



Cultural and Media Studies of *Fusarium oxysporum* f. sp. *elaeidis* of the oil palm from African Countries

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Abstract

Fusarium oxysporum f. sp. *elaeidis* species from African countries produced visible growths in culture media. The objective of this study was to determine the best supporting medium for the growth and sporulation of *F. oxysporum* f. sp. *elaeidis*. The four species were grown on different media and at six days produced various morphological structures. Microscopic view of *Fusarium* species shows macro conidia were absent on sporodochia, macro conidia small, macro conidia elongated and slender on sporodochia and macro conidia large on aerial mycelia. In some cases, it was straight on the right (ventral) and slightly curved on the left (dorsal), the apical cell was blunt. The overall mean of spore counts on best supporting media for *F. oxysporum* f. sp. *elaeidis* species, Malachite Green had the highest with 11.94. These were followed by Home Green (11.13); V8 Juice medium and Potato Dextrose Agar (6.56); Malachite Green Captan Medium (2.31) and Park's Medium (2.13) respectively. Mean counts of *Fusarium oxysporum* f. sp. *elaeidis* species in broth sporulation in different media, *F. oxysporum* isolate 1 had the highest counts. This was followed by *F. oxysporum* isolate 4. The least isolate was *F. oxysporum* CRT. Six days after incubation in different selective media, the highest mean linear growth in centimeter (cm) was recorded by Malachite Green (4.5). These were followed by Malachite Green Captan and V8 Juice medium with 4.3 each, Home and Mitters (4.26), Komada medium (4.23), PDA (3.6) and Park medium (3.25). Malachite Green was found to be most suitable for spores production.

Key words: Oil Palm, Culture, Media, Spore, *Fusarium oxysporum*

INTRODUCTION

A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Pradeep *et al.*, 2013)

Fusarium species have been important for many years as plant pathogens causing diseases such as crown rot, headblight, and scab on cereal grains, vascular wilts on a wide range of horticultural crops, root rots, and other diseases (Gilardi *et al.*, 2007; Kausar *et al.*, 2009). *Fusarium* is a difficult genus taxonomically. Its identification based on morphological features only is not reliable; thus the identification of different *Fusarium* species requires special culture media, some physiological criteria or molecular methods. The high variability in species, especially under different environmental conditions, has led taxonomists to consider some special criteria for the classification of species (Booth, 1975). There are many isolation media for recovering and identifying *Fusarium* species, such as Czapek Dox agar (Raper and Thom 1949) and Czapekiodinedichloran agar (Abildgren *et al.* 1987). Selective media for isolation and identification of *Fusarium* species were developed and found to be helpful, permitting rapid identification of *Fusarium* isolates on such

media (Vázquez *et al.*, 1993; Thrane 1986).

Fusarium oxysporum f. sp. *elaeidis* is a major fungal parasite. It is a pervasive soil and plant inhabiting pathogens causing wilt disease of some plant species (Michielse and Rep, 2009). It has a broad host range and includes both non-pathogenic and pathogenic strains

Fusarium oxysporum is known to exhibit cultural and morphological variations (Chittem and Kulkarni, 2008). However, sub culturing of the genus consecutively may lead to many more variants (Bai, 1996). In most of the species small patches or sectors were formed and they differed from their parental colony (Ho and Varghese, 1988). Different isolates may show cultural and morphological differences when grown on the same media (Leslie and Summerell, 2006). This type of work will help to know the extent of variations a pathogen exhibits and also the variations existing among the isolates which are collected from a particular geographical area (Dudley *et al.*, 2005; Shenoj *et al.*, 2004)..

Vegetative compatibility in fungi relates to its morphological differences and inter-relationships among the individuals and population respectively. Tests in Petri dishes make it relatively easy to determine the population of isolates and this provides a robust view of population diversity (Burgess *et al.*, 2001; van Heerden *et al.*, 2001). This approach also provides a means of understanding the genetic diversity in a fungal population where molecular markers and the concomitant sophisticated facilities may not be readily available. Although the taxonomy of plant pathogenic fungi mainly depends on morphological and pathological criteria (Kistler, 1997). The objective of this study was to determine the best supporting medium for the growth and sporulation of *F. oxysporum* f. sp. *elaeidis*

Materials and Methods

Survey and samples collection

The survey was carried out in three African countries, namely Nigeria, Ghana and Cameroon. In Nigeria, samples were collected from diseased oil palm at Abak sub-Station of the Nigerian Institute for Oil Palm Research (NIFOR). In Ghana, samples were collected from Oil palm Research Institute (OPRI), Kusi, Benso Oil Palm Plantation and Ghana Oil Palm Development Company (GOPDC); and also in Cameroon, oil palm trees showing acute and chronic symptoms belonging to the Institute of Agricultural Research for Development (IRAD) Ekona (South West Region) and Cameroon Development Company (CDC) of the same South-West region were also explored. Diseased samples of infected petioles, roots and soil were collected in separate polythene bags during the survey (Plate 1). The collected samples were brought to the laboratory for the isolation of pathogens. The samples were stored in a refrigerator at 4°C before processing.

Isolation, purification and preservation of *Fusarium oxysporum* f. sp. *elaeidis*

Fusarium oxysporum f. sp. *elaeidis* species were isolated from the vascular bundles, petioles and soil samples of adult oil palms showing chronic vascular wilt symptoms from different locations in Nigeria, Ghana and Cameroon. Vascular bundles and petioles of chronic vascular infected oil palms were cut into smaller bits using a sterile knife to expose the infected internal portions (Plate 2). They were aseptically extracted using a flamed sterile scalpel, and plated on already prepared mycelium medium (MM) that had been cooled and added streptomycin antibiotic. Incubation was done at room temperature of 24-26°C. Root samples were also plated out, cut portions of the roots were surfaced sterilized with 1% sodium hypochlorite mixture. One gramme of the soil samples were weighed and transferred to McCartney bottles containing 9 ml sterile distilled water. The bottles were shook using an orbit shaker for 15 minutes. One milliliter of the solution was obtained and transferred to a waiting nine milliliter of sterilized distilled water. They were serially diluted from 10⁻¹ to 10⁻⁵ diluting factors. Using a sterile syringe, one milliliter each of the serially diluted samples were dispensed into each sterile Petri dishes, then nine milliliter of 45°C cooled mycelium medium was poured into the dishes and swirled. The plates were incubated at ambient temperature for 48 hours. The emerging *Fusarium* fungi colonies were sub cultured aseptically into solidified PDA plates until pure cultures were obtained.

Morphological identification of fungi

After 3 to 4 days of incubation growth of *Fusarium* species were observed under light microscope, the photographs of the organisms were taken using Motic Camera 2000. The *Fusarium* species identified were confirmed by comparing their morphology using the descriptions of Talbot, 1972; Deacon, 1980; Bryce, 1992; Booth, 1977.

Preparation of conidial suspensions

Preparations of conidia suspensions were done by cutting 3mm portions of the already grown *Fusarium oxysporum* f. sp. *elaeidis* species in Petri dishes. The portions were inoculated into 250ml conical flasks containing sterilized 50ml of Armstrong medium. At each preparation, four flasks were inoculated for each isolate of *Fusarium oxysporum* f. sp. *elaeidis* and incubated for 10 days at room temperature (26-28°C). At the end of the incubation period, contents in the flasks were poured out into a warren blender and macerated. The macerated mixture of each isolate was then filtered through muslin cloth. The supernatants were decanted and the suspensions were made up with sterile water to the required spore concentrations.

Determination of spore count

A drop of the inoculum of was pipetted on a haemocytometer and covered with a cover slip. Using a phase contrast illumination microscope, the spores were counted with a 10x ocular and 4mm objective and this was done for at least 20 squares. The average number per square was determined. Three counts of diluted spore suspension were made, and their average was taken. The number per millilitre of the original spore suspension (undiluted) was calculated as follows:

(Average No. per square) x y x 4,000,000

Odigie (1994).

Measurement of Fungal Growth

The pure cultures of *Fusarium oxysporum* species were prepared using 7 days old mycelia. The *Fusarium* species grown on Malachite Green forming colonies of the different shapes, sizes and colours were then transferred aseptically to the centre of Petri dishes containing PDA medium and incubated at 25°C. The linear growth of the fungus was assessed in cm after 48h.

Selection of medium for the growth of *Fusarium oxysporum* f. sp. *elaeidis*

Eight media, Park's medium (PM) Potato dextrose agar (PDA), V8 juice medium (V8JM), Komada medium (KO), Malachite Green medium (MG), Malachite green captan medium (MGCM) and Horne and Mitters (H&M) were selected to determine the best supporting medium for the growth of different species of *Fusarium oxysporum* f. sp. *elaeidis*. All the media were prepared according to the manufacturer instructions (Hi Media) and sterilized at 120 C for 15 minutes. Twenty milliliters of each already sterilized medium was poured into Petri dishes. They were shaken uniformly and the plates were allowed to solidify. They were each inoculated with a 5 mm mycelia disc cut from the margin of 5-day-old culture using a 2mm cork borer and placed at the middle of each Petri dishes They were incubated at 26±2°C for 10 days. Each treatment was replicated four times.

Radial Growth and Pigment Production of the different isolates of *Fusarium oxysporum* f. sp. *elaeidis* on different Solid Media

The diameter of each *Fusarium* species were recorded in centimeters in two directions at right angles to each other and then average colony diameter in centimeter was calculated and recorded. Measurement of growth was made at the interval of 24 h, till the full expansion of growth and studies of sporulation on different solid media was also undertaken.

RESULTS

The results revealed that *Fusarium oxysporum* f. sp. *elaeidis* species. produced visible growths in culture media. The four species (Herbarium IMI No. KP942906.1, species (1) from Cameroon); *F. oxysporum* (Herbarium IMI No. AY928419.1, species (4) from Cameroon); *F. oxysporum* (Herbarium IMI No..KR094464.1, species (CRT) from Ghana and *F. oxysporum* (Herbarium IMI No..JF807394.1, species (13) from Nigeria) were grown on PDA and at 14 days produced various

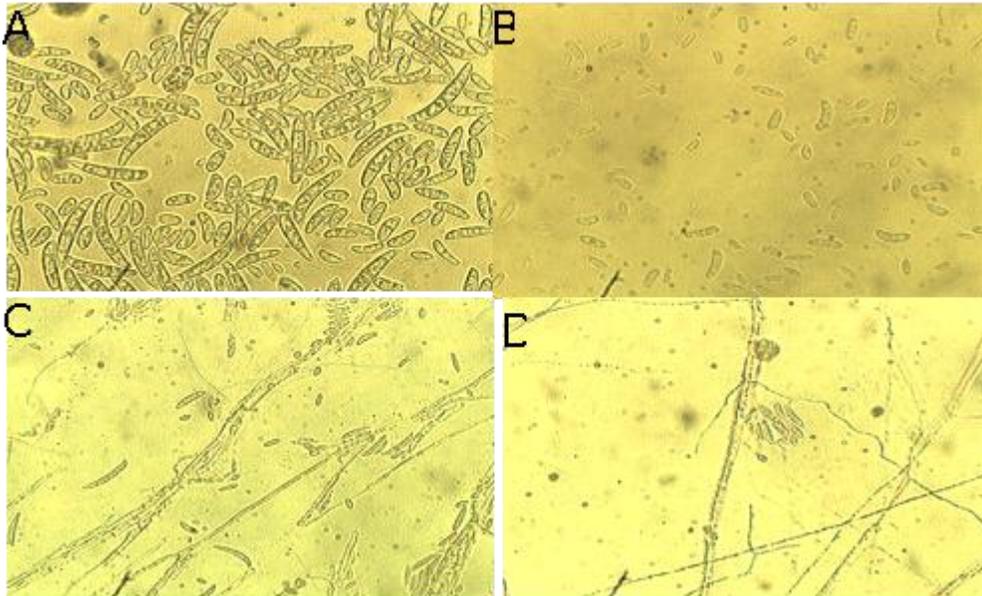


Figure 1. Shows the microscopic view of *Fusarium oxysporum* f. sp. *elaeidis* cultures from different African countries on PDA at 14 days

A. Shows the conidia of *Fusarium oxysporum* f. sp. *elaeidis* (1) from Cameroon (X4)

B. Shows the conidia of *Fusarium oxysporum* f. sp. *elaeidis* (4) from Cameroon (X4)

C. Shows the conidia of *Fusarium oxysporum* f. sp. *elaeidis* (CRT) from Ghana (X4)

D. Shows the conidia of *Fusarium oxysporum* f. sp. *elaeidis* (13) from Nigeria (X4)

morphological structures. *F. oxysporum* (1) and (4) both from Cameroon showed cream and coloured surfaces, convex in shape with cottony or ropey texture. Colony centimetre after 10 days was greater than 7cm. Reverse pigmentation on PDA was colourless to cream, aerial mycelium was abundant; *F. oxysporum* (CRT) from Ghana presented a pink coloured surface, convex in shape with cottony or ropey texture sporodochia present at the centre of the aerial mycelium, colony centimetre after 10 days was greater than 7cm. Reverse pigmentation on PDA was red. *F. oxysporum* (13) from Nigeria showed a cream coloured surface, convex in shape with cottony or ropey texture. Colony centimetre after 10 days was greater than 7cm. Reverse pigmentation on PDA was colourless to cream. (plate 1A-H).

Microscopic view of *Fusarium* sp. 1. shows macro conidia was absent on sporodochia, macro conidia is large on aerial mycelium. It was straight on the right (ventral) and slightly curved on the left (dorsal), the apical cell was blunt while the basal cell was barely notched with 4-5 septa. *Fusarium* sp. 4. shows macro conidia was absent on sporodochia, macro conidia was medium size on aerial mycelium. It was straight on the right (ventral) and slightly curved on the left (dorsal), the apical cell was blunt while the basal cell is equally blunt with 3-4 septa. *Fusarium* sp. CRT shows macro conidia was present on sporodochia, macro conidia was elongate and slender on sporodochia. It was straight on the right (ventral) and so slightly curved on the left (dorsal), the apical cell was papillate while the basal cell was barely notched with 4-5 septa. *Fusarium* sp. 13. Shows macro conidia was absent on sporodochia, macro conidia was medium sized on aerial mycelium. It was straight on the right (ventral) and very slightly curved on the left (dorsal), the apical cell was blunt while the basal cell was equally blunt with 3-4 septa (figure 1A-D). The colour characteristics of four *Fusarium oxysporum* f. sp. *elaeidis* isolates on different selective growth media are shown in table 1.

Out of the overall mean of spore counts on best supporting media for *F. oxysporum* f. sp. *elaeidis* species, Malachite Green had the highest with 11.94. These were followed by Home Green (11.13); V8 Juice medium and Potato dextrose agar (6.56 each); Malachite Green Captan Medium (2.31) and Park's Medium (2.13) respectively (table 2). Mean counts of *Fusarium oxysporum* f. sp. *elaeidis* isolates in broth sporulation in different media broth, *F. oxysporum* isolate 1 had the highest counts. This was followed by *F. oxysporum* isolate 4. The least isolate was *F. oxysporum* CRT (figure 2).

Six days after incubation of *F. oxysporum* f. sp. species in different selective media, the highest mean linear growth in centimeter (cm) was recorded by Malachite Green (4.5). These were followed by Malachite Green Captan and V8

Table 1. Colour characteristics of four *Fusarium oxysporum* f. sp. *elaeidis* isolates on different selective growth media

Fusarium species	Park's medium (pm)	V8 juice medium (v8jm)	Komada medium (ko)	Malachite green medium (mg)	Malachite green captan medium (mgcm)	Horne and mitters (h&m)	Potato dextrose agar (pda)
1	White	White	White	White	White	White	White
4	White	White	White	White	White	White	White
13	White	White	White	White	White	White	White
CRT	White	Pink	White / Pink	Pink	White / Pink	Pink	Pink

Table 2. Mean of spore Counts of *Fusarium oxysporum* f. sp. *elaeidis* isolates

	Overall counts	<i>Fusarium oxysporum</i> f. sp. <i>elaeidis</i> 1	<i>Fusarium oxysporum</i> f. sp. <i>elaeidis</i> 4	<i>Fusarium oxysporum</i> f. sp. <i>elaeidis</i> 13	<i>Fusarium oxysporum</i> f. sp. <i>elaeidis</i> CRT
Media	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Park's Medium (PM)	2.13 ± 0.56 ^a	4.25 ± 0.63 ^a	3.50 ± 1.26 ^b	0.5.0 ± 0.29 ^a	0.25 ± 0.25 ^a
Komada Medium KO)	0.81 ± 0.37 ^a	3.25 ± 0.25 ^a	0 ^a	0 ^a	0 ^a
Malachite Green (MG)	11.94 ± 1.44 ^c	19.75 ± 1.75 ^b	6.00 ± 0.71 ^c	13.5 ± 0.87 ^c	8.50 ± 0.50 ^d
Horne &Mitters (H/M)	11.13 ± 1.19 ^c	17.00 ± 1.22 ^b	13.00 ± 1.00 ^e	7.75 ± 1.31 ^b	6.75 ± 1.18 ^c
Malachite Green Captan Medium (MGCM)	2.31 ± 0.62 ^a	4.25 ± 0.75 ^a	5.00 ± 0.00 ^{bc}	0 ^a	0 ^a
V8 Juice Medium (V8JM)	6.56 ± 1.68 ^b	14.25 ± 3.38 ^b	10.00 ± 0.00 ^d	0 ^a	2.00 ± 0.41 ^b
Potato Dextrose Agar (PDA)	6.56 ± 1.68 ^b	14.25 ± 3.38 ^b	10.00 ± 0.00 ^d	0 ^a	2.00 ± 0.41 ^b
F – Statistics	F _{6,105} = 13.745; p < 0.001	F _{6,105} = 11.713; p < 0.001	F _{6,21} = 45.378; p < 0.001	F _{6,21} = 79.667; p < 0.001	F _{6,21} = 40.888; p < 0.001

Juice medium with 4.3 each, Home and Mitters (4.26), Komada medium (4.23), PGA (3.6) and Park medium (3.25). On day ten after incubation, there was no significant difference among the isolates (table 3).

Discussions

The various isolates from West African countries revealed that the *Fusarium oxysporum* f. sp. *elaeidis* species had different morphological structures and growth rates in media which was in agreement with the study of Jalander and Gachande, 2015 which stated that growth of *Fusarium oxysporum* species on CDA medium revealed variety in growths.

The pigmentation characteristics of the different *Fusarium oxysporum* f. sp. *elaeidis* species had revealed that the different species produced different colour pigmentations in different media which is in line with the report of Ismail *et al.*, 2013. The number of spores of the *Fusarium* species produced by the different media from abundant to few revealed that some of the media caused some *Fusarium* species to produce more spores than the other.

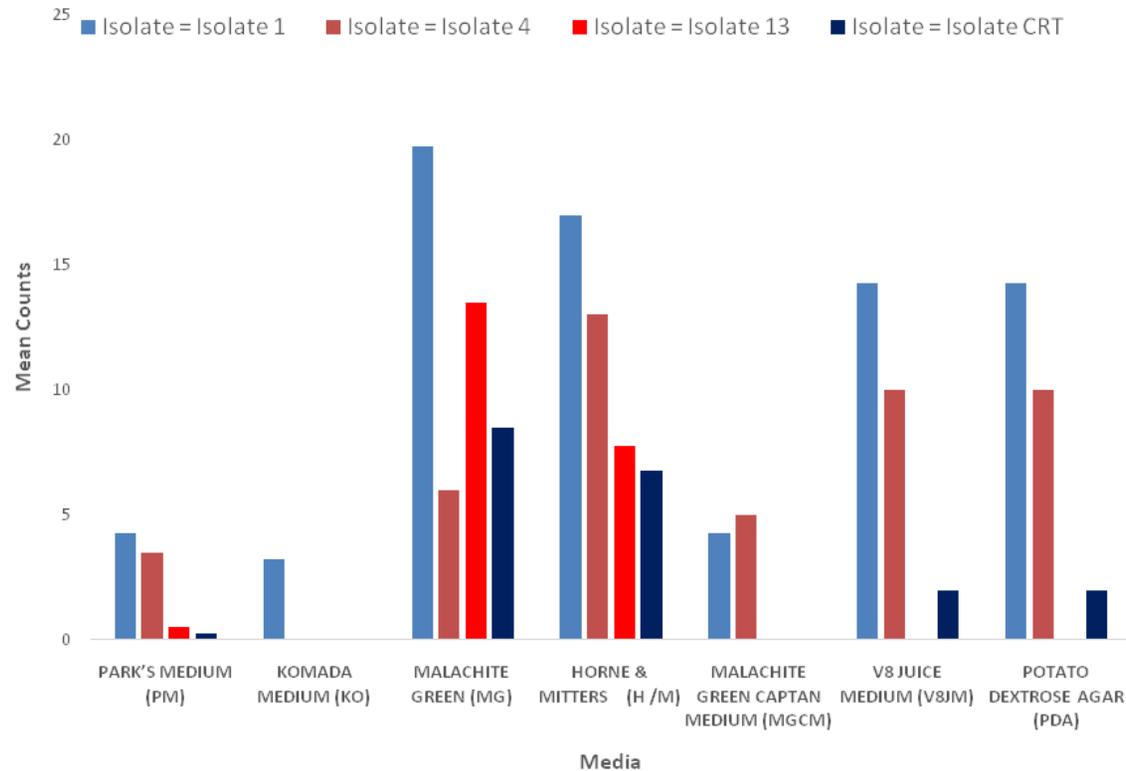


Figure 2. Mean counts of *Fusarium oxysporum* f. sp. *elaedis* species in media broth

This was important because spores were effective agents of pathogenesis, so the best media that gave sporulation and pathogenicity would be adopted for culturing and future studies.. This was in agreement with the work of Gupta *et al.*, 2010).

Malachite green agar broth sporulated *Fusarium oxysporum* f. sp *elaedis* more when compared with the other media. Komada medium was the least. This report was supported with the work of Gupta *et al.*, 2010

Conclusion

The cultural variation of four species of *Fusarium oxysporum* f. sp. *elaedis* from Africa expressed variations in respect to cultural and morphological characters. The study revealed that the culture media influenced sporulations of *Fusarium oxysporum* f. sp. *elaedis*. Out of the seven tested media, Malachite Green (MG) was found to be most suitable for spores production.

Table 3. Mean linear growth (cm) of isolates of *Fusarium oxysporum* f. sp. *elaedis* in different selective media

Days	Park's Medium (PM)	Komada Medium (KO)	Malachite Green (MG)	Horne &Mitters(H/M)	Malachite Green Captan Medium (MGCM)	V8 Juice Medium (V8JM)	Potato Dextrose Agar (PDA)
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Day 1	0.08 ± 0.08 ^a	0.08 ± 0.08 ^a	0.08 ± 0.08 ^a	0.09 ± 0.09 ^a	0.1 ± 0.10 ^a	0.08 ± 0.08 ^a	0.06 ± 0.06 ^a
Day 2	0.55 ± 0.1 ^b	0.73 ± 0.11 ^b	1.1 ± 0.19 ^b	0.96 ± 0.1 ^b	1.18 ± 0.09 ^b	1 ± 0.06 ^b	0.78 ± 0.06 ^b
Day 3	1.17 ± 0.07 ^c	1.46 ± 0.09 ^c	2.33 ± 0.33 ^c	1.81 ± 0.08 ^c	1.95 ± 0.13 ^c	1.6 ± 0.06 ^c	1.21 ± 0.12 ^{bc}
Day 4	1.43 ± 0.1 ^c	2.2 ± 0.07 ^d	3.95 ± 0.15 ^d	2.29 ± 0.15 ^d	2.84 ± 0.12 ^d	2.25 ± 0.06 ^d	1.65 ± 0.13 ^c
Day 5	1.92 ± 0.24 ^d	2.89 ± 0.11 ^e	4.41 ± 0.07 ^e	3.02 ± 0.22 ^e	3.53 ± 0.12 ^e	2.9 ± 0.18 ^e	2.28 ± 0.22 ^d
Day 6	3.25 ± 0.22 ^e	4.23 ± 0.04 ^f	4.5 ± 0 ^e	4.26 ± 0.05 ^f	4.43 ± 0.04 ^f	4.43 ± 0.04 ^f	3.6 ± 0.24 ^e
Day 7	3.51 ± 0.22 ^{ef}	4.36 ± 0.03 ^{fg}	4.5 ± 0 ^e	4.35 ± 0.04 ^f	4.47 ± 0.02 ^f	4.47 ± 0.02 ^f	3.76 ± 0.2 ^{ef}
Day 8	3.79 ± 0.19 ^f	4.49 ± 0 ^g	4.5 ± 0 ^e	4.47 ± 0.02 ^f	4.5 ± 0 ^f	4.5 ± 0 ^f	4.06 ± 0.19 ^{efg}
Day 9	4.04 ± 0.15 ^{fg}	4.5 ± 0 ^g	4.5 ± 0 ^e	4.5 ± 0 ^f	4.5 ± 0 ^f	4.5 ± 0 ^f	4.16 ± 0.17 ^{fg}
Day 10	4.35 ± 0.08 ^{gh}	4.5 ± 0 ^g	4.5 ± 0 ^e	4.5 ± 0 ^f	4.5 ± 0 ^f	4.5 ± 0 ^f	4.37 ± 0.13 ^g
F - Statistics	F _{9,109} = 95.644; p < 0.001	F _{9,109} = 677.450; p < 0.001	F _{9,109} = 153.806; p < 0.001	F _{9,109} = 272.090; p < 0.001	F _{9,109} = 417.940; p < 0.001	F _{9,109} = 627.825; p < 0.001	F _{9,109} = 94.956; p < 0.001

NB: Days with different superscripts are not significantly different at 5% level of significance

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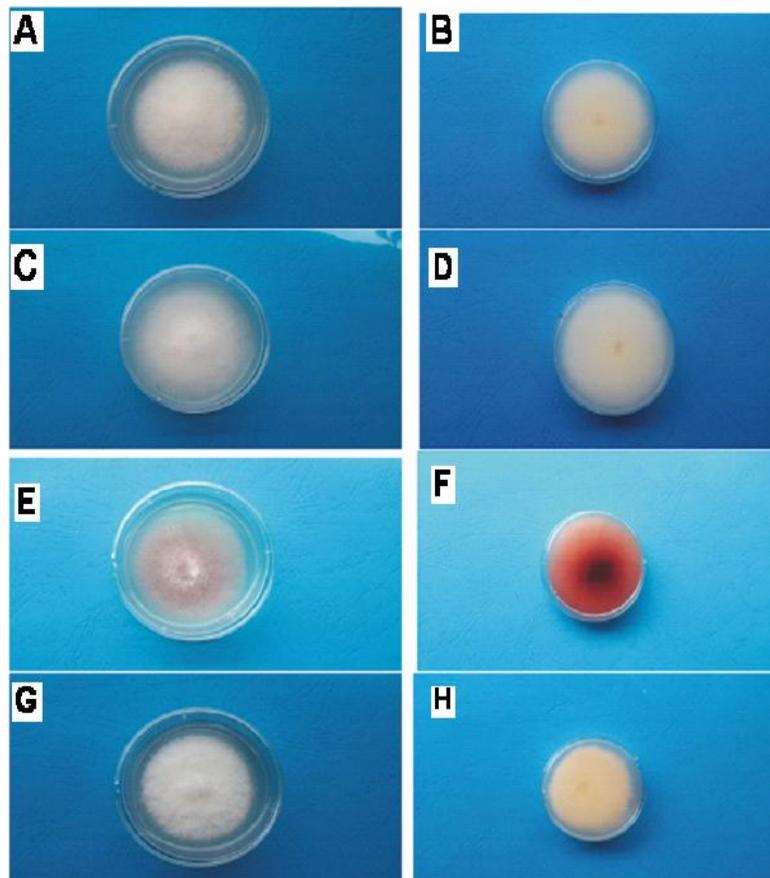


Plate 1A-H: Shows the photographs of *Fusarium oxysporum* f. sp. *elaedis* cultures from different West African countries on PDA at 14

A-B: Shows top and reverse photographs of *Fusarium oxysporum* f. sp. *elaedis* culture (1) from Cameroon (x3)

C-D: Shows top and reverse photographs of *Fusarium oxysporum* f. sp. *elaedis* culture (4) from Cameroon (x3)

E-F: Shows top and reverse photographs of *Fusarium oxysporum* f. sp. *elaedis* culture (CRT) from Ghana (x3)

G-H: Shows top and reverse photographs of *Fusarium oxysporum* f. sp. *elaedis* culture (13) from Nigeria (x3)

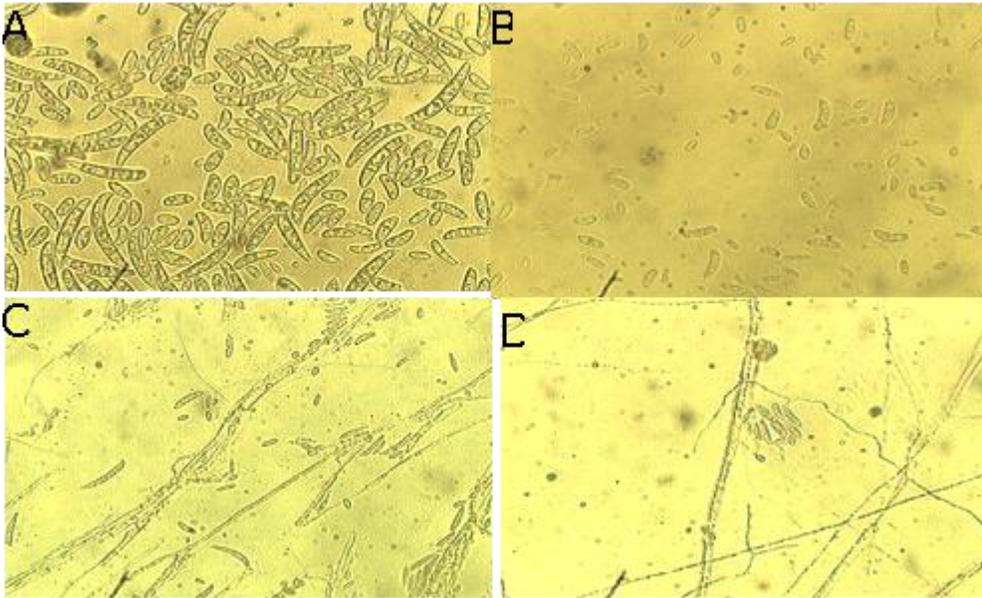


Plate 2A-D: Shows the microscopic view of *Fusarium oxysporum* f. sp. *elaeidis* cultures from different West African countries on PDA at 14 days

- A.** Shows the conidia of *Fusarium oxysporum* f. sp. *elaeidis* (1) from Cameroon (X4)
- B.** Shows the conidia of *Fusarium oxysporum* f. sp. *elaeidis* (4) from Cameroon (X4)
- C.** Shows the conidia of *Fusarium oxysporum* f. sp. *elaeidis* (CRT) from Ghana (X4)
- D.** Shows the conidia of *Fusarium oxysporum* f. sp. *elaeidis* (13) from Nigeria (X4)