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Soursop (*Annona muricata* L.) seed extracts: phytochemical composition and biocontrol potential against cercosporiosis in okra (*Abelmoschus esculentus* L. Moech)

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Abstract

Background: *Annona muricata*, or soursop, is a tropical plant known for antimicrobial and antiparasitic properties, widely used in traditional remedies. Extracts from leaves, seeds, and bark show activity against pathogens in humans and plants, making it a promising candidate for natural biopesticide development as safer alternatives to chemical controls. This study evaluates Annona muricata seed extracts as bio-fungicides against *Cercospora* leaf spot in okra under field conditions.

Methods: Capillary gas chromatography and mass spectrometry (GC-MS) analyzed aqueous and organic (acetone, ethyl acetate, and methanol) extracts. Two okra varieties (V1 and V2) were tested in field trials during 2019 and 2020. Six treatments included control, synthetic fungicide (3.33 g/L), aqueous extract (33.33 g/L), and methanol, acetone, and ethyl acetate extracts (1:600 v/v). Disease incidence, severity, and yield were measured.

Results: GC-MS identified eight antifungal compounds (hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, hexadecane, n-hexadecanoic acid, octadecanoic acid methyl ester, 9-octadecanoic acid ethyl ester, and 10-octadecadienoic acid (*Z*,*Z*)-methyl ester), though these were not directly tested in this study. The Cercospora incidence at 12 weeks after sowing was significantly reduced from 51.11 % and 47.82 % in control plots during 2019 and 2020, respectively, to 8.88 % and 6.64 % in plots treated with aqueous extract and 9.99 % and 9.76 % in plots treated with methanol extract. Yields were highest with aqueous extract (6.48 and 5.22 t/ha), outperforming methanol extract, synthetic fungicide, and control.

Conclusion: Bio-fungicides from *Annona muricata* extracts offer environmentally friendly options for managing okra cercosporiosis.

Keywords: *Annona muricata* extracts; Metabolic profile; Biological control; *Cercospora malayensis*; Antifungal properties; *Abelmoschus esculentus.*

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Background

Agriculture remains the primary activity in Cameroon, as it employs 62% of the active population [1]. Among the crops grown in Cameroon, okra (*Abelmoschus esculentus* L.) is an exceptional and original plant because all its parts (roots, stem, leaves, fruits, seeds) are valued in food, medicinal, artisanal, and even industrial terms [2]. Its high contents of carbohydrates, proteins, vitamins A and C, iron, phosphorus, potassium, and magnesium have been demonstrated [3]-[4]. Additionally, its therapeutic virtues have been discussed [5]. Roasted okra seeds are used in certain regions of Nigeria as a substitute for coffee [6].

Global okra production was estimated at nine million tons in 2023 [7]. India secures first place in okra production with 72% of the global output. In Africa, Nigeria is the largest producer (1,039,000 t/ha), followed by Ivory Coast and Ghana [7]. In Cameroon, okra is the second most represented vegetable on the market after tomatoes, with an average annual production of 104.216 tons for an area of 24.004 hectares [7]. All regions of Cameroon offer climatic conditions conducive to okra cultivation. There are, nevertheless, areas with high production and marketing potential, such as: the regions of the North-West (4.9 t/ha), the West (3.1 t/ha), and the Far North (2.3 t/ha) approximately [1]. Despite its multiple uses, its proven nutritional contribution, its financial value [7]-[9], and the revival interest in market gardening in general and the cultivation of okra in particular in Cameroon [10], the quantity of okra produced remains very low. The major causes of this insufficient production are, among other things, the low availability on the market of improved varieties adapted to the conditions of the hot and humid agro-ecological zone, as well as the presence of diseases and pests that can cause a significant reduction in its production [11]-[12].

Cercosporiosis disease of okra, caused by *Cercospora malayensis* is of fungal origin and one of the major diseases of okra. Cercosporiosis disease of okra has been observed in the tropical and subtropical zones of Asia and Africa, where okra is cultivated during rainy seasons [13]-[14]. Symptoms seen on okra leaves are usually irregular, brown in color, and then turn reddish brown with a yellowish margin [15]. In the field, cercosporiosis disease of okra follows a polycyclic process characterized by a chain of infection cycles interrupted during the hot season. The pathogen is an ascomycete of the Mycopharellaceae family. *Cercospora malayensis* is a biotrophic fungus that develops fruits only on living plant fragments. This fungus causes damage to leave, leading to a reduction in green leaf surface area and consequently a reduction in photosynthetic activity. In situations of strong attacks, it can cause a drop in yield of more than 60% in the absence of appropriate protection measures [16]. The use of improved varieties and/or the use of synthetic chemical pesticides constitutes the most widely used means of combating this pathogenic fungus [17]. However, synthetic chemical pesticides are still not within the reach of farmers, on the one hand, and on the other hand, have harmful effects on human health and the environment [18]-[19]. Numerous studies are being carried out to be able to reduce as much as possible the use of chemical pesticides and to promote the use of biocides of plant origin [20]. Plant extracts rich in secondary metabolites (phenolic compounds, terpenoids, and nitrogen compounds) for their pesticidal properties as a means of combating crop diseases have already successfully demonstrated their effectiveness. Several studies have shown the fungicidal effect of *Jatropha curcas* seeds [21]-[22] and the antifungal effect [23], [24], [25]-[26] of *Thevetia peruviana* seeds. Like most products with a biodegradable pesticide effect, the seeds of *Annona muricata* have been the subject of numerous studies, which have demonstrated insecticidal, fungicidal, and bactericidal properties [27], [28], [29]-[30]. Additional research efforts must be deployed to explore the potential as a biological control agent due to both the ease of its mass production and its formulation, as well as its specificity of action [31], and could therefore constitute an alternative to chemical products in the fight against these plant pathogens. The present study aimed to characterize the extracts of *Annona muricata* seeds and evaluate their bio-fungicidal potential in controlling cercosporiosis of okra in the field.
the field.

Methods

Study site

The test was carried out over two successive campaigns, 2019 and 2020, in the locality of Akonolinga, located in the bimodal rainfall forest zone of the Central Region of Cameroon. The geographical coordinates of the locality are 3°46′00″ N latitude and 12°15′00″ E longitude, with an altitude of approximately 669 ± 3 m above sea level. The local population mainly lives from agriculture, particularly the cultivation of cocoa, coffee, and food crops, as well as fishing and trade.

Method for obtaining extracts

The identification of *Annona muricata* plants was carried out using a species-specific botanical identification key, in accordance with the current version of the International Code of Botanical Nomenclature [32]. The mature fruits, collected from the Manjo locality (agro-ecological zone 4 with monomodal rainfall regime; coordinates: N 04°51'00", E 09°49'00"), were then submitted to the National Herbarium for taxonomic confirmation.

The organic solutions of *A. muricata* were produced according to the process described by [33]. Using the SCALTEC brand precision balance (0.01 g) (SPB55), 500 g of seed powder were weighed and macerated in 2 liters of solvent represented here by acetone, methanol, and ethyl acetate for 72 hours. After filtration using filter paper, the filtrate is transferred to the rota-vapor, in order to eliminate the extraction solvent. The extracts obtained were weighed and subsequently stored cool at 4°C until use.

The aqueous solution of *Annona muricata* was produced according to the method used by [34]. Five hundred grams (500 g) of seed powder were weighed and introduced into a container containing 5 liters of water, then macerated for 12 hours and filtered using a muslin cloth. The filtrate obtained was ready for use.

Determination of extraction yields and chemical screening of Annona muricata extracts

The extract yields (Yield) were evaluated in relation to the mass of the powdered plant material used, according to the formula opposite [35].

Yield (%) = $\frac{\text{Mass of extract }(g)}{\text{Mass of powder }(g)} x\ 100$

The classes of secondary metabolites present in the organic and aqueous extracts of *Annona muricata* seeds were determined by standard procedures described for alkaloids, anthocyanins, flavonoids (Shinoda test), phenols, saponins, and triterpenes (Liebermann-Burchard test) [36], [37], [38], [39]. These techniques are based on turbidity, precipitation, and foam of the extracts in the presence of different reagents, characterizing each class of secondary metabolites.

Gas chromatography coupled with mass spectrometry (GC-MS) of Annona muricata extracts

The *Annona muricata* seed extracts were also analyzed by capillary gas chromatography followed by mass spectrometry (GC-MS), using an Auto system XL gas chromatograph (Agilent GC 7890A) equipped with a split mode vaporization injector (1:50) coupled to a Perkin-Elmer Turbomass mass spectrometer (Agilent Technologies 5975 C TAD VL MSD). The analytical parameters were helium as the carrier gas with the column flow rate of 1.21 ml/min. The oven temperature program was 40°C for 3 min, then increased at 5°C/min to 180°C, followed by 15°C/min to 240°C and finally to 300°C at 10°C/min (15 min isothermal). A fused silica capillary column, 30 x 25 mm internal diameter and 30 x 32 mm (DB-1; 100% di-15099. Methylpolysiloxane) was used. The ion source and transfer line were maintained at 200 and 280 °C, respectively. The diluted extracts were drawn into a syringe and injected into an injector in split mode. Electron ionization mass spectra in the range 40- 500 Da were recorded at an electron energy of 70 eV. The scanning time was 1 ms, the multiplier potential 430 V, and the source pressure 10 Torr. A computer recorded all the data and compounds.

Evaluation of the fungicidal potential of Annona muricata seed extracts

Preparation of study plots and experimental setup

To properly carry out this work in the field, an experimental design in double factorial or subdivided plots of the split-plot type, consisting of four blocks spaced 1.5 m apart, was used [40]. Varieties were the primary randomized factor in two-level blocks: V1 (Hire) and V2 (Clemson). The treatments represented the secondary factor randomized in the main plots at six levels: T0 (control), T1 (synthetic fungicide containing Metalaxyl 80 g/kg and Mancozebe 640 g/kg), T2 (aqueous extract of *Annona muricata* seeds), T3 (methanol extract of *Annona muricata* seeds), T4 (acetone extract of *Annona muricata* seeds), and T5 (ethyl acetate extract of *Annona muricata* seeds). The elementary plots or experimental units measuring approximately 3 m x 2 m were separated by aisles of 0.5 m.

Obtaining concentrations of extracts and chemicals

For the different field treatments, the concentrations of the aqueous and organic extracts that were used were those that provided good laboratory results on cercosporiosis [29]. For this, regarding the aqueous extract, the filtrate was mixed with 10 liters of water to obtain a final concentration of 33.33 g/L. Concerning the organic extracts, 25 mL of each extract was measured using a syringe and introduced into a 15 L backpack sprayer [33]. Powdered soap (10 g) was added to the extracts (aqueous and organic) as a wetting agent to strengthen the adhesion of the products to the parts of the plant to be treated and thus limit their leaching by rainwater. The concentration of the fungicide was recommended by the manufacturer, i.e., 0.27 g/l [41].

Method of application of different phytosanitary substances

The seed extracts of *A. muricata* and the synthetic fungicide were applied using a 15-liter backpack sprayer to the leaves and stems of okra. The plot coverage of each extract was 50 mL/m^2 as recommended [42]. The applications began 28 DAS and took place weekly until the fruits were ready to harvest.

Evaluation of the effectiveness of Annona muricata seed extracts

The evaluation of the effectiveness of *Annona muricata* seed extracts was based on the incidence and severity of the fungal disease. This evaluation was made on 27 plants taken at random in each subplot. It started 28 days and ended 70 days with a periodicity of 7 days.

Disease Identification

The identification of cercosporiosis was made by a precise visual diagnosis of the symptoms appearing naturally on the leaves compared to healthy plants and the evolution in time and space according to [43]. Confirmation of the identification was made in the laboratory by culture of the infected fragments on PDA medium and macroscopic observation of the isolates obtained, and microscopic observation of the spores of the pathogen.

Pathogenicity test

Koch's postulate was carried out to establish the pathogenicity of isolated fungi. For this in vitro test, six isolates were tested from two localities (Akonolinga and Yaoundé). To prepare hyphal suspensions, three-week-old colonies grown on PDA medium at 25℃ were homogenized in distilled water. Twelve (12) apparently healthy leaves were inoculated with hyphal suspensions and twelve (12) leaves with sterile distilled water on young leaves of healthy okra plants until water began to drain. The leaves were individually covered with plastic bags to maintain 100% relative humidity for 24 h, and then kept in a greenhouse at 28 ± 2 ℃ with a 12-h photoperiod. Necrotic spots appeared on inoculated leaves after 10 days and compared to those observed in the field. Subsequently, re-isolation of symptomatic leaf tissues was carried out to confirm the morphological characters of the original isolate. The pathogenicity test was performed twice and showed similar results, meeting Koch's postulates [44]. The isolate was considered more aggressive, moderately aggressive, and less aggressive if the spot diameter (SD) was greater than 1 cm and, between 0.7 and 1 cm, and less than 0.7 cm, respectively 10 days after inoculation (DAI).

Assessment of the incidence of cercosporiosis

Incidence measures the proportion of diseased plants within a given experimental unit, independently of the severity or attack of each plant. It was obtained by the formula proposed by [44] as $\mathrm{I}(\%) = \frac{\mathrm{NDP}}{\mathrm{TNP}} \times 100$ Where I (%) = frequency of disease or pest attack on a study plot (incidence); NDP = Number of diseased plants or plants attacked by pests in the plot; TNP = Total number of plants (diseased or attacked + healthy).

Assessment of the severity of cercosporiosis

Severity is the degree to which an organ or entire plant is attacked by a disease. It measures the amount of disease on an organ of the plant. It was given by the formula of [44] $\mathrm{S}\,(\%) = \frac{\sum(\mathrm{ab})}{\mathrm{N}} \times 100$, with: S (%) = attack intensity (severity); $\sum(\mathrm{ab}) =$ sum of multiplications of the number of plants attacked (a) by the corresponding degree of infection (b) given in %; N = total number of plants attacked. The visual rating scale from 1 to 9 to estimate the disease was established by [46]. where: 1 = no symptoms, 2 = 1-4%; 3 = 5 – 9%; 4 = 10 – 19%; 5 = 20 – 29%; 6 = 30 – 44%; 7 = 45 – 59%; 8 = 60 – 75%; 9 = > 75% average percentage of lesions per infected plant was used to measure the intensity of disease symptoms.

Principal component and cluster analysis of screened treatments

Principal component analysis (PCA) and hierarchical ascending classification were carried out to define the links that exist between the variable's incidence and severity of the disease were chosen for the construction of the PCA. The number of axes to be retained for the analysis was determined using the Kaiser criterion (or "absolute" criterion), which consists of retaining only the axes whose eigenvalues are greater than 1. This makes it possible to measure the reliability of the reading and the overall explanatory quality of the analysis.

Yield

Mature fruits were harvested 80 DAS; then introduced into bags previously labeled according to the different treatments and varieties and brought back to the laboratory, where the weighing was carried out using a 0.01 g precision electronic balance. The yield in kg/ha was determined using the following formula from [47]:

$$\text{Yield}\left(\frac{\text{kg}}{\text{ha}}\right) = \frac{PFfp\ x\ 81}{\boldsymbol{parcel\ area}\ (\text{m}^2)}\,x\,\frac{10000\ \text{m}^2}{1\ ha}\,x\,\frac{1\ \text{kg}}{1000\ g}$$

PFfp = weight of fresh okra fruits per plant weighed using a brand scale: Satlec, precision 0.01 g; 81= number of okra plants per treated elementary plot; 10,000 m2 = 1 hectares; 1000g =1kg

Statistical analyzes

At the end of the experimental studies, the data collected on the epidemiological parameters (incidence and severity) of the disease and the yield were entered, processed, and calculated in the Excel 2013 spreadsheet for each treatment and for each variety. One-way and two-way analysis of variance (ANOVA) was performed using R software version 3.5.1. Differences between means were compared by the Tukey test at ($P \leq 0.05$) when the normality of the data (Shapiro-Wilk test; $P > 0.05$) and the homogeneity of variance (Levene test; $P > 0.05$) were verified. Pearson correlations between the different epidemiological parameters studied and yields were carried out using IBM SPSS version 20.0 software. Principal component analysis (PCA) was carried out using R software version 3.6.2 between varieties, treatments, and epidemiological parameters (incidence and severity), to detect connections between varieties and treatments.

Results

Chemical characterization of compounds from Annona muricata seed extracts

Extraction yield and phytochemical screening

The use of different organic extraction solvents (methanol, ethyl acetate, and acetone) and water made it possible to obtain *A. muricata* seed extracts of varying volumes and appearance. The result obtained shows that the highest yield is obtained with methanol as extraction solvent (39.8%), followed by acetone (38.02%). Extraction with ethyl acetate presents the lowest yield (26.02%) (Table 1). Phytochemical screening of different extracts from *Annona muricata* seeds revealed the presence of several compounds belonging to various chemical classes. Alkaloids, terpenes, coumarins, sterols, phenols, flavonoids, oils, sugars, saponins, and tannins are present in the extracts. Alkaloids, flavonoids, sterols, and terpenes are the most abundant. The methanol and aqueous extracts are the richest in compounds. The acetone extract and ethyl acetate are the poorest in the chemical compound family (Table 1).

GC-MS analysis of different extracts of Annona muricata

The result of the GC-MS analysis led to the identification of a number of compounds from the extracts of *A. muricata* seeds. The active compounds with their formulas, molecular weights and bioactivities are shown in Table 2. Table 2 presents the bio-fungicidal compounds of the different aqueous extracts, acetone, ethyl acetate, and methanol of the seed extracts of *A. muricata*, the seed extracts of *Annona muricata* obtained a diversity of molecules of different molecular weights and redemption times depending on the type of extract. Among the compounds detected and according to the literature, for the aqueous extract, six (06) compounds present bio-fungicidal activity, and five (05) compounds present bio-fungicidal and bioinsecticide activity. For the methanol extract, six (06) compounds present bio-fungicidal activity, and two (02) compounds present bio-fungicidal and bioinsecticide activity. The different compounds detected by GC-MS for the acetone extract are six (06) exhibiting bio-fungicidal activity, and two (02) compounds exhibiting bio-fungicidal and bioinsecticide activity. For the ethyl acetate extract among the compounds detected and according to the literature, six (06) present bio-fungicidal activity, and two (02) compounds present bio-fungicidal and bioinsecticide activity. Likewise, in the methanol extract of *Annona*

Field evaluation of the fungicidal potential of Annona muricata seed extracts on Cercospora malayenyis

Cercospora malayensis observed on different okra varieties

During the two years of experimentation, the disease was identified on the site by a visual diagnosis in comparison with the expected phenotype on okra leaves. The symptoms observed on the plants were reddish spots of variable shape on the majority of the upper surface of the leaves (Figure captions). After isolation of the pathogen from the leaves showing symptoms of the disease on PDA medium, observation of the macroscopic and microscopic characters revealed that it is the leaf spot caused by *Cercospora malayensis.* At 6 days after incubation on PDA medium, the macroscopic (Figure 2A and B) and microscopic (Figure 2C) characteristics of pure isolates were observed in the laboratory. The macroscopic observation of pure isolates showed whitish mycelia on the upper side of the Petri dish with a cottony appearance (Figure 2B) and yellowish on the lower side (Figure 2A). After successive subcultures, a total colonization of the Petri dishes was noted, six days after seeding. Under the light microscope (magnification X100). The culture showed the presence of elongated conidiospores with a few successive conidia, straight and slightly curved (Figure 2C). This fruiting could be that of *Cercospora malayensis*, the causal agent of cercosporiosis.

Pathogenicity test using isolated fungi

The results of the pathogenicity test were positive for all fungal isolates tested (Figure 3) and were found to be pathogenic. After two weeks of observation, okra leaves inoculated with sporangial suspensions developed disease symptoms. When symptoms were observed on the inoculated leaves, the fungus was re-isolated and cultured on PDA medium for confirmation. All *Cercospora malayensis* isolates tested were pathogenic. Isolate AKO1 and AKO 2 were more aggressive (lesion diameter greater than 1 cm, 10 DAI (day after incubation) on leaves, while isolate AKO 3 was moderately aggressive (lesion diameter between 1 and 0.7 cm, 10 DAI). YDE isolates 1, 2, and 3 were less aggressive, the lesions were less than 0.7 cm in diameter, 10 days after treatment (Table 3).

Effect of treatments and varieties on epidemiological parameters

Effect of treatments on the incidence of Cercospora malayensis

The results relating to the effect of phytosanitary treatments on the incidence of *Cercopora malayensis* throughout the experiment of the 2019 campaign reveal that there is a significant difference ($P < 0.05$) between the different treatments during the weeks after application of fungicidal products (6; 8 and 10 WAS) (Figure 4A). The incidence of the disease decreases from 4 to 10 WAS in all treated plots. At 8 WAS, a significant difference is observed which results in a considerable decrease in the degree of infection in the different treatments, i.e. 8.88; 4.44 and 2.04% for T4; T3 and T2 respectively. In T0, on the other hand, the disease incidence increased continuously between 4 and 10 WAS. At the 10th WAS, it was 2.04 and 4.44% in T1 and T2, on the other hand, in T0 the disease incidence was 72.21 and 21.98%. The disease incidence dropped after the treatment application intervals (6th to 10th WAS). In other words, during these time intervals, the disease no longer affected any other okra plant in the fungicide, aqueous and methanol treatments.

During the 2020 campaign, the incidence of the disease varied over time in the different treatments (Figure. 4B). A significant difference ($P < 0.05$) was recorded between the treatments at 6, 8 and 10 WAS. To this end, after application of the aqueous, organic and chemical extracts, the incidence of the disease significantly decreased compared to the initial situation, reaching low rates of around 27.67; 24.88; 21.44; 8.88 and 4.28% respectively in the plots treated T5, T4, T3, T2 and T1. On the other hand, in T0 the incidence of the disease was high by around 33.33% at 8 WAS. At 10 WAS, the control treatment (51.10%) recorded the highest incidence followed by the ethyl acetate extract (24.88%), the acetone extract (21.44%), the methanol extract (10.99%), the aqueous extract (8.88%) and finally the synthetic fungicide treatment (4.28%) which recorded the lowest incidence.

Effect of treatments on the severity of cercosporiosis

For the 2019 campaign at 8 WAS, the severity of the disease increased considerably in the control plots 33.82 ± 1.04 and remained constant or even fell in the treated plots, i.e. 8.38 ± 1.04 and 14.45 ± 1.98, respectively for T1 and T2 (Figure 5A). Significant differences are observed between the T0 plots and the other plots. However, for the T1, T2, and T3 treatments, the severity was less pronounced and remained low over time. At 10 WAS, the statistical analysis reveals significant differences between the treatments ($p < 0.05$). The arithmetic value of the treatment means show that the control treatment recorded the highest disease intensity (48.19 ± 11.17), the fungicide and aqueous extract treatments of respective severity T2 (1.65 ± 0.52) and T3 (5.57 ± 6.35) obtained the lowest disease intensities. The severity of the disease according to the treatments over time during the 2020 campaign is illustrated in Figure 5B.

At 4 WAS, there were no significant differences. On the other hand, at 6, 8, and 10 WAS, significant differences were observed. Between 4 and 8 WAS, the severity of the disease increased in treatment T0 and gradually decreased in treatments T1; T2; T3; T4 and T5. Indeed, at 6 WAS, a high severity of 25.88% was recorded in T0. At T2 and T3, on the other hand, they were low, at around 10.59 and 10.9% respectively. Regarding the observations made for the incidence of the disease at 8 WAS, it was observed that during this same time interval, the degree of infection of plants by cercosporiosis was considerably high in T0, i.e., an increase rate of 12%. The decrease rates of 0.3 and 0.6% were obtained respectively in T1 and T2, where the degree of infection of plants by the disease decreased considerably. Moreover, it emerges that the severity of the disease was higher in T0 (47.82%), T1, and T2; on the other hand, recorded the lowest severities (1.70 and 6.64%) at the 10th WAS. Figure 6 illustrates the phytosanitary status of okra plots in the field, seven (7) weeks after sowing (WAS). It is observed that young okra leaves are severely attacked by *cercospora* leaf spot in untreated plots (control, (T0) Figure 6A and Figure 6B). On the other hand, the plants which received the extracts Figure 6C and 6D appear free from this disease, presenting a healthy appearance.

Influence of varieties on the incidence and severity of cercosporiosis

The incidence and severity of cercosporiosis during the 2019 and 2020 campaigns increased over time (Table 4). It appears that during the 2019 campaign, no significant difference ($P > 0.05$) for the incidence and severity of the disease was recorded between varieties at 4 WAS. On the other hand, a significant difference ($P < 0.05$) for both parameters was observed at 6, 8 and 10 WAS. The incidence was higher in Clemson (22.55; 20.63 and 22.47%) compared to Hire (19.92; 18.25 and 20.47%) and the disease severity was higher in Hire (17.27; 20.20 and 18.57%) than in Clemson (15.53; 14.73 and 12.57%) at 6; 8 and 10 WAS respectively.

During the 2020 campaign, a significant effect ($P < 0.05$) was recorded between varieties at 6, 8, and 10 WAS for incidence and at 4, 6, 8, and 10 WAS for severity of cercosporiosis. The Hire variety had recorded incidence and severity rates of 7.93 and 10.63%, respectively, compared to the Clemson variety, which had recorded an incidence of 9.57% and a severity of 5.58% at 10 WAS.

Principal component analysis and dendrogram between the studied parameters

Principal Component Analysis

The variability of the treatments was described using the principal component analysis (Figure7 A) and the dendrogram (5% dissimilarity) (Figure 7B) from the okra varieties screened in the different treated plots according to the epidemiological parameters. The disease variables made it possible to discriminate the treatments. During the two campaigns (2019 and 2020), the system provides fairly reliable information (80.8%) of variance for axis 1; (76.9%) of variance for axis 2, respectively. Based on four variables, including IM (disease incidence 2019 and 2020) and SM (disease severity 2019 and 2020), at very specific observation periods, 4WAS, 6WAS, 8WAS, and 10WAS. The first two components of the PCA, explaining 98.13% of the information, were retained and allowed to group similar treatments according to their effectiveness over time. Treatments T1 T2 T3 are grouped because of their similar effectiveness against *Cercospora malayensis* with a reduction in incidence and severity of the disease of 70-80%, Treatments T4 and T5 are grouped because of their moderate effectiveness with a reduction in incidence and severity of the disease of 40-60% and treatment T0 is isolated because of its low effectiveness, with a reduction in incidence and severity of the disease of less than 20%. A dendrogram with automatic truncation (Figure 7B) allowed the obtaining of three (3) groups (I, II, and III) composed respectively of 1, 2, and 3 treatments respectively and allowed the grouping of treatments according to their similarity in terms of efficacy. The first group (I) formed is composed of treatment T0 which is a treatment with low efficacy lower than 20% reduction in the incidence and severity of the disease is composed of susceptible treatments. The second group (III) is made up of treatments T4 and T5 which is a group with moderate efficacy to the infestation to the disease with a reduction of 40-60% of the incidence and severity. Finally, the third group (II) high-efficiency treatment with a reduction of more than 70%, which is distinguished from the other two groups due to their non-susceptibility to infection for the disease in terms of incidence and severity, is formed by treatments T1, T2 and T3.

Yield of okra varieties according to treatments during the 2019 and 2020 campaigns

The analysis of variance reveals a significant difference between the different treatments. The observation of the results shows that the application of the treatments significantly improved the yield of okra compared to the control during the two years of experimentation. During the first year (2019) the control obtained the lowest yield (2.35 and 3.51 t / ha) followed by the fungicide (3.27 and 4.33 t / ha), the acetate extract (3.45 and 4.38 t / ha), the acetone extract (3.71 and 4.38 t / ha), the methanol extract (4.11 and 5.22 t / ha) and finally the aqueous extract (4.59 and 5.84 t / ha) which obtained the highest yield respectively for the Hire and Clemson varieties. During the second campaign, the same observations were made; the control obtained the lowest yield (2.85 and 3.66 t/ha), and the best yield was obtained in the T2 treatment (5.81 and 7.15 t/ha). The extracts significantly increased the okra yield compared to the controls during the two growing campaigns. The analytical comparison of the results highlights the different variations in yield between the two years of experiments. Thus, all treatments showed higher yields in the second year of experimentation than in the first year of experimentation. Aqueous extracts recorded significantly higher yields than organic extracts. However, seed extracts significantly increased yields, as did chemical treatment (fungicide), compared to the control (Table 5). Table 5 illustrates the influence of the variety on fruit yield. Analysis of variance reveals a significant difference between the two varieties at the 5% threshold. Comparison of variety means shows that the Hire variety recorded an average yield of 3.61 and 4.56 t/ha compared to 4.52 and 5.87 t/ha for the Clemson variety for the 2019 and 2020 campaigns, respectively.

Analysis of the correlation matrix between the different parameters studied in the field

A Pearson correlation matrix between certain parameters studied in the field made it possible to show the existence or not of correlations between the epidemiological parameters and the yield parameters studied (Table 6). Positive correlations (**) (1, 0.978, 0.995, and 0.969) are observed between the incidences (I10 C1 and I10 C2) and the severities (S10 C1 and S10 C2) of the disease, respectively, during the two growing seasons. However, strong negative correlations were recorded between the two yield parameters fruit length and fruit diameter LF and DF (-.783; -.834) and (-.787 and -.818) and the yield Y V1C1 and Y V2C1 (-.800 and -.740) and Y V1C2 and Y V1C2 (-.877 and -.877) obtained respectively during the two growing seasons. Thus, the incidence and severity of the two campaigns increase proportionally and are inversely proportional to fruit yields.

Discussion

The extraction of 500 g of *A. muricata* seeds produced different yields. Depending on the solvents used, including 39.02% with the methanol extract; 38.03% with the acetone extract; 26.02% with the ethyl acetate extract, and 30.04% with the aqueous extract. These different yields obtained can be attributed, on the one hand, to the nature of the solvents (aqueous or organic). Indeed, organic solvents would fix more compounds compared to water and would consequently increase the extraction yield [60], similarly the solvent such as methanol with its high polarity allows a more efficient extraction of many compounds [61]. On the other hand, the different yields obtained can be attributed to the extrinsic factors of the plant (such as storage conditions and age of the plant) and to the plant species and/or organ considered. Indeed, [62]-[63] reported that environmental conditions, the state of the plant material at the time of its harvest, the harvest period and the age of the plant material can considerably influence extraction yields.

The results of the phytochemical screening carried out showed that the aqueous and organic extracts of *Annona muricata* exhibit several families of compounds which are natural bioactive substances such as essential oils, coumarins, sterols, saponins, sugars, terpenes and flavonoids. Several of these similar phytochemicals have also been reported by [64]-[65] on the organic and aqueous extracts of the leaves, roots and seeds of *Annona muricata*.

Gas chromatography mass spectrometry GC-MS allowed, from the different chromatographic profiles of the extracts obtained, to confirm the richness of these extracts. The main constituents were terpenes, terpenoids, alkaloids, flavonoids and phenolic acids, which are the main secondary metabolites. Secondary metabolites are molecules whose nature and content vary according to biotic factors, environmental or temporal factors [66]. They intervene in the most diverse plant processes [67]. Several biological activities according to the literature have been reported in its compounds that act as bio-fungicide (hexadecanoic acid, hexadecanoic acid ethyl ester, phytol and 9,12 octadecadienoic acid methyl ester (Z, Z) etc. [68], The activities of aqueous and organic extracts are often reduced to the activity of their major compounds, or those likely to be active; however, some minor compounds may act in synergy with the major compounds or others [68]. The fungicidal activity observed in this study could be attributed to the presence of the identified major compounds.

The results on the antifungal potential of *Annona muricata* seeds on okra plants during the two campaigns showed that the incidence and severity of *Cercospora* leaf spot recorded in the experimental sites were variable over time. The treatments had a significant effect on the incidence and severity of *Cercospora* leaf spot, with very low rates obtained in the plots treated with synthetic pesticide (fungicide), aqueous and organic extracts of *A. muricata,* unlike the control, where the disease had evolved considerably and during the two crop campaigns. The antifungal power of this plant is thus close to that obtained by [18] who showed that aqueous extracts of *Thevethia puriviana* reduced the incidence and severity of *Cercospora* leaf spot in peanuts in the field by approximately 32.61 to 37.25% compared to the control.

During the experiment, no significant differences were recorded for the incidence and severity of the disease between the aqueous extract, the methanol extract of the pesticide plant used and the synthetic fungicide. [69]-[70] showed respectively that the rate of cocoa pods infected by *Phytophthora* spp in the neem seed oil treatment was statistically similar to that of the Ridomil 66 WP (chemical fungicide) treatment and that the severity of black nightshade on potato was statistically identical between the acetone extract of *Callistemon viminalis* and maneb (chemical fungicide). Thus, plant extracts are as effective as synthetic fungicides for the control of plant disease. The antifungal performance of *Annona muricata* extracts would be attributable to the presence of terpenes (pulegone, spathulenol, citronellol, carvacrol, nerolidol) and fatty acids (linoleic acid, palmitic acid, oleic acid, caprylic acid) in these extracts, as observed by [71]. Similarly, phytochemical screening of *A. muricata* seed extracts showed that they are rich in secondary metabolites, including polyphenols, terpenoids, alkaloids, saponins, flavonoids, some of which are known to have fungicidal, inhibitory, and antioxidant activity that could be attributed to the presence in these extracts of aporphinoid alkaloids consisting of (annonaine, annomontine, asimilobine and nornuciferine), terpenes (limonene and y-terpinene), and phenolic acids (caffeic acid and ferulic acid) as reported by [72]. Therefore, all these molecules deemed bioactive by GC-MS (9,12 methyl Z, Z 3,13 octadecadienal, 9,17 octadecadienal (Z etc.) would also be responsible for the fungicidal potential.

Although the Clemson variety had the highest incidence, the Hire variety was the most affected by the disease (higher severity) during both growing seasons. This could be explained by the fact that the Hire variety would have more sophisticated external defense barriers to prevent the entry of *Cercospora* into its leaves; but once infected, the internal defense mechanisms would not be strong enough to stop the progression of the pathogen. This could be the opposite case for the Clemson variety. These results are similar to those of [73], working on plant defense strategies, reported that plants, during their evolution, have set up protective barriers against bio aggressors: cuticle, pectocellulosic wall. But if the pathogens succeed in crossing them, they will have to deal with active (internal) defense mechanisms.

The okra yields obtained in the plots show that the extracts (*Annona muricata*) improved okra production compared to the phytosanitary products and the control during the two growing seasons; the significant differences between the treatments and the control suggest that the *Annona muricata* seeds would stimulate the biomass and yield of okra. These results would probably be the result of the effectiveness that the aqueous and organic extracts showed both as insecticide and fungicide in the experiments.

The yield is significantly higher in the Clemson variety compared to the Hire variety. This could be due to the effectiveness of the aqueous extracts combined with the genetic capacities of the Clemson variety to produce high yields and its ability to adapt to environmental conditions. These results are in line with those of [74] working on the cultivation of soybeans and other legumes, mentioned that the different varieties of soybeans each have their own genetic characteristics resulting either from the adaptation of the plant to different environmental conditions, or from artificial crosses and genetic manipulations. Furthermore, the second explanation lies in the fact that the Hire variety in the present experiments was severely more attacked by the disease than the Clemson variety; indeed, the disease can cause heavy losses when the severity is high [75].

Susceptibility tests for *C. malayensis* on young okra plants highlighted variations in infestations depending on the treatments (T0, T1, T2, T3, T4, and T5). Thus, the incidence and severity of the disease during the 2019 campaign on the one hand made it possible to obtain good yields per treatment compared to the 2020 campaign on the other hand. Treatment T1 and T2 are the least susceptible (more effective) to the disease, followed by treatment T3, T4 and T5 are moderately susceptible, while the control treatments were the most susceptible to cercosporiosis.

The correlation matrix obtained by factor analysis of the components of quantitative variables shows the existence of a significant correlation between several variables. Indeed, the analysis showed that there is a strong negative correlation between yield (y) and fruit length (LF) and fruit diameter (DF). The same is true for incidence (I) which is strongly and positively correlated with fruit diameter (DF). Furthermore, there is a significant negative correlation between yield and fruit length. This is an important indicator in the selection of okra oriented towards early okra sought by producers and consumers. Similar results were obtained by [76].

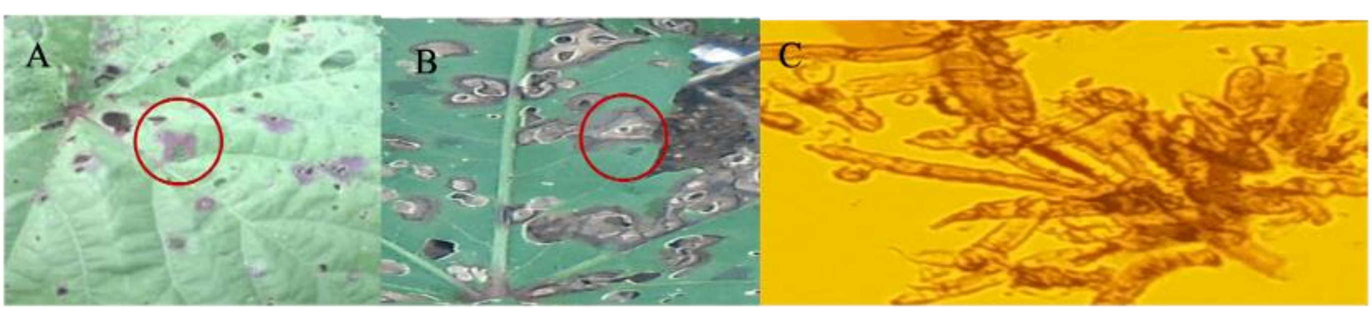

Figure 1. Okra leaf showing symptoms of Cercospora leaf spot
(A): Hire variety, (B): Clemson variety, (C): spores; red circle indicates disease symptoms.

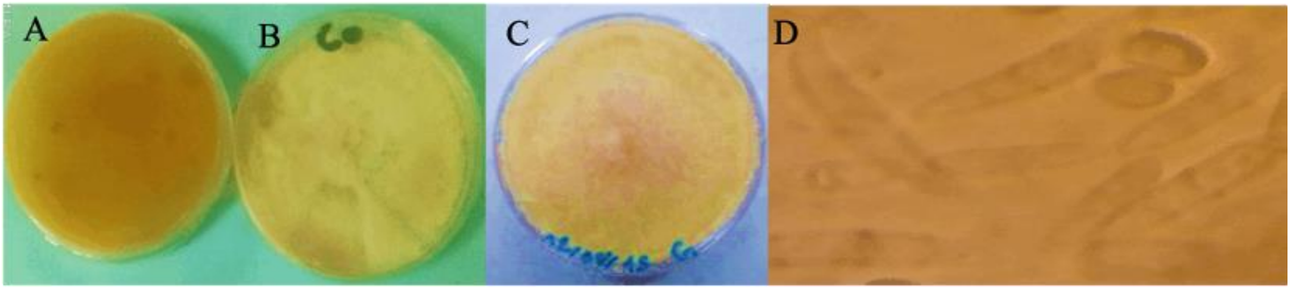

Figure 2. Pure isolates and spores of *Cercospora malayensis*
A: back of the Petri dish; B: upper side of the Petri dish; C: Pure strains; D: spores.

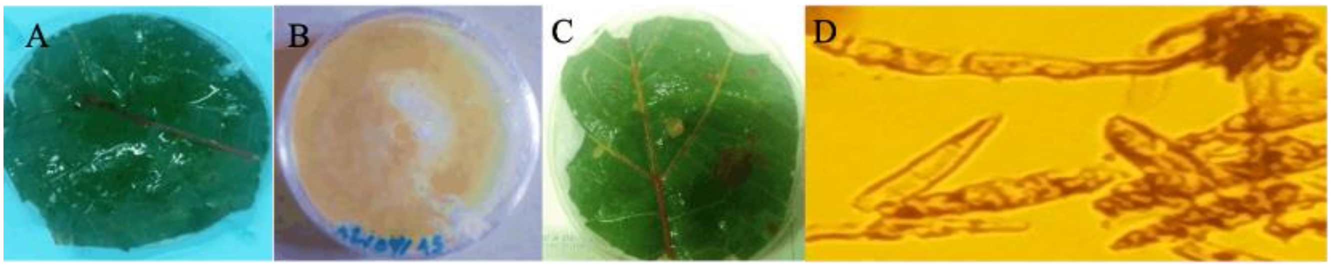

Figure 3. Pathogenicity test
A: infected leaf; B: Pure strains; C: leaf fragment on a Petri dish; D: spores on leaf fragment.

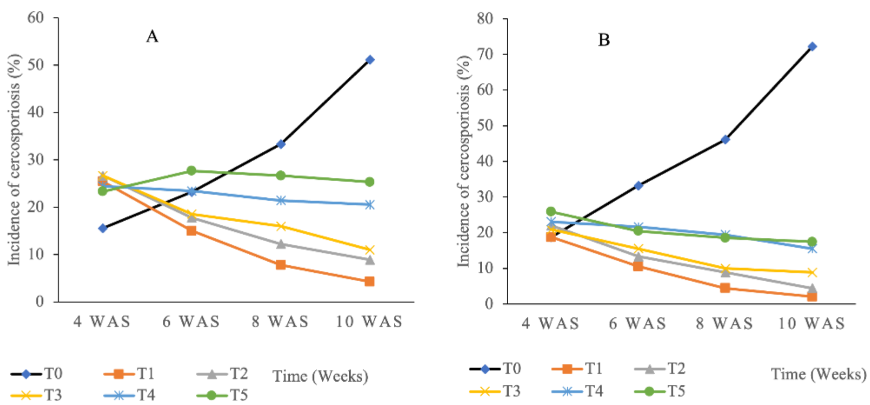

Figure. 4. Effect of treatments on the incidence of cercosporiosis as a function of time.
A: 2019 campaign, B: 2020 campaign, WAS: Weeks after sowing; T0: control, T1: synthetic fungicide, T2: aqueous extract, T3: methanol extract, T4: acetone extract, T5: ethyl acetate extract.

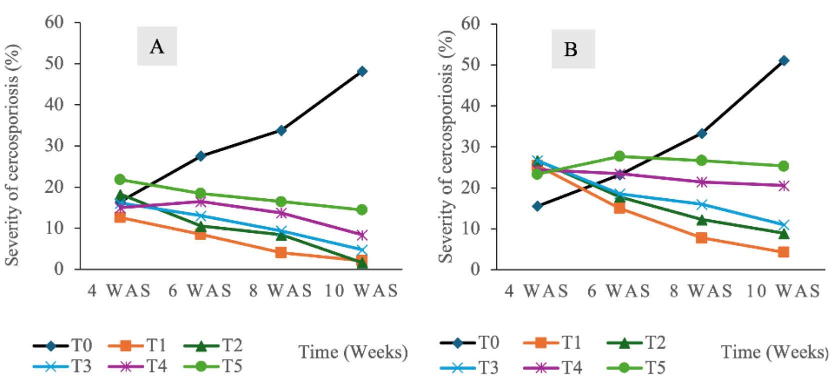

Figure 5. Effect of treatments on the severity of cercosporiosis as a function of time.
A: 2019 campaign, B: 2020 campaign, WAS: Weeks after sowing; T0: control, T1: synthetic fungicide, T2: aqueous extract, T3: methanol extract, T4: acetone extract, T5: ethyl acetate extract

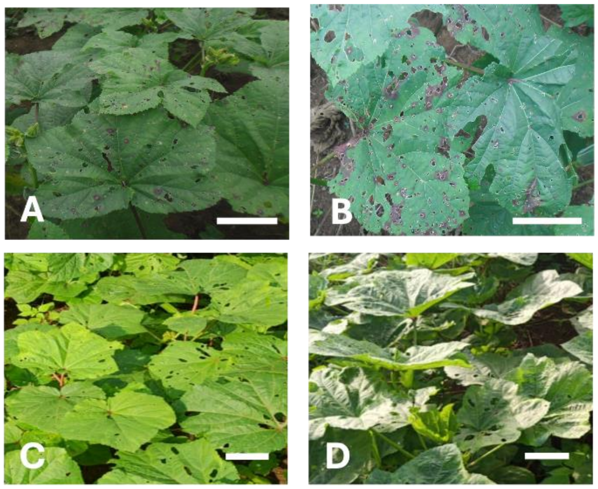

Figure 6. Phytosanitary status of different okra plots at 7 WAS.
A: Untreated plot of Hire variety; B: Untreated plot of Clemson variety; C: Plot of Hire variety treated with aqueous extracts; D: Plot of Hire variety treated with methanol extract. Scale bar = 5cm

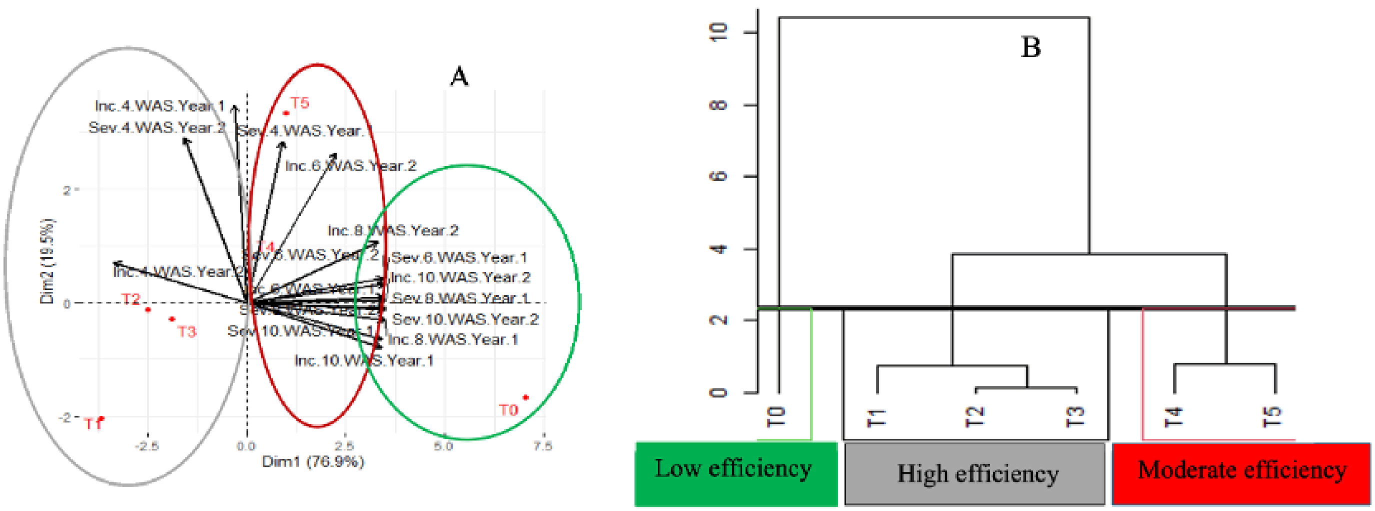

Figure 7. Different okra varieties and the parameters studied in the field.
A: Principal component analysis, B: Dendrogram of reconciliation of Campaign 2019 and 2020; IM: disease incidence, T0: control, T1: synthetic fungicide, T2: aqueous extract, T3: methanol extract, T4: acetone extract, T5: ethyl acetate extract; V1: Hire and V2: Clemson; Year 1: 2019 campaign; Year 2: 2020 campaign; WAS: Weeks after sowing; Inc: incidence; Sev: severity.

Table 1. Different bioactive compounds present in *Annona muricata* extracts

Characteristics and bioactive compounds	EA	ME	EEA	Eaq
Extraction yield	38.2%	39.8 %	26.02 %	29.32 %
Color and appearance	blackish oily	blackish oily	blackish oily	colorless liquid
Oil (Oleic acid, Linoleic acid, Palmitic acid, Stearic acid)	++	++	++	+
Coumarins (7-hydroxycoumarin; 5,7-Dihydroxycoumarin	+	+	+	+
Alkaloids (annonacin, annomontine, Annopurine)	++	++	++	+++
Sterols (β-sitosterol, Stimasterol, Campesterol	+	+	++	+
Terpenoids (limonene, y-terpinene)	+	+	++	++
Flavonoids (quercetin, Kaempferol)	+	+	++	++
Tannins (Gallic acid, Ellagic acid, Caffeic acid)	-	-	+	+++
Saponins (Annonin, Annonin A, Annonin B)	++	-++	+	+++
Sugar (Sucrose, Glucose, Fructose)	+	+	T	T
Phenols (caffeic acid, phenolic acid)	+	+	++	++
Carbohydrate (Starch, cellulose, Hemicellulose)	+	-	+	++

-: Absence, +: presence, +++: abundant presence, T: presence in the form of traces, ME: methanol extract, ACE: ethyl acetate extract, EA: acetone extract, Eaq: aqueous extract.

Table 2. Different compounds identified by GC-MS

MW	Compounds	Structures	BA	References	EA	EAE	EAq	Me
296.53	3,7,11,15-Tetramethyl-2 hexadecen-1-ol	$C_{20}H_{40}O$	F and I	[48]	A	A	P	A
256.42	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	F and I	[49]	P	P	P	P
130.18	Pentanoic acid ethyl ester	$C_7H_{14}O_2$	F	[50]	P	P	P	A
270.45	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	F	[51]	P	P	P	P
298.50	Heptadecanoic acid ethyl ester	$C_{15}H_{30}O_2$	F	[52]	A	A	P	A
294.47	9, 12-Octadecadienoic acid (Z, Z)-methyl ester	$C_{19}H_{34}O_2$	F and I	[53]-[54]	P	P	P	P
312.53	Octadecanoic acid ethyl ester	$C_{20}H_{40}O_2$	F	[48]	P	A	P	A
264.40	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	F and I	[55]	A	A	P	A
176	Propane, 1,1,3-triethoxy	$C_9H_{20}O_3$	F	[56]	A	A	P	A
354.52	9,12- Octadecadienoic acid (Z,Z)- 2 hydroxy 1 (hydroxymethyl) ethyl ester (2 Monolinoleic)	$C_{21}H_{38}O_4$	F and I	[57]	A	P	P	P
280.50	2-methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	F and I	[55]	A	A	A	P
296.4879	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	F	[48]	A	A	A	P
312.5	9-Octadecenoic acid, 12-hydroxy-ester	$C_{18}H_{36}O_3$	F	[58]	P	A	A	A
314.50	Ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	F and I	[48]	A	A	A	A
296.48	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	F	[53]	A	A	P	A
296.48	15-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	F and I	[59]	A	P	A	P
298.50	Octadecenoic acid, methyl ester	$C_{19}H_{38}O_2$	F	[59]	A	P	A	A

MW: Molecular weight g/mol; P: Presence of the molecule; A: Absence of the molecule. EA: Acetone; EAQ: Aqueous; EAE: Ethyl acetate; ME: Methanol.

Table 3. *Cercospora malayensis* pathogenicity test

Harvest area	Isolates	Leaves (dl)
	Akonolinga 1	+++
Akonolinga	Akonolinga 2	+++
	Akonolinga 3	++
	Yaoundé 1	+
Yaoundé	Yaoundé 2	+
	Yaoundé 3	+

Aggressiveness: +++= more aggressive; ++=moderately aggressive and += less aggressive; dl: lesion diameter

Table 4. Variety effect on the incidence and severity of cercosporiosis during the 2019 and 2020 campaigns

Campaigns	Periods	Incidence %		severity %	
		Hire (V1)	Clemson (V2)	Hire (V1)	Clemson (V2)
2019 campaigns	4 WAS	19.44 ± 8.23a	24.66 ± 11.82 a	16.73 ± 6.23a	17.35 ± 6.93a
	6 WAS	19.92 ± 10.11a	22.55 ± 12.95b	17.27 ± 6.72b	15.53 ± 9.40a
	8 WAS	18.25 ±14.06a	20.63 ± 14.75b	20.20 ± 16.07b	14.73 ± 12.32a
	10WAS	20.47 ± 20.71a	22.47 ± 22.40b	18.57 ± 8.18 b	12.57 ± 2.10a
2020 campaigns	4 WAS	18.41 ± 9.39a	23.22 ± 10.31a	22.06 ± 5.80 b	15.80 ± 4.86 a
	6 WAS	16.59 ± 7.36a	19.24 ± 7.34b	17.17 ± 3.67b	12.67 ± 1.85a
	8 WAS	12.82 ±3.25a	15.73 ± 3.87b	12.37 ± 1.15b	9.21 ±2.76a
	10WAS	7.93 ± 2.92a	9.57 ± 2.3b	10.63 ± 1.42b	5.58 ± 0.59 a

Values followed by the same letter on the same line are not significantly different at the 5% threshold (Tukey test). WAS: week after sowing

Table 5. Influence of treatments on the yield of the two okra varieties during the 2019 and 2020 campaigns

Fruit yield (t/ha)				
	2019 Campaign		2020 Campaign	
Treatments	Hire	Clemson	Hire	Clemson
Control	2.35 ± 1.54a	3.51 ± 1.54a	2.85 ± 1.54a	3.66 ± 1.54a
Synthetic fungicide	3.27 ± 1.54b	4.33± 1.54b	4.69 ± 1.54bc	6.71 ± 1.54b
Aqueous extracts	4.59 ± 1.54c	5.84 ± 1.54d	5.81 ± 1.54d	7.15 ± 1.54b
Methanol extracts	4.11 ± 1.54c	5.22 ± 1.54c	5.24 ± 1.54cd	6.99 ± 1.54b
Acetone extracts	3.71 ± 1.54bc	4.63 ± 1.54b	4.61 ± 1.54bc	5.39 ± 1.54b
Ethyl acetate extracts	3.45 ± 1.54b	4.38 ± 1.54b	4.05 ± 1.54b	4.98 ± 1.54b
Varieties	3.61 ± 1.54 a	4.52 ± 1.54b	4.56 ± 1.54a	5.87 ± 1.54b
Interactions	* * *			**
Treatments	*			*
Varieties	*			*

Values followed by the same letter are not significantly different at the 5% threshold between rows for treatments and between columns for varieties. *: Significant at the 0.05 probability level. * *: Significant at the 0.01 probability level; * * * Significant at the 0.001 probability level.

Table 6. Correlation matrix between quantitative variables.

	I10C1	S10C1	I10C2	S10C2	YV1C1	YV2C1	YV1C2	YV2C2	LFC1	DFC1	LFC2	
I10C1	1											
S10C1												
S10 C1	,995**	1										
I10C2	,000											
I10 C2	,978**	,975**	1									
S10C2	,001	,001										
S10 C2	,969**	,974**	,997**	1								
YV1C1	,001	,001	,000									
Y V1C1	-,800	-,830*	-,755	-,766	1							
YV2C1	,056	,041	,083	,076								
Y V2 C1	-,740	-,774	-,720	-,736	,987**	1						
YV1C2	,093	,071	,106	,095	,000							
Y V1C2	-,877*	-,907*	-,879*	-,896*	,958**	,956**	1					
YV2C2	,022	,013	,021	,016	,003	,003						
Y V2C2	-,877*	-,897*	-,932**	-,949**	,832*	,844*	,948**	1				
LFC1	,022	,015	,007	,004	,040	,035	,004					
LF C1	-,783	-,812*	-,754	-,764	,993**	,995**	,962**	,843*	1			
DFC1	,065	,050	,083	,077	,000	,000	,002	,035				
DF C1	-,787	-,822*	-,771	-,787	,985**	,992**	,977**	,869*	,995**	1		
LFC2	,063	,045	,073	,063	,000	,000	,001	,024	,000			
LF C2	-,834*	-,864*	-,798	-,807	,987**	,973**	,971**	,848*	,988**	,990**	1	
DFC2	,039	,026	,057	,052	,000	,001	,001	,033	,000	,000		
DF C2	-,818*	-,851*	-,775	-,785	,981**	,964**	,958**	,819*	,981**	,983**	,998**	1
	,047	,032	,070	,064	,001	,002	,003	,046	,001	,000	,000	

V1: Hire; V2: Clemson; LFC1: fruit length 2019 campaign; LFC2: fruit length 2020 campaign; DFC1: fruit diameter 2019 campaign; DFC2: fruit diameter 2020 campaign; YV1C1: Hire variety yield 2019 campaign; YV1C2: Hire variety yield 2020 campaign; YV2C1: Clemson variety yield 2019 campaign; YV2C2: Clemson variety yield 2020 campaign; I10C1: disease incidence 10 WAS 2019 campaign; I10C2: disease incidence 10 WAS 2020 campaign; S10C1: disease severity 10 WAS 2019 campaign; S10C2: disease severity 10 WAS 2020 campaign; WAS: week after sowing.

Conclusion

This study evaluated the efficacy of aqueous and organic extracts of *Annona muricata* seeds against okra leaf spot in the bimodal rainfall forest zone. Four extracts were profiled using GS-MS, revealing several fungicidal compounds: hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, hexadecane, n-hexadecanoic acid, octadecanoic acid ethyl ester, phytol, and 12-octadecadienoic acid (*Z, Z*)-methyl ester. Field trials over two seasons showed that both aqueous and organic extracts significantly reduced disease incidence and severity, demonstrating strong fungicidal activity comparable to the standard synthetic fungicide. The aqueous extract was particularly effective on the Clemson variety. Yields were significantly higher in treated plots. These results demonstrate that *Annona muricata* seed extracts are promising for integrated management of okra leaf spot. However, organic solvents can be toxic to humans and animals if mishandled. To limit these dangers, it is recommended to use less toxic solvents, such as water, or to handle other organic solvents carefully, wearing personal protective equipment (gloves, goggles). Training and risk awareness are also essential. Appropriate technologies could be developed to enable the production of organic solvent extracts safely and efficiently, even in resource-limited settings.

Abbreviations

ACE: Ethyl acetate extract
C1: 2019 campaign
C2: 2020 campaign
DAI: Day After Inoculation
DF: Fruit diameter
EA: Acetone extract
Eaq: Aqueous extract
GC-MS: Gas Chromatography Mass Spectrometry
I or Inc: Disease incidence
LF: Fruit length
ME: Methanol extract
PCA: Principal Component Analysis
PDA: Potato Dextrose Agar
S or Sev: Severity
SAS: Weeks after sowing
T: Presence in the form of traces
T0: Control treatment
T1: Treatment with synthetic fungicide
T2: Treatment with aqueous extract of *Annona muricata* seeds
T3: Treatment with methanol extract of *Annona muricata* seeds
T4: Treatment with acetone extract of *Annona muricata* seeds
T5: Treatment with ethyl acetate extract of *Annona muricata* seeds
t/ha: ton/hectare,
V1: Variety Hire
V2: Variety Clemson
Year 1: 2019
Year 2: 2020
Y: Yield

Authors' Contribution

Conceptualization: BN and LB; Methodology, data collection: HB, PZN, SLLD; Data Analysis: HB; Original draft preparation: HB; Writing –Review- Editing: LBT, SLLD, CTT, EBN, JPA, TT.

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Conflict of interest

The authors declare no conflict of interest

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