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Research Article

Biological activity of *Callistemon viminalis* and *Chenopodium ambrosioides* ethanolic extract on banana Fusarium wilt in screen house conditions

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Abstract

Plantain (*Musa* spp.) is one of the most important food crops in Cameroon, however its production is threatened by many fungal diseases, such as banana Fusariumwilt a siol born fungal causing heavy losses.The objective of this work was to evaluate the biological activity of *Callistemon viminalis* and *Chenopodium ambrosioides* ethanolic extract against this disease in screen house. 42314Infected roots and leaves samples were collected from banana plantations in five localities of the West Region of Cameroon and brought to the laboratory for pathogen isolation and identification. Soil samples collected from the wilted banana rhizosphere were analysed to determine the edaphic factors influencing the growth of the pathogen. Two incoculation techniques were tested in the screen house prior to the pathogenicity test and the bioassay. All *F. oxysporum*isolates identified were pathogenic and the Foumbot isolate was more agressive. Clay-rich and slightly acidic soils favored the growth of *F. oxysporum*, while soils rich in organic matter and phosphorus inhibited its development. The drenching inoculation technique was more favorable for the inoculation of the parasite on banana seedlings. Ethanolic extracts of *C. viminalis* and *C.ambrosioides*, significantly reduced disease incidence and severity. Efficiency of *Callistemon* ethanolic extract (20 mL) was highly and significantly comparable to Eagrow Care (20 mL) in controlling banana Fusarium wilt. Ethanolic extracts of *C. viminalis*and to some extend *C.ambrosioides* could serve as a basis for a biofungicide formulation as alternative to chemical fungicides. Complementary molecular and ecological studies are essential to optimize disease management strategies

Keywords: *Banana plantain, Biocontrol, C.viminalis, Drenching inoculation technique ethanolic extract, Fusarium wilt.*

Introduction

Host plants of Fusarium wilt are many in Cameroon. In the highland and monomodal rain forest agro ecological zones, the most cultivated host plants are bananas, oil palm, tomato, pepper, potato, water melon and eggplant. Their economic values are high in Cameroon and are staple food with high nutritional values. These crops are export commodities. Soil-borne fungus, *Fusarium oxysporum* is the causal agent of

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Fusariumwilt which is a vascular disease that affects a large variety of economically important crops worldwide (Ortoneda *et al.*, 2004). The morphology of *Fusarium* species is notoriously difficult to identify. Even from one formaespecialis to another, the environment can readily alter *Fusarium* morphology, notably its conidiogenesis, especially when it comes to the composition of the growth medium (Donnell, 2000). As a result of its metabolism, a fungal culture's appearance is often controlled by pH in relation to the medium's nitrogen source, Kwaśna and Bateman (2005).

The most popular and widely disseminated fruit in the world, the banana (*Musa acuminata* L.) significantly improves food security in developing nations. Evidence of banana (including plantain) production dates back to 4000 BCE in New Guinea, making it one of the most significant and ancient human food crops (Denham *et al.,* 2004) with 55% of total exports, Cameroon's agricultural sector continues to lead the country's economy in terms of GDP contribution. It also serves as the primary employment and source of foreign exchange, employing 62% of the country's active population. Over 70% of the primary sector's GDP is made up of food crops, which are the primary driver of this increase (Nkapnang, 2011). Three agro-ecological zones in Cameroon are used for banana cultivation: the Western High Land, which includes the West and North-West Regions; the Humid Forest which has unimodal rainfalls covers the Littoral and South-West Regions; and the Humid Forest, which has bimodal rainfalls and covers the Centre, South, and East Regions of the country. Loum-Penja, Njoumbe, Kribi, Tiko, Mutengene, Ekona, Kumba, Foumbot, Bangante, Balatchi, Boyo, Manfe, Tubah, Bali, Widikum, and Batibo are Cameroon's principal production locations.

The production of bananas worldwide has decreased from 145 million tonnes in 2017 to 119 million tonnes in 2020 (FAO, 2017). According to current figures, Central Africa's food security will be questionable for the next 20 years. The productivity of local food crops, such as plantains, macabo, and cassava, has decreased. This could be due to a variety of stresses, including pest and disease attacks, as well as a decrease in fruit yield and quality by 26.5%.

The country's West Region is where banana Fusarium wilt is most prevalent. Smallholder farmers throughout the nation continue to produce very little bananas, with the exception of agro-industrial firms like *Plantation du Haut Penja* (PHP), which produce a lot of them, and research facilities like the African Centre for Research on Bananas and Plantains (CARBAP). Local consumption accounts for between 70 and 80 percent of banana production. Due to strong demand in local, regional, and global markets, this production which was formerly mostly for self-consumption and the growth of urban markets is increasing today.Despite efforts to revitalise the sector through the distribution of technologies that should have led to the intensification of this commodity, a number of studies demonstrate that plantain banana yields in Cameroon's traditional family farms (EFA) hardly ever surpass 4 to 10t.ha^{-1}.Conversely, on accessible and well-monitored routes, research stations with the same types can generate well over 30 t. ha^{-1} (Dépigny *et al.,* 2011; Tomekpe *et al.,* 2019). This is caused by a number of limitations, including the choice of varieties that are not well suited, a lack of technical farming expertise, and a cropping system that uses inadequate fertilisation and disease and pest management. Of these, Fusarium wilt, often known as panama disease, is extremely harmful to plantain farming. It lowers the yield and causes moderate to severe severities based on the production systems and agroclimatic zones. For disease management, producers typically apply synthetic fungicides to the soil. Nevertheless, there are disadvantages to the growing usage of these compounds, such as the emergence of pathogen strains resistant to the products, contamination of the environment, and toxicity to living things (Cissokho *et al.,* 2015; Massi *et al.,* 2021). Since these natural compounds are less harmful to both individuals and the environment, it appears intriguing to look for a chemical substitute utilising natural means in order to address these issues.

Materials and Methods

Collection, conservation and preparation of roots and soil samples

Root and soil samples from plants exhibiting Fusarium wilt symptoms were collected in February 2022 and 2023, respectively, from five locations in five divisions of the West Region of Cameroon: Banekane (Nde Division), Kekem (Haut-Nkam Division), Foumbot (Noun Division), Dschang (Menoua Division), and Balatchi (Bamboutos Division) for isolation and soil characterization. Root samples from Foumbot (Noun Division) of the West Region of Cameroon were collected for the *in vivo* test.The soil samples were taken from the rhizosphere of infected banana plants in the field and brought to the lab in plastic bags. Phytoextracts were made from the aerial portions of the leaves of the weeping bottle brush (*Callistemon viminalis*) and the

Mexican tea (*Chenopodium ambrosioides*) that were gathered from Dschang. Based on their demonstrated antibacterial capabilities against plant or animal diseases, these plant species were selected (Sousa *et al.,* 2012; Salem *et al.,* 2017).

Physical analysis, Particle size determination and chemical analysis

The soil were ground in a ceramic mortar to grind it into fine soil to ease particle size determination, allowed to air dry in a ventilated area, and then sieved through a 2 mm screen. In order to determine the moisture correction factors, portions of the samples were subsequently oven dried at 105 ^{o}C. For further analysis, the remaining less than 2 mm portion was gathered and homogenised. Part of it, was used to analyse routine parameters (organic carbon, total nitrogen, CEC, available phosphorus, etc.) at the Environmental and Analytical Chemistry Laboratory of the University of Dschang (Yerima and Van Ranst, 2005).

Particle size determination allows us to know the percentage of different textural classes (sand, silt, and clay), found in a < 2 mm mineral fraction of soil (Yerima and Van Ranst, 2005). It was determined by the Pipette method. Hydrogen peroxide (H_2O_2, 35% p/p) was added to the samples placed on a hot plate to eliminate organic cementing materials on a hot plate. This was followed by the addition of hydrochloric acid (HCl, 0.02 N) to destroy sesquioxides binding the colloidal fraction. The samples were then washed with distilled water. Sodium hexametaphosphate was added to the samples for dispersion. The clay, silt and sand fractions were separated using the Robinson–Köhn pipette. The time and depth of pipetting is determined by Stocks law. Textural classes were determined using the USDA textural triangle.

Preparation of culture media

The culture medium of Potatoe Dextrose Agar (PDA) was prepared from 200 g of peeled potatoes, cut and boiled in 500 mL distilled water for 20 minutes. After cooling and filtration, 15g of agar was then added, 20g of Dextrose and 1g of chloramphenicol to prevent the development of bacteria. After homogenization, the mixture was adjusted to 1000 mL with the distilled water in an Erlen-meyer flask of 1000 mL and sealed before being sterilized in the autoclave at a temperature of 121° C for about 15 min (Djeugap *et al*., 2013). After sterilization and cooling, a volume of 15mL of the medium was poured into Petri dishes.

Isolation and identification of the pathogen

The diseased samples (roots) were cleansed with tap water to get rid of any dirt or other visible particle.After that, they were sterilised in a 1% sodium hypochloride solution for 30 seconds and then rinsed three times in sterile distilled water to get rid of any remaining bleach residue. Samples were next sliced into tiny pieces and plated on potato dextrose agar (PDA) medium supplemented with ampiciline (20 mg/L) or chloramphenicol. Hyphae from diseased tissue were sub-cultured on fresh PDA and purified following three days of incubation at 25±2°C (Djeugap *et al.,* 2011). The occurrence (%) or frequency of isolation (FI) of each fungus was calculated using the following formula, FI = (NF÷NT) × 100, where NF is the total number of samples from which a particular fungus was isolated and NT is the total number of samples from which isolations were carried out (Djeugap *et al.,* 2017). Mycelium and spore morphological traits, including spore size and shape, the presence or absence of septa etc, were used for identification (Rossman *et al.*, 1987; Dugan, 2006).

Preparation of plant extracts

The leaves of *Callistemon viminalis* and *Chenopodium ambrosioides* were separately ground in an electric grinder after two weeks of air drying. Following that, 100 g of the finely powder were macerated in 1000 mL (corresponding at 0.1g/mL) of water or ethanol for 72 hours at room temperature. The solution was then filtered through the muslin cloth. While the ethanol extract was evaporated in a Buchner brand rotary evaporator at 67° C and then dried in an oven (brand Koeltenhiek-ElectriciteitCornelis) at 40°C, the aqueous extract was oven dried for seven days at 40°C (Djeugap *et al.*, 2017).

Deep wound inoculation technique

Inoculation by transferring healthy banana plantlets into spore suspensionof the fungal isolates (pre-planting inoculation). In deed,two months-old healthy banana seedlings were completely uprooted from the polythene pot, roots washed and root tips cut 1 cm and then dipped for two hours in 10 mL and 20 mL infested suspension of FO conidia (10^6 conidia/mL) [Figure 1A]. For control, two month old healthy banana seedlings were dipped in sterile water without FO. After inoculation, plant extracts test to control Fusarium wilt in the screen house was carried out by adding the same volume of extract into the beaker containing the inoculated seedlings. The plants were grown in the screen house at the laboratory of plant Pathology of FASA University

of Dschang Cameroon. Temperature during the trial was 23 ± 2 °C and plants were maintained under high relative humidity about 96% and a 12-h photoperiod. The experiment was conducted in a randomized complete design with five replicates per isolate. The remaining suspention after deep inoculation was collected in a syringe and applied at the base of the treated crops per treatment (Dita *et al*., 2011).

Drenching inoculation technique (post-planting inoculation)

Plants already established and meeting the requirements such as disease-free, raised under the same conditions, acclimatized for at least 60 days before inoculation, at the same physiological stage, ideally ranged between 20–25 cm in height (from soil surface to lowest leaf axil) and had five leaves and a healthy root system were inoculated by drenching (liquid) [Figure 1B, 1C]. Following the steps described below:

1. Three days before the inoculation, soil at the base of two months old banana seedlings were completely removed to exposed the base in the polythene pot, the base was washed, dried and four equidistant hollows (3–5 cm depth) around the plant base on each pot were made using a sharp knife. Making the hollows in advance speeded up inoculation process and minimized the effect of any root wounding that may occurred.
2. Drenching - the inoculum produced in liquid medium adjusted to a suspension of 10^6 conidia/mL then,10mL and 20 mL of suspension was poured into each hollow and covered with sterilized soil (potting soil) removed from the base of the plant in the pot. Five replications were used in a complete randomized experiemental design.
3. Plants were maintained in the screenhouse (25-28°C) with a 12-h light/8-h dark photoperiod) until disease symptoms appeared in the controls. Plantlets were watered regularly to maintain the substrate at field capacity (Dita*et al.*, 2011).

Figure 1: Deep wound (A) and drenching (B and C) inoculation techniques.A= plantlets in spore's suspension, B= Creatingfour equidistant hollows around, C = four equidistant hollows around the plantlets base.

Evaluation of the biological activity of *C. viminalis*and *C. ambrosioides* extracts on Fusarium wilt in the screen house

Pathogenicity test

After the previous inoculation techniques, the best one was used to evaluate the pathogenicity of isolates of interest (those which were positive to deep wound and drenching inoculation) at the rate of one isolate per locality. Indeed, seedlings of the French giant plantains variety were inoculated using the drenching inoculation technique as described above. A fungal conidia suspension (10^6conidia/mL) of each isolate of a 10-day-old *F. oxysporum* culture was applied to the healthy roots of two-month-old seedlings. Sterile water served as the control treatment.Plants were maintained in the screenhouse (25-28°C) with a 12-h light/8-h dark photoperiod until disease symptoms appeared in inoculated plantlets. Inoculated plantlets were watered regularly to maintain a suitable environment for pathogen disease development (Dita *et al.*, 2011). Five replications were used under complete randomized experimental design. Plants were observed of disease incidence and severity every two days after inoculation.

Application of plant extracts on inoculated banana plantlets in the screen house

Using the drenching inoculation techniques, a fungal conidia suspension (10^6conidia/mL) of 10-day-old fungal cultures of *F. oxysporum* obtained from the pathogenicity test (Foumbot isolate, FO_FBT) was applied on two-month-old seedlings of the French giant plantains variety as described above. Sterile water served as the control treatment. Only ethanolic extracts were applied on plants. In fact a study on the efficiency of aqueous and ethanolic extract of theses plants was first carried out in the laboratory on the growth of the

pathogen which reveals that the activity of the aqueous extract of the two plants was lower compared to ethanolic extract (Djeugap *et al.*, 2023). This explains why aqueous extracts were no more tested *in vivo*. Two hours after inoculation, 10 and 20 mL ethanolic extracts of *C. viminalis* and *C. ambrosioides* at 0.1g/mL were taken up from a flask with a syringe and applied on the roots in the beaker containing the uprooted seedling and allowed for two hours. The positive control was the fungicide Eagrow Care 720WP (Dimethomorph and Copper Oxide) with at the rcommended concentration of 3.33g/L and the volume applied were 10 and 20 mL. The treated plants were then introduced in the pots and the ground part recovered with soil previouslty removed from the pot. The experimental set-up included 7 treatments presented as followed: To = 0 mL of extract, negative control, T1= 10 mL of *C. ambrosioides*, T2= 10 mL of *C. viminalis*, T3 20 mL of CA, T4=20 mL of CV (0.1g/mL), T5=10 mL of Ea, T6=20 mL of Ea (3.33g/L). Plants were maintained in the screenhouse at the Applied Research Farm of the Faculty of Agronomy and Agricultural Sciences in a complete randomized design for 9 weeks and were irrigated according to their moisture requirements Steinkellner *et al.,* (2005). Banana wilt incidence and severity were measured weekly.Incidence (%) = (number of infected plantlets/total number of plantlets considered) x 100. Severity was estimated using the following 5 levels- scale (Ung *et al.*, 2021):

1. No streaking or yellowing of leaves. Plant appears healthy. All leaves green = 0%;
2. Slight streaking and/or yellowing of lower leaves. Lower leaves yellow= 0 - 25%;
3. Streaking and/or yellowing of lower leaves. Lower leaves dead= 25 -50%;
4. Extensive streaking and/or yellowing on most or all of the leaves. Lower leaves dead and upper leaves wilted=50 -75%;
5. Dead plant = 75 -100%.

Data analysis

The Rstudio version 4.4.1 software was used for data analysis. Prior to analysis, data expressed in percentage underwent arsine transformation. Due to the data's noncompliance with the normal law and disregard for variance homogeneity, an analysis of variance (ANOVA) could not be conducted. Means were separated using the Turkey test at 5%. Spearman correlation matrix was used to evaluate correlation between physico-chemical properties and disease variables.Simple linear regression analysis was carried out to determine the influence of total nitrogen (N %) on disease severity.

Results

Occurence of fungi identified from the various locations

A total of 114 fungi isolates in [Table 1] were identified in the five locations. They were *Trichoderma* sp, *Rhizoctonia solani, Aspergillus niger, Pythium* sp and *Fusarium oxysporum.* A total of 54 *Fusarium oxysporum* isolates were identified with at least ten isolates identified in each location and Foumbot had the highest number of *Fusarium oxysporum* identified followed byBalatchi and Kekem (12, 11, 11, respectively).

Table1: Occurence (%) or isolation frequency (IF) of fungi identified from the various locations.

	Kekem		Foumbot		Balatchi		Dschang		Banekane	
Fungi	**NF**	**IF (%)**	**NF**	**IF (%)**	**NF**	**IF (%)**	**NF**	**IF (%)**	**NF**	**IF (%)**
*Trichoderma*sp	4	17.4	5	20	3	13.0	4	18.2	2	9.1
Rhizoctoniasolani	3	13.0	2	8.3	2	8.7	2	9.1	3	13.6
Aspergillusniger	2	8.7	3	12.5	4	17.4	3	13.6	3	13.6
*Pythium*sp	3	13.0	2	8.3	3	13.0	3	13.6	4	18.2
Fusariumoxysporum	11	47,9	12	50.9	11	47.9	10	45,5	10	45.5
Total (NT)	23	100	24	100	23	100	22	100	22	100

***NF is the total number of samples from which a particular fungus was isolated and NT, the total numbers of samples from which isolations were carried out.**

Physico-chemical compositionof soil of the different locations

Soil which was slightly acidic with high percentage of clay favoured the development of the fungus while soil with high percentage of organic matter and phosphorus inhibited the growth of the fungus [Table 2]. The soil type of study sites was sandy-clay-silt types with Foumbot having the highest organic carbon value (6.15%). The pH varied between (6.1) and (6.8). The CECand total potassium varied with Foumbot having the highest values.

Table 2: Physico-chemical characterization of soil from the five sites of collection.

Soil composition	Kekem	Balatchi	Menoua	Foumbot	Banekane
Sand	41.5	29.5	53.0	52.5	49
Silt	16.5	33.5	17	30	28
Clay	42.0	37	29.5	17.5	23
pH	6.8	6.8	6.8	6.5	6.1
CO (%)	2.69	1.60	2.69	6.15	2.74
Mo (%)	4.63	2.76	4.63	10.61	4.72
N (%)	0.23	0.79	0.223	0.385	0.153
C. / no	22	20	12	16	18
Ca (Méq / 100g)	9.60	8.72	12.40	25.76	8.32
Mg (Méq / 100g)	2.72	3.44	5.92	11.34	10.96
K (Méq / 100g)	2.20	1.71	2.46	2,02	1.37
Na (Méq / 100g)	0,23	0.23	0.33	0.57	0.45
SBE	14.74	14.11	21.11	40,20	21.10
CEC (Méq / 100g)	28.80	21.64	39.19	59.79	35.15
P (mg / kg)	20.09	30.52	25.58	47.15	27.16
Cond (mS/cm)	0.04	0.08	0.07	0.06	0.17

Correlations betweenphysico-chemical properties and disease variables

The multidimensional interaction between the physico-chemical properties of Foumbot soils and Fusarium wilt incidence and severity in banana plantations. There is a highly significant positive correlation between organic matter (OM %), nitrogen (N %), and disease severity ($r>0.85$, $p<0.001$) There is a negative correlation significant between (pH, CO %, C. / N, Ca, Mg, K, Na, SBE, CEC, P, Cond) and disease severity ($r>0.85$, $p<0.001$) (Figure 2).

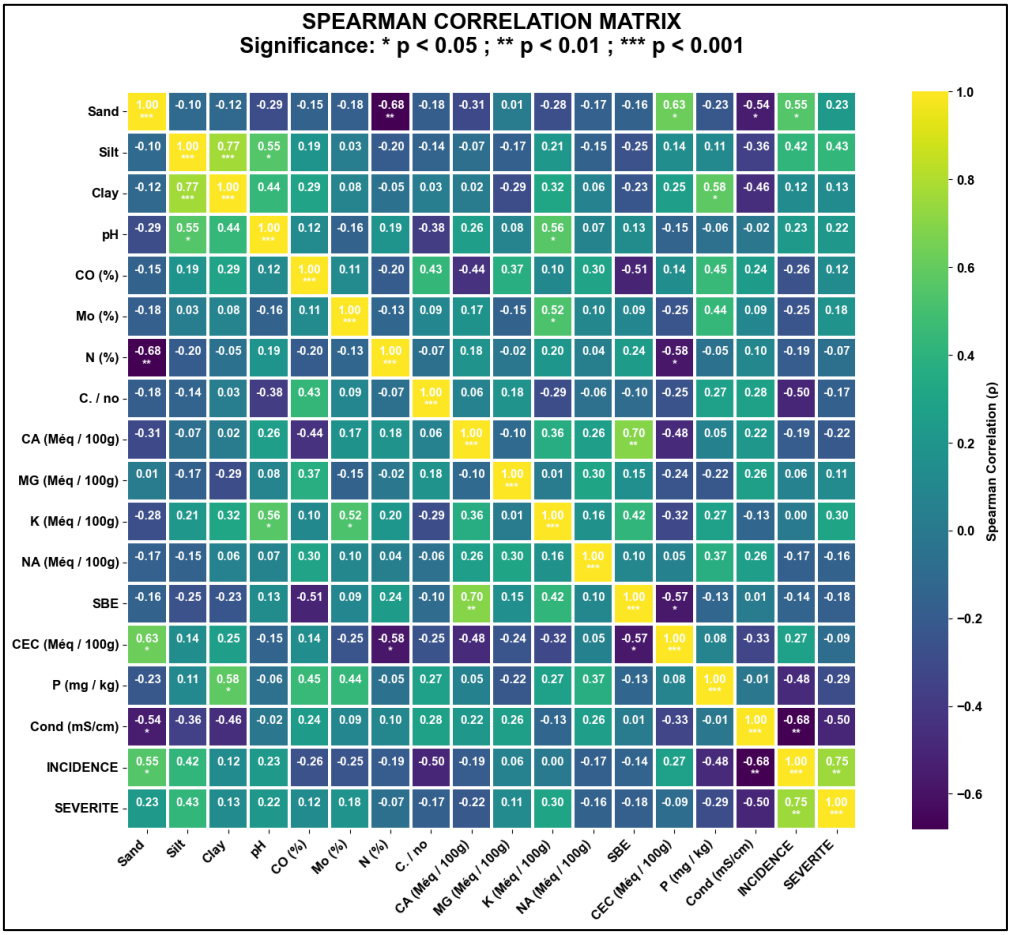

Figure 2: Pearson correlation matrix between physico-chemical properties and disease variables (incidence and severity).

Linear regression

The predictive modeling of Fusarium wilt severity as a function of the nitrogen gradient in soils from Foumbot is shown in [Figure 3]. The model's statistical robustness (R^2=0.82) demonstrated that nitrogen availability alone explains 82% of the exacerbation of tissue necrosis in banana. The positive slope of the line ($p<0.0001$) revealed the dose-response effect. Indeed, the nitrogen enrichment of volcanic soils in West Cameroon appears to correlate with increased severity of banana Fusarium wilt. The low dispersion of data points around the trend line validated the reliability of nitrogen as a bio-indicator of phytosanitary risk for banana plantations in this area. This model thus provided a scientific basis for adjusting balanced fertilization as leverage for integrated management against Fusarium wilt.

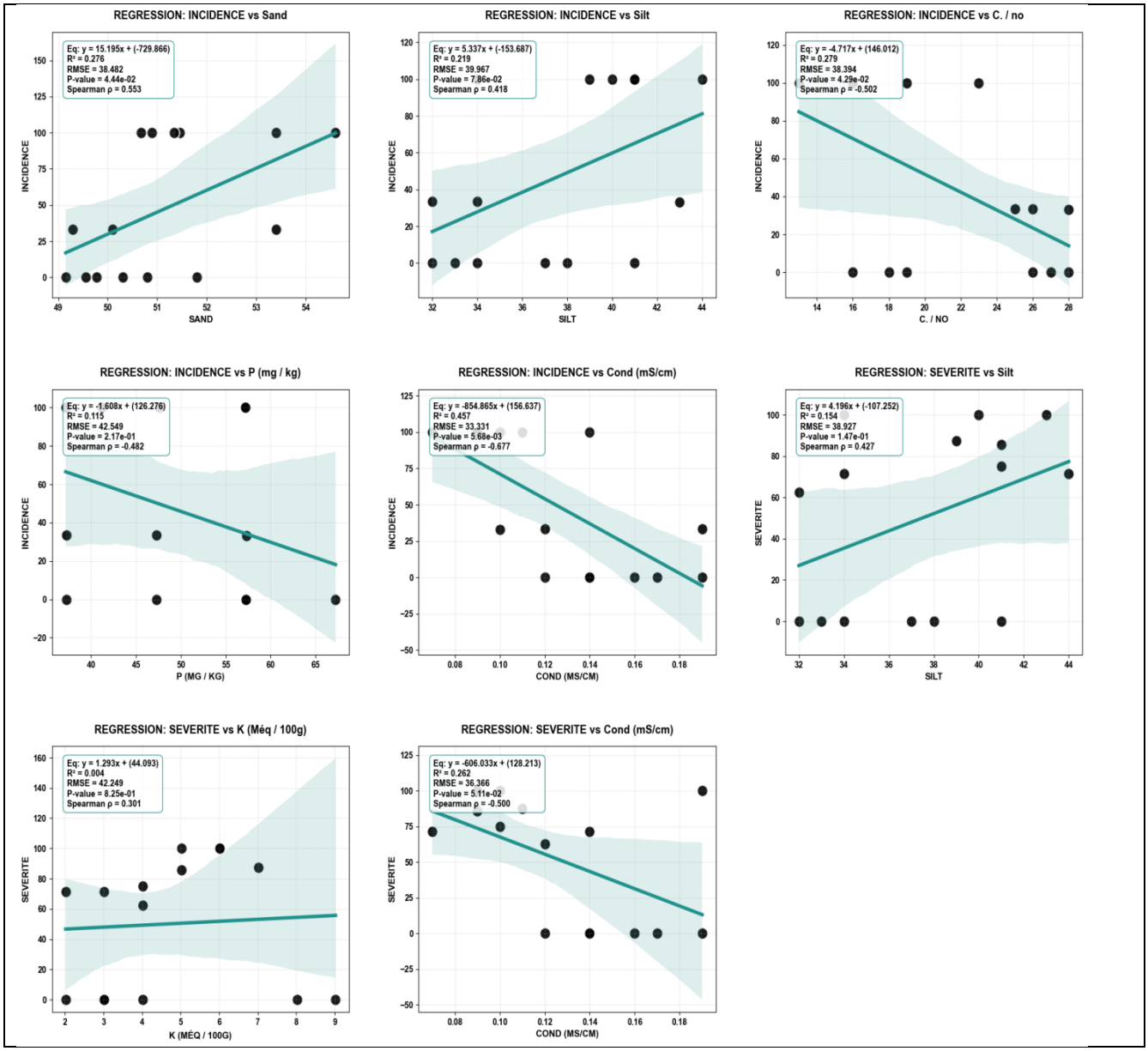

Figure 3: Simple linear regression showing the influence of total nitrogen (N %) on disease severity.

Biological activities of *Callistemon viminalis*and *Chenopodium ambrosioides* ethanolic extracts on Fusarium wilt

Banana plantlets response to inoculation techniques

The experimental data from inoculation tehniques revealed that drenching inoculation significantly increased both the incidence and severity of *Fusarium oxysporum* (FO) infection in banana seedlings compared to deep wound inoculation, with statistically significant differences (p = 0.00). Specifically, drenching inoculation resulted in a high incidence of 69.57%, markedly higher than the 28.97% observed with deep wound inoculation [Table 3]. Similarly, disease severity was notably higher with drenching inoculation at 39.81%, compared to 20.23% for deep wound inoculation, suggesting more extensive tissue damage or pathogen proliferation under drenching conditions.

Figure 4: Seedlings of banana treated with extract of *C. viminalis*: A = healthy plant with no disease and B = infected plant, 30 DAI.

Table 3: Effect of type of inoculation on banana Fusarium wilt incidence and severity.

Isolates	Incidence (%)	Severity (%)
Deep wound inoculation	28.97±44.96^{b}	20.23±33.30^{b}
Drenching inoculation	69.57±46.27^{a}	39.81±27.96^{a}
p value	0.00	0.00

Pathogenicity in the screenhouse

During this trial, isolates were identified only from Foumbot one of the most aggressive isolates. Banana plant inoculated with FO_FBT isolate under screenhouse conditions developed Fusarium wilt symptoms (wilting symptoms) of different severity with time. This confirmed that FO isolate used was very aggressive and produced the highest severity of the disease [Figue5A, 5B, 5C].

Figure 5: Pathogenicity of FO_FBT isolate on banana plantlets, A= Control (healthy banana plantlets). B and C =Infected banana plants (30 and 60 DAI, respectively).

Extraction yield of *Callistemon viminalis* and *Chenopodium ambrosioides*

Extraction yields varied depending on the solvent and the plant used. The aqueous extract of *C. ambrosioides* gave a better performance with 9.28% and ethanolic extract of *C. viminalis* gave the lowest yield of 2.85% [Table 4].

Table 4: Physical aspect and extraction yield of C*allistemon viminalis* and *Chenopodiumambrosioides* aqueous and ethanolic extracts.

Plant extract	Organ used	Extraction solvent	Physical aspect	Color	Yield (%)
C. viminalis	Leaves	Distilled water	Creamy	Blackish	3.64
C. viminalis	Leaves	Ethanol	Powdery	Greenish	2.85
C. ambrosioides	Leaves	Distilled water	Creamy	Blackish	9.28
C. ambrosioides	Leaves	Ethanol	Creamy	Blackish	4.66

Banana Fusarium wilt incidence and severity after treatment with *C. viminalis* and *C. ambrosioides* ethanolic extracts

A preliminary efficiency test was carried out in the laboratory with both aqueous and ethanolic extracts on the pathogen which reveals that the activity of the aqueous extract was low compared to ethanoloci extract. This explains why only the ethanolic extracts were tested in plant. Result showed that *C. viminalis* at 0 mL had an incidence of 100.00% and a severity of 55.60% compared to the control (0 mL FO) with 0.00% incidence and severity [Table 5]. The ethanolic extract of *C. viminalis* (20 mL) completely eliminated the infection in (0.00% incidence and severity) while ethanolic extract of *C. ambrosioides* (20 mL) significantly reduced the incidence to 39.75% and the severity to 19.20%. In comparison, Eagrow Care at 10 mL is ineffective (20.06% incidence, 8.04% severity), but at 20 mL, it completely suppressed the infection in both provenances (0.00% incidence and severity). There was no significant different using *C. viminalis* at 20 mL and Eagrow Care at 20 mL, thus our study demonstrated that the extracts of *C. viminalis* have a marked antifungal effect, comparable to Eagrow Care eliminating FO infection compared to the untreated control.

Table 5: Banana Fusariumwilt incidence (%) and severity (%) 60 days after treatmentwith plant extract.

Plant extracts (0.1 g/mL)	Incidence (%)	Severity (%)
FO – FBT(0.1g/ mL)		
Control (0 mL)	100.00 ± 0.00^{a}	55.60 ± 11.50^{a}
C. viminalis (10 mL)	16.71 ± 4.28^{d}	5.74 ± 2.45^{c}
C. ambrosioides (10 mL)	62.25 ± 11.26^{b}	24.20 ± 4.4^{b}
C. viminalis (20 mL)	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}
C. ambrosioides (20 mL)	39.75 ± 7.26^{c}	19.20 ± 4.4^{b}
Eagrow Care (10 mL)	20.06 ± 5.2^{d}	8.04 ± 3.06^{c}
Eagrow Care (20 mL)	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}
FO – FBT (10^{6}conidia/ mL)		
0 mL of FO	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
p value	**0.00**	**0.00**

Discussion

Occurence of fungi from different localities

Fusarium wilt, also known as Panama disease, is a devastating vascular wilt disease that affects banana crops worldwide. It is caused by the soilborn fungal pathogen Foc, which infects banana roots and colonizes the plant's vascular system, leading to wilting, yellowing and eventual plant death, Ploetz (2015); Pegg *et al*., (2019). The disease is highly persistent in soil, where Foc chlamydospores can survive for decades in the absence of host, making eradication extremely challenging and posting a severe threat to global banana production, Dita *et al*., (2018). The first recorded outbreak of Fusarium wilt occurred in the early 20th century, devastating plantations of Gros Michel variety in Central America and Caribbean. Foc race 1 was responsible for the widespread collapse of Gros Michel plantations, as the monoculture-based industry struggled to contain the disease. By the mid-20th century, Fusarium wilt had spread throughout major banana-producing regions, including South America and Africa, leading to significant economic losses. The cisis forced the banana industry to transition to Cavendish bananas, which exhibited resistance to Foc race 1 and could be propagated through tissue culture, ensuring large scale production, Ploetz (2005, 2006).

Physico-chemical properties of soil in different locations

Biological activities and physical-chemical properties of the soil have a strong interdependence, Budoudou *et al., (*2009). Indeed, studies have shown that chemicals favor or inhibit growth, germination and sporulation of

Fusarium (Nyiransengiyumva (2007). Sandy soil with 30% with a high soditation has low fungal load while clay soils with high cationic exchange capacity has a high fungal load (Budoudou *et al.,* 2009). This explained why *Fusarium oxysporum* from Foumbot with the highest CEC (59.79) and Kekem with the highest clay content (42) had the most aggressive *Fusarium oxysporum*. Poor organic soils have a low reception capacity of *Fusarium (*Mahdi, 2011). Organic fertilization leads to the increase in organic carbon content in the soil (Mohamed (2006), which promotes the development of antagonistic microorganisms to *Fusarium,* Alabouvette *et al., (*2009). This is why Foumbot with the most favourable soil characteristics for *Fusarium oxysporum* growth can tolerate because of high carbon and organic matter content (6.15 and 10.61).

The analysis of the physico-chemical characterization of soil in different location where disease samples were collected showed that the pH was slightly acidic and varied between (6.1 and 6.8) which explains the presence of *F.oxysporum* in all the locations. Mycelian growth and germination of conidia were restricted to a certain range for each species. This range of pH could be broad or restricted according to the species as well as for the species *Fusarium oxysporum* that develops best in soils with pH between 4 and 7, Oritsejafor (1986a). This is in line of the work of Balasu that shows that *Fusarium oxysporum* f. sp.*glycine*can grows on environments whose pH varies respectively between 4 and 7. In the agroecological context of West Cameroon, this result suggests that the nitrogen-rich soils of Foumbot, while fertile, may promote vigorous mycelia growth of the pathogen or increased host susceptibility. Furthermore, the observed relationship with pH and CEC indicatested that the soil's mineral balance modulates the receptivity of the environment to *Fusarium* infection, confirming that local edaphic characteristics are major determinants of parasitic pressure.

Relationship between soil physico-chemical properties and banana Fusarium wilt development

The results show that the severity of *Fusarium oxysporum*-induced wilt strongly linked to the edaphic properties of Foumbot soil, particularly total nitrogen and organic matter, with high correlation coefficients ($r > 0.85$; $p < 0.001$). Comparable results were reported by Pérez-Vicente et al., (2014), who observed a 40-60% increase in the severity of Fusarium wilt in banana plots grown on soils with high nitrogen and organic matter content. In these rich volcanic soils, the abundance of nutrients promotes intense microbial activity and increased availability of mineral nitrogen, creating conditions conducive to pathogen growth and symptom expression. Nitrogen, particularly in the form of ammonia, is known to stimulate the mycelia growth of *F. oxysporum* and increase the susceptibility of banana root tissues. Similarly, Muhamad *et al.* (2018) showed that high levels of easily mineralizable organic matter were associated with a significant increase in *Fusarium* incidence, with correlation coefficients greater than 0.78. These observations confirmed that, in soils from Foumbot, chemical fertility acts as a determining factor in the aggressiveness of the pathogen and the intensity of the disease.

The linear relationship observed between the severity and the nitrogen gradient ($R^2 = 0.82$; $p < 0.0001$) indicated that nitrogen is a major limiting factor in the expression of the disease in this agroecosystem. The gradual increased in severity with nitrogen content suggesting a direct dependence of *F. oxysporum* development on soil nutrient availability. Furthermore, the significant correlation with cation exchange capacity highlights the role of the chemical stability of Foumbot's volcanic soils in maintaining high pathogen pressure by ensuring the long-term retention of nutrients essential to the fungus. Dita *et al.,* (2018) reported that soils with high CEC promote the persistence of *F. oxysporum* inoculum over time, with severity levels exceeding 70% in susceptible banana plantations. These results suggested that the severity of Fusarium wilt is not a random phenomenon, but an integrated biological response to the physicochemical conditions of the soil, making the management of nitrogen fertility and soil chemical balance an essential lever for the integrated control of banana disease in Foumbot.

Biological activities of *C. Viminalis* and *C. Ambrosioides* ethanolic extracts on banana Fusarium wilt

The experimental results revealed significant variation in the efficacy of treatments against *Fusarium oxysporum* (FO) infection in banana isolates from Foumbot, with statistical significance ($p = 0.00$) indicating robust differences among treatments. It exhibited 100.00 % incidence and high severity (55.60 % to 65.89 % when treated with 10 or 20 mL pesticides, confirming the pathogen's high virulence, consistent with its known ability to cause *Fusarium* wilt in banana through vascular colonization and toxin production (Cramer, 2000). The absence of infection in 0 mL FO controls (0.00 % incidence and severity) validated the experimental setup, ensuring that observed effects were attributed to treatments. The ethanolic *C. ambrosioides* extract (20 mL) significantly reduced FO incidence to 39.75% and severity to 19.20% in

Foumbot isolates, demonstrating moderate antifungal activity. However, the variability in severity suggests inconsistent performance, potentially due to variations in bioactive compound concentrations (e.g., alkaloids phenoids, flavonoids, tannins, sterols, and triterpenoids). Previous studies have reported *Chenopodium* species' antifungal properties, attributed to essential oils like ascaridole, which disrupt fungal cell membranes (Pârvu*et al.,* 2009). The inconsistency observed here may reflect differences in extract preparation or environmental interactions affecting compound stability. In contrast, the ethanolic *Callistemon* extract (20 mL) completely suppressed FO incidence and severity (0.00%), highlighting its higher efficacy as a biocontrol agent. This aligns with findings reported by Seyedjavadi *et al., (*2017) who showed that *C. viminalis* extracts, has higher concentration in phenols, flavonoids and tannins (45.46, 4 and 1.99, respectively) exhibit strong antifungal activity by inhibiting ergosterol biosynthesis and disrupting fungal hyphal growth. The complete suppression suggests that *Callistemon*'s bioactive compounds are highly effective against FO in the screenhouse, possibly due to synergistic effects among its phytochemicals, warranting further chemical profiling to identify key active components. EagrowCareat 10 mL was ineffective, with 20.06% incidence and 8.04% severity, indicating insufficient active ingredient concentration to inhibit FO. Conversely, 20 mL Eagrow Care completely suppressed both incidence and severity (0.00%), suggesting a dose-dependent response. This is consistent with studies on commercial biocontrol agents containing microbial antagonists (e.g., *Bacillus* or *Trichoderma* spp.), which require optimal concentrations to outcompete pathogens or induce systemic resistance in plants, Shoresh *et al.,* (2010). The high efficacy at 20 mL suggests Eagrow Care's active components, likely microbial or chemical, effectively target FO's mycelial growth or spore germination, though its exact composition requires further investigation. Genetic diversity in *Fusarium oxysporum* populations is well-documented, with vegetative compatibility groups (VCGs) influencing pathogenicity, Leslie (1993). Environmental factors, such as soil pH or organic matter content may also enhance FO virulence, as reported by Weller *et al.* (2002). The differential response to 10 mL Eagrow Care (ineffective) suggests isolate-specific resistance or interactions between Eagrow Care's active agents and local soil microbiota, necessitating further molecular and ecological studies. Compared to other studies, the efficacy of *C. viminalis* extract aligns with research on plant-based antifungals, such as neem (*Azadirachta indica*) extracts, which reduced *F. oxysporum* incidence by up to 90% in tomato crops (Guleria and Kumar, 2006). However, *Callistemon'scomplete* suppression is notable, suggesting superior potency. The *C. ambrosioides* extract's moderate efficacy was also reported by El-Mougy *et al*. (2004), due to some environmental factors. Eagrow Care's dose-dependent efficacy parallels results from commercial biocontrols like Serenade ® (*Bacillus subtilis*), which achieved 80–100% FO suppression at higher doses (Gordon, 2017).

Conclusion

This study shows that physicochemical factors influence the growth of the Fusarium wilt agent in the banana plantations of the West Region of Cameroon. Clay-rich and slightly acidic soils favored the growth of *F. oxysporum,* while soils rich in organic matter and phosphorus inhibited its development. The drenching inoculation technique gave the highest disease incidence and severity and was therefore suitable for inoculation of the parasite. Ethanolic extracts of *C. viminalis* (and in some extend *C.ambrosioides*) applied on inoculated banana seedlings significantly reduced disease.

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Author's contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data.

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