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Invitro Evaluation of the Antifungal Effect of the Aqueous Extract of *Erigeron floribundus* on *Colletotrichum gloeosporioides*, the Causal Agent of Anthracnose in Pepper (*Piper nigrum*)

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A B S T R A C T

Penja white pepper produced in Cameroon is subject to numerous attacks, the most severe of which is parasites, which are one of the causes of the drop in production. The chemical control used is profitable but harmful, not only to health, and not in line with IPG specifications. This study was conducted with the aim of contributing to the improvement of the control of the pepper pathogen through the use of aqueous extract of *Erigeron floribundus*. To achieve this objective, leaves showing symptoms of anthracnose were harvested for isolation of the pathogen on PDA medium, the pathogenicity test was carried out on the plants, the inhibitory potential of the extracts was assessed using five concentrations (7.5; 15; 30; 60; 120 mg/ml), and the minimum concentrations inhibiting 50 and 90% of the growth of the phytopathogen (MIC50 and MIC90) were evaluated. The results show that two fungi are responsible for anthracnose in pepper: *Colletotrichum gloeosporioides* and *Colletotrichum necator*, the pathogenicity test carried out with the two pathogens revealed that *Colletotrichum gloeosporioides* produced characteristic symptoms with proven severity after 08 days. Inhibition of radial growth of the strain on culture medium was a function of the concentration applied during the study. This inhibition varied from 0% for low concentrations (7.5; 15; 30 mg/ml) to 28.26% for the 60 g/l concentration. For high doses (120 mg/ml) inhibition was 100%. The concentrations inhibiting 50 and 90% of growth were 82.42 g/l and 113.33 mg/ml. This extract showed fungicidal activity at the highest dose and could therefore be used as an alternative method for controlling plant pathogens.

Introduction

Agriculture remains Cameroon's main activity, employing more than 70% of the working population (Abdoul *et al.,* 2010). Despite this, agricultural production is insufficient because it does not meet the needs of all consumers. In this sector, food crops play an important role in human nutrition (FAO, 2012).

Defined as highly specialised agriculture, food crops are one of the most productive agricultural systems in Africa and are considered a food sovereignty activity (FAO, 2012). Food crops play a key role in most nutrition and poverty alleviation programmes and make a significant contribution to family incomes (James *et al.,* 2010).

Among food crops, pepper (*Piper nigrum*) originated in Asia, more specifically on the Malabar coast of India, and is now grown in many tropical countries. Since ancient times, pepper, renowned for its many uses, has spread from India to Europe, eventually conquering the whole world. The most important, popular and widely used spice, pepper is the most traded spice in the world (Gadekar *et al.,* 2006; Yogesh and Mokshapathy, 2013; Damanhouri and Ahmad, 2014). Pepper is used in cooking to enhance the flavour of many dishes. Vietnam has been the world leader in pepper production for several years, with an average production of 201,265 tonnes in 2021, followed over the same period by Brazil with 89,954 tonnes, India with 66,000 tonnes, Indonesia with 52,758 tonnes and in fifth place by China with 24,684 tonnes.

In Africa, the biggest pepper producers are: Madagascar, Ghana and Ethiopia with respectively 4532, 4409, 3732 tonnes produced in 2020 (IPC, 2022; FAOSTAT, 2022). With marginal production (<0.02% of world production) (FAOSTAT, 2017), Cameroon is classified as a low-production country. However, its production has increased tenfold in recent years, due to the growing interest that pepper has aroused among local farmers since the labelling and international recognition of Penja pepper. In 2013, these rare qualities earned it, alongside 'Oku white honey' and 'Guinea coffee', the 'Penja pepper' label, the first Protected Geographical Indication (PGI) in Sub-Saharan Africa to be awarded by the European Union (Nzenowo, 2017). In Cameroon, the pepper production area par excellence is the Moungo department, particularly Njombé-Penja, a district in the area.

In 2015, production in the Njombé-Penja area reached 300 tonnes a year, and one of the major difficulties facing pepper cultivation worldwide today is its vulnerability to pests and diseases (FAOSTAT, 2022).

In Cameroon, as elsewhere in the world, Penja pepper production faces numerous production constraints, the most severe of which is parasitic attack; as a result, little or nothing is known about the studies and pathogens causing the diseases observed in Cameroon. Annual losses of 1 to 5%, and even 50%, have been recorded in Penja pepper plantations (personal communication, PHP). The distressed growers we met, out of concern not to lose any more of their production, used a number of control methods to reduce pest pressure. The use of chemical products on unidentified pathogens is not only costly for the grower but often pointless, and chemical products used haphazardly could lead to the appearance of resistant pathogens (Conway, 2004), In addition to increasing the presence of toxic residues in food and the environment, it is also necessary to comply with the cultivation and processing techniques set out in the specifications in order to achieve the qualities required to qualify as 'Penja pepper'. Alternative control methods, such as the use of plant extracts and antagonistic micro-organisms, can be envisaged for the sustainable production of Penja pepper. Extracts have the advantage not only of being cheaper, but also of being non-toxic, biodegradable and environmentally friendly. Some progress has indeed been made in this area, despite the difficulties. Numerous studies have been carried out on the use of plant extracts in the fight against pathogenic micro-organisms. Numerous authors (Wang *et al.,* 2007; Djeugap *et al.,* 2011; Galani *et al.,* 2013; Petchayo *et al.,* 2013; Mekam *et al.,* 2019) have demonstrated the efficacy of *Phyllanthus amarus, Commelina benghalensis* and *Chromolaena odorata* extracts in controlling slow decline and root rot in pepper. Furthermore, Ouattara *et al.,* (2019); demonstrated the fungicidal effect of extracts of *Erigeron floribundus* (Kunth.) Sch. Bip. (Asteraceae) on *Sclerotium rolfsii* and *Colletotrichum musae*, two phytopathogenic fungi, with fairly satisfactory results. These plant extracts produce secondary metabolites whose antimicrobial activities have already been proven (Mazid *et al.,* 2011); studies on the secondary plant metabolites responsible for their antimicrobial activities are still topical. Hence the general objective of the study, which is to contribute to improving control of the pepper pathogen (*Colletotrichum*

gloeosporioides) through the use of plant extracts. Specifically, it will involve:

Characterise the pathogen responsible for the disease (Anthracnose);

Assess the effect of the extract on the fungal strain responsible for anthracnose in pepper (*P. nigrum*);

Determine the minimum inhibitory concentrations.

Materials and Methods

Presentation of the study area

This study was conducted in the Moungo department in the Littoral region, which is one of the most important Penja pepper production basins for the collection of leaves showing disease symptoms. Laboratory tests were carried out at the University of Dschang (Cameroon), more specifically in the Phytopathology and Agricultural Zoology Research Units of the FASA and the Applied Botany Research Unit (Figure 1).

Plant material and choice of pesticide Plant material

The plant material consisted of *Erigeron floribundus* and *Piper nigrum* leaves. The *E. floribundus* leaves were harvested early in the morning on the campus of the University of Dschang and dried in the shade to prepare the extract for the test. The *Piper nigrum* leaves were harvested in Njombé-Penja, a pepper production area in Cameroon. The chemical used was a synthetic fungicide, PENNCOZEB 80 WP. It is a contact fungicide that inhibits spore germination, and its efficacy is independent of temperature, with 80% Mancozeb and 10% Metalaxyl as the active ingredient (Figure 2, 3).

Preparation of plant extracts

Harvesting, drying and preparation of extracts

The leaves were harvested on the campus of the University of Dschang, washed with tap water and dried in a room at room temperature away from the sun for 14 days.

The dried leaves were finely ground in a mill and used for extraction using the method of Zirihi *et al.,* (2003). 200 g of powder were mixed in 1 L of distilled water, and the mixture was stirred three times a day for 48 h. The homogenate was filtered using coffee filter paper to remove debris; the filtra obtained was dried in an oven at 50 °C for five days. The material thus obtained represented the pure extract and was subsequently weighed to assess the extraction yield.

Determination of extraction yield

The crude extract obtained from 200 g of leaf powder was weighed in order to assess the extraction yield. The yield will be determined by the ratio of the mass of the dry extract after evaporation to the mass of the dry plant matter powder used for extraction, according to the formula:

$$R\,dt(\%) = \frac{\text{Mass of the extract}}{\textit{Total mass of the vegetal}} \times 100$$

Isolation, purification and identification of anthracnose pathogens in pepper

Isolation of micro-organisms associated with diseases

The parts of the leaves showing disease symptoms were cut into 5 mm pieces, disinfected in a 1% sodium hypochlorite solution for 5 min, rinsed three times with sterilised distilled water for 5, 10 and 15 minutes. The pieces were placed in petri dishes containing culture medium (PDA), which were then sealed and incubated in the dark at 25°C ± 2 for two days. Leaves showing microscopically visible structures (mycelium, spores, etc.) were then aseptically removed and placed on another PDA culture medium (Potato Dextrose Agar). Two days after incubation, the mycelia that had emerged from the leaves were isolated and then transferred to other culture media. Purification was carried out by successively transferring an agar fragment taken from the mycelium growth front onto the culture medium. This operation is repeated as many times as necessary until pure cultures are obtained, which are then stored in sterile distilled water in pillboxes.

The fungal species isolated were identified on the basis of the morphological characteristics of the mycelium (septate or nonseptate) and the fruiting bodies of the colonies observed under the microscope, using mycological identification keys (Warham *et al.,* 2008).

Pathogenicity test

The pathogenicity test is a cause-and-effect relationship linking a microorganism and a disease. It makes it possible to ascertain the role played in the disease by the microorganisms (fungi and Oomycetes) isolated from plants that have shown symptoms of the disease. This pathogenicity test was carried out on six-month-old P. nigrum plants obtained from cuttings and stored under natural conditions.

The test was carried out in a nursery set up at the FASA Phytopathology and Agricultural Zoology Research Laboratory (Figure 4) by spraying. This spraying method involves spraying solutions containing fungal strains onto the leaves of young pepper plants.

According to Lepoivre (2003), Koch's postulate stipulates that to be considered a pathogenic agent, a microorganism must be able to reproduce the same symptoms observed in the field in a controlled environment.

Evaluation of the effect of extracts on the radial growth of the strain

Preparation of different concentrations of plant extracts and synthetic fungicide (penncozeb)

Preparation of fungicide-supplemented media

The medium enriched with the synthetic fungicide (PENNCOZEB) was prepared according to the manufacturer's instructions (3.33 g/l). A stock solution of PENNCOZEB (50 mg/ml) was prepared by mixing 500 mg of PENNCOZEB with 10 ml of sterile distilled water. A volume of 2 ml was then taken from this stock solution and mixed with 28 ml of PDA medium to give a final volume of 30 ml. This proportion was obtained using the relationship Ci Vi = Cf. Vf.

Preparation of media supplemented with plant extract

For the aqueous extracts, a stock solution of 250 mg/ml was prepared by mixing 50 mg of extract with 200 ml of sterile distilled water. Culture media of 7.5, 15, 30, 60 and 120 mg/ml were prepared by successively taking 0.9, 1.8, 3.6, 4.1 and 8.2 ml of this stock solution and adding 29.1, 28.2, 26.4, 25.9 and 20.2 ml of PDA respectively, for a final volume of 30 ml each. These volumes were obtained using the relationship Ci Vi = Cf Vf.

Inoculation of culture media and incubation

After thorough mixing of the PDA culture medium with the plant extracts and fungicide, the corresponding volumes were poured into 90 mm Petri dishes. After solidification, the PDA plates were inoculated with 0.5 cm discs of pure 5-day-old *C. gloeosporioides* culture. Three replicate plates were used for the fungicide, as well as for each concentration of plant extract. Replicate PDA media that had received no fungicide or extract were used as negative controls.

Inoculated plates were incubated at 25°C ± 2°C and radial colony diameter data were recorded from the second day after incubation, with measurements ending when growth on the control plates completely covered the plate. Colony diameter on PDA with and without fungicide was measured from the bottom of the petri dishes.

Evaluation of the inhibition of the radial growth of the strain

The radial growth of the fungus was measured from the second day after inoculation until complete colonisation of the control boxes, by measuring the two perpendicular diameters of each culture. It is given by the formula of Singh *et al.,* (1993):

D = ((d1+d2))/2 - d0 (Singh *et al.,* 1993). With:

D = radial growth;

d0 = diameter of the explant;

d1 and d2 = perpendicular crop diameters.

Evaluation of the in vitro antifungal activity of the various extracts

The antifungal activity of the various extracts was determined by tests which verified whether the extracts had a fungicidal or fungistatic effect on the strains. In this case, the explants used to inoculate the media and which showed total inhibition were recovered and transferred to culture media without additives.

A treatment is said to be fungicidal if the fungus grown there does not resume its growth when transferred to a nutrient medium without additives. Otherwise, the treatment is said to be fungistatic (Pandey *et al.,* 1982).

Determination of MIC50 and MIC90 of the different extracts

The MIC50 and MIC90 (Minimum concentration inhibiting 50 and 90% of growth) of the different extracts with respect to the fungal strain was determined by comparing the values of the percentage inhibition (PI) with those of the natural logarithm of the corresponding concentrations (Ci): PI = f (ln Ci).

The percentage inhibition (PI) was determined for each treatment relative to the control, using the formula of Singh *et al.,* (1993):

$$PI(\%) = \frac{Dt - Dx}{Dt} \times 100$$

Where: Dt = mean diameter without extract; Dx = mean diameter with extract.

The linear regression line of type Y = aX + b derived from the function PI = f (ln Ci) will be used to determine the MIC50 and MIC90, where Y = percentage of inhibition, a = slope of the line, b = constant (Dohou *et al.,* 2004).

Correlation between concentration and percentage inhibition

Correlation tests were carried out to determine the relationship between the concentrations used and the percentages of inhibition obtained for each extract. In each case, the correlation coefficient was determined in order to provide information on the degree of linear dependence between the two variables. In this case, if $a < 0$ then the relationship is inversely proportional and the correlation is negative.

If $a > 0$ then the relationship is positive; if r is between 0.8 and 1 then the correlation is perfect and positive; if r is between -0.8 and -1 then the correlation is perfect and negative; if r is greater than -0.8 then the correlation is negative but imperfect.

Statistical analysis

Relative data from radial growth inhibition were statistically analysed. The results of the inhibition tests on extracts of *Erigeron floribundus* against the *C. gloeosporioides* strain were expressed as a percentage of inhibition, the means were separated and subjected to an analysis of variance (ANOVA) processed by XLSTAT software, with a 95% confidence interval criterion followed by a Newman-Keuls test (tolerance: 0.0001). The MIC50 and MIC90 were calculated using Excel 2007.

Results and Discussion

Identification and characterisation of the '*C. gloeosporioides*' microorganism

Isolation and purification of fungi from leaves showing disease symptoms enabled two fungi to be identified as responsible for anthracnose. Koch's postulate was used to justify the role played by microorganisms in the onset of the disease. The test identified *C. gloeosporioides* and *C. necator* as the responsible pathogens (Figure 5). The pathogenicity test carried out in the laboratory on detached leaves showed that *C. gloeosporioides* exhibited characteristic symptoms with proven severity, rapid growth and high production of infective structures (Oospore, Sporange). *Colletotrichum necator*, on the other hand, showed little severity, slow growth on detached leaves and few infecting structures.

Colletotrichum gloeosporioides is a fast-growing fungus on Potato Dextrose Agar (PDA) culture medium. Isolations of isolates on PDA medium after three days' incubation show mycelial colonies with a brown coloration that intensifies with time. These isolates are characterised by dense aerial mycelia with a contoured appearance, varying in colour from grey to

brown bordered by a whitish colour, but also cottony and covered with black conidia diffusing onto the PDA medium (Figure 6A).

On PDA medium the mycelia are single acervuli (Figure 6A), rarely in groups, sometimes appearing as pycnidial bodies emerging from broken, greyish black spots. The mycelia may or may not be septate, or may be discrete at the base of the condial mass. This condial mass can be dull white to dull orange or sometimes bright orange. In conditions of high humidity, conidia form false heads; these are cylindrical and curved conidia. Cylindrical conidia are characteristic of the species *C. gloeosporioides* (Figure 6 B).

Extraction yield

Extraction of 200 g of *E. floribundus* leaves yielded a crude aqueous extract with a variable yield of 5%.

Effect of aqueous extract of *E. floribundus* leaves on inhibition of radial growth of *C. gloeosporioides* strain

In the presence of aqueous extracts of *E. floribundus*, *C. gloeosporioides* exhibited higher radial growth than the negative control. The low concentrations (7.5; 15; 30 mg/ml) had a stimulatory effect on the radial growth of the strains with mean values of 6.80; 6.65; 6.28 cm respectively for concentrations C1 (7.5 mg/ml); C2 (15 mg/ml) and C3 (30mg/ml). No significant difference was observed between these doses and the negative control according to the Newman-Keuls tests at the 5% threshold.

However, at the 60 mg/ml dose, radial growth was already inhibited, with a diameter of 2.93±0.78 cm at the end of the experiment. At the highest dose (120 mg/ml), mycelial growth was completely inhibited and no growth was observed during the experiment, so there was no significant difference between these concentrations and the positive control (Figure 7).

Percentage inhibition by extract at different concentrations Test with E. floribundus

The test carried out with the different doses of aqueous extract of *E. floribundus* showed a variable inhibition rate depending on the dose. For the low doses (7.5; 15; 30 mg/ml), the percentage of inhibition was 0% as there was stimulation of radial growth in these boxes during the experiment. For the dose (60 mg/ml), mycelial growth was inhibited with a rate of 28.26. The percentage inhibition was 100 % for the concentration (120 mg/mg), comparable to that of the synthetic fungicide (Figure 8).

Fungicidal activity of the extract and PENNCOZEB

The test carried out on the explants contained in the boxes where the inhibition was total revealed that at this dose (120 mg/mg) the extract of *E. floribundus* proved to be fungicidal in the same way as the synthetic fungicide (Table 2).

Correlation test between concentrations and inhibition percentages of extracts

The aim of this test was to see if there is a linear relationship between the decrease or increase in inhibition with the different concentrations of extract on the radial growth of *C. gloeosporioides* strains, the regression line obtained after analysis revealed the similar behaviors of the strain towards the plant extract. It appears that the line obtained suggests a positive slope and perfect correlations between the concentrations and the different percentages of inhibition except with doses C1, C2 and C3. The regression equation obtained with the different doses of extracts tested show increasing linear relationships with straight lines with a positive slope: $y = 1.2941x - 56.657$ (Figure 9). A perfect and positive correlation was obtained between the different concentrations and the percentage of inhibition obtained, the correlation coefficient (r^2) being between 0.85 and 1, i.e. $r = 0.97$.

Minimum inhibitory concentrations MIC

From the regression line obtained after the correlation tests, the concentrations of the extract inhibiting the growth of the strain by 50 and 90 % were determined. The MIC50 and MIC90 values were 82.42 mg/ml and 113.33 mg/ml respectively (Table 3).

Identification and characterization of *C. gloeosporioides*

The isolation on culture medium (PDA) of the pathogen responsible for anthracnose and the pathogenicity test

carried out on detached leaves made it possible to identify two fungi as responsible for the disease in the pepper plant: *Colletotrichum gloeosporioides* and *Colletotrichum necator*. These results corroborate those of Anandaraj *et al.,* (2000) who working on pepper in Brazil identified the same pathogens as causing anthracnose.

Furthermore, these results are contrary to those of Sarma *et al.,* (2010) who identified *Cephaleurus virescens* and *Colletotrichum gloeosporioides* as the pathogen responsible for anthracnose; this difference could be due to the study area, the microclimate and the different cultivars.

Extraction yield

The quantities of raw extracts obtained from 200 g of extract powder of the plant tested presented extraction yields of 5%. This low yield can be attributed to extrinsic factors, of the organ considered and/or depending on the nature of the plants used. The low rate recorded by the plant could be due to the period of collection of the sample, to the family to which the species belongs, or to the age of the plant. Indeed, Svoboda and Hampson (1999) and Smallfield (2001) reported that environmental conditions, the harvest period and the age of the plant material can influence the extraction yield. In addition, we note differences in plants in terms of their potential; certain botanical families offer higher yields than others.

Effect of *E. floribundus* aqueous extract on mycelial growth of *C. gloeosporioides*

The various antifungal tests carried out on the strain during this study revealed that the aqueous extracts possess antifungal activities whose intensity varies depending on the concentrations of the extract and the strain tested.

The different concentrations of the extract exhibited varied inhibitory effects on *C. gloeosporioides* strains. The reduction in the diameter of the mycelial colonies of these isolates by the extracts of *E. floribundus* shows that this extract at high concentration would possess active molecules with antifungal properties which would inhibit the growth of the fungus. Indeed, Pamo *et al.,* (2003) and Ling *et al.,* (2003) reported that plant extracts from a number of plants contain compounds such as tannins, flavonoids and alkaloids which are endowed with fungicidal properties. Leaves represent the most used parts of plants because they represent the ideal place for storing secondary metabolites responsible for the biological properties of plants (Orsot *et al.,* 2015). These groups of metabolite in plant leaves contain particularly antifungal and antimicrobial properties (N'Guessan *et al.,* 2009), this is how the work of Okigbo and Ajalie (2005) showed the antimicrobial effect of leaf extracts of *Chromolaena odorata and Citrus aurantifolia* on four pathogens (*Bacillus cereus, Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*). Furthermore, the work of Ngono *et al.,* (2006) made it possible to fight against pathogenic fungi via *C. odorata* leaf extracts. Given the role it plays, the fungicidal power of these extracts could be due to all the secondary metabolites contained in the plant (Doga *et al.,* 2017).

Application of *E. floribundus* extract at low dose 7.5; 15; 30 mg/ml on the *C. gloeosporioides* strain during the test rather shows a stimulation of the pathogen compared to the control, this stimulation of the growth of the fungus could be explained by the low levels of secondary metabolites contained in the extract. Mohapotra *et al.,* (2000) showed that low concentrations of the metabolite would promote normal growth of fungi and are only phytotoxic when the concentration is high. Recent studies by Galani *et al.,* (2013); Petchayo *et al.,* (2013) showed that aqueous extracts in media could instead stimulate the mycelial growth of fungi at low concentrations. Tests on the strains show that these concentrations cannot act on inhibition and that an increase in the concentration could have an effect.

From 60 mg/ml we already note an inhibitory effect of plant extracts on the growth of fungus isolates. This effect could be due to a high concentration of secondary metabolites which give these extracts their antifungal power. Analysis of the results shows that the *C. gloeosporioides* strain presented sensitivity at high doses with total inhibition of the strains at concentrations of 120 mg/ml. these could be due to the majority of compounds found and which are responsible for the antifungal activities of these extracts. These results corroborate of Biyiti *et al.,* (2004) who showed during their numerous works carried out that the antimicrobial or antifungal activity of plant extracts required in the most cases of high concentrations.

Table.1 Plant extraction yields

Plant species	Mass (g)	Type Appearance	Performance (%)
E. floribundus	200	Aqueous solid	5

Table.2 Antifungal activity of the extract and the synthetic fungicide

Aqueous extract	Concentration	Effect
E. floribundus **Penncozeb**	**120 mg/ml** **3,33 mg/ml**	**fongicide fongicide**

Table.3 MIC50 and MIC90 values mycelial growth

EA	Regression line	Slope of the right (a)	CI_{50}	CI_{90}
E. floribundus	**y = 1,2941x - 56,657**	**a = 0,9708**	**82,42 mg/m**	**113,33 mg/ml**

Figure.1 Location of the study area.

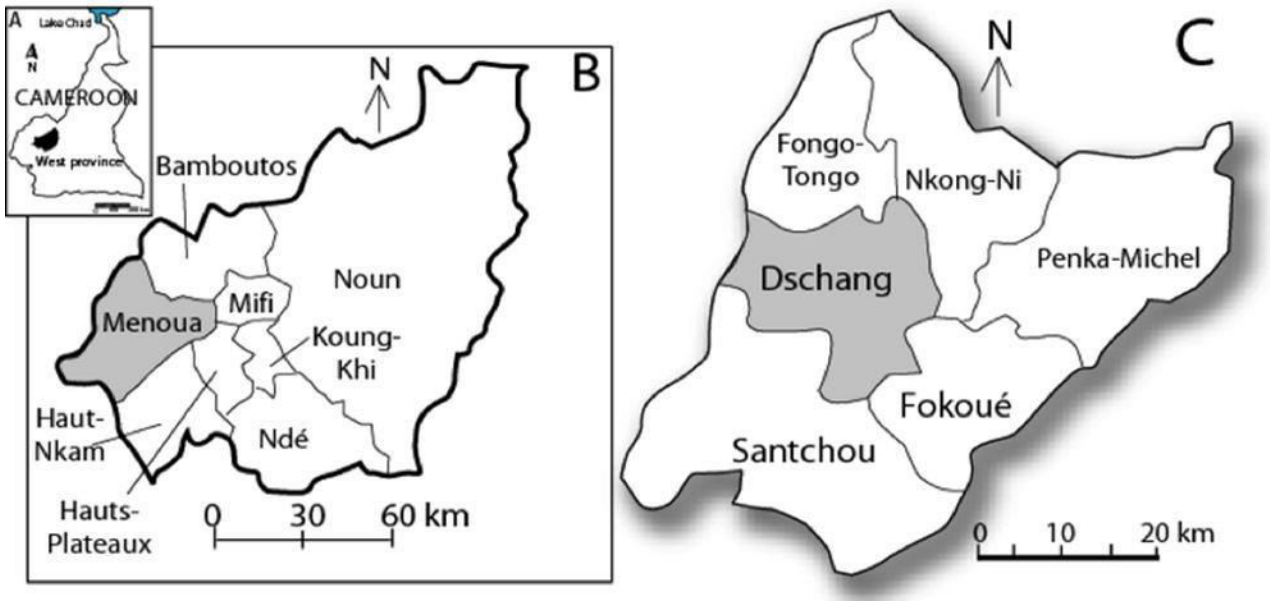
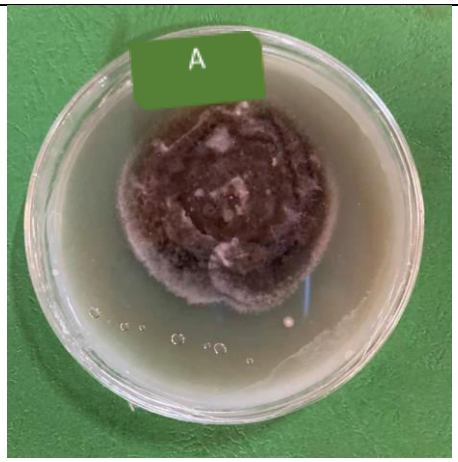


Figure.2 Penncozeb 80 WP	**Figure.3** Plant of Erigeron floribundus

Figure.4 Device for pathogenicity testing; A: six-month-old pepper plant, B: leaf inoculation by spraying.

Figure.5 Pathogens responsible for the disease

A: *Colletotrichum gloeosporioides*	B: *Colletotrichum necator*



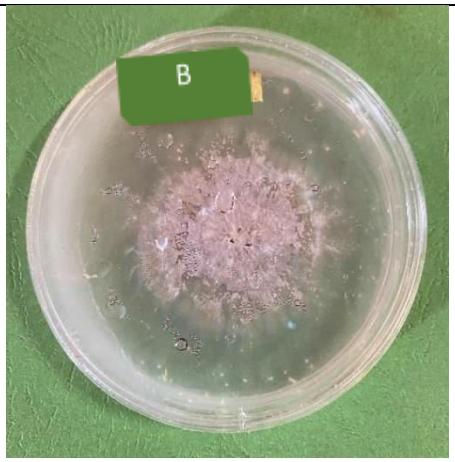


Figure.6 Microscopic appearance of *C. gloeosporioides*

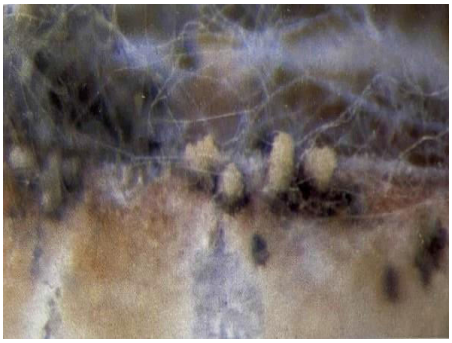
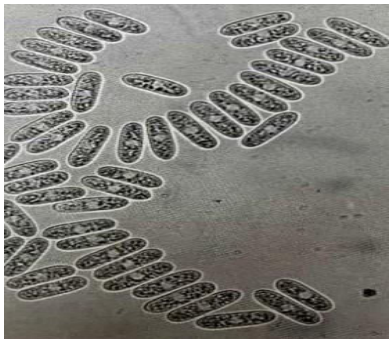
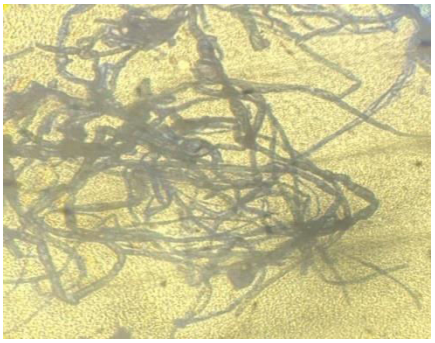
A: habit character of the fungus	B: *C. gloeosporioides* conidia	C: *C. gloeosporioides* mycelium
		

Figure.7 Radial strain growth on the effect of *E. floribundus* extract at different doses.

		
(7,5mg/ml)	*(15 mg/ml)*	*(30 mg/ml)*

		
(60mgm/l)	***(120mg/ml)***	***Témoin négatif***

Figure.8 Percentage (%) inhibition of *E. Floribundus*

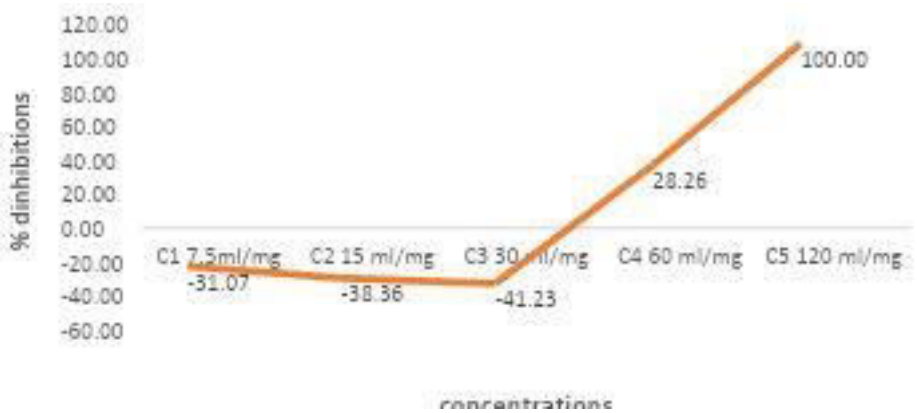
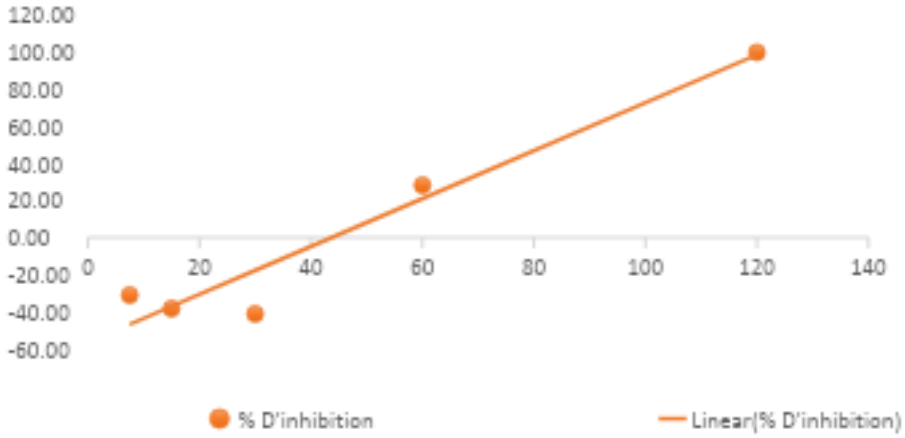


Figure.9 Regression line of the *E. Floribundus* extract



The inhibition obtained with high doses would be due to the richness of metabolites contained in the extracts. These results confirm those of Giordani and Sadoux (2008); Benattia and Bettayeb (2015) who demonstrated that the effect of extracts can be attributed to their richness in phytochemical substances as well as secondary metabolites, giving them their antifungal power).

Percentage inhibition of extracts

The correlation tests used between the concentrations and the different percentages of extract obtained on the growth of the strain also varied with the increase in concentrations. These tests revealed positive and perfect correlations for all the extracts tested. These extracts in high doses are as effective as the synthetic fungicide. Similar results were obtained by Mboussi *et al.,* (2016) when they evaluated in vitro the effect of *Thevetia peruviana* extracts on the *Phytophthora megakarya* strain.

Equivalent concentrations MIC50 and MIC90 of the extract

Inhibition of radial growth of the pathogen was dose dependent. The analysis of the regression line revealed the similar behavior of the strains with respect to extract concentrations. The various tests obtained made it possible to determine the minimum concentrations reducing mycelial growth by 50 and 90% (MIC50 and MIC90). The high MIC50 and MIC90 values for the

plant highlight the low effectiveness of the extracts against the strain. These results are contrary to those obtained by Doumbouya *et al.,* (2012), working on the effectiveness of *Ocimum graticinum* extracts on phytopathogenic fungi, showed that low MIC values highlight the effectiveness of an extract.

At the end of this work, the general objective of which was to contribute to the improvement (through the use of plant extracts) of the fight against the pepper pathogen (*Colletotrichum gloeosporioides*) responsible for anthracnose and to propose as an alternative solution the use of extracts. The following results were obtained:

Colletotrichum gloeosporioides and *Colletotrichum necator*, have been identified as pathogenic agents responsible for anthracnose, these fungi are imperfect fungi where the asexual form is observed on affected organs collected in the field.

The preparation of the aqueous extract by maceration of 200 g presented a low extraction yield of 5%.

At doses of 60 mg/ml, the extracts showed inhibitory effects on the growth of *C. gloeosporioides* strains but with total inhibition at high concentrations, i.e. 120 mg/ml.

The percentages of inhibition of plant extracts on the growth of the pathogen varied with increasing concentrations with an inhibition rate of 100% for the 120 g/l dose.

The MIC50 and MIC90 values obtained from the regression lines were 82.42 mg/ml and 113.33 mg/ml respectively. The biological extracts obtained from the plant, although effective at high concentrations, exert effects on the strains of *C. gloeosporioides* tested in vitro and could therefore be used as an alternative means of combating fungal diseases.

Author Contributions

Kone Nsangou Abdou Nourou: Investigation, formal analysis, writing—original draft. Mboussi Serge Bertrand: Validation, methodology, writing—reviewing. Alain Heu:—Formal analysis, writing—review and editing. Kenmoe Tchouakam Patrice: Investigation, writing—reviewing. Jules Patrice Ngoh Dooh: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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