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Isolation and *in vitro* Characterization of *Fusarium oxysporum* f. sp. *elaeidis*, Causal Agent of Oil Palm (*Elaeis guineensis* Jacq.) Vascular Wilt

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Abstract Vascular wilt disease caused by the fungus *Fusarium oxysporum* f. sp. *elaeidis* (F.o.e) is one of the major diseases of oil palm (*Elaeis guineensis* Jacq.) in the South-West region of Cameroon. In order to document the F.o.e isolation and characterization procedures, a 3 months study was carried out in palm plantations around IRAD Ekona and in the palm pathology laboratory located in the South-West region of Cameroon. Some 30 isolates of F.o.e and 10 g of infected soil were collected on and around infected oil palm trees from the field and used to appreciate various morphological characters that influence their aptitude to persist in the soil and on the host. The *in vitro* mycelial growth, sporulation (conidia), growth rate, biomass and the other characteristics as well as the correlation of the isolates in the culture media were recorded. Results showed that the isolates develop very quickly in the Medium for Mycelium (MM) contrary to PDA medium. In MM, the conidial stocks developed and filled the Petri dish in about 3 days contrary to the filamentous fungal stocks which took about 7 days to fill the Petri dish. These results can improve understanding of the procedures used and devise a selection strategy of the antagonistic microbial stocks for a biological control against oil palm vascular wilt disease.

Keywords: *Fusarium oxysporum f. sp. elaeidis, oil palm, vascular wilt, Elaeis guineensis Jacq., in vitro characterization*

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1. Introduction

The oil palm (*Elaeis guineensis* Jacq.) with chromosome number 2n = 32 belongs to the family Arecaceae. It is a perennial monocot currently considered to be the most important and highest producing oil crop in the world [5,22]. The fruits of this crop produce two types of oil: crude palm oil (CPO) extracted from the mesocarp and palm kernel oil (PKO) extracted from the kernel. Palm oil is used for food purposes (cooking oil, margarine, vanaspati, and shortenings), oleochemicals (detergents, cosmetics) and pharmaceutical purposes (carotenes), health supplement and as a source of biofuel [34].

Endemic diseases of the oil palm (*Elaeis guineensis* Jacq.) are a major threat to its culture around the world. Research endeavors have been geared towards the development of appropriate crop management approaches like lower cost cropping systems and non-chemical control of endemic diseases [16,21,43]. Among oil palm diseases are Ganoderma common in South East Asia (highest oil palm production zone in the world) [10], bud rot (Latin America) and vascular wilt (Africa) that all affect growth and reduce oil yields [34].

Fusarium wilt is endemic in several African countries where thousands of palms have been lost and yield markedly reduced [6]. Vascular wilt caused by *Fusarium oxysporum* f. sp. *elaeidis* is the most damaging disease of oil palm in Africa [9,17,39,42], causing up to 70% mortality (Renard et Ravise, 1986; [34]). The expression of the disease depends on the age of the plant, the stage of infection, susceptibility of the plant material and environmental factors [34,39].

The prevalence of oil palm vascular wilt remains important despite the much effort to control it. Expansion of this disease is favoured by climatic conditions, exchange of sensitive planting material, transport of contaminated tools and inappropriate cultural techniques. By 1986, vascular wilt concerned about 600 000 hectares of palm plantations in Africa [40]. Yield losses due to vascular wilt range between 25% and 50% and even more (Flood 1986; [34,48]).

Fusarium oxysporum f. sp. *elaeidis* is specific to oil palm. The morphology and anatomy of this parasite have been described by Ravisé (1965). Its infection is observed as brown fibres in the stem or the leaf petioles and it has same characteristics as all of the *Fusarium oxysporum* fungi: macroconidia, microconidia and chlamydospores (Flood, 2006). The systematic classification of *Fusarium oxysporum* f. sp. *elaeidis* Toovey is as follows (Brayford, 1992): Kingdom Fungi; Phylum: *Ascomycota*; Subphylum *Pezizomycotina*; Class *Sordariomycetes*; Sub-class Hypocreomycetidae; Order *Hypocreales*; Family *Nectriaceae*; Genus *Fusarium*; Species *oxysporum*; Common name vascular wilt.

Symptoms of fusarium wilt disease occur when oil palm seedlings are inoculated with the fungus, [20,39]. *Fusarium oxysporum* f. sp. *elaeidis* (F.o.e) is a soil-borne pathogenic fungus that invades intact roots then the xylem to cause water stress and hormonal imbalance, with consequent severe yield loss and even death of the palm tree [6]. The pathogen penetrates the roots, develops in the xylem vessels, and causes gum and thyloses which block the vessels [17,48]. Internal vascular browning is diagnostic for F.o.e and the pathogen can easily be re-isolated from xylem [6]. Since F.o.e can contaminate seed and pollen, it is considered that isolated outbreaks in South America appear to have resulted from inter-continental seed movement [6]. Moreover, spores of *Fusarium oxsporum* have been found in batches of lyophilized pollen with some isolates shown to be pathogenic [6,18]. Plant quarantine measures should therefore constitute a very imperative component of the fight against oil palm vascular wilt [6,7,36].

Movement from tree to tree is supported by the occurrence of infected palms in pairs or groups and the greater infection of palms with missing neighbours than those without [6,7].

Figure 1. Healthy (A) and wilted (B) oil palm seedlings

Some oil palm strains are tolerant to vascular wilt [47] [40]. Research on defense mechanisms of the oil palm in reaction to *Fusarium oxysporum* f. sp. *elaeidis* has revealed the production of phytoalexines by the plants which accumulate in the tissues of the roots and pseudo bulbs of resistant plants in vitro [40]; Diabate *et al*., 1990). Moreover, dosage of the rooting and pseudobulb extracts of oil palm, after infection by F.o.e. has revealed the presence of phenolic compounds in the tolerant crosses and clones (Diabaté *et al*., 2010). The symptoms triggered by *Fusarium oxysporum* were first described by Wardlaw (1946a, 1946b; [34,39]). Later on, the disease was observed in numerous plantations in Congo, then in Nigeria, Cameroon, Ivory Coast, and throughout West Africa (Renard et al., 1972; [39]; Cochard et al., 2005).

Two forms of symptoms are usually observed on vascular wilted palms [17,41]: the typical or acute symptoms through which the palm dies three to four months after their appearance, and the chronic symptoms which maintain the palm alive, weaken and render it almost unproductive [34,48].

In Cameroon, oil palm cultivation is very important, practiced by both agro-industries and smallholders. Smallholder plantations are mostly constituted of either improved or unimproved (wild) planting materials or both types. Cameroon produced 1 600 000 tonnes of CPO in 2009 from a surface area of about 190 000 ha [25]. This production has been envisaged to increase from 300 000 tonnes in 2005 to 450 000 tonnes en 2020 [24,25]. Moreover, there is currently regain of interest in oil palm cultivation in Cameroon by smallholders and big local and foreign investors.

A major obstacle to the growth of the oil palm industry in Cameroon could be vascular wilt disease and Ganoderma incidence if nothing is done to improve on the current control strategies. The South West, Littoral, North West, Centre, West and South regions of Cameroon with best ecology for oil palm are particularly concerned by this Fusarium wilt disease [33,34]. Also, previous work in Cameroon has led to the collection of several strains of *Fusarium oxysporum* f. sp. *elaeidis*, currently available in the phytopathology laboratory of IRAD Dibamba located at Ekona in the South West region of Cameroon. Moreover, a test of aggressiveness for these local strains of Fusarium isolated from wilted oil palms in Cameroon has been realized with results revealing variability of aggressiveness between strains [48]. Due to this variability, it is advised to use vascular wilt tolerant planting material in Cameroon.

To the best of our knowledge, no study has so far done with the objective of standardizing and documenting field isolation and laboratory characterization procedures for *Fusarium oxysporum* f. sp. *elaeidis.* To this effect, the objective of this work was to experiment and document the procedures for field isolation and laboratory characterization of *Fusarium oxysporum* f. sp. *elaeidis*. The documented procedures here will certainly facilitate the work of breeders in selecting tolerant varieties through laboratory and field/nursery experiments requiring isolation of *Fusarium oxysporum* f. sp. *elaeidis* or other similar micro-organisms. The study thus involved the mastery of symptoms identification on infected palms in the field and the collection, *in vitro* culture, identification and characterization of F.o.e. in the laboratory. The

identification of isolated fungi was done on the basis of cultural and morphological characteristics [8,28,51].

2. Materials and Methods

2.1. Study Site

Figure 2. Map showing **g**eographical locations from where F.o.e. samples were collected

The study was realized from May to July 2014 at Ekona in the South West Region of Cameroon. Identification of field symptoms and collection of F.o.e. samples were done on a 16.86 ha oil palm plantation near the S. W. Regional Research Centre, IRAD Ekona (Figure 2). *In vitro* culture (Figure 3) and characterization of the fungal samples (Annex 2 and 3) were realized in the IRAD La Dibamba Phytopathology laboratory located in IRAD Ekona.

2.2. Materials

Biological materials used in this study were palm fronds and F.o.e isolates. The palm fronds were cut at the level of petioles from infected *Dura*, *Pisifera* and *Tenera* in three blocks of palm trees at the IRAD Ekona research site. F.o.e isolates were collected from brown fibres in the petioles of these palm fronds cut from 30 palm trees infected by *fusarium* wilt. Soil samples were also collected from around the infected trees. The collected isolates are presented in Table 1.

Technical materials and reagents included harvesting knives, laboratory material and glassware, reagents for mycelium culture and PDA media. The following items were necessary for the isolation: Bunsen burner, scalpel, laminar Hood, Knives, Petri dishes, alcohol 50%, cotton to cover the glassware and avoid contact with the exterior.

Table 1. F.o.e isolates collected from three blocks at Ekona

Plot	Isolates	Variety	Date
1	092746R, 093658R, 094840R, 095110R, 035200R, 035244R, 035633R, 035728R, 040353R, 040539R,	Dura	15/05/2014
2	040958R, 041416R, 041528R, 041746R, 042649R, 042746R, 042904R, 043012R, 043110R, 043204R,	Pisifera	22/05/2014
3	043252R, 043428R, 043536R, 043639R, 043834R, 043916R, 044015R, 044745R, 044838R, 045216R	Tenera	22/05/2014

2.3. Methods

2.3.1. Identification of F.o.e through Symptoms

The symptoms of the oil palm vascular wilt disease caused by F.o.e are typical and chronic. Pure cultures of *Fusarium oxysporum* are generally salmon-pink. When oil palm seedlings are inoculated with the cultures, symptoms of the disease are reproduced [20,39,40]. Identification of the fungus only from external and internal symptoms is not completely reliable. In this study, identification and enumeration of F.o.e was done through its isolation from the rachis or petioles of infected palms and soil samples and the in vitro identification of its pure culture by assessment of growth rate and enumeration after suspension-dilution ([50]) through the microbiological analysis of the soil respectively. The optical microscope was used for the visualization of different forms of the pathogen.

The goal was to determine the special form *elaeidis* of the collected isolates of F.o.e. The aggression of its specific host - the oil palm - is manifested by the appearance of external symptoms such as yellowing of the leaflets from the petiole of the palm progressively toward the apex, until the death of the entire palm. The fronds which have external symptoms have a brown color and their vessels are also observed to be coloured when dissected (Figure 1B). So there is continuity of vascular symptoms from the roots up to the apical leaves of the palm tree [14].

2.3.2. Isolation, Purification and Determination of Strains

For F.o.e isolation, the surface of the explant of vascular tissue from the rachis or petiole was sterilized with ethanol to 50% for one minute [15]. The bench top, sampling and cutting equipment were flamed each time by soaking in alcohol and passage over the flame.

2.3.2.1. Composition and Preparation of Culture Media

The medium for Mycelium (MM), NASH and Potato Dextrose Agar (PDA) were used in this study. The reagents of the medium for Mycelium (MM) and Potato Dextrose Agar (PDA) were weighed on an electric balance and introduced into 500 mL of distilled water. The reagents were then supplemented with 500 mL of distilled water and mixed thoroughly. This was heated to 400°C on a magnetic heat stirrer for 30 min. After cooling, the medium was distributed in volumetric flasks of 250 mL and the flasks were covered with parafilm [31]. The culture media used were made up of the following composition.

PDA Medium (Potato Dextrose Agar): 4 g potatoes, 20 g Dextrose (glucose), 15 g Agar-Agar, 1000 mL of distilled water, the whole was autoclaved for one hour at 120°C (sterilisation) and then distributed in Petri dishes [37].

MM Medium (Medium for Mycelium): 1g dipotassium hydrogen phosphate, 0.5 g magnesium sulfate, 0.1 g iron sulphate, 1.5 g asparagine, 1g yeast extract, 25 g Agar Agar, 20 g glucose, 1L distilled water

NASH Medium: 15 g peptone, 20 g Agar Agar, 1 g monopotassium phosphate, 0.5 g magnesium sulfate, 1000 mL of distilled water. After sterilization, dihydrostreptomycine (10 mL) are added in order to eliminate any bacteria present. At times, some bacteria are resistant to the antibiotics. In such a case, chloramphenicol and pentachloronitrobenzene (1g), previously ground in a few drops of di-ethyl ether and Triton x 100 are added to the medium (Smith et al., 1975).

The first two solid media were used for the evaluation of growth rate while the third was used to count the fusarium flora (Olutiola and Ayres, 1973). The composition of Mycelium medium (MM) and PDA for 1 litre used in this study are presented in Table 2 and Table 3.

Table 2. Composition of Mycelium medium (MM) for 1 litre

Constituent	Amount
Dipotassium Phosphate	1g
Magnesium Sulfate	0,5 g
Iron Sulfate	0,1 g
Asparagine	1,5 g
Yeast extract	1g
Agar agar	25g
Glucose	20g
Distilled water	1l
Autoclave at 120°C for 50 min (pH = 6.5)	

Table 3. Composition of Potato Dextrose Agar medium (PDA) for 1 litre

Constituent	Amount
Irish potato	4g
Glucose	20g
Agar	15g
Distilled water	1l
Autoclave at 120°C for 50 min (pH = 6.5)	

2.4.2 Isolation and Purification of F.o.e Strains from Leaf Explants

Petri dishes and other glassware were previously sterilized by autoclaving (120°C). Twenty (20) mL of the culture medium was poured into the Petri dish in a fume hood. After a short while, explants (infected leaf fiber) were cut longitudinally into small fragments of 1 cm length with sterile equipment and put in the center of the Petri dish (70 x 15 mm) containing 20 mL of the solid PDA culture medium and MM medium.

After 8 days of incubation in the Petri dishes and for purification, the F.o.e colonies obtained were sub cultured separately in Petri dishes on the same media 2 to 3 times.

Figure 3. *In vitro* culture of F.o.e

2.4.3. Isolation and Purification of F.o.e Strains in Soil Samples

Suspension-dilution technique was used to count and quantify the microflora of collected soil samples (Figure 4). Using an electric balance, 10 g of soil was weighed into a Petri dish. This was dried in an oven at 104°C for 24 hour. Each soil sample was diluted (1/10) in conical flasks. For this, 10 g of soil were introduced with a syringe into a test tube containing 90 mL of distilled water: this corresponds to 10^{-1} dilution. Then, 5 mL of the first dilution was introduced in 45 mL of distilled water. The flask was then covered with parafilm paper and agitated with a top mix (vortex) for 20 minutes for better suspension of micro-organisms and soil particles. For the same soil sample, the least diluted flasks were chosen (-1, -2, -3).

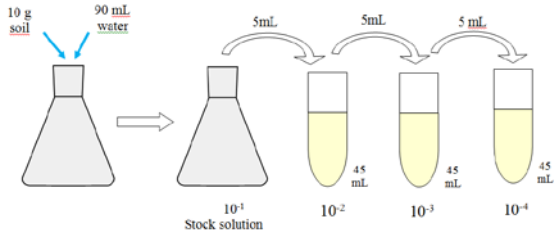

Figure 4. Suspension-dilution technique for microflora quantification

NASH medium (10 mL) was poured in Petri dishes and 1 mL of the least dilutions was introduced into the Petri dishes in three repetitions per sample and 5 Petri dishes per dilution (Figure 3). This was homogenized and incubated for 7 days [32] at 27°C. The production of F.o.e flora occurred when the Petri dishes were placed in the dark. After 24 hours, the quantity of F.o.e per gram of soil was counted on the back of the Petri dishes. Counting was done following the solid medium counting technique [37] on the 3 repetitions for 7 days.

According to this technique, 1 mL of stock or diluted solution is placed in the Petri dish before the NASH medium is poured in the dish. This is done under a laminar flow hood. The mixture is homogenized for uniformity. The pipette used for reseeding is sterilized between reseeding operations by rapid immersion in ethyl alcohol followed by passing it over a flame.

2.4.3.1. Counting of Fusarium Flora

Most microbes found in the soil can develop on specific culture media forming separate colonies within 24 to 48 hours at 27°C. It is thus possible to count the number of mycelial colonies found in a known volume of soil. For our experiment, counting was done with dilutions from 10^{-1} to 10^{-3}. Three Petri dishes each received 1 mL of the solution (stock or diluted solutions) and 20 mL of NASH for the counting. The dishes were inverted and incubated at 27°C for 8 days on the lab bench.

Interpretation of colony counts was done by calculating the number of colonies per dilution. Petri dishes with same dilution whose number of colonies is between 30 and 50 were retained to this effect. Counting obeys the law of Poisson; mycelial density, dilution and the volume reseeded are such that an average x of colonies in NASH medium is obtained. The confidence threshold at 95 % is given by:

$$Confidence\ threshold\ at\ 95\% = \frac{1.96\sqrt{N}}{n}$$

The number of UFC per dilution was obtained using the formula:

$$Number\ of\ UFC\ per\ dilution = \frac{N}{n} + \frac{1.96\sqrt{N}}{n}$$

Where: N= Total number of colonies counted in a given dilution; n= number of dishes counted for that dilution.

To obtain the microbial load of the sample in a given dilution, multiply the number of colonies found for that dilution by its inverse and by the volume of sample reseeded. Isolation and purification of F.o.e strains in soil samples was done using NASH medium. Its composition for one litre used in this study is presented in Table 4.

Table 4. Composition of NASH medium for 1 litre

Constituent	Amount
Peptone	15 g
Agar agar	20 g
Monopotassium Phosphate	1 g
Magnesium Sulfate	0,5 g
Distilled water	1 l
Dihydrostreptomycine	1 ml
Pentachloronitrobenzene	1 g
Autoclave at 120°C for 50 min (pH = 6.5)	

2.4.4. Fungal Growth Rate Measurement

Isolated F.o.e samples were cultured on mycelium (MM) and Potato-Dextrose-Agar (PDA) media. Fungal growth rate was evaluated by daily measurements of the diameter of the zone occupied in Petri dish by the fungus.

To measure the mycelia growth rate of F.o.e in this study, 20 mL per Petri dish of solid media were used. From cultures obtained on this medium, the diameters of small spots representing the starting point of growth were measured with a graduated ruler. Four measurements were done for each Petri dish. This measurement of the evolution of the fungus was done on the back of the dish on two perpendicular lines and averages were calculated ([29]; Osagie and Obuekwe, 1991; Mitchell et al. 1997). Measurements were done after every 24 hours for a period of 8 days. The collected soil samples were evaluated on liquid medium with counting of *fusarium* done every 24 hours.

2.4.5. Production of Microconidia and Macro-Conidia

Petiole fibre explants were deposited at the centre of Petri dishes and after 10 days incubation at 28°C, a 4 cm sector of the medium was cut (from exterior to interior part of the colony) and introduced into 10 mL distilled water. This was then agitated for 3 min. After inoculation, conidia were observed in the Petri dish with the optical microscope (40X) after 12 days when the mycelium had covered the whole dish. This was to evaluate morphological characteristics (conidial form and the structure). Conidia in the NASH medium were counted for 7 days by spotting with bold marker on the back of the dish in the presence of light at 27°C. Microscopic observation revealed macro-conidia as conidia with at least two septa. Other fungal characteristics like colour and growth habit were also recorded.

2.4.6. Production of Chlamydospores

Explant were deposited at the centre of Petri dishes and after incubation for 20 days at 28°C, a section of the medium was cut and placed on a glass slide in a few drops of distilled water and then covered with a slide cover before microscopic observation.

2.4.7. Statistical Analysis

F.o.e strains from leaf petiole were cultured in two repetitions each while those from soil samples were cultured in three repetitions. The Microsoft Excel STATVIEW was used for statistical analysis. Analysis of variance (ANOVA) was used to compare means of diameters of fungal (mycelium) colonies. Student-Newman-Keuls test at 5% significance was used to distinguish diameter means.

3. Results

3.1. Fungal Isolation and Mycelial Growth

After incubation on the culture media, mycelia gradually appeared around the explants fragments. Linear growth of the 30 F.o.e isolates on the two culture media (PDA and MM) increased with incubation duration. By the 8th day, maximum growth of 70 mm was reached by 26 isolates on MM, and 28 isolates on PDA (Table 5).

Table 5. F.o.e samples with maximal mycelial growth

Media	N° of Isolates	Maximum growth on day 8
MM	26	092746R, 093658R, 094840R, 095110R, 035200R, 035244R, 035633R, 035728R, 040353R, 040958R, 041416R, 041528R, 041746R, 042904R, 043012R, 043110R, 043252R, 043428R, 043536R, 043639R, 043834R, 043916R, 044015R, 044745R, 044838R, 045216R
PDA	28	092746R, 093658R, 094840R, 095110R, 035200R, 035244R, 035633R, 035728R, 040353R, 040539R, 040958R, 041416R, 041528R, 041746R, 042649R, 042746R, 042904R, 043012R, 043110R, 043204R, 043252R, 043428R, 043536R, 043834R, 043916R, 044015R, 044745R, 044838R, 045216R,

In the PDA culture medium, the isolates 035728R, 042904R, 043204R, 043252R, 043536R, 043834R, 044838R reached maximum growth on the 5th day of incubation while the isolates 035633R, 040539R, 043428R, 044745R, 045216R, 095110R attained maximum growth on the 7th day (Kaur et al., 2012). The only isolate 093658R in this medium attained maximum growth on the 3rd day. It was also noted that the isolates 094840R, 095110R, 035200R, 035244R, 040958R, 041746R and 043110R expressed exponential growth in the two culture media as well as 035728R, 040539R, 041416R, 041528R and 042904R in the MM medium. Annex 1 indicates that the mycelial front of the 30 F.o.e isolates vary between 9 and 70 mm in 7 days in the MM culture medium meanwhile in the PDA medium, the variation was between 8 and 70 mm.

3.2. Macroscopic Characterization of F.o.e

After purification of strains by successive reseeding of monosporous cultures, several morphological types were observed. Observation with the naked eyes permitted to appreciate and identify aspects of mycelia. The following types were identified (Figure 5):

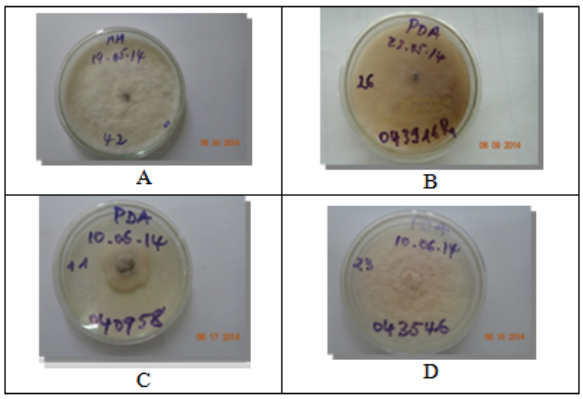

Figure 5. Pure cultures of F.o.e showing some morphological types observed

- The cotton-like type of aerial mycellium was identified in 7 days with a dense, consistent aspect and whitish pigmentation (Figure 5A).
- Less dense aerial mycellia with little consistence in 7 days (Figure 5B).
- Senescent type with less consistent aerial mycellia and slow growth rate (Figure 5C).
- Less consistent aerial mycellia with grayish-violet-rose pigmentation (Figure 5D).

3.3. Microscopic Characterization of F.o.e

Observation under the optical microscope (40X) helped to identify the morphological structure (Wassim, 2010) of fungal spores (micro-conidia, macro-conidia and chlamydospores) (Annex 2 and 3).

3.4. Biomasse of Soil Mycelia in Solid Medium

Mycelia biomass was evaluated through 1/10, 2/10 and 3/10 dilutions (Figure 6).

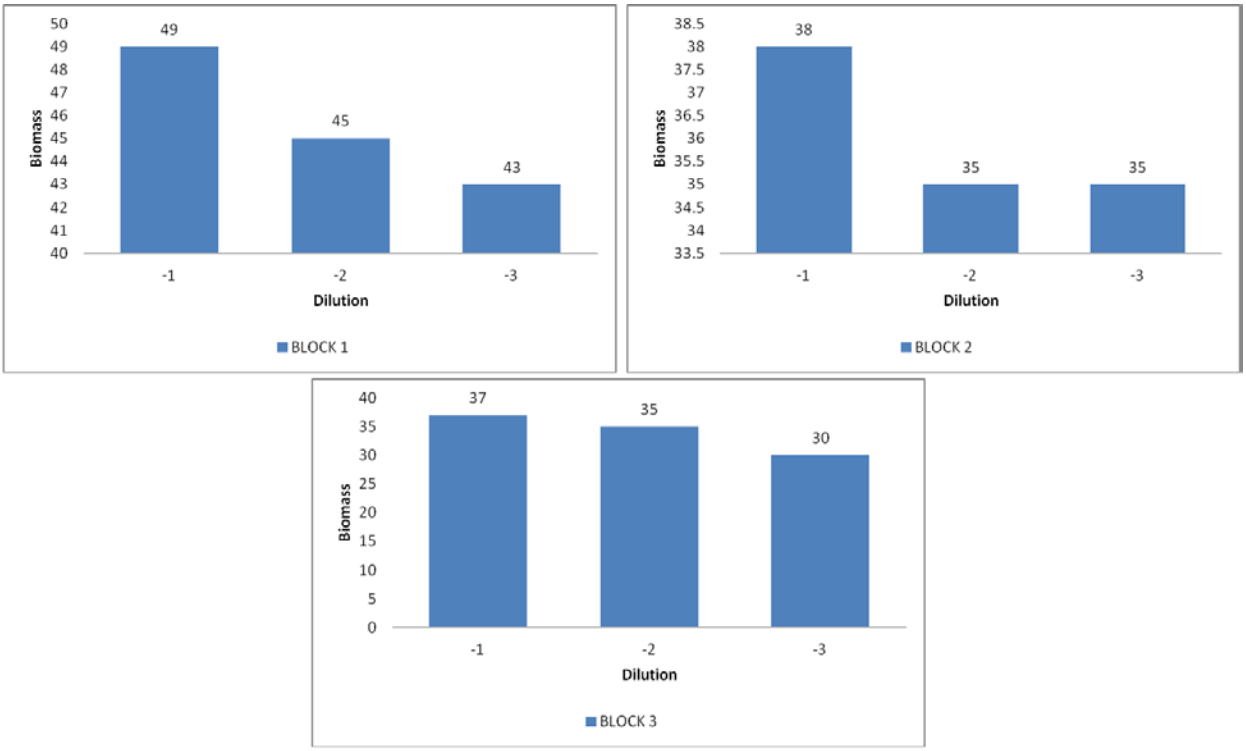

Figure 6. Quantitative analysis of F.o.e colonies

Results show that Blocks 1 and 3 present considerable reduction of the mass of colonies as dilution proceeds while Block 2 shows an almost constant mass of colonies. The interval of colony production in all blocks is between 34.00 ± 3.61 mg/day and 45.67 ± 3.06 mg/day. Statistical analysis with the aid of STATVIEW software show that there is a significant difference of colony amounts at the threshold of 5% between the three blocks (Table 6): there is a difference between Block 1 and 2; between Block 1 and 3 and between Block 2 and 3.

Table 6: Fusarium count per block

Bloc	Colonie	F	P
Block 1	45.67 ± 3.06		
Block 2	36.00 ± 1.73	13.83	0.01
Block 3	34.00 ± 3.61		

Moreover, soil microbial analysis showed that the number of UFC vary between 10.00 ± 3.59 and 16.33 ± 2.33 (Table 7) and the mycelia loads are (14.33 ± 4.28). 103 UFC/g, (11.66 ± 3.86). 103 UFC/g and (10.00 ± 3.59). 103 UFC/g respectively for *Dura*, *Pisifera* and *Tenera* for the 10^{-3} dilution. Thus there is a significant difference of the number of UFC between the three palm varieties.

Table 7. Number of UFC per dilution in the 3 blocks

Block	Number of UFC per dilution		
	Dilution -1	Dilution -2	Dilution -3
1	16.33 ± 2.33	15.00 ± 4.38	14.33 ± 4.28
2	12.66 ± 4.02	11.66 ± 3.86	11.66 ± 3.86
3	12.33 ± 3.97	11.66 ± 3.86	10.00 ± 3.59

3.5. Behaviour of Isolates in Culture Media

Measurements of mycelia growth (diameter on Petri dish in the MM and PDA media revealed significant differences between isolates (Table 8). There is however a similarity of behaviour between the 7th and 8th day.

Table 8. Behaviour of isolates in the two culture media

Culture medium	Diametre (mm)	F	P
MM	42.892 ± 20.381	9.689	0.0019
PDA	44.356 ± 20.610		

4. Discussion

It is important to master the life cycle and characteristics of a pathogen as a move towards devising a strategy to fight against it more efficiently through possible development of microbial antagonism (Arfaoui *et al*., 2006) or targeted pesticide application. Contaminated seeds and debris have been suggested as alternative sources of F.o.e ([17]). Irrespective of source, movement of breeding materials constitutes a major threat to regions without F.o.e ([6]). The pathogen is a soil-borne fungus that produces macro and microconidia and chlamydospores that survive in soil and debris. All these forms were revealed by our study. In addition, our study also confirmed the fact that F.o.e is a very successful colonizer in plantations and easy-to-re-isolate fungus for study in the laboratory ([6]), contrary to *Ganoderma boninense*, which grows slowly in vitro and is also a very weak competitor in plantation soil and organic debris (Rees et al., 2007; [6]).

Results of this study show that the number of F.o.e colonies and their behaviour among palm blocks in our study area are variable. Sporulation ability is also variable among isolates. Some colonies do not produce macroconodia in culture medium while others produce very few chlamydospores. Given that the mode of resistance and dissemination of the fungus in the soil corresponds to chlamydospores, it is possible that this fructification develops best in nature (soil) where conditions are harsh and variable, than on artificial media. Macro-conidia on their part are capable of changing into chlamydospores (Booth, 1971; Rouxel, 1978). The short culture duration which however corresponds to the life span of the fungus, coupled with the favourable culture media are seemingly reasons for the lack of macrocodidia production in some colonies. Meanwhile, microconidia are dissemination spores which are however less resistant in the soil (Rouxel, 1978). Concerning fungal growth rate, net differences were observed in the two culture media. Growth was found to be influenced by the effect of day where strains behaved differently as a function of day!

The morphology of F.o.e isolates studied was unstable possibly due to the concentration and quantity of culture media. Microscopic observation revealed diversity of colonies. This could be a reflection of the heterogeneity of thalli in the soil. However, morphological variability is a constitutive characteristic of isolates (Henri et al., 1994).

F.o.e strains were obtained from soil sample collected from the field. It is generally admitted that Fusarium flora should be between 20 and 50 colonies per Petri dish (Tengoua, 1993). This is in line with our results of soil microbial analysis which showed that F.o.e varied from 30.10^3 to 45.10^3 UFC/g. The study of mycelia growth revealed four types of isolates differing from each other by their aspect in the Petri dish. On the overall, these results seemingly indicate that the population of F.o.e is homogeneous under the same experimental conditions.

The abundance of the microbe in soil samples also confirms the soil borne nature of this pathogen ([6]). The location of the parasite both in the soil and in the plant renders it difficult to fight against the disease and the application of fungicide is expensive for large areas ([40]; [34]. Hence the importance of our study that helped in mastering the life cycle and characteristics of the pathogen as a move towards devising a strategy to fight against it more efficiently through possible development of microbial antagonism (María *et al*., 2008) or targeted pesticide application. This is possible since the pathogen is known to spread according to soil type, sandy soils favoring its development [34]. It also exists on rich volcanic soils like that of our study area and clay soil [39].

This study also revealed that the three palm varieties are strongly affected by the pathogen though only Tenera presents regressive attitude towards the disease.

Growth and morphology of all 30 strains studied varied with time and culture medium. However, this variability is inevitable and not quite impressive as it could be influenced by light, quantity and composition of culture medium, laboratory conditions, and size of Petri dishes etc. This study would have been more consistent if the application of molecular techniques such as RAPD PCR (Diana *et al*., 1998; Said *et al*., 2014) were applied to assess genetic variations between the F.o.e. isolates studied.

5. Conclusion

This study had as objective to master the isolation and *in vitro* characterization procedures of *Fusarium oxysporum* f. sp. *elaeidis* strains from soil samples and plant tissue. With the current rapidly growing interest in the oil palm sector in Cameroon, there is no control of movement of planting material with regards to disease control. It is thus essential to anticipate on the most efficient ways of tackling the vascular wilt disease which is the major threat, besides Ganoderma, to the industry in this context. The procedures presented in this work could be used to the study of other important microbes. This work can help in devising a strategy towards the mastery of microbial stocks for a biological control against oil palm vascular wilt disease. It is in this light that this study was done to pave the way for a project for the assessment of the effect of several pesticides and plant extracts on the *in vitro* growth of F.o.e. in our laboratory.

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