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Morphological and Cultural Characterization of *Fusarium proliferatum* Associated with the Heart Rot of Abaca (*Musa textilis* Nee) in Baybay City, Leyte, Philippines

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ABSTRACT

Abaca (*Musa textilis* Nee) of the Musaceae family is grown in the Philippines by small-holder farmers, but the country is considered as the world's leading abaca producer contributing 87.5% of the global market demand. However, abaca production has been declining mainly due to diseases. In Leyte, one of the recent observed diseases is abaca heart rot that exhibits symptoms different from the one caused by bacteria. Infected plants exhibited leaf wilting, petiole breaking, and vascular discoloration without foul odor. For any disease management program to be effective, it is necessary to diagnose the causal pathogen correctly, hence this study aims to (a) isolate and observe the pathogen colony characteristics in pure culture; and (b) examine its microscopic characteristics. The cross- and longitudinal section examinations of the pseudostem show a rotten purplish vascular bundle. Cultures of the fungal pathogen in potato dextrose agar (PDA) developed white colony which turns light purple at about 7 days in culture. It has floccose white cottony aerial mycelia towards the margin of the colony. Moreover, microscopic examination of the pathogen at 7 days in culture, and 3 days in agar block produces aseptate hyaline microconidia with tapered lower end and blunt top portion. The one-week-old conidia measure 6.790µm. The conidiophore of the pathogen is in upright position at about 45º and measures about 12.897 µm. At three weeks in culture, the macroconidia became apparent with apical cell slightly curved like a slender sickle, measuring 136.680 um having one to three transverse septa. The obtained biological characteristics of the isolated heart rot pathogen are similar to that of *Fusarium proliferatum* and *Fusarium moniliforme*. Compared to published articles, the isolate from Baybay City is confirmed to be *Fusarium proliferatum*. The results of the study have implicated that there is another disease in abaca, heart rot disease, that needs to be given with attention to increase abaca farm productivity.

Keywords: abaca, abaca heart rot, *Fusarium proliferatum*, agar-block technique, vascular discoloration

INTRODUCTION

Abaca, (*Musa textilis* Nee), known as Manila hemp of the Musaceae family, is valued for its fibers derived mainly from the plant's sheath. The fibers are used as

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raw materials in a variety of specialized paper products, tea bags, banknotes, filter paper, and is very important in the handicraft industries. It is classified as hard fiber, along with coir, henequin, and sisal (Britannica 2014). The Philippine abaca industry is vital to the country's economy with an annual proceeds of P4.7 billion from abaca production and importation alone about half a decade ago providing income to small-holder farmers. The Philippines is considered as the world's leading abaca producer, especially Visayas and Mindanao islands, providing 87.5% of the market demand (PhilFIDA 2022).

Despite the high demand and the Philippines enjoying the monopoly of supplying abaca fiber, there is a downfall trend of abaca productivity. One of the important factors for this downfall trend are biotic agents causing diseases infecting abaca which decreases both the volume of production and quality of fiber obtained . Bunchy-top virus, *Fusarium* wilt, heart rot caused by bacteria, and heart rot caused by fungi are some of the identified diseases which menace abaca production.

Among the abaca farms in Leyte, the pathogenic fungus *Fusarium oxysporum* f. sp. *cubense,* is the proven causal agent of *Fusarium* wilt also known as Panama wilt infecting the hemp. Severe infection in abaca has been observed due to this pathogen. Abaca growing municipalities in the said province accumulated an almost 65% disease severity (Borines & Baliad 1994). The *Foc* wilt pathogen can survive in the infected area for years. If left unchecked, the pathogens' inoculum level can multiply exponentially with virulence that can potentially wipe out the entire area. With the said risk, it is crucial to take into consideration the ways to control the *Foc* wilt pathogen and minimize its expanding geographic probable density in the Philippines (Magnaye 1996).

In 2012, Borines et al reported a pathogenic bacterium that was proven to be pathogenic to abaca. It causes foul odor, and soft rotting symptoms of the plant's central cylinder, thereby it is named as bacterial heart rot. The pathogenic bacterium was identified as *Dickeya* sp. for it gave positive amplicons once subjected to PCR using *Dickeya* specific primers. Currently, a disease with similar symptoms causing heart rot was observed in Baybay Leyte. The heart rot is purplish in color, has no foul odor, and is not water-soaked (dry). The heart rot recently observed is suspected to be *Fusarium* heart rot. Aside from the heart rot symptom, the abaca plant also showed wilting of the older leaves which progresses to the younger-leaves. Breaking of the leaf base was also observed which are characteristic symptoms of *Fusarium* heart rot infection.

Fusarium sp. infection is manifested by chlorosis, which begins in the older leaves progressing to the younger ones . Vascular bundles develop discoloration, contributing to lower yield and quality of the crop's produced textile (Bastasa & Baliad 2005).

Since *Fusarium* control management is a battle against time, once the pathogen is established no known management practices will eradicate it. *Fusarium* species in abaca is also attributed on the occurrence of the different species, and some species can infect humans (Antimicrobe 2023). Different species of *Fusarium* calls for different management strategies for effective control . Since rapid and reliable pathogen identification is key to the development or use of effective management control, this study was conducted to identify the specific species of *Fusarium* causing the newly observed heart rot in abaca.

MATERIALS AND METHODS

Field Collection of Heart Rot Infected Abaca

The infected abaca plant was collected from an identified farm of Barangay Kabungaan, Baybay City, Leyte where the disease was known to be prevalent. In the field, a thorough inspection of the abaca plants was done accurately to determine those exhibiting the typical symptoms of fungal heart rot disease. When confirmed that it is affected by the *Fusarium* heart rot pathogen, using a bolo knife, the diseased plant was uprooted. The used bolo was disinfected before and after usage. Proper precaution was observed to not further disseminate the pathogen in transporting the infected plant. The uprooted abaca plant was immediately brought to the Plant Disease Diagnostic Laboratory (PDDL) of Visayas State University for the pathogen isolation and characterization.

Laboratory Characterization of Inner Symptom Characterization

Characterization of the pathogen started already prior to its isolation. The vascular bundles of collected diseased plant was examined by cutting the pseudostems to expose their longitudinal and cross section Observed abnormalities were documented as characteristic of the disease. The typical symptoms caused by *Fusarium* heart rot in abaca includes wilting of the older leaves, petiole breaking, and inner core rotting without odor. In the abaca field, the beforementioned symptoms serves as basis in singling out the diseased abaca plant to be collected. After symptoms inspection of the outer and inner parts of the infected abaca plant, it was then prepared for pathogen isolation.

Pathogen Isolation and Culture identification

Identification of the fungal pathogen associated with the heart rot disease of abaca can be identified by isolation and culture purification. Pathogen isolation was conducted at the Plant Disease Diagnostic Laboratory (PDDL), and Mycology Laboratory, Department of Pest Management, Visayas State University. In isolating the pathogen, using a sharp cutter, tissue sections were acquired by thinly slicing the advancing region of the outer sheath next to the rotted core tissues of about $3mm^2$ size.

Sample tissues were surface sterilized with 1% NaOCl for 1 minute, and blot-dried. Air-dried sectioned tissues were rinsed with sterile distilled water and soaked in the lactic acid for three to five minutes to prevent the growth of contaminants.

Tissue sections were equidistantly and aseptically planted on plated PDA culture medium and sealed with parafilm. The cultures were incubated in a dark place to excite sporulation in an inverted position at room temperature. Observation for the mycelial outgrowth was monitored for seven days after isolation. Mycelial outgrowth was monitored seven days after isolation, and sub-cultured for further purification. Pure cultures on slant was kept for future use by periodic sub-culturing, or by storing a 7-day old slant in the refrigerator (Borines & Baliad 1994).

Fungal Colony Characterization

The pure cultures in the PDA slant and petri plates serves as the source for the further sub-culturing of the *Fusarium* pathogen. Using a 3mm cork borer, the fungal colony characterization was accomplished by getting a mycelial plug from a 7-day old petri plate pure culture, then the mycelial plug was placed the center of the freshly plated PDA medium. The plate was sealed with parafilm after the sub-culture process. The growth of the pure culture *Fusarium* mycelial plug in the petri plate was kept under observation for more than 5 days. During incubation, the sub-cultures were observed daily to determine the number of days from initial sub-culture to colony color change from white floccose mycelia to purplish in color colony, and the form of colony margin. The pure culture of the heart rot pathogen was maintained on PDA slant.

Morphological Characterization of the Heart Rot Pathogen

Microscopic characterization can also serve as basis in fungi identification. Morphological characteristics such as conidial form, shape, size, and septation of the *Fusarium* heart rot pathogen were observed under the compound microscope following the agar block technique. In doing the agar block technique aseptically, a mycelial block from the representative 1-week-old, 2-week-old, and 3-week-old culture from the PDA plate were obtained using a 3 mm cork borer. The obtained mycelial plugs were placed on the glass slide topped with cover slip and laid over the capillary tube placed inside a sterile petri plate. An agar block was incubated inside the sterile petri plate at room temperature provided that it is lined with moistened tissue paper to prevent the block from drying up. After three days the top cover slip was detached, and then placed on top of another glass slide with plain lactophenol as the staining agent. It was then viewed under the compound microscope.

Since the cultures in this study were slow-growing, observations on conidia were done on three-week old cultures. The isolate's fungal structures including its hyphal branching, and conidial forms were documented and measured using the dino-lite.

The pathogen *Fusarium proliferatum* is the suspected fungal pathogen that causes heart rot of abaca in Brgy. Kabungaan, Baybay City, Leyte. To confirm the identity of the pathogen in this study, the morpho-cultural characteristics of the isolate were compared to the published characteristics of *Fusarium proliferatum* in the study of Barcos et al 2016; Mohd et al 2013; and Ye et al 2022.

RESULTS AND DISCUSSION

Characteristic Symptoms of Abaca Fusarium Heart Rot

In a farm in Barangay Kabungaan, Baybay City, Leyte (Fig.1) where the disease was previously reported, the heart rot infected standing abaca plant exhibited wilting and premature dying of leaves (Figs. 2A & B). Wilt and death of infected tissues were more observed in older leaves progressing upward to the younger ones resulting in defoliation of the plant with severe infection.

Examinations of the cross and longitudinal sections of the infected pseudostem show vascular rotting and discolorations (Fig. 2 C & Fig. D). Cross sections of the infected abaca plant showed that rotting which started at the inner core which had darker color than the surrounding outer sheaths.

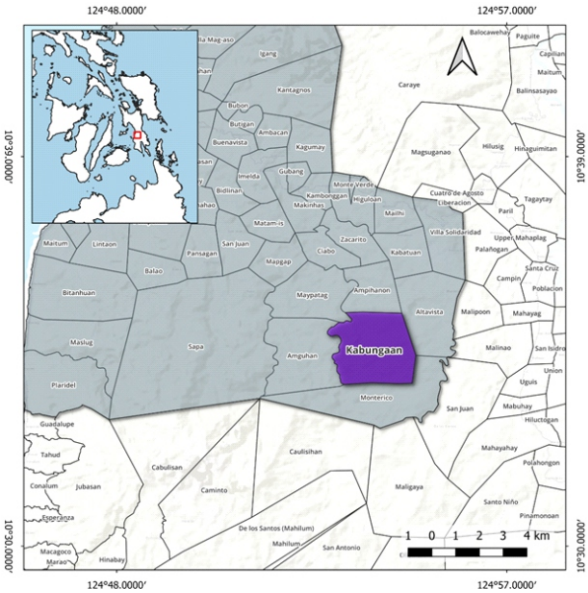

Figure 1. Location of site collection. GIS map with the coordinates where the sample was collected

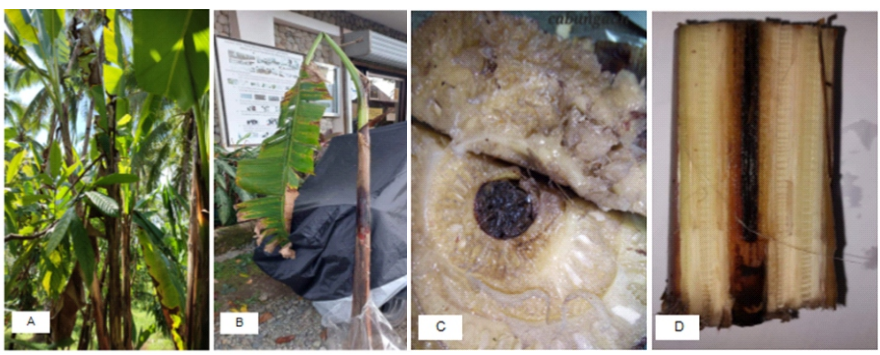

Figure 2. Visual symptoms of Fusarium heart rot in standing abaca plants: (A) wilting and (B)premature dying of leaves, and (C) breaking of petiole and vascular rotting in the inner parts of the pseudostem, (D) longitudinal cross-section of pseudostem showing rotting in the inner core which is more advanced at the base

pineapple which causes lesions, later turned into brown, necrotic tissues at the base of infected pineapple leaves, while advanced symptoms showed infected tissues with dark brown to black margins, that later dried up the leaves.

Environmental conditions may favor the growth of *Fusarium*, the heart rot pathogen. The abaca farm where the diseased abaca plants were collected has an estimated elevation of 300.3 meters above sea level (Philatlas 2023) and a GPS coordinate shown in Figure 1. Generally, it is mountainous having a plateau like topography with an estimated temperature range of 22ºC to 27ºC. In relation to the temperature, *Fusarium* species favored alternating high and low temperatures. It can be inferred that the topography, weather, and temperature range of Brgy. Kabungaan favors *Fusarium* infection. The temperature range in the area is very favorable to the growth of *Fusarium* spp. as shown by the study of Chakrapan et al (2023) confirming that a temperature range of 23–27°C favored the growth of all *Fusarium* isolates studied with the highest growth at 25°C.

Cultural Characteristics of Fusarium Heart Rot Pathogen in Abaca

The fungal colony on plated PDA medium initally started as white cottony mycelia (Fig. 3A). After 2 days of incubation the colony remained white in color spreading having a lobate colony margin. At five-days-old, the mat of colony started to appear as purplish, while the aerial mycelia are white. Thus, from sub-culturing, the heart rot pathogen colony color and pigmentation is the same. Pigmentation starts off as white cottony, based on the advancing region of the young mycelium, which turns to floccose bright purple as it ages, about 7 days post subculture, with lobate colony margin as observed on its underside (Fig. 3B). The colony characteristics of *F. proliferatum* in this study coincides with the isolated *F. proliferatum* of Guevarra et al (2014) (Fig. 3C). Colony characteristics including color are vital in *Fusarium* species identification since colony pigmentation varies from pale, rose, burgundy to bluish violet depending on species (Haruhisa & Mitsuro 2004).

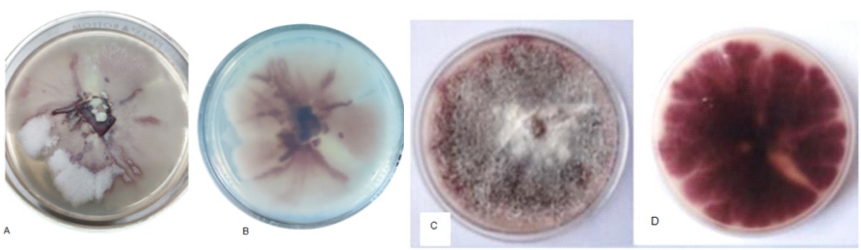

Figure 3. Cultural characteristics of *Fusarium* heart rot pathogen in plated PDA: (A) Cottony white colony with lobate margin at 7 days old, (B) Floccose, bright pruple colony with lobate margin observed on its underside at 7 days after sub-culture, (C and D) surface and underside view of *F. proliferatum* from the study of Guevara et al (2014) as basis for comparison in identifying the *Fusarium* heart rot identification

Morphological Characteristics of Fusarium Heart Rot in Abaca

Further confirmation of the identity of the abaca heart rot pathogen was done through distinctive shapes and sizes of macro- and microconidia, presence and absence of chlamydospores, as well as colony form, pigmentations, and growth rates on agar media. Traditional mycological characterization based on its morphological features such as conidial forms, sizes, septation and the degree (º) of its hyphal branching can be serve as basis.

The morphology of the *Fusarium* heart rot pathogen was studied following the agar block technique which permits observation of a complete fungal structure which can be seen in Figure 5 (A & B), blunt end microconidia, 7 days old, of *Fusarium* measures 6.792 micrometer one week after transferring it to a new plate, with its conidiophore in 45º measuring about 12.897 micrometer. Microscopic observations revealed that the isolates produced microconidia without septa, and its macroconidia measuring 136.680 µm was obtained.

The obtained cultural and morphological characteristics in this study coincides with the study of Barcos et al (2016). In their study, cultures of *F. proliferatum* had initially white, abundant aerial mycelium which turned purple-violet in color as it ages. Also, the fungal pure culture isolated in this study is highly like to be *Fusarium proliferatum* isolated by Hernandez-Arenas et al (2014) from maize (Figs. 3 & Fig. 4).

Figure 4. *Fusarium proliferatum*
pure culture isolate on PDA slant:
(A) surface view (B) underside

Furthermore, with reference to its conidia, as shown in Figure 5, the *Fusarium* heart rot pathogen of abaca produced microconidia without septa in monophialides, and its macroconidia with transverse septa and slightly curved apical cell.

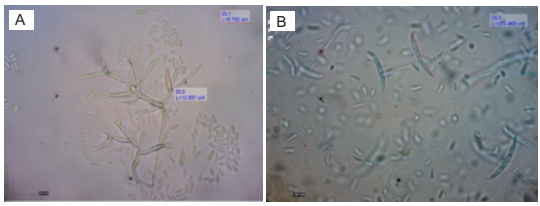

Figure 5. Distinctive characteristics of the fungal heart rot pathogen: (A) phialide with conidial spores (400x), and (B) macroconidia (400x)

The *Fusarium* sp. in this set-up is slow growing, thus microconidia observed were from the 1-week-old culture. According to Mohd et al (2016), it was observed in their study that the microconidia of *Fusarium proliferatum* are hyaline, and aseptate. Also, according to the study of Barcos et al (2016) *Fusarium proliferatum* have polyphialides bearing aseptate, club shaped microconidia on PDA, which measures up to 4.25 to 8.57 × 1.7 to 3.21 µm (average 5.90 × 2.34 µm). With regards to the macroconidia characterization of this study, observations were obtained from the representative 3-week-old culture. The macroconidia are yellowish in color having one to three transverse septa with apical cell slightly curved. The sparse falcate macroconidia with 3-4 septa were 19.4 to 39.2 × 1.9 to 5.2 µm in size (Barcos et al 2016; Mohd et al 2013; Ye et al 2022).

Fusarium proliferatum and *Fusarium moniliforme* have very similar colony morphology, having floccose mycelium which may become greyish violet or magenta with age. The formation of polyphialides by *F. proliferatum* distinguishes the two species. Polyphialides by *P. proliferatum* appeared as false heads (Burgess et al 1994). Also, the microconidia are are oval to club-shaped with blunt end, produced from the branched conidiophore (Fig. 6A).

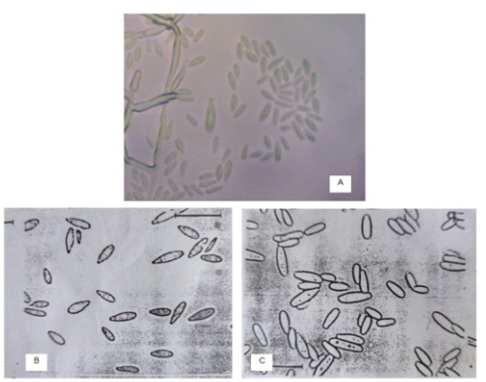

Figure 6. Morphology of *Fusarium* spp. microconidia: (A) Conidia of isolates obtained from this study, (B) conidia of *Fusarium proliferatum* and (C) *Fusarium moniliforme as* illustrated in a manual for *Fusarium* research (Burgess et al 1994)

Incidence of *F. proliferatum* infecting banana was first reported by Jiminez et al (1992) in Malaysia, who isolated the fungal pathogen from the part of the fruits attached to the plant. Banana and abaca belong to genus Musa. According to Zakariah (2022), *Fusarium proliferatum,* which is a member of the *Fusarium fujikoroi* species complex (FFSC), has a range of infection strategies. This pathogen initially infects the roots by entering the plant through wounds or openings progresses upward to the above-ground parts. During the early stage of infection, *F. proliferatum* acts as a biotroph depending on the *Musa* species as host for growth and development, but later turns into a necrotroph which eventually kills the plant host by vascular rotting and leaf petiole wilting.

F. proliferatum belong to the *Liseola* group of the FFC together with *F. verticilliodes* previously *moniliforme*. According to Burgess et al (1994), these species are distinguished by their morphology, the mode of formation of their microconidia, and the morphology of their microconidiophores. *F. proliferatum* has the same characteristics as *F. verticilliodes* except that its microconidia are borne in short chains and possess false heads on both monophialides and polyphialides.

Fusarium heart rot pathogen detection was found ~~to be~~ reliable if it is assessed biologically. The heart rot pathogen causes dry rot which will turn the central vascular bundle to be purplish in color. When cultured as isolate~~d~~ it produces white floccose aerial mycelia which turns purplish as it ages. *Fusarium* is characterized as a large genus of filamentous fungi, part of a group often referred to as hyphomycetes, widely distributed in soil having a wide host range. These fungal species releases toxins such as fumonisins and trichothecenes that ~~can~~ harm host plants. With the right identification, effective management practices cab be developed and employed against the pathogen to minimize or prevent yield loss. Based on the visual symptoms and results of the cultural and morphological studies on the isolates obtained from diseased plants, the pathogen causing the odorless heart rot in abaca is *Fusarium proliferatum*. Thus, the fungal pathogen causing leaf wilting, petiole breaking, and vascular rots in Baybay Leyte which when isolated manifest a purplish mycelium with aseptate blunt end microconidia and sepatated macrocondia is *Fusarium proliferatum*. It is recommended to subject the isolated *Fusarium proliferatum* from abaca in Baybay City, Leyte to sequence the highly conserved gene region for the determination of its heterogenetic ancestor, design PCR primers based on the obtained sequences, and validate the primer for reliable pathogen detection.

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Frontiers in Microbiology 14

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