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CULTURAL CHARACTERIZATION OF FIVE ISOLATES OF *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* (BANANA FUSARIUM WILT) AND ANTIFUNGAL ACTIVITY OF PLANT EXTRACTS

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ABSTRACT

Plantain (*Musa* spp.) is one of the most important food crops in Cameroon and elsewhere in Central and West Africa. However, its production is threatened by many diseases, including banana Fusarium wilt. The objective of this work was to contribute to the improvement of plantains production through the assessment of the behavior of the pathogen isolates to some environmental factors and the biological management of Fusarium wilt in Lab conditions. To achieve this, roots and leaves samples were collected from infected plantain plantations in five divisions of the West Region of Cameroon and brought to the laboratory for isolation and identification of the pathogen. Then, the pathogenicity test was carried out and the optimal growth conditions of *Fusarium oxysporum* f. sp. *cubense* (FOC), were evaluated on three culture media (V8, PDA and Banana Leaf Extract Dextrose Agar), five pH (5, 6, 7, 8 and 9) and two temperatures (18 and 25°C). Finally, antifungal activities of aqueous and ethanolic extracts of *Callistemon viminalis* and *Chenopodium ambrosioides* were evaluated on the growth of the five isolates (FOC-DSC, FOC-BAL, FOC-FBT, FOC-BAN and FOC-KEK) at 7.5 mg/mL and 15mg/mL. The results showed that all the five isolates were pathogenic. However, the isolates from Foumbot (FOC-FBT) and Kekem (FOC-KEK) were more aggressive. The V8 and BLEDA media at the pH 6 and 25°C were more suitable for the growth of all the isolates of FOC. The efficiency of ethanolic extract of *C. ambrosioides* was significantly comparable to the chemical fungicide (Eagrow Care) at 15mg/mL. Isolates of the Fusarium wilt pathogen in the West Region of Cameroon are phenotypically different and due to the efficiency of the ethanolic extract of *C. ambrosioides*, it may be exploit for its antifungal properties on the management of the disease.

Keywords: Antifungal activity, Banana *Fusarium* wilt, Morphological characterization, Plant extracts.

INTRODUCTION

In Cameroon, the rural sector remains the leading sector of the national economy for its contribution to Gross Domestic Product (with 55% of total exports), the leading employer and provider of foreign currency (with 62% of the active population found there). Food crops are the main driver of this growth and represent 70% of primary sector Gross Domestic Product (Nkapnang, 2011). The world's total banana production has dropped from 145 tons in 2017 (FAO, 2017) to 119 million tons in 2020.In Central and West Africa, the plantain (*Musa* spp.) is one of the most important food crops due to its role in the daily diet, its nutritional potential, its economic role for small producers and its place in the scale of starches. Food security in Central Africa would be subject in the next 20 years to many

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uncertainties with regard to current statistics which would reveal a drop in productivity for local food crops: plantain, macabo, cassava. This could be due to many stresses such as attacks by pests and diseases; including Fusarium wilt which reduces fruit yield and quality by 26.5%. World banana production is estimated at nearly 30.5 million tons and has changed little over the past fifteen years (Olumba and Onunka, 2020). Plantain, with an overall production of 8 million tons in Central and West Africa, and 1.2 million tons in Cameroon has a central place in achieving regional food security and more particularly that of cities (Dury, 2002). In Cameroon, it is grown in three agro ecological zones (the Western High Plateau which include the West and North-west regions, The banana Fusarium wilt is more spread in the west region of the country and the Littoral region where agro-industrial companies like Plantation du Haut Penja (PHP) with high banana production are located, Despite the governmental efforts to promote the banana sector through the dissemination of innovations, considerations which should have led to the intensification of this commodity, several studies show that plantain banana yields in traditional family farms (EFA) in Cameroon hardly exceed 4 to 10 t. ha^{-1}) whereas in research stations one can obtain well over 30 $t.ha^{-1}$) with the same varieties on well-monitored and accessible itineraries (Tomekpe *et al.,* 2011; Dépigny *et al.,* 2019).This is due to many constraints such as; the use of poorly adapted varieties, lack of mastery of the technical route and the cropping system used, poor fertilization and management of diseases and pests. Among these diseases, panama disease or Fusarium wilt is dreadful for plantain cultivation. It causes moderate and severe severities depending on the agro-climatic zones and the production systems, which would reduce the yield. Producers generally use synthetic fungicides which are applied in the soil to control the disease. However, the increased use of these chemicals has drawbacks, including the appearance of strains of pathogens resistant to the products, environmental pollution and the toxicity of living beings (Cissokho *et al.,* 2015; Massi *et al.,* 2021). To overcome these problems, the search for alternative to chemicals by using natural methods seems interesting since these natural products are less toxic for humans and the environment. This objective of this study was to improve plantain production in Western highlands of Cameroon through biological management of Fusarium wilt disease after biology cultural characterization of the aggressive isolates of the pathogen (*Fusarium oxysporum* f. sp. *cubense*).

METHODOLOGY

Collection of plant materials: Root and leaf samples with symptoms of Fusarium wilt were collected in February 2022 in five localities of five divisions of the West Region of Cameroon: Banekane (Ndé Division), Kekem (Haut-Nkam Division), Foumbot (Noun Division), Dschang (Menoua Division) and Balatchi (Bamboutos Division). These samples were put in plastic bags labeled, sealed and brought to the laboratory and stored at 4°C in the laboratory prior to isolation and characterization of *F. oxysporum* f. sp. *cubense* isolates. Leaves of Weeping bottlebrush (*Callistemon viminalis*) and aerial parts of the Mexican tea (*Chenopodium ambrosioides*) collected from Dschang were used to prepare phytoextracts. These plant species were chosen based on their proven antimicrobial properties against plant and animal pathogens (Sousa *et al.,* 2012; Salem *et al.,* 2017).

Isolation and identification of the pathogen: Infected samples (roots and leaves) were washed with tap water to removed soil and others visible particles and sterilized in 1% sodium hypochloride solution for 30 secs and rinsed three times, in sterile distilled water to remove bleach residues. Then, samples were cut into small fragments and plated on potato dextrose agar (PDA) medium amended with chloramphenicol (20mg/L) to avoid bacterial contamination. The plates were incubated at 25±2°C and three days later, hyphae that grew from diseased tissue were sub-cultured on fresh PDA and purified Djeugap *et al.* (2011). The identification was based on the morphological traits of the mycelium and spores such as the presence or absence of septa, type and size and morphology of spores, etc. (Rossman *et al.,* 1987; Dugan and Dugan, 2006).

Pathogenicity test: Fungal conidia suspension (1×10^6 conidia/mL) prepared from 10-days-old fungal cultures of each *Fusarium oxysporum* f. sp. *cubense* isolates grown on PDA was applied to healthy roots and leaves from two-month-old seedlings of French giant plantains variety using a sterile brush through the wounded inoculation technique. Control treatment was done with sterile water and the inoculated samples were incubated 25°C. Five replications were

considered. The plates were examined every day for fungal development and each lesion diameter (LD)was measured using a transparent graduated ruler using the following formula LD = (d1+d2)/2 where d1 was the diameter of the lesion according to the length of the root and d2, the diameter obtained according to the width of the root (Singh *et al.,* 2009; Djeugap *et al.,* 2011). The inoculated fungus was re-isolated and compared with the parental pathogen from the rotted fruits tested, and its identity was confirmed by microscopic observation. The isolate was considered as more aggressive, moderate aggressive and less aggressive if the LD was greater than 2 cm, between 1 and 2 cm and smaller than 1 cm respectively, 7 days after inoculation (DAI).

Cultural characterization of *Fusarium oxysporum* f. sp. *cubense* isolates: A mycelia disk of5 mm in diameter of pure culture of each isolate was placed in the centre of the Petri dishes containing15 ml of the three culture media: PDA, banana leaf extract dextrose agar (BLEDA) and V8 with pH 5, 6, 7, 8 and 9 incubated separately at18 and 25 °C (Tabuc, 2007; Tsopmbeng *et al.,* 2012). Diameter of the fungal colony formed at each temperature, pH and culture medium was measured after every three days (Djeugap *et al.,* 2011). Morphological characteristics of the fungi such as the colour of the mycelium, the production of pigments in the culture medium, were recorded. The radial growth (RG) of the fungal isolates was measured as follow: RG= (d1+d2/2)-2d0 with d0 = initial diameter of the mycelial disc, d1 first diameter and d2 second diameter measured at n^{th} day (Keuete *et al.,* 2018).

Evaluation of the *in-vitro* antifungal activity of plant extracts against *F. oxysporum* f. sp. *cubense*

Preparation of plant extracts: The leaves of *Callistemon viminalis* and *Chenopodium album* were drying and ground separately in an electric grinder. Then, 100 g of the fine powder obtained were macerated in 1000 ml of water or ethanol for 72 hours at room temperature. The solution was then filtered through the muslin cloth. Aqueous extract was oven dried at 40°C for 7 days while ethanol extracts were evaporated in a Buchner brand rotary evaporator at 67° C then, the evaporate was dried in an oven (Brand Koeltenhiek-Electriciteit Cornelis) at 40°C (Djeugap *et al.,* 2017a).

Antifungal activity of plant extracts: The activity of the plant extracts (aqueous and ethanolic) was evaluated at 7.5 mg/ml and 15 mg/ml on the radial growth of *Fusarium oxysporum* f. sp. *cubense* isolates on PDA at pH6 using the poisoning solid medium method (Keuete *et al.,* 2016; Yaouba *et al.,* 2017). In fact, after solidification of the medium, a 5 mm in diameter mycelial explant was removed using a cookie cutter, and aseptically placed in the center of each Petri dish supplemented with plant extracts. The negative and positive control plates were supplemented with sterilized distilled water and synthetic fungicide (Eagrow Care) respectively. Three repetitions were considered per treatment and the plates were sealed with para-film paper and incubated at 25°C. The radial growth (RG) of the pathogen was measured as previously explained (Keuete *et al.,* 2018). The percentage of inhibition (PI) of the fungal growth by the plant extracts was obtained as follow: PI = (DC−D/DC) × 100, where DC is the growth diameter of the fungus in the negative control plates and D is the growth diameter of the fungus in the plates supplemented with the plant extract or the synthetic fungicide Eagrow Care (Keuete *et al.,* 2018).

DATA ANALYSIS

Data were analyzed using software R version 3.5.1 at the 5% probability threshold. Given the fact that data were not followed the normal law and the homogeneity of the variance not being respected, the ANOVA (Analysis of Variance) could therefore not be carried out. The Kruskal Wallys test was used for separation of means. Data in percentages were previously submitted to arsine transformation before analysis.

RESULTS

Morphological description of *Fusarium oxysporum* f. sp. *cubense* isolates: Five isolates of *F. oxysporum* f. sp. *cubense* were selected based on their geographic origin and the morphology of their colony on the culture medium. There was a high phenotypic variability among the five isolates considered. For example, the mycelium of the isolate of *F. oxysporum* f. sp. *cubense* from Foumbot locality (FOC_FBT) was cottony, flat and light pink in appearance at 18°C; the isolate color the culture medium with a pink pigment and brownish at 25°C (Figure 1). Radial growth was low on PDA medium. The mycelium of the isolate from Kekem locality (FOC_KEK) was brownish yellow and produced bright red pigment at 18°C and creamy white with dark red pigment at 25°C.The isolate from Balatchi (FOC_BAL) has a whitish sparse mycelium

producing brown pigment at 18°C while at 25°C the mycelium is whitish with the medium color with a greyish pigment (Figure 1). There was also a morphological variation among isolate as far as culture medium is concerned. For example, for the isolate FOC_FBT, the mycelium is light pink when young and turns brownish when mature (presence of spores) in the center part of the culture on PDA while on V8, the mycelium is cottony, whitish with greyish coloration (sporulation) on older part of the culture. On BLEDA, the medium is dotted with whitish tufts of mycelium (Figure 2).

Pathogenicity test of *Fusarium oxysporum* f. sp. *cubense* isolates: All *Fusarium oxysporum* f. sp. *cubense* tested were pathogenic. Isolates FOC_FBT and FOC_KEK were more aggressive (lesion diameter greater than 2 cm, 7 DAI) both on the leaves and roots while isolates FOC_BAL was moderately aggressive (lesion diameter between 1 and 2 cm, 7 DAI). Isolates FOC_DSC and FOC_BAN were less aggressive (lesion diameter smaller than 1 cm) (Table 1). Seven days after inoculation, wilt and rot symptoms were developed on leaves and roots, respectively while the control samples do not present any infection (Figure 2).

Table 1. Pathogenicity of *Fusarium oxysporum* f. sp. *cubense* isolates.

Isolates	Roots	Leaves
FOC_DSC	+	+
FOC_BAL	++	++
FOC_FBT	+++	+++
FOC_BAN	+	+
FOC_KEK	+++	+++

Aggressiveness: +++= more aggressive, ++= moderate aggressive and + = less aggressive. FOC = *F. oxysporum* f. sp. *cubense.* Origin of isolates: DSC=Dschang, BAL=Balatchi, FBT=Foumbot, BAN= Banekane and KEK= Kekem.

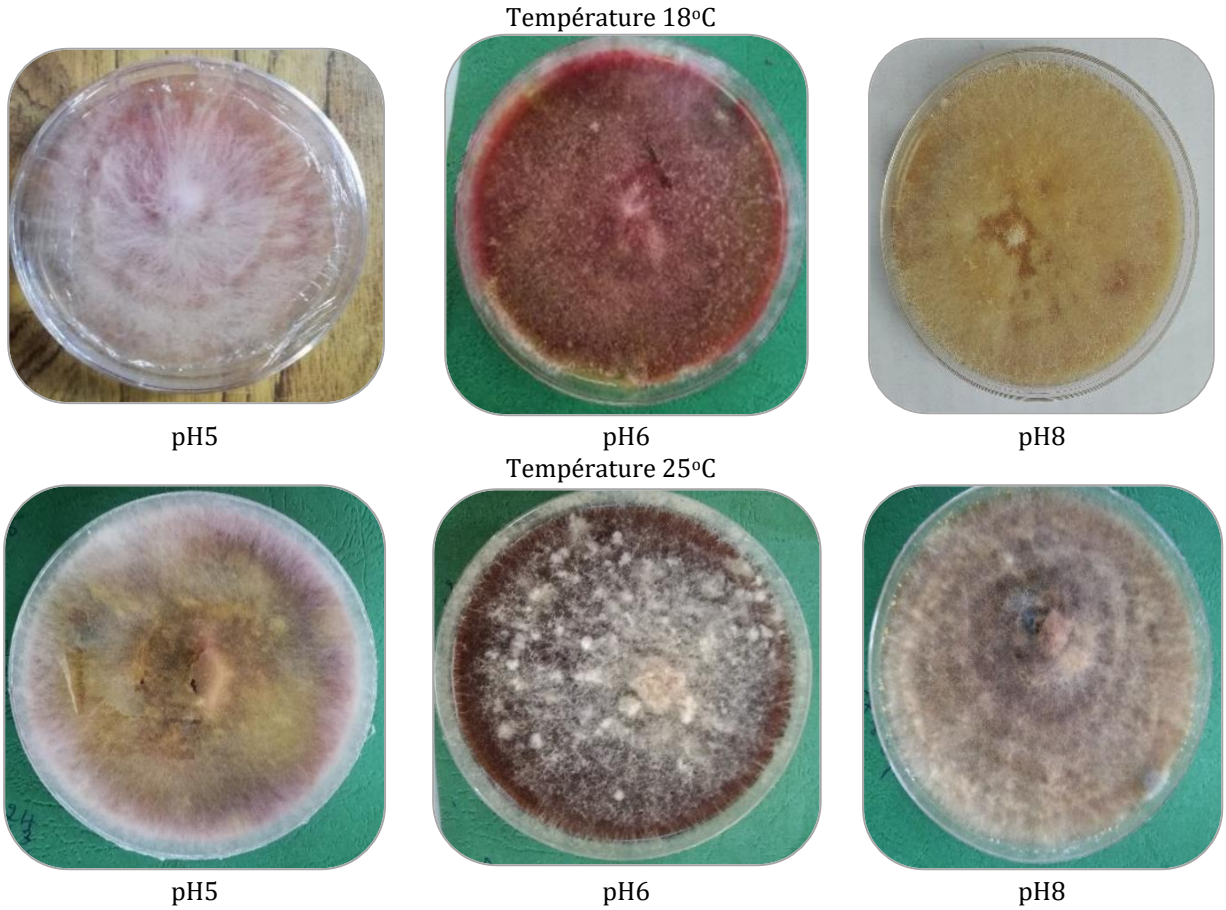
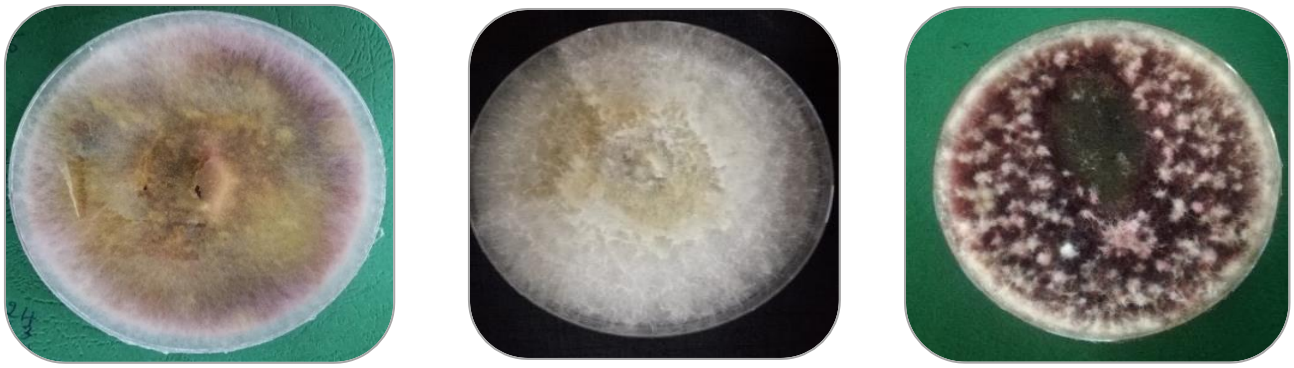


Figure 1. Variation of the morphological traits of the three aggressive *Fusarium oxysporum* f. sp. *cubense* isolates on PDA at pH6 and temperatures 18 and 25°C. FOC_FBT (left), FOC_KEK (center) and FOC_BAL (right).



A B C

Figure 2. Variation of the morphological traits of isolate FOC_FBT based on culture media: PDA (A), V8 (B) and Banana leaf extract dextrose agar (C).

A B C

D E F

G H

Figure 3. Wilted leaves and roots rot after Lab inoculation with isolates of *F. oxysporum* f. sp. *cubense*: Control (A and B), samples inoculated with isolates FOC_BAL (C and D), FOC_FBT (E and F) and FOC_KEK (G and H) at 7 days after inoculation.

Effect of culture medium, temperature and pH on the morphological characterization of *F. oxysporum* f. sp. *cubense* isolates: The radial growth of FOC isolates varied depending on culture media, temperature and pH. The V8medium was suitable for the rapid growth for all the FOC isolates at all temperatures (18 and 25°C). Indeed, the radial growth was statistically higher on V8 (8.20 and 8.47 cm) and lower on BLEDA both at 18 and 25°C respectively. The isolate from Kekem grow faster on V8 than on other media (Table 2). Isolates of *F. oxysporum* f. sp. *cubense* gave the best and rapid mycelia growth on V8 and PDA culture media. This difference on mycelia growth was because V8 contained more nutritive elements that positively influence the growth of *F. oxysporum* f. sp. *cubense.*

Table 2. Radial growth (cm) of *F. oxysporum* f. sp. *cubense* isolates at different culture media and temperatures.

Culture medium	FOC-DSC	FOC-BAL	FOC-FBT	FOC-BAN	FOC-KEK
Temperature 18°C					
PDA	7.50 ± 0.52^{aA}	7.95 ± 0.14^{aA}	7.50 ± 0.34^{bA}	7.80 ± 0.22^{aA}	7.78 ± 0.28^{bA}
V8	7.86 ± 0.51^{aB}	7.85 ± 0.51^{aB}	7.91 ± 0.19^{aB}	6.57 ± 0.23^{aC}	8.20 ± 0.11^{aA}
BLEDA	6.33 ± 1.49^{bA}	6.65 ± 1.51^{aA}	6.73 ± 1.65^{bA}	6.57 ± 1.23^{aA}	5.65 ± 1.49^{cA}
Temperature 25°C					
PDA	5.44 ± 1.60^{bB}	6.30 ± 1.90^{aA}	6.32 ± 1.64^{aA}	6.96 ± 1.59^{aA}	6.67 ± 1.38^{aA}
V8	7.99 ± 0.24^{aA}	7.87 ± 0.34^{aA}	7.61 ± 0.51^{aA}	7.80 ± 0.41^{aA}	8.47 ± 0.72^{aA}
BLEDA	3.63 ± 1.42^{cB}	5.17 ± 1.63^{aA}	4.50 ± 1.38^{bA}	4.87 ± 1.48^{bA}	5.67 ± 0.99^{bA}

*For each temperature, means followed by the same letter in the column or in the line (capital letter) are not significantly different according to the Kruskal-Wallis test at 5%. BLEDA = Banana Leaf Extract Dextrose Agar. Origin of isolates: DSC=Dschang, BAL=Balatchi, FBT=Foumbot, BAN= Banekane and KEK= Kekem.

All *F. oxysporum* f. sp. *cubense* isolates grew at all incubation temperatures and pH tested (Table 3). However, the optimum growth temperature and pH for all *F. oxysporum* f. sp. *cubense* isolates was 25°C and pH 6. This highest mycelial growth showed that the development of isolates is best in a culture media towards neutral pH.

Table 3. Effect of pH and temperature on fungal growth (cm).

Isolates	pH5	pH6	pH7	pH8	pH9
Temperature 18°C					
FOC_DSC	6.71 ± 0.45^{ab}	6.63 ± 0.45^{c}	6.50 ± 1.24^{b}	6.83 ± 1.38^{c}	6.70 ± 1.53^{a}
FOC_BAL	6.41 ± 0.55^{b}	7.07 ± 1.53^{b}	6.67 ± 1.72^{ab}	7.09 ± 1.14^{bc}	6.68 ± 1.62^{a}
FOC_FBT	7.43 ± 0.8^{a}	7.88 ± 0.35^{ab}	7.72 ± 0.42^{ab}	7.52 ± 0.7^{a}	7.52 ± 0.87^{a}
FOC_BAN	7.94 ± 0.16^{a}	8.01 ± 0.12^{a}	8.02 ± 0.31^{a}	7.11 ± 0.21^{bc}	7.07 ± 1.84^{a}
FOR_KEK	7.7 ± 0.30^{a}	7.8 ± 0.51^{ab}	7.77 ± 0.34^{a}	7.83 ± 0.35^{a}	7.74 ± 0.39^{a}
Temperature 25°C					
FOC_DSC	4.97 ± 2.32^{b}	7.05 ± 2.37^{b}	4.81 ± 2.24^{b}	5.07 ± 1.68^{b}	5.65 ± 0.72^{b}
FOC_BAL	4.68 ± 2.57^{b}	8.35 ± 1.52^{a}	5.56 ± 1.42^{ab}	6.36 ± 1.51^{ab}	5.97 ± 1.42^{ab}
FOC_FBT	8.85 ± 1.12^{a}	9.28 ± 1.4^{a}	7.80 ± 0.93^{a}	7.28 ± 1.27^{a}	7.38 ± 0.58^{a}
FOC_BAN	6.65 ± 2.08^{ab}	8.02 ± 1.2^{ab}	6.58 ± 2.13^{ab}	6.45 ± 2.31^{a}	6.99 ± 1.51^{a}
FOR_KEK	7.39 ± 1.40^{a}	9.45 ± 0.59^{a}	7.97 ± 1.15^{a}	7.54 ± 6.69^{a}	7.01 ± 1.15^{a}

$^{(a-c)}$ For each temperature, means followed by the same letter in the column are not significantly different according to the Kruskal-Wallis test at 5%. Origin of isolates: DSC=Dschang, BAL=Balatchi, FBT=Foumbot, BAN= Banekane and KEK= Kekem.

The interaction between culture media, temperature and pH on the radial growth of *F. oxysporum* f. sp. *cubense* isolates shows that the isolates FOC_FBT and FOC_KEK grow faster both on V8 and BLEDA media at temperature 25°C and pH6 while at temperature 18°C, there was no significant difference on the growth between the five isolates at the same culture media and pH (Table 4). The unsuitable interaction for all the *F. oxysporum* f. sp. *cubense* isolates were BLEDA x pH5x 25°C followed by PDA x pH9 x 25°C.

Table 4. Interaction effect between culture media, temperatures and pH on the radial growth (cm) of *F. oxysporum* f. sp. *cubense.*

Isolates	pH5			pH6			pH7			pH8			pH9		
	PDA	V8	BLEDA	PDA	V8	BLEDA	PDA	V8	BLEDA	PDA	V8	BLEDA	PDA	V8	BLEDA
								Température 18°C							
FOC_DSC	5.1± 1.1^{e}	8.0± 0.1^{a}	7.1± 0.7^{cd}	4.9± 0.6^{c}	8.7± 1.8^{a}	8.9± 0.5^{a}	5.3± 0.8^{d}	8.2± 0.2^{a}	7.1± 0.3^{cd}	5.1± 0.3^{e}	8.4± 0.6^{a}	7.5± 0.3^{cd}	4.8 ± 0.1^{c}	8.1± 0.3^{a}	7.4± 0.1^{b}
FOC_BAL	4.2± 0.3^{e}	7.3± 1.1^{ab}	7.04± 0.4^{cde}	5.2± 1.2^{c}	8.6± 0.4^{a}	8.7 ± 0.3^{a}	4.7± 1.8^{d}	7.7± 0.4^{ab}	7.5± 0.5^{bc}	5.6± 0.2^{e}	8.2± 0.3^{a}	7.7± 0.8c	4.5± 0.5^{c}	8.8± 0.5^{a}	7.5± 0.3^{b}
FOC_FBT	6.4± 0.5^{de}	8.1± 0.2^{a}	7.9± 0.4^{ab}	7.6 ± 0.6^{ab}	8.9± 0.1^{a}	8.5 ± 0.2^{a}	7.5± 0.7^{d}	8.1± 0.3^{a}	7.6± 0.2^{a}	6.5± 0.5^{de}	8.2± 0.5^{a}	8.3± 0.4^{a}	6.6± 1.1^{bc}	8.7± 0.5^{a}	8.2± 0.3^{a}
FOC_BAN	8.2± 0.1^{a}	8.2± 0.3^{a}	7.8± 0.28^{ab}	8.3± 0.6^{a}	8.5± 0.2^{a}	8.6 ± 0.2^{a}	8.2± 0.1^{ab}	8.0± 0.2^{a}	8.3± 0.4^{a}	8.1± 0.1^{a}	8.6± 0.4^{a}	8.7± 0.1^{a}	5.2± 2.4^{bc}	8.6± 0.2^{a}	8.1± 0.4^{a}
FOR_KEK	7.3± 0.1^{cd}	8.3± 0.5^{a}	7.66 ± 0.14^{bc}	7.48 ± 0.2^{ab}	8.7± 0.1^{a}	8.8 ± 0.7^{a}	8.1± 0.3^{a}	8.3± 0.1^{a}	7.3± 0.2^{a}	7.6± 0.5^{b}	8.1± 0.1^{a}	7.8± 0.3^{ab}	7.3 ± 0.2^{c}	8.1± 0.7^{a}	8.8± 0.3^{a}
								Temperature 25°C							
FOC_DSC	4.1± 0.6^{ef}	7.9± 0.5^{a}	2.8± 0.2^{fg}	5.1± 2.5^{cd}	8.2± 0.5^{b}	8.5± 0.4^{b}	4.8± 0.1^{efg}	7.6± 0.8^{ab}	4.7± 0.5^{g}	4.7± 0.4^{ef}	7.1± 0.2^{b}	5.5 ± 0.7^{fg}	4.3± 0.9^{efg}	6.3± 0.4^{def}	6.3± 0.5^{efg}
FOC_BAL	3.7± 0.3^{ef}	8.3± 0.1^{a}	2.3± 0.2^{g}	5.5 ± 1.1^{de}	8.4± 0.2^{ab}	8.2± 0.4^{b}	4.9± 0.2^{de}	7.3± 0.3^{b}	6.3± 0.4^{ef}	6.8± 1.8^{bc}	8.2± 0.4^{a}	7.9± 1.4^{de}	5.1± 1.6^{cde}	7.2± 0.6^{bcd}	5.6± 0.2^{g}
FOC_FBT	5.3± 0.1^{bc}	8.1± 0.4^{a}	4.3± 0.5^{cde}	7.8±0.2^{ab}	8.9± 0.2^{a}	8.8± 0.8^{a}	7.5± 0.8^{ab}	7.1± 0.7^{bc}	7.8± 0.4^{d}	8.2± 0.4^{a}	8.8± 0.3^{a}	7.8± 1.4^{cde}	6.6± 0.4^{abc}	7.8± 0.3^{ab}	7.7± 0.3^{cde}
FOC_BAN	8.4± 0.3^{a}	8.4± 0.6^{a}	3.9± 0.9^{def}	6.4± 2.8^{abc}	8.3± 0.4^{ab}	8.3± 0.8^{b}	8.1± 0.3^{a}	8.1± 0.3^{a}	5.7± 0.6^{fg}	8.3± 0.7^{a}	8.5± 0.4^{a}	5.4± 0.2^{g}	7.2± 0.5^{a}	8.3± 0.6^{a}	5.9± 0.2^{fg}
FOR_KEK	6.1± 0.4^{b}	8.1± 0.1^{a}	4.8± 0.5^{bcd}	7.3 ± 0.7^{abc}	8.7± 0.1^{a}	8.9± 0.4^{a}	7.1 ± 0.8^{b}	8.2±0.3^{a}	7.9± 1.2^{cd}	8.1± 0.1^{a}	8.4± 0.7^{a}	5.4± 0.2^{bcd}	5.3± 1.6^{def}	8.2± 0.7^{a}	7.5± 0.5^{cd}

$^{(a-f)}$ For each temperature, means followed the same letter in the column are not significantly different according to the Kruskal-Wallis test at 5%. Origin of isolates: DSC=Dschang, BAL=Balatchi, FBT=Foumbot, BAN= Banekane and KEK= Kekem.

Bioactivity of aqueous and ethanolic extracts of *C. viminalis* and *C. ambrosioides* on the growth of *F. oxysporum* f. sp. *cubense* isolates: The effect of aqueous and ethanolic extracts of *C. viminalis* and *C. ambrosioides* on the growth of all the five isolates vary from one concentration to another and from one plant extract to another. The effect of ethanolic extract was higher compared to the effect of the aqueous extract. However, the effect of the aqueous extract of the two plant species was significantly higher (p<0.05) than their effect in the control (water) but not statistically comparable (p>0.05) to the effect of the reference fungicide which totally inhibited the growth of all the isolates at recommended concentration (Table 5). The highest growth inhibition of the aqueous extract was obtained on FOC_DSC (87%) and FOC_BAL (82.33%) with extract of *C. ambrosioides* at 15 mg/mL. The effect of ethanolic extract of *C. viminalis* was not significantly comparable to the synthetic fungicide both at 7.5 and 15 mg/mL on all the five isolates of *F. oxysporum* f. sp. *cubense* tested. In contrast, the growth inhibition of FOC_DSC (97%), FOC_BAL (96.65%) and FOC_KEK (98.27%) isolates at 15 mg/mL by ethanolic extract of *C. ambrosioides* was statistically comparable to the fungicide (100%) (Table 6).

Table 5. Effect of aqueous extracts of *C. viminalis* and *C. ambrosioides* on growth inhibition (%) of isolates of *F. oxysporum* f. sp. *cubense*.

Concentration (mg/mL)	FOC-DSC	FOC-BAL	FOC-FBT	FOC-BAN	FOC-KEK
Callistemon viminalis					
Control (SDW)	0 ± 0[a]	0 ± 0[a]	0 ± 0[a]	0 ± 0[a]	0 ± 0[a]
7.5	39.66±2.30[b]	33.66±3.05[b]	47.66±5.77[b]	35.66±3.21[b]	44.01±3.3[b]
15	54.66±3.57[c]	58.66±2.51[c]	59.66±3.51[c]	60.33±2.52[c]	69.5±3.53[c]
Eagrow Care (fungicide)	100[d]	100[d]	100[d]	100[d]	100[d]
Chenopodium ambrosioides					
Control (SDW)	0 ± 0[a]	0 ± 0[a]	0 ± 0[a]	0 ± 0[a]	0 ± 0[a]
7.5	61.33±3.78[b]	68.00±3.14[b]	57.66±4.04[b]	60.66±3.21[b]	63.3±3.06[b]
15	87.00±5.32[c]	82.33±3.71[c]	75.33±3.86[c]	78.00±2.73[c]	81.33±4.04[c]
Eagrow Care (fungicide)	100[d]	100[d]	100[d]	100[d]	100[d]

[(a-d)] For each plant extract, means followed the same letter in the column are not significantly different according to the Kruskal-Wallis test at 5%. SDW= sterilized distilled water. Origin of isolates: DSC=Dschang, BAL=Balatchi, FBT=Foumbot, BAN= Banekane and KEK= Kekem.

Table 6. Effect of ethanolic extracts of *C*. *viminalis* and *C. ambrosioides* on growth inhibition (%) of isolates of *F. oxysporum* f. sp. *cubense.*

Concentration (mg/mL)	FOC-DSC	FOC-BAL	FOC-FBT	FOC-BAN	FOC-KEK
Callistemon viminalis					
Control (SDW)	0 ± 0[a]	0 ± 0[a]	0 ± 0[a]	0 ± 0[a]	0 ± 0[a]
7.5	74.33±2.51[b]	66.24±3.12[b]	52.37±5.29[b]	56.16±3.73[b]	61.33±2.66[b]
15	94.33±2.30[c]	78.33±4.61[c]	76.66±2.08[c]	78.66±2.51[c]	88.33±2.18[c]
Eagrow Care (fungicide)	100[d]	100[d]	100[d]	100[d]	100[d]
Chenopodium ambrosioides					
Control (SDW)	0 ± 0[a]	0 ± 0[a]	0 ± 0[a]	0 ± 0[a]	0 ± 0[a]
7.5	76.50±2.12[b]	72.66±2.88[b]	65.00±2.4[b]	68.66±3.41[b]	76.00±3.16[b]
15	97.00±3.24[c]	96.65±3.36[c]	67.15±3.29[b]	79.66±3.15[c]	98.27±2.09[c]
Eagrow Care (fungicide)	100[c]	100[c]	100[c]	100[d]	100[c]

[(a-d)] For each plant extract, means assigned the same letters in the same column are not significantly different according to the Kruskal-Wallis test at 5%. SDW= sterilized distilled water.

DISCUSSION

Pathogenicity of *Fusarium oxysporum* f. sp. *cubense* isolates: All isolates of *F. oxysporum* f. sp. *cubense* induced lesions on two-month-old banana roots and leaves confirming that these isolates tested were pathogenic and therefore responsible to banana wilt observed in the fields. This is in line with the work of (Thangavelu *et al.,* (2019). Similar results on the pathogenicity of *Fusarium oxysporum* species inoculated on both seedlings and detached leaves of some crop and forest tree species like *Moringa oleifera*, *Ricinodendron heudelotii* and banana (Takuete, 2016; Thangavelu *et al.,* 2019). The differences on aggressiveness of *F. oxysporum* isolates from banana was also reported with 67 isolates of *F. oxysporum* species complex after Lab inoculation of detached yam leaves (Dongzhen *et al.,* 2020).

Cultural characterization of *F. oxysporum* f. sp. *cubense* isolates: V8 culture medium exhibited high radial growth and in some extend BLEDA medium at pH6. This is in contradiction to Djeugap *et al.* (2017b) findings who reported that PDA and MEA were suitable for the growth of *Fusarium oxysporum* causing wilt on *Ricinodendron heudelotii* seedlings. Poorvasandhya *et al.* (2020) also reported that the growth and development of *Fusarium oxysporum* f. sp. *udum* pathogen to Pigeon pea (*Cajanus cajan*) was higher on PDA. Though V8 culture medium gave a rapid radial mycelia growth on this activity of the study, PDA was the best culture medium to characterized the morphological traits of the different isolates of *F. oxysporum* f. sp. *cubense*. This variability could be explained by the variability on the chemical composition of the culture media used. These media are organic substrates rich in carbohydrates which are source of energy for cell metabolism, growth and sporulation of fungi. Previous workers have recognized the importance of various organic media for mycelial growth in fungi (Kim *et al.,* 2005; Zhao *et al.,* 2010; Pradeep *et al.,* 2013). This difference could be also

explained by the high variability that exist in the member of the *Fusarium oxysporum* complex. In fact there is a high phenotypic and genetic variability (at least 300 phylogenetically distinct species/species complexes) among the individuals from this species (O'Donnell *et al.,* 2015).The members from this species also possess a high range of host plants and at the same time also have a high host specificity (Edel-Hermann and Lecomte, 2019; Dongzhen, 2020). They cause many diseases such as wilts, rots, and damping-off. The radial growth was significantly high at pH6 which is in line with Tyagi and Paudel (2014) and Cruz *et al.* (2019)findings who reported that the optimal pH growth conditions for *F. oxysporum* causing seedling disease and root rot of soybean was at 6 and 6.3 respectively. Temperature influences the development of *Fusarium,* and generally constitutes an essential parameter in the development of pathogenic fungi. It strongly influences spore production in *Fusarium* (Doohan *et al.,* 2003). The greatest growth were obtained at a temperature of 25°C. Similar results were reported by Ramirez *et al.*(2006) and Cha *et al*. (2007), who showed that the temperature of 25°C was suitable for the growth and sporulation of *F. graminearum* from wheat and *F. oxysporum* from *Capsicum annuum* respectively. However, some authors showed that members of this species have a range of favorable growth temperature from 15 °C to 28°C (Khilare and Rafi, 2012; Djeugap *et al.,* 2017b; Poorvasandhya *et al.,* 2020).

Antifungal activity of plant extracts: The aqueous and ethanolic extracts of *Callistemon viminalis* and *Chenopodium ambrosioides* significantly inhibited the radial growth of the *F. oxysporum* f. sp. *cubense* isolates compared to the negative control and only the ethanolic extract of *C. ambrosioides* at 15 mg/mL gave an efficiency which was comparable to the reference synthetic fungicide. This activity could be due both the extract concentration and chemical composition. These plants extract contain compounds with antifungal properties as previously reported by some researchers on others fungal plant pathogens. This was the case of Djeugap *et al.* (2011) and Tsopmbeng *et al.*(2014) on the *in vitro* efficacy of plant extracts including *C. viminalis* against *Phytophthora infestans* (huckleberry late blight) and *Phytophthora colocasiae* (taro late blight), respectively. Concerning *C. ambroisoides* ethanolic extract, Sousa *et al.* (2012) reported that *C. ambrosioides* significantly inhibited the growth of many pathogenic fungi like: *C. gloeosporioides*, *Cladosporium oxysporum, Aspergillus flavus* and *Penicilium* spp.

CONCLUSION

At the end of this study, the main findings are: (i) the aggressiveness of the *Fusarium oxysporum* f. sp. *cubense* isolates vary among the different localities of the west region of Cameroon. (ii) The most suitable culture medium for the growth of *F. oxysporum* f. sp. *cubense* was V8; the BLEDA medium at pH6 could be used as alternative to V8. (iii) The optimum pH and temperature for the growth of *F. oxysporum* f. sp. *cubense* was pH6 and 25°C respectively. (iv) Only the ethanolic extracts of *Chenopodium ambrosioides* at 15mg/mL totally inhibited he growth of the pathogen; this inhibition was comparable to the reference synthetic fungicide. Another study is being carried out to test higher concentrations of the ethanolic extract of *C. ambrosioides* and assess their effectiveness on infected seedlings in the greenhouse before making a recommendation of this extract as an alternative to the chemical for sustainable management of banana Fusarium wilt.

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Arlette M. Sonkoue	:	Analyze data and review the manuscript
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Joliesse N. K. Nouteka	:	Supervised the experiment in the Laboratory