



Antifungal Activity of Certain Plants of Benin on *Fusarium graminearum* Cereals Pathogene

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Phytopathogenic fungi decrease crop yield and quality and cause crop damage. The present work aims to study the effectiveness of certain extracts of medicinal and food plants (EtOH, DCM) that are present in the Republic of Benin on the mycelial growth of toxigenic molds responsible for the degradation of cereals.

Ethanol, dichloromethane and aqueous extracts were studied in vitro for their antifungal activities against *Fusarium graminearum* using microdilution methods. The results show that the minimum inhibitory concentration (MIC) of *Ocimum gratissimum* essential oil is 2.5µl/ml. Regarding the essential oils of *Cymbopogon citractus* and *Eucalyptus globulus* they have a complete inhibition at 0.3µl / ml which is the most concentration tested.

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Ethanol and dichloromethane extracts from different plants showed relative growth of about 77.54% for trunk bark and 41.7% for root bark of *Anogeissus leiocarpus*. The DCM extract of *Momordica charantia* showed an inhibition of approximately 59.675% at 800µg/ml. The essential oils *Cymbopogon citractus*, *Eucalyptus globulus*, *Ocimum gratissimum* and trunk bark of *Anogeissus leiocarpus* show promise as an antifungal agent.

Keywords: *Anogeissus leiocarpus*; *Cymbopogon citractus*; plant extracts; inhibition; antifungal.

1. INTRODUCTION

Fusarium graminearum is a plant pathogenic Ascomycota that can cause Fusarium head blight caused by Fusarium from wheat and other grains worldwide, Fusarium head blight is a disease that affects all straw cereals. It leads to yield losses and lower grain quality, which causes the most problems for grain production [1,2]. These losses are estimated at about 30 and 70% in the case of wheat contamination by various fungal mycotoxins, including deoxynivalenol (DON) and zearalenone, harmful to humans and animals. pose a significant threat to human and animal health [3]. the presence of this mycotoxin in cereal-derived food and feed poses a serious threat to public health [4, 5] and is the cause of more than 17 million deaths per year worldwide and more than half occur on the African continent [6]. About 17 *Fusarium* species are associated with the disease and *Fusarium graminearum* causes the most damage [7].

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 cereal production [8]. However, synthetic fungicides are not economical for long-term use, and cause a range of adverse environmental effects [9, 10]. The use of plant extracts, as biopesticides as an alternative to synthetic fungicides, has recently been explored as a solution [11, 12].

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

2. MATERIALS AND METHODS

2.1 Ethnobotanical Survey

In the Republic of Benin, as in most African countries, traditional medicine is the first line of defence and is integrated into the health system. According to the World Health Organization,

more than eighty percent of the population depends on traditional medicine, particularly because of the high cost of allopathic pharmaceuticals [13, 14, 15]. In order to obtain and document information on the traditional therapeutic uses of the plants under our study, an ethnomedical survey was conducted among traditional practitioners in February 2019 in northern Benin. The survey was conducted in several regions and, in total, seventy-five (75) Aboriginal traditional medicine practitioners were identified on the basis of recommendations from older community residents (leaders, community workers and representatives of rural associations) and interviewed using a pre-prepared questionnaire. All participants were informed of the survey and their written consent was obtained prior to the personal visits. The questionnaires were designed in French and addressed to traditional healers in their local dialect for those who did not speak French. The main questions focused on the general knowledge of plants with high antibiotic potency used for the treatment of mycoses and plants used in the management of pests and fungi that attack crops, including vernacular names and method of application.

The identification of plant material was made in the field using the Analytical Flora of Benin [16] and verified at the Herbarium of the University of Abomey-Calavi (UAC) Benin.

2.2 Selection of Locations, Characteristics, Environment 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 healers. For this work, eleven (11) localities were selected.

During May 2019. The samples were authenticated at Abomey Calavi University (UAC), Benin. Voucher specimen (ID-number AB-05-2019) have been deposited at the Inter-Regional University of Industrial Engineering

Biotechnologies and Applied Sciences obtained from the Ministry of Agriculture and (IRGIB Africa University), Cotonou, Husbandry. Benin. A certificate for exportation was

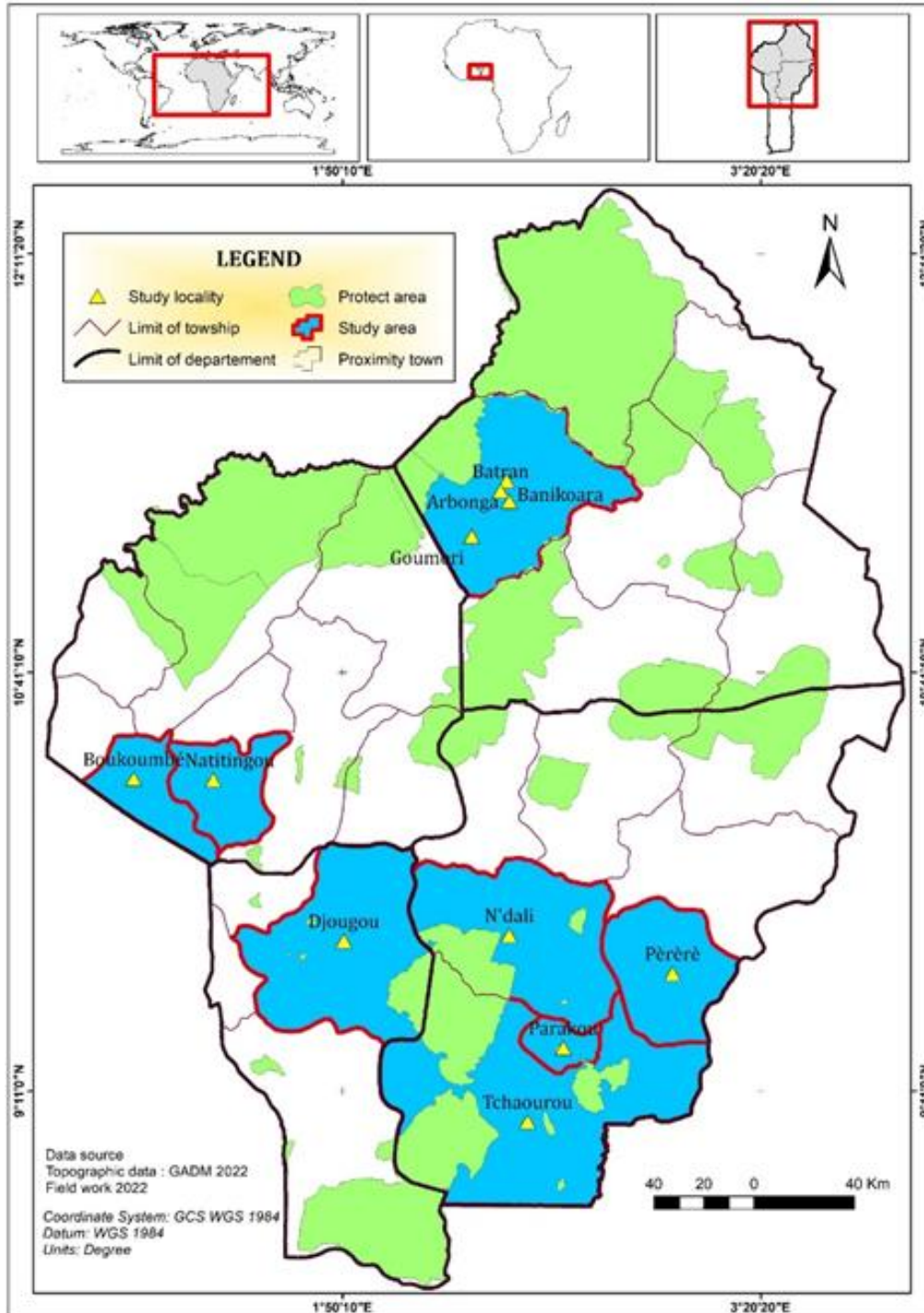


Fig. 1. Geographical description of the study area. Map of septentrional region showing the location of Boukoubé, djougou, N'dali, Batran, Arbonga, Goumori, Banikoara, Kérou, Pépère, Parakou, and Natitingou districts

2.3 Sampling Technique

Our study sample consisted of 75 people. Before going to meet some traditional healers in Alibori, Donga, Atacora and Borgou, a symbolic contribution of kola nuts was prepared and given to the interviewee. The interview was conducted to obtain information on the conditions treated, knowledge of plants, method of identification, methods of preparation, methods of administration, frequency of administration and duration of treatment. The name of the practitioner, age, the number of patients treated on average per month, the number of years of practice were also taken into account.

2.4 Preparation of Extracts

The plants used were harvested in the Republic of Benin in the area indicated (Fig. 1). The bark of the trunk, the bark of the roots and the leaves of the plants were collected and dried at a laboratory temperature of 25°C, protected from the sun. The dry specimens were then ground into powder using an electronic mill and packed in jars [17,18].

Preparation of ethanolic extracts and dichloromethane: Ten grams (10 g) of powdered substance from each part of the plant were macerated in 100 ml of ethanol (96 % V/V) or dichloromethane for 24 hours. The macerate was filtered with Whatman filter paper. The filtrate was concentrated under vacuum at 30 °C and stored at 4 °C until further use.

Preparation of essential oils: The extraction of essential oils (E.O) was carried out by hydro-distillation using the apparatus described by Sibel karaya et al, [19]. Distillation was carried out using a dry leaf/water ratio of 1:10 for 4 hours at least. The essential oil was collected in amber-colored vials and stored at 4°C for future use. The yield depends on the plants and the rest period [20].

2.5 Fungal Material

2.5.1 Culture of Fungi on Potatose Dextrose Agar (PDA) Petri Dish

Preparing PDA support:

24 g of potato dextrose (Sigma-Aldrich) were mixed with 15 g of Aga agar (ROTH) ® in 1 liter of distilled water. the medium was autoclaved for

30 minutes at 121 ° C and poured into Petri dishes (Greiner bio-one®)

Inoculation of Petri dishes:

Place an agar of an active growing fungus (4 to 6 days old) with a diameter of about 5 mm on a PDA plate. The fungal culture is done in the dark at 23-25°C and the agar plates prepared without fungal culture have been stored at 4°C.

Fusarium graminearum conidia preparation

Preparation of MBB Medium (Mung Bean Broth)

For the preparation of the MBB medium, distilled water was brought to a boil, then mung (organic) beans were added. They were soaked for 15 minutes before filtering the macerate which was Autoclaved the middle for 30 minutes at 121°C. After cooling it was stored at 4°C.

Inoculation of MBB Medium

For the production of conidia, (05) five agar discs (of an actively growing fungal culture (aged 4 to 6 days) are added to 50 ml of MBB medium (300 ml flask) and incubated on a stirrer at 115 rpm and 25 °C in the dark. After 3 to 5 days, the MBB medium with the formed conidia was filtered (3 Mirachloth® layers) and centrifuged at 4000 rpm for 10 midnight at room temperature. The supernatant was discarded and the conidia in the pellet was counted. For cell count, conidia were diluted with 0.3% Tween 80 (Sigma Aldrich®) (1:10, 1:100) and counted in the Neubauer® count chamber with quadruple determination. The number of conidia was calculated according to the following formula.

Number of conidia per ml = conidia counted* 10⁴
* dilution

2.6 Antifungal Activity

2.6.1 Microbial strains

Antimicrobial activity was evaluated using the Fusarium graminearum strain provided by the Fraunhofer Institute for Cell Therapy and Immunology, Antimicrobial Agents Unit (Leipzig, Germany).

2.6.2 Chemicals and reagents

Potato dextrose broth (PDB, Sigma-Aldrich), potato dextrose agar (PDA, Sigma-Aldrich) and

Agar (ROTH)[®] purchased and prepared and used according to the manufacturer's instructions. (DMSO; ≥ 99.7%), dimethylformamide (DMF) and Tween 80 were obtained from Sigma-Aldrich, Steinheim, Germany and Mung Bean Broth prepared in the laboratory.

2.6.3 Determination of the minimum inhibitory concentration (MIC)

The determination of MIC plant extracts on strains used in bioassays was determined by the broth microdilution method [17, 21, 22, 23]. One hundred millilitres (100 µ L) of liquid PDBs were transferred to the wells of a 96-well microdilution plate. For the control medium, 200 µl of PDB were pipetted into the wells and for the positive control 100 µl of PDB and 100 µl of conidial suspension containing 10³ conidia per ml of PDB. The remaining wells were each filled with up to 100 µl of conidia suspension and 100 µl of prediluted extract in PDB at different concentrations using the dual concentration method. Some wells are filled with 100 µl of

prediluted extract mixed with 100 µl of PDB. These correspond to the context of the absorbance measurement and were included in the assessment as a correction factor. The microtiter plates were sealed and incubated in the dark at room temperature. According to the growth curve, absorbance was then measured at 72 and 96 hours and at 620 nm using the microplate reader; Epoch2 BioTek.

2.7 Data Analysis

Data analysis was performed using GraphPad Prism version 9.3.1 for Windows (GraphPad Software, San Diego, California, USA). Data are expressed as an average ± SEM; n is the number of independent experiments/ different animals used. Statistical analysis was performed by unidirectional analysis of variance (ANOVA). The concentration-response curves were obtained by nonlinear least squares adjustment analysis, in which IC_x describes the extract concentration that induced x% inhibition.

3. RESULTS AND DISCUSSIONS

3.1 Ethnobotanical Survey

Table 1. Results of the survey in relation to the number of localities by department, the number of respondents and the distance from the economic capital of Benin

Department	Locality	Number of respondents	Distance from Cotonou
BORGOU	Parakou	08	419 km
	N'Dali	06	480 km
	Péréré	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

Table 2. List of medicinal plants and frequency of use

Plants	Parts used	Directions	Frequencies
<i>Aerva lanata</i>	Whole plant	Antimicrobial, hepatoprotective, nephroprotective, stranguria, anti-inflammatory, hypoglycemic, antidiabetic	2,30%
<i>Anogeissus leiocarpus</i>	Steam bark	Aphrodisiac, fungicidal, antibacterial, anti-inflammatory	6,50%
<i>Annona senegalensis</i>	Root	Antifungal, analgesic	2,80%

Plants	Parts used	Directions	Frequencies
<i>Bridelia ferruginea</i>	Steamed leaves, bark and root	Antidiabetic, antibacterial, anti-inflammatory, antifungal	1,80%
<i>Cassia alata</i>	leaves	Antibacterial, fungicidal, analgesic, anti-inflammatory, hypoglycemic	2,65%
<i>Clerodendrum capitatum</i>	leaves	Antibacterial, analgesic,	6,41%
<i>Crateva adansonii</i>	leaves; steam	Anti-inflammatory, antimicrobial, antioxidant and anticancer	1,20%
<i>Crossopteryx febrifuga</i>	Bark, steam, branch, trunk	anti-inflammatory, antipyretic, antiplasmodial, analgesic, antibacterial	2,83%
<i>Turmeric longa</i>	Rhizome	Anti-inflammatory, antioxidant, antineoplastic, antiviral, antibacterial, antifungal, antidiabetic, anticoagulant, anti-fertility,	0,50%
<i>Flueggea virosa</i>	Root	Anti-inflammatories, urinary and venereal diseases, antivirals, antibacterials, antifungals, sterility, aphrodisiacs, respiratory infections, analgesics,	5,50%
<i>Hyptis suaveolens</i>	Leaves	Anti-plasmodium, antifungal, antibacterial, anticonvulsant	3,50%
<i>Jatropha multifida</i>	Leaves	Anti-infective, antioxidant, antimicrobial, anti-inflammatory, healing, purgative.	2,10%
<i>Kalanchoe crenata</i>	Leaves	Analgesic, anticonvulsant, antimicrobial	0,50%
<i>Khaya senegalensis</i>	Steam bark	Anti-inflammatory, anti-hyperglycemic, antibacterial, antimalarial	3,50%
<i>African Kigelia</i>	Steam bark	Fungicidal, antibacterial, analgesic; tonic	2,20%
<i>Lannea barteri</i>	Steam bark, leaves	Antimicrobial, anticholinestrage, anticonvulsant, antioxidant, anti-inflammatory and anticancer	1,20%
<i>Lantana camara</i>	leaves	insecticide, antibacterial, herbicide,	3,50%
<i>Lippia multiflora</i>	leaves	Antiparasitic, antibacterial, insecticide,	3,10%
<i>Melia azedarach</i>	leaves	Insecticide, antibacterial, antiviral, fungicidal, antioxidant	0,60%
<i>Mitracarpus scaber</i>	leaves	Antioxidant, fungicidal, anti-inflammatory,	2,20%
<i>Guiera senegalensis</i>	leaves	Antibacterial, anticancer, antioxidant, fungicidal,	1,20%
<i>Momordica charantia</i>	Whole, fresh and dried fruit, seeds	deworming, eczema, galactagogue, gout, jaundice, abdominal pain, kidney, laxative, leprosy, leucorrhoea, hemorrhoids, pneumonia, psoriasis, purgative, rheumatism, fever, scabies, antibacterial and antiviral diabetes	6,10%
<i>Monodora myristica</i>	Seeds	Antioxidant, antibacterial, anti-inflammatory	0,20%
<i>Nymphaea lotus</i>	Leaves	anti-inflammatory, antimicrobial, fungicidal, anxiolytic, antidepressant,	1,10%
<i>Ocimum basilicum</i>	Whole plant	Analgesic, anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, immunomodulatory and antivenomous activities	2,12%
<i>Ocimum gratissimum</i>	leaves	insecticide, antibacterial, fungicide, cold treatment	7,20%
<i>Pavetta</i>	Aerial parts	Antibacterial, antimalarial,	1,30%

Plants	Parts used	Directions	Frequencies
<i>corymbose</i>			
<i>Pericopsis laxiflora</i>	Steam bark	Antioxidant, fungicide,	0,45%
<i>Piliostigma thonningii</i>	Leaves	Treatment of wounds cough fever and various ulcerations; Antibacterial, antifungal.	3,85%
<i>Pseudocedrela kotschyi</i>	Steamed bark and root	Antimicrobial, antipyretic, antiplasmodial, antioxidant, antifungal	2,64%
<i>Pteleopsis suberosa</i>	Leaves, steam bark	Antimicrobial, anticancer, antiulcer, anti-inflammatory, antioxidant, fungicidal.	2,10%
<i>Rytigynia canthioides</i>	Leaves	Antiplasmodial, anti-inflammatory, antimicrobial	1,50%
<i>Sansevieria liberica</i>	Root, leaves	Anti-inflammatory, anticancer, antimicrobial, snake venom, antiplasmodial, antiviral	1,20%
<i>Sclerocarya bierra</i>	Leaves, steam bark, steam	antibacterial, hypoglycemic, fungicidal, anti-inflammatory, analgesic	3,20%
<i>Securinega virosa</i>	Leaves, steam bark, root bark	Anti-inflammatory and analgesic, antidiarrheal, antidiuretic	0,68%
<i>Terminalia glaucensceus</i>	Bark of young shoots, leaves	Antibacterial, antiulcer, deworming and purgative; Antifungal	5,20%
<i>Thapsia transtagana</i>	Vegetable	Tonic, against female sterility	1,50%
<i>Uvaria chamae</i>	leaves	Analgesic, stomach cramps, edema, antianemic, febrifuge, wound healing; antifungal, antibacterial.	2,24%
<i>Xylopi aethiopica</i>	Roots, leaves and epicarp	Analgesic, antibacterial, antifungal, antiviral	1,33%

Table 3. List of plants tested with extraction yield [24]

Plant name	Family	Coins	Extraction	Yield
<i>Terminalia glauscenceus</i>	Combretaceae	Steam bark	EthOH	12,0
			DCM	2,3
<i>Lannea acida</i>	Anacardiaceae	Seeds	DCM	2,3
			EthOH	1,83
<i>Mucuna sloanei</i>	Fabaceae	Seeds	EthOH	2,45
<i>Momordica charantia</i>	Cucurbitaceae	Whole plant	EthOH	2,1
			DCM	2,9
		Seeds	EthOH	21,2
			DCM	11,8
		Nut	EthOH	14,8
			DCM	34,6
<i>Anogeisus leiocarpus</i>	Combretaceae	Steam bark	EthOH	8,7
			DCM	0,4
		Root bark	EthOH	19,9
			DCM	0,2
		Leave	EthOH	3,7
			DCM	1,4
<i>Hyptis suaveolens</i>	Lamiaceae	Leave	EthOH	4,5
			DCM	3,5
<i>Terminalia aviennioides</i>	Combretaceae	Root bark	EthOH	11,7
			DCM	0,3
<i>Cymbopogon citractus</i>	Poaceae	leave	Essential oil	1,3
<i>Ocimum gratissimum</i>	Lamiaceae	Leave	Essential oil	1,38
<i>Eucalyptus globulus</i>	Myrtaceae	leave	Essential oil	0,65

Plant name	Family	Coins	Extraction	Yield
<i>Monodora myristica</i>	Annonaceae	Seeds	EthOH DCM	26,2 31,1
<i>Ocimum Basil</i>	Lamiaceae	Leave	EthOH DCM	3,4 3,2

3.2 Extraction Yield

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

3.3 Minimum Inhibitory Concentration of Plant Extracts

3.3.1 Essential oils

The essential oils of *Ocimum gratissimum*, *Cymbopogon citractus* and *Eucalyptus globulus* were tested on *Fusarium graminearum* to evaluate their antifungal activity.

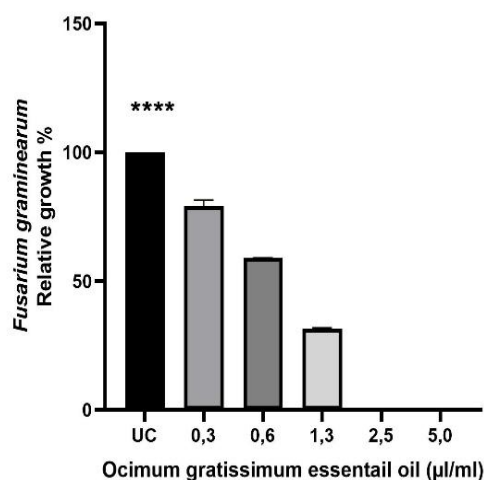


Fig. 2. Influence of *Ocimum gratissimum* essential oil on the growth of *Fusarium graminearum* mycelium

Relative growth was determined by microdilution in 96-well plates. essential oil was applied from 0.3 µl/ml to 5 µl/ml. Data are presented as ±SEM, ****P<0.0001vs control, n=7. The reading took 72 hours after the application of the extracts, the MIC is 2.5µl/ml.

There was complete inhibition at all concentrations tested (0.3 to 5µl/ml) as shown in the different images above. In relation to the control, we notice that the conidia have not undergone any change.

The various results obtained from the in vitro study of the essential oils of *Ocimum gratissimum*, *Cymbopogon citractus* and *Eucalyptus globulus* prove that they have great potential in the development of a new type of fungicide as an alternative to synthetic fungicides [25]. Essential oils have demonstrated antimicrobial activity, their broad spectrum of actions on fungi has already been highlighted [26]. *Eucalyptus globulus* essential oil in the gas phase showed anti-rot activity before and after harvest, which is confirmed by our results on the germ studied [27]. Some essential oils have shown activity against *Fusarium graminearum* with a MIC = 200µl/ml which is far superior to the results we obtained which is 2.5µl/ml maximum found in the essential oil of *Ocimum graminearum* [28].

3.4 Ethanolic Extracts and Dichloromethanes

Relative growth was determined using microdilution in 96-well plates, ethanolic and dichloromethane extracts were applied from 1.6 µg/ml to 800 µg/ml. Data are presented as ±SEM, ****P<0.0001vs control, n=6. The reading took 72 hours after the application of the extracts.

Inhibition is approximately 77.54% for barter bark and 41.7% for root bark for *Anogeisus leiocarpus* at a concentration of 800µg/ml. The ethanolic extract of *Hyptis suaveolens* showed an inhibition of approximately 48.55% and the extract of *Momordica charantia* DCM showed an inhibition of approximately 59.675% at 800µg/ml on *Fusarium graminearum*.

From these successes we can deduce that the ethanolic extracts of the bark of the trunk, the bark of the root of *Anogeisus leiocarpus* and the ethanolic extract of *Hyptis suaveolens* are more effective than extracts of dichloromethane. About *Momordica charantia* there is an inhibition of the order of 33.9% for the ethanolic extract which is lower than that shown by the DCM extract [29]. All these extracts with a MIC > 625µg/ml allow us to say that they have a low antifungal activity [30].



Fig. 3. Influence of essential oils of *Cymbopogon citractus* (B) and *Eucalyptus globulus* (C) on the growth of *Fusarium graminearum* mycelium. (A) Control

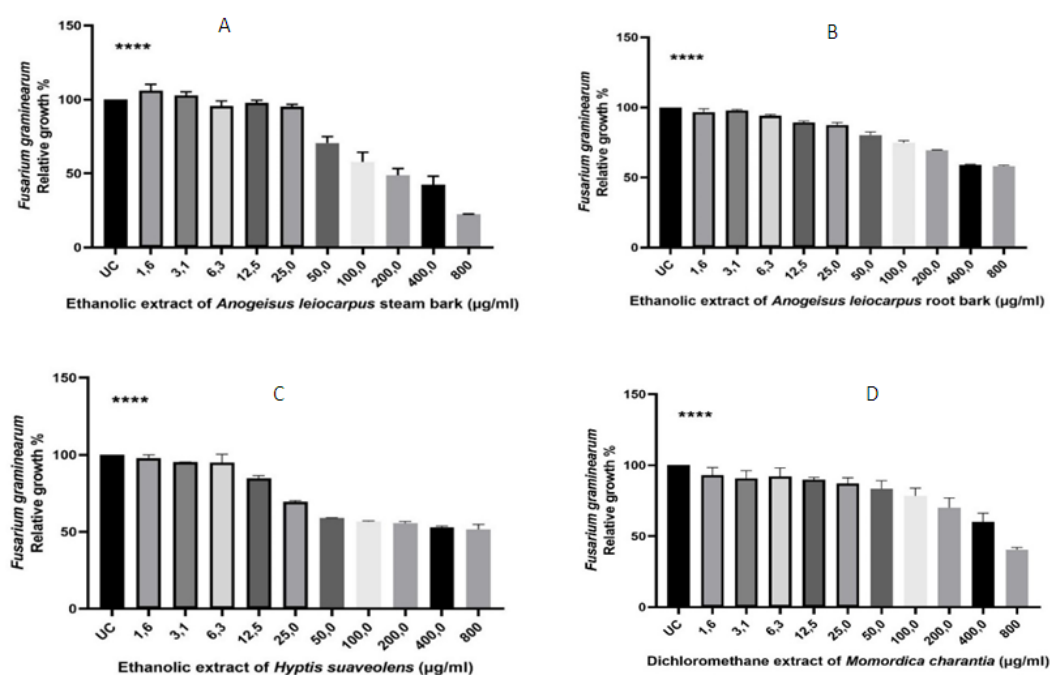


Fig. 4. Influence of the vapor bark of *Anogeisus leiocarpus* (A); Root bark of *Anogeisus leiocarpus* (B), ethanolic extracts of *Hyptis Suaveolens* (C) and extract of *Momordica charantia* DCM (D) on growth of *Fusarium graminearum* mycelium

4. CONCLUSION

For ecological and sustainable management of *Fusarium graminearum* wilt disease, the present study tested the antifungal activity of ethanol, dichloromethane and aqueous extracts of medicinal plants selected according to their availability in BENIN and their traditional use against fungal pathogens. Among these plants 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 a different percentage of

inhibition on mycelial growth of *F. graminearum*. Other plant extracts showed no efficacy on the fungus studied.

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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CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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