

 RESEARCH PAPER

Bio-effectiveness of *Balanites aegyptiaca* (L.) Del. seed extracts against *Colletotrichum capsici* (Syd.) Butler & Bisby causal agent of anthracnose in cowpeas (*Vigna unguiculata* (L.) Walps.) under natural conditions

Tize Tize¹, Patrice Zemko Ngatsi¹, Sylvère Landry Lontsi Dida¹, Apollinaire Njome Toka¹, Thierry Songwe Atindo¹, Eric Biyo'a Ndongo¹, Charles Sale Essome¹, Bekolo Ndongo¹

¹ Department of Plant Biology, Phytopathology Laboratory, University of Yaounde I, Yaounde, Cameroon

Corresponding author: Tize Tize (tize5@gmail.com)

Academic editor: Habib Ali ♦ Received 11 July 2024 ♦ Accepted 4 November 2024 ♦ Published 22 November 2024

Abstract

Anthrachnose disease is one of the major causes of yield losses around 45 to 85% in cowpea crops production in Cameroon. Synthetic fungicides are commonly used for diseases management, yet concerns exist regarding their cost effectiveness and environmental impact. This study aimed to control the development of cowpea anthracnose by using extracts from the seeds of *Balanites aegyptiaca* (L.) Del. The experimental design was a “split-plot” with four replicates. The varieties constituting the main plots randomly in replication with two variants: V1 (Tiligré) and V2 (Lori 24-130) and treatments represented sub-plots randomized within the main plot (T0: control; T-AqE: aqueous extract; T-AcE: acetone extract; T-ME: methanol extract; T-Fong: synthetic fungicide containing 80% of maneb) during two campaigns (2020 and 2021). As results, the plots treated with synthetic fungicide T-F: (9.78 and 9.1%), aqueous extracts (10.6 and 20.48%) and acetone extracts (12.2 and 12.3%) recorded lower severity than control plots (64.14 and 59.82%) for varieties V1 and V2, respectively. The yields were higher in plots treated with aqueous extract (794.05 and 926.63 kg ha⁻¹) and lower in the control plots (518.03 and 545.055 kg ha⁻¹) respectively for the varieties V1 and V2. *Balanites aegyptiaca* (L.) seed extracts could be used in the integrated control of cowpea anthracnose disease.

Keywords

Vigna unguiculata, Biopesticide, *Balanites aegyptiaca*, *Colletotrichum capsici*

Introduction

Cowpea, *Vigna unguiculata* [L] Walp., is one of the most widely grown grain legumes in all hot, zones, arid and semi-arid zones of sub-Saharan Africa where other crops can fail, due to their poor adaptation to drought, high temperatures and poor soils (Lalsaga and Drabo 2017; Hamidou et al. 2018). The species *V. unguiculata* plays an important role in food security, as cowpea is used in human and

animal food. It constitutes an important income resource for producers, thanks to the marketing of seeds and tops (Remond and Walrand 2017). This legume is an excellent source of protein (23 to 25% on average), carbohydrates (64%), and dietary fiber. Other nutrients such as thiamin, niacin and riboflavin are present in grain (Jackson 2009; Modu and al. 2010). In addition, cowpea provides the human diet with the content of its proteins in essential amino acids such as lysine, tryptophan, phenylalanine, valine,

threonine and methionine (USDA 2004; Neya et al. 2019). Agronomically, cowpea is well known for its ability to fix atmospheric nitrogen up to 240 kg/ha, thanks to a symbiotic association with bacteria of the genus *Rhizobium* sp., which are found in the nodules of its roots and contributes to improving soil fertility (Kaboré 2004; Husson et al. 2010).

Despite the multiple roles played by this legume, cowpea cultivation is faced with numerous biotic (pathogenic diseases, weeds, insect pests) and abiotic (drought due to very high temperatures, poor soils, very unfavorable sowing dates) problems (Hartman and Leandro 2015; Maimouna et al. 2020). Cowpea production is low, due to attacks by fungal, bacterial, viral and nematological diseases which attack different parts of the plant at all stages of its growth. Among the fungal diseases, anthracnose, caused by *Colletotrichum capsici* (Syd.) Butler & Bisby, is the most devastating, with a yield loss of up to 85% during periods of high humidity (Alabi 1994).

Faced with these biotic constraints, producers apply synthetic chemical pesticides for their effectiveness. But these chemicals have harmful consequences on the environment, human and animal health (Adam et al. 2010). Bio-pesticides can provide an alternative, as they have the advantage of being locally available, biodegradable, and having low toxicity for humans and the environment (Faye 2010; Elfeel and Warrag 2011; Sane et al. 2018; Traoré et al. 2019). Like most plant products with a biopesticidal effect, the antiparasitic activity of the bark, roots and seed extracts of *Balanites aegyptiaca* has already been the subject of numerous studies, which have identified insecticidal, fungicides, antimicrobial, vermicides and vermicide, and faciolicidal (Dwivedi et al. 2009; Al Ashaal et al. 2010; Emad et al. 2012; Elamin and Satti 2013; Haruna et al. 2020; Toka et al. 2023). The objective of this work was to control the development of cowpea anthracnose by using extracts from the seeds of *Balanites aegyptiaca* (L.) Del.

Material and methods

Study site

The field trials were carried out in the locality of Akonolinga, located in agro-ecological zone V of Cameroon (forest zone with bimodal rainfall). The geographical coordinates of the site recorded using a GPS (Garmin model Etrex Legend HCX) are: 03°48.136'N, 012°15.518'E, for an average altitude of 671 m (accuracy of ± 3 m). The site was left fallow for four years with the previous crops: cassava, peanuts, cocoyam and plantain. The climate of Akonolinga is a sub-equatorial climate of the Congo-Guinean type, with two dry seasons alternating with two rainy seasons. The average rainfall is 1633 mm/year, divided into a major rainy season, from March to June, and a short rainy season, from September to November. The average annual temperature is relatively constant, around 23 to 27 °C. The average annual humidity is around 80%. The soil belongs to the group of ferrallitic soils of acidic, lateritic, sandy loam and marshy rocks. It is characterized by outcrops of

the hardened horizon in the form of slabs or gravel, which sterilize large areas of land (Moudingo 2007).

Plant material

The plant material consists of the seeds of two improved cowpea varieties. The V1 variety: Tiligré (KVBX-775-33-2G), whose development cycle is 70 days, and the V2 variety (Niébé Lori 24-130), very resistant to attacks by insect pests, supplied by Institute of Agricultural Research for Development (IRAD) of Maroua and the choice of these varieties is linked to their good yield potential (1500–2000 kg ha⁻¹) (Ouedraogo et al. 2011). As a biopesticide plant, the seeds of *Balanites aegyptiaca* (almonds) were used for the tests.

Experimental design

The split-plot experimental design with four replicates was used with two varieties constituting the main plots randomly in replication (V1: Tiligré) and V2: Lori 24-130) and five treatments represented sub-plots randomized within the main plot (T0: control; T-AqE: aqueous extract; T-AcE: acetone extract; T-ME: methanol extract; T-Fong: synthetic fungicide containing 80% of maneb). Each block is composed of 10 sub-plots, for a total of 40 experimental units. The experimental units, which measure approximately 3 m $\times$ 2 m, are separated from each other by paths of 0.5 m. The blocks, for their part, are 1 m apart from each other. Each experimental unit was made up of 3 rows, sown with a spacing of 80 cm $\times$ 40 cm, three seeds per plot (Lalsba and Drabo 2017; Traoré et al. 2019). The experimental design was spread over an area of 400 m².

Preparation of treatments

The aqueous extract of *Balanites aegyptiaca* was prepared according to the method proposed by Zhang et al. (2018). The mature fruits are collected under the desert date tree. They were freed of their epicarps and mesocarps by soaking in water. After drying in the sun, the fruits containing the endocarp were crushed manually using a hammer against a stone to obtain the kernels. Using an electric mill, the almonds were crushed, and 500 g of almond powder were weighed using a precision balance from the "Sartorius Ag Gottingen" brand and macerated in 5 L of water for at least 24 hours (Gayathri and Sahu 2015). After filtration using a muslin cloth, the solution obtained with a concentration of 100 g L⁻¹ was poured into a 15 L backpack sprayer, to which 10 g of powdered soap was added as a wetting agent, to reinforce the adhesion of the products to the parts of the plant to be treated and limiting their leaching by rainwater. The mixture thus obtained is ready for the treatment of the 8 sub-plots concerned for this extract. The preparation of organic extracts was made by maceration of the almond powder of *Balanites aegyptiaca* seeds, at a rate of 500 g in 2 L (concentration: 250 g

L⁻¹) of Acetone and Methanol at 70 °C for 48 hours. The products obtained were filtered using filter paper and concentrated in a Rota-vapor (Büchi R-200 Rotary Evaporator at 60 °C), until the essential oils were obtained. The extracts obtained were placed in a desiccator to eliminate the remaining solvent. Subsequently, they were weighed and stored in the refrigerator at 4 °C until use (Gata-Gonçalves et al. 2003). *Balanites aegyptiaca* seed extracts were diluted at a rate of seven (7) mL for 1 L of water, 35 mL for 5 L of water to treat the 8 sub-plots concerned (Dabiré-Binso et al. 2008). Fungicide with active ingredient 80% Maneb was used following the dose recommended by the manufacturer 3.33 g L⁻¹. The fungicide Plantineb 720 WP with active ingredient 80% Maneb was used.

Pathogen identification

The identification of pure isolate was made by observing the cultural and microscopic characteristic of *Colletotrichum capsici* and his comparison to a reference *C. capsici* isolate. In culture on PDA culture medium, *C. capsici* has a gray mycelial mass characterized by an absence of sclerotia. Under the microscope, the acervuli are made up of conidiophores producing conidia and numerous long, black brown to black bristles protruding from the conidial mass. The conidia are unicellular, hyaline, fusoid with rounded and slightly hooked ends, most often falcate. The identification key r described by Sérémé et al. (2001) was used.

Disease incidence and severity measurement

Incidence is the proportion of diseased plants within a given experimental unit, independently of the severity of the attack on each plant; the number of plants attacked by diseases out of the total number of plants in the plot. The incidence is evaluated every two weeks interval from the first week of application of the treatments, on the 10 plants labeled randomly in each experimental unit (leaves, stems, pods) (Aroga 2007). The incidence is determined according to the formula of Tchoumakov and Zaharova (1990).

$$I(\%) = \frac{N_{pa} \times 100}{N_{pt}}$$

Where I (%) : is the incidence of diseases in the plot expressed as a percentage, N_{pa}: is the number of plants attacked by diseases in the plot and N_{pt} the total number of plants in the plot.

Severity of disease on pods

Severity is the degree to which an organ or entire plant is attacked by a disease. Severity is assessed visually every two weeks interval as soon as the first symptoms of the disease appear, on the 10 plants randomly labeled in each experimental unit (leaves, stems, pods). (Aroga 2007).

Severity is determined using the formula proposed by Tchoumakov and Zaharova (1990).

$$S(\%) = \frac{\sum (ab)}{N} \times 100$$

Where: S (%) is the severity, is the sum of the products of the number of diseased or attacked plants (a) by the degree of infection (b) given in % and N is the number of sick or attacked plants.

Evaluation of the effect of different extracts of *Balanites aegyptiaca* seeds on yield

The production parameters are measured considering the number of mature pods at 80 days after sowing (DAS) for the 2 varieties of cowpea per labeled plant and per experimental unit of each block. The grains obtained after shelling the pods were dried and weighed, then the weight was determined in each elementary plot according to the treatments and varieties. The values obtained were subsequently estimated per hectare using the formula from Zakari (2003).

$$\text{Yield kg/ha} = \frac{\text{weight/plot (g)}}{\text{Surface parcellaire (m}^2\text{)}} \times \frac{10\,000\text{ m}^2}{1\text{ ha}} \times \frac{1\text{ kg}}{1000\text{ g}}$$

Statistical analysis

The data from field observations were subjected to one-way and two-way ANOVA using R software version 4.0.1. Then multiple comparisons of means were determined on data, and the Tukey test was used to separate them when the analysis of variance was significant. Principal component analysis (PCA) and cluster were performed using RStudio interface between varieties, treatments, epidemiological parameters (incidence and severity), and yield, with a view to detecting the correlation between varieties and treatments less susceptible to anthracnose.

Results

Morphological characterization of *Colletotrichum capsici* isolate

In PDA medium, *C. capsici* has a gray mycelial mass characterized by an absence of sclerotia. This species of *Colletotrichum* produces rounded or elongated acervula. From these acervuli arise abundant septate brown-black bristles, having a lanceolate terminal cell or having the same diameter as the central cells, numerous small black dots corresponding to the fruiting bodies, isolated or in groups (the acervuli). Under the microscope, the acervuli are made up of conidiophores producing conidia and numerous long, black brown to black bristles protruding from the conidial mass. The conidia are unicellular, hyaline, fusoid with rounded and slightly hooked ends, most often falcate (Fig. 1).



Figure 1. Microscopic and macroscopic observation of the conidia of *Colletotrichum capsici*; **A.** Pure culture; **B.** Conidia.

Effect of *Balanites aegyptiaca* seed extracts and varieties on the incidence of cowpea anthracnose

During the 2020 and 2021 campaigns, a significant difference at ($P < 0.001$) is recorded between the different treatments for both varieties of cowpea. In 2020, the plot treated with aqueous (T-AqE) and organic extracts of *B. aegyptiaca* seeds reduced the incidence of anthracnose at 5, 7 and 9 weeks after sowing (WAS). However, at 9 WAS, the disease incidence rate is higher in the control plots (T0: 75.38% and 76.01%) respectively for V1 and V2 and lower in the plots treated with the aqueous (T-AqE: 20.91% and 28.24%) and acetone (T-AcE: 22.75% and 29.28%) extract, respectively for V1 and V2 during the 2020 campaign. Fungicide treatment (T-F: 14.6% and 14.19%) recorded lower disease incidence respectively for V1 and V2 (Table 1).

As for the 2021 campaign, the plots treated with aqueous (T-AqE: 19.13% and 20.81%); acetone (T-AcE: 19.25% and 22.21%) and methanol (T-ME: 17.93% and 24.15%) extract recorded the lower incidence of anthracnose compared to the control treatment which recorded the higher incidence (T0: 79.06 and T0: 72.43%) respectively for

varieties V1 and V2 (Table 1). Fungicide treatment (T-F: 25.54 and 37.6%) recorded a higher incidence of anthracnose than *B. aegyptiaca* seed extracts respectively for V1 and V2 at 9 WAS.]

Effect of *Balanites aegyptiaca* seed extracts and variety on the severity of cowpea anthracnose

A significant difference was recorded between treatments and varieties ($P < 0.001$) during 2020 and 2021 campaigns (Table 2). Thus in 2020 campaign at 7 WAS, the rate of anthracnose severity was more reduced in plot treated with *B. aegyptiaca* seeds extract (T-AqE: 22.7%; T-AcE: 25.24% and T-ME: 27.69%) compared to the control plot which recorded the highest severity (T0: 49.83%) for variety V1. For V2 variety at the same period, the rate of anthracnose severity in the control plot was higher (T0: 44.31%), compared to the plot treated with aqueous (T-AqE: 15.61%), acetone (T-AcE: 22.44%) and methanol (T-ME: 22.75%) extracts. But fungicide treatments recorded the higher rate of severity due to anthracnose (T-F: 19.63% and 18.8%) for V1 and V2 respectively. During the 2021 campaign at 9 WAS, plot treated with aqueous (T-AqE: 10.6% and 20.48%), acetone (T-AcE: 12.2% and 12.3%) and methanol (T-ME: 11.63% and 14.39%) extracts recorded the lower rate of disease severity compared to the control plot (T0: 64.14% and 59.82%), respectively for the V1 and V2 variety (Table 2).

Effect of different treatments on yield during the 2019/2020 and 2020/2021 campaigns

During the two campaigns, a significant difference ($P < 0.001$) was recorded between the treatments $\times$ varieties (Table 3). In the 2020 campaign, plot treated with aqueous extract, acetone and methanol recorded a better yield

Table 1. Effect of *Balanites aegyptiaca* seed extracts on the incidence of the disease as a function of time during two campaigns.

Varieties	Treatments	2020			2021		
		5 WAS	7 WAS	9 WAS	5 WAS	7 WAS	9 WAS
V1	T0	67.80 $\pm$ 0.62a	72.80 $\pm$ 0.52a	75.38 $\pm$ 0.70b	61.38 $\pm$ 0.72a	69.95 $\pm$ 0.54a	79.06 $\pm$ 0.48a
	T-AqE	27.83 $\pm$ 0.41g	22.78 $\pm$ 0.53h	20.91 $\pm$ 0.42h	54.08 $\pm$ 1.17c	32.78 $\pm$ 0.26f	19.13 $\pm$ 0.33g
	T-AcE	32.83 $\pm$ 0.32e	23.88 $\pm$ 0.43g	22.75 $\pm$ 0.53e	58.83 $\pm$ 0.21b	31.38 $\pm$ 0.75f	19.25 $\pm$ 0.87g
	T-ME	33.55 $\pm$ 0.33e	27.78 $\pm$ 0.63e	17.83 $\pm$ 0.42f	58.80 $\pm$ 0.48b	34.78 $\pm$ 0.26de	17.93 $\pm$ 0.67g
	T-Fong	30.52 $\pm$ 0.40h	20.78 $\pm$ 0.30k	14.60 $\pm$ 0.10h	50.50 $\pm$ 0.54g	38.61 $\pm$ 0.26g	25.54 $\pm$ 0.14h
V2	T0	65.90 $\pm$ 0.20a	72.83 $\pm$ 0.20a	76.01 $\pm$ 0.4a	50.90 $\pm$ 1.23d	61.15 $\pm$ 0.85b	72.43 $\pm$ 0.87b
	T-AqE	35.65 $\pm$ 0.20h	28.19 $\pm$ 0.60i	28.24 $\pm$ 0.6g	49.64 $\pm$ 0.5e	38.33 $\pm$ 1.15c	20.81 $\pm$ 0.56f
	T-AcE	38.45 $\pm$ 0.50f	30.12 $\pm$ 0.20f	29.28 $\pm$ 0.4d	48.45 $\pm$ 0.48ef	34.62 $\pm$ 0.63e	22.21 $\pm$ 1.00f
	T-ME	39.88 $\pm$ 0.25f	29.30 $\pm$ 0.30d	26.69 $\pm$ 0.2 ^c	47.55 $\pm$ 0.71f	36.30 $\pm$ 0.29d	24.15 $\pm$ 0.54e
	T-Fong	28.29 $\pm$ 0.20g	21.31 $\pm$ 0.30j	14.19 $\pm$ 0.5g	41.92 $\pm$ 1.11h	37.43 $\pm$ 0.83d	37.60 $\pm$ 0.42h
	P (V)	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***
	P (T)	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***
	P (V $\times$ T)	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***

P: 0 ***; V: Varieties; T: Treatments; V $\times$ T: Interaction; Means followed by the same letter in the columns are not significantly different in the Tukey test at ($P < 0.05$), T0 = Control treatment, T-AqE = Aqueous extract treatment, T-AcE = Acetone extract treatment, T-ME = Methanol extract treatment, T-Fong = Fungicide treatment, V1 = Tiligre, V2 = Lori 24-130, WAS = Week after sowing.

Table 2. Effect of *Balanites aegyptiaca* seed extracts on disease severity as a function of time during two campaigns.

Varieties	Treatments	2020			2021		
		5 WAS	7 WAS	9 WAS	5 WAS	7 WAS	9 WAS
V1	T0	42.94 ± 0.25a	49.83 ± 0.46a	54.64 ± 0.65a	45.83 ± 0.53a	51.08 ± 0.55a	64.14 ± 0.98a
	T-AqE	29.63 ± 0.32ef	22.70 ± 0.06f	14.6 ± 0.12f	38.83 ± 0.54b	18.45 ± 0.91hi	10.60 ± 0.12h
	T-AcE	32.95 ± 0.5d	25.24 ± 0.62d	16.2 ± 0.78e	32.83 ± 0.73de	22.99 ± 0.91ef	12.20 ± 0.55gh
	T-ME	34.80 ± 0.30c	27.69 ± 0.43c	20.51 ± 0.55d	36.05 ± 0.78c	19.69 ± 0.43gh	11.63 ± 0.72gh
	T-Fong	28.77 ± 0.5fg	19.63 ± 0.46g	9.78 ± 0.09h	32.59 ± 0.41de	30.32 ± 0.6d	27.03 ± 0.82e
V2	T0	36.01 ± 0.50c	44.31 ± 0.40b	48.77 ± 0.30b	38.54 ± 0.8b	48.06 ± 0.66b	59.82 ± 0.13b
	T-AqE	28.24 ± 0.33g	15.61 ± 0.54h	4.35 ± 0.44j	29.71 ± 0.81f	28.55 ± 0.56d	20.48 ± 0.80f
	T-AcE	30.28 ± 0.82e	22.44 ± 0.31f	12.10 ± 0.50g	33.79 ± 0.57e	21.19 ± 1.14fg	12.3 ± 0.56gh
	T-ME	28.69 ± 0.43fg	22.75 ± 0.19f	12.76 ± 0.30g	32.50 ± 0.51de	23.25 ± 1.06e	14.39 ± 0.83g
	T-Fong	28.96 ± 0.80efg	18.80 ± 0.16g	9.10 ± 0.13h	32.74 ± 0.71de	17.61 ± 0.45ij	9.47 ± 0.37hi
	P (V)	< 0.001***	< 0.001***	< 0.001***	0.02727 *	< 0.001***	< 0.001***
	P (T)	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***
	P (VxT)	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***

P: 0 '***', V: Varieties; T: Treatments; V × T: Interaction; Means followed by the same letter in the columns are not significantly different in the Tukey test at (P < 0.05), T0 = Control treatment, T-AqE = Aqueous extract treatment, T-AcE = Acetone extract treatment, T-ME = Methanol extract treatment, T-Fong = Fungicide treatment, V1 = Tiligre, V2 = Lori 24-130, WAS = Week after sowing.

Table 3. Yield of two cowpea varieties (kg/ha) depending on treatments during two campaigns.

Treatments	2020		2021	
	V1	V2	V1	V2
T0	518.03 ± 3.25j	545.05 ± 1.95i	385.37 ± 1.82j	760.47 ± 1.34h
T-AqE	794.05 ± 3.33h	926.63 ± 1.67c	852.97 ± 1.85f	935.79 ± 2.09d
T-AcE	859.15 ± 4.52e	860.23 ± 1.04d	823.69 ± 1.78g	961.68 ± 1.56c
T-ME	804.55 ± 2.91g	866.15 ± 1.82d	658.77 ± 1.11i	885.74 ± 2.04e
T-Fong	894.94 ± 2.05a	901.23 ± 2.99b	850.1 ± 1.48e	1050.68 ± 1.87a
Pr (> F) V	< 0.001***	< 0.001***	< 0.001***	< 0.001***
Pr (> F) T	< 0.001***	< 0.001***	< 0.001***	< 0.001***
Pr (> F) VxT	< 0.001***	< 0.001***	< 0.001***	< 0.001***

P: 0 '***', V: Varieties; T: Treatments; V × T: Interaction; Means followed by the same letter in the columns are not significantly different in the Tukey test at (P < 0.05), T0 = Control treatment, T-AqE = Aqueous extract treatment, T-AcE = Acetone extract treatment, T-ME = Methanol extract treatment, T-Fong = Fungicide treatment, V1 = Tiligre, V2 = Lori 24-130, WAS = Week After Sowing.

respectively (T-AqE: 794.05 ± 3.33 kg.ha⁻¹ and 926.63 ± 1.67 kg.ha⁻¹); (T-AcE: 859.15 ± 4.52 kg.ha⁻¹ and 860.23 ± 1.04 kg.ha⁻¹) and (T-ME: 804.55 ± 2.91 kg.ha⁻¹ and 866.15 ± 1.82 kg.ha⁻¹) compared to the control plot (T0: 518.03 ± 3.25 kg.ha⁻¹ and 545.05 ± 1.95 kg.ha⁻¹) which recorded the lower yield, respectively for V1 and V2 (Table 3). During the 2021 campaign, the yield was higher in the aqueous extract treatments (T-AqE: 852.97 ± 1.85 kg.ha⁻¹ and 935.79 ± 2.09 kg.ha⁻¹), at acetone (T-AcE: 823.69 ± 1.78 kg.ha⁻¹ and 961.68 ± 1.56 kg.ha⁻¹) and methanol (T-ME: 658.77 ± 1.11 kg.ha⁻¹ and 885.74 ± 2.04 kg.ha⁻¹) compared to the control plot (T0: 385.37 ± 1.82 kg.ha⁻¹ and 760.47 ± 1.34 kg.ha⁻¹) which recorded the lower average yield respectively for the V1 and V2 varieties. At the end of the observations from the point of view of yield and other parameters evaluated, the most productive variety is the V2 variety (cowpea Lori 24-130).

Multivariate analysis

Principal component analysis

During the campaign 2020, principal component analysis (PCA) made it possible to group the parameters studied and the two cowpea varieties according to their proximity on the axes. The different treatments for each cowpea

variety (T0V1, T1V1, T2V1, T3V1, T4V1 and T0V2, T1V2, T2V2, T3V2, T4V2) are screened based on six variables including the total number of leaves, the diameter at crown, plant height, incidence of anthracnose, severity of anthracnose and yield (Yield) for 5, 7 and 9 weeks of observation. The system provides reliable information with a good rate of restitution of information on the total variability on axes 1 and 2 of (86.45%) with two groups trained (Fig. 2A). Group 1 consists of control plot of the two cowpea varieties V1 (Tiligré) and V2 (Lori 24-134). They are very close to the incidence and severity of anthracnose. Group 2 is composed of treatments T4, T3, T2 and T1 respectively the fungicide treatment (T4 = T-Fong), the methanol extract treatment (T3 = T-ME), the extract treatment with acetone (T2 = T-AcE) and treatment with the aqueous extract (T1 = T-AqE) of the two varieties V1 (Tiligré) and V2 (Lori 24-130). They are closer to growth parameters including total number of leaves, plant height and diameter at the collar and characterized by high yield.

During the 2021 campaign, the dispersion visualized represents approximately 91.27% (axis 1 and axis 2) of the variation of the system studied (Fig. 2B). The first group is made up of control treatment T0 of the two varieties V1 and V2 which is distant from the growth parameters and yield but closer to the incidence and severity of

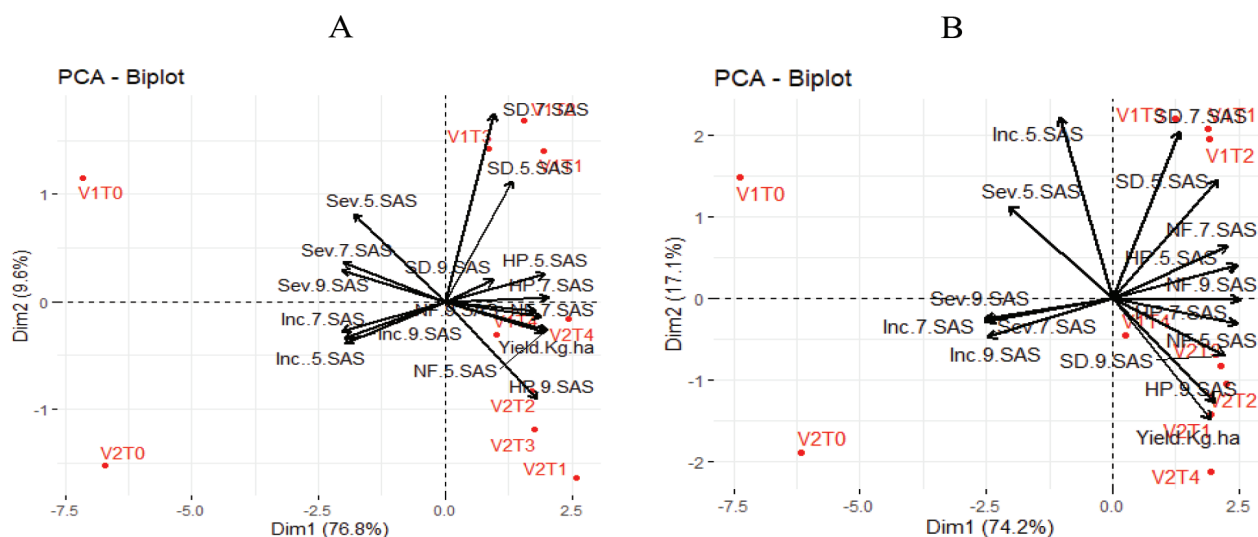


Figure 2. Principal component analysis between the treatments and the parameters studied in the two cowpea varieties during the 2020 and 2021 campaigns. T0 = Control treatment, T-AqE = Aqueous extract treatment, T-AcE = Acetone extract treatment, T-ME = Methanol extract treatment, T-Fong = Fungicide treatment, V1 = Tiligré, V2 = Lori 24-130.

anthracnose. Group 2 consists of the extract treatment (T1 = T-AqE); of the acetone extract treatment (T2 = T-AcE) and the methanol extract treatment (T3 = T-ME) of the V1 variety which is far from the incidence and severity of the anthracnose and closer to the growth parameters. Group 3 consists of fungicide treatment (T4 = T-Fong.), treatment with aqueous extracts (T1 = T-AqE), treatment with acetone extract (T2 = T-AcE), from the treatment with the methanol extract (T3 = T-ME) of the two varieties V1 and V2 which is far from the incidence and severity of anthracnose and closer to the growth parameters and yield.

Cluster analysis

The dendrogram (dissimilarity of 5%) of the different treatments and the variety according to the different parameters studied shows two groups formed (Fig. 3). Group 1 is made up of the control treatment T0 of the two varieties V1 (Tiligré) and V2 (Lori 24-130) and group 2 is made up of the treatments T1, T2, T3 and T4 of the

two varieties (V1 and V2). The dendrogram (dissimilarity of 5%) of the different treatments and the variety according to the different parameters studied shows four groups of treatments (Fig. 3B). The first group is made up of the control treatment T0 of the two varieties V1 (Tiligré) and V2 (Lori 24-130); the second is made up of the T4 treatment of the varieties V1 and V2 and the third is made up of the T3 treatment of the two varieties (V1 and V2) and the fourth group is made up of the treatments T1, T2, T3 and T4 of the two varieties V1 and V2.

Discussion

Cowpea (*Vigna unguiculata* L.) is the third most widely grown legume after groundnuts and beans, due to its high protein content. However, its productivity remains limited each year due to several biotic and abiotic constraints that farmers face on a daily basis. To overcome

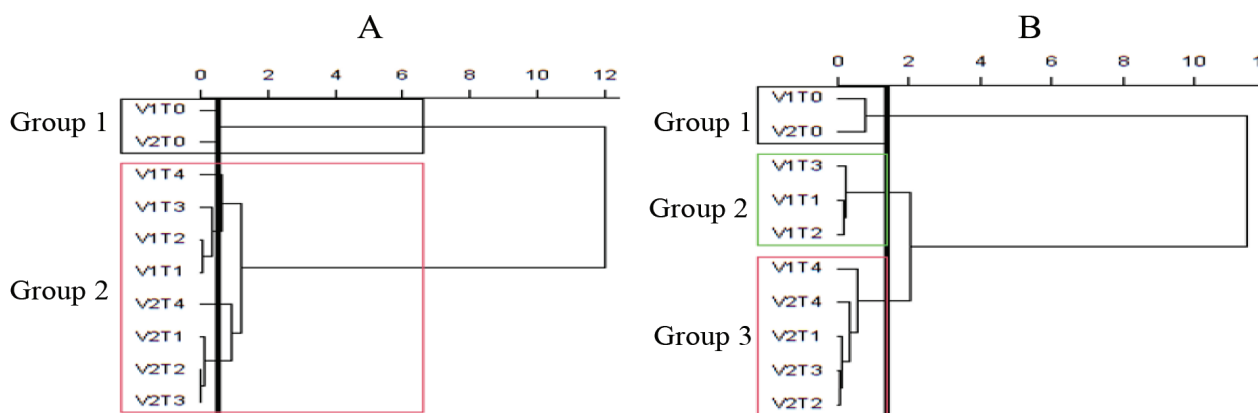


Figure 3. Dendrogram of reconciliation between the treatments and the parameters studied in each variety of cowpea during the 2020 and 2021 campaigns. T0 = Control treatment, T-EAq = Aqueous extract treatment, T-AcE = Acetone extract treatment, T-ME = Methanol extract treatment, T-Fong = Fungicide treatment, V1 (Tiligré) and V2 (Lori 24-130).

biotic constraints and ensure food security, many countries use synthetic pesticides. Although effective, their intensive and uncontrolled use has many drawbacks for the environment and human health (Gueye et al. 2011; Belkebir 2018).

Morphological characterization was used to identify isolates using infected cowpea pods on PDA medium. Under the microscope, the acervuli are made up of conidiophores producing conidia and numerous long, black brown to black bristles protruding from the conidial mass. The conidia are unicellular, hyaline, fusoid with rounded and slightly hooked ends, most often falcate (Emechebe 1981; Sérémé et al. 2001).

The use of plant extracts as a phytosanitary product could offer a solution as an alternative to synthetic pesticides, especially as these extracts are biodegradable and locally available (Sane et al. 2018; Sherin 2018). Several studies have shown the biopesticidal effect of plant extract rich in naturally occurring compounds (Djeugap et al. 2023; Dida et al. 2024). Like most products with biodegradable pesticide effect, the biological activity of *B. aegyptiaca* seeds has already been the subject of numerous studies, at the end of which it has been attributed, among other things, fungicidal properties (Toka et al. 2023). The present work examined the fungicidal properties of these aqueous and organic extracts of *Balanites aegyptiaca* seeds on the development of *Colletotrichum capsici* (agent responsible for brown rot of cowpea). The results obtained on the evaluation of the epidemiological parameters (incidence and severity) of anthracnose during the 2020 and 2021 campaigns showed a positive action of the treatments on the brown spot disease in cowpea crops, characterized by a gradual decline in the incidence and severity of the disease. These two parameters depend on the stage of development of the plants; the type of treatment, the variety and the growing season. These results are similar to those of (Godlewska et al. 2021; Flore et al. 2023) who showed the importance of plant extracts in protecting crops against fungal diseases for sustainable agriculture; similarly, Bolou Bi-Bo-

lou (2015), showed that extracts of *Xylopi aethiopica* strongly reduce the incidence of disease caused by *Sclerotium rolfsii* on treated tomato plants and moreover (Sané et al. 2018; Djeugap et al. 2023). Also showed the effectiveness of aqueous extracts of *Artemisia annua*, *Commelina benghalensis* and *Euphorbia hirta* on growth, yield and incidence of brown spot disease in rice crops. The aqueous and organic extracts of *B. aegyptiaca* seeds have an effect on the incidence and severity of diseases that is significantly greater than that observed in the control plots. Bioactive compounds in *B. aegyptiaca* seed extracts have been identified by several researchers as having antifungal, antimicrobial, insecticidal and antibacterial activity (Rubila and Ranganathan 2014; Habiebballa et al. 2021). The specific mechanism of action of biochemical compounds in plant extracts is unclear, but it is likely that these biochemical compounds form complexes with polysaccharides and proteins associated with an outer layer of fungal cells, which can lead to destabilisation of cell membrane function, resulting in pathogen death (Rongai et al. 2017).

The Principal Analyses Component reveal that treatments with *B. aegyptiaca* extracts (AqE and ME) reduced the incidence and severity of *Colletotrichum capsici* and increased yields in the same way as the chemical fungicide treatment during both campaigns.

Conclusion

In this study, the aim was to control the development of cowpea anthracnose by using extracts from the seeds of *Balanites aegyptiaca* (L.) Del. in field. The evolution of epidemiological parameters showed a positive effect in plots treated with *B. aegyptiaca* extracts. This was characterized by a progressive reduction in the incidence and severity of anthracnose on cowpea plants compared with the control during the 2019–2020 and 2020–2021 seasons. The best yields were obtained in the treated plots of the two cowpea varieties.

References

- Abdallah EM, Hsouna ABS (2012) Antimicrobial, antioxidant and phytochemical investigation of *Balanites aegyptiaca* (L.) Del. edible fruit from Sudan. African Journal of Biotechnology 11(52): 11535–11542. <https://doi.org/10.5897/AJB12.1102>
- Adam S, Edorh PA, Totin H, Koumoulou L, Amoussou E, Aklilikou K, Boko M (2010) Pesticides and heavy metals in drinking water, soils and sediments of the cotton belt of Gogounou, Kandi and Banikoara (Benin). International Journal of Biological and Chemical Sciences 4(4): 1170–1179. <https://doi.org/10.4314/ijbcs.v4i4.63054>
- Al Ashaal HA, Farghaly AA, Abd El Aziz MM, Ali MA (2010) Phytochemical investigation and medicinal evaluation of fixed oil of *Balanites aegyptiaca* fruits (Balantiaceae). Journal of Ethnopharmacology 127: 495–501. <https://doi.org/10.1016/j.jep.2009.10.007>
- Alabi O (1994) Epidemiology of cowpea brown blotch induced by *Colletotrichum capsici* and assessment of crop losses due to the disease. PhD thesis, Ahmadou, Bello University. Zaria, Nigeria, 95 pp.
- Aroga R (2007) Main insect pests of corn and their antagonists in forest areas.
- Belkebir S (2018) Evaluation of the insecticidal effect, on aphids, of the aqueous extract of leaf and flower of *Moringa oleifera* Lam. Master's thesis in Agricultural Science, 79 pp.
- Bolou Bi BA (2015) Inhibition of *Sclerotium rolfsii* Sacc. (Corticaceae), causal agent of tomato stem crown rot, by *Xylopi aethiopica* (Dunal) A. Rich. (Annonaceae) and *Trichoderma* sp. European Scientific Journal, ESJ 11(12): 61–85.
- Dabiré/Binsou C, Ba NM, Sanon A (2008) Training module on insect pests of cowpea in cultivation. 9 pp.

- Dida SLL, Kuate WNT, Ngatsi P, Mboussi SB, Kone NAN, Gbaporo GCF, Ambang Z (2024) First report of different mating types of *Phytophthora infestans* isolates from potatoes in Cameroon and their control using *Thevetia peruviana* (Pers.) K. Schum. seeds extracts analyzed by GC–MS. *Indian Phytopathology* 77: 363–375. <https://doi.org/10.1007/s42360-024-00720-6>
- Djeugap JF, Mbatkam B, Sonkoue MA, Dida SL, Galani HJY, Mbi C, Lapa M, Anchang KY (2023) Field efficacy of aqueous extracts of *Artemisia annua*, *Commelina benghalensis* and *Euphorbia hirta* on rice growth, yield and brown spot disease incidence. *Agriculture and Natural Resources* 57: 417–426. <https://doi.org/10.34044/jan-res.2023.57.3.05>
- Dwivedi A, Joshi V, Barpete PK, Akhtar AK, Kaur A, Kumar S (2009) Anthelmintic activity of root bark of *Balanites aegyptiaca* (L.) Del. *Ethnobotanical Leaflets* 13: 564–567.
- Elfeel AA, Warrag EI (2011) Conservation status of *Balanites aegyptiaca* (L.) Del. (Hegleig Tree) in Sudan: Local people perspective. *Journal of Agricultural Science* 3(4): 386–390.
- Elamin MM, Satti AA (2013) Insecticidal potentialities of *Balanites aegyptiaca* extracts against the khapra beetle (*Trogoderma granarium*). *Global Advanced Research Journal of Environmental Science and Toxicology* 2: 005–010.
- Emebebe AM (1981) Brown blotch of cowpea in northern Nigeria at Samaru. *Journal of Agricultural Research* 1(1): 20–26.
- Faye M (2010) New process for splitting Senegalese neem seeds (*Azadirachta indica* A. JUSSI): production of an oil and cake bio-pesticide. Doctoral thesis from the University of Toulouse, France, 267 pp.
- Flore M, Martial T, Ebenezer F, Aristide D (2023) Formulation of Bio-fungicides from *Cymbopogon citratus* and *Tithonia diversifolia*: Evaluating its antimicrobial activities against *Pythium myriotylum*, the causal agent of root rot of *Xanthosoma sagittifolium* (L.) Schott. *American Journal of Plant Sciences* 14: 896–914. <https://doi.org/10.4236/ajps.2023.148060>
- Gata-Gonçalves L, Nogueira JMF, Matos O, De Sousa B (2003) Phytoactive extract from *Thevetia peruviana* with antifungal properties against *Cladosporium cucumerinum*. *Journal of Photochemistry and Photobiology* 70(1): 51–54. [https://doi.org/10.1016/S1011-1344\(03\)00024-1](https://doi.org/10.1016/S1011-1344(03)00024-1)
- Gayatri N, Sahu RK (2015) Biopesticidal effect of leaf extract of neem (*Azadirachta indica* A. Juss) on growth parameters and diseases of tomato. *Journal of Applied and Natural Science* 7(1): 482–488. <https://doi.org/10.31018/jans.v7i1.636>
- Ghosh S, Tiwari SS, Srivastava S, Sharma AK (2013) Acaricidal properties of *Ricinus communis* leaf extracts against organophosphate and pyrethroids resistant *Rhipicephalus (Boophilus) microplus*. *Veterinary Parasitology* 192: 259–267. <https://doi.org/10.1016/j.vet-par.2012.09.031>
- Godlewska K, Ronga D, Michalak I (2021) Plant extracts – Importance in sustainable agriculture. *Italian Journal of Agronomy* 16(2). <https://doi.org/10.4081/ija.2021.1851>
- Gueye MT, Seck D, Wathelet JP, Lognay G (2011) Control of pests of cereal and legume stocks in Senegal and West Africa: synthesis. *Bio-technology and Agronomy Society of Environment* 15(1): 183–194.
- Habiebballa AG, Alebead HE, Koko MK, Ibrahim AS, Wady AF (2021) Antimicrobial activity and physicochemical properties of *Balanites aegyptiaca* seed oil. *European Journal Chemistry* 12: 450–453. <https://doi.org/10.5155/eurjchem.12.4.450-453.2142>
- Hamidou F, Harou A, Achirou B, Halilou O, Bakasso Y (2018) Nitrogen fixation in peanuts and cowpeas under drought conditions for improving productivity in the Sahel. *Tropicicultura* 36(1): 63–79.
- Hartman GL, Chang HX, Leandro LF (2015) Research advances and management of soybean sudden death syndrome. *Crop Protection* 73: 60–66. <https://doi.org/10.1016/j.cropro.2015.01.017>
- Haruna A, Jada MY, Gali AI, Abaka, AK (2020) Antifungal activities and phytochemical analysis of *Balanites aegyptiaca* seed kernel extract against *Colletotrichum gloeosporioides* causing anthracnose disease in mango. *Dutse Journal of Agriculture and Food Security (DUJAFS)* 7(1): 48–54.
- Husson O, Charpentier H, Razanamparany C, Moussa N, Michellon R, Naudin K, et al. (2010) Corn or sorghum associated with a twining food legume (Cowpea or *Vigna umbellata*). Practical manual for direct sowing in Madagascar, Volume I: Chapter I. growing cowpea on.
- Jackson JC (2009) Protein nutritional of cowpea quality and navy bean residue fractions. *African Journal of Food, Agriculture, Nutrition and Development* 9: 764–778. <https://doi.org/10.4314/ajfand.v9i2.19225>
- Kaboré (2004) The nitrogen contributions of legumes and organomineral amendments in cropping systems: impact on cereal yields and long-term soil fertility. Memory IDRI/Agronomy/Polytechnic University of Bobo Dioulasso, Burkina Faso, 77 p.
- Maimouna AH, Ibrahim B, Mahamane MR, Abdoul Aziz S, et al. (2020) Diagnostic study of the main constraints of cowpea cultivation (*Vigna unguiculata* L. Walp) in the regions of Maradi and Zinder in Niger. *Africa SCIENCE* 16(5): 32–43.
- Modu Y, Putai AJ, Petu-Ibikunle AM (2010) An economic analysis of cowpea production among women farmers in Askira/Uba Local Government Area Borno State Nigeria. *African Journal of Agricultural Research* 6: 7–17.
- Moudingo EJ (2007) Situation of the forests in Cameroon. *Cameroon Wild life Society Conservation*, 24 pp.
- Néya JB, Zida EP, Zinga I, Zemba P, TRAORÉ O (2019) Pathogenic, serological and molecular characterization of the cowpea mosaic virus (*Vigna unguiculata* (L.) Walp.) transmitted by aphids (CABMV) in isolates from Burkina Faso, Cameroon and the Central African Republic. *International Journal of Biological Chemistry*.
- Ouédraogo TJ, Tignégré JB, Drabo I, Dabiré C, Ba NM, Néya BJ, Batieno B, Sankara WO, Ilboudo D, Tapsoba B, Ouédraogo L, Bambio K, Herve B (2011) Sheet technique: Variety of tiligré cowpea. 5 pp.
- Remond D, Walrand S (2017) Legume seeds: nutritional characteristics and effects on health. *Agronomic Innovations*, INRA, 60.
- Rongai DP, Pulcini BP, Milan F (2017) Antifungal activity of pomegranate peel extract against *Fusarium* wilt of tomato. *European Journal of Plant Pathology* 147: 229–238. <https://doi.org/10.1007/s10658-016-0994-7>
- Rubila S, Ranganathan TV (2014) Evaluation of phytochemical constituents in various heat treatments of *Zingiber officinale* Roscoe and In vitro antioxidant assay systems. *International Research Journal of Biological Sciences* 3: 54–58.
- Sané B, Badiane D, Gueye MT, Faye O (2018) Evaluation of the biological efficacy of extract of neem (*Azadirachta indica* Juss.) as an alternative to pyrethroids for the control of the main cotton pests (*Gossypium hirsutum* L.) in Senegal. *International Journal of Biological and Chemical Sciences* 12(1): 157–167. <https://doi.org/10.4314/ijbcs.v12i1.12>

- Saraka AI, Abo K, Coulibaly K, Zirihi GN (2018) Phytochemical study and antifungal activity of extracts of some medicinal Euphorbiaceae used among the Baoulé of the Yamoussoukro District (Ivory Coast). *European Scientific Journal* 14(30): 1857–7881.
- Séréme P, Diasso GA, Ake S (2001) Identification in Burkina Faso of a new taxon of *Colletotrichum* in the etiology of cowpea brown spot disease. *Tropicultura* 19(3): 156–160.
- Sherin MMYH (2018) Insecticidal and biological effects of four plant oils on the cowpea beetle, *Callosobruchus maculatus* (F.). *Journal of Entomology and Zoology Studies* 6(2): 3111–3118.
- Tchumakov EE (1990) and Zaharova I. Influence and statistics of disease development. In: disease damage to agricultural crops. Edition Agroprom, Moscow, 5–60.
- Toka NA, Ngatsi ZP, Dida LSL, Tayo TPM, Kuate NWT, Boli H, Atindo TS, Tize T, Ndong B (2023) Phytochemical analysis and antifungal activity of *Azadirachta indica* and *Balanites aegyptiaca* seed extracts against *Fusarium oxysporum* isolate on tomatoes. *Journal of Global Innovations in Agricultural Sciences* 11(3): 293–304. <https://doi.org/10.22194/JGIAS/23.1159>
- Traoré F, Waongo A, Drabo E, Yamkoulga M, Dabire-Binso C, Sanon A (2019) Effet des périodes d'épandage des huiles de neem (*Azadirachta indica* L.) sur les populations de *Megalurothrips sjostedti* Trybom et de *Maruca vitrata* Fabricius dans la culture de niébé. *International Journal of Biological and Chemical Sciences* 13(3): 1300–1307. <https://doi.org/10.4314/ijbcs.v13i3.7>
- USDA (2004) USDA national nutrient database for standard reference, release 17. (Internet) U.S. Department of Agriculture, Agricultural Research Service, Nutrient Data laboratory, Beltsville Md, United States.
- Zakari AH (2003) Effects of seed quality on peanut production in Senegal. Thesis of the Agricultural Engineering Diploma, Specialization in crop production, ENSA, 59 pp.
- Zhang QW, Lin LG, Ye WC (2018) Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine* 13: 20. <https://doi.org/10.1186/s13020-018-0177-x>