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RESEARCH ARTICLE OPEN ACCESS

ANTIFUNGAL EFFECT OF AQUEOUS EXTRACTS OF *CHROMOLAENA ODORATA* AND *OCIMUM GRATISSIMUM* ON TWO PATHOGENIC FUNGI OF *VIGNA UNGUICULATA* (L.) WALP, IN DALOA (CENTER-WEST, CÔTE D'IVOIRE)

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ABSTRACT

This study is a contribution to the search for alternative solutions based on plant extracts to fight against phytopathogenic fungi. The objective is to test the effectiveness of doses of aqueous extracts of *C. odorata* and *O. gratissimum* on the development of two species of phytopathogenic fungi. The results showed that the extracts inhibit mycelial growth. These extracts significantly reduced the progression of two fungi, *Colletotrichum* sp. and *Rhizoctonia* sp. The severity averageratings of the two accessions ranged from 0.16 to 0.82. *Rhizoctonia* sp caused the most severe symptoms with an average severity of 0.76 and 0.82 respectively in the white and red accession. *Colletotrichum* sp was less severe on all two cowpea accessions studied with an average severity of 0.28. On the other hand, these extracts significantly reduced the infection of cowpea plants and the development of symptoms. The best yields were obtained with the lowest doses (25 g/l). They could be used in the treatment of cowpea plants and seeds. Research could also be carried out on its herbicidal properties on certain invasive legumes close to cowpeas.

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INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is the main food legume rich in protein after peanuts. The annual world production of cowpea varies between 3 and 5.5 million tonnes of dry seeds, of which Africa produces more than 60 % (FAOSTAT, 2013; Shankoetal, 2014). In Côte d'Ivoire, according to agricultural statistics, the annual production of cowpea is between 20.000 and 30.000 tonnes/year (Soule, 2002). Cultivated for its nutritional and economic benefit, the young, immature leaves and pods are eaten as a vegetable, while the seeds rich in protein and vitamins are used primarily as a pulse. Cowpeas are also important as fodder for livestock (Diawara, 2015). Thanks to its high protein content (25 %), cowpea is the primary source of vegetable protein. The seeds are used in the preparation of many dishes such as donuts, cookies and various pastas. Cowpea fans as well as pod pods are appreciated by livestock and sell well in the market, thus providing added value for producers. Indeed, the fixing capacity of atmospheric nitrogen gives cowpea an important place in the crop rotation system, especially in areas characterized by low soil fertility. The inclusion of cowpea in crop rotations makes it possible to meet crop nitrogen fertilizer needs (Diawara, 2015). Despite this importance, cowpea remains a marginal crop in Côte d'Ivoire. Yields rarely exceed 400 to 500 kg of seeds per hectare in traditional cultivation (Shanko *et al*., 2014). The constraints to cowpea production, like other crops, are mainly poor soil, irregularity and low rainfall. Add to this, the high pressure of pathogens, pests and weeds greatly reduce production. This is because pathogens can attack different parts of the plant at all stages of its development. The most important are of fungal origin and are the cause of diseases that can cause heavy losses (INRA, 2018). To eradicate these phytopathogens, several control methods, including chemical control, are being considered. In addition, although effective, chemicals can be toxic to both users and the environment. Therefore, the need to find an alternative to synthetic chemicals through the use of plant extracts minimizing the dangers both for producers and for the environment is imperative (Doumbouya *et al*., 2012). Research has successfully tested plant extracts with antifungal properties against fungal diseases of major crops. Plants such as *Azadirachta indica* and *Balanites aegyptiaca* have shown efficacy against the main pathogenic fungi sorghum and millet (Zida *et al*., 2008). This study is part of this dynamic and aims to use extracts from the leaves of *Ocimum gratissimum* and *Chromolaena odorata* to fight against fungi. The objective of this study is to improve the productivity of *Vigna unguiculata*, by using aqueous extracts of *Chromolaena odorata* and *Ocimum gratissimum* on the activity of two fungal strains.

MATERIAL AND METHODS

Study Site

The study was carried out on the site of the University Jean Lorougnon Guédé located in the department of Daloa. The city is located in the region of Haut Sassandra, in the center-west of Côte d'Ivoire between 6° and 7° North latitude and 7° and 8° West longitude. The Daloa soil substrate belongs to the old Precambrian basement composed of granites, migmatites. These soils, leached and deep (20 m) are due to abundant precipitation and rapid weathering of rocks. The soil is ferralitic with a 20 m deep leaching and a high rate of organic matter due to abundant precipitation (1276 mm) and rapid weathering of rocks (Tra Bi *et al*., 2014).

MATERIAL

Plant Material

The plant material used consisted on the one hand of plants from two accessions White (Figure 1A) and Red (Figure 1B) of cowpea, less than one year old and bought in a market in Daloa, and on the other hand share of leaves of *C. Odorata* (Figure 1C) and *O. gratissimum* (Figure 1D) collected at Jean Lorougnon Giédé University in Daloa. The choice of these two species is based on their inhibitory effect on thr growth of certain fungal strains (Oussou *et al*., 2004; Ngono *et al.,* 2006).

A: White accession of Cowpea; B: Red accession of Cowpea, C: *C. odorata* Plant; D: *O. gratissimum* Plant

Figure 1. Different plant materials

Fungal Material

The fungal material consisted of a strain of *Colletotrichum* sp. (Figure 2A and 2B) and a strain of *Rhizoctonia* sp. (Figure 2C and 2D). These two-week-old strains were provided by the Plant Pathology Laboratory of Jean Lorougnon Guédé University in Daloa, Côte d'Ivoire.The choice of these two species is based on their ability to cause various diseases many cultivated plant (Zida *et al*., 2008).

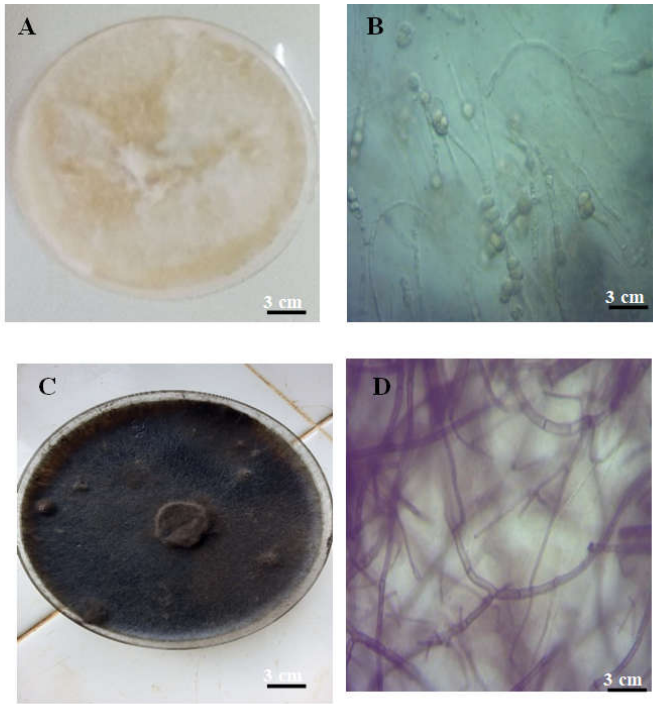


Figure 2. Different biological materials

METHODS

Experimental device: The experimental set-up consisted of two Fisher blocks with 5 repetitions. Each block (8 m x 3 m) represents a cowpea accession and is made up of 10 elementary plots one meter apart, or a total of 20 elementary plots. Each plot of 2.4 m^2 corresponds to one treatment and was made up of 5 bags of cultures, in a total of 50 bags of cultures per block. The distance between the bags of cultures of the same block was 0.6 m and the 5th bag was placed in the center of each elementary plot. In each bag, two cowpea seeds were sown, for a total of 200 seeds (Figure 3).

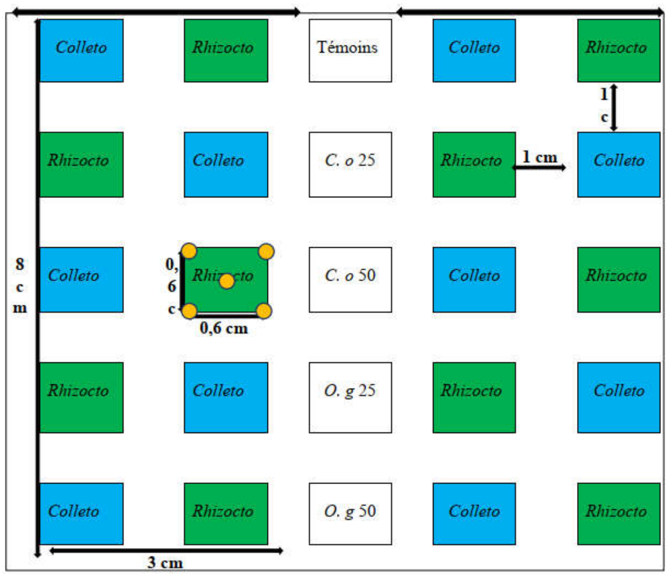


Figure 3. Experimental device

Preparation of the growing medium and sowing: The substrate was made up of soil taken from the cocoa trees located within the University Jean Lorougnon Guédé. The soil was heated to a temperature of 100 °C. for 50 min and allowed to cool on sheet metal placed under shelter for 24 hours. After filling the bags of cultures, a cowpea seed was sown in each bag at a depth of 2 cm and then regular watering was carried out.

Preparation of fungal inocula and inoculation of plants: The fungal inocula of *Colletotrichum* sp. and *Rhizoctonia* sp. were prepared according to the protocol of Nirina *et al*. (2014). For this purpose, the surface of each fungal strain contained in the Petri dishes was scraped under a hood using a sterile spatula, then 325 g of each of the strains was weighed using a balance. precision and suspension in an Erlenmeyer flask with a capacity of 1 L. After 24 h, the fungal suspension was filtered through filter paper and the resulting filtrate was used for inoculation of the cowpea accessions plants. Two weeks later, at the 3 to 5 leaf stage, the cowpea plants were thinned in order to keep the most vigorous. The inoculation consisted of spraying the cowpea leaves of the different elementary plots with 5 ml of each fungal filtrate and the controls were sprayed with 5 ml of the distilled water.

Preparation of different doses of aqueous extracts and plant spraying: The preparation of the plant extracts was done according to the protocol of Ackah*et al.* (2008). The fresh leaves of *C. odorata* and *O. gratissimum* were collected and brought to the laboratory. They were rinsed with tap water and dried in the shade at 25 ° C. After two weeks of drying, the leaves of each species were finely ground and the resulting powder stored in the dark and dry in sterile, hermetically sealed bottles for later use. The powder of each plant obtained was weighed in two different quantities (25 g and 50 g) and mixed separately with 1 liter of distilled water to obtain the concentrations of 25 g/l and 50 g/l. The resulting solutions were infused for 72 hours and filtered through cotton wool and then through wattman paper. After the first fungal symptoms appear on the leaves of cowpea accessions, the different doses of *C. odorata* and *O. gratissimum* were pulverized. For this purpose 5 ml of each dose of extracts was sprayed on the leaves of each accession of cowpea. Controls were sprayed with 5 ml of distilled water.

Assessment of pathogenicity parameters

Observation of the incubation period and description of symptoms: The incubation period, which is the period between inoculation and the appearance of the first symptoms, was determined for each cowpea accession and for each fungal strain. The various symptoms observed on the plants were noted and described on the basis of regular observations. Their appearance, shape, coloration and evolution with different fungal strains were noted during the experiment.

Infection rate of cowpea plants according to the fungal strains inoculated during the treatment with aqueous extracts of the two plants: During the experiment the infected plants were counted and the infection rate was calculated using the following formula:

$$\text{TI (\%)} = \frac{\text{NIP}}{\text{TNP}} \quad (1)$$

TI (%): Infection rate as a percentage
NIP: Number of infected plants
TNP: Total number of plants

Severity of symptoms: The severity of symptoms was noted for 10 days. The disease severity index caused by each fungal strain on infected leaves was determined by the percentage of diseased leaf area (S.F.M) estimated according to the scale of Vakalounakis & Fragkiadakis (2000). The leaves were assigned a score of 0 to 3 depending on the symptom condition.

$$\text{Pr} = \frac{\text{W}}{\text{s}} \quad (2)$$

Pr :Production in grams of cowpea seed per m^2
W : weight in g
S: Surface in m^2

0: No symptoms visible on the leaves;
1: slight yellowing, slight rot of taproot, crown and secondary roots;
2: severe yellowing of leaves with or without wilting, stunting of plants, severe rot of taproot and secondary roots, severe rot of crown and browning of stem vessels;
3: death of the plant.

Seed production evaluation: The seed production was evaluated by summing the values obtained at the 1st, 2nd and 3rd harvest as a function of the fungal strains and the doses of plant extract.

Statistical analysis of the data: A two-way analysis of variance (ANOVA 2) was used at threshold α = 5 %. To assess incubation time, infection rate, disease severity induced by fungal strains and yield. If there was a significant difference between the means, they were classified using Fisher's LSD test. These tests were carried out using Statistica 7.1 software.

RESULTS AND DISCUSSION

RESULTS

Incubation Period: The incubation period of the fungal strains varied from 1.20 to 2.80 days depending on the cowpea accessions studied. Statistical analysis showed a significant difference (P < 0.05) between the incubation period of the two fungal strains on the leaves of the two accessions (Table 1).

Table 1. Incubation period of fungal strains depending on cowpea accessions

Accessions of cowpea		Incubation Period (days) ± standard deviation	
		Colletotrichum sp.	*Rhizoctonia* sp.
Red Accession		1,20 ± 1,15b	1,20 ± 1,50b
White Accession		1,50 ± 1,50a	2,80 ± 1,18a
Statistics	F	0,212	6,777
	P	0,019	0,002

On the same line, the averages affected by the same letter are equal to the threshold α = 5 % according to Fisher's LSD test : F : Fisher frequency ; P : probability ; *C. odorata* 25 : *Chromolaena odorata* 25 g/l ; *C. odorata* 50 : *Chromolaena odorata*50 g/l ; *O. gratissimum*25 : *Ocimum gratissimum*25 g/l ;*O. gratissimum*50: *Ocimum gratissimum*50 g/l

The longest incubation period (2.80 days) was observed on the white accession of cowpea inoculated with Rhizoctonia sp. and the shorter (1.20 days) was observed on the red lifetime cowpea accession of the two fungal strains.

Table 2. Infection rate of the leaves of the red accession of cowpea inoculated with the fungal strains and treated with the different doses of aqueous extracts

Doses (g/l)	Infection rate (%) ± standard deviation		Statistics	
	Colletotrichum sp.	*Rhizoctonia* sp.	F	P
C. odorata 25	3,24 ± 1,62a	3,12 ± 2,29a	0,192	0,825
C. odorata 50	0,24 ± 1,54b	3,12 ± 0,59a	20,185	0,001
*O. gratissimum*25	2,04 ± 1,86b	3,46 ± 1,76a	6,866	0,001
*O. gratissimum*50	3,48 ± 1,78a	1,04 ± 1,97b	16,013	0,001
Control	5,59 ± 2,26b	7,55 ± 2,45a	14,037	0,003

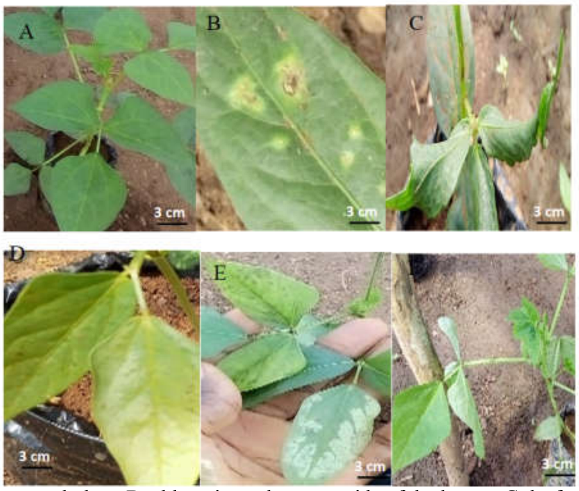
On the same line, the averages affected by the same letter are equal to the threshold α = 5 % according to Fisher's LSD test : F : Fisher frequency ; P : probability ; *C. odorata* 25 : *Chromolaena odorata* 25 g/l ; *C. odorata* 50 : *Chromolaena odorata* 50 g/l ; *O. gratissimum* 25 : *Ocimumgratissimum* 25 g/l ;*O. gratissimum* 50: *Ocimum gratissimum*50 g/l

Table 3. Infection rate of leaves of cowpea white accession inoculated with fungal strains and treated with different doses of aqueous extracts

Doses (g/l)	Infection rate (%) ± standard deviation		Statistics	
	Colletotrichum sp.	*Rhizoctonia* sp.	F	P
C. odorata 25	4,52 ± 2,35a	4,22 ± 1,97a	0,857	0,426
C. odorata 50	3,40 ± 4,05a	3,15 ± 1,96b	7,555	0,001
*O. gratissimum*25	4,16 ± 2,67a	4,10 ± 0,70a	1,585	0,208
*O. gratissimum*50	4,68 ± 3,25a	3,90 ± 2,49a	1,475	0,232
Control	6,86 ± 1,38a	5,68 ± 1,25b	8,270	0,012

On the same line, the averages affected by the same letter are equal to the threshold α = 5 % according to Fisher's LSD test: F: Fisher frequency; P: probability; *C. odorata* 25: *Chromolaena odorata* 25 g/l; *C. odorata* 50 : *Chromolaena odorata* 50 g/l; *O. gratissimum* 25: *Ocimumgratissimum* 25 g/l; *O. gratissimum* 50: *Ocimumgratissimum* 50 g/l

Description of symptoms caused by fungal strains inoculated on the leaves of cowpea accessions: The two fungal strains caused similar symptoms on the leaves of the two cowpea accessions compared to the control leaves (Figure 4A). Thus, five types of symptoms were observed on the leaves of cowpea accessions. The first and second type of symptom were caused by *Colletotrichum* sp. These symptoms manifest as chlorosis on the upper side of the leaves (Figure 4B) and leaf curl towards the upper side (Figure 4C), respectively. The third, fourth and fifth type of symptom was caused by *Rhizoctonia* sp. These are manifested respectively by yellowing of the entire leaf (Figure 4D), a white coloration on the upper surface of the leaves (Figure 4E) and wilting of the young leaves (Figure 4F).



A: control plant, B: chlorosis on the upper side of the leaves, C: leaf curl toward the upper side, yellowing of the whole leaf, E: white coloration on the upper side of the leaves, F: wilting of the young leaves

Figure 4. Symptoms observed on inoculated cowpea leaves

Effect of different doses of plant extracts on the leaves of cowpea accessions depending on the strain inoculated

Leaf infection rate of the two cowpea accessions: Infections caused by the two fungal strains on the leaves of the cowpea red accession varied with the type and dose of aqueous extract compared to controls (Table 2). Statistical analysis revealed that there is no significant difference (P = 0.825) in the level of infections on the leaves treated with the dose of 25 g/l of *C. odorata* extract, whatever the fungal strain. inoculated. However, there was a significant difference (P = 0.001) in the level of infections on the leaves of red cowpea treated with the 50 g/l doses of *C. odorata* extract then the 25 g/l and 50 g/l doses of *O. gratissimum*. Indeed, infections caused by *Rhizoctonia* sp. were more important on leaves treated with 50 g l extract of *C. odorata* (3.12 %) and that treated with 25 g/l extract of *O. gratissimum* (3.46%). However, on leaves treated with the dose of 50 g/l of *O. gratissimum*, infections were more severe on leaves inoculated with *Colletotrichum* sp. (3.48 %) than with *Rhizoctonia* sp (1.04 %). Regarding the white accession of cowpea, the infections caused by the two fungal strains on the leaves varied according to the type and the dose of aqueous extract only for the control (P = 0.012) and the leaves treated with the dose. 50 g/l of *C. odorata* extract (P = 0.001). For these two doses of extract, infections progressed more on the leaves inoculated with *Colletotrichum* sp. (3.40 % and 6.86 %) respectively for the 50 g/l dose of *C. odorata* extract and the control than for those inoculated with *Rhizoctonia* sp. (Table 3). However, statistical analysis revealed that there was no significant difference (P ≥ 0.05) regarding the change in percentages of infection on leaves inoculated with *Colletotrichum* sp. and *Rhizoctonia* sp. regardless of the other doses of extracts of the two plants.

Averages of symptom severity caused by the fungal strains on the leaves of the two cowpea accessions: The means of symptom severity of the two fungal strains on the leaves of the red cowpea accession varied according to the types and doses of extract plant (Table 4). The means of infection severity showed a significant difference (P <0.05). Indeed, they were higher on leaves inoculated with *Colletotrichum* sp. and treated respectively with 25 g/l of *C. odorata* extract (0.56) and 50 g/l of *O. gratissimum* (0.50). The average severity of leaves inoculated with *Rhizoctonia* sp. was only elevated with the 50 g/l dose of *C. odorata* extract (0.82). However, the mean severity of infections treated with 25 g/l extract of *O. gratissimum* did not vary (P ≥ 0.05) regardless of the strain inoculated. On the leaves of cowpea white accession, the mean severity of symptoms caused by fungal strains varied between the two types of extract (Table 5). Statistical analysis showed that only the severity averages of the leaves inoculated with the two fungal strains and treated with the two doses of *O. gratissimum* and control varied (P <0.05).

Table 4. Average severity of fungal symptoms on the leaves of the cowpea red accession depending on the type of aqueous exteact

Doses (g/l)	Severityaverage± standard deviation		Statistics	
	Colletotrichum sp.	*Rhizoctonia* sp.	F	P
C. odorata 25	0,56 ± 0,50a	0,24 ± 0,43b	4,588	0,011
C. odorata 50	0,28 ± 0,53b	0,82 ± 0,48a	13,408	0,000
*O. gratissimum*25	0,42 ± 0,49a	0,42 ± 0,57a	0,081	0,921
*O. gratissimum*50	0,50 ± 0,50a	0,16 ± 0,42b	5,330	0,005
Control	0,71 ± 0,63b	0,80 ± 0,40a	15,132	0,001

On the same line, the averages affected by the same letter are statistically equal to the threshold α = 5 % according to Fisher's LSD test: F: Fisher frequency; P: probability; *C. odorata* 25: *Chromolaena odorata* 25 g/l; *C. odorata* 50: *Chromolaena odorata* 50 g/l; *O. gratissimum* 25: *Ocimumgratissimum* 25 g/l; *O. gratissimum* 50: *Ocimumgratissimum* 50 g/l

Table 5. Average severity of fungal symptoms on the leaves of the cowpea white accession depending on the type of aqueous extract

Doses (g/l)	Severityaverages± standard deviation		Statistics	
	Colletotrichum sp.	*Rhizoctonia* sp.	F	P
C. odorata 25	0,56 ± 0,50a	0,50 ± 0,58a	1,919	0,150
C. odorata 50	0,28 ± 0,45a	0,30 ± 0,49a	0,517	0,597
*O. gratissimum*25	0,44 ± 0,54b	0,76 ± 0,47a	8,909	0,000
*O. gratissimum*50	0,28 ± 0,45b	0,54 ± 0,50a	3,831	0,023
Control	0,72 ± 0,34b	0,83 ± 0,48a	10,659	0,003

On the same line, the averages affected by the same letter are statistically equal to the threshold α = 5 % according to Fisher's LSD test: F: Fisher frequency; P: probability: *C. odorata* 25: *Chromolaena odorata* 25 g/l ; *C. odorata* 50: *Chromolaena odorata* 50 g/l ; *O. gratissimum*25: *Ocimumgratissimum* 25 g/l ; *O. gratissimum* 50: *Ocimumgratissimum* 50 g/l

Indeed, the mean severity was greater: Average severity of fungal symptoms on the leaves of the cowpea red accession depending on the rype on the leaves inoculated with *Rhizoctonia* sp. And treated with the respective doses of 25 g/l of *O. gratissimum* (0.76) and 50 g/l of *O. gratissimum* (0.54) than those inoculated with *Colletotrichum* sp. at the same doses.

Production of cowpea accessions depending on the fungal strains and the type and dose of aqueous extract: The grain yields of cowpea accessions as a function of fungal strains and the type and dose of aqueous extract varied Concerning the red accession of cowpea, except for the control which showed no significant difference (P = 0.329) , statistical analysis revealed that there is a significant difference (P < 0.05) between the yields depending on the type and all the doses of aqueous extracts (Table 6). Regardless of the dose and type of treatment, the leaves inoculated with *Colletotrichum* sp. had the best yields (18.80 g, 17.74 g, 5.05 g and 6.12 g) respectively with the doses 25 g/l of extract of *C. odorata*, 25 g/l of extract of *O. gratissimum*, 50 g/l of *C. odorata* extract and 50 g/l of *O gratissimum*extract. than those inoculated with *Rhizoctonia* sp. Doses 25 g/l of extract from both plants increased the seed production of cowpea plants. For the white cowpea accession, the doses of 25 g/l of C. odorata extract (P = 0.245) and the control (P = 0.214) had no significant effect on the yield whatever the fungal strain inoculated (Table 7).

However, analysis showed high yields on leaf plants inoculated with *Colletotrichum* sp. and treated with doses of 50 g / l extract of C. odorata (0.53 g) and 25 g/l extract of *O. gratissimum* (13.46 g).

Table 6. Production of red cowpea accession depending on the fungal strains and the doses of aqueous extracts

Doses (g/l)	Production (g) ± standard deviation		Statistics	
	*Colletotrichum*sp.	*Rhizoctonia*sp.	F	P
C. odorata 25	18,80 ± 16,81a	4,20 ± 5,17b	8,909	0,001
C. odorata 50	5,05 ± 4,59a	2,21 ± 2,19b	3,517	0,021
*O. gratissimum*25	17,74 ± 9,14a	6,53 ± 8,76b	5,059	0,001
*O. gratissimum*50	6,12 ± 8,33a	4,86 ± 3,35b	2,281	0,001
Control	2,30 ± 1,53a	2,37 ± 1,02a	3,450	0,329

On the same line , the averages affected by the same letter are statistically equal to the threshold α = 5 % according to Fisher's LSD test : F : Fisher frequency; P : probability; *C. odorata* 25: *Chromolaena odorata* 25 g/l; *C. odorata* 50: *Chromolaena odorata* 50 g/l; *O. gratissimum* 25: *Ocimumgratissimum*25 g/l; *O. gratissimum* 50: *cimumgratissimum* 50 g/l

Table 7. Production of white cowpea accession depending on the fungal strains and the doses of aqueous extracts

Doses (g/l)	Production (g) ± standard deviation		Statistics	
	Colletotrichum sp.	*Rhizoctonia* sp.	F	P
C. odorata 25	2,57 ± 3,16a	2,26 ± 1,39a	1,324	0,245
C. odorata 50	0,53 ± 0,46a	0,16 ± 0,28b	0,547	0,001
*O. gratissimum*25	13,46 ± 13,42a	6,26 ± 5,42b	6,140	0,001
*O. gratissimum*50	2,07 ± 1,80b	3,87 ± 4,89a	2,031	0,042
Control	2,42 ± 1,83a	2,56 ± 2,24a	1,259	0,214

Figure 5. Burns caused by the 50 g/l dose of *C. odorata* on cowpea accessions inoculated with the two fungal strains

Only leaf plants inoculated with *Rhizoctonia* sp. and treated with 50 g/l extract of *O. gratissimum* had their high yields (3.87 g). For the two cowpea accessions inoculated with the two fungal strains, the 50 g/l dose of *C. odorata* caused leaf burns. To this end, the recorded yields were the lowest (Figure 5).

DISCUSSION

The evaluation of the incubation time, the rate of infection, the severity of the infections as well as the evaluation of the yield of cowpea accessions according to the fungal strains and the type and dose of aqueous extract was made. The study of the two cowpea accessions showed that the red accession had the shortest incubation time and the white accession the longest. In the red accession, the short incubation marked by the rapid onset of symptoms indicates its greater sensitivity to the fungal strain compared to the white accession. This finding could be due to the action of biochemicals secreted by the pathogen in the intracellular environment of the host. These substances are generally hydrolytic enzymes (cellulases, pectinases and proteases) and toxins, which are secreted in order to degrade the wall of plant cells which acts as a natural and preformed physical barrier against pathogens. These statements confirm those of Rezzonico *et al*. (2005) who claim that hydrolases are responsible for the host infection process during plant-pathogen interaction.

The two fungal strains inoculated caused symptoms on the leaves of the two cowpea accessions. *Colletotrichum* sp. induced chlorosis and leaf rolls. In contrast, *Rhizoctonia* sp. caused yellowing, wilting and white discoloration of the leaves. These results could be explained by the predisposition of cowpea plants to be infected with a diverse range of pathogens. Also, Buruchara *et al*. (2010) observed in their study on cowpea diseases the same fungal symptoms caused by these fungi. Also, this observation could be explained by the fact that the fungi studied can develop on several host plants as well as on organic matter. This corroborates the results of Achbani & Tourvielle (2000) who qualified these fungi as polyphagous and ubiquitous. Indeed, the infection rate recorded could explain that the pathogen was able to cross, thanks to the degradation enzymes, physical barriers (cuticle and cell wall) which would prevent the penetration of most pathogenic fungi. This penetration of the pathogen into its host results in the onset of symptoms, which means that the host is infected (susceptible) and the interaction is said to be compatible, Soro (2014). Plants of the two inoculated cowpea accessions exhibited varying severities depending on the dosage and types of aqueous extracts.
This could be explained by the fact that at the seedling stage some cultivars would be vulnerable to attack by pathogens, especially those in the soil. Indeed, at this stage, the natural barriers of young plants may not yet be put in place to fight against potential aggressors. Our results agree with those of Aka *et al*. (2009) who showed that 50% of infections due to the mosaic virus from cucumber to banana occur at the seedling stage. The effectiveness of plant extracts differs from one fungal species to another. Indeed, the inoculated fungi may not exhibit the same levels of sensitivity to aqueous extracts. These results are in agreement with those obtained by Adekunle & Ikumapayi (2006).

These authors have shown in their work that *Aspergillus flavus*, *Candida albicans*, *Microsporium andouinii*, *Penicillium* sp. and *Trichoderma* sp. showed different sensitivity levels depending on the aqueous extracts of *Funtumia elastica* and *Mallotus oppositifolus*. Also, the aqueous extracts reduced the infections at the plant levels and the severity of the strains at the leaf level compared to the controls. This reduction could be due to the properties of the substances contained in the aqueous extracts of the leaves of *C. odorata* and *O. gratissimum*. These substances could be very active on the development of *Colletotrichum* sp. and *Rhizoctonia* sp. According to Avlessi *et al*. (2012), the aqueous extract of *C. odorata* contains the antifungal substances such as sesquiterpenes, monoterpenes, flavonoids and tannins which are said to inhibit the effect of fungi. Regarding the extract from *O. gratissimum*, the work carried out by Oussou *et al*. (2004), have shown that it is predominantly rich in carvacrol, para-cymene, γ-terpinene and thymol, the main antifungal substances. In addition, the work of Soro etal. (2011) have shown that thymol and carvacrol would be the most active essential compounds against fungi. Also, the work of Chebli *et al*. (2003) and Ajouri *et al*. (2008) highlighted the antifungal efficacy of terpene phenols, thymol and carvacrol. For these authors, these substances have shown efficacy on many fungal strains such as *Aspergillus ochraceus*, *Fusarium oxysporum* and *Penicillium digitatum.*

However, in spite of their antifungal properties, these aqueous extracts stimulated at low dose (25 g/l), the grain production of cowpea plants. The yield was high at this dose compared to 50 g/l and the control. This stimulation of grain production could be explained by the fact that the aqueous extracts sometimes as nutrients when the active compounds are at low doses. Our results are in the same direction as those obtained by Conventry & Allan, (2001) as well as those of Alkhail, (2005). These authors have shown that aqueous extracts such as *C. odorata* and *Diversifolia tithonia* stimulate the production of tomato plants at low doses. Also, previous work by Maliki cc. (2017) proved that the leaves of *C. odorata* contain mineral elements such as carbon, potassium, nitrogen, calcium and phosphorus, which are essential for plant life. In addition, these minerals participate in the nutrition of the plant while improving the physicochemical properties of the soil. As for the extract from *O. gratissimum* thymol and carvacrol could stimulate plant growth and promote better production of cowpea plants. Soro *et al.* (2011) also found similar results with the *O. gratissimum* extract. on the growth and production of tomato plants. Indeed they have shown in their work that thymol and carvacrol stimulate the height growth of tomato plants as well as fruit production. Finally, the 50 g/l concentration of *C. odorata* extract caused burns on the leaves of certain cowpea plants, hence its phytotoxic effect on these plants.

CONCLUSION

The present study on the use of aqueous plant extracts in the fight against pathogenic fungi of cowpea was carried out under cultivation conditions under shelters on the site of the University Jean Lorougnon Guédé. This study investigated the effect of extracts from the leaves of *C. odorata* and *O. gratissimum* on *Colletotrichum* sp. and *Rhizoctonia* sp, two pathogenic fungi of cowpea. Most cowpea plants showed infections by day 2 after inoculation. Of the two cowpea accessions inoculated, the red accessions were the most susceptible to pathogens. In fact, the plant extracts made it possible to reduce the pathogenicity of the two fungal strains on the inoculated plants. However, the best yields were obtained with the 25 g/l concentration of the two plant extracts tested.

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