Original Research Article

Spécific diversity and physiological caracterization of Fusarium *spp.* isolates causing Potato (*Solanum tuberosum* L.)Fusariosis (Far-North, Cameroon).

ABSTRACT

Introduction : Potato production in the Far North Region, Cameroon is hampered by a wide range of fungi of the genus Fusarium.

Aims : The aim of this work was to isolate and conduct physiological characterization of Fusarium spp. isolates responsible for potato fusarium disease in the Far North Region, Cameroon.

Methodology : Samples were collected from 15 villages in the district of Mogodé, Mokolo and Koza. Fusarium incidence and rainfall were assessed. Isolates were obtained, the species responsible for fusariosis and the parameters of physiological characterization such as growth rate, sporulation and pathogenicity of the isolates were evaluated.

Results : The overall average incidence of Fusarium head blight in the Far North Region, Cameroon is 29.36%. Furthermore, out of the 20 isolates obtained, six Fusarium species *(Fusarium oxysporium, F. solani, F. equiseti, F. avenacearum, F. colmorum and F. sambicum)* were identified. The highest growth rate (8.1 cm), the highest spore production (8 × 105) and the highest severity index (3%) were respectively obtained with the isolates FUROM 2 and FUTEK 3, FUMOG 1, FUMOG 2, FUROM 1 and FURAF from Mogodé District on PCA medium.

Conclusion : Potato production in the Far North Region, Cameroon is confronted by various Fusarium species which have physiological characteristics that vary according to the area of origin of the isolates and the growing media.

Key word : *Solanum tuberosum* L, Fusarium, Incidence, Isolates, Sporulation, Pathogenicity.

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is a tuberous herbaceous plant native to Peru and belonging to the Solanaceae family [1]. It is a vegetatively propagated species that produces more

nutritious food on less cultivated area and in the harshest of climates than any other crop [2, 3]. It has become one of the staple foods of humanity and thus occupies a prominent place in the diet of many countries in terms of the land it occupies, the jobs it provides and the production volumes it generates. World production was estimated at more than 376 million tonnes on 19.4 million hectares spread over 151 producing countries in 2014 [4]. Which makes the potato the fourth most important non-cereal food crop in the world after wheat, rice and maize [4].

In Cameroon, it is grown in high altitude areas (1000 to 3000 m) [5,6] and extensively in six of the ten regions of Cameroon (North-West, South-West, West, Adamaoua, Littoral and Far-North), mostly by rural people and mainly by women [7]. It is a staple food for the populations of these regions (annual consumption of 4 to 10 kg per capita/year) [5,6] and an important source of income for producers. Yields are generally very low and range from 3 to 11 tonnes per hectares in the Far North Region of Cameroon, while those in European countries average 25 tonnes per hectares and reach 60 t/ha [7,8,9]. The low yields observed in this production zone are associated with poor farming practices such as the absence of crop rotation, the semi-open system, the poor choice of varietal material and chemical fertilisation methods [7,10,6] pests and especially diseases that hamper potato development in all potato production areas in the world [11,8,6].

In almost all potato production areas, Fusarium head blight appears to be the most important foliar and post-harvest disease causing the highest yield losses estimated at more than 25% per year [12,13,14]. Various studies have also shown that the severity of diseases caused by Fusarium varies from 6 to 25% and even 60% in some cases [12,14]. In Great Britain, the national average incidence of affected tubers could be as high as 1.4% and 50-100% of tubers are attacked [13]. More than 50% of tubers are infected with Fusarium in Michigan [15]. Other studies have shown that the severity of diseases caused by Fusarium phytopathogens are among the most common fungi found on potato crops in the Far North Region, Cameroon [6].

It is estimated that there are thirteen species of Fusarium causing potato fusariosis depending on agro-ecological zones [16] edaphic factors, environmental factors [17,18], climatic factors [18,19] and cultivars. Nevertheless, *Fusarium sambicum* is the most widespread fungus in North America, China and parts of Europe [21,22,23]. *Fusarium coeruleum* is the most

widespread agent in the United Kingdom [22]. In addition, F. *oxysporium*, F *avenaceum*, F. *acuminatum*, F. *equiseti*, F. *sulphureum* and F. *solani* have been recorded on potato [17,24]. In Africa, *Fusarium sambicum*, F. *oxysporium*, F. *verticillioides* and F. *incarnatum* predominate in Egypt [14]. To the best of our knowledge, no study has revealed the specific diversity of fungi of the genus Fusarium in Cameroon in general and in the Far North Region in particular. However, the knowledge of the diversity and aggressivity of these species constitute the diagnostic gateways to master the biology, the epidemiological mechanisms and especially the setting up of biological control methods. The present review provides an update on the status of the genus Fusarium of potato in the Far North Region, Cameroon, and opens up avenues of investigation. The aim of this work is to carry out a physiological characterization of Fusarium isolates and to evaluate the degree of aggressiveness of the species.

2. MATERIALS AND METHODS

2.1 Area of study

The study was carried out in two Departments : the Department of Mayo-Tsanaga with geographical coordinates 10°24'23" N and 13°49'17" E, and the Department of Diamaré with coordinates between 10°44'33" N and 14°16'81" E [25], all located in the Far North Region, Cameroon.

2.2 Plant Material

The plant material used for this study consisted of two potato varieties, namely the resistant local variety called Dosa and the improved susceptible variety called Spunta.

2.3 Sample collection, incidence assessment and rainfall

Samples were collected over a period of two years (June 2020 to October 2022) in fifteen villages spread over three district namely Mogodé, Mokolo and Koza all located in the Mayo-Tsanaga Department, Far North Region of Cameroon. The samples collected were coded and then sent to the IRAD plant pathology laboratory in Maroua for isolation and specific tests. The incidence was evaluated by counting the number of diseased plants out of the total number of plants present in the fields surveyed per village and per District. Rainfall data were collected from the stations of the Agricultural Delegations of each District surveyed [26]. Incidence of diseases were evaluated using the following formula [26]: $\boldsymbol{I}(\%) = \frac{\boldsymbol{np}}{\boldsymbol{N}} \times \mathbf{100.}$

where, I is incidence; n p number of plants showing symptoms per plot ; and N total number of plant in plot.

2.4 Obtention of isolates

Obtaining isolates consisted firstly of preparing the culture media, then culturing the samples, purifying the strains and finally isolating them.

2.5 Culture and isolation

The culture of the samples consisted first of the preparation of the culture media PDA (200g potato, 20g D-Glucose and 15g Agar), PPA (70 grams of Petit Pois and 15g agar), PSA (200g potato, 20g sucrose and 20g Agar) and PCA (40g potato, 40g carrot and 20g Agar) all dissolved in 1L distilled water as defined by [27,28,29]. Next, the samples were disinfected. For this purpose, the leaves with disease symptoms were cleaned three times in running tap water to remove saprophytic flora and debris, then with 1% sodium hypochlorite for one minute, further with 70% alcohol for one minute and finally rinsed with running tap water. The disinfected organs were dried on a white hygienic cloth for 30 minutes to remove or absorb the water on the samples. Finally, the parts of the samples showing symptoms of the disease were cut into squares using a bustori blade. The samples were taken with the help of a coin lifter and aseptically and individually placed in petri dishes containing culture media previously prepared in the fume hood. The petri dishes were closed, sealed with parafilm and incubated in a climate chamber at 22-27 °C under a 12/12h photoperiod. The mycelium developed from the explant and after 2-4 days reached sufficient growth to proceed to purification.

The purification of the strains consisted of three successive subcultures on the PDA medium, by taking fragments of mycelial discs of 08 mm in diameter at the growth front of the 10-day-old cultures to be sown in the centre of each petri dish containing the medium. Isolates were obtained by taking a mycelial disc at the growth front from each pure culture obtained after three successive purifications. The isolates thus obtained were directly used for species identification and partially preserved in sterile distilled water at 4°C for further use.

2.6 Species identification

The identification of the species was based on the identification keys as defined by [30,1214,6] this was done by comparing the symptoms on different organs (leaves and tubers)

in the field and the microscopic structures in the laboratory. The identification of the different species in the laboratory was done by determining the presence or absence of filamentous structures such as macroconidia, microconidia and chlamydospores characteristic of each Fusarium species on 20-day-old culture media.

2.7 Physiological characterization of isolates

Four culture media, namely PDA (Potato Dextrose Agar), PCA (Potato Carrot Agar), PSA (Potato Sucrose Agar), and PPA (Pea Agar) were used to evaluate the parameters of physiological characterisation, including growth rate, sporulation and pathogenicity.

2.7.1 Evaluation of the cultural characteristics of the isolates

The parameters of morphological characterisation, i.e. the colour, shape, appearance and relief of the colonies grown on PPA, PCA, PSA and PDA culture media were evaluated 7 days after subculturing, by macroscopic observation in order to determine the specific cultural characteristics of each isolate according to the culture media and the origin of the strains. The whole set was incubated at a photoperiod of 12H/12 and at the laboratory's ambient temperature, which varied between 25° and 30°C.

2.7.2 Measurement of radial growth of isolates

Growth rate was assessed by measuring the perpendicular diameters previously traced on the back of the petri dishes and inside which a fragment of mycelium disc of 08 mm diameter of each isolate was placed. Measurements were made every two days and at the same time by simple measurement with a 30 cm ruler. The measurement was done after 2, 4, 6 and 6 days of culture to evaluate the growth rate of the different strains under the influence of the different culture media. Three replicates were made for each isolate and culture medium. The Petri dishes containing the culture media were incubated at a photoperiod of 12/12h and at room temperature of the laboratory, ranging between 25° and 30°. The formula of [31] opposite was used to calculate the growth rate of each isolate.

$$\mathbf{D} = \frac{d1 + d2}{2} - d_0$$

D = diameter of growth ; do = diameter of mycelial disc ; d1 and d2 = two perpendicular diameters.

2.7.3 Sporulation measurement of isolates

Spores production was evaluated on the 20-day-old cultures, incubated at a 12H/12 photoperiod and at room temperature. For this purpose, four 08 mm mycelial discs of each isolate were collected and placed in tubes containing 1 mL of sterile distilled water. The solutions were filtered through muslin cloths to remove unwanted fragments. Next, 0.1mL of solution from each isolate was withdrawn with an insulin syringe and then calibrated with a malasssez cell or hematimeter for spore enumeration. Each operation was repeated three times for each isolate [32].

2.7.4. Pathogenicity test of isolates

The detached leaf disc test was used to assess the pathogenicity of the different isolates. For this purpose, young healthy leaves of two varieties, namely Dosa, a resistant local variety, and Spunta, a susceptible introduced variety, one month old, were collected very early in the morning in the field at about 6 a.m. and individually placed in white plastic bags and transported to the laboratory for conditioning and testing.

2.7.4.1. Conditioning of detached leaves and incubation

Conditioning consisted of carefully washing the leaves in running tap water, rinsing them with 70° alcohol for one minute, and finally drying them on white absorbent paper for 30 minutes with their petioles inserted in the cotton wool to maintain moisture (Ganie et al., 2013). Then white toilet paper of 110 mm length and width dimension was cut with the scissors and placed in sterile petri dishes of 150 mm diameter. Four millilitres (4ml) of sterile distilled water were sprayed into each petri dish using a sterile propette and the leaves were transferred into each dish. Finally, sporal suspensions obtained from the 20-day-old pure cultures were calibrated at 3-4×105 spores/ml using the Malassez cell. The suspensions were spread on the four cardinal points of the upper surface of the leaf using a micropipette. Three replicates were made for each isolate and according to the potato varieties. The media were regularly humidified every two days to maintain the relative humidity of the medium. Incubation was done at a 12H/12 photoperiod and at room temperature [33].

2.8. Data Analysis

The data obtained were subjected to an analysis of variance using SPSS 25.0 software. The DUNCAN test was used to compare the means of the measured parameters at the 5% threshold.

3. RESULTS

3.1. Evolution of the incidence of fusariosis in the study area

The incidence of Fusarium wilt varied considerably between villages and study Districts. The average incidence of fusariosis in the Far North Region, Cameroon is 29.36%. However, it is in the district of Mogodé that the highest average incidence was obtained, 51.08%, contrary to the district of Koza where the lowest incidence was obtained, 6% (Table 1). The intermediate average incidence was obtained in the Borough of Mokolo, 19.5% (Table 1).

Table 1: Evolution of the incidence of fusariosis according to the villages and Districts surveyed in two years.

	Means Incidence 2020-2021			Means Incidence 2021-2022			
Areas	Villages	Incidence%	Means	Villages	Incidence%	means	Means 2020-2022
MOGODE	Mogodé	45%	**39,58%**	**Mogodé**	**75%**	**62,58%**	**51,08%**
	Rhomuzou	35%		Rhoumzou	45%		
	Ndegvaya	**67%**		Ndegvaya	57%		
	Teki	25%		Teki	28%		
	Zimi	51%		Zimi	53%		
	Sirakoti	20%		Sirakoti	40%		
	Raffa	49%		Raffa	69%		
	Karanti	32%		Karanti	42%		
	Rhumsiki	23%		Rhumsiki	33%		
	Gouria	**15%**		Gouria	35%		
	Mouvou	32%		Mouvou	62%		
	Migi	34%		**Migi**	**24%**		
MOKOLO	Kosehone	20%	14,5%	Kosehone	25%	**24,5%**	**19,5%**
	Gawar	**15%**		**Gawar**	**24%**		

KOZA	**Ziler**	**5%**	**5%**	**Ziler**	**7%**	**7%**	**6%**
Overall average incidence in the study area: 29.36							

3.2 Rainfall trends in the study area

Rainfall varies over time and space depending on the weeks, months and Study Districts. The highest average rainfall, the average or intermediate rainfall, and the lowest average rainfall were respectively obtained in the district of Mogodé, Koza, and Mokolo: 1,988 mm, 1,024 mm and 828.5 mm (Fig 1).

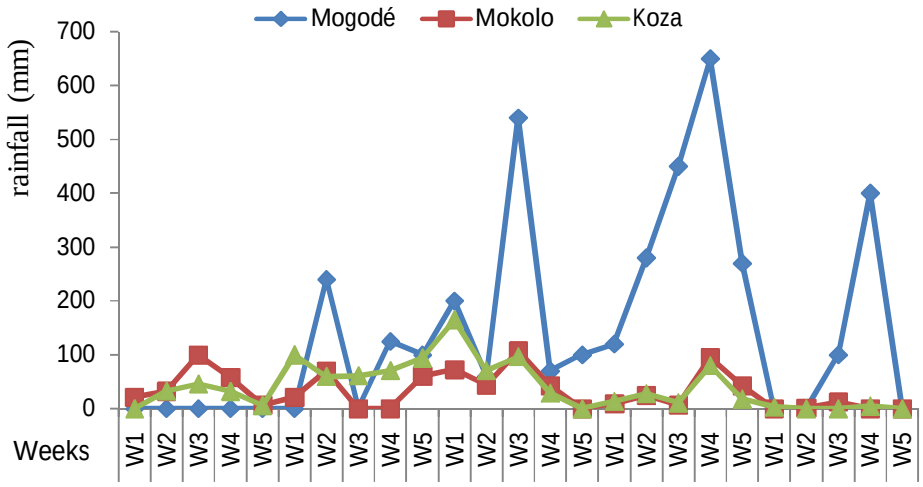


Fig 1: Changes in rainfall in the districts of Mogodé, Mokolo and Koza.

3.3. Physiological characteristics of the isolates

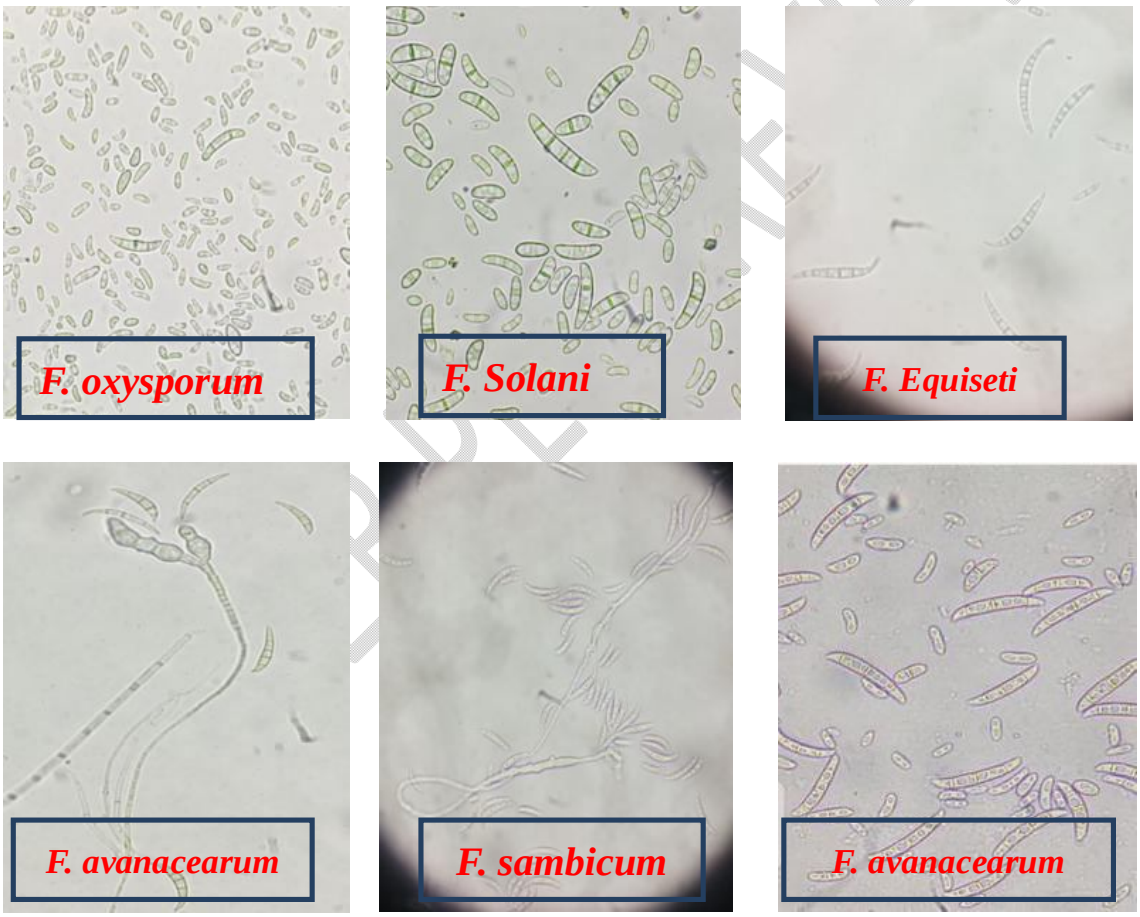
3.3.1. Isolates obtained

Twenty isolates were obtained in the district of Mogodé, Mokolo and Koza. Of the Twenty isolates, fifteen isolates,75% (FUMOG 1, FUMOG 2, FUROM 1, FUROM 2, FUROM 3, FUNDG 5, FUTEK 3, FUZIM 1, FUSRA 1, FUSRA 2, FURAF 12, FUKAR 5, FUSIK 8, FUGOU 1 and FUMOV) were obtained respectively in the villages (Mogodé, Rhoumzou, Ndegvaya, Teki, Zimi, Sirakoti, Raffa, Karanti, Rhumsiki, Gouria and Mouvou) of Mogodé District; three isolates, 15% (FUKOS 1, FUKOS 2 and FUGAW 4) were obtained in two

villages (GAWAR and KOSEHONNE) in the of Mokolo and two isolates, 10% (FUKOZ 1 and FUKOZ 2) were obtained in the village ZILER in the district of Koza.

3.3.2. Species identified

In general, 6 species were identified from the samples collected in the three study Districts (Mogodé, Mokolo and Koza*): Fusarium oxysporium, Fusarium solani, Fusarium equiseti, Fusarium avenacearum, Fusarium colmorum and Fusarium sambicum.* Clamydospores, macroconidia and microconidia characteristic of the genus Fusarium were also obtained (Fig 2). All the 6 species inventoried are present in Mogodé District unlike Mokolo and Koza Districts where only *Fusarium oxysporium* and *Fusarium solani* were specifically identified.



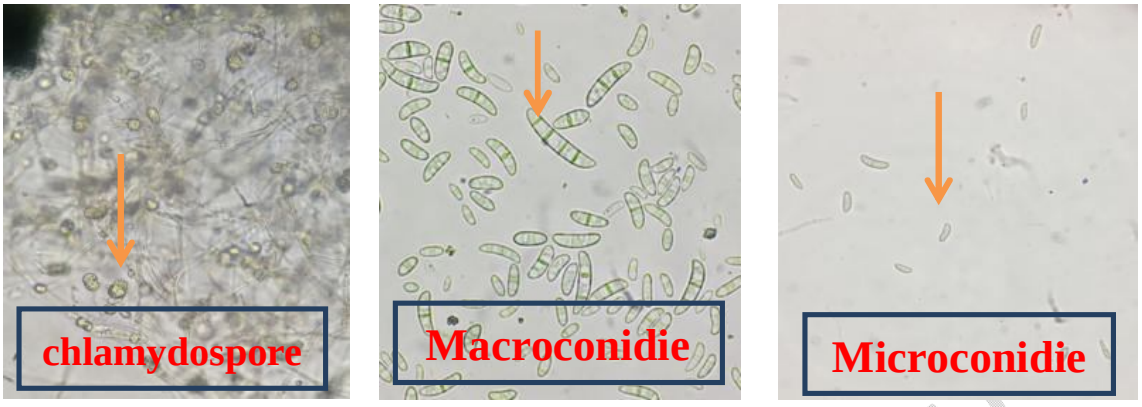


Fig 2: Fusarium species diversity in the are study

3.3.3. Cultural characteristics of the isolates obtained

The results of the morphological characterization of the colonies grown on PPA, PCA, PSA and PDA media revealed considerable variation according to the above media and the areas of origin of the isolates (Fig 3). In general, the isolates obtained in the three study Districts all show a white colour as a common morphological feature, as opposed to the bulging relief feature not observed in Koza District with isolates (FUKOZ 1 and FUKOZ 2).

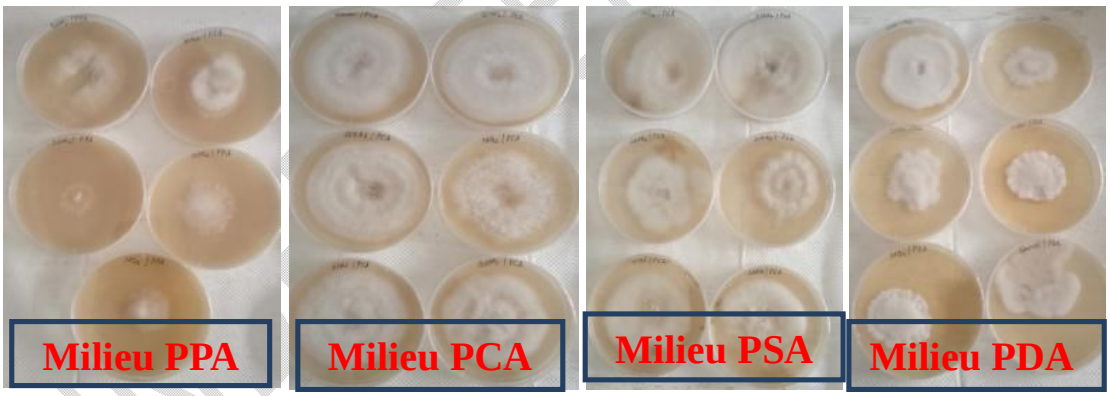


Fig 3: Cultural characteristics of some Fusarium isolates on culture media.

3.3.4. Radial growth diameters of isolates

Four statistical groups were obtained from the evaluation of the growth diameter at 6 days after transplantation (6 DAR). Statistical analyses (P = 0.000) showed that growth diameters varied considerably between isolates and culture media. Nevertheless, the highest growth diameters (8.1 cm) were obtained mainly in Mogodé District with isolates FUROM 2, FUTEK 3 and FUKOS1, on PCA medium, in contrast to isolates from Mokolo and Koza Districts (Table 2).

3.3.5. Sporulation rate of isolates

Statistical analysis revealed a highly significant difference (P=0.000) in spore production. Isolates from Mogodé were the most abundant in spore production compared to isolates from the other district. (Table 3). Spores were most abundant in PCA and PSA media respectively with isolates FUMOG 1 and FUMOG 2 at 8 × 105 in Mogodé District as opposed to isolate FUKOZ 1 from Koza District at 1 × 105 spores (Table 3).

Table 2: Growth diameter and spore production of isolates

		Number of spores×10^5 ml					Growth diameter (cm)			
		Culture media					Culture media			
Areas	Isolats	PCA	PSA	PPA	PDA	Isolats	PCA	PSA	PPA	PDA
MOGODE	FUMOG1	8	7	5	2	FUMOG1	8	8	7,5	4,2
	FUMOG2	8	8	5	3	FUMOG2	8	8	8,1	6,9
	FUROM1	5	6	4	2	FUROM1	6,2	5,9	7	5,3
	FUROM2	5	6	5	4	FUROM2	8,1	7,6	8	6,1
	FUROM3	4	7	4	3	FUROM3	8	8	8	8
	FUNDG5	3	6	3	3	FUNDG5	6,6	6	7,9	6,5
	FUTEK3	6	4	4	4	FUTEK3	8,1	8	8	8
	FUZIM1	5	4	5	2	FUZIM1	8	8	8	8
	FUSRA1	4	4	4	2	FUSRA1	7,6	7,4	7,8	6
	FUSRA2	5	6	4	3	FUSRA2	6,2	5,5	7,5	6
	FUKAR5	7	6	8	3	FUKAR5	6,8	7,9	8	6,5
	FURAF12	5	5	5	2	FURAF12	7,5	7,8	7,1	5,1
	FUSIK8	5	4	5	2	FUSIK8	7,3	8	7	6
	FUGOU1	6	6	5	4	FUGOU1	6,5	5,4	6,8	6
	FUMOV1	4	6	3	2	FUMOV1	7,6	4,2	7	5,5
MOKOLO	FUKOS1	6	5	4	2	FUKOS1	8,1	7,9	8	7
	FUKOS2	6	4	4	3	FUKOS2	6,9	6,6	8	7
	FUGAW4	5	4	5	2	FUGAW4	7,7	5,8	7,7	6
KOZA	FUKOZ1	4	5	2	1	FUKOZ1	7,8	7,6	7,8	6',4
	FUKOZ2	4	5	3	3	FUKOZ2	7,7	7,7	7,5	6,7

Probability P=0,000	Probability P=0,001

3.3.6. Pathogenicity of isolates

Statistical analysis of the severity index carried out by artificial inoculation of potato leaves of the Dosa and Spunta varieties from Fusarium spore suspensions revealed a significant difference (P=0.000) in the degree of aggressivity of the different isolates (Fig 4). The severity indices of the isolates in Mogodé District ranged from 0.92% to 2% for the Dosa variety and from 1% to 3% for the Spunta variety. However, in Mokolo district, the index varied from 1.5% to 2% for Dosa and from 1% to 3% for Spunta. No variation in the severity index was observed across the two potato varieties in Koza. However, the index was 1.5% for the Dosa variety and 2% for the Spunta variety. Overall, the isolates with the highest severity index were obtained in Mogodé district with isolates FUROM 1 and FURAF 12, i.e. 3%, 2.5%, in contrast to Koza district, i.e. 1.5% obtained with isolate FUGAW 4 (Fig 5). The highest severity index (3%) was obtained with isolate FUROM 1 on the variety Spunta, which confers the status of susceptible variety on Spunta and resistant variety on Dosa.

Fig 4: Leaf appearance 10 days after inoculation

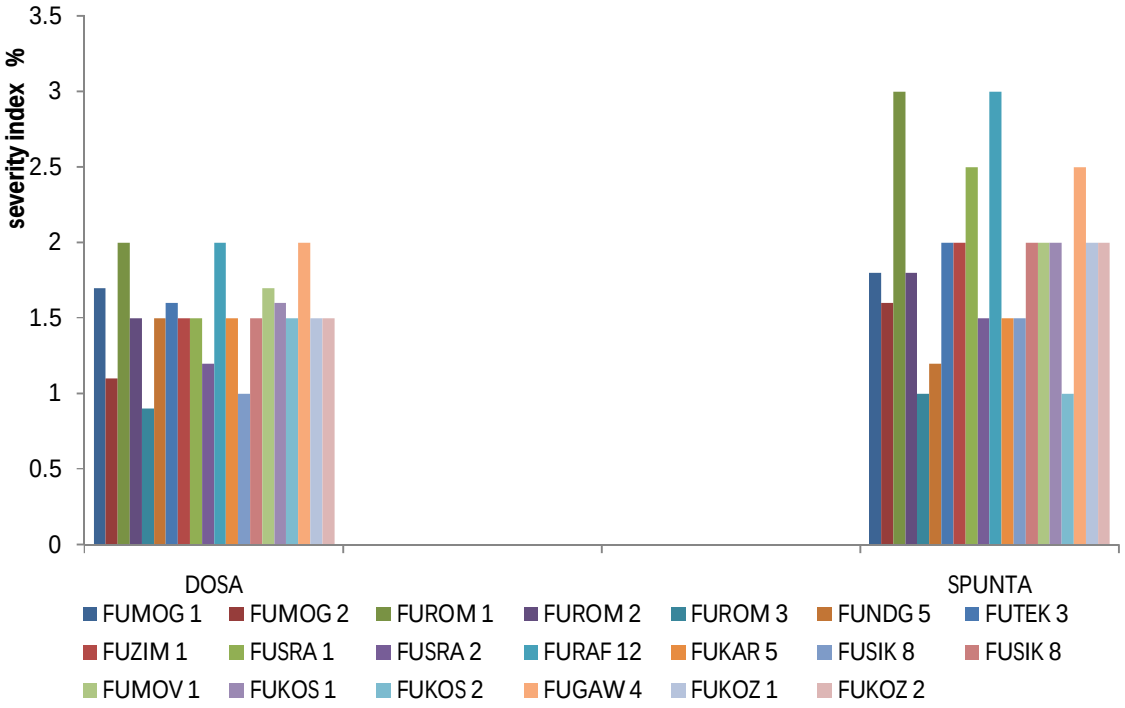


Fig 5: Severity index of isolates according to varieties.

4. DISCUSSION

The aim of this work was to isolate and conduct physiological characterization of Fusarium spp. isolates causing Fusarium on potato in the Far North, Cameroon. The results of the survey work showed that the overall average incidence of Fusarium spp. is 29.36%. This percentage shows that Fusarium head blight is a cosmopolitan fungal disease that significantly affects the potato crop in the Far North Region, Cameroon. These results are in agreement with the work of [13,14] who reported that in Great Britain, the national average incidence of affected tubers was as high as 1.4% and 50-100%. Furthermore, [15] also showed that more than 50% of tubers are infected by Fusarium in Michigan ; it is 43.7% in the Oued Tafna region of Algeria [34]. The random distribution of Fusarium lobtene incidence in Mogodé (51.08%), Mokolo (19.5%) and Koza (6%) correspond to the rainiest months respectively obtained during September in Mogodé and August in Mokolo and Koza : this shows that the intensity of the disease varies according to the rainfall prevailing in a given geographical area. These findings are in agreement with those of [17,18,35] who showed that Fusarium head blight is a disease that adapts rapidly to climatic conditions to develop on the host plant.

Regarding the isolation itself, it should be noted that twenty (20) isolates were obtained from samples collected from potato fields in the Far North Region, Cameroon. This result is in line with those reported by [36]. Indeed, the latter was able to isolate 120 isolates of Fusarium responsible for yield losses on cowpea in the Far North Region, Cameroon. Various of Fusarium (*Fusarium oxysporium, Fusarium solani, Fusarium equiseti, Fusarium avenacearum, Fusarium colmorum and Fusarium sambicum*) were identified as being responsible for potato fusariosis in the Far North Region. These results are in agreement with those identified in North America, China and parts of Europe by [22,23]. These authors reported the presence of Fusarium sambicum as the most prevalent fungus in these regions. In addition, F. *oxysporium,* F *avenaceum,* F. *equiseti*, and F. *solani* have been recorded on potato by [17, 24]. In Africa, *Fusarium sambicum*, F. *oxysporium*, predominate in Egypt [14].

The results of the morphological characterization of the colonies of the isolates grown on PPA, PCA, PSA and PDA culture media revealed that the majority of the colonies had a white colour, a regular growth, a cottony aspect on the different culture media with a relief varying from Flat to Domed. The results obtained corroborate with those of [36]. Who showed that the white colour of the colonies is widely observable on most of the PDA culture media. The large variability observed in the growth rate and spore production of the isolates is due to the fact that the isolates tested came from three different districts, all of which were subject to the influence of climatic, soil and environmental factors prevailing in each district. Furthermore, the nature of the isolates, the nutritional elements contained in the culture media, the temperature of the reaction medium, and the humidity of the air that accompanied the experiment would be at the origin. This same finding was reported by [34,37,36]. For them, the growth rate varies according to the culture media. However, [38] reported that PDA medium is not conducive to the production of Fusarium spores isolated from cowpea in the Far North Region, Cameroon in contrast to potato isolates of Fusarium that produced spores in low quantities. The variation in the degree of aggressiveness of the isolates is thought to be due to the fact that the isolates do not all come from the same area and that the two potato varieties used do not have the same genetic make-up or ability to respond to phytopathogenic stresses. This was also reported by [20] who showed that the aggressiveness of the isolates varies between potato cultivars.

5. CONCLUSION

The objective of this work was to isolate and conduct a physiological characterization of isolates of Fusarium spp. agents responsible for potato fusariosis in the Far North, Cameroon. It was found that six different species of Fusarium (*Fusarium oxysporium, F. solani, F. equiseti, F. avenacearum, F. colmorum and F. sambicum*) not only cause potato fusariosis in the Far North Region, Cameroon but also cause an estimated 29.39% damage. Furthermore, isolates from Mogodé District have a higher growth rate, sporulation rate and degree of aggressiveness than the preventive isolates from Mokolo and Koza Districts. The Dosa variety is the one that is resistant to Fusarium fungi, while the Spunta variety is not.

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