

## Antifungal Activity of Some Plant Extracts Against Morphotypes of *Fusarium spp.* Associated with Tomato (*Solanum Lycopersicum L.*) Fruit Rots in The West-Region of Cameroon

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### Abstract

Tomato fruit rot caused by species of the genus *Fusarium* is one of the major constraints to the production and marketing of this fruit in the West-region of Cameroon. To reduce this rot, a study evaluating the antifungal activity of aqueous and ethanolic extracts of *Cyathea maniana*, *Cupressus lusitanica*, *Psychotria peduncularis* and *Cymbopogon citratus* at different concentrations on the development of *Fusarium spp.* morphotypes associated with tomato fruit rot was realised. To achieve this aim, extracts of these four plants were prepared and tested for their antifungal activities on the *In vitro* growth of *Fusarium spp.* morphotypes and their ability to protect tomato fruit against rot caused by these fungi was evaluated. The results obtained showed that the ethanolic extracts of *Cupressus lusitanica* and *P. peduncularis* at a concentrations of 2.048 and 1.024 mg/ml were most actives on both F1FBT and F3BD *Fusarium spp.* morphotypes on four tested with 100 % inhibition percentages. At the concentrations of 16.384 and 8.192 mg/ml, the aqueous extract of these plants totally inhibited the growth of the same *Fusarium* morphotypes. The results of *in vivo* test showed that fruits inoculated with these two morphotypes and treated with the extracts of *C. lusitanica* and *P. peduncularis* did not develop any lesion 7 days after inoculation. These results suggest a possibility of using these extracts as alternatives to the chemical control against Fusariosis of tomato fruits caused by these *Fusarium spp.* morphotypes.

**Keywords:** Antifungal activity; *Fusarium spp.*; Plant extracts; Tomato

## Introduction

Tomato (*Solanum Lycopersicum L.*) is an annual plant of the Solanaceae family widely cultivated in many regions of world for its nutrient-rich fruits and medicinal properties [1,2]. In Cameroon, tomato production is increasing over the years. From a production of 488 790 tons in 2005, it increased to 954 384 tons in 2013 [3], an increase of 465 594 tons with the main production area being in the West-region of Cameroon [4]. Despite this increase in production, this crop is facing many difficulties, including fungi disease, namely fungi belonging to the genus *Fusarium* [5]. *Fusarium* rot caused by *Fusarium spp.* is one of the diseases responsible for most pre- and post-harvest losses of tomato fruits [6]. This disease in farms is characterised by leaves and stems discoloration and fruits changing from brown to black. The fruits of the affected plants are flaccid. These symptoms vary depending on the *Fusarium spp.* morphotypes involved in the disease, their aggressiveness and their level of crop loss [7]. Although, several works show the existence of various morphotypes of *Fusarium spp.* that can attack a large number of plants with great economic importance [8]. Among some morphotypes of *Fusarium oxysporum f. sp. radialis-lycopersici* were responsible for root rot, affects xylem of young tomato seedlings and causes up to about 90 % of losses [9]-[10]. These damages are high on plants that bear fruits. Similarly, vascular fusariosis can lead to the formation of adventitious roots and leaves necroses, causing the death of plants [11]. Some morphotypes of *Fusarium spp.* such as *F. oxysporum f. sp. Cubense* were totally ended the banana industry in 1960 [12], and others like *F. oxysporum f. sp. graminearum* resulted in the loss of billions of dollars by attacking wheat and barely in the United States [13]. In addition to production losses, some *Fusarium spp.* morphotypes can lead to the contamination of tomato fruits with various mycotoxins. These secondary metabolites produced by fungi have proven to be toxic to many organisms.

Face to pre and post-harvest rots that limit the shelf life and marketing of tomato fruit, control of tomato fruit rot has been by the application of chemical fungicides. However, these days, consumers request less use of chemicals and still want food devoid of contaminations, microbial growth, toxins as well as other qualities deteriorating factors [14]. Added to this, is the hazard involved in using chemical fungicides and the development of resistance to synthetic fungicide by plant pathogenic organisms, hence needing an alternative control. Natural plant products and their analogs are important sources of agricultural bio-pesticide which serves as antimicrobial properties [15]. Several antifungal activities of some plant extracts against *Fusarium spp.* have been reported [16,17]. In Cameroon, information on antifungal activity plants products against different *Fusarium spp.* morphotypes, associated with tomato fruit rot, is lim-

ited. The objective of this study was to evaluate the *In vitro* and on the tomato fruit efficacy of extracts of *Cyathea maniana*, *Cupressus lusitanica*, *Cymbopogon citratus* and *Psychotria pedoncularis* on the growth of *Fusarium spp.* morphotypes, associated with tomato fruits rot in the West-region of Cameroon.

## Materials and Methods

### Study area

This study was conducted at the University of Dschang, Cameroon, in the Plant Pathology and Agricultural Zoology Research Unit and in the Microbiology and Antimicrobial Substances Research Unit. Dschang is located at 5°26' N latitude and 10°26' E longitude and at altitude 1400 m. The temperature ranges from 11.8 to 26.8 °C and with a relative humidity of 90 % and a mean rainfall of 1566.8 mm per annum [18].

### Collection of tomato fruits

Tomatoes fruits showed the symptoms of rot and others apparently healthy were collected from farms in Fombot and Mbouda localities in West-region of Cameroon. These fruits were packed into a labeled sterile polyethylene bags already lined with soft paper and taken to the Research Unit of Pathology and Agricultural Zoology for isolation of *Fusarium spp.* morphotypes.

### Preparation of plants extracts

Plant extracts were prepared from young leaves of *Cupressus lusitanica*, *Cymbopogon citratus*, *Psychotria pedoncularis* and *Cyathea maniana* which were collected in the locality of Dschang. The collected samples of each plant species were washed under tap water and surface disinfected with 2 % sodium hypochlorite solution followed by thorough rinsing with sterile water. The plant samples were air-dried at room temperature and pounded in a mortar with the use of a pestle. Thereafter, 100 g of powder of each plant were macerated in 500 ml of distilled water or ethanol and mixed thoroughly. The mixture was filtered using cheese cloth followed by Whatman filter paper N°.1 after 48 hours of maceration. The ethanol extracts were evaporated using a Rota vapor at 67 °C. The extracts were transferred into labeled sterile bottles and store at 4 °C [19].

### Isolation of *Fusarium spp.* morphotypes associated with tomato fruits rots

Tomatoes fruit showing symptom of rot were thoroughly washed with tap water and separately cut into small fragments of about 5 mm<sup>2</sup>, showing half healthy and half infected tissue, with the help of a previously sterilized blade. The surfaces of the fragment were

disinfected with 5 % sodium hypochlorite solution for 2 minutes, followed by 3 rinses with sterilized distilled water. These fragments were then aseptically transferred separately into Petri dishes containing 20 ml Potato Dextrose Agar (PDA) medium supplemented with 1 g/l of chloramphenicol (to prevent bacterial contamination) and then incubated at 25° C. After 2 to 3 days of incubation, the growing mycelium was sub-cultured on fresh PDA medium until pure cultures were obtained. In this way, pure cultures of different isolates were obtained. Identification of *Fusarium spp.* was carried out based on the cultural characteristics and with the help of identification keys of mycology [20] and pure cultures were maintained in a refrigerator at 4° C. A summary of morphotypes of *Fusarium spp.* used in this study is listed in Table 1.

**Table 1:** Different morphotypes of *Fusarium spp.* associated with tomatoes fruits rots

Locality	Code of morphotypes
Foumbot	F1BT
Mbouda	F2BD
	F3BD
	F4BD

### *In vitro* antifungal activity of plant extracts

The *In vitro* antifungal activity of plant extracts was assessed using the agar dilution method on PDA [21]. Ethanolic extracts were dissolved in dimethyl sulphoxide (DMSO) and then diluted at concentrations of 16.384, 8.192 and 4.096 mg/ml for aqueous extracts and 2.048, 1.024 and 0.512 mg/ml for ethanolic extracts. The choice of the different concentrations was made on the basis of the results of the preliminary tests. 1 ml of these extracts at different concentrations were incorporated into 19 ml of warm PDA and poured into sterile Petri dishes. After solidification, A 5 mm diameter of the actively growing mycelium disc of the *Fusarium spp.* of 8 days old culture was placed in the center of the Petri dishes. PDA plates mixed with fungicide (Mancozeb) at 1 mg/ml and sterile distilled water served as positive and negative controls, respectively. The Petri dishes were incubated at 25 °C. The experiment was repeated three times. The radial growth was measured in two perpendicular directions on the reverse side of the Petri dishes every day and was expressed a percentage of inhibition of radial mycelia growth using the following formula:

$$PI(\%) = \frac{C - T}{C} \times 100$$

Where, PI is the percent of inhibition; C and T, are the radial growth measurements in the negative control and treatment in Petri dishes respectively.

### Antifungal activity of plant extracts on the tomato fruits

Based on *In vitro* test results, a concentration of 16.384 mg/ml for aqueous extracts and 2.048 mg/ml for ethanolic extracts were used for antifungal assays on tomato fruits. Conidial suspensions were prepared from a pure culture of different morphotypes of *Fusarium spp.* and adjusted to a concentration of  $5 \times 10^4$  conidia/ml using a hemocytometer [23]. Apparently healthy tomato fruits collected from the farm in, Mbouda and Foumbot were washed with tap water, dried and surface disinfected by alcohol at 70°. These fruits were received simultaneously on the epicarp 50 µl of conidial suspension and 50 µl of plant extract. These fruits were placed in plastic plates containing cotton soaked with sterile distilled water to maintain humidity during the experiment. Seven days after incubation at room temperature, the lesion areas induced on the fruits by each isolate of *Fusarium spp.* were measured using a graph paper [24]. The experiment was repeated thrice.

### Statistical analysis

Data collected on percentage inhibition of radial growth and lesion area induced by *Fusarium spp.* morphotypes were subjected to analysis of variance (ANOVA) using SPSS software version 20. The mean was separated by Duncan Multiple Range Test (DMRT) at 5 %.

## Results

### *In vitro* antifungal activity of plant extracts on the development of different morphotypes of *Fusarium spp.*

The different plant extracts had a depressant effect on the growth of the different *Fusarium spp.* morphotypes. This depressant effect was all the more important with increasing concentration applied and depended on the type of plant extract, the plant, the concentration applied, and the *Fusarium spp.* morphotypes (Table 2 & Table 3).

### *In vitro* antifungal activity of aqueous extracts

Table 2 shows the percentage of inhibition of the different *Fusarium spp.* morphotypes by the aqueous extracts of the four plants. Petri dishes enriched with extracts from each of the four plants showed higher percentages of inhibition of the different *Fusarium spp.* morphotypes at all concentrations than the negative control Petri dishes. Aqueous extracts of *C. maniana* at 16.384 mg/ml and 8.192 mg/ml exhibited significantly identical and significantly lower percentages of inhibition of the F1FBT morphotype compared to the positive control in the Duncan test at the 5% probability level. With

the F2BD morphotype, extracts of *C. lusitanica* and *P. pedoncularis* from the 8.192 mg/ml concentration showed significantly identical percentages of inhibition to the positive control. Extracts from the four plants at the 8.192 and 16.384 mg/ml concentrations completely inhibited the growth of the F3BD morphotype (100%). Extracts of

*P. pedoncularis* and *C. maniana*, showed significantly identical percentages of inhibition on the development of the F4BD morphotype, regardless of the concentration applied. These percentages of inhibition with *P. pedoncularis* ranged from 85.65 to 91.29% and those of *C. maniana* from 94.12 to 94.51%.

**Table 2:** Effect of aqueous plant extracts and synthetic fungicide on the inhibition of radial mycelia growth of *Fusarium spp.* morphotypes recorded in percentage

Morphotypes	Concentration (mg/ml)	Percentage inhibition (%)			
		<i>C. lusitanica</i>	<i>P. pedoncularis</i>	<i>C. maniana</i>	<i>C. citratus</i>
F1BT	0 (T-)	00.00±00.00e*	00.00±00.00 <sup>e</sup>	00.00±00.00 <sup>d</sup>	00.00±00.00 <sup>e</sup>
	16.384	94.12±01.00 <sup>b</sup>	96.47±00.02 <sup>b</sup>	82.39±00.07 <sup>b</sup>	87.45±00.68 <sup>b</sup>
	8.192	91.76±00.03 <sup>c</sup>	95.29±01.00 <sup>c</sup>	80.78±00.68 <sup>b</sup>	85.88±00.17 <sup>c</sup>
	4.096	89.92±00.14 <sup>d</sup>	93.92±00.01 <sup>d</sup>	78.82±02.04 <sup>c</sup>	82.94±01.02 <sup>d</sup>
	1 (T+)	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>
F2BD	0 (T-)	00.00±00.00 <sup>c</sup>	00.00±00.00 <sup>c</sup>	00.00±00.00 <sup>d</sup>	00.00±00.00 <sup>c</sup>
	16.384	96.08±06.79 <sup>a</sup>	100.00±00.00 <sup>a</sup>	91.76±07.16 <sup>b</sup>	90.20±08.51 <sup>b</sup>
	8.192	95.29±08.15 <sup>a</sup>	100.00±00.00 <sup>a</sup>	90.59±08.15 <sup>b</sup>	88.24±10.20 <sup>b</sup>
	4.096	82.35±00.02 <sup>b</sup>	87.45±01.36 <sup>b</sup>	82.16±00.34 <sup>c</sup>	81.37±00.68 <sup>b</sup>
	1 (T+)	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>
F3BD	0 (T-)	00.00±00.00 <sup>c</sup>	00.00±00.00 <sup>c</sup>	00.00±00.00 <sup>c</sup>	00.00±00.00 <sup>c</sup>
	16.384	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>
	8.192	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>
	4.096	83.33±00.34 <sup>b</sup>	85.18±00.61 <sup>b</sup>	87.84±00.68 <sup>b</sup>	82.75±00.34 <sup>b</sup>
	1 (T+)	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>
F4BD	0 (T-)	00.00±00.00 <sup>d</sup>	00.00±00.00 <sup>c</sup>	00.00±00.00 <sup>c</sup>	00.00±00.00 <sup>d</sup>
	16.384	90.00±00.20 <sup>b</sup>	91.29±00.11 <sup>b</sup>	94.51±00.34 <sup>b</sup>	93.53±01.02 <sup>b</sup>
	8.192	74.78±01.65 <sup>c</sup>	90.24±00.25 <sup>b</sup>	94.31±00.02 <sup>b</sup>	92.35±00.01 <sup>c</sup>
	4.096	73.33±01.78 <sup>c</sup>	85.65±07.95 <sup>b</sup>	94.12±00.01 <sup>b</sup>	92.35±00.03 <sup>c</sup>
	1 (T+)	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>

\*Values in the same column followed by different letters are significantly different ( $p \leq 0.05$ ) according to Duncan Multiple Range Test. T- = negative control (Distilled water); T+ = positive control (Mancozeb).

### *In vitro* antifungal activity of ethanolic extracts

**Table 3:** Effect of ethanolic plant extracts and synthetic fungicide on the inhibition of radial mycelia growth of *Fusarium spp.* morphotypes recorded in percentage

Morphotypes	Concentration (mg/ml)	Percentage inhibition (%)			
		<i>C. lusitanica</i>	<i>P. pedoncularis</i>	<i>C. maniana</i>	<i>C. citratus</i>
F1BT	0 (T-)	00.00±00.00 <sup>d</sup> *	00.00±00.00 <sup>e</sup>	00.00±00.00 <sup>c</sup>	00.00±00.00 <sup>c</sup>
	2.048	97.65±00.08 <sup>b</sup>	96.47±00.68 <sup>b</sup>	90.04±00.07 <sup>b</sup>	94.50±01.68 <sup>b</sup>
	1.024	94.12±00.01 <sup>c</sup>	95.29±00.01 <sup>c</sup>	86.28±00.68 <sup>c</sup>	92.94±00.01 <sup>c</sup>
	0.512	93.73±00.68 <sup>c</sup>	91.73±00.07 <sup>d</sup>	84.71±00.00 <sup>d</sup>	91.76±00.01 <sup>d</sup>
	1 (T+)	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>
F2BD	0 (T-)	00.00±00.00 <sup>c</sup>	00.00±00.00 <sup>c</sup>	00.00±00.00 <sup>e</sup>	00.00±00.00 <sup>d</sup>
	2.048	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	89.57±0.07 <sup>c</sup>	100.00±00.00 <sup>a</sup>
	1.024	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	90.00±00.10 <sup>b</sup>	91.76±00.02 <sup>b</sup>
	0.512	95.29±00.01 <sup>b</sup>	85.49±00.68 <sup>b</sup>	88.82±00.00 <sup>d</sup>	88.31±00.07 <sup>c</sup>
	1 (T+)	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>

<b>F3BD</b>	<b>0 (T-)</b>	00.00±00.00 <sup>c</sup>	00.00±00.00 <sup>c</sup>	00.00±00.00 <sup>c</sup>	00.00±00.00 <sup>c</sup>
	<b>2.048</b>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>
	<b>1.024</b>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>
	<b>0.512</b>	92.94±00.04 <sup>b</sup>	94.51±00.68 <sup>b</sup>	81.18±00.03 <sup>b</sup>	82.76±01.36 <sup>b</sup>
	<b>1 (T+)</b>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>
<b>F4BD</b>	<b>0 (T-)</b>	00.00±00.00 <sup>e</sup>	00.00±00.00 <sup>e</sup>	00.00±00.00 <sup>e</sup>	00.00±00.00 <sup>e</sup>
	<b>2.048</b>	95.10±00.34 <sup>b</sup>	97.06±01.02 <sup>b</sup>	85.64±00.12 <sup>b</sup>	86.47±00.33 <sup>b</sup>
	<b>1.024</b>	93.73±00.68 <sup>c</sup>	91.69±00.14 <sup>c</sup>	83.53±00.21 <sup>c</sup>	85.88±00.01 <sup>c</sup>
	<b>0.512</b>	90.59±00.03 <sup>d</sup>	87.06±00.00 <sup>d</sup>	81.57±01.36 <sup>d</sup>	84.31±00.68 <sup>d</sup>
	<b>1 (T+)</b>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>	100.00±00.00 <sup>a</sup>

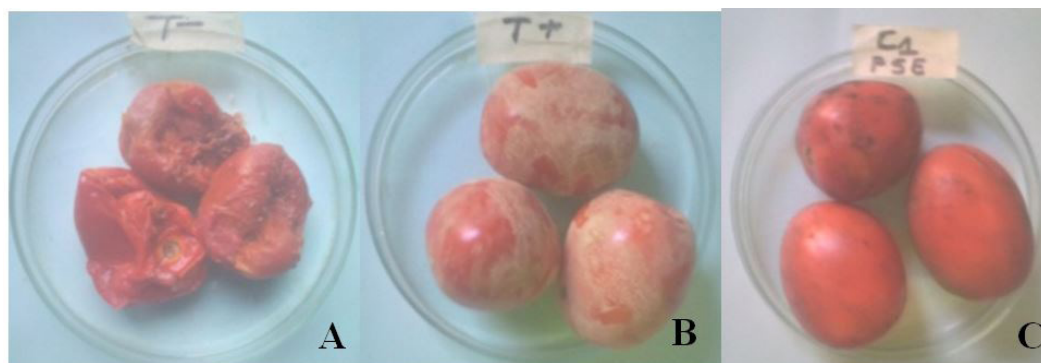
\*Values in the same column followed by different letters are significantly different ( $p \leq 0.05$ ) according to Duncan Multiple Range Test. T- = negative control (Distilled water); T+ = positive control (Mancozeb).

Table 3 shows the percentages of inhibition of the different morphotypes of *Fusarium spp.* against extracts of *C. lusitanica*, *P. peduncularis*, *C. maniana* and *C. citratus*. The ethanolic extracts of the four plants at the concentration of 2.048 mg/ml, showed significantly higher percentages of inhibition of the growth of F1FBT and F4BD morphotypes than the other concentrations applied, except for the positive controls which completely inhibited the growth of these morphotypes. With the F3BD morphotype, the various plant extracts from the concentration of 1.024 mg/ml and above, completely inhibited its growth. The same observation was

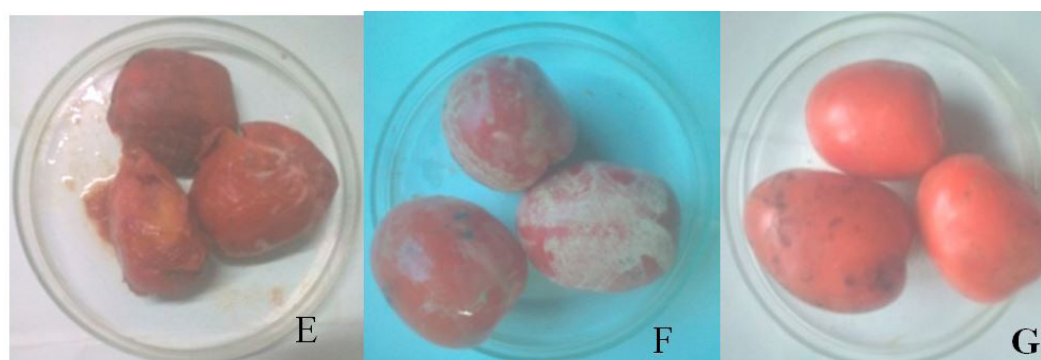
observed with extracts of *C. lusitanica* and *P. peduncularis* on the development of the F2BD morphotype.

#### Antifungal activity of plant extracts on the development of *Fusarium spp.* morphotypes inoculated on tomato fruits

The tomato fruits that received the aqueous and ethanol extracts from the different plants had smaller lesion areas than those presented by the negative control fruits (Figures 1 and 2).



**Figure 1:** Effect of ethanolic extracts of *P. peduncularis* on tomatoes rot, 7 days after fruits inoculation by *Fusarium spp.* A: negative control (distilled water), B: positive control (mancozeb) and C: Plate treated with extracts of *P. peduncularis*



**Figure 2:** Effect of aqueous extracts of *P. peduncularis* on tomatoes rot, 7 days after fruits inoculation by *Fusarium spp.* E: negative control (distilled water), F: positive control (mancozeb) and G: Plate treated with extracts of *P. peduncularis*

## Antifungal activity of aqueous plant extracts on the tomato fruits

Table 5 shows the lesions developed by the different morphotypes of *Fusarium spp.* on tomato fruits treated with aqueous extracts. The F1FBT and F4BD morphotypes developed significantly identical lesions in the 5% Duncan's test on tomato fruits treated with extracts. Lesion areas developed ranged from 84.33 to 90 mm<sup>2</sup> for the F1FBT morphotype and from 97 to 103.67 for the F4BD morphotype. The F2BD and F3BD morphotypes did not induce any lesions on tomatoes treated with ethanol extracts in the same way as the tomato fruits used as a positive control. However, the F2BD morphotype induced a lesion area of 105.79 mm<sup>2</sup> on tomato fruits treated with aqueous extracts of *C. maniana*.

**Table 4:** Effect of plant aqueous extracts on the lesion area developed by *Fusarium spp.* morphotypes on tomato fruits at 16.384 mg/ml (mm<sup>2</sup>)

Morpho types	Treatments	Concentration (mg/ml)	Lesion area (mm <sup>2</sup> )
F1FBT	T-	0	193.33 ± 05.78 <sup>a*</sup>
	<i>C. lusitanica</i>	16.384	90.00 ± 00.01 <sup>b</sup>
	<i>P. pedoncularis</i>	16.384	85.00 ± 00.02 <sup>b</sup>
	<i>C. maniana</i>	16.384	84.33 ± 01.73 <sup>b</sup>
	<i>C. citrus</i>	16.384	89.00 ± 01.73 <sup>b</sup>
	Mancozebe	1	00.00 ± 00.00 <sup>c</sup>
F2BD	T-	0	138.00 ± 01.73 <sup>a</sup>
	<i>C. lusitanica</i>	16.384	00.00 ± 00.00 <sup>c</sup>
	<i>P. pedoncularis</i>	16.384	00.00 ± 00.00 <sup>c</sup>
	<i>C. maniana</i>	16.384	105.79 ± 00.82 <sup>b</sup>
	<i>C. citrus</i>	16.384	00.00 ± 00.00 <sup>c</sup>
	Mancozebe	1	00.00 ± 00.00 <sup>c</sup>
F3BD	T-	0	202.67 ± 02.31 <sup>a</sup>
	<i>C. lusitanica</i>	16.384	00.00 ± 00.00 <sup>b</sup>
	<i>P. pedoncularis</i>	16.384	00.00 ± 00.00 <sup>b</sup>
	<i>C. maniana</i>	16.384	00.00 ± 00.00 <sup>b</sup>
	<i>C. citrus</i>	16.384	00.00 ± 00.00 <sup>b</sup>
	Mancozebe	1	00.00 ± 00.00 <sup>b</sup>
F4BD	T-	0	181.33 ± 02,31 <sup>a</sup>
	<i>C. lusitanica</i>	16.384	100.67 ± 00.91 <sup>b</sup>
	<i>P. pedoncularis</i>	16.384	97.00 ± 06.08 <sup>b</sup>
	<i>C. maniana</i>	16.384	101.71 ± 00.49 <sup>b</sup>
	<i>C. citrus</i>	16.384	103.67 ± 00.58 <sup>b</sup>
	Mancozebe	1	00.00 ± 00.00 <sup>c</sup>

\*Values in the same row followed by different letters are significantly different (P < 0.05). Data are means ± SD of three experiments, T- = negative control (Distilled water) ; M = mancozebe

## Antifungal activity of ethanolic plant extracts on the tomato fruits

Table 4 shows the areas of lesion induced by different *Fusarium spp.* morphotypes on tomato fruits treated with ethanolic extracts. The F1FBT and F4BD morphotypes induced significantly identical areas of the lesion on extract-treated tomato fruits in the 5% Duncan test. These lesion areas ranged from 75.20 mm<sup>2</sup> to 80.33 mm<sup>2</sup> for the F1FBT morphotype and from 87.51 mm<sup>2</sup> to 93.67 mm<sup>2</sup> for the F4BD morphotype. Similarly, these lesions were significantly smaller than those of the negative control fruit and significantly larger than those of the positive control fruit. The F2BD and F3BD morphotypes did not induce any lesions on tomatoes treated with ethanol extracts in the same way as the tomato fruits used as a positive control.

**Table 5:** Effect of plant ethanolic extracts on the lesion area developed by *Fusarium spp.* morphotypes on tomato fruits at 2.048 mg/ml (mm<sup>2</sup>)

Morpho types	Treatments	Concentration (mg/ml)	Lesion area (mm <sup>2</sup> )
F1FBT	T-	0	193.33 ± 05.78 <sup>a*</sup>
	<i>C. lusitanica</i>	2.048	80.33 ± 00.58 <sup>b</sup>
	<i>P. pedoncularis</i>	2.048	75.20 ± 00.17 <sup>b</sup>
	<i>C. maniana</i>	2.048	76.00 ± 01.29 <sup>b</sup>
	<i>C. citrus</i>	2.048	79.27 ± 01.50 <sup>b</sup>
	Mancozebe	1	00.00 ± 00.00 <sup>c</sup>
F2BD	T-	0	138.00 ± 01.73 <sup>a</sup>
	<i>C. lusitanica</i>	2.048	00.00 ± 00.00 <sup>b</sup>
	<i>P. pedoncularis</i>	2.048	00.00 ± 00.00 <sup>b</sup>
	<i>C. maniana</i>	2.048	00.00 ± 00.00 <sup>b</sup>
	<i>C. citrus</i>	2.048	00.00 ± 00.00 <sup>b</sup>
	Mancozebe	1	00.00 ± 00.00 <sup>b</sup>
F3BD	T-	0	202.67 ± 02.31 <sup>a</sup>
	<i>C. lusitanica</i>	2.048	00.00 ± 00.00 <sup>b</sup>
	<i>P. pedoncularis</i>	2.048	00.00 ± 00.00 <sup>b</sup>
	<i>C. maniana</i>	2.048	00.00 ± 00.00 <sup>b</sup>
	<i>C. citrus</i>	2.048	00.00 ± 00.00 <sup>b</sup>
	Mancozebe	1	00.00 ± 00.00 <sup>b</sup>
F4BD	T-	0	181.33 ± 02,31 <sup>a</sup>
	<i>C. lusitanica</i>	2.048	90.34 ± 00.78 <sup>b</sup>
	<i>P. pedoncularis</i>	2.048	87.51 ± 06.43 <sup>b</sup>
	<i>C. maniana</i>	2.048	91.42 ± 01.15 <sup>b</sup>
	<i>C. citrus</i>	2.048	93.67 ± 00.58 <sup>b</sup>
	Mancozebe	1	00.00 ± 00.00 <sup>c</sup>

\*Values in the same row followed by different letters are significantly different (P < 0.05). Data are means ± SD of three experiments, T- = negative control (Distilled water) ; M = mancozebe

## Discussion

Aqueous and ethanolic extracts of *Cyathea maniana*, *Cymbopogon citratus*, *Cupressus lusitanica* and *Psychotria peduncularis* significantly reduced the radial growth of the different morphotypes of *Fusarium spp.* compared to the negative control. This reduction may be because the plants used could contain compounds or substances that would inhibit or retard the radial growth of the different *Fusarium spp.* morphotypes. The work of Saraka, *et al.* (2018), Kossonou, *et al.* (2019) and Ouattara, *et al.* (2020) has shown that some plants contain compounds with antifungal properties (alkaloids, sterols, terpenoids, flavonoids, anthraquinone phenols, saponins or tannins) in their organs, which is why they are sometimes used in traditional medicine. This inhibition was important with the increase in concentration. Similar results were reported by Keuete, *et al.* (2015) and Yemo, *et al.* (2017) who showed that *C. papaya* extracts further inhibited the radial growth of *Colletotrichum gleosporoides* and *C. kahawae* respectively with increasing concentration. Ethanolic extracts were more effective than aqueous extracts. This difference could be attributed to a difference in the concentration of chemical compounds during the extraction process. The work of Bagré, *et al.* (2011) reported that ethanol allows better extraction of compounds such as flavonoids and terpenoids, which are molecules known for their antifungal activity. In addition, water would act much more on inactive than active substances while more selective ethanol would act more on active substances against the various *Fusarium spp.* morphotypes. This hypothesis corroborates with the work of Ruffini *et al.* (2020) who reported that ethanol extracts from *Terminalia ivorensis* were more effective on *Fusarium spp.* morphotypes than aqueous extracts. Similarly, the work of Bakari *et al.* (2019) on the *In vitro* evaluation of the antifungal activity of *Mallotus oppositifolius* leaf extracts on *Fusarium sp.* and *Phytophthora sp.* showed that the antifungal activities were greater with ethanol extracts than with aqueous extracts.

Radial growth of the F3BD morphotype was completely inhibited by the aqueous and ethanol extracts of the four plants at concentrations of 8.192 mg/ml and 1.024 mg/ml, respectively. This would mean that this *Fusarium spp.* morphotype would be sensitive to the active compounds present in the extracts of the four plants. With the F2BD morphotype, the aqueous extracts of *C. lusitanica* and *P. peduncularis* completely inhibited its radial growth. While the aqueous extracts of *C. maniana* and *C. citratus*, did not inhibit 100% the development of this morphotype. This difference in the efficacy of the extracts with respect to one *Fusarium spp.* morphotype to another would be due to the fact that some morphotypes would be more sensitive to the

active compounds of the plant extracts and others less sensitive. These results suggest that the control of *Fusarium spp.* morphotypes would require several approaches. Similar work has been reported by Farah, *et al.* (2018) which showed that extracts of *Allium sativum* and *Petroselinum crispum* were 100% inhibitory to radial growth of some *Fusarium Oxysporum* morphotypes and not others. On the other hand, those of Anil Khumar, *et al.* (2015) showed that extracts of *Solanum indicum*, *Azadirachta indica* and *Oxalis latifolia* completely inhibited the growth of all *Fusarium oxysporium* morphotypes. This difference in results would be justified by the fact that the different plants used by these authors would not contain the same active ingredients.

The different plant extracts significantly inhibited the development of the different *Fusarium spp.* morphotypes on fruits compared to the negative control. This suggests that on tomato fruits, the bioactive molecules present in these extracts would also have been metabolized and would have protected these tomato fruits against the invasion of the different *Fusarium spp.* morphotypes. Similar results were reported by Keuete, *et al.* (2015) who showed that extracts of *Cupressus lusitanica* would inhibit the development of fungi in preserved avocado fruits. On the other hand, work by Mugao (2015) investigating the causes of post-harvest tomato rots and the control of pathogens associated with these fruits in Kenya reported that extracts of *Azadirachta indica* can extend the shelf life of tomato fruits after harvest. Similarly, Kasmi, *et al.* (2018) evaluating the efficacy of aqueous extracts of aromatic and medicinal plants against gray mold in Morocco showed that extracts of *Cymbopogon citratus* were the most effective against tomato fruit gray mold.

## Conclusion

To contribute to the discovery of new substances that can be used to fight against *Fusarium spp.* with tomatoes, antifungal activity extracts of four plants against different morphotypes of *Fusarium spp.* associated with tomato fruits rot in the West-region of Cameroon was evaluated in this study. The results of the inhibition test showed that the four extracts tested reduced to varying degrees the development of all *Fusarium spp.* morphotypes. However, extracts of *C. lusitanica* and *P. peduncularis* were the most effective. This study suggests that the extracts of these plants could be used as an alternative to chemical control in the fight against fusariosis of tomato. However, evaluation of the synergistic effect of these extracts to better potentiate their activities against other morphotypes of *Fusarium spp.* will be the subject of future research.

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