



ISOLATION AND CHARACTERIZATION OF FUNGAL DISEASES IN CACTI

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ABSTRACT

Fungal diseases affecting cacti were identified and isolated through in vitro culture using Potato Sucrose Agar (PSA) medium. Cultures were incubated under controlled conditions of 12-hour light-dark cycles at 25°C for seven days to facilitate purification, following the Saccardo classification system. The results revealed symptoms consistent with suspected anthracnose, characterized by brown to black cankers or lesions on globose stems and roots. Additionally, an unidentified fungal disease was observed, presenting as pinkish discoloration across the epidermal surface and periphery of affected tissues. However, further taxonomic verification is required to confirm its identity.

The cactus garden is currently colonized by both suspected anthracnose and an unidentified fungal pathogen. These diseases are disseminated through multiple vectors, including air, water, soil, and contaminated biological materials. While some infections may be attributed to human negligence, others appear to be naturally occurring and unavoidable.

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INTRODUCTION

Cacti, members of the flowering plant family within the order Caryophyllales, comprise nearly 2,000 species across 139 genera. These plants are native to a vast range extending from British Columbia and Alberta in North America to the southernmost regions of Chile and Argentina in South America. Mexico hosts the greatest diversity and abundance of cactus species. Interestingly, the only cacti potentially native to the Old World belong to the genus *Rhipsalis*, found in East Africa, Madagascar, and Sri Lanka.

Although some cactus species thrive in tropical and subtropical climates, the majority are well adapted to arid environments. Their evolutionary adaptations such as the loss of leaves, development of a succulent cortex for water storage, and transformation of leaves into spines have enabled them to survive in desert conditions. These spines not only protect stored water but also contribute to photosynthesis, as the stem becomes the primary photosynthetic organ. While cactus wood supports water transport and structural integrity, globose cacti rely on internal turgor pressure

for support. Root succulence is also common, with many species storing water in both shoots and roots. The cephalium, a specialized growth form in some cacti, is a permanent structure, unlike the seasonal inflorescences of other perennial plants (Mauseth Research).

Cacti were among the earliest plants brought back to Europe by explorers in the 15th century (Howard & Touw, 1981), quickly becoming popular in botanical collections and gardens (Anderson, 2001). Over time, the horticultural trade in cacti has grown into a global industry, facilitating the intercontinental spread of numerous species (Walters et al., 2011). In the Philippines, Benguet—once known as the "rose capital" has seen a shift in agricultural trends due to climate change, market competition, and the influence of social media. This transformation has given rise to a thriving cacti and succulent economy, contributing to the region's agritourism (Potpot, 2020).

Despite their reputation for drought and disease resistance, cacti are not immune to pests and pathogens. Diseases caused by bacteria, viruses, fungi, and other microorganisms can severely affect their health. While some infections result from human neglect, others are naturally occurring and difficult to prevent. Regular monitoring of growing conditions is essential, as prevention is more effective than treatment (Cactusway, 2022; Plantinterrarium).

Currently, the cactus garden at the Children's Joy Foundation, Incorporated in Indangan, Davao City, Philippines is in critical condition, with fungal infections affecting even the juvenile plants (pups) in containers. This study was therefore conducted to isolate and identify the fungal pathogens affecting cacti through *in vitro* culture techniques. The goal is to characterize the diseases and contribute to effective management strategies for cactus cultivation.

Objectives of the Study

Generally, this study aimed to identify and characterize fungal diseases derived from cactus pots from the cactus garden and brought to the laboratory at Indangan, Davao, Philippines; specifically this study aimed to:

- a. Isolate and identify the suspected fungal pathogens associated to the cacti plant at Indangan, Davao City, Philippines condition; and
- b. Classify and characterize the isolated fungal pathogens associated in cacti *in vitro* culture.

METHODOLOGY

Location and Plant Material

This study was conducted at Children's Joy Foundation Incorporated Indangan, Davao City, Philippines from March to June 2023 (Fig. 1). The cactus pots with severe disease from the cactus garden were isolated and brought into the crop protection laboratory.



Figure 1: The samples of infected cactus from Cactus garden.

Laboratory Preparation

The isolation room was maintained in aseptic condition or free from any contamination. The laminar floor was sprayed with 10% zonrox/ ethanol. The UV light was turned on for 30 minutes before use of the isolation room (Cobrado and Fernandez 2016).

Culture Media

This media and glassware sterilized for about 15 minutes using a pressure cooker at 5 psi. Potato sucrose agar (PSA) was used in this study.

Isolation of Fungal Disease

Each of the infected plant parts was isolated using PSA media culture. Explants were surface sterilized by soaking them in 1% Sodium hypochlorite for 10 minutes before inoculating into PSA media taped and labeled each sampling as demonstrated by MichiganStateUni with 12 Light-dark cycle, 25°C for 12 hours cycle (Fig. 2).



Figure 2: The isolation was done using a laminar flow hood.

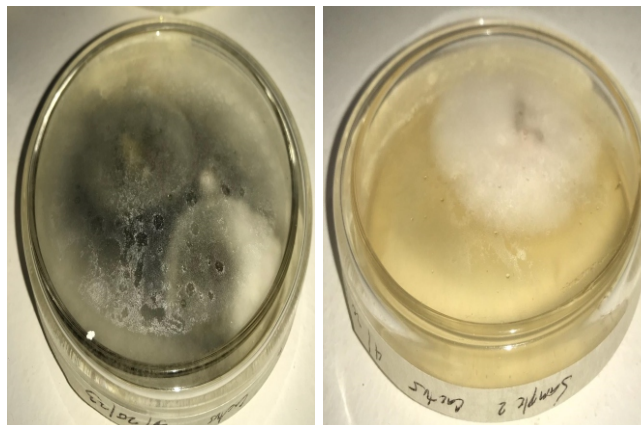


Figure 3: The isolation of suspected fungal diseases is shown (a) sample 1 and (b) sample 2.

After three to seven days, pathogens exhibiting fungal characteristics were inoculated into the PSA media and then put into successive new cultures until purification is obtained at 7 days (Fig. 3).

Identification of Fungal Pathogens

The identification of fungal pathogens associated with the cactus garden was conducted using the Saccardo System, a classical taxonomic framework for fungi, in conjunction with light microscopy. This method remains a standard approach for identifying imperfect fungi and common fungal diseases in plants, including cacti. Familiarity with fungal morphological structures such as conidiophores, conidia, and mycelial characteristics is essential for accurate identification using taxonomic keys.

A small portion of mycelium or conidia was carefully extracted from sporulating conidiophores using a sterile needle tip embedded with a fragment of agar. The sample was then transferred onto a microscope slide, gently covered with a cover slip, and examined under a compound microscope to observe diagnostic features (Barnett & Hunter, 1998). This technique allows for precise morphological characterization, which is critical for distinguishing among fungal species and understanding their pathogenic roles in cactus health.

RESULTS AND DISCUSSION

Isolated fungal diseases of cacti

An assessment was conducted at the Children's Joy Foundation Incorporated, located in Indangan, Davao City, Philippines, from March to June 2023. During this period, cactus plant samples exhibiting symptoms of fungal infection were collected and cultured on Potato Sucrose Agar (PSA) medium. The incubation period ranged from three (3) to seven (7) days to allow for sufficient fungal growth and sporulation.

Purification and microscopic identification were performed for each sample. A portion of conidia was carefully lifted from the sporulating conidiophores using a sterile needle tip embedded with a small fragment of agar. The sample was then mounted on a microscope slide, gently covered with a cover slip, and examined under a compound microscope to observe key morphological features. This procedure follows the methodology described by Barnett and Hunter (1998), and identification was guided by the Saccardo System, which remains a foundational framework for classifying imperfect fungi and diagnosing common fungal diseases in cactus plants.

Table 1: The isolated fungi from cacti.

Sample	Literature cited	From the cactus plant
1. Order: <i>Melanconiales</i> Family: <i>Melanconiaceae</i> (anthracnoses)	<ul style="list-style-type: none"> ● Develop below the cuticle or below the epidermis ● White, cream-colored, pink, orange or black brown with black lesions in stem and the root 	<ul style="list-style-type: none"> ● Brown and black canker or lesions through the periphery/epidermis of stem and roots ● White cottony and later on becomes gray to black
2.	No evident of existing symptoms	Pinkish discoloration Color white cottony.

As described in University of California that *Melanconiales* spores are borne on an acervulus means erupts through the epidermis, that *Melanconiaceae* only one form-family has been designated. Many species are parasitic on plants and cause a group of diseases called anthracnoses. Anthracnose in nature, usually develop below the cuticle or below the epidermis they release their conidia/spores which may be white, cream-colored, pink, orange or black depending on the pigmentation. Symptoms of anthracnose in cactus exhibit dry roots based on shutterstock. The epidermis of the stem and roots become brown with black lesions.

Sample 1 (Figure 4 a) showed the symptoms on the cacti had a brown to black canker or lesions through

the periphery/epidermis and root, after 2 days of isolation that mycelium (colony) spreads outward from the infected tissue in cultured media. its color appeared to be white cottony and later on becomes gray to black.

For common fungal pathogens associated in cactus there is no evidence of existing symptoms of pinkish discoloration.

Sample 2 (Figure 4 b) the symptoms had pinkish discoloration throughout the periphery/epidermis after 3 days of isolation. The mycelium (colony) spreads outward from the infected tissue in cultured media and a white cottony appearance showed.

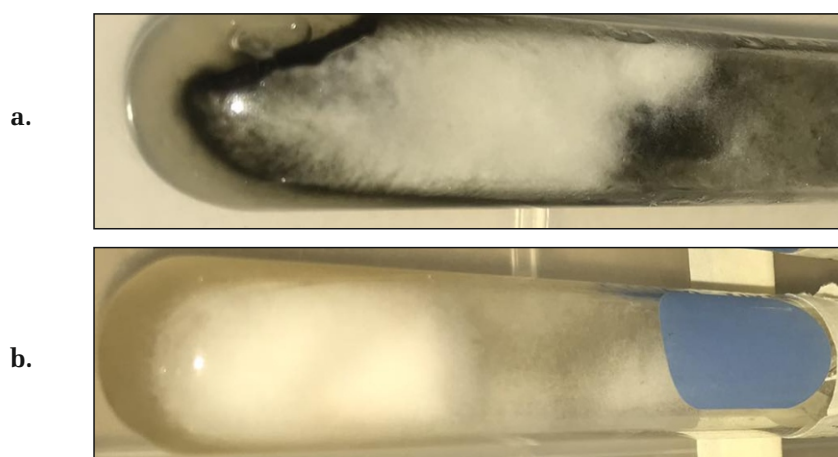


Figure 4: Cultural features of fungal pathogens A and D isolated from cactus stem and b, colonies respectively, on PSA after 7 days of incubation at 12 Light-dark cycle, 25°C for 12 hours cycle.

Identification of fungal Pathogens from cacti

Table 2: Characteristics of isolated fungi from cacti.

Sample	Literature cited	From the cactus plant
1. Order: <i>Melanconiales</i> Family: <i>Melanconiaceae</i> (anthracnoses) Genus: <i>Gloeosporium</i> sp.	<ul style="list-style-type: none"> ● University of California, Riverside ● Melanconiaceae cause a group of diseases called anthracnoses ● usually develop below the cuticle or below the epidermis. ● white, cream-colored, pink, orange or black. ● Shutterstock The stem and root of the cactus had brawn and black lesions. ● Barnett and Hunter 1998 conidiophores are simple conidia ovoid to oblong 	In nature/symptoms: <ul style="list-style-type: none"> ● The symptoms show that the globose stem of Cactus and pups had a brown with black canker or lesions through the periphery/epidermis. ● parasitic In Culture: <ul style="list-style-type: none"> ● white cottony and later on becomes gray to black In Microscopic view <ul style="list-style-type: none"> ● conidiophores are simple and conidia ovoid to oblong poorly formed
2. Order: <i>Mycelia Sterilia</i>	University of California, Riverside no presence conidia or other reproductive Sclerotia are formed, but there are no fruiting bodies.	In nature/symptoms: <ul style="list-style-type: none"> ● pinkish discoloration throughout the periphery (epidermis) In Culture: <ul style="list-style-type: none"> ● white, cottony, reverse white off. In Microscopic view: <ul style="list-style-type: none"> ● conidiophores were ramified, forming tree-like structures ● Septate hyphae Absence of conidia

Sample 1

Based on the Saccardo system that forms order Melanconiales or called Melanconiaceae. Two common forms of genera are *Gloeosporium* and *Colletotrichum*. They were both very similar in appearance, except that the latter has prominent dark

setae associated with the conidiophores. The many species of the two genera have conidia which are hyaline, one-celled, and ovoid to oblong. Under certain cultural conditions, however, the setae of *Colletotrichum* fail to form, thereby making it impossible to distinguish between the two genera. A

conidiophore is a simple or branched septate/etae consisting of cells separated from each other by cell walls on which conidia are produced.

Mycelium (colony) is a branch of fungal threads called hyphae/conidiophore on which conidia are produced. Conidiophores are attached directly to the epidermis of plants. In isolation it is attached directly in culture media in which absorption of nutrients occurs. If the conidia are dispersed by air, water, soil, or cross contamination with favorable conditions it becomes a mycelium.

Sample 1 (Figure 5 b) in microscopic view exhibited the hyphae as a septate which has a cell wall and conidiophores appear simple or straight at the edge. Attached spores called conidia ovoid showed ovoid to oblong poorly formed but no dark setae have been found and therefore classified as *Gloeosporium* sp.

Sample 2

Based on University of California taxonomy of fungi, Mycelia Sterilia are known as no presence conidia or other reproductive sclerotia formed, but there are no fruiting bodies. A compact mass of hardened mycelium stored with reserve food material found in some higher form of fungi becomes detached and remains dormant until a favorable opportunity for growth occurs. The mycelium (colony) is described as white and cottony.

Sample 2 (Figure 6) in microscopic view showed only the presence of mycelium fungal threads but the absence of conidia. Its septate has been found and the conidiophores were ramified, forming tree-like structures.

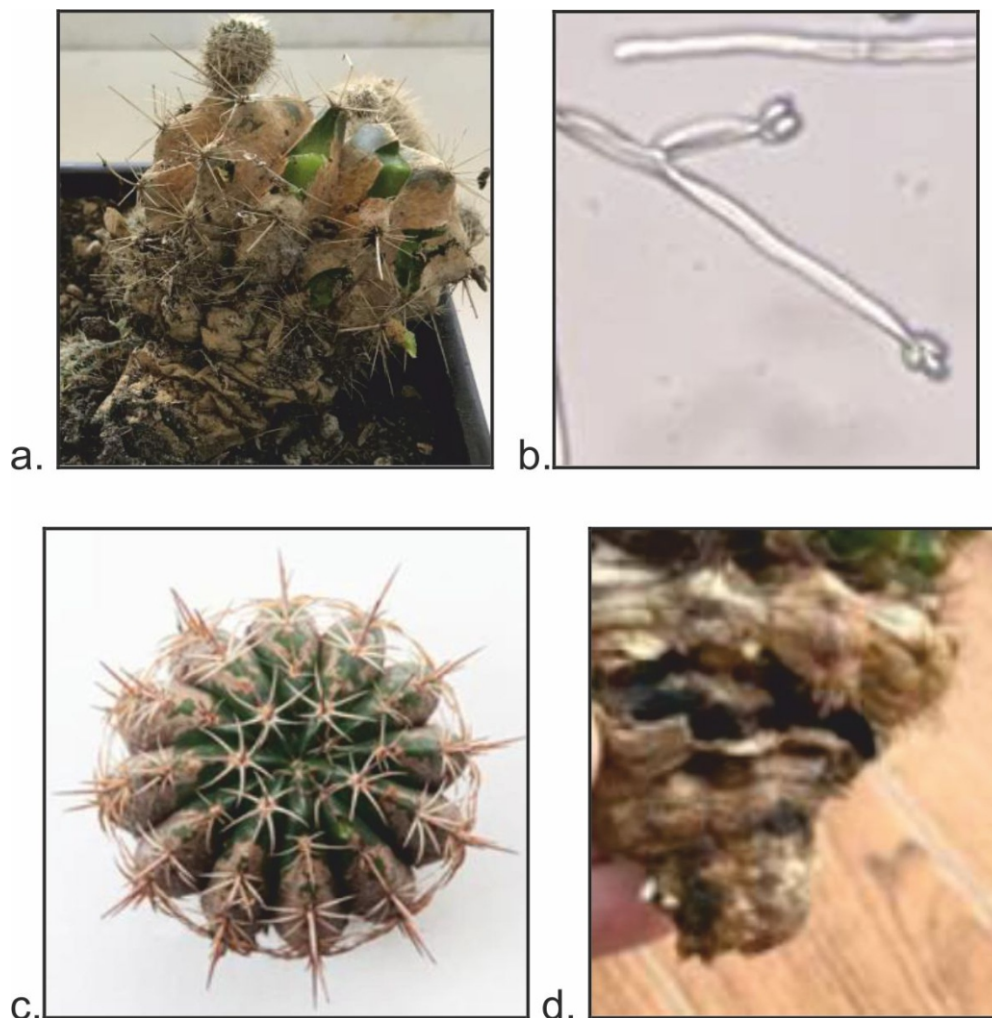


Figure 5: Suspected anthracnose in Sample 1 (a) symptoms from the cactus plant (b) Microscopic view 100x, (c)(d)symptoms from literature cited.

In sample 1 suspected *Gloeosporium* sp. (Anthracnose) as described in table 1 and 2 show the symptoms on plants as shown in (Figure 5 a) that the globose stem of Cactus and pups had a brown with black canker or lesions through the periphery/epidermis of stem and roots. When isolated from the infected plants in PSA media (Figure 4 a) fungal growth is characterized by white cottony and later on becomes gray to black. Under 100x microscopic view (Figure 5 b) conidiophores were simple and conidia were ovoid to oblong poorly formed. It is similar to the Saccardo system of classification (Barnett and Hunter 1998; University of California, Riverside). This pathogenic fungus was characterized as *Gloeosporium*

sp. in common name called anthracnose. In (Figure 5 c and d) on the authority of shutterstock showed that this symptom has been reported also.

Furthermore, These findings have been investigated by Texas A&M University that this fungal disease commonly affects several species of cacti. Initial infections appear as small spots that later enlarge into a moist, light brown rot and become covered by small, light pink spore-producing structures. The infection spread to large areas, sometimes eventually destroying the plant. Yet, its further identification is subject for molecular verification.

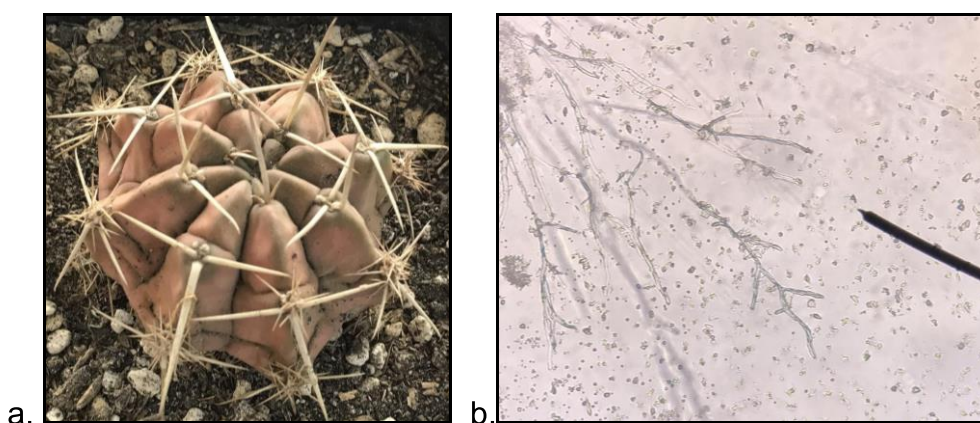


Figure 6: Unidentified fungal disease in Sample 2 (a) symptoms from the cactus plant (b) microscopic examined at 100x magnification.

In sample 2 the unidentified fungus of cacti resembling as described in table 1 and 2 has pinkish discoloration throughout its periphery/epidermis (Figure 6 a), when isolated from the infected plants in PSA media (Figure 4 b). Fungal growth is characterized as white and cottony. (Figure 6 b) Under 100x microscopic view, it contains septate hyphae and conidiophores were ramified, forming tree-like structures, however its further identification is subject for verification.

SUMMARY, CONCLUSION AND RECOMMENDATION

The Children's Joy Foundation, Inc., in Davao City, Philippines assessed samples of fungal pathogens from a Cactus Plant from March to June 2023. After purification, each sample was mounted under a microscope, and conidia were lifted from sporulating conidiophores using a needle and compound microscope.

In relation to the Saccardo system and the common fungal pathogens (4 to 18) identified anthracnose as the common fungi found in the samples under study. Although further identification is subject for molecular verification. The unidentified fungal pathogens have not been identified under those two bases, therefore, further identification is subject to verification.

The cactus garden at Children's Joy Foundation, Inc at Indangan, Davao, Philippines is colonized by Unidentified fungal disease and suspected anthracnose. It is dispersed by air, water, soil, and also by tainted pathogens. Although some are a result of negligence of people while others are unavoidable and natural.

Based on the findings of the investigation. Some basic management practices can help prevent pest problems from occurring in the first place. The following

precautions can reduce the likelihood of plant disease development and spread of anthracnose. and any fungal pathogens for that matter.

Regular monitoring and evaluation of cacti is crucial for early detection of anthracnose or other fungi diseases, enabling quicker response and improved disease management. Maintaining excellent garden sanitation involves removing and destroying contaminated plant components. Proper soil drainage and allowing soil to dry out between waterings reducing fungal growth. Increased air circulation between cacti helps lower humidity and disease risk. Fungicides should be used for severe anthracnose control, following manufacturer's instructions. Choosing disease-resistant cactus cultivars is recommended to reduce infection risk. Maintaining optimal growing conditions with proper temperature, light, and soil pH is a must. As a matter of protocol the need to quarantine new cacti to check for disease symptoms before integration to the garden should be practiced.

Cactus growers can lessen the effects of anthracnose and other fungal diseases by employing good agricultural practices to improve the growth environment for their plants. To keep cacti healthy and free of disease, regular maintenance, preventive measures, and prompt response should be implemented.

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