Cameroon Journal of Experimental Biology (2024) Vol. 18, N°01, 58-63. DOI: https://dx.doi.org/10.4314/cajeb.V18i1.8

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Cameroon Forum for Biological Sciences

Cameroon Journal of Experimental Biology

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Susceptibility of two cowpea varieties (*Vigna unguiculata* (L.) Walp.) to fungal infection in field in the western highlands (Dschang)

Abdou Nourou Nsangou Kone [1,*] , Serge Bertrand Mboussi [2], Alain Heu [3], Ambang Lucy Agyingi [1], David MBainarem [1], Jules Patrice Ngoh Dooh [4]

[1] *University of Dschang. Department of Plant Biology, Applied Botanic Research Unity Po Box 67 Dschang;*
[2] *University Institute of Technology of the University of Douala. Laboratory of Biotechnology, P.O Box.8698 Douala;*
[3] *Higher Technical Teachers Training College, Ebolowa, Department of Agriculture and Agropastoral, University of Ebolowa, Po Box 886 Ebolowa;*
[4] *Department of Biological Sciences, Faculty of Science, University of Maroua Po Box 814 Maroua Cameroon.*

Keywords

Symptomatology;
Diseases;
Vigna unguiculata;
Growth.

Historic

Received : 08 March 2024
Received in revised form : 10 May 2024
Accepted : 25 May 2024

Abstract

Cowpea is an important food and socio-economic crop in West Africa. However, its yield is low due to biotic and abiotic constraints. The present work was carried out in the field and in the laboratory at the University of Dschang, Cameroon, with the general aim of identifying disease symptoms on cowpea (*Vigna unguiculata L.*) in the field during the growth stages. Two varieties were used (MTA-22 and TN5-78) in a completely randomized block design with three replications. The various symptoms of the diseases identified were described, their incidence and severity assessed, and a morphological characterization of the pathogens responsible for fungal diseases based on microscopic observation of fruiting bodies was carried out. At the end of this work, seven fungal diseases were identified and described. Four (4) fungal disease pathogens were clearly isolated and characterized: *Fusarium oxysporum, Fusarium moniliforme, Cercospora canescens* and *Rhizoctonia solani* These results will contribute to the development of a control program and the improvement of cowpea productivity with a view to combating malnutrition and reducing poverty in rural areas.

1. Introduction

As population growth accelerated in the second half of the 20th century, fears of a global food crisis reappeared. The world's population is set to rise from 7.6 billion to 9.7 billion by 2050 [1]. New data show that the number of hungry people in the world is on the rise.

Cultivated in almost all tropical and subtropical regions, cowpea is the most important grain legume crop in sub-Saharan Africa. Efficiently exploited and valorized, this staple could be a real bulwark against malnutrition and dependence on products such as rice, animal proteins and wheat [2].

Its production is estimated at 9.7 million tonnes on around 15 million hectares globally [3].

Cowpea offers many benefits to small-scale farmers in terms of food, cash income, livestock feed and improved soil fertility. In Cameroon, most cowpea is produced in the Far North region [4]. Cowpea production in Cameroon is estimated at around 177,722 tonnes, or 2 % of world production. This low yield is attributed to abiotic, biotic and agro-climatic constraints. Although production has improved, it remains well below that of countries such as Nigeria and Niger, the continent's top producers with 3,656,617 and 2,642,219 tonnes per year respectively [3].

Cowpea is a fast-growing plant, and the creeping varieties common among farmers quickly cover the soil and protect it from erosion [5]. During its vegetative cycle, cowpea is particularly vulnerable to insect pests such as aphids, thrips, pod borers and pod-sucking bugs [6].

Leaf spot disease, zonal spot, pod anthracnose and yellow mottle of cowpea are the most dreaded diseases in cowpea, as they inhibit the plant's photosynthetic activity and deteriorate seed quality, contributing to weevil infestation. These risks of major production losses are what discourage farmers from growing cowpeas. In this context, good disease management is essential if cowpea cultivation is to become profitable and sustainable. As a result, research programs have focused on varietal selection, plant protection and post-harvest technologies with a view to improving production of this legume [7]. Despite cowpea's importance in a number of areas, its production faces various constraints. Variety development by institutes was based on the yields while neglecting the aspect of abiotic and biotic constraints. In the face of climate change, these varieties are no longer adapted and yields are increasingly low, as certain varieties that were once adapted have become vulnerable (variety tomato). Faced with all these phenomena, it was important to assess the resistance of two varieties to diseases that are an obstacle to yield. It was also important to assess the resistance of varieties to changing climate condition, so that research institutes could take account some of these constraints when improving varieties. Changes in climatic conditions have other consequences, notably favoring the development of certain diseases. The general objective of this study was to identify and describe cowpea disease symptoms in the field during growth. Specifically, it aimed to:

Describe the various disease symptoms identified in the different varieties used;

Evaluate the incidence and severity of diseases in the field;

Morphologically characterization fungal disease pathogens identified.

*Corresponding author: *University of Dschang. Department of Plant Biology, Applied Botanic Research Unity Po Box 67 Dschang. Email: konensangouabdou@yahoo.fr*, Tel.: +237 6 99 12 36 13.

2. Material and methods

2.1. Study area

The experiments were carried out in the highlands of western Cameroon, more precisely in Dschang (cowpea cultivation is increasingly being abandoned by farmers in the area because it is too susceptible to disease) (figure 1). Dschang is located between 700 and 2740 m above sea level, with geographic coordinates of Latitude: 5°26'38''North and Longitude: 10°03'11'' East [8]. Microscopic manipulations and observations were carried out at the Phytopathology and Agricultural Zoology Research Unit (UR_PHYZA) and the Applied Botanical Research Unit (URBOA) of the University of Dschang

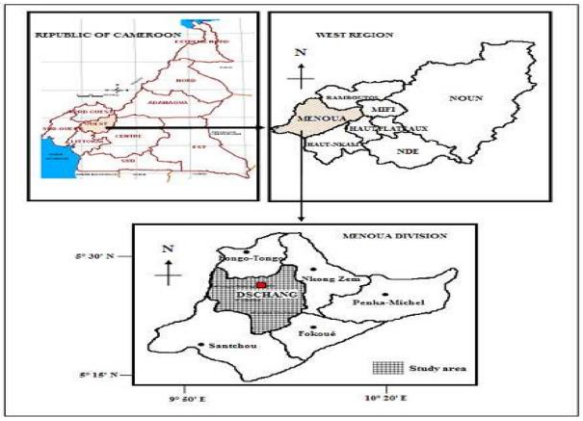

Figure 1: Location for the town of Dschang in the Western region [8].

2.1.1. Climate and rainfall

Dschang is characterized by an equatorial monsoon climate with mountain facies. This climatic configuration gives rise to mean annual temperatures ranging from 18.9°C to 21.1°C (minimum 13.4°C) for an average rainfall of 1,900 mm/year. The annual temperature range is small, around 3°C. Relative humidity is consistently high, with an annual average of 83% [8].

2.1.2. Plant material

Two (2) cowpea varieties (MTA-22 and TN5-78) whose characteristics are listed in Table I were used during the experiment (MTA-22) is an improved variety from the Agricultural Research Institute for Development (IRAD) station in Foumbot (Cameroon) and (TN5-78) is imported from the Chadian Agricultural Research Institute for Development (ITRAD) in Chad.

Table 1: Characteristics of the varieties used (MTA-22 and TN5-78)

Varieties	Features			
	Seed color	Seed habit	Cycle (days)	Yield (t/ha)
TN5-78	Brown	Semi-creeping	90-95	3
MTA-22	Brown-tinted	Creeping	70-75	1.8

2.2. Preparing the experimental field and setting up the experimental set-up

The site was weeded, deseeded and ploughed, and the experimental set-up set up. The experiment began in february and will end in june 2023. The set-up consisted of two (02) completely randomized blocks with six replicates each and containing 120 plants, i.e. 720 plants per variety. In all, some 960 cowpea plants were tested on the site. Each replication had a surface area of 5 m^2 and seedlings were sown directly with spacing of 0.30 cm between liners and 0.60 cm between poquet. Each poquet contained three seeds. Emergence occurred 5-10 days after sowing. Plot maintenance consisted of dematting, watering, weeding when necessary and ridging. Dematting consists in removing the plants, leaving only one or two (the most vigorous) in each cluster. This operation was done when the plants were 5 to 10 cm tall.

2.3. Observations of symptoms in the field

Observations of disease symptoms began as soon as the first symptoms appeared on the various organs of the cowpea varieties studied (two weeks after sowing). These observations focused on the number of plants attacked and the degree of attack. These observations made it possible to determine the presence of all symptoms of pathological origin.

2.3.1. Disease identification

2.3.1.1. Identification of disease symptoms

On each experimental plot, ten (10) plants labelled by variety were used to take parameters (leaves showing symptoms of disease) during the experimentation period.

Diseases were identified using several identification keys [11.9]. For fungal diseases, in addition to symptoms observed in the field using an identification key [9]. samples of infected, marked plants were collected and taken back to the laboratory for purification and characterization of the pathogen. Isolated fungal species were identified on the basis of morphological characteristics of the mycelium (septate or nonseptate) and fruiting bodies (conidia) observed under an ordinary Olympus microscope (name:Olympus; brand: 400X), using mycological identification keys [10]. and growth rate assessment. Identification consisted of scraping the surface of pure cultures and taking fragments of mycelial growth with a scalpel, spreading these fragments on a slide containing a drop of dye (methyl blueto expose spore outlines) and cover with a coverslip before microscopic observation.

2.3.2. Assessing disease incidence and severity

2.3.2.1. Disease incidence

Incidence is the proportion of diseased plants out of the total number of plants. Assessment began as soon as symptoms appeared. For fungal diseases, identified infected organs were marked after counting, to avoid double counting. The formula [11]. was used.

$$I\ (\%) = np/N \times 100$$

Where: I: Incidence of disease, np: number of plants attacked; N: total number of plants.

2.3.2.2. Disease severity

Severity is the degree of attack of the disease. Observations on the severity index have been quantitatively estimated. They are made visually on the plants tagged in the replicates. It was determined using the formula of [12].

$$Severity = (\sum n \times I) / N \times 100$$

With: n= the number of attacked leaves; N= the total number of leaves. I= severity index with estimation scale [13].

Table 2: Severity assessment scale [13]

Scale	Symptoms	Degree of disease
1	No symptoms	healthy plants
2	25 % of leaves are diseased	Slight symptoms of the disease
3	50 % of leaves attacked	The disease is less severe
4	75 % of diseased leaves	Severe illness
5	100 % of diseased leaves.	The disease is very severe

2.3.3. Morphological characterization of fungi

2.3.3.1. Pathogen purification and observation

In the laboratory using a scalpel, the part of the organs showing symptoms of the disease (leaves, stems and pods) were disinfected in a 2% sodium hypochlorite solution for 2 min, then followed by a triple rinse with sterilized distilled water at 5, 10 and 15 min respectively to remove traces of the disinfectant and subsequently these parts were placed on hydrophilic paper to absorb excess water and dried over a flame [14]. Sterilization of the culture media was carried out in an autoclave for 20 min at 121°C. After sterilization, the culture media Potato Dextrose Agar (PDA) were placed in Petri dishes to cool for 30 min. The various disinfected parts were aseptically seeded into Petri dishes containing 20 ml of culture medium at a rate of 5 fragments per Petri dish under a microbiological hood close to a Bunsen burner flame and placed in an incubator at a temperature of 18-20 °C.

2.4. Data analysis

Incidence and severity assessment data were entered into Excel 2013 and subjected to analysis of variance (ANOVA). Duncan's test at the 5 % threshold was used to compare different means when differences were significant, using GenStat Release 12.1 software.

3. Results

At the end of this study, several diseases were identified on the basis of their symptoms, identification keys and fruiting bodies: Four (4) fungal diseases (two types of wilt, cercosporiosis, leaf blight).

3.1. Fungal diseases

a. Fusarium wilt

Fusarium wilt (FW), caused by a pathogenic agent, was identified from the 14th day after sowing (DAS), causing the death of several plants in the experimental plots on the MTA-22 variety and TN5-78 (Figure2). Typical symptoms of this disease are premature and rapid wilting of some or all plants, due to the action of soil-borne fungi invading and clogging the vascular system. The causal agent of this disease is *Fusarim moniliforme.*

Figure 2: Symptome of Fusarium wilt caused by *F. moniliforme* on variety MTA-22 and TN5-78.

The other manifestation of fusarium wilt is the desiccation or death of a seedling or plant following root or crown necrosis (Figure 3). It is caused by *Fusarium oxysporum*. The disease appears in foci, where plants rapidly wither. Infection begins with punctate, moist, light-brown, irregularly-shaped leaf lesions with dark-brown margins. Symptoms of this disease were observed on the TN5-78 variety.

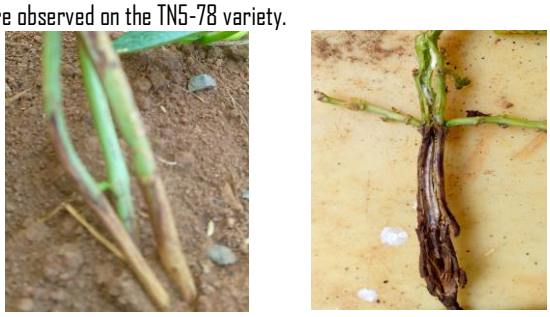

Figure 3: Fusarium wilt caused by *Fusarium oxysporum*

b. Cercosporium leaf spot disease

Infected plants show symptoms of the disease in the form of necrotic spots on the upp
fully developed. Cercosporiosis is seed-borne. Symptoms of this disease were observed on the MTA-22 variety.

Figure 4: Symptom of cercosporiosis on cowpea leaves A: upper surface; B: lower surface

c. Rhizoctonia or leaf blight

This disease is caused by a fungus called *Rhizoctonia solani*. During the vegetative growth phase, basal leaves are affected, sometimes forming a spider-web-like mycelial network (Figure 5). These fungi can be recognized by the mycelium and the numerous rounded sclerotia which appear on the lesions. Symptoms of this disease have been observed on the TN5-78 variety.

Figure. 5: Leaf burn caused by *Rhizoctonia solani* : A : Lower side ; B : Upper side

3.2. Evaluation of the incidence and severity of the various diseases identified in the field

3.2.1. Incidence of fungal diseases

3.2.2. Incidence according to variety

Analysis of variance of the incidence of fungal disease on the two varieties showed a significant difference (P ≤ 0.05) between the means of these two varieties (P=0.03). Incidence varied from 53.89 ± 1.1 and 70.90 ± 2.77 respectively for varieties TN5-78 and MTA-22. MTA-22 was more susceptible to fungal diseases than TN5-78.

III.5.1.2 Incidence between pathogens (Fungi).

The analysis of variance of the incidence of diseases caused by fungi isolated during the field experiment shows that there is a significant difference between the averages P ≤ 0.05. The disease caused by *R.solani* had an incidence of 42.71 ± 1.7, while that caused by *F.moniliforme* had an incidence of 45.71 ± 2.5. No significant difference exists between the diseases caused by these two pathogens according to Duncan's test at P ≤ 0.05. The incidence of disease caused by *C. canescens* and *F. oxysporum* ranged from 49.66 ± 5.19 to 51.5 ± 1.95 respectively.

3.2.3. Fungal disease severity

Analysis of variance of fungal disease severity shows that there are significant differences (P≤0.05) between the varieties used (Table 3). The degree of fungal disease severity differs according to the virulence of the pathogens. Analyses show that the MTA-22 variety was more susceptible to Cercosporiosis (*C. canescens*) and to wilting than the TN5-78 variety. The severity of leaf scorch due to *R. solani* was significantly different for

the varieties used and ranged from 50.9 ± 2.77 to 24.38 ± 0.9 respectively for varieties MTA-22 and TN5-78 (Table 3).

Table 3: Disease severity by variety and pathogen

Variétés	*F.moniliforme*	*F. oxysporum*	*C. canescens*	*R. solani*
MTA-22	33,019±3,17a	29,13±2,15b	53,9±1,7b	50,9±2,77a
TN5-78	23,98±1,41b	16,89±2,28a	35,77±3,4a	24,38±0,9b

The values on the same line for the two varieties with different letters are significantly different at the 5% threshold.

3.2.4. Morphological characterization of pathogens of identified (fungal) diseases

Fusarium moniliforme

Fusarium moniliforme develops rapidly, with a growth rate of 15 mm/d, and produces flat colonies with a woolly to cottony texture; these colonies tend to spread. The surface of the colony may be white, creamy and/or light brown (Figure 6 B). The basic characteristics of Fusarium species are the presence of septate hypae, conidiopores, macroconidia and microconidia observable under the microscope. Fusarium is a member of the Nectreaceae family, order Hypocreales.

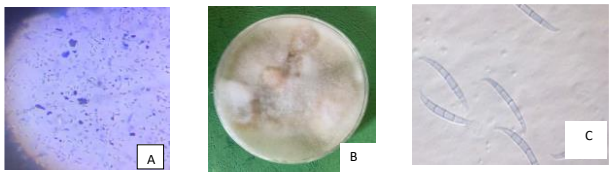

Figure 6: Microscopic observations of *Fusarium moniliforme* (A and C), pure strain (B).

Fusarium oxysporum

Morphological and macroscopic characteristics were determined from fungal colonies by observing texture, color and growth on PDA medium, on the one hand and the microscopic parameters of spore shape (Figure7). The color of the mycelium ranges from whitish to grayish, and its growth rate in the Petri dish is 11.25 mm/dr. It is a fungus of the Nectreaceae family and Hypocreales order.

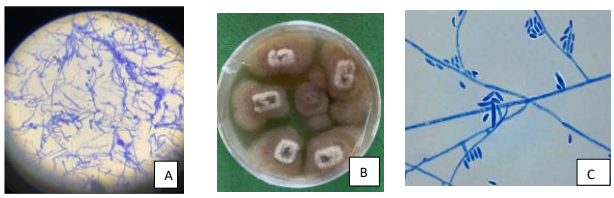

Figure 7: Microscopic observations (A and B), mycelial growth (C).

Cercospora canescens

C. canescens is a slow-growing pathogen; spore production on culture media (PDA) takes 10-12 days, and spore numbers are lower due to the fungus' slow growth rate of 4.28 mm/dr. Electron microscope observations show that *C. canescens* spores are compartmentalized (Figure 8). This fungus belongs to the Mycosphaerellaceae family and the Capnodiales order.

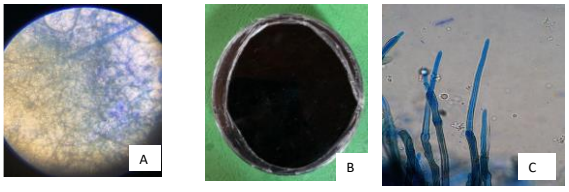

Figure 8: Microscopic observations (A and C), mycelial growth (B).

Rhizoctonia solani

R. solani is the pathogenic fungus of the Rhizoctonia genus. Under the electron microscope, the mycelium of *R. solani* is quite characteristic: fairly wide, compartmentalized, with branches. *R. solani* produces no spores. Sclerotia germinate and produce mycelia (Figure 9). Enlarged microscopic observations show partitioned mycelia. The fungus has a growth rate of 11.25 mm/dr, enabling it to cause rapid tissue destruction when germinated.

Figure 9: *Rhizoctonia solani* microscopic observations (A and C), pure strain (B)

4. Discussion

The results obtained in the field showed that cowpea is confronted with various fungal diseases mainly during the vegetative growth phase. This phase is critical for successful cowpea yield.

Cercosporiosis symptoms observed on varieties during the experiment are caused by *Cercospora canescens*. It is a serious disease in growing areas of the country where high humidity prevails during the growing season, and causes considerable damage if no protective measures are taken. It was observed during of plant growth, considerably reducing the photosynthetic surface area of the leaves and consequently yield. These results are similar to those of [15]. who identified Cercosporiose spots on beans caused by *C. canescens* in ten regions of Rajasthan (India), where the region falls within the semi-arid eastern plain. However, these results do not contradict those obtained by [15]. where this disease was identified only during flowering.

Wilt, root rot, collar rot and stem rot are usually caused by *Furasium oxysporum*. It is widely distributed naturally in soils. *F. oxysporum* can cause fusarium wilt, when the vascular system is affected. This disease was present with varying degrees of severity, depending on the variety. It is a serious disease that can lead to the death of all plants aleading to total yield loss. These same symptoms were identified by [17], on *Solanum* spp in Lomé, who showed that *F. oxysporum* is a soil fungus that lives as a facultative saprophyte. In addition, it behaves as a pathogen that causes vascular wilt under appropriate humidity and temperature conditions.

The Fusarium pathogen is responsible for fusariosis of higher plants. Typical symptoms of this disease are premature and rapid wilting of part or all of the plant. These results are similar to those obtained by [18] on oil palm fusariosis in Benin. Climatic factors, in particular humidity and temperature, play a key role in the development of *Fusarium moniliforme* by conditioning their germination.

The blight symptoms identified in the field are characteristic of wetland diseases that attack several susceptible species of the Fabaceae family. These results are similar to those obtained by [19]. In Niger and Burkina Faso on groundnuts, where the disease is caused by the same pathogen (*Rhizoctonia solani*) under high humidity conditions.

The results show that the MTA-22 variety developed by IRAD is a susceptible variety, compared with the TN5-78 variety imported by ITRAD, which is adapted to a dry climate. Analysis of data on the incidence of fungal diseases showed a significant difference between varieties. This proves that in the field, the local improved variety (MTA-22) shows more

symptoms of fungal diseases than the imported but resistant variety (TN5-78). These results are not similar to those obtained by [7] in Nigeria. *F. oxysporum* reproduces via microconidia, macroconidia and chlamydospores, which are responsible for infecting new plants and spreading throughout the crop. In particular, chlamydospores confer resistance and remain viable in the soil for long periods. Macroscopic morphological identification of mycelial colony growth ranging from 8 to 21 days, i.e. a minimum of 11.25mm/dr. These results are comparable to those obtained by [20], [17]. For mycelial color, our results differ with those obtained by [21], [22], who assert that mycelial colors are a characteristic trait of *F. oxysporum* as it displays a large color variation on the PDA support.

F. monoliforme is a pathogen responsible for wilting in young plants. Plants affected by wilt wither and die quickly by turgidity. These results corroborate those obtained by [7]. Once germinated, the fungus grows very fast, with a growth rate of 15 mm/dr. These results are not similar to those obtained by the National Plant Protection Laboratory (LNPV) in which is 2 mm/dr.

Cercospora canescens isolate sporulation in PDA medium has an average growth rate of 4.25 mm/dr. These results are close to those obtained by [23]. which is 4.8 mm/dr.

Foliar blight or aerial Rhizoctonia: aerial Rhizotonia occurs when foliage is dense and plants are not sufficiently spaced. In this case, there is a lack of damping-off and root rot. Infections occur when soil temperature varies between 15 and 27 °C and soils are moist. These results are similar to those obtained by [24]. its growth rate is 11.75 mm/dr, well above that obtained by [25], which is 3 mm/dr on the same culture medium (PDA). These species do not produce spores, but consist of hyphae and sclerotia. These results are similar to those of [26] on the morphological characterization of *R. solani* on *Solanum* spp.

5. Conclusion

This study gave a general idea of the different diseases that infected the two varieties tested. Its objective was to identify the different diseases in the field and the associated fungi. The laboratory results confirmed that the four fungi characterised were respectively responsible for the disease symptoms described, namely wilt, cercosporiosis and rhizoctonia of cowpea. However, these results show that the MTA-22 variety is susceptible to fungal diseases because it is a local variety. On the other hand, the TN5-78 variety showed tolerance to fungal diseases. At an advanced stage of its growth (end of cycle), it showed symptoms of fungal diseases.

The severity of the symptoms expressed by the varieties made it possible to determine the resistance and susceptibility of all the varieties tested.

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