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Pathogenicity of *Fusarium semitectum* and *Fusarium chlamydosporum* associated with pineapple fusariosis

Nurul Faziha Ibrahim[1,2], Masratul Hawa Mohd[1], Nik Mohd Izham Mohamed Nor[1] and Latiffah Zakaria[1*]

[1]School of Biological Sciences, Universiti Sains Malaysia, 11800 USM Penang, Malaysia.
[2]School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia.
Email: Lfah@usm.my

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ABSTRACT

Aims: Symptoms of pineapple fusariosis has been detected in several pineapple plantations in several states in Peninsular Malaysia. The main aim of this study was to identify *Fusarium* species associated with pineapple fusariosis based on their morphological characteristics and to test their pathogenicity.
Methodology and results: Based on morphological characteristics, two *Fusarium* species were identified, namely *F. semitectum* (41 isolates) and *F. chlamydosporum* (13 isolates). The isolates were isolated from symptoms of pineapple fusariosis. Representative isolates of *F. semitectum* and *F. chlamydosporum* were evaluated for pathogenicity test and the results showed different pathogenic levels on pineapple leaves and fruits of Gandul, Josapine and Moris varieties. *Fusarium semitectum* isolates appeared to be more virulent (D.S.I = 38.89 – 50.00) compared to *F. chlamydosporum* on both pineapple leaves and fruits.
Conclusion, significance and impact of study: The present study indicated that the isolates of *F. semitectum* and *F. chlamydosporum* can cause fusariosis on three pineapple varieties, namely Gandul, Josapine and Moris in Peninsular Malaysia. The presence of both *Fusarium* species causing fusariosis at local pineapple plantations should be monitored and controlled as it would affect the trading of pineapples.

Keywords: *Fusarium semitectum*, *Fusarium chlamydosporum*, fusariosis, pineapple, pathogenicity

INTRODUCTION

Fusariosis is one of the most serious diseases of pineapple cause by *Fusarium* species. The disease infected primarily the fruit but can also affect the whole pineapple plant. Symptoms on the fruits include a light to dark brown discolouration of the fruitlet and dry, rotten or sunken of the fruit skin. Infected leaves showed dry rot, necrosis, bending of the stem and chlorosis (Rohrbach and Schmitt, 1998). Fusariosis of pineapple can spread through infected planting materials and injuries by insects may promote infection of the inflorescence and fruit (Rohrbach and Schmitt, 1998). However, the severity of fusariosis symptoms depends on the inoculum potential of the fungus, pineapple growing region and the weather during harvesting (Matos and Reinhardt, 2009).

Three *Fusarium* species, *F. ananatum*, *F. subglutinans* and *F. guttiforme* have been reported to be associated with fusariosis of pineapple (Rohrbach and Schmitt, 1998; Nirenberg and O'Donnell, 1998; Jacobs *et al*., 2010). In Malaysia, there is no report on the causal pathogen of fusariosis of pineapple although symptoms of fusariosis have been observed in pineapple plantations. Therefore, the present study was conducted to identify *Fusarium* species associated with pineapple fusariosis based on their morphological characteristics and their pathogenicity. Thus, identification of other *Fusarium* spp. as potential fusariosis pathogen is crucial for managing fusariosis at the plantations, and controlling the pathogen movement through quarantine procedure for international trade.

Infected pineapple plant showing one or more fusariosis symptoms as described by Ploetz (2001), Matos (1995), and Rohrbach and Schmitt (1998) were collected from pineapple plantations in the states of Perak, Kedah, Penang, Selangor, Negeri Sembilan and Johor. Common symptoms of pineapple fusariosis observed at the sampling locations were brown discoloration of fruitlet and water-soaked appearance (Figures 1A and B). On the leaves, rotting at the base, necrotic spot, dry rot on the roots and wilting were observed (Figures 1C, 1D, 1E).

*Corresponding author

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Figure 1: Fusariosis symptoms caused by *Fusarium* species observed in the field. A-B, Brown discoloration of fruitlet with water soaked-symptoms; C, Wilt symptom on the leaves; D, Rotting at the leaves base and E, Necrotic leaves spot.

Infected fruit, leave and root were randomly sampled and about 20 – 40 diseased samples were collected from each plantation. Soil samples around the disease plant were also collected for fungal isolation. The diseased tissues were cut and surface disinfected with 1% sodium hypochlorite. The tissues were then rinsed in three changes of sterile distilled water, blotted dry on sterile filter paper (Whatman® No. 1) before plated on peptone pentachloronitrobenzene agar (PPA). Soft and necrotic tissues showing symptoms of fusariosis were directly plated onto PPA. For isolation of *Fusarium* from soil samples, approximately 0.01 g of the soils was evenly distributed on PPA using sterile spatula. The Petri dishes were incubated under alternate light-dark, 12:12 h at 27±1 °C until visible growth of mycelia were observed. The colonies were sub-cultured onto PDA and single spored to obtain a pure culture.

Fusarium isolates were identified based on morphological characteristics according to the method and species descriptions in *Fusarium* Laboratory Manual (Leslie and Summerell, 2006). The main morphological characters observed were the shape and size of macroconidia, microconidia, type of conidiogenous cells and presence of chlamydospores as well as the pigmentation of the colony.

From symptomatic plant parts, a total of 38 isolates of *F. semitectum* were isolated from fruit (n = 15), leaves (n = 11) and root (n = 12), and three isolates were recovered from the soil. Thirteen isolates of *F. chlamydosporum* were isolated from infected roots (n = 7) and soil (n = 6).

In the present study, identification of *F. semitectum* and *F. chlamydosporum* was done using morphological characteristics as both species are listed in 'species list A' of Leslie and Summerell (2006) of which the species in the list can be identified based on morphological characteristics. The morphological and cultural characteristics observed are listed in Table 1 and these characteristics conform to the descriptions of *F. semitectum* and *F. chlamydosporum* in the *Fusarium* Laboratory Manual (Leslie and Summerell, 2006).

Table 1: Morphological characteristics of *F. semitectum* and *F. chlamydosporum* from pineapple fusariois symptoms and soil.

Fusarium species	Microconidia / Mesoconidia	Macroconidia			Conidiogenous cell	Chlamydospore	Pigmentation
		Shape	Apical cell	Basal cell			
F. semitectum	- microconidia are scarce and almost absent - fusoid mesoconidia, 3 septa, appearance of rabbit ears	spindle-shaped, straight to slightly curve ventral-dorsal surface, 3-5 septa	tapered and pointed	foot-shaped	monophialide and polyphialide	abundant, singly and in-pairs	- pale cream, white to brown and brown colony colours - pale cream, pale peach, pale orange to dark brown pigmentation
F. chlamydosporum	- microconidia abundant, singly - straight to comma-shaped microconidia, 0-1 septa	Macroconidia were not found			elaborate polyphialides, branching conidiophores with a tree-like appearance	abundant, singly and in-pairs	- pale peach to grayish rose colony colours - grayish rose, some isolates have pale peach to pink pigmentation

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Figures 2 and 3 show the morphological characteristics of *F. semitectum* and *F. chlamydosporum* isolated from diseased pineapple plant parts and soil. Isolates of *F. semitectum* were identified based on the abundance of spindle-shaped mesoconidia with rabbit ear appearance (Figures 2D and E). As for *F. chlamydosporum*, the isolates were identified based on branching of conidiophores with tree-like appearance (Figures 3C, D, E, F).

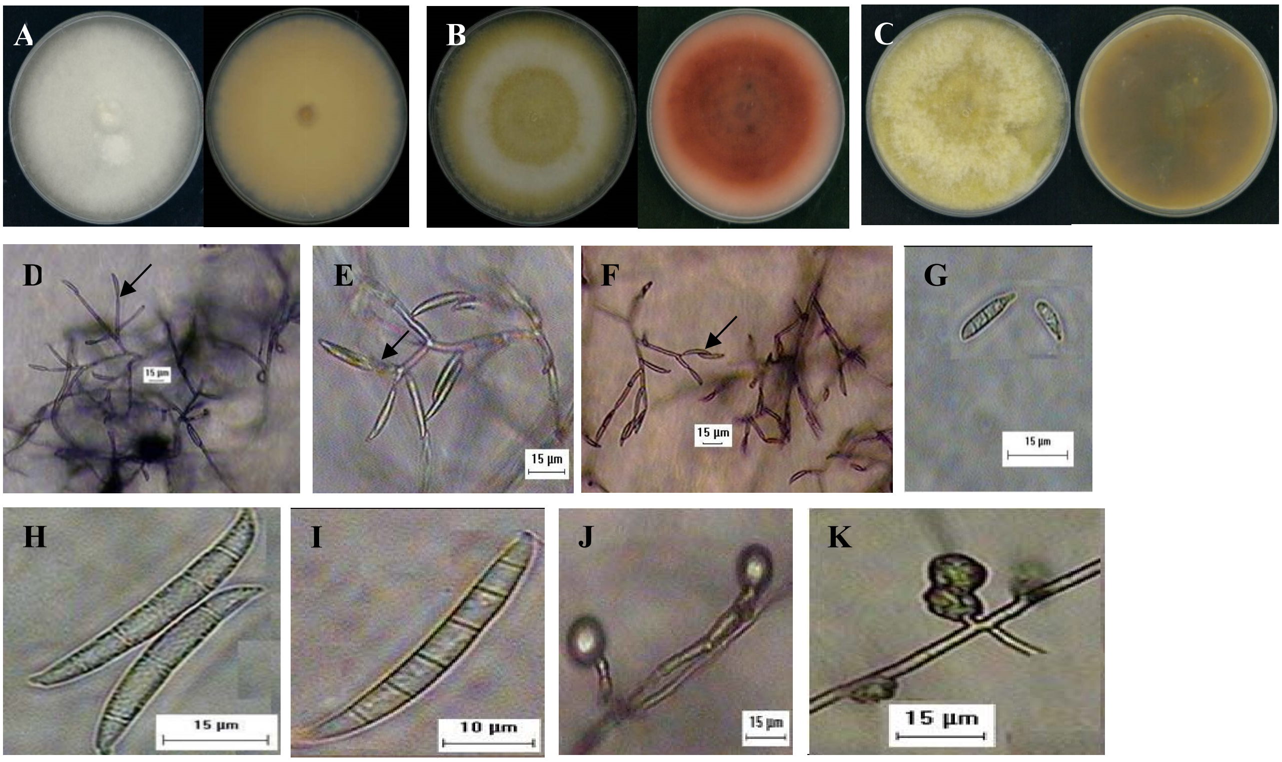

Figure 2: Colony colour, pigmentation, macroscopic and microscopic characteristics of *F. semitectum* isolates. A, White colony with pale cream pigmentation; B, White to brown colony colour with pale peach pigmentation; C, Brown colony with dark brown pigmentation; D-F, Mesoconidia with 'rabbit ear' appearances (arrow); G, Mesoconidia; H, Macroconidia with 3- to 5-septa; I, Macroconidia with 6-septa; J, Singly chlamydospores and K, Chlamydospores formed in pair.

Pathogenicity test was conducted using representative isolates of which four isolates of *F. semitectum* and three isolates of *F. chlamydosporum* were chosen based on different plant parts where the isolates were isolated and locations of the pineapple plantations.

Three pineapple varieties, Gandul, Josapine and Moris were used for pathogenicity test as these varieties are widely planted in Malaysia for direct consumption and processing into canned pineapple. Agar plug technique was applied on the leaves while pricking technique was used on the fruits (Dianese *et al*., 1981). For agar plug technique, mycelia plug (5 mm) was taken from 7 days old culture on PDA and placed on the wounded site of the leaf surface. Sterile fine needle was used to wound the healthy leaf surface. The inoculations were repeated three times at three different locations on the same leaf started from 4 cm from the base. Control was inoculated with PDA plug without the mycelia.

For pricking technique, three sterile tooth picks were placed on PDA plates before sub-cultured with *Fusarium* isolates. The plates were incubated at 27±1 °C for 7 days until the tooth picks were fully colonized by the mycelia. Detached matured fruits were surface sterilized before wounded with sterile tooth pick at approximately one inches deep. The colonized tooth picks were inserted into the wounded area and left intact until the end of the experiment. Control was pricked with sterile tooth pick without mycelia. All inoculated fruits were arranged in sterile container and wrapped with plastic wrapper to maintain moisture content. After two weeks, the fruits were cut vertically and appearance of lesion at the inoculation point was recorded.

The pathogenicity tests were conducted using completely randomized design with three replicates. Appearance of fusariosis symptoms were scored according to scoring scales of 0 to 6 based on Rohrbach and Pfeiffer (1976) with some modifications.

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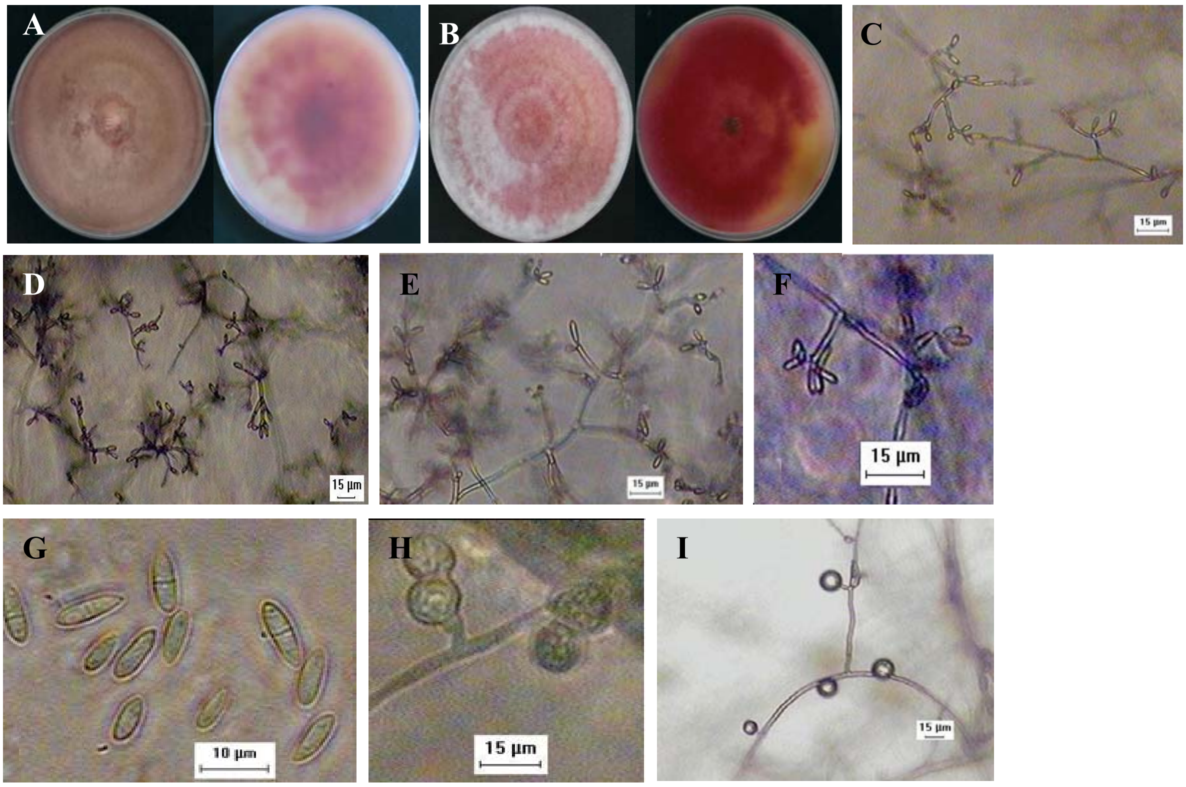

Figure 3: Colony colour, pigmentation and microscopic characteristics of *F. chlamydosporum* isolates. A, Pale grayish rose colony with grayish rose pigmentation; B, Pale grayish rose colony with pale peach to pink pigmentation; C-F, Branching conidiophores with tree-like appearance; G, Microconidia; H, Chlamydospores formed in pair and I, Singly chlamydospores.

Disease severity index (D.S.I) was calculated according to the following formula with n=number of replicates, d= scoring of disease scale and d_{max}= maximum number of disease scale.

$$D.S.I = \frac{\Sigma (n \times d) \times 100}{d_{max} \times \Sigma n}$$

At the end of the pathogenicity test, re-isolation of *Fusarium* isolates from infected fruits and leaves were carried out. The isolates were morphologically identified and compared with the original isolates to confirm Koch's postulates.

Table 2 shows the results of pathogenicity test on pineapple fruits and leaves. On fruits, fusariosis symptoms were observed after 2 weeks of inoculation and produced brown lesion around inoculated area (Figures 4A and B). Representative isolates of *F. semitectum* were pathogenic to the three pineapple varieties (D.S.I = 38.89 – 50.00) except isolate A10137I which was non-pathogenic to Gandul variety. *Fusarium chlamydosporum* isolates were also pathogenic to the three pineapple varieties (D.S.I = 11.11 to 22.22), except isolate A10304I which was non-pathogenic to Gandul variety (Table 2). Inoculated *Fusarium* isolates were re-isolated from the inoculated areas and the control leaves and fruits remain healthy, therefore Koch's Postulates were fulfilled.

For pathogenicity test on the leaves, formation of necrotic spot of brown and dark brown lesion indicating fusariosis symptoms were observed after 35 days after inoculation (d.a.i) on Gandul, and after 21 d.a.i on Josapine and Moris varieties (Figures 4C and D). On Gandul variety, only two isolates of *F. semitectum* (K10124I and A10152I) were pathogenic to the leaves with D.S.I=11.11. On Josapine and Moris varieties, all representative *F. semitectum* isolates were pathogenic to the leaves with D.S.I ranged from 16.67 to 50. None of the representative isolates of *F. chlamydosporum* were pathogenic to Gandul and Moris's leaves, but all the isolates were pathogenic to Josapine's leaves with D.S.I from 27.78 to 38.89 (Table 2).

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Table 2: Disease severity index (D.S.I) on pineapple fruits and leaves.

Isolates	D.S.I on leaves			D.S.I on fruits		
	Gandul	Josapine	Moris	Gandul	Josapine	Moris
F. semitectum						
J10130I	0	44.44	50	5.56	22.22	33.33
K10124I	11.11	50	38.89	5.56	27.78	33.33
A10152I	11.11	44.44	22.22	5.56	38.89	27.78
A10137I	0	33.33	16.67	0	11.11	16.67
F. chlamydosporum						
K10237I	0	27.78	0	11.11	22.22	16.67
A10304I	0	33.33	0	0	16.67	11.11
A10305I	0	38.89	0	16.67	16.67	22.22
Control	0	0	0	0	0	0

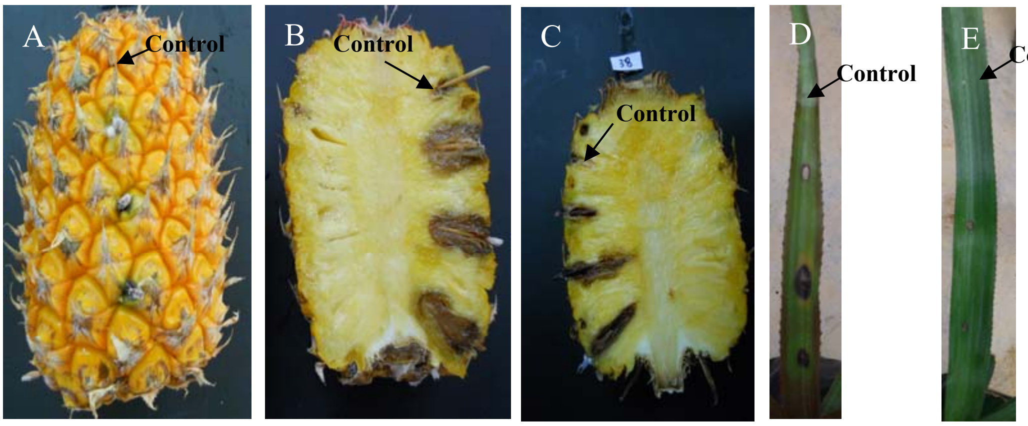

Figure 4: Pathogenicity test on fruits and leaves of pineapple. A, Pathogenicity test on fruit; B, Cross section of pathogenicity test on fruit inoculated with *F. semitectum* isolate; C, Cross section of pathogenicity test on fruit inoculated with *F. clamydosporum* isolate; D, Cross section of pathogenicity test on leaf inoculated with *F. semitectum* isolate; E, Cross section of pathogenicity test on leaf inoculated with *F. clamydosporum* isolate.

Based on pathogencity test, several isolates of *F. semitectum* and *F. chlamydosporum* isolated from diseased fruits, leaves, roots as well as soils are pathogenic causing pineapple fusariosis. Although, *F. semitectum* and *F. chlamydosporum* are not regarded as important plant pathogens, both species have been reported to cause diseases on several crops. *Fusarium chlamydosporum* has been reported causing fruit rot of chili (Krishna *et al*., 2012), wilt of guava (Gupta and Misra, 2012) and damping-off of Aleppo pine (Lazreg *et al*., 2013). Meanwhile, *F. semitectum* has been reported causing blight of kangaroo paw (Satou *et al*., 2001), twig canker of Persian walnut (Belisario *et al*., 2010) and blight and rot of bamboo (Gogoi *et al*., 2013).

So far, fusariosis is attributed to three species in the *Fusarium fujikuroi* species complex namely, *F. guttiforme*, *F. subglutinans* and *F. ananatum.* However, the pathogenicity of *F. ananatum* is not known (Jacobs *et al*., 2010). The symptoms of fusariosis on the fruit caused by *F. guttiforme* and *F. subglutinans* were similar with the symptoms observed in the present study of which the infected fruits produced light to dark brown discolouration and the infected areas become sunken with appearance of mycelia and gum exudation (Rohrbach and Schmitt, 1998).

From our pathogenicity test, pathogenic variations were observed among seven representative isolates inoculated on leaves and fruits which indicated differences in their virulence. The differences in virulence are commonly related to genetic factors which may be conditioned by environmental factors (Narayanasamy, 2011). Between the two species, *F. semitectum* isolates appeared to be more virulent compared to *F. chlamydosporum* isolates on both leaves and fruits. Different levels of susceptibility of the three pineapple varieties, Gandul, Josapine and Moris indicate differences in the ability of the *Fusarium* isolates to colonize the host and establish the disease. Cabral and Coppens (1997) suggested that each pineapple variety have different susceptibility and resistance to fusariosis, therefore, planting of susceptible variety might favor the occurrence of fusariosis. In the present study, Gandul's leaves showed low infection level to fusariosis which was also reported by Aquije *et al.* (2010) on Victoria pineapple

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leaves. A comparison study between tolerant (Victoria) and susceptible leaves (Perola) showed that tolerant leaves have thicker cell wall with high cicatrisation process after inoculation test (Aquije *et al*., 2010) while susceptible leaf possessed higher scales which were suitable for *Fusarium* colonization (Aquije *et al*., 2011). From this study, Gandul variety seems to show better resistance to fusariosis.

Several isolates of *F. semitectum* and *F. chlamydosporum* tested in the pathogencity test were not pathogenic to pineapple leaves and fruits. Presence of non-pathogenic isolates could indicate that these isolates might be saprophyte or endophyte. Saprophytic *F. semitectum* and *F. chlamydosporum* have been regularly recovered from diseased plant parts (Summerell *et al*., 2003). Both *F. semitectum* and *F. chlamydosporum* were commonly isolated as endophytes on many plants. Endophytic *F. chlamydosporum* has been isolated from leaf and stem of a medicinal plant, *Tylophora indica* (Chaturvedi *et al*., 2014) and root of *Dendrobium crumenatum* (Siddiquee *et al*., 2010). Meanwhile, endophytic *F. semitectum* has been recovered from seeds of cowpea (Rodrigues and Menezes, 2005), roots of wild banana (Latiffah and Nur Hidayah, 2011) and from medicinal plant, *Ricinus communis* (Sardul *et al*., 2014).

The results of the present study provide useful information on the occurrence and pathogenic variations of *F. semitectum* and *F. chlamydosporum* associated with pineapple fusariosis on Gandul, Josapine and Moris varieties in Peninsular Malaysia. Due to the ability of these species to produce chlamydospores, efficient strategies should be developed to control and manage pineapple fusariosis. To our knowledge, this is the first report of pineapple fusariosis on Gandul, Josapine and Moris varieties caused by *F. semitectum* and *F. chlamydosporum*.

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