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MORPHOLOGICAL IDENTIFICATION OF *PHYTOPHTHORA* A CAUSAL ORGANISM OF PINEAPPLE HEART ROT DISEASE IN UGANDA

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A B S T R A C T

Pineapple (*Ananas comosus* L. Merr) is an economic horticultural crop in Uganda. However, pineapple production is currently being threatened by the latest outbreak of pineapple heart rot disease in Uganda. Yet, information on pineapple heart rot disease causal pathogen is unknown. Therefore, the objective of this study was to identify the pathogen causing pineapple heart rot disease (PHRD) in Uganda. Samples of pineapple leaves with symptoms of PHRD were collected from four districts of Masaka, Luwero, Mukono and Kayunga. Pathogen isolation was done using corn meal agar (CMA) amended with Pimaricin, Ampicillin, Rifampicin Pentachloronitrobenzene, Hymexazol and Benomyl (PARPHB). Macro- and micro- morphological characteristics of the isolates were assessed using Potato dextrose agar (PDA) and V8 media, respectively. Pathogenicity of the isolates was tested using healthy pineapple plants based on Koch's postulate. The results showed a significant difference ($P<0.05$) in the growth rates, hyphae diameter and asexual structure dimensions of the isolates. Isolates were characterized by a dense rosette and stoloniferous mycelial growth pattern in PDA media. Although, sporangia were non-caduceus, terminal, papillate and mostly ovoid, obpyriform and limoniform sporangia (32-56µm) with a mean length/breadth ratio of 1.32:1µm were observed. Chlamydospores were spherical and thick-walled (25-42µm diameter) while Sporangiophores branching was sympodial. Based on the morphological characteristics of isolates, it was concluded that *Phytophthora nicotianae* is the species causing pineapple heart rot disease in Uganda. This study, therefore, represents the first comprehensive information in Uganda. However, molecular identification of the pathogen is recommended to confirm the genetic identity of the species.

Keywords: *Ananas comosus,* Heart rot, Morphological identification, *Phytophthora.*

INTRODUCTION

Pineapple (*Ananas comosus* L. Merr) is a significant horticultural crop in tropical and subtropical areas of the world. With global production estimated at more than 19 million metric tonnes (MT) in 2011, Thailand is the largest producer of pineapple in the world (FAO, 2013). However, in East Africa, Kenya (539,151MT) is the leading producer country in the region followed by Tanzania (235,000MT), Rwanda (42,800MT) and Uganda (31,000MT), respectively. Though being the lowest producer in East Africa, pineapple is by far the most developed and widely grown commodity in the fruit crop range and value chain in Uganda (Evers *et al.*, 2014). Varieties of pineapple currently grown in Uganda include Smooth Cayenne, Red Spanish and Sasirimu. However, in central Uganda, Majority of farmers grow Smooth Cayenne (Bua *et al.*, 2013). The preference for Smooth cayenne is the ability to ratoon and produce larger juicier and sweet fruits (MUZARD, 2010).

However, the recent outbreak of pineapple heart rot disease in Uganda has been reported to threaten pineapple industry (Ocwa *et al.*, 2016; NARO, 2012). In fact, all areas around Lake Victoria basin Crescent which is a major pineapple producing area in Uganda are at risk. Elsewhere, pineapple heart rot disease is reported to be devastating with the incidence of 25-45% (Shen *et al.*, 2013; Shreenivasa *et al.*, 2015) and associated yield loss of up to 100% (Rohrbach and Schenck, 1985). Several publications indicate that *Phytophthora cinammomi* and *Phytophthora nicotianae* cause pineapple heart rot

disease (Joy and Sindhu, 2012; Shen *et al.*, 2013; Shreenivasa *et al.*, 2015; Rodríguez *et al.*, 2015).

Pineapple heart rot disease manifests as water-soaked lesions with brown streaks on the base of the heart leaves; light brown exudates with foul smell emerging from lesions as rotting occurs and heart leave getting off when pulled. At advanced stages, infected plants collapse, fail to produce fruits and die (Ocwa *et al.*, 2016). Moreover, no sources of resistance to pineapple heart rot disease have been reported nor the identity of the causal organism in some areas established (Rodríguez *et al.*, 2002; Green and Nelson, 2015). This, therefore, makes *Phytophthora* to be the most dangerous pathogen to pineapple (Green and Nelson, 2015). Reports from several authors from India, China and Mexico show that pineapple heart rot disease caused by *Phytophthora cinammomi* and *Phytophthora nicotianae* is responsible for severe economic losses to farmers and Government, respectively (Shen *et al.*, 2013; Rodríguez *et al.*, 2015; Shreenivasa *et al.*, 2015). Whether these two *Phytophthora* species are also involved in pineapple heart rot disease epidemic Uganda is not known (NARO, 2012). Yet, correct identification of the pathogen is critical to the development of appropriate disease control packages (Mbaka *et al.*, 2010; Akrofi, 2015).

Due to lack of information on presence of *Phytophthora,* limited precision of *Phytophthora* species causing heart rot disease of pineapple crop in Uganda and high susceptibility of pineapple varieties, as well as the high risks to pineapple industry, this study focused on determining the presence of *Phytophthora* on pineapple plants and identifying specific *Phytophthora* species causing pineapple heart rot disease in Uganda based on morphological identification of obtained isolates.

MATERIALS AND METHODS

Study area and collection of samples: The study was conducted from April 2016 to August 2016 in the laboratory at the National Crops Resource Research Institute (NaCRRI) Namulonge, National Agricultural Research laboratory (NARL) Kawanda and in the screen house at the Department of Agriculture, Kyambogo University Kampala (1189meters above sea level, 00°20′54″N, 32°37′49″E). All the experiments were repeated twice.

One hundred twenty (120) symptomatic pineapple leaf samples were collected from the four districts of Masaka, Luwero, Mukono and Kayunga in the month of April 2016. The collected samples were packed in paper bags and taken to the laboratory for isolation of possible causal pathogen.

Isolation of *Phytophthora* species: Isolation of pineapple heart rot disease causal organisms was done using cornmeal agar amended with 10mg Pimaricin, 250mg Ampicillin, 10mg Rifampicin, 10mg Benomyl, 25mg Pentachloronitrobenzene (PCNB) and 50mg Hymexazol (PARBPH) as described by Drenth and Sendall (2001). The symptomatic pineapple leaves were washed under running water to eliminate soil. Five (5) mm pieces were cut off the disease lesions between healthy and diseased tissue. The cut tissue pieces were disinfected by immersion in a solution of 70 % ethanol for 3 minutes, rinsed three times with sterile distilled water and dried with sterile paper towels. The dried tissue fragments were plated on cornmeal agar (CMA) amended as above (Drenth and Sendall, 2001; Mounde *et al.*, 2012; Rodríguez *et al.*, 2015). The Petri-plates were incubated at 25 ^{o}C in the dark for 2-3 days (Drenth and Sendall, 2001; Mbaka *et al.*, 2010; Mounde *et al.*, 2012). Pure cultures of *Phytophthora* species were obtained by sub-culturing hyphal tips onto freshly prepared corn meal agar as described above for 2-3 days.

Inoculum preparation and pathogenicity test: *Phytophthora* isolates were induced to sporulate following the protocol described by Jeffers (2006). Zoospore release was induced by incubating agar plugs with sporangia in non-sterile soil extract solution (NS-SES) at 4 °C for 30 minutes to shock the sporangia. Isolates were later placed at room temperature for 10-20 minutes to burst the sporangia so as release zoospores (Saadoun and Allagui, 2008). Required zoospores concentration was adjusted using the method described by Rodríguez *et al.* (2002); (2015).

The pathogenicity of *Phytophthora* isolates recovered from the infected plant tissues was confirmed by inoculating them on two-month-old healthy smooth cayenne pineapple plants (Shen *et al.*, 2013), grown out in the screen house to confirm that they were healthy (Palmucci *et al.*, 2013; Shen *et al.*, 2013). The base of the heart leaves of pineapple plants were surface sterilized with 70% alcohol and blot dried. The disinfected basal heart leaves were inflicted with four wounds and inoculated with 10^{8} zoospores ml-1 (Rodríguez *et al.*, 2015). Control pineapple leaves were wounded and inoculated with 4 ml of sterile distilled water. The inoculated pineapple plants were laid out in a complete randomized design with three replications. Pineapple plants were left under normal day

and night cycle of illumination for three months in a screen house. Pineapple plants were monitored on a daily basis for one month (Rodríguez *et al.*, 2015) for pineapple heart rot disease symptom appearance. Once the symptoms appeared, information was recorded and plants left in the screen house.

Morphological identification: Pathogenic *Phytophthora* isolates were identified based on macro morphological characteristics (growth rate and colony morphology) on PDA media and micro morphological characteristics (sporangia shape and size, chlamydospore diameter, sporangiophore branching and hyphae diameter) on V8 media induced using Non sterile soil extract solution (NS-SES) (Drenth and Sendall, 2001; Hüberli *et al.*, 2001; Mbaka *et al.*, 2010). Recorded features were compared with known characteristics in published identification keys (Waterhouse, 1963; Hall, 1993; Erwin and Ribeiro, 1996; Drenth and Sendall, 2001) as well as with the data from recently published papers describing *Phytophthora* (Mbaka *et al.*, 2010; Mounde *et al.*, 2012; Pao-Jen *et al.*, 2015; Palmucci *et al.*, 2013; Rodríguez *et al.*, 2015).

Radial growth rate and mycelium growth pattern: This was done using potato dextrose agar (PDA) as described by Drenth and Sendall (2001). Individual hyphal tips were cut from the edge of 37 actively growing colonies that caused pineapple heart rot disease symptoms, plated on corn meal agar (CMA) and grown for 3 days following the procedure of Mbaka *et al.* (2010). After growth, 5mm agar discs were cut from the edge of actively growing colonies using a sterile cork borer and placed with the mycelia facing downwards in the centre of Petri plates containing 10 ml of Potato Dextrose Agar (PDA). The plates with mycelial plugs were sealed with parafilm and incubated at 25°C in the dark for 7 days (Mounde *et al.*, 2012). The plates were arranged in a complete randomized design (CRD) with three replications for each isolate. Radial growth of the growing colonies was measured daily for one week along two lines intersecting at right angles at the centre of the inoculum disc (Fenn, 1984).

Sporangia production and morphology characterization: Test isolates were induced to sporulate by floating agar plugs of each isolate in non-sterile soils extract solution (NS-SES) (Jeffers, 2006). Non-sterile soils extract solution was prepared by mixing fifteen grams (15grams) of the loam soil (free from *Phytophthora* and collected from a field where trees were previously growing) with one litre of distilled water in a bottle and shaken for five hours using a rotary shaker. The suspension was allowed to settle overnight. The supernatant was decanted and centrifuged in falcon tubes of 50mls for 6 minutes at 6000rpm. Later, the supernatant was filtered through Whatman filter paper to remove floating organic debris. The non-sterile soil extract soil solution (NS-SES) was stored for 3days in a glass bottle to allow it to age in the refrigerator at 4 ˚C (Jeffers, 2006).

Each *Phytophthora* isolate was grown on 10% V8 juice agar at 25 °C in the dark for 3 days (Jeffers, 2006). The agar plugs (5mm) of young actively growing mycelia were cut, plated in 9 cm diameter petri dish floated covered with NS-SES (Drenth and Sendall, 2001; Jeffers, 2006). The flooded plates were incubated under continuous fluorescent light (18W, cool light) suspended 18cm above the cultures at room temperature (25˚ C) in order to induce production of sporangia. After 24hours, individual plugs of each isolate were mounted on glass slides and observed under a light microscope (Zeiss, German) for the presence of sporangia. Sporangia associated features for characterization like sporangia shape, sporangia papillation and sporangiophores branching were examined at ×400 magnification and recorded following the descriptors of Erwin and Ribeiro (1996) and Drenth and Sendall (2001). Chlamydospore and hyphae diameter was also examined (Palmucci *et al.*, 2013; Rodríguez *et al.*, 2015).

Sporangia length and width/breadth, chlamydospore and hyphae diameter were measured and recorded using the Zeiss camera (German) and software motic images (Asia) (Rodríguez *et al.*, 2015). Thirty to fifty sporangia per isolate were selected and their sizes measured (Milenković *et al.*, 2013).

Data analysis: Data on radial growth rate, colony diameter and asexual structure dimensions were summarized and their means subjected to analysis of variance (ANOVA) using Genstat (15th edition). Where there were significant differences, means were separated using Least Significant Difference (LSD) test at 5% probability level. Sporangia shape, papillation and sporangiophores branching frequencies were also recorded.

RESULTS AND DISCUSSION

All isolates recovered were identified as *Phytophthora nicotianae* (Figure 1). The highest (42%) number of *Phytophthora nicotianae* isolates was recovered from Kayunga district followed by Luwero district (39.5%), Mukono (10.5%) and lastly Masaka (8%) (Table1). The cause of the variation in prevalence was not clear but could have been attributed to varying soil drainage

conditions of the four districts.

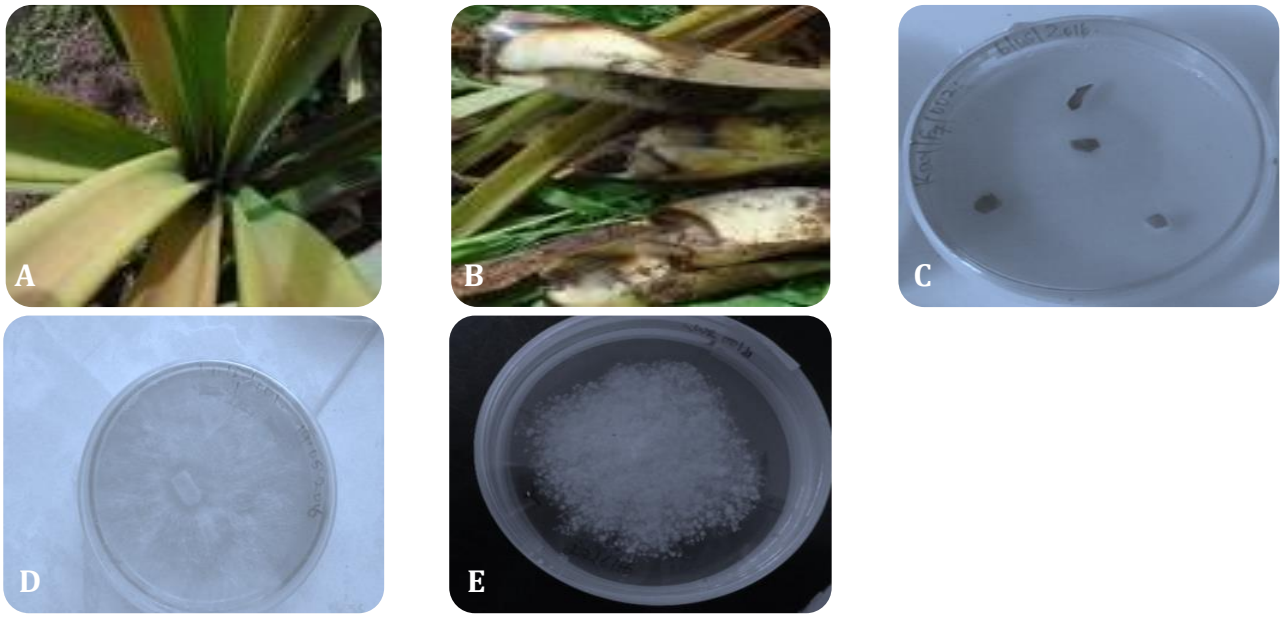


Figure 1. Isolation of PHRD causal organisms. A) Symptomatic pineapple plant in the field. B) Pineapple leaves extracted from PHRD infected plants in the field during survey. C) Infected pineapple leaf fragments plated on amended CMA. D) Pure culture of *Phytophthora* species growing in CMA. E) Isolate of *Phytophthora* species growing in PDA media.

Table 1. Origin and pathogenicity of *Phytophthora* isolates in the screen house at Kyambogo University, 2016.

District	Isolate	Pathogenicity	District	Isolate	Pathogenicity
Kayunga	KAY 01	++	Luwero	LUW 04	++
Kayunga	KAY 02	++	Luwero	LUW 05	++
Kayunga	KAY 03	++	Luwero	LUW 06	++
Kayunga	KAY 04	++	Luwero	LUW 07	++
Kayunga	KAY 05	++	Luwero	LUW 08	++
Kayunga	KAY 06	++	Luwero	LUW 09	++
Kayunga	KAY 07	++	Luwero	LUW 10	++
Kayunga	KAY 08	++	Luwero	LUW 11	++
Kayunga	KAY 09	++	Luwero	LUW 12	++
Kayunga	KAY 10	++	Luwero	LUW 13	++
Kayunga	KAY 11	++	Luwero	LUW 14	++
Kayunga	KAY 12	++	Luwero	LUW 15	++
Kayunga	KAY 13	++	Masaka	MAS 01	++
Kayunga	KAY 14	++	Masaka	MAS 02	++
Kayunga	KAY 15	++	Masaka	MAS 03	++
Kayunga	KAY 16	++	Mukono	MUK 01	++
Luwero	LUW 01	++	Mukono	MUK 02	++
Luwero	LUW 02	++	Mukono	MUK 03	++
Luwero	LUW 03	++	Mukono	MUK 04	--

++ Pathogenic, -- Non-pathogenic, MAS: Masaka, KAY: Kayunga, MUK: Mukono, LUW: Luwero

Mounde *et al.* (2012) from Kenya reported that prevalence of *Phytophthora nicotianae* depends on the conditions from the geographical locations where the pathogens in found. Symptoms characteristic of pineapple heart rot disease were observed within seven days after inoculation of the pineapple plants. The

disease manifested as a pale green colour and necrosis on the tips of the leaves followed by heart rot, browning of the base of the middle leaves coupled with the foul smell (Figure 2). Of the 114 inoculated pineapple plants, 111 (97%) developed the disease. However, control plants did not develop the disease (Figure 2). When re-isolated from the infected plants, the pathogens were able to re-infect the inoculated plants (healthy pineapple plants) producing symptoms as above in accordance with Koch's postulates. Additionally, 97% (37/38) isolates inoculated in pineapple plants produced symptoms characteristic of pineapple heart rot disease (Table 1).

High pathogenicity was due to high aggressiveness by *Phytophthora* isolates. This in agreement with a report by Rodríguez *et al.* (2015) that the majority of *P. nicotianae* isolates were pathogenic to pineapple plants upon inoculated.

The water soaked lesions at the base of the leaves and the heart of the pineapple plants foul smell was a result of physical hyphae penetration that allows entry of other secondary organisms (fungi and bacteria) which cause oxidation hence accumulation of cellular degradation residues (Rodríguez *et al.*, 2015). *Phytophthora* zoospores are attracted to invade elongation and differentiation zones producing progressive symptoms (Galiana *et al.*, 2005; Attard *et al.*, 2010; Rodríguez *et al.*, 2015).

Figure 2. Pathogenicity testing of *Phytophthora* isolates causing PHRD in the screen house, Kyambogo University, 2016. A) Asymptomatic control pineapple plant month after inoculation. B) Asymptomatic pineapple plant close to three months after inoculation (Non-pathogenic). C) Pineapple plants showing pale green colour and heart rot disease signs seven days after inoculation. D) Pineapple plant exhibiting symptoms of PHRD 21 days after inoculation. E) Water soaked pineapple plant heart leaves exhibiting symptoms of PHRD 21 days after inoculation. F) Completely infected pineapple plants close to three months after inoculation.

Pathogenicity of the pathogen isolates relates to the ability to produce high sporangia numbers. According to Shearer and Shea (1987), sporangial size and number have a relationship with pathogenicity. Thus, the ability of an isolate to produce greater numbers of sporangia may provide it with the potential to release more zoospores making it more virulent (Mbaka*et al.,* 2010). This coincides with the results of this study where all isolates produced a considerable number of sporangia (Figure 3). Significant differences ($P<0.05$) in growth rates amongst the isolates on PDA media was observed. In the first day, the highest and lowest colony

diameters of 4.0 mm (KAY 10) and 1.2mm (LUW 11) were recorded respectively.

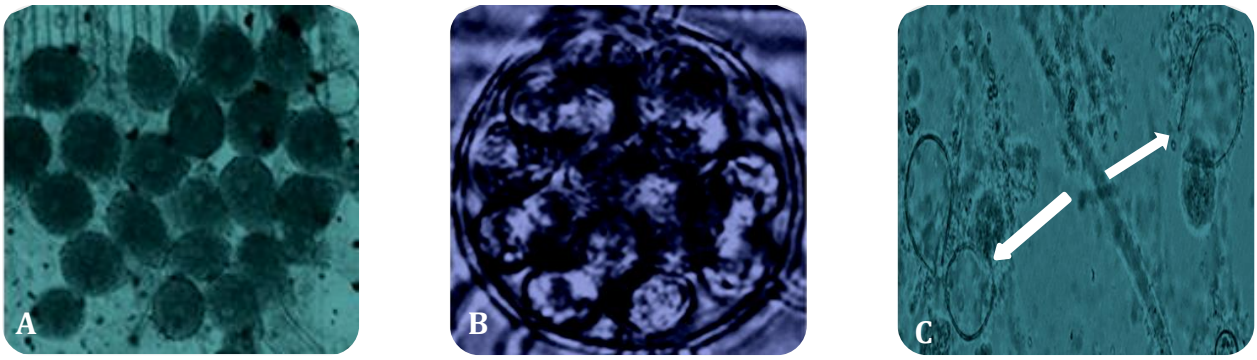


Figure 3. Zoospore release by *Phytophthora nicotianae* isolates A) Multiple sporangium production by isolates after being flooded with NS - SES. B) Differentiation of the sporangium cytoplasm to release zoospores. C) Empty sporangia that released zoospores

By the seventh day, the highest and lowest colony diameters of 42mm (KAY13) and 20.17mm (LUW 12) respectively were recorded. Overall, the average colony diameters were 2.65mm and 25.83mm for the first and seventh days, respectively (Table 2). Results for the second and sixth days followed a similar trend (Table 3). All pineapple heart rot disease isolates formed a dense rosette growth pattern with white stoloniferous colonies on PDA (Figure 4). This agrees with the report of Drenth and Sendall (2001). Similarly, Palmucci *et al.* (2013) reported that *P. nicotianae* produces a dense rosette growth pattern on PDA. The sporangia dimensions (length and width), as well as the chlamydospore and hyphal diameters, were significantly different ($P<0.001$) amongst all the isolates from the four districts.

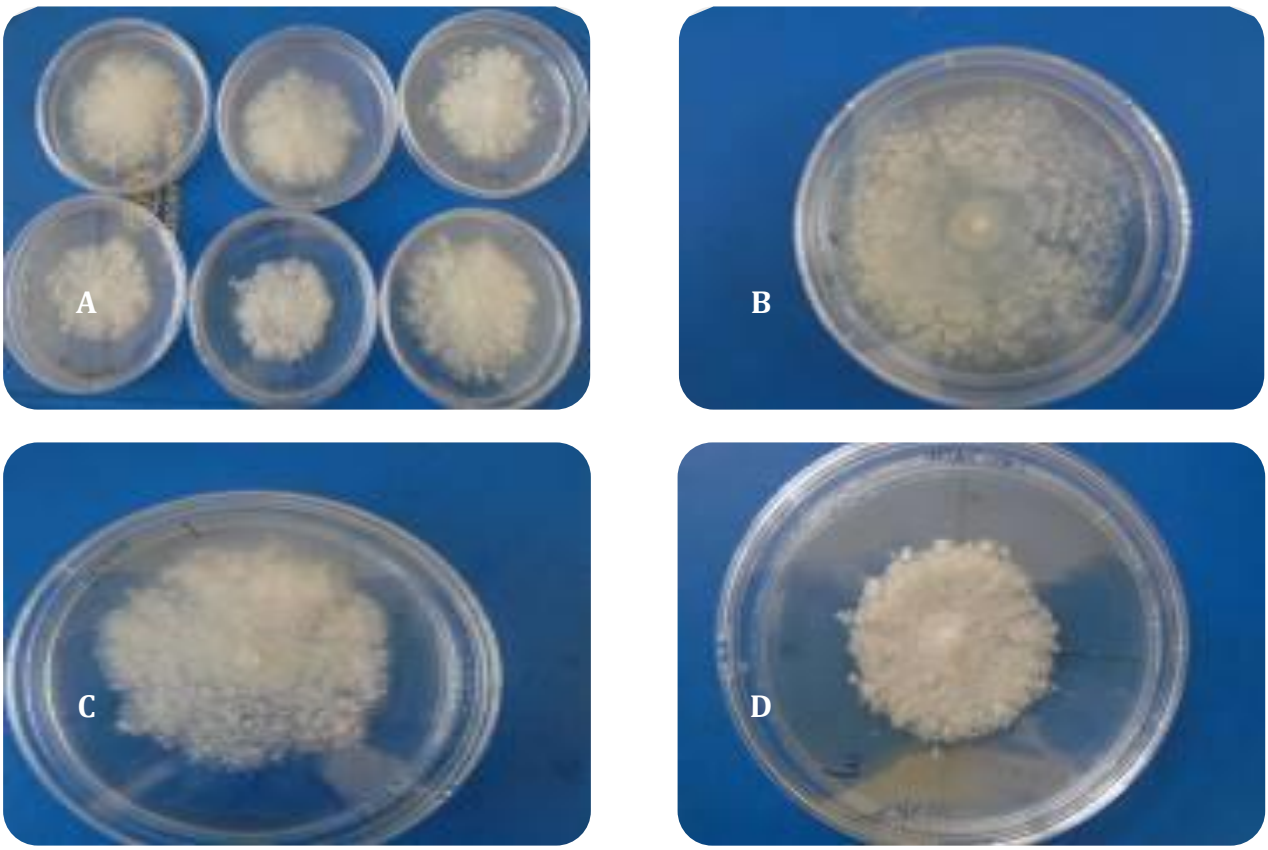


Figure 4. Colony characteristics of selected isolates of *Phytophthora nicotianae* growing in PDA. **A -D)** Isolates showing dense growth pattern in PDA.

Table 2. Colony diameter of *Phytophthora nicotianae* isolates grown on PDA for a period of seven days at National Agriculture Research Laboratories, 2016.

Isolates	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
KAY 01	2.17	4.00	7.67	12.00	16.67	22.67	29.00
KAY 02	1.83	4.83	7.50	12.00	14.33	18.00	21.33
KAY 03	2.33	4.83	6.50	10.33	13.50	16.83	21.17
KAY 04	2.67	5.17	8.00	12.33	15.83	21.83	23.83
KAY 05	2.33	6.00	8.67	12.50	19.17	33.00	20.50
KAY 06	1.83	4.33	7.33	12.33	17.50	17.50	24.17
KAY 07	2.67	5.33	7.50	13.70	16.37	18.33	25.00
KAY 08	1.17	4.50	6.33	8.33	11.33	22.00	19.17
KAY 09	2.17	5.33	7.00	13.50	17.17	23.50	29.00
KAY 10	4.00	8.00	17.00	22.00	27.17	35.00	39.00
KAY 11	2.63	4.50	6.67	13.67	14.50	17.33	22.33
KAY 12	3.33	11.4	16.20	20.33	25.2	28.83	31.83
KAY 13	4.50	13.2	18.30	15.50	31.00	36.83	42.00
KAY 14	1.65	4.51	6.50	13.01	15.17	18.33	22.17
KAY 15	3.18	5.50	8.50	12.33	15.33	18.67	21.17
KAY 16	2.17	4.83	7.00	11.17	13.17	18.17	22.50
LUW 01	3.67	6.00	8.00	11.00	14.00	18.17	21.33
LUW 02	1.83	4.67	6.33	9.83	13.33	16.50	20.33
LUW 03	2.17	4.83	7.67	10.83	17.00	17.33	26.80
LUW 04	1.83	6.67	8.88	13.67	16.00	21.33	26.50
LUW 05	1.67	4.50	6.50	13.00	15.17	18.33	22.17
LUW 06	2.00	5.33	8.17	16.00	18.00	22.00	25.67
LUW 07	2.00	5.5	7.00	12.67	16.83	21.33	25.50
LUW 08	3.83	8.33	10.2	16.83	20.50	24.83	27.67
LUW 09	1.87	5.50	7.83	15.17	18.17	22.90	27.00
LUW 10	1.17	5.50	7.67	12.67	17.17	18.33	22.17
LUW 11	1.20	7.83	13.0	21.85	26.67	32.67	34.83
LUW 12	2.00	4.67	6.67	11.83	14.83	16.67	20.17
LUW 13	1.67	4.17	7.50	13.17	17.2	18.33	22.83
LUW 14	2.50	6.00	8.83	15.17	18.33	21.5	24.67
LUW 15	8.00	13.00	15.00	19.00	22.00	24.00	28.00
MAS 01	5.00	10.00	14.00	18.00	22.00	24.00	25.00
MAS 02	5.00	11.00	15.00	19.50	23.00	26.50	28.00
MAS 03	2.13	5.17	9.00	15.33	18.50	21.00	25.50
MUK 01	3.00	10.1	15.2	20.83	26.26	31.50	35.27
MUK 02	2.67	5.17	6.83	13.50	15.00	17.50	20.50
MUK 03	1.33	7.67	10.00	13.17	22.67	26.23	27.83

Mean	2.65	6.48	9.43	14.31	18.36	22.48	25.83
LSD $_{(5\%)}$	1.05	1.38	1.62	2.33	2.48	2.39	3.38
CV(%)	10.1	4.8	6.5	3.6	2.5	3.6	2.2

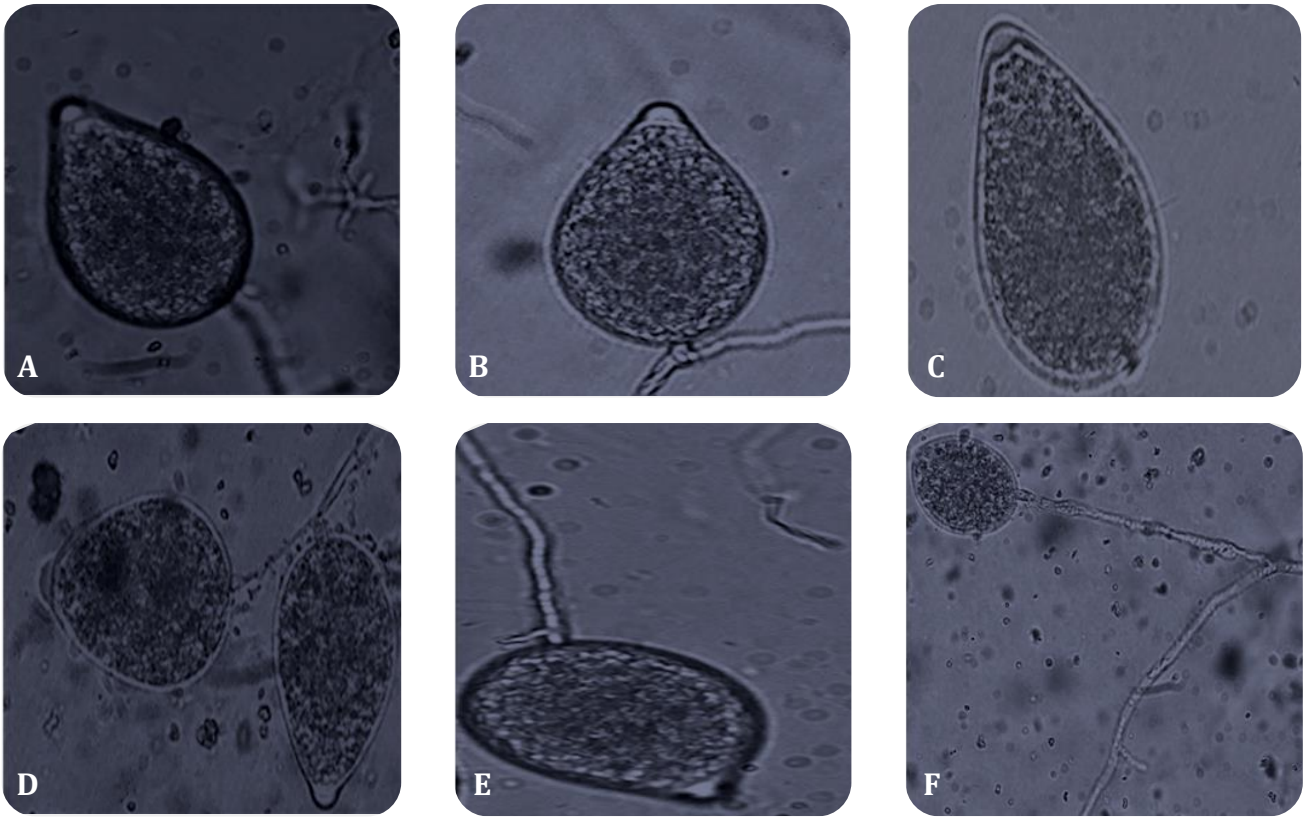
Significant differences (P<0.05).

Table 3. Micro morphological characteristics of 37 *Phytophthora nicotianae* isolates isolated from pineapple heart rot disease infected pineapple leaves at National Agricultural Research Laboratories, 2016.

Isolate	Sporangia length (µm)	Sporangia breadth (µm)	Sporangia Length/breadth ratio	Chlamydospore diameter (µm)	Hyphae diameter (µm)
KAY 01	51.1	37.6	1.35 : 1	32.0	6.0
KAY 02	51.7	40.4	1.27 : 1	27.0	7.5
KAY 03	46.1	34.0	1.36 : 1	37.5	5.0
KAY 04	56.0	40.0	1.40 : 1	38.0	6.0
KAY 05	55. 0	41.5	1.33 : 1	42.0	6.0
KAY 06	45.7	37.5	1.22 : 1	41.0	6.7
KAY 07	52. 0	37.5	1.38 : 1	32.0	6.0
KAY 08	33.2	30.85	1.11 : 1	41.0	6.5
KAY 09	52. 0	37.5	1.39 : 1	32.0	6.0
KAY 10	42. 0	30.3	1.39 : 1	40.0	3.0
KAY 11	54. 0	42.0	1.29 : 1	No	4.0
KAY 12	47.3	37.2	1.27 : 1	37.5	5.7
KAY 13	46.7	33.0	1.42 : 1	24.7	6.0
KAY 14	54.7	42.0	1.30 : 1	30.7	6.0
KAY 15	50.3	39.0	1.28 : 1	40.0	3.5
KAY 16	50.3	39.0	1.28 : 1	40.0	3.5
LUW 01	47.6	34.0	1.40 : 1	25.0	6.0
LUW 02	50. 0	38.5	1.29 : 1	28.0	5.5
LUW 03	51.8	35.5	1.46 : 1	30.0	4.5
LUW 04	51.8	35.5	1.46 : 1	30.0	4.5
LUW 05	56. 0	40.0	1.40 : 1	38.0	6.7
LUW 06	57.5	39.0	1.47 : 1	40.0	3.3
LUW 07	52.3	42.0	1.25 : 1	40.0	6.7
LUW 08	51.8	35.5	1.46 : 1	40.0	4.5
LUW 09	51.3	39.5	1.29 : 1	38.5	4.0
LUW 10	52. 0	41.0	1.27 : 1	39.5	7.5
LUW 11	45.7	36.0	1.27 : 1	41.0	6.5
LUW 12	45.7	35.7	1.28 : 1	39.0	6.2
LUW 13	51.7	40.7	1.27 : 1	39.0	7.0
LUW 14	51.3	40.3	1.27 : 1	33.7	6.0
LUW 15	47.5	37.0	1.28 : 1	33.5	5.0
MAS 01	46. 0	39.0	1.17 : 1	37.0	5.0
MAS 02	47. 0	39.5	1.21 : 1	37.0	4.5
MAS 03	52. 0	38.5	1.35 : 1	32.0	6.0
MUK 01	42. 0	30.0	1.40 : 1	38.0	5.0
MUK 02	52.3	41.0	1.29 : 1	38.6	6.0

MUK 03	52. 0	44.5	1.17 : 1	41.0	8.6
Means	49.8	38.1	1.32:1	37.6	5.6
LSD (5%)	6.79	0.75		5.91	0.72
CV (%)	8.30	1.22		10.17	7.75

The widest and narrowest hyphae diameters were recorded from isolates MUK 04 (8.6 µm) and LUW 01 (3.0µm). Overall, the average hyphae diameter was 5.6µm (Table 3). The isolates produced coralloid aseptate hyphae (Figure 5). Although sporangia shapes were mostly ovoid, other shapes such as limoniform and obpyriform were also common. Additionally, sporangia were all non-caducous and papillate, with prominent pedicels. Sporangiophores branching was compound sympodium for all the isolates (Figure 5). The longest and shortest isolate sporangia lengths were 57.5µm (LUW 06) and 33.2µm (KAY 08) respectively. The average sporangia length was 49.8µm. In contrast, the widest and narrowest sporangia breadths were 44.5µm (MUK 03) and 30µm (MUK 01), respectively. The average sporangia breadth was 38.1µm. Accordingly, the length to breadth ratio was 1.32:1. Overall, the highest proportion (97%) of the isolates produced chlamydospores with a diameter ranging from 25-42µm in contrast to 3% that did not produce chlamydospores (Table 3).



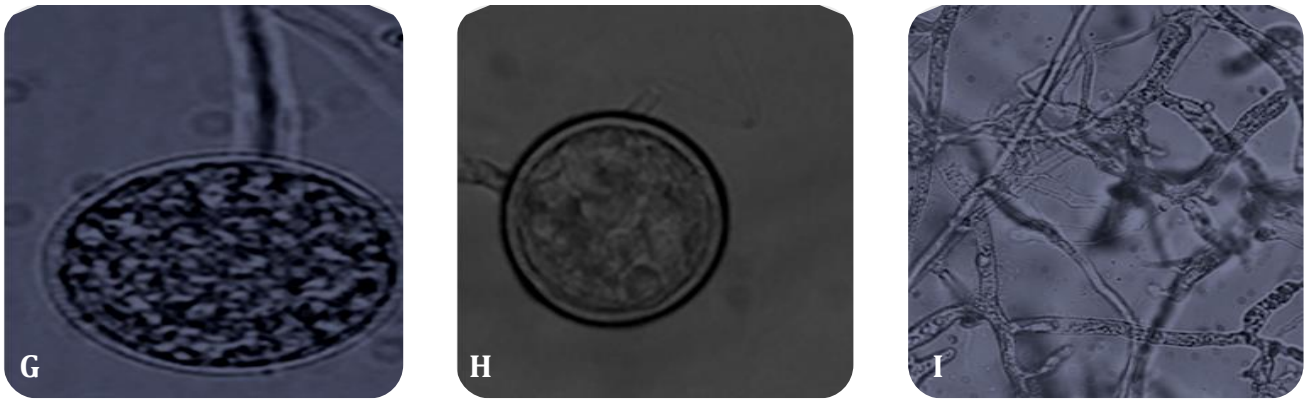


Figure 5. Micro morphological features of *Phytophthora nicotianae* isolates. A and B) Limoniform Sporangia. C) Ovoid sporangium. D and E) Papillate sporangia. F) Sympodium sporangiophore branching. G and H) Terminal chlamydospores. I) Aseptate hyphae.

Phytophthora nicotianae was characterized mostly by ovoid papillate non caduceus sporangium (32.4-56×30-41.5µm, length/breadth ratio of 1.32:1). Hyphae were coralloid and sporangiophores were sympodially branched. Also, the largest proportion of isolates (97%) produced terminal chlamydospores (25 to 42 µm in diameter) with the exception of one isolate (KAY 11). Earlier, reports indicated the presence of intercalary and terminal chlamydospores in *P. nicotianae (Hall, 1993)*. Similarly, Palmucci *et al.* (2013) reported that *P. nicotianae* produced persistent, mono- and sometimes bipapillate, spherical to ovoid, ellipsoid, obpyriform sporangia (28–54×42–46 µm; length/breadth ratio of 1.3:1). However, terminal and intercalary chlamydospores (25 to 48 µm in diameter) and sexual structures were not observed in Argentina. Similarly, a report from Taiwan indicated that *P. nicotianae* produced sporangia with length 40 (48.2) - 55µm breadth of 30 (36.6) – 45µm and length to breadth ration of 1.06 – 1.43µm. The chlamydospores diameter was between 30-45 µm (Pao-Jen *et al.*, 2015). Gallup *et al.* (2006) in North Carolina indicated that hyphae of *P. nicotianae* were irregular with the width of 3-11µm with few numerous hyphal swellings. Sporangia were ovoid, pear-shaped, or spherical, very conspicuous papillae (Sizes of sporangia vary (18-70 x 14-39 µm) with isolate and the growth medium and chlamydospores range from 13 to 60 µm in diameter which coincides with the findings of this study. In China, Tao *et al.* (2011) reported that *P. nicotianae* produced non-caduceus, terminal, papillate and mostly obpyriform sporangia, av. 46.2x34.9 µm with the mean length/breadth ratio as 1.34:1Chlamydospores were spherical, thick-walled (av. 30.2 µm diameter. All this is in agreement with the findings of this study.

The variation in macro-morphological and micro-morphological characteristic was due to variation in temperature and soil drainage conditions where samples were picked. Earlier, Mbaka *et al.* (2010) in Kenya and Bernadovicová and Juhásová (2005) in Slovakia reported phenotypic variations and sporangial dimensions within isolates of *Phytophthora.* These variations aid better understanding of the causal pathogen population for the development of management strategies for a disease (Mbaka *et al.*, 2010).

The fact that isolates tested induced characteristic heart rot disease symptoms on inoculated pineapples confirms that in particular, *Phytophthora nicotianae* is a principal causative agent of pineapple heart rot disease in Masaka, Mukono, Luwero and Kayunga districts of central Uganda. This study confirms previous studies from other parts of the world that pineapple heart rot disease is caused by *Phytopthora* species (Shen *et al.*, 2013; Rodríguez *et al.*, 2015; Shreenivasa *et al.*, 2015).

The identification of all isolates from the four districts of Uganda as *Phytophthora nicotianae* has confirmed that the pathogen is wide spread in central Uganda. The high prevalence of *P. nicotianae* could be attributed to favourable temperature and poorly drained soils coupled with high rainfall in these areas that favours the survival and spread of *Phytophthora*. Shreenivasa *et al.* (2015) in India reported that inoculum levels of *Phytophthora* are usually very high during the rainy season.

Phytophthora nicotianae being reported as one of the most destructive pathogens that cover a wide ecological habit calls for an immediate intervention because the

tropical regions where Uganda falls are among the major pineapple production areas. According to many authors, *Phytopthora* pathogens are very destructive to crops and their wide spatial distribution makes management difficult (Joy and Sindhu, 2012).

CONCLUSION AND RECOMMENDATION

Phytophthora nicotianae is the causal organism of pineapple heart rot disease in Lake Victoria Crescent basin in Uganda. This, therefore, provides a baseline on the development of management of options for pineapple heart rot disease in Uganda. Because morphological characteristics are plastic and influenced by environment, it is recommended that molecular identification of *Phytophthora nicotianae* be done.

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