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Pythium aphanidermatum root rot of pawpaw (*Carica papaya* L.) in Nigeria

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Abstract. Root rot of pawpaw (*Carica papaya* L.) reported in Nigeria is caused by *Pythium aphanidermatum* which was consistently isolated from diseased plant parts and highly pothogenic. Out of 16 different media tested, it grew best on corn-meal-agar (CMA) and CMA supplemented with cellulose and sucrose. The highest number of oospores/ml was on CMA with average diameter of 19.9 ± 0.1 μm. The symptom is characterized by dark brown rot of roots, absence of secondary roots and disintegration of internal tissue of the main root. These cause the progressive decline of the aerial parts of the tree untill it dies.

Key words: Pawpaw (*Carica papaya* L.), Root rot, *Phythium aphanidermatum*, Nigeria

Introduction

Pawpaw (*Carica papaya* L., fam. Caricaceae) is an important component of diet in the tropics, containing proteins, vitamins A and C [1, 2] and is also a commercial source of papain [3] used in brewing and leather industries. Pawpaw is the third most important horticultural fruit consumed in Nigeria after citrus and Plantain [4]. In the National Fruit Development Project, Pawpaw is a selected crop, but production falls short of supply compared with other horticultural crops. This setback may be directly linked with root rot of pawpaw in Nigeria.

Root rot of pawpaw caused by *Phytopthora nicotianae* van Breda de Haan, *Phytophthora palmivora* (Butler) Butler and *Pythium aphanidermatum* (Edson.) Fitz Pat., has been cited as the most important disease of root rot of pawpaw in Hawali [5–7], Costa Rica [8], Mexico [9], and in India [10], where it is widely distributed and caused severe damage.

Recently, there has been a serious outbreak of pawpaw root rot in Nigeria which has not yet been investigated, and which led to high prices of pawpaw products in the market. This work investigates the causal organism and its pathogenicity using different media.

An infected part of the root of pawpaw is dark brown without secondary roots. The internal tissues breakdown leading to the formation of spongy, water soaked areas on the back at the region of infection. The tissues soften, and a copious exudation of latex with foul odour takes place. These dried tissues are usually exposed through wounds on the covering skin. The disease gradually spreads above and below the region of infection. Affected roots deteriorate and some of them may be entirely destroyed. Simultaneously, it causes the progressive decline of the aerial parts of the tree. Usually, the terminal leaves begin to droop, wilt, becoming yellow and drop prematutredly. The internal tissues of the stem disintegrated and a copious exudation as in roots takes place. The skin peels off and the plant finally collapses.

Materials and method

Isolation of the causative organism. Diseased samples of infected roots of pawpaw were surface sterilized in 70% alcohol (v/v) for 1 min., rinsed in several changes of sterile distilled water, blotted dry and plated on potato-dextrose-agar (PDA), and incubated at 25 ± 1 °C for 3 days. The baiting technique of Sastry and Hedge (1988) using castor seeds (*Ricinus communis* L.) was also employed in isolating the organism from soil collected from the rhizosphere of diseased roots of the plants. Soil samples (500 g each) were collected randomly from the rhizopsphere of infected roots of pawpaw. Thirty such samples were powdered and sieved through 0.2 cm sieve. Castor seeds 25 in number, were surface sterilized and buried in 100 g of the soil taken in 100 mm Petri plates. The soil was then moistened. After incubation for 48 h at 25 ± 1 °C, the castor seeds were removed from the soil, washed in running tap water to dislodge soil particles. Some of the seeds were then plated on 2% water agar to minimize contaminants. The remaining ones were surface sterilized, rinsed several times in sterile distilled water, and plated on PDA. Both diseased tissues and the seeds used for baiting were incubated at 25 ± 1 °C for 3 days. Pure cultures were obtained by subculturing colonies growing from the infected tissues and the seeds, and sent to International Mycological Institute, Kew, Surrey, England, for identification.

Pathogenicity

Pawpaw seedlings (cv. Homestead) employed for the pathogenicity tests were raised in pasteurized soil in 5L plastic buckets. Soil surrounding the seedlings was removed and 50 ml suspension containing 4×10^5 oospores/ml from a 3 day-old-culture was poured around 20 day-old seedlings in the pot and covered with the same soil. Each pot comprised of 4 seedlings/pot. The treatment which was replicated 3 times was repeated twice. Spore suspension was prepared by macerating 3 plates of 3 day-old culture in 150 ml of sterile distilled water in a waring blender. This was filtered through 3 layers muslin cloth. The resulting suspension was homogenized in a glass tissue grinder, passed 10 times through a single layer of cheesecloth to remove mycelia fragments. The appropriate concentration of oospores in the suspension was determined by counting six fields for each samples in a standard Hawskley Haemocytometer. Also, seedlings of different ages, 45, 65 and 90 days old were inoculated and the percentage of dead pawpaw seedlings was recorded.

Effect of media on cultural characteristics and pathogenicity of Pythium aphanidermatum. The 16 different media mentioned below were employed to study cultural characteristics and pathogenicity of *P. aphanidermatum*. Corn and cassava extracts were prepared by boiling 200 g ground corn in 1 L of distilled water, and 200 g freshly peeled and sliced cassava (*Manihot utilissima* L.) tuber in 250 ml distilled water for 30 and 15 min. respectively. Pawpaw-leaf- and tomato-carrot-extract were obtained by boiling 200 g chopped young pawpaw leaf in 500 ml water for 1 h. Each extract was made to 1 L on cooling and supplemented with 20 g plain agar to make corn-meal-agar (CMA), cassava-extract-agar (CACA), pawpaw-leaf-extract-agar (PLEA) and tomato-carrot-agar (TCA). Of all these media, variants were produced by supplementing each with 20 g glucose-resulting in CMGA, CAEGA, PLEGA, TCGA; cellulose-resulting in CMCA, CAECA, PLECA, TCCA; and sucrose-resulting in CMSA, CAESA, PLESA, TCSA. All these media were autoclaved at 121 °C for 15 min.

Each medium was inoculated with the isolate of *P. aphanidermatum* in the centre of the petri dish from a 3 day-old culture and replicated 3 times. The bottom of each plate was marked in four places at right angles to each other from the inoculum point. Radial growth at 3 hourly intervals was measured until the plates (9 cm diam) were covered. The plates were left for 3 days to allow oospores formation, and oospores counted in each plate. The diameter of 100 oospores in each plate were measured. The culture

Table 1. Effect of media on formation of oospores, oospores size, growth rate (mm/h) and pathogenicity (20 days-old seedlings)

Media	Pathogenicity % dead seedling within 3 days	Growth rate (mm/h)	Number of oospores ($\times 10^5$/ml)	Oospore dimension (μ)
CMA	98.33 (0.17) a	4.14 a	4.0 a	18–22 (19.9 ± 0.1)
CMGA	95.00 (0.17) a	3.13 ef	0.08 fg	10–22 (17.7 ± 0.3)
CMCA	73.33 (0.15) c	4.14 a	0.10 f	10–21 (14.4 ± 0.4)
CMSA	91.67 (0.17) a	4.14 a	0.08 fg	10–22 (15.2 ± 0.4)
TCA	96.67 (0.17) a	2.3 g	0.80 c	17–25 (20.5 ± 0.2)
TCGA	100.00 (0.17) a	2.07 h	0.05 fg	10–20 (15.9 ± 0.4)
TCCA	78.33 (0.15) c	2.2 gh	0.10 f	10–25 (18.2 ± 0.3)
TCSA	98.33 (0.17) a	1.81 i	0.11 f	10–22 (16.2 ± 0.4)
PLEA	58.33 (0.13) d	3.03 f	0.03 g	10–22 (15.5 ± 0.3)
PLEGA	56.67 (0.13) d	3.41 bcd	0.31 fg	10–22 (16.7 ± 0.3)
PLECA	61.67 (0.13) d	3.17 def	0.03 g	10–20 (16.2 ± 0.4)
PLESA	90.00 (0.16) b	3.29 bcde	0.02 g	10–20 (14.6 ± 0.4)
CAEA	96.67 (0.17) a	3.48 b	0.80 b	10–22 (16.3 ± 0.4)
CAEGA	95.00 (0.17) a	3.22 b	0.20 e	10–20 (16.2 ± 0.3)
CAECA	91.67 (0.17) a	3.44 bc	0.30 e	10–20 (16.2 ± 0.3)
CAESA	86.67 (0.16) b	3.37 bcde	0.50 d	10–20 (15.1 ± 0.3)

Those figures followed by the same letter(s) are not significantly different ($p \leq 0.05$) according to Duncan's New Multiple Range Test. Figures in paretheses are arc sin transformed values, $\sin \sqrt{x}$.

plates of each medium were used to test for the pathogenicity of the fungus as described above.

Results

The causal organism was identified as *Pythium aphanidermatum* (Edson.) Fitz Patrick (IMI 333810). The organism was pathogenic on 16 different media to pawpaw but there were variations in pathogenicity between media. The highest percentage mortality was tomato-carrot-glucose-agar: 100% followed by corn-meal-agar 98.3% seedling mortality 3 days after inoculation. The least was in pawpaw-leaf-extract-agar 56.1% and pawpaw-leaf-glucose-agar 58.3% 3 days after inoculation respectively (Table 1).

P. aphanidermatum grew best on corn-meal-agar, and corn-meal-cellulose-agar with growth rate of 4.14 mm/h. This was significantly different from growth in other media ($p \leq 0.05$). The highest growth rate was followed by growth on cassava-extract-agar with 3.48 mm/h, and slowest was 1.81 mm/h in tomato-carrot-sucrose-agar (Table 1).

The highest oospore production was on corn-meal-agar with 4.0×10^5 oospores/ml and the lowest in pawpaw-leaf-extract-agar. In the media supplemented with glucose, sucrose or cellulose, oospore production were usually lower than when the media were not supplemented with any carbon source except in pawpaw-leaf-extract. The differences between supplemented and non-supplemented media were highly significant from each other ($p \leq 0.05$) (Table 1).

The largest oospores were on tomato-carrot-agar with diameter of 17–25 (mean 19.9 ± 0.2) followed by corn-meal-agar with 18–22 (mean 19.9 ± 0.1). The smallest oospores were in corn-meal-cellulose-agar, 10–21 (mean 14.4 ± 0.4). With the exception of pawpaw leaf-extract derived media, the oospores in non-supplemented media were smaller than in supplemented media.

Effects of *P. aphanidermatum* on pawpaw seedlings of different ages. The collapse of infected seedlings was retarded with increase in ages (Table 2).

Discussion

The establishment of *Pythium aphanidermatum* as the causal agent of root rot of pawpaw in

Table 2. Cumulative percentage of dead pawpaw seedlings of different ages inoculated with equal mycelia/oospore suspensions (4.0×10^5/ml) of *P. aphanidermatum*

Day	Age (days)					
	40–45	Control	60–65	Control	85–90	Control
2	3.33 (0.03) f	0	0	0	0	0
3	13.00 (0.06) e	0	0	0	0	0
4	20.00 (0.08) d	0	0	0	0	0
5	30.00 (0.01) d	0	6.67 (0.05) c	0	0	0
6	46.67 (0.12) c	0	16.67 (0.07) c	0	0	0
7	50.00 (0.12) c	0	23.33 (0.08) b	0	0	0
8	61.67 (0.14) b	0	48.33 (0.12) a	0	0	0
9	70.00 (0.15) a	0	48.33 (0.12) a	0	0	0
10	75.00 (0.15) a	0	48.33 (0.12) a	0	0	0
11	75.00 (0.15) a	0	53.33 (0.13) a	0	11.67 (0.06) a	0
12	75.00 (0.15) a	0	53.33 (0.13) a	0	15.33 (0.07) a	0

Those figures followed by the same letter(s) are not significantly different ($p \leq 0.05$) according to Duncan's New Multiple Range Test. Figures in paretheses are arc sin transformed values, $\sin \sqrt{x}$.

Nigeria agrees with the work of Trujillo & Hine [6], Ko [7] and Rao & Bhat [10] that this organism caused root rot of pawpaw.

There are reported cases of failure of *P. aphanidermatum* to infect susceptible cowpea if grown on potato-dextrose-agar or decoctions prepared from stem, leaf and seed of cowpea plus agar (Oladiran, unpublished).

The isolate of *P. aphanidermatum* reported in this investigation was pathogenic whatever its growth medium. However, it was less pathogenic when pawpaw-leaf-extracts were used as media for inoculum than with other media except PLESA. Different carbon sources in the medium have little effect on Pathogenicity. Death of zoospores of *Phytophthora nicotianae* by papain, naturally occurring in abundance in pawpaw leaves and unripe fruits that has been implicated by Hine et al. [11] may probably be responsible for these findings.

Media had a significant effect on growth and oospore formation. The highest growth rate and oospore count were obtained on CMA but did not correspond to the highest percentage dead seedlings when culture medium was used as inoculum source. TCGA gives the highest percentage of dead seedlings (Table 2). It is possible that some oospores in CMA probably failed to germinate and infect the seedlings.

The slower collapse of seedlings caused by *P. aphanidermatum* with increased age (Table 2) is similar to that reported for *phytophthora nicotianae* on pawpaw [7] and *P. aphanidermatum* on seedlings of tobacco [12] which these authors attributed to the development of lignin. Garret [13] noted that *Pythium* and *Rhizoctonia* caused damping off by attacking the immature tissues. These fungi, however continue to do a certain amount of damage to the older plants because new roots are being produced throughout the life of the plant. Indeed, this investigation was provoked by heavily attacked adults in the field. Decrease in death with increased age may not therefore be a control measure of any economic importance.

Further work will be carried out on the use of pawpaw leaves as a possibility for control of root rot of pawpaw. Also, the effect of the media used in this work on zoospore production will be investigated.

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