Original Research Article

ANTIFUNGAL ACTIVITY OF CERTAIN PLANTS FROM BENIN ON *Fusarium graminerarum* CEREALS PATHEGENE

Abstract

Ethanolic, Dichloromethane and aqueous extracts of some plants were investigated in vitro for antifungal activities against *Fusarium graminearum* using microdilution methods. The minimum inhibitory concentration (MIC) of *Ocimum gratissimum* essential oil is 2.5 µl/ml when *Cymbopogon citractus* and *Eucalyptus globulus* essential oils showed complete inhibition at 0.3 µl/ml. The ethanolic and dichloromethane extracts of different plant showed the relative growth about 77.54% for steam bark and 41,7% for root bark of *Ano geisus leiocarpus* 48,55% and *Momordica charantia* DCM extract showed inhibition about 59,675% at 800 µg/ml. *Anogeissus Leiocarpus steam* bark appears to be more effective as an antifungal agent.

Key words: *Anogeissus leiocarpus, Cymbopogon citractus*, , extracts, inhibition, antifungal

Introduction

Fusarium graminearum is a phytopathogenic Ascomycota that can cause Fusarium head blight of wheat and other cereals worldwide, Fusarium head blight is a disease that affects all straw cereals. It causes yield losses as well as reduced grain quality, which cause the most problems for the cereal crop (Nadia Ponts, 2015). These losses are evaluated at about 30 and 70% for the case of wheat (source Arvalis, 2017b) contamination by various fungal mycotoxins, including deoxynivalenol (DON) and zearalenone, which are harmful to humans and animals. pose a significant threat to human and animal health (Fen Yang et al, 2013). the presence of this mycotoxin in food and feed derived from cereals poses a serious threat to public health (Wu et al., 2014) and is the cause of more than 17 million deaths per year worldwide and more than half occur on the African continent (Bourgeois, 2016). There are about 17 species of Fusarium are associated with the disease and *Fusarium graminearum* the one that causes the most damage (Pasquali et al., 2016).

Currently, some effective measures including crop rotation, selection of resistant wheat lines, application of fungicides and biological control agents have been implemented to control mycotoxin contamination in grain production (Wegulo et al., 2015). However, synthetic fungicides are not economical for long-term use, in addition to causing a range of

adverse environmental effects (Schoneberg et al., 2015). The use of plant extracts, as biopesticides as an alternative to synthetic fungicides has recently been explored as a solution (Tian et al., 2016).

The present study consisted in evaluating the in vitro antifungal activity of ethanolic, dichloromethane and essential oil extracts of some plants from Benin on the growth of *Fusarium graminearum*, a pathogen of cereal crops.

Materials and Methods

In the framework of this study, an ethnobotanical survey on the plants used in the treatment of fungal and bacterial diseases in the Republic of Benin by traditional practitioners. The method used is that of the questionnaire which allows to apprehend the informations on the plants and the affections. The approach used is the semi-direct interview. The identification of plant material was done in the field using the Analytical Flora of Benin (Akoègninou et al., 2006) and a verification using the Herbarium of the University of Abomey-Calavi (UAC) Benin. The frequency of use of the medicinal plants identified was determined. The documentary research allowed to have a better knowledge of the listed plants.

Choice of localities, characteristics, setting and study population

The study was carried out in several departments of Benin, the areas were chosen according to accessibility, the size of the agricultural population, the number of traditional practitioners. 11 localities were selected.

Survey instruments

We interviewed traditional therapists and farmers. We conducted individual interviews with those who agreed to answer our questions using an interview guide. In each locality the study population was interviewed by the survey team composed of the researcher and a translator when necessary.

Sampling Technique

Our study sample consisted of 75 individuals. Before going to meet with certain traditional therapists in Alibori, Donga, Atacora and Borgou, a symbolic contribution of kola nuts was prepared and given to the interviewee. The interview was conducted in such a way as to obtain information on the ailments treated, the knowledge of the plants, the method of identification, the methods of preparation, the methods of administration, the frequency of administration and the duration of treatment. The name of the practitioner, the age, the

number of patients treated on average per month, the number of years of practice were also taken into account.

Phytopathogenic fungus

Our study focused on the phytopathogenic fungus *Fusarium graminearum*. Before each series of experiments, the microorganisms undergo rejuvenation on PDA medium. Incubation was done at 25 °C ± 1 and in the dark for five days. The antifungal activity of the natural extracts was tested in vitro on PDB medium. The culture medium alone is used as a control. Each test was repeated five times.

Preparation of extracts

The plants used were harvested in the Republic of Benin. The bark of the trunk, root bark and leaves of the plants were collected and dried. The dried organs were then ground to a fine powder using an electronic grinder. The powders were packed in jars. The preparation of plant extracts was done by three methods: maceration with absolute ethanol, dichloromethane and hydrodistillation**.**

Preparation of ethanolic extracts and Dichloromethane: Ten grams (10 g) of powder of each plant was macerated in 100 ml of 96% alcohol or dichloromethane for 24 hours. The preparation was filtered with N1 wattman paper; and the macerate was evaporated.

Preparation of essential oils: The extraction of essential oils (E.O) was done by hydro-distillation. We used 10 Kilograms (Kg) of fresh plant material.

Culture of fungi

Culture of fungi on Potatose Dextrose Agar (PDA)

The fungal culture was done on Potatose Dextrose Agar (PDA) medium prepared with 24 g potato dextrose (Sigma-Aldrich), 15 g of Aga agar (ROTH®), 1 liter of distilled water. The resulting broth was autoclaved for 30 minutes at 121°C and poured into Petri dishes (Greiner bio-one®). They are then inoculated by placing in the center of the Petri dish a disk of about 5 mm diameter of the actively growing fungus (4 to 6 days old) and incubated at 23-25°C in the dark. Agar plates prepared without fungal culture were stored at ***4°C.***

Preparation of conidia

For the conidiation a medium MBB (mung bean broth) was used to prepare it, 1 liter of distilled water was boiled, then 40 g of mung beans (organic) were added and let soak the mung beans for 15 minutes, filter the water and autoclave for 30 minutes and finally let cool and keep at 4 °C. The obtained medium (05) five agar discs (of an actively growing fungal

culture (4-6 days old) are added to 50 ml of MBB medium (300 ml bottle) and incubated on a shaker at 115 rpm and 25 °C in the dark. After 3 to 5 days, the MBB medium with the formed conidia was filtered (3 layers of Mirachloth®) and centrifuged at 4000 rpm for 10 min at room temperature. The supernatant was discarded and the conidia in the pellet were counted. For cell counting, conidia were diluted with 0.3% Tween 80 (Sigma Aldrich®) (1:10, 1:100) and counted in the Neubauer® counting chamber with quadruple determination. The number of conidia was calculated according to the following formula.

Number of conidia per ml = conidia counted* 〖10〗 ^ 4 * dilution.

Microdilution assay

For the determination of the minimum inhibitory concentration microtiter plates (96 wells) and PDB medium were used. Each well was filled with 200 µl. For the control medium, 200 µl PDB were pipetted into the wells and for the positive control 100 µl PDB and 100 µl conidial suspension containing 10^3 conidia per ml PDB. The rest of the wells were filled each up to 100 µl of conidia suspension and 100 µl of prediluted extract in PDB at different concentrations according to the double concentration method. Some wells are filled with 100 µl of prediluted extract were mixed with 100 µl of PDB. These correspond to the background of the absorbance measurement and were included in the evaluation as a correction factor. The microtiter plates were sealed and incubated in the dark at room temperature. According to the growth curve, the absorbance was then measured at 48, 74 and 96 hours and at 620 nm using the microplate reader; Epoch2 BioTek.

Results and discussions

Table 1: Results of the survey in relation to the number of localities per department, the number of people surveyed and distance from the economic capital of Benin

Department	Locality	Number of persons surveyed	Distance from Cotonou
BORGOU	Parakou	08	419 Km
	N'Dali	06	480 Km
	Pérédé	12	518 Km
	Tchaourou	06	364 km
Alibori	Banikoara	05	701 Km
	Goumori	11	721 Km
	Batanan	07	735Km
	Arbonga	04	704 Km
Atacora	Natitingou	08	541 Km

	Boukoumbé	06	590 Km
Donga	Djougou.	02	464 Km

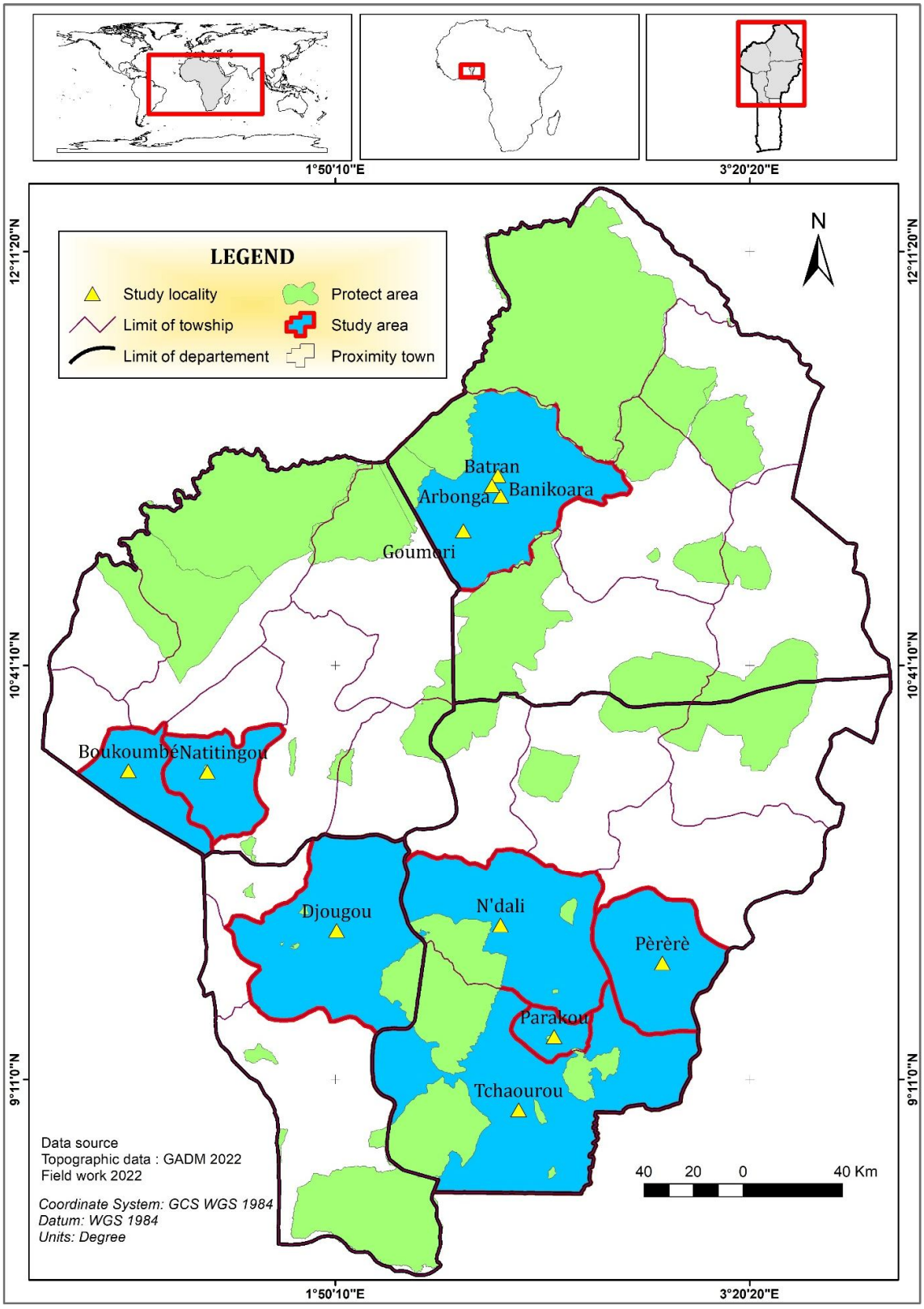

Fig 1: Map of the study area

Table 2: List of plants identified and frequency of use

Plantes	Parties utilisées	Indications	Fréquences
Aerva lanata	Whole plant	Antimicrobial, hepatoprotective, nephroprotective, stranguria, anti inflammatory, hypoglycemic, antidiabetic	2,30%
Anogeissus leiocarpus	Steam bark	Aphrodisiac, fungicide, antibacterial, anti-inflammatory	6,50%
Annona senegalensis	Root	Antifungal, analgesic	2,80%
Bridelia ferrurginea	leaves, Steam bark and root	Antidiabetic, antibacterial, anti-inflammatory, antifungal	1,80%
Cassia alata	leaves	Antibacterial, fungicidal, analgesic, anti-inflammatory, hypoglycemic	2,65%
Clerodendrum capitatum	leaves	Antibacterial, analgesic,	6,41%
Crateva adansonii	leaves; steam	Anti-inflammatory, antimicrobial, antioxidant and anticancer	1,20%
Crossopteryx febrifuga	Bark, steam , branch, trunk	anti-inflammatory, antipyretic, anti-plasmodial, analgesic, antibacterial	2,83%
Curcuma longa	Rhizome	Anti-inflammatory, anti-oxidant, antineoplastic, antiviral, antibacterial, antifungal, antidiabetic, anticoagulant, anti-fertility,	0,50%
Flueggea virosa	Root	Anti-inflammatory, urinary and venereal diseases, antiviral, antibacterial, antifungal, sterility, aphrodisiac, respiratory infections, analgesic,	5,50%
Hyptis suaveolens	Leaves	Anti-plasmodium, antifungal, antibacterial, anticonvulsant	3,50%
Jatropha multifida	Leaves	Anti-infectious, anti-oxidant, antimicrobial, anti-inflammatory, healing, purgative.	2,10%

Kalanchoe crenata	Leaves	Analgesic, anti convulsant, antimicrobial	0,50%
Khaya senegalensis	Steam bark	Anti-inflammatory, anti-hyperglycemic, antibacterial, antimalarial	3,50%
Kigelia africana	Steam bark	Fungicide, antibacterial, analgesic; tonic	2,20%
Lannea barteri	Steam bark, leaves	Antimicrobial, anticholinestrase, anticonvulsant, antioxidant, anti-inflammatory and anticancer	1,20%
Lantana camara	leaves	insecticide, antibacterial, herbicide,	3,50%
Lippia multiflora	leaves	Antiparasitic, antibacterial, insecticide,	3,10%
Melia azedarach	leaves	Insecticide, antibacterial, antiviral, fungicide, antioxidant	0,60%
Mitracarpus scaber	leaves	Anti-oxidant, fungicide, anti-inflammatory,	2,20%
Guiera senegalensis	leaves	Antibacterial, anticancer, antioxidant, fungicide,	1,20%
Momordica charantia	Whole fresh and dried fruit, seed	worming, eczema, galactagogue, gout, jaundice, abdominal pain, kidney, laxative, leprosy, leucorrhea, hemorrhoids, pneumonia, psoriasis, purgative, rheumatism, fever, scabies, diabetes antibacterial and antiviral	6,10%
Monodora myristica	Seeds	Anti-oxidant, antibacterial, anti-inflammatory	0,20%
Nymphaea lotus	Leaves	anti-inflammatory, antimicrobial, fungicide, anxiolytic, antidepressant,	1,10%
Occimum basilicum	Whole plant	Analgesic, anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, immunomodulatory and antivenom activities	2,12%
Occimum gratissimum	leaves	insecticide, antibacterial, fungicide, cold treatment	7,20%
Pavetta corymbosa	Aerial parts	Antibacterial, antimalarial,	1,30%
Pericopsis laxiflora	Steam bark	Anti-oxidant, fungicide,	0,45%
Piliostigma	Leaves	Treatment of wounds coughs fever and	3,85%

thonningii		various ulcerations; Antibacterial, antifungal.	
Pseudocedrela kotschyi	Steam bark and root	Antimicrobial, antipyretic, antiplasmodial, antioxidant, antifungal	2,64%
Pteleopsis suberosa	Leaves, steam bark	Antimicrobial, anticancer, antiulcer, anti-inflammatory, anti-oxidant, fungicide.	2,10%
Rytigynia canthioides	Leaves	Antiplasmodial, anti-inflammatory, antimicrobial	1,50%
Sansevieria liberica	Root, leaves	Anti-inflammatory, anticancer, antimicrobial, anti-snake venom, antiplasmodial, antiviral	1,20%
Sclerocarya bierra	Leaves, steam bark, steam	antibacterial, hypoglycemic, fungicidal, anti-inflammatory, analgesic	3,20%
Securinega virosa	Leaves, steam bark, root bark	Anti-inflammatory and analgesic, antidiarrheal, antidiuretic	0,68%
Terminalia glaucensceus	Bark of young shoots, leaves	Antibacterial, antiulcer, vermifuge and purgative; Antifungal	5,20%
Thapsia transtagana	Roots	Tonic, against female sterility	1,50%
Uvaria chamae	leaves	Analgesic, stomach cramps, edema, antianemic, febrifuge, wound healing; antifungal, antibacterial.	2,24%
Xylopia aethiopica	Roots, Leaves, and Epicarp	Analgesic, antibacterial, antifungal, antiviral	1,33%

Evaluation of antifungal activity

For the evaluation of the antifungal activity, plants were selected on the basis of the ethnobotanical study to be tested on the different fungal strains.

Table 3: List of plants tested with extraction yield

Plant's name	Family	Parts	Extraction	Yield
Terminalia glauscenceus	Combretaceae	Steam Bark	EthOH	12,0
			DCM	2,3
Lannea acida	Anacardiaceae	Seed	DCM	2,3
			EthOH	1,83

Mucuna sloanei	Fabaceae	Seed	EthOH	2,45
Momordica charantia	Cucurbitaceae	Whole plant	EthOH	2,1
			DCM	2,9
		seed	EthOH	21,2
			DCM	11,8
		Nut	EthOH	14,8
			DCM	34,6
Anogeisus leiocarpus	Combretaceae	Steam Bark	EthOH	8,7
			Dcm	0,4
		Root bark	Ethoh	19,9
			Dcm	0,2
		Leave	Ethoh	3,7
			Dcm	1,4
Hyptis suavelensis	Lamiaceae	Leave	Ethoh	4,5
			Dcm	3,5
Terminalia aviennioides	Combretaceae	Root bark	Ethoh	11,7
			Dcm	0,3
Cymbopogon citractus	Poaceae	leave	Essential oil	1,3
Ocimum gratissimum	Lamiaceae	Leave	Essential oil	1,38
Eucalyptus globulus	Myrtaceae	leave	Essential oil	0,65
Monodora myristica	Annonaceae	Seed	Ethoh	26,2
			Dcm	31,1
Ocimum Basilicum	Lamiaceae	Leave	Ethoh	3,4
			Dcm	3,2

Minimum Inhibitory Concentration of plants extracts

Essential oils

Three different essential oils were tested on *Fusarium graminearum* to evaluate their antifungal activity

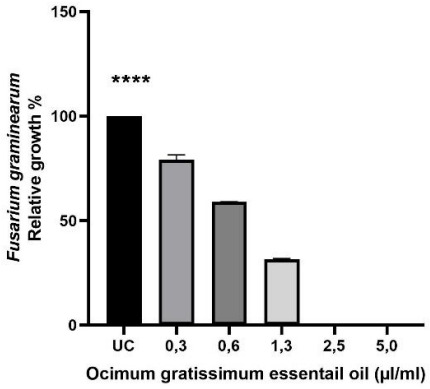

Figure 2: Influence of *Ocimum gratissimum* essential oil on *Fusarium graminearum* mycelium growth

The relative growth were determine using microdilution in 96 wells plates essential oil was applied from 0.3µl/ml to 5µl/ml. Data are presented as ±SEM, ****P<0.0001vs control, n=7. The reading was taking 72H after extracts application, the MIC is 2.5µl/ml.

Figure 3: Influence of *Cymbopogon citractus* (B) and *Eucalyptus globulus* (C) essential oils on *Fusarium graminearum* mycelium growth. (A) control

There was complete inhibition in all wells at all concentrations tested (0.3 to 5µl/ml) as shown in the different images above. In comparison to the control we notice that the conidia did not undergo any changes.

The different results obtained from the in vitro study of the essential oils of *Ocimum gratissimum, Cymbopogon citractus* and *Eucalyptus globulus* prouve that they have a great potential in the development of new type of fungicide as an alternative to the synthetic ones (Nikos G.Tzortzakis et al, 2007). The essential oils have demonstrated an antimicobial activity, their wide spectrum of actions on the fungi has already been evidenced (Semdé Z et al, 2018). The essential oil of *Eucalyptus globulus* in gas phase showed activity against rot before and after harvest which is confirmed by our results on the studied germ (Tyagi AK, 2011). Some essential oils showed activity against *Fusarium graminearum* with a MIC= 200µl/ml which is largely above the results we obtained which is 2.5µl/ml at the maximum found in the essential oil of *Ocimum graminearum* (Amini M, 2012).

Ethanolic and dichloromethane extracts

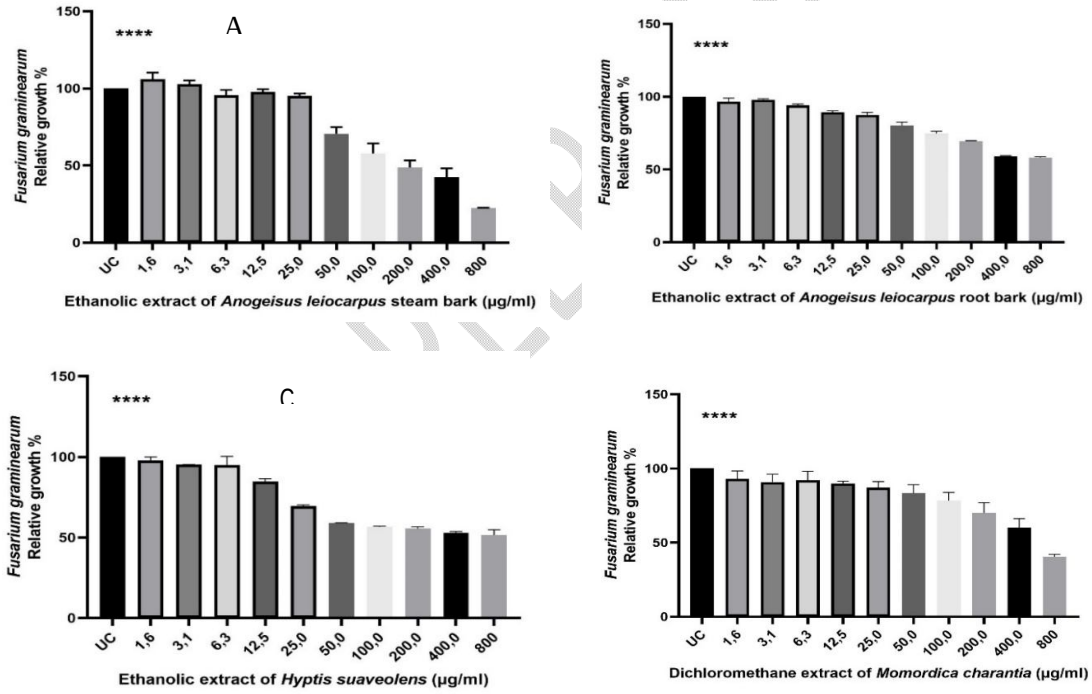

Figure 4: Influence of *Anogeisus leiocarpus* steam bark (A); *Anogeisus leiocarpus* root bark (B), *Hyptis Suaveolens* (C) ethanolic extracts and *Momordica charantia* DCM extract (D) on *Fusarium graminearum* mycelium growth

The relative growth were determine using microdilution in 96 wells plates ethanolic and dichloromethane extracts was applied from 1.6µg/ml to 800µg/ml. Data are presented as ±SEM, ****P<0.0001vs control, n=6. The reading was taking 72H after extracts application, the inhibition was about 77.54% for steam bark and 41,7% for root bark for *Anogeisus leiocarpus*. *Hyptis suaveolens* ethanolic extract showed inhibition about 48,55% and

Momordica charantia DCM extract showed inhibition about 59,675% at 800µg/ml on *Fusarium graminearum*.
The ethanolic extract of the trunk bark and root bark of *Anogeissus leicarpus* and the ethanolic extract of *Hyptis suaveolens* are more effective than the dichloromethane extracts. For *Momordica charantia* we notice an inhibition of the order of 33.9% for the ethanolic extract which is lower than that shown by the DCM extract which is more effective on the germ. All these extracts having MIC > 625µg/ml they have a low activity (Kuete V. 2010). As for EthOH and DCM extracts of *Anogeisus leiocarpus* leaves, they did not show inhibition on *F. graminearum.*

Conclusion

Among the plant extracts used, the essential oils of *Cymbopogon citractus*, *Eucalyptus globulus* and *Ocimum gratissimum* showed strong antifungal activity against *F. graminearum*. Ethanolic and dichloromethane extracts of *Momordica charantia, Hyptis suaveolens and Anogeisus leiocarpus* showed different percentage inhibition on mycelial growth of *F. graminearum*. The other plant extracts did not show any efficacy on the studied fungus. The essential oils of *Cymbopogon citractus* and *Eucalyptus globulus* showed the best antifungal activity. They showed a total inhibition up to the minimal concentration of 0.3µl/ml tested and the essential oil of *Ocimum gratissimum* had a MIC= 2.5µl/ml for *F. graminearum*.

Finally, it would be interesting to verify the effect of these different extracts in vivo for the formulation of a fungicide capable of reducing pre- and post-harvest losses in cereal crops. This study allows us to say that the Beninese flora has good candidates for the development of biopesticides

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