X=400); (**D**) Circular to oblong, necrotic and sunken lesions incited by *Colletotrichum* sp. 2 days after inoculation (x=1.5); (**E**) Whitish, straight hyphae of *Colletotrichum* sp. w/ orange-colored droplets visible after 3-4 days in PDA (X=1); (**F**) *Colletotrichum* sp. w/ hyaline, one-celled, rod shape conidia still attached to simple and elongated conidiophores (X=400); (**G**) Watersoaked spot as initial symptom incited by *Phytophthora* sp. 9 days after inoculation; (**H**) Irregular, blackish and sunken lesion w/ yellowish margin caused by *Phytophthora* sp. 16 days after inoculation (X=15); (**I**) *Phytophthora* sp. exhibiting whitish & cottony mycelium with radial growth pattern (X=1); (**J**) Brownish, mature and papillate sporangium of *Phytophthora* sp. (X=100).

Phytophthora sp. The pathogen had suppressed growth in PDA and did not produce spores even though incubation time was extended. Palomar *et al.* (1995) discovered that *Phytophthora colocosiae* Rac. grew and reproduced on onion agar (OA), yielding large number of sporangia after immersion in sterile water for several days. Hence, instead of PDA, OA was used as the artificial culture medium. The mycelium was whitish, cottony with radial or circular growth pattern thick towards the center compared to the growth near the edge of the plate (Fig 1I). Growth was faster at the early stage compared to the slower growth during the later stage. About 4-7 days old *Phytophthora* sp. grown on OA gave abundant sporangia after an agar disc of mycelia was transferred to sterile water for 2-4 days under laboratory condition. The sporangiophores were produced sympodially on sporangius that differed little from vegetative hyphae. They were spherical hyaline at first but later turned brownish. Mature spores generally had a papillate form or shape (Fig. 1J).

Symptoms appeared 9-12 days after inoculation consisting initially of 3 mm diameter watersoaked spots (Fig. 1G) which then gradually changed to dark brown areas. The lesion became more pronounced on either leaf surfaces 5 days after the incubation period. Afterwards, the lesions turned blackish and sunken with more or less circular to irregular

Table 1. Pathogenicity and percentage infection exhibited on butterfly orchid plantlets inoculated with different fungal organisms[1/]

Casual Organisms	Inoculum	% Infection[2/]	Rating Scale[3/]
Sclerotium rolfsii	10 slerotial bodies	100	9
Collettotrichum sp.	1.9×10^3 spores/ml	100	3
Phytophthora sp.	5.2×10^4 spores/ml	100	3

1/ Taken at 9 days after inoculation

2/ Ten (10) plantlets per pathogen

$$\% \text{ infection} = \frac{\text{no. of plants infected}}{\text{no. of plants inoculated}} \times 100$$

3/ Rating Scale : 0 = no infection; 3 = 1-5% leaf area infected;
5 = 6-25% leaf area infected; 7 = 26-50% leaf area infected;
9 = 51-100% leaf area infected

shape (Fig. 1H). As the spot enlarged, dead portions had defined yellowish margins. Furthermore, watersoaking often persisted at the border of the lesions.

Of the three (3) fungal pathogens, *S. rolfsii* was the most destructive with 100% infection and the highest disease severity (Table 1). Both *Colletotrichum* sp. and *Phytophthora* sp. had minimal infection of the leaf area, hence as the plantlets matured the plant simply recovered.

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