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RESEARCH ARTICLE

Cultural characterization of *Fusarium oxysporum* forma specialis melongenae from Guinea savannas of Nigeria

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ABSTRACT

The probability of two isolates of *Fusarium oxysporium* from two climatic regions being different was investigated *in vitro* using cultural characterization techniques. The specific aim of this experiment was to determine the characteristics of this *Fusarium* that may be consistent in spite of the medium used. The experiment was set up using a completely randomized design with factorial arrangement. The factors comprised of four culture media and two *Fusarium* isolates from Southern and Northern Guinea Savannas (i.e. from Benue and Kaduna States respectively). The eight treatments were replicated three times. The experimental units consisted of glass Petri dishes. The media were autoclaved at 121 ^{o}C for 15 minutes at 15 psi and pour plate carried out after supplying 50 mg/L streptomycin sulphate into each medium. Data were collected at three, six and nine days after inoculation (DAI) and subjected to analysis of variance using Genstat® statistical software. The means were separated using Fisher's Least Significant Difference (P≤0.05). At 9 days after inoculation (DAI), the diameters of the *Fusarium* colonies could be arranged in order of decreasing magnitude for both Kaduna and Benue States (FoiK and FoiB respectively) as follows: czapeck dox agar (CDA), potato dextrose agar (PDA), acetate differential agar + dextrose (ADAD) and corn meal agar (CMA). Based on number of whorls produced the order was CDA>ADAD>CMA>PDA. FoiB had the highest number of spores on ADAD then CDA, CMA and PDA (in decreasing order). For FoiK, the order was ADAD>CMA>CDA>PDA. The best medium was ADAD while PDA was the least suitable medium for macroconidia production. Based on their area of origin, the isolates were not different. The different media did not influence the elevation, edge type and the shape of the colony. All the media utilized were excellent for culturing *Fusarium* and are recommended for research on this pathogen however, the responses were parameter dependent.

Keywords- *Fusarium* Characterization, Culture Media, Guinea savannas, *Solanum aethiopicum*.

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INTRODUCTION

Solanum aethiopicum L. is commonly called jaxatu, mock tomato or African garden egg plant. It is an herbaceous perennial plant in the Family Solanaceae (Anonymous, 2006). The crop is believed to have originated from tropical Africa (Norman, 1992; Grubben and Denton, 2004). Mock tomato is widely cultivated in West and East Africa and is a highly valued constituent of diet and herbal medicine wherever it is cultivated. It is part and parcel of sub-Saharan African culture as the fruit is said to represent blessings and fruitfulness. The fruits are also offered as a token of goodwill during visits, marriages and other social events. They are eaten raw, boiled, fried or ground as condiments for stews, soups and vegetable sauces in some parts of Nigeria (Chinedu *et al*., 2004).

Solanum aethiopicum is a fruit and leafy vegetable that is rich in vitamins, minerals, fibre, antioxidants (cancer-preventing antioxidants), carotene and nasunin (lowers cholesterol level and promotes a healthy heart) (Sikora and Fernandez, 2005; Sunseri *et al*., 2010; Tyree, 2012). The crop is used for the treatment of many diseases such as diabetes, bronchitis, asthma, dysuria, dysentery and rheumatoid arthritis. Its pharmacological properties are likely due to its high fiber, ascorbic acid, phenols, anthocyanin, glycoalkaloids, chlorogenic acid and α-chaconine content (Chinedu *et al*., 2004).

The production of this crop in West Africa is hampered by many constraints like inadequate water supply for irrigation, low soil fertility, inadequate access to capital, poor transportation system, lack of processing and storage facilities, and pests and diseases. Serious post-harvest losses of mock tomato tend to compound the problems being faced by farmers (Nsiah-Gyabaah, 2003; Ozobia *et al*., 2013). *Solanum* species (including *S. aethiopicum)* are affected by many pathogens. Among these pathogens are *Fusarium* root rot and wilt which are among the most important pathogens of solanaceae (Kukogho, 1988; Nelson et al., 1994; Osuinde and Ikediugwu, 2002; Rivard, 2006; Back *et al*., 2002 and Betrand *et al*., 2000). This wilt disease affects plant-water relations, nutrient uptake, light interception and assimilate formation thereby causing heavy yield loss. Crop losses depend on pathogen population density, soil type, soil water, yield potential of site and cultivar (Trudgill, 1967).

In Nigeria, infestation of tomato by *Fusarium* specie was reported (Anonymous, 2006). Information on the effects of *Fusarium* on mock tomato in Nigeria is highly limited. The rate at which pathogen populations tend to became acclimatized, adapted to specific localities or co-evolve with their host populations is becoming clearer as global warming is making researchers to look closely at differences in the populations they are dealing with. It was deemed necessary to investigate the likelihood of the isolates of *Fusarium oxysporium* Schelecht ex Fries (Syn. & Hans) f.sp melongenae Mauto and Ishigami turning out to be different in any significant way. Any significant difference in pathogen populations in a locality can make control of the pathogen more difficult in future.

The aim of this experiment was to determine the characteristics of this *Fusarium* species that may be stable in spite of the medium used. The results may show which of the media is most suitable for culturing the pathogen in future. This may help in identification of the pathogen with certainty whenever it is encountered. The findings may be used to compare any new isolates of the pathogen being investigated. Two

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isolates of this pathogen from Kaduna State (northern Guinea savanna) and Benue State (southern Guinea savanna) were obtained and employed during this experiment.

MATERIALS AND METHODS

Culturing of the isolates of *Fusarium* species

This experiment was carried out in Makurdi (07^{0}45' N and 08^{0}37' E at altitude of 101 m above sea level) with annual rainfall range of 1000 – 1600 mm spread out between 190 – 250 days. Makurdi lies within the southern Guinea savanna zone of Nigeria. The media utilized in this study included Acetate Differential Agar enriched with Dextrose (ADAD), Potato Dextrose Agar (PDA), Corn Meal Agar (CMA) and Czapeck Dox Agar (CDA). The dehydrated culture media were weighed and prepared as recommended by their manufacturers. The media were each separately dissolved in distilled water in a conical flask as required. Each medium was heated using the S-and-J electric hot plate till boiling. This heating helped dissolve the medium. Each medium was then autoclaved for 15 minutes at 121 ^{o}C. Each medium was allowed to cool to about 50 ^{o}C at which point 50 mg/L streptomycin sulphate was added to the media standing in the Holten laminar airflow hood unit. The content of the flask was swiveled around to mix without creating bubbles. Pouring plates was carried out at about 45 ^{o}C and the agar allowed to set (Stapp, 1961; Taylor and Sasser, 1978).

The experiment was set up in the laboratory using sterilized glass petri dishes (9 cm diameter) under aseptic conditions. Autoclaving was utilized for disinfecting distilled water and glassware. Glassware and metallic equipment were autoclaved for 20 minutes at 121 ^{o}C. Flaming of metal equipment in the process of working was adhered to strictly. Chemical sterilization of work surfaces was achieved using sodium hypochlorite (3.5%), plant tissue surfaces and glassware were surface sterilized when working using sodium hypochlorite (1%) and 70% alcohol as the need arose (Stapp, 1961; Taylor and Sasser, 1978). The autoclaved media were poured (i.e. plate pouring) inside the laminar flow hood. Enough medium (15 ml) was poured per plate. The experimental layout utilized was the completely randomized design (CRD) and each of the eight treatments was repeated three times. PDA - a popular general purpose medium - was considered as the standard check. The media were combined as follows: Kaduna isolate in PDA = T2, Kaduna isolate in CDA = T3, Kaduna isolate in ADAD = T7, Benue isolate in PDA = T1, Benue isolate in CDA = T4, Benue isolate in CMA = T6 and Benue isolate in ADAD = T8.

The pathogen was cultured for 9 days under room temperature and the morphological characteristics listed below recorded. The data collected included the colony diameter, number of whorls / concentric rings, front and back colours of Petri dish, texture, elevation, edge type, shape, opacity and production of smells. Colony diameter (in mm) was obtained by measuring the diameter using a divider and a transparent ruler. The number of whorls was counted visually. The Front and Back colours of the colony/ growth in Petri dishes were obtained using the Royal Horticultural Colour Chart series as recommended in the chart's manual. Data collection was carried out on the 3rd, 6th and 9th day (DAI). For each parameter per Petri dish, two measurements were taken and the average recorded.

The texture, elevation, edge type, shape, opacity and production of smells were

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obtained by applying standard mycological terms to describe them. The average number of spores per unit area on a medium was obtained on the 9th DAI. To determine the number of spores/unit area, a 0.5 cm cork borer was used and the surface of the medium midway in the growth radius cut. The extracted medium was placed in 1 ml of water and 0.5% sodium hypochlorite dropped into the suspension to free the spores. It was agitated and a hemocytometer used to determine the number of spores in the suspension.

Slide culture for microscopy

This experiment was set up at the same site in order to record the data on morphometric features of this *Fusarium* species. The experiment was laid out using CRD layout and with the same treatments as in section 2.1. The treatments were replicated three times. The slide culture was carried out for 48 hours so as to record the morphometric characteristics listed below. The culture slides were prepared using cotton wool to suspend the slide, cover-slips to place on the agar blocks, glass slides for placing agar blocks, square blocks of agar medium and second set of glass slides for subsequently mounting cover-slips. The inoculation of the agar on the slides was performed using inoculating loop brushed on the surface of the growth produced 5 days prior to commencement of the slide culture work. Microscopy was utilized to measure the length of macroconidia and microconidia per treatment. The stage micrometer (Graticules Ltd, Kent, England), the eyepiece reticule (Erma Inc. Tokyo, Japan) and the microscope (Olympus 100 compound microscope) were used during the experiment.

The data collected were subjected to analysis of variance using Genstat® statistical software to determine the significance of the cultural characteristics of *Fusarium oxysporum* f.sp melongenae from the two locations. The means were separated using Fisher's LSD (P≤0.05).

RESULTS AND DISCUSSION

Main effects of isolates and media on Cultural characterization of *F. o.* f.sp. melongenae the causal agent of *S. aethiopicum* wilt disease

The main effects of effects of isolates and type of media on cultural characteristics of two isolates of Fusarium oxysporum from African garden egg plants cultured on four culture media are presented in the Table 1. It was observed that colony diameter was significantly lower in CMA and macroconidia length was significantly lower in PDA. Macroconidia length was significantly lower in CDA and the highest number of spores was produced on ADAD. There was no significant difference between the media in terms of number of whorls likewise there was no significant difference between isolates FoiK and FoiB.

The interaction effects of isolates and type of media on cultural characteristics of two isolates of Fusarium oxysporum from African garden egg plants are presented in the Table 3. Colony diameter was significantly lower on FoiK in CMA compared with the other treatments. The number of whorls was significantly higher in FoiB in ADAD compared with FoiK in CMA which had the lowest number of whorls. Macroconidia length was significantly longer in FoiB in ADAD compared with FoiK in CMA. Number of spores/ml was significantly higher in FoiK in ADAD compared with other treatments except FoiB in ADAD and FoiB in CDA. The lowest number of spores was recorded in FoiK in PDA and FoiK in CMA. The front color was generally greyed green while

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the back color was generally white on the media. The texture was floccose, while the elevation was low throughout in all the media. The edge of the growth was entire for all media. The opacity of the growth was not consistent for all the media except for CDA where the growth was consistently transluscent.

Table 1: Main effects of isolates and type of media on cultural characteristics of two isolates of *Fusarium oxysporum* from African garden egg plants cultured on four culture media

Medium	diameter/plate (cm)	No. of whorls/ plate	macroconidia/ plate (µ)	Length of microconidia/ plate (µ)	spores/plate (1000s/ml)
PDA	7.9	2.9	198.6	104.9	533.5
ADAD	7.7	3.4	257.7	95.9	2812.2
CMA	5.9	2.7	222.2	104.2	742.8
CDA	8.6	3.3	229.2	52.8	2068.8
FLSD (P≤0.05)	1.3	ns	49.8	24.6	626.8
Isolate					
FoiK	7.1	2.9	217.7	100.4	1238.1
FoiB	8.0	3.2	236.1	78.5	1840.6
FLSD (P≤0.05)	ns	ns	ns	ns	ns
Interactions	*	*	*	ns	*

FoiK = *Fusarium oxysporum* isolate from Kaduna State.
FoiB = *Fusarium oxysporum* isolate from Benue State
Czapeck dox agar (CDA), Potato dextrose agar (PDA),
Acetate differential agar + dextrose (ADAD) and Corn meal agar (CMA)

Interaction effects of isolates and media on Cultural characterization of *F. o.* f.sp. *melongenae* the causal agent of *S. aethiopicum* wilt disease

The results of the cultural characterization of *F. o.* f.sp melongenae isolates - the causal agent of S. *aethiopicum* wilt disease - from Kaduna and Benue States are presented in Table 2. The results showed that there were highly significant differences (P≤0.05) between the diameters of the fungal growths *in vitro* at 3 DAI. The largest diameter was obtained from the Benue isolate cultured using PDA followed by that from the same isolate cultured using CDA. The first four

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largest diameters were from the isolate from Benue State. These four fungal growths on the different media were all at par. There was no significant difference (P≤0.05) in the number of whorls produced 3 DAI. There was no clear difference among the diameters of colonies at this stage.

At 6 DAI, it was observed that for both states the fungal growth on CMA had significantly (P≤0.05) lower diameters than all other media (Table 2). The first three largest diameters were from the isolate from Benue State at 6 DAI. The largest growth from Kaduna State was that cultured on PDA which was at par with the largest growths from Benue State. At this point the highest number of whorls was from the Benue isolate cultured on ADAD which was at par with the isolate from Kaduna raised on PDA, ADAD and CDA. The isolate from Benue when cultured on CMA had a small diameter but with a high number of whorls which compared favorably with the highest media/colonies at this stage. The smallest numbers of whorls were from the Benue isolate x PDA and Kaduna isolate in CMA.

At 9 DAI the diameters of the fungal growths could be arranged in order of magnitudes and region as follows: for Benue State from largest to the smallest, we have CDA, PDA, ADAD and CMA. While for Kaduna State we had the following: CDA, PDA, ADAD and CMA. In terms of number of whorls produced we had from Benue State; CDA, ADAD, CMA and PDA. While for Kaduna we had CDA, ADAD, CMA and PDA in descending order. The highest number of spores produced from Benue isolate was from ADAD followed by CDA then CMA and finally PDA. Meanwhile for Kaduna we had ADAD, CMA, CDA and PDA least.

At 9 DAI, the average length of macroconidia revealed significant differences between the media, unlike the results of microconidia that showed that there were no significant differences (Table 3). When each macroconidium for a specific medium was compared to that from the other state, it was observed that there were no clear cut differences between means. The mean length of the Kaduna isolate of *F. o.* f.sp melongenae macroconidia was 217.7µ and that of the microconidia was 100.15 µ. For Benue isolate the mean macroconidia length was 236.1 µ and that of the microconidia was 103.5 µ.

There were positive correlations between the parameters throughout the experiment (Table 4). The number of spores was positively correlated with diameters of colony and whorls at 6 DAI. Correlation of the cultural characteristics of *F. o.* f.sp. melongenae showed that number of spores was highly positively correlated with growth diameter and number of whorls at 9 DAI. The production of spores was independent of the number of whorls or diameter of colony produced this may imply that very young colonies of this fungus may be infective. Besides this, the size of growth that can start an infection is independent of the number of days that the colony has been cultured. In fact the spores were highly significantly correlated with fungal growth diameters at 9 DAI. Diameters 2 (6 DAI) was highly positively correlated with Diameter 3 (9 DAI) and Whorls 2 (6 DAI). Diameters 1, 2 and 3 were highly positively correlated.

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Table 2: Interaction effects of isolates and media on cultural characterization of *F. o.* f.sp. melongenae the causal agent of *S. aethiopicum* wilt disease

TREATMENT 3 DAI	COLONY DIAMETER 1 (cm)	WHORLS 1	FRONT COLOUR	BACK COLOUR	TEXTURE	OPACITY
FoiK in PDA	2.33	1	greyed green	greyed green	floccose	translucent
FoiK in ADAD	1.93	1	greyed green	greyed green	floccose	translucent
FoiK in CMA	1.81	1	greyed green	greyed green	floccose	translucent
FoiK in CDA	2.30	1	greyed green	greyed green	floccose	translucent
FoiB in PDA	3.10	1	greyed green	greyed green	floccose	translucent
FoiB in ADAD	2.80	1	greyed yellow	greyed green	floccose	translucent
FoiB in CMA	2.66	1	greyed green	greyed green	floccose	translucent
FoiB in CDA	3.03	1	greyed green	greyed green	floccose	translucent
SED	0.34					
FLSD (P≤0.05)	0.73					

6 DAI	COLONY DIAMETER2 (cm)	WHORLS 2	FRONT COLOUR	BACK COLOUR	TEXTURE	OPACITY
FoiK in PDA	5.60	4.66	orange white	orange white	grainy floccose	translucent
FoiK in ADAD	5.23	4.00	greyed green	greyed green	smooth floccose	translucent
FoiK in CMA	3.30	2.66	white group	white group	smooth floccose	translucent
FoiK in CDA	5.03	5.00	orange white	orange white	smooth floccose	translucent
FoiB in PDA	6.60	2.66	greyed green	orange white	smooth floccose	translucent
FoiB in ADAD	5.90	5.33	greyed green	blue green	grainy floccose	translucent
FoiB in CMA	4.43	4.00	yellow white	yellow white	grainy floccose	translucent
FoiB in CDA	6.96	3.33	yellow white	yellow white	grainy floccose	translucent
SED	0.77	0.66				
FLSD(P≤0.05)	1.65	1.42				

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Table 3: Interaction effects of isolates and media on cultural characterization of *F. o.* f.sp. melongenae the causal agent of *S. aethiopicum* wilt disease

TREATMENT 9 DAI	COLONY DIA METER 3 (cm)	WHORLS 3	MACRO CONIDIA (µ)	MICRO CONIDIA (µ)	SPORES (1000s)	FRONT COLOUR	BACK COLOUR	TEXTURE	OPACITY
FoiK in PDA	7.67	2.80	213.9	104.2	158.67	orange white	orange white	grainy floccose	opaque
FoiK in ADAD	7.50	3.40	223.6	88.9	2917.00	greyed green	greyed green	smooth floccose	opaque
FoiK in CMA	4.77	2.25	200.0	102.8	181.67	orange white	orange white	smooth floccose	translucent
FoiK in CDA	8.37	3.22	233.3	105.6	1695.00	greyed green	orange white	smooth floccose	translucent
FoiB in PDA	8.30	2.95	183.3	105.6	908.33	greyed green	orange white	smooth floccose	translucent
FoiB in ADAD	7.97	3.43	291.7	102.8	2707.33	greyed green	blue green	grainy floccose	opaque
FoiB in CMA	6.97	3.11	244.4	105.6	1304.00	yellow white	yellow white	grainy floccose	translucent
FoiB in CDA	8.80	3.38	225.0	100.0	2442.67	yellow white	greyed white	grainy floccose	opaque
SED	0.77	0.08	34.3	14.5	235.7				
FLSD ($P \leq 0.05$)	1.66	0.17	73.5	31.1	505.6				

FoiK = *Fusarium oxysporum* isolate from Kaduna State.
FoiB = *Fusarium oxysporum* isolate from Benue State
Czapeck dox agar (CDA), Potato dextrose agar (PDA), Acetate differential agar + dextrose (ADAD) and Corn meal agar (CMA)

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Table 4: Correlation of the cultural characteristics of *F. o.* f.sp. melongenae the causal agent of *S. aethiopicum* wilt Disease

	DIAMETER 3 (9 DAI)	WHORLS 3 (9 DAI)	SPORES	DIAMETER 2 (6 DAI)	WHORL 2 (6 DAI)	DIAMETER 1 (3 DAI)
DIAMETER 3 (9DAI)	1					
WHORLS 3 (9DAI)	0.792	1				
SPORES	0.517*	0.895*	1			
DIAMETER 2 (6DAI)	0.889*	0.623*	0.4188	1		
WHORL 2 (6DAI)	0.373	0.523*	0.367	0.069*	1	
DIAMETER 1 (3DAI)	0.683*	0.448	0.207	0.786*	-0.046	1

** Correlation is significant at the P0.01 level (2-tailed).
* Correlation is significant at the P0.05 level (2-tailed).

The isolate of *Fusarium* from Benue grew faster in all the different media utilized during the first part of the trial which could be a good attribute for its survival. The Kaduna isolate of *Fusarium* seemed to be slow in establishing but it equally had good adaptability to all the four media utilized. This led to significant gains in terms of diameter of growth/colony during at 6 DAI. The least suitable medium used was CMA for culturing both isolates. The reason for this is not clear however, some nutrient deficiency may be suspected. The results showed that the frequency for the production of rings of growth (whorls) was very high at 6 DAI. This may be linked to an increase in pigment production (as both front and back colours on the Petri dishes were becoming highly pigmented). The normal texture of *F. oxysporum* is usually floccose but in this trial efforts were made to determine the texture in different media by adding adjectives to the word floccose. This strategy was difficult to sustain because any change in properties of a medium could cause changes in the culture. The ability to produce spores in different media was tested and PDA was shown to be the least suitable medium for culturing this pathogen for conidia production. The fungus produced spores in all media utilized and the best medium was ADAD medium. The different media did not affect the elevation, edge type and shape of the colony which were

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consistently low, entire and circular respectively. The results confirmed that the same pathogen was being screened and that they may be differences in virulence in the field based on the ecological zone where an isolate was obtained. It is recommended that this kind of trial be carried out in future at different locations or growth conditions.

The least suitable medium used for culturing both isolates was CMA. The reason for this is not clear but some specific nutrient deficiency or pH may be suspected. The ability to sporolate in different media was tested and PDA was shown to be the least suitable medium for culturing this pathogen for conidia production. All the same, the fungus sporolated in all media utilized and the best medium was ADAD may be due to its rich nutrient profile. The lack of clear-cut significant differences between the isolates in terms of macroconidia and microconidia affirms the fact that the same causal agent was involved in causing the wilt disease on African garden egg plants in Benue and Kaduna States.

The fungus may have adapted to different regions of the savanna hence the slight to significant differences here and there in the results obtained. Concerning the use of different media to produce standard length conidia, no medium was consistent so none can be recommended as the sole medium to be used in micrometry of *F. oxysporum* f.sp melongenae. However, since all the media enhanced conidia production anyone of them can be used for future research on the pathogen. The different media did not affect the elevation, edge type and shape of the colony. The production of spores was independent of the number of whorls or diameter of colonies produced which may imply that very young colonies of this fungus may be infective. Besides this, the size of growth that can start an infection is independent of the number of days that the colony has been cultured or the physical expanse of the colony produced. Common knowledge has it that conidia of *Fusarium* spp can survive for above 14 years in the absence of the host as saprophytes.

CONCLUSION

An *in vitro* experiment was carried out to compare two isolates of *Fusarium oxysporum* f.sp. melongenae using cultural characterization. This trial was carried out on the isolates obtained from Kaduna and Benue States. There were highly significant differences between the diameters of the fungal colony *in vitro* at 3 DAI. Meanwhile 6 DAI it was observed that for both regions the colonies on CMA had significantly smallest diameters. The three largest diameters were from the *Fusarium* isolate from Benue State. At this point the highest number of whorls was from the Benue isolate cultured on ADAD whose number of whorls was at par with those of the isolate from Kaduna raised on PDA, ADAD and CDA. At 9 DAI, the diameters of the colonies could be arranged in order of decreasing magnitudes for both regions as follows: CDA, PDA, ADAD and CMA. In terms of number of whorls produced we had CDA, ADAD, CMA and PDA. In terms of number of spores produced we had from Benue isolate; the highest medium being ADAD followed by CDA then CMA and finally PDA. Meanwhile for Kaduna isolate we have ADAD, CMA, CDA and finally PDA.

The results clearly showed that the two isolates of *Fusarium* utilized in this research had significantly different cultural characteristics no matter the medium used to culture the pathogenic fungus. The isolate from Benue grew faster in all the different

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media utilized during the first part of the trial which could be a good attribute for survivability. The Kaduna isolate seemed to be slow at establishing but it equally had good adaptability to all four media utilized. It thus made significant gains in terms of diameter of colony during the second data collection period. The least suitable medium for culturing both isolates was CMA. The reason for this is not clear, studies should be carried out on long term storage of this fungus at room temperature and pressure. The fungus produced spores in all media utilized and the best medium was dextrose enriched ADA medium.

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