

## Original research article

### ANTIFUNGAL ACTIVITY OF CERTAIN PLANTS OF BENIN ON *Fusarium graminearum* PATHOGENE CEREALS

#### 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.

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

#### 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 (Nadia Bridges 2015). These losses are estimated at about 30 and 70% in the case of wheat contamination (source Arvalis, 2017b) by various fungal mycotoxins, including deoxynivalenol (DON) and zearalenone, 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 cereal-derived food and feed 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). About 17 Fusarium species are associated with the disease and *Fusarium graminearum* 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 cereal production (Wegulo et al., 2015). However, synthetic fungicides are not economical for long-term use, and cause 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 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.

## **Materials and methods**

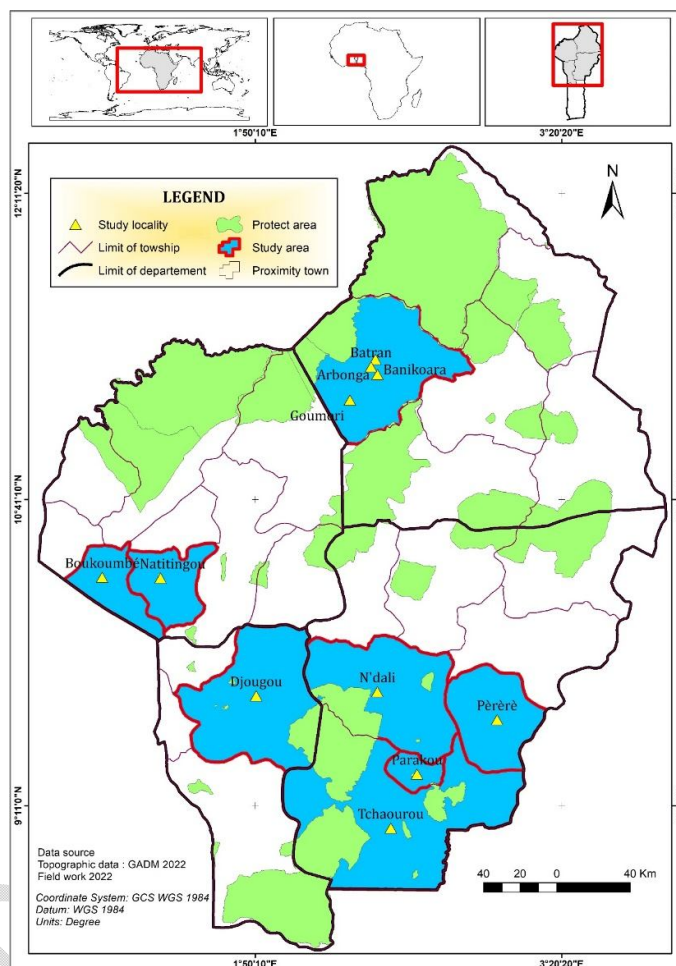
### **Ethnobotanical survey**

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 per cent of the population depends on traditional medicine, particularly because of the high cost of allopathic pharmaceuticals (World Health Organization, 2001). 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 (Akoègninou et al., 2006) and verified at the Herbarium of the University of Abomey-Calavi (UAC) Benin.

## 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.



**Figure 1.** Geographical description of the study area. Map of septentrional region showing the location of Boukoumbé, djougou, N'dali, Batran, Arbonga, Goumori, Banikoara, Kérou, Pèréré, Parakou, and Natitingou districts.

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

## **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.

## **Preparation of extracts**

The plants used were harvested in the Republic of Benin in the area indicated ( **Figure 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.

***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 Sedef nehir el et al, 2014. 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.

## **Fungal material**

### **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 macerated 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\* 10<sup>4</sup>\* dilution

### **Antifungal activity**

#### **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).

#### **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.

### **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 (R. Cleeland and E. Squires, 1991). 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 10<sup>3</sup> 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.

### 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<sub>x</sub> describes the extract concentration that induced x% inhibition.

## Results And Discussions

### 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
	Pérére	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
	Boukoubé	06	590 km

Donga	Djougou.	02	464 km
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**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%
<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, anticholinestrase, 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 corymbosa</i>	Aerial parts	Antibacterial, antimalarial,	1,30%
<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>Xylopia aethiopica</i>	Roots, leaves and epicarp	Analgesic, antibacterial, antifungal, antiviral	1,33%

### Extraction yield

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

**Table 3: List of plants tested with extraction yield**

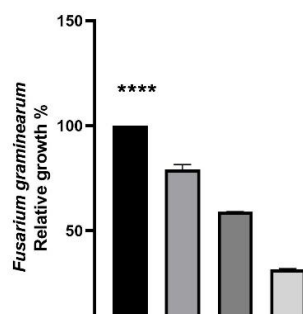
Plant name	Family	Coins	Extraction	Yield
<i>Terminalia glaucensceus</i>	Combretaceae	Steam bark	EthOH	12,0
			DCM	2,3
<i>Lannea acida</i>	Anacardiaceae	Seeds	DCM	2,3
			EthOH	1,83
<i>Mucuna sloanei</i>	Fabaceae	Seeds	EthOH	2,45
		Whole plant	EthOH	2,1

<i>Momordica charantia</i>	Cucurbitaceae	Seeds	DCM	2,9
			EthOH	21,2
		Nut	DCM	11,8
			EthOH	14,8
<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
<i>Monodora myristica</i>	Annonaceae	Seeds	EthOH	26,2
			DCM	31,1
<i>Ocimum Basil</i>	Lamiaceae	Leave	EthOH	3,4
			DCM	3,2

## Minimum inhibitory concentration of plant extracts

### Essential oils

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



**Figure 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  $\mu\text{l/ml}$  to 5  $\mu\text{l/ml}$ . Data are presented as  $\pm\text{SEM}$ , \*\*\*\* $P < 0.0001$  vs control,  $n=7$ . The reading took 72 hours after the application of the extracts, the MIC is 2.5 $\mu\text{l/ml}$ .

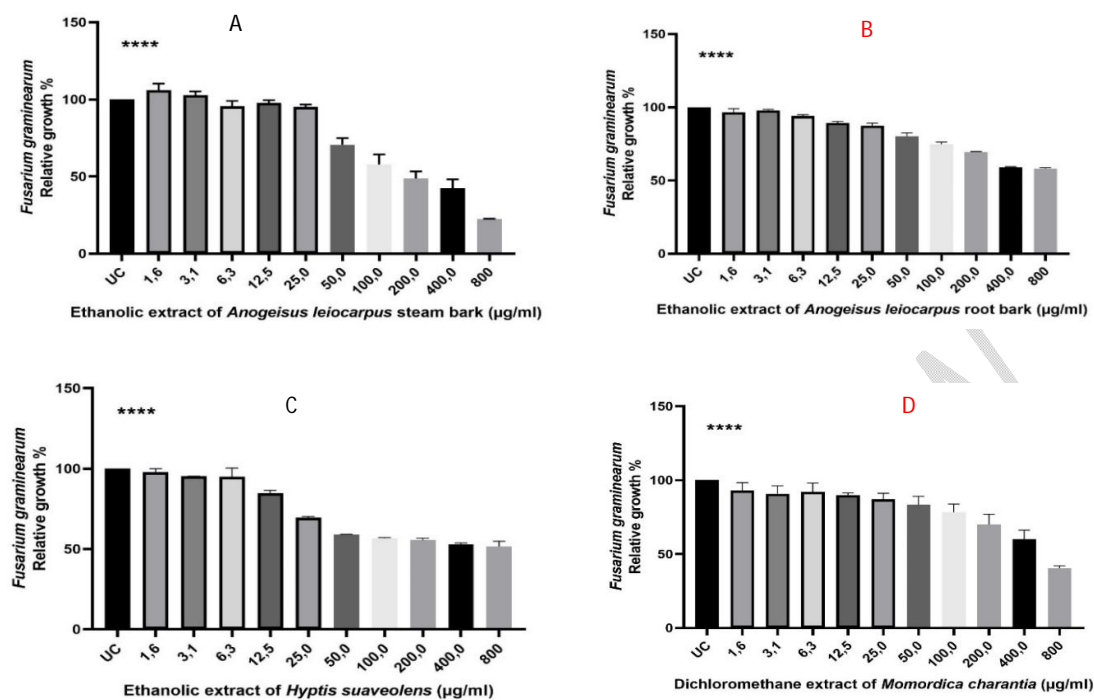


**Figure 3:** Influence of essential oils of *Cymbopogon citractus* (B) and *Eucalyptus globulus* (C) on the growth of *Fusarium graminearum* mycelium . (A) Control

There was complete inhibition at all concentrations tested (0.3 to 5 $\mu\text{l/ml}$ ) 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 (Nikos G. Tzortzakis et al, 2007). Essential oils have demonstrated anti-microbial activity, their broad spectrum of actions on fungi has already been highlighted (Semd  Z et al, 2018). *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 (Tyagi AK, 2011). Some essential oils have shown activity against *Fusarium graminearum* with a MIC = 200 $\mu\text{l/ml}$  which is far superior to the results we obtained which is 2.5 $\mu\text{l/ml}$  maximum found in the essential oil of *Ocimum graminearum* (Amini M, 2012).

## Ethanolic extracts and dichloromethanes



**Figure 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

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 *Anogeissus 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 *Anogeissus 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. All these extracts with a MIC > 625µg/ml allow us to say that they have a low antifungal activity (Kuethe V. 2010).

## **Conclusion**

For ecological and sustainable management of fusarium fusarium wilt disease, the present study tested the antifungal activity of ethanol, dichloromethane and aqueous extracts of medicinal plants selected according to their availability at BENIN and their traditional use against fungal pathogens. Among these 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 *Anogeissus 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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## **Declaration of competing interests**

The authors state that there is no conflict of interest

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## **Consent**

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

## **Reference**

Pasquali M, Beyer M, Logrieco A, Audenaert K, Balmas V, Basler R, Boutigny A-L, Chrpová J, Czembor E, Gagkaeva T, González-Jaén MT, Hofgaard IS, Köycü ND, Hoffmann L, Lević J, Marin P, Miedaner T, Migheli Q, Moretti A, Müller MEH, Munaut F, Parikka P, Pallez-Barthel M, Piec J, Scauflaire J, Scherm B, Stanković S, Thrane U, Uhlig S, Vanheule A, Yli-Mattila T, Vogelgsang S (2016). A European database on trichothecene genotypes of *Fusarium graminearum* and *F. culmorum*. *Forehead. Microbiol.* 7:406.

Fen Yang,<sup>1</sup> Susanne Jacobsen, Hans J. L. Jørgensen,<sup>1</sup> David B. Collinge,<sup>1</sup> Birte Svensson and Christine Finnie; *Fusarium graminearum* and its interactions with cereal heads: studies in the era of proteomics; *Plant Sci.*, February 28, 2013.

Arvalis-Institut du Végétal (2017a) "Choose & decide – Spring operations – Straw cereals – National synthesis 2016/2017", page 9. Excerpt from <https://www.arvalis-infos.fr/view-23265-arvarticle.html>.

Nadia Bridges. Mycotoxins are a component of the stress response system *Fusarium graminearum*, 2015.

Bourgeois A.L., Wierzba T.F., Walker R.I. (2016) Status of vaccine research and development for enterotoxigenic *Escherichia coli*, *Vaccine*, 34 (26): 2880-2886.

Tian, Y., Tan, Y., Liu, N., Liao, Y., Sun, C., Wang, S., Wu, A., 2016. Functional agents for biologically controlling deoxynivalenol contamination in cereal grains. *Forehead. Microbiol.* 7, 1–8. <https://doi.org/10.3389/fmicb.2016.00395>

Wegulo, S. N., Baenziger, P. S., Hernandez Nopsa, J., Bockus, W. W., and Hallen Adams, H. (2015). Control of ear blight caused by *Fusarium* of wheat and barley. *CropProtect.* 73, 100–107. doi: 10.1016/j.cropro.2015.02.025

Nikos G.Tzortzakis Costas D.Economakis, Antifungal activity of lemongrass essential oil (*Cymbopogon citratus* L.) against major post-harvest pathogens. Volume 8, Number 2, June 2007, pages 253-258.

Semdé Z, Koudou J, Figueredo G, Zongo C, Somda KM, Sawadogo/Lingani H, et al. Chemical composition, antioxidant and antimicrobial activities of *Lantana camara* Essential oil of Linn leaves from Burkina Faso. *CGC Biol Pharm Sci.* 2018;5(3):124-35.

Tyagi AK, Malik A. Antimicrobial potential and chemical composition of *Eucalyptus globulus* oil in liquid and vapor phase against food spoilage microorganisms. 2011;126(1):228-35.

Amini M, Safaie N, Salmani MJ, Shams-Bakhsh M. Antifungal activity of three essential oils of medicinal plants against certain phytopathogenic fungi. *Trakia J Sci.* 2012;10(1):1-8.

Kuete V. 2010. Potential of Cameroonian plants and derived products against microbial infections: an assessment. *Planta Med.*, 76: 1479-1491. DOI: <http://dx.doi.org/10.1371/journal.pone.0078085> Sep 09, 2014.

Adeleye, I.A., C.C. Onubogu, C.I. Ayolabi, A.O. Isawumi and M.E. Nshiogu, 2008. Screening of crude extracts of twelve medicinal plants and "wonder-cure" concoction used in Nigeria Unorthodox medicine for activity against *Mycobacterium tuberculosis* isolated from sputum of tuberculosis patients. *Afr. J. Biotech.*

Akande, J.A. and Hayashi, Y., 1998. Potency of the extract content of selected tropical chewing sticks against *Staphylococcus aureus* and *Staphylococcus auricularis*. *World Journal of Microbiology and Biotechnology*

Awor and Samseny R-R. Contribution to the phytochemical study of a plant traditionally used as a test poison in Gabon: *Strychnos Icaja* Baillon (Mbundu), Loganiaceae. Thesis, University of Bamako, Faculty of Medicine, Pharmacy and Odonto - Stomatology, Mali. 2003

AYEPOLA O.O; 2009. Evaluation of the Antimicrobial Activity of Root and Leaf Extracts of *Terminalia glaucescens*

Ayman Al-Mariri, and Mazen Safi, 2014, In vitro antibacterial activity of several plant extracts and oils against certain gram-negative bacteria

Azzi R. Contribution to the study of medicinal plants used in the traditional treatment of diabetes mellitus in western Algeria: ethnopharmacological investigation, pharmacotoxicological analysis of fig tree (*ficus carica*) and colocynth (*citrulluscolocynthis*) in the WISTAR rat. PhD thesis 2012.

Badiaga M. Study of the ethnobotanical, phytochemical and biological activities of *Nauclea latifolia* Smith, an African medicinal plant collected in Mali. Doctoral thesis 2011.

Bao, B.; Chen, Y.G.; Zhang, L.; Na, X.Y.; Wang, X.; Liu, J.; Qu, W. 2013. Correction: *Momordica charantia*, (bitter melon) reduces the infiltration of macrophages and mast cells associated with obesity as well as the expression of inflammatory cytokines in adipose tissue.

Basavraj Nagoba and Milind Davane, 2018, Natural immunomodulators. *iMed ad reviews.*

Batawila, K., Kokou, K., Koumaglo, K., Gbeassor, M., de Foucault, B., Bouchet, P. & Akpagana, K., 2005. Antifungal activities of five Combretaceae used in traditional Togolese medicine. *Fitoterapia*

Bentabet Lasгаа N. Phytochemical study and evaluation of the biological activities of two plants *Fredoliaaretioides* and *echiumvulgare* of western Algeria. Doctoral thesis 2015,

Berghioua A., Cheriti A. Belboukhari A. & Marouf A. Ethnopharmacological and chemical overview of Brassicaceae ah. 2009. Annals of Bechar University. #5

Boitard C, Revillard JP. Induce or restore immune tolerance? Therapeutic Medicine 1999; 5:115-22.

Chao CY, Sung PJ, Wang WH, Kuo YH, 2014. Anti-inflammatory effect of *Momordica charantia* in mouse sepsis. *Molecules*.

Chaturvedi, P.; George, S. 2010, *Momordica charantia* maintains normal glucose levels and lipid profiles and prevents oxidative stress in diabetic rats with chronic sucrose load. *J.Med. food*,

Che-Yi Chao, Ping-Jyun Sung, Wei-Hsien Wang and Yueh-Hsiung Kuo, 2014. Anti-inflammatory effect of *Momordica Charantia* in sepsis mice.

D. Sathish Kumar; 2010, A MEDICINAL POTENCY OF *MOMORDICA CHARANTIA*, *International Journal of Pharmaceutical Sciences Review and Research*.

Dohou N. Floristic, ethnobotanical, phytochemical approach and study of the biological activity of *thymelelythroids*. Doctoral thesis 2015,

Dzoyem J.P., Tangmouo J.G., Manfouo J.R., Lontsi D., Etoa F. X, Lohouc P.J. « Antifungique activity of extracts of some CAMEROUNIAN medicinal plants » *Nig. J.Nat. production and medical flight*. 10 2006

E. Attard and A. Cuschieri, 2009, In vitro immunomodulatory activity of various Maltese plant extracts of the Asteraceae family.

Esnouf C., Huyghe C., 2015, Socio-economic issues and impacts of agricultural and food losses.

Gbala, I.D. and Anibijuwon, I.I., 2016. antibacterial activity of *Terminalia glaucescens*, *mangifera indica* and *mitracarpus villosus* on carbapenem-resistant enterobacteriaceae

Goossens H, Guillemot D, Ferech M, Schlemmer B, Costers M, van Breda M, Baker LJ, Cars O, Davey PG (2006) National campaigns to improve antibiotic use. *Eur. J. Clin. Pharmacol*.

Grover, J.K.; Yadav, S.P. 2004, Pharmacological actions and potential uses of *Momordica charantia*: A review. *J. Ethnopharmacol*.

Ibiri PM. C.2005. Microbiological sanitation of air and ventilation systems using essential oils. Thesis. Swiss Federal Institute of Technology, EPFL. Lausanne, Switzerland.

Jean-louis Pousset, 1989, *Plantes médicinales africaines*, ellipse.

Karla K.A. Santos, Edinaldo F.F. Matias, Celestina E. Sobral-Souza, Saulo R. Tintino, Maria F.B. Morais-Braga, Glauca M.M. Guedes, Francisco A.V. Santos, Ana Carla A. Sousa,

Miriam Rolón, Celeste Vega, Antonieta Rojas de Arias, José G.M. Costa, Irwin R.A. Menezes & Henrique D.M. Coutinho, 2012, Trypanocide, cytotoxic, and antifungal activities of *Momordica charantia*.

Kumar D, Arya V, Kaur R, Bhat ZA, Gupta VK, et al., 2012, A review of immunomodulators in the Indian traditional health care system. *J Microbiol Immunol Infect.*

Liaw CC, Huang HC, Hsiao PC, Zhang LJ, Lin ZH, Hwang SY, Hsu FL, Kuo YH (2015). *Momordica charantia* 5 $\beta$ ,19-epoxycucurbitan triterpenoids and their anti-inflammatory and cytotoxic activity. *PlantaMed.*

Mada, S.B.; Garba, A.; Mohammed, H.A.; Muhammad, A.; Olagunju, A.; Muhammad, A.B. Antimicrobial activity and phytochemical screening of aqueous and ethanolic extracts of *Momordica charantia* leaves L. *J.Med. Plants Res.* 2013.

Mahmood, A. Isolation and characterization of antimicrobial activity conferring components from bitter pumpkin seeds (*Momordica charantia*). 2012.

Manabe, M.; Takenaka, R.; Nakasa, T.; Okinaka, O. 2003. Induction of anti-inflammatory responses by *Momordica charantia* L. food (bitter squash). *Biosci. Biotechnol. Biochemistry.*

Ming-Feng Lin et al, 2014, Minimal Inhibitory Concentration (MIC) Assay for *Acinetobacter baumannii*

Mishra, A.; Gautam, S.; Pal, S.; Mishra, A.; Rawat, A.K.; Maurya, R.; Srivastava, A.K., 2015. Effect of *Momordica charantia* fruits on streptozotocin-induced diabetes mellitus and its associated complications. *Int. J.Pharm. Pharma.*

Mukherjee, P.K., Chandra, J., Kuhn, D.M. and Ghannoum, M.A. (2003) "Mechanism of fluconazole resistance in *Candida albicans* biofilms: phase specific role of efflux pumps and membrane sterols". *Infection and Immunity* 71(8), 4333-4343.

Nesar Ahmad, 2016; *Momordica charantia*: for traditional uses and pharmacological actions; *Journal of Drug Delivery and Therapeutics.*

Neuwinger, H.D., 2000. *Traditional African Medicine: A Dictionary of the Use and Applications of Plants.* Medpharm Scientific, Stuttgart, Germany.

Normak HB, Normak S (2002) Evolution and spread of antibiotic resistance. *J. Intern. Medi.* 252: 91-106

Philip P. Gerbino. *The science and practice of pharmacy.* American Journal of Pharmaceutical Education 2006.

PROBST A, CARTRON G and WATIER H (2006). Mechanisms of action of recombinant monoclonal antibodies used in oncology. *Cancer Newsletter*, April Special Issue

Reyes, B.A.S.; Bautista, N.D.; Tanquilut, North Carolina; Anunciado, R.V.; Leung, A.B.; Sanchez, G.C.; Maeda, K.I. 2006. Antidiabetic potentials of *Momordica charantia* and *Andrographis paniculata* and their effects on oestrous cyclicity in alloxane-induced diabetic rats. *J. Ethnopharmacol.*

Saeed, S.; Tariq, P. Antibacterial activities of *Mentha piperita*, *Pisum sativum* and *Momordica charantia*. *Pack. J. Bot.* 2005.

Santos KKA, Matias EFF, Sobral-Souza CE, Tintino SR, Morais-Braga MFB, Guedes GMM, Santos FAV, 2012. Trypanocide, cytotoxic and antifungal activities of *Momordica charantia*. *Pharmaceutical. Biol.*

Savard, P.Y. (2003). Structural and dynamic characterization of TEM-1 beta-lactamase of *Escherichia coli* bacteria by liquid NMR, *Philosophiae Doctor of Biochemistry and Microbiology*, Faculty of Science and Engineering, Université Laval, Québec, 224 p.

Sheehan, D.J., Hitchcock, C.A. and Sibley, C.M. (1999) "Current and emerging azole antifungal agents". *Blinking. Microbiol.* 12(1), 40-79

Slimani Amina, Djaalane Bakhta, 2013. In vitro study of the antibacterial activity of *Citrus aurantium* essential oil against *Erwinia carotovora* and *Erwinia carotovora subsp. atropurpurea*, bachelor's thesis in biochemistry and natural substance, AbdAlhamid Ibn Badis Mostaganem University.

Texas, A. A., N. Guessennd, C. Baby, A.M. Kra, D. A. Otokore and M. and Dosso (2013). In vitro evaluation of the antibacterial activity of the aqueous extract of the trunk bark of *Harungana madagascariensis* on the growth of multidrug-resistant strains. *Bulletin de la Société Royale des Sciences de Liège.*

Wang, S.; Zheng, Y.; Xiang, F.; Li, S.; Yang, G.; 2016. Antifungal activity of *Momordica charantia*, seed extract to the pathogenic fungus *Fusarium solani* L. *J. Food Drug Anal.*

World Health Organization. *World Health Report 2007: A Safer Future: Global Public Health Security in the 21st Century.* Geneva: World Health Organization; 2007.

Yam M.F, Eng L.F, Ameer O.Z, Salman I.M, Aziz H.A, Asmawi M.Z. Anti-inflammatory and analgesic effects of ethanolic extract *Elephantopus scaber*. *Journal of acupuncture and meridian studies.* 2009.

Young, L.Y., Hull, C.M. and Heitman, J. (2003) "Distribution of ergosterol biosynthesis confers resistance to amphotericin B in *Candida lusitanae*". *Antimicrobe. Chemother Agents.*