Journal of Plant Sciences
2026, Vol. 14, No. 1, pp. 38–50
https://doi.org/10.11648/j.jps.20261401.13

Research Article

Main Diseases of Pepper (Piper nigrum L.) in the Localities of Njombe-Penja (Cameroon)

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Abstract

Piper nigrum is an important crop. However, this crop is very vulnerable to pests and diseases, which can cause a drop in production if no control methods are applied. The general objective of our study is to contribute to a better understanding of diseases and the various associated pests and diseases in the study sites (Njombe and Penja). To achieve this objective, the main diseases of pepper in the field were identified and described. In each site, four quadrats of twenty pepper plants out of twenty were planted; the different diseases were identified and described according to their characteristic symptoms. Their incidence and severity were assessed along the diagonal of each quadrat. The pathogens responsible for these diseases were characterized macroscopically and microscopically using several identification keys. The results showed that in both study areas, several symptoms were observed, including necrosis, scorching, diffuse spots, sparse spots, blistering on the leaves and leaf yellowing corresponding to diseases such as: Anthracnose, Cercosporiosis, Mildew, Rust, Galle and slow decline. In both sites, the incidence varied from 65 to 100% and the severity from 25 to 75%. In the Petri dishes, the colour of the mycelium varied between black, white, pink and brown and had a milky, cottony appearance. Microscopically, the shape of the spores varied from round, oblong, fusiform, reniform and falciform. Some hyphae were septate and others were non-septate. Species of fungi such as *Colletotrichum gloeosporioides*, *C. necator*, *Cercospora* sp., *F. solani*, *F. oxysporum*, *Rhizoctonia* sp. and *Cephaleurus virescens* were identified as being responsible for these diseases. Knowledge of these pathogens could contribute to the development of more environmentally-friendly control methods.

Keywords

Piper Nigrum, Disease, Symptom, Characterisation

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Received: 19 January 2026; **Accepted:** 29 January 2026; **Published:** 11 February 2026

1. Introduction

The Department of Mungo (Cameroon) has a favourable climate for market gardening and food crops, in particular pepper growing. The pepper plant, *Piper nigrum* L., is a perennial climber in the Piperaceae family. It belongs to the genus Piper, which comprises more than 1,000 species [1]. It is mainly grown for its fruits, which are used as spices and condiments. Pepper, also known as 'black gold' by the traders of the time, is now used in cooking on every continent [2]. In addition to its culinary properties, the different parts of the pepper plant, namely the leaves, fruits and seeds, have been attributed numerous biological properties, including antihypertensive, analgesic, antifungal, anti-inflammatory, liver-protective, antimutagenic, antibacterial, menorrhagia, dysmenorrhoea and many others [1, 3, 4]. Global pepper production has fallen from 633,000 tonnes in 2019 to 497,000 tonnes in 2021, a decline of 16%.

Worldwide, Vietnam is the leading producer, with an average output of 201,265 tonnes in 2021, followed by Brazil with 89,954 tonnes, India with 66,000 tonnes, Indonesia with 52,758 tonnes and in fifth place China with 24,684 tonnes. In Africa, the biggest pepper producers are Madagascar, Ghana and Ethiopia, with 4,532 tonnes, 4,409 tonnes and 3,732 tonnes respectively produced in 2020 [5, 6]. With marginal production (<0.02% of world production) [7], Cameroon is classified as a low-production country. However, its production has increased tenfold in recent years, due to the growing interest in pepper among local farmers since the labelling and international recognition of Penja pepper. This pepper, grown in the Mungo region of Cameroon, has a pungent, full-bodied character, a fragrant taste and a unique aroma. In 2013, these rare qualities earned it, alongside 'Oku white honey' and 'Guinea coffee', the 'Penja pepper' label, the first Protected Geographical Indication (PGI) in Sub-Saharan Africa to be awarded by the European Union [8]. In addition to enhancing the status of producers of this spice, the labelling of this pepper since 2013 has stimulated interest in this crop, characterised by an increase in the number of producers, from around ten at the outset to more than 200 after 2013, but also and above all in the area under cultivation [9]. Pepper cultivation has enormous potential in Cameroon, and it is necessary to promote its dissemination and development in order to give it a further boost. Production to meet ever-increasing demand. However, the major difficulty with pepper cultivation worldwide is its vulnerability to pests and diseases, which can cause losses of 1-5% or even 50% if no precautions are taken.

To deal with these problems and guarantee satisfactory production, some growers around the world have opted for the extensive use of synthetic plant protection products (fungicides, insecticides, nematicides). The heavy use of these products is considered a prerequisite for the success of a strategy of rapid agricultural development and intensive production. Given their toxicological properties, these products are a real danger and are currently considered to be among the main environmental pollutants responsible for the toxic residues that cause numerous human diseases [10]. Since 2013, when Penja pepper was awarded the PGI label, producers have had to comply with a set of specifications that require them to grow pepper without chemical inputs, or in very limited doses. In view of climate change, the constraints involved in complying with the specifications and the proliferation of diseases in the fields, there is an urgent need to characterise the pathogens responsible for diseases in pepper-growing areas in Cameroon, so that they can be better managed. Hence, the general objective of our study, which is to contribute to a better understanding of diseases and the various associated bio-aggressors in the localities of Njombe and Penja. More specifically, the aim is to identify and describe the main diseases of pepper in the field and to characterize the pathogens responsible for the diseases identified.

2. Materials and Methods

2.1. Materials

Study area

The study was carried out in the Njombe-Penja district, Moungo department, and in the Phytopathology and Agricultural Zoology research units of the FASA and Applied Botany research units of the Plant Biology department of the University of Dschang. The Njombe-Penja district belongs to zone IV, a humid forest zone with monomodal rainfall [11]. Rainfall is abundant, averaging between 2,500 and 4,000 mm, the temperature varies between 22 and 29 ℃ and air humidity is between 61 and 90%, making for a heavy atmosphere. The altitude of the whole area varies between 150 m and 600 m. The district covers an area of 260 km ², with a hot, humid equatorial climate. The soils in this locality are volcanic and fertile, eutrophic brown, ferralitic soils typical of less evolved types [12].

2.2. Methods

2.2.1. Identification and Description of the Main Diseases of Pepper in the Field

2.2.2. Establishment of Quadrats

An orchard was selected in each of the two localities (Njombe and Penja), and four quadrats of 20 pepper plants each, 20 wide and 20 long, were delineated within each orchard. Several identification keys were used to identify and define the diseases that were present [13].

Disease incidence and severity were assessed on the 20 feet along the diagonal using the following formulae:

I (%) = $\frac{Xi}{Xt} \times 100$ [14] where: I: Incidence; Xi: Number

of infected trees per quadrat; Xt: Total number of trees sampled.

The degree of disease severity on infected plants in the field was assessed by observation of pepper plants and a score of 0 to 4 was assigned to each percentage using a scale adapted from [15]:

0 = no symptoms;
1= 25%: [0- 1/4] of the foot has symptoms;
2 = 50%: [1/4-2/4] of the foot has symptoms;
3=75%: [2/4-3/4] of the foot has symptoms;
4=100%: [3/4 -4/4] of the foot has symptoms

The disease severity index (%) was calculated using the formula [15].

$ISM(\%) = \frac{\Sigma n \times v}{N \times V} \times 100$ With: ISM: disease severity index: number of infected plants for each score; v: rating index (0-4); N: total number of plants; V: highest rating index.

2.2.3. Sample Collection

Sampling was carried out on vine (pepper plants) and stakes bearing leaves, stems, roots and pods (pepper plants) showing typical disease symptoms. In order to better observe the spatial distribution of diseases in the study sites, samples of infected leaves, stems, roots and pods were taken from each vine along the diagonal of each quadrat. The samples were collected and wrapped in blotting paper soaked in distilled water, labelled and transported to the laboratory.

2.2.4. Isolation, Purification and Identification

The various parts of the pepper plant (leaves, roots, stems and pods) showing typical disease symptoms were cut and disinfected in a 5% sodium hypochlorite solution for 2 minutes, then rinsed with sterile distilled water for 5, 10 and 15 minutes respectively to remove traces of the disinfectant. These parts were placed on hydrophilic paper to absorb the excess water [16]. They were then aseptically seeded into sterile Petri dishes containing 20 ml of PDA culture medium at a rate of 10 fragments per Petri dish and incubated at a temperature of 18-20 ℃.

Fungal species were identified on the basis of the morphological characteristics of the mycelium (septate or nonseptate) and fruiting bodies (conidia) observed under the microscope, using mycological identification keys [17].

2.2.5. Characterisation of Pathogens

Pure isolates of the different fungi collected from samples of leaves, stems, and roots from the study locations were used to characterize the fungal species.

Parameters such as the number of sporangiophores, the length and width of sporangiophores, the number of sporangia, the length and width of sporangia and the number of partitions were assessed. The number of rust lesions was assessed by counting the leaves sampled at each site, and the mean and coefficient of variation were calculated using the following formulae:

Mean

$\mu = \frac{\Sigma x}{N}$ with: μ: mean; x: Number of lesions per leaf; N: total number of leaves.

Coefficient of variation

$CV = \sqrt{\sigma}$ with: CV: Coefficient of variation

2.2.6. Pathogenicity Test

Koch's postulate calls for a set of techniques necessary for the detection and identification of the pathogen in situ. To identify the various pathogens responsible for the disease symptoms observed in *P. nigrum*, two (02) techniques were used, namely: inoculation on young pepper plants for pathogens that attack the roots and inoculation on detached apparently healthy leaves for pathogens that attack the leaves more.

2.2.7. Inoculation on Detached Leaves

Inoculation was carried out on apparently healthy *P. nigrum* leaves (Figure 1). These leaves were rinsed with tap water and then disinfected with 1% sodium hypochlorite for 2 minutes and rinsed with sterile distilled water. The leaves were then placed in sterile Petri dishes containing blotting paper soaked in sterile distilled water.

Figure 1. *Inoculated detached leaves.*

Mycelia from pure isolates of each fungus were scraped into Petri dishes containing distilled water and drops of inoculum were placed on the upper surface of each leaf. The dishes were then labelled (name of isolate and date of inoculation). The boxes containing the leaves were incubated for 7 days at a photoperiod of 12/12.

2.2.8. Inoculation of Young Plants

Inoculation was carried out by extracting the main root of the pepper plants from the pot and soaking the roots in flasks containing spore suspensions (10^3spores/suspension) for 10

min (Figure 2). After inoculation (by dipping), the roots were reintroduced into the pots. Control plants underwent the same treatment but with distilled water instead of inocula. Three batches of three pepper plants were inoculated respectively with *Fusarium* sp, *Rhizoctonia* sp and *Colletotrichum* sp. Each batch was identified by a label bearing: the date of inoculation, the type of inoculation, the pot number and the name of the microorganism. Observations focused on the description of symptoms observed in relation to those observed in the field.

Figure 2. *Young plants inoculated.*

Data analysis

The data collected on incidence, severity, rust symptomatology and microscopic characteristics of the pathogens were entered into Microsoft Excel 2013 and then subjected to analysis of variance (ANOVA). Means were separated using Duncan's test at the 5% probability threshold.

3. Results

3.1. Description of Pepper Disease Symptoms in the Field

A total of six (06) diseases were identified at the two sites (Njombe and Penja). These were: gall, anthracnose, Cercosporiosis, mildew, rust and yellowing.

3.1.1. Anthracnose

Plants affected by anthracnose show circular or angular water-soaked lesions on the leaves, which then become soft and slightly sunken (Figure 3). The centres of the lesions are either orange or brown and then turn black, while the surrounding tissue is lighter in colour.

Figure 3. *Symptom of anthracnose.*

3.1.2. Cercosporiosis

In the early stages of infection, infected pepper leaves show brownish circular spots with light-grey centres and reddish-brown margins (Figure 4). In severe cases, spots can also be seen on the stem and calyx, often resulting in rotting of the stem tip or leaf.

Figure 4. *Symptom of Cercosporiose.*

3.1.3. Rust

Rust on plants appears in the form of pustules or elliptical blisters (Figure 5A). They result from the development of urediniospores which multiply along the vein of the leaf blade or sheath. These pustules are scattered, rounded then linear in shape and can reach 10 to 12 mm in length. They frequently become confluent and form irregularly wide streaks. The pustule-covered epidermis eventually ruptures irregularly, revealing a powdery mass of brick-red urediniospores. It then takes on the appearance of jagged shreds, roughening the surface of the organ.

When the plant is close to maturity, the pustules turn black with the production of teliospores. The first symptoms are

bulging, yellowish or orange spots on the upper surface of the leaves (Figure 5B).

Figure 5. *Symptoms of red rust on pepper plants; Lesions on leaves (A); bulging spots (B).*

3.1.4. Downy Mildew

Downy mildew on pepper leaves presents diffuse spots, with a white conidial felting on the upper and lower surfaces of the leaves (Figure 6). A heavy attack can lead to the total loss of the plant. The first symptoms appear on the upper surface of the leaves in the form of pale yellow to crimson-red spots, which gradually become necrotic. They are irregular or angular and often delimited by the veins.

Figure 6. *Downy mildew on P. nigrum leaves.*

3.1.5. Gall

At both sites, gall appeared as abnormal swellings, bulges or outgrowths on the upper surfaces of pepper leaves, stems and pods. These blisters were generally round or elongated in shape and whitish in colour (Figure 7).

Figure 7. *Gall on pepper leaves.*

3.1.6. Leaf Yellowing (Slow Decline of the Pepper Plant)

Pepper plants generally affected by slow decline show a reduction in leaf area, followed by a progressive loss of leaves, which may lead to complete desiccation of the plant after a few years (Figure 8).

Figure 8. *Symptom of slow dieback of the pepper plant.*

3.2. Incidence and Severity of the Diseases Identified

Disease Incidence

Figure 9 shows the incidence of the various diseases observed at the two (02) sites which are the two main pepper production basins in Cameroon. The incidence of the diseases varied according to the site. At Njombe, these values were 100; 97; 95 and 80% respectively for gall, rust, cercosporiosis, anthracnose, mildew and leaf yellowing. Statistical analysis showed that there was no significant difference between the incidences of anthracnose in the two study sites at the 5% threshold according to Duncan's test. On the other hand, in Njombe, only leaf yellowing showed a low incidence (65%).

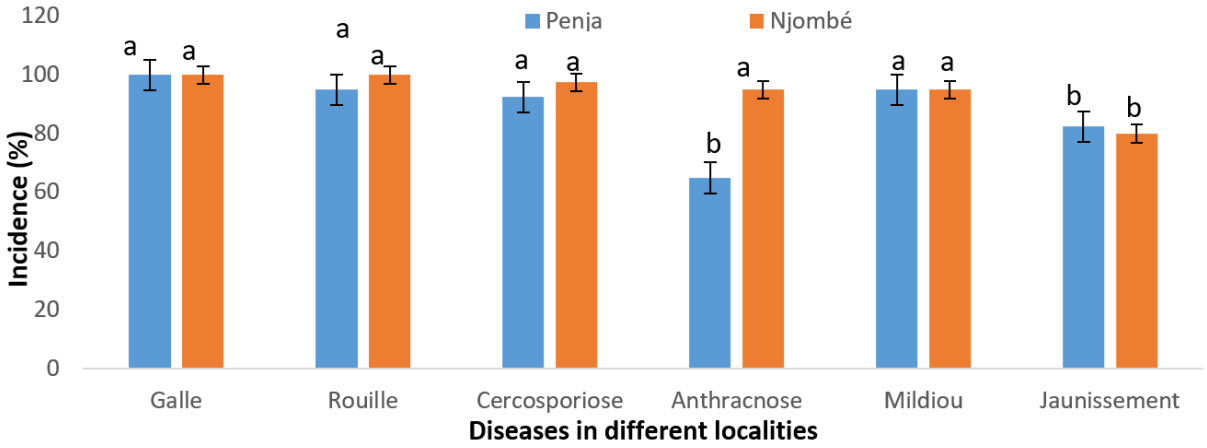

Figure 9. *Incidences of different diseases in different localities.*

3.3. Disease severity

Table 1 shows the severity of the various diseases in Njombe and Penja. Statistical analysis shows that: Gall, downy mildew and leaf yellowing (slow dieback) had the highest severity values in Penja: 60.00 ±19.75; 48.75 ±7.75 and 35.00 ±8.00 respectively. A significant difference was observed between these diseases in the two sites. At Njombe, on the other hand, only cercosporiosis showed a high level of severity (46.25 ±13.50). Disease trends on pepper were significantly different at the two sites, with the exception of red rust, which showed no significant difference according to the Duncan test at the 5% probability threshold.

Table 1. *Severity (%) of the different diseases in the two study areas.*

	Gall	Rust	Cercospora	Anthracnose	Mildew	Yellowing
Njombe	31.25 ±9.75 b	39.25 ±13.25 a	46.25 ±13.50 a	23.75 ±13.00 a	28.00 ±21.5 b	20.00 ±18.50 b
Penja	60.00 ±19.75a	34.25 ±14.50 a	31.75 ±17.25 b	16.25 ±2.50 b	48.75 ±7.75 a	35.00 ±8.00 a
Pr > F	0.000	0.147	0.001	0.003	0.000	0.002
Significant	Yes	No	Yes	Yes	Yes	Yes

Numbers followed by the same letter in the same column are not significantly different according to the Duncan test at the 5% threshold.

3.4. Characterisation of the Pathogens Associated with the Diseases Identified

A total of seven (07) fungal species grouped into 04 classes and one algal species were identified as the main causal agents of pepper diseases. These are mainly the Sordariomycetes class (*Colletotrichum gloeosporioides, Colletotrichum necator, Fusarium oxysporum, and Fusarium solani*), the Dothideomycetes class (*Cercospora* sp), the Eurotiomycetes class (*Aspergillus* flavus) and the Basidiomycetes class (*Rhizoctonia* sp). In terms of algal species, only *Cephaleurus virescens* belonging to the Ulvophyceae class has been identified as the causal agent of pepper rust.

Macroscopic and Microscopic Description of Fungal Species Identified

3.4.1. *Colletotrichum gloeosporioides*

In 14 day-old pure culture, *C. gloeosporioides* has a black mycelium and mycelial growth is very slow (Figure 10). Microscopically, single acervuli, rarely in groups, sometimes resemble pycnidial bodies emerging from broken greyish-black spots. Setae absent or barely visible at the base of the conidial mass. Conidial mass dull white to dull orange or sometimes bright orange, mycelium usually absent, when present white and shiny.

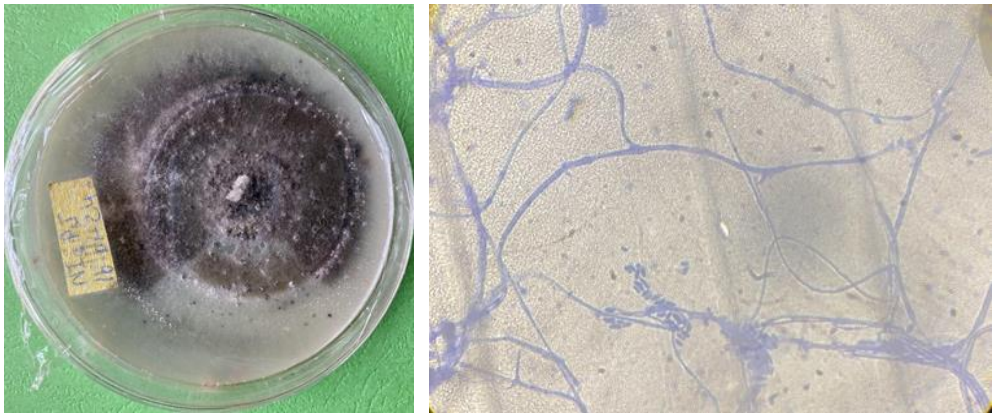

Figure 10. *Colletotrichum gloeosporioides; Macroscopic appearance (A); Microscopic appearance (B).*

3.4.2. *Colletotrichum Necator*

In pure culture, *C. necator* has a milky white mycelium with very slow mycelial growth. The acervuli are usually in groups, coalescing and covering the seed (Figure 11), rarely isolated. Setaceae few in number, longer than the conidial mass. Conidial mass orange to bright orange. Mycelium sparse, white. Conidia hyaline, oblong to round, unicellular with rounded ends.

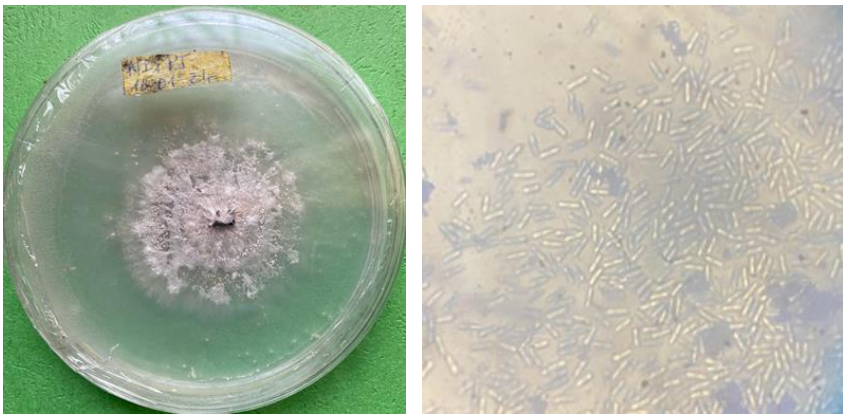

Figure 11. *Colletotrichum Necator; Macroscopic appearance (A); Microscopic appearance (B).*

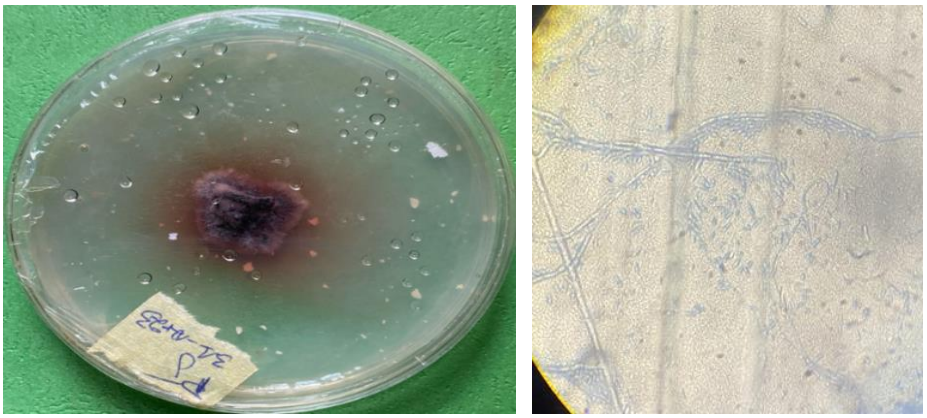

Figure 12. *Fusarium oxysporum; Macroscopic appearance (A); Microscopic appearance (B).*

3.4.3. *Fusarium Oxysporum*

The fungus produces sparse to abundant growth, covering part or all of the sown sample. The mycelium can be pink to cream in colour. Microconidia are generally produced in abundance, they vary greatly in size and are oval, elliptical or kidney-shaped, generally non-septate but septate conidia can be found.

3.4.4. *Fusarium Solani*

The fungus generally produces a white to cream mycelium, which is generally sparse and flaky (Figure 13). These become translucent to opaque and milky white as the fungus grows. Microconidia are 1-2-celled, hyaline, oval, ellipsoid or kidney-shaped. Macroconidia are produced in abundance in cream-coloured sporodochia. They are 3-4 septate, hyaline, thick-walled with a short, rounded, and sometimes hooked apical cell and a serrated base in the basal cell.

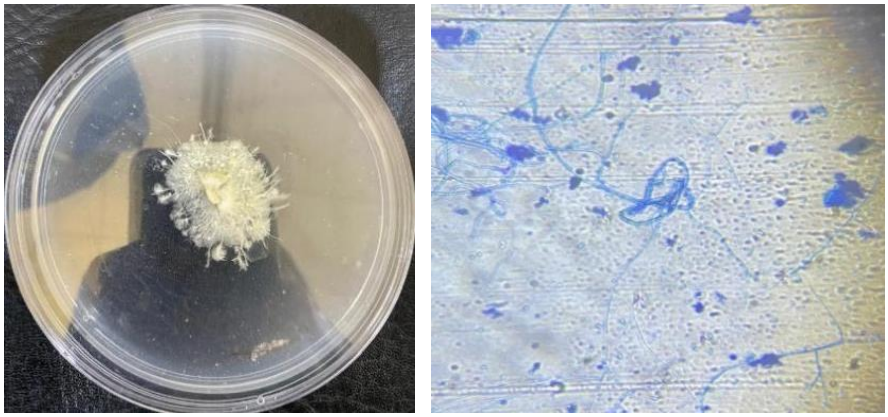

Figure 13. *Fusarium solani; Macroscopic appearance (A); Microscopic appearance (B).*

3.4.5. *Cercospora* Sp

On a macroscopic scale, the fungus produces a loose, gray mycelium in which light brown to brown conidia are observed on many hyphae (Figure 14A). Conidia appear to be “thicker parts of the hyphae.” It is only from the preparation of the slides that we can be sure that these thick parts of hyphae are indeed conidia of the fungus. Hyaline conidia, elongated to fusiform, tapering towards the tip (Figure 14B).

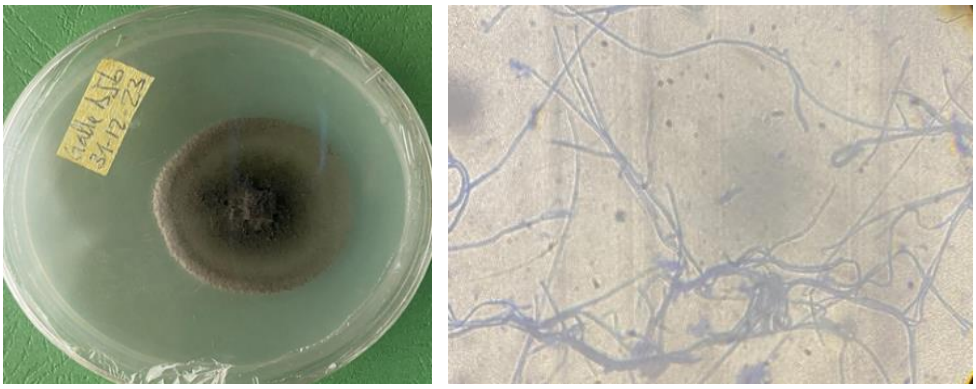

Figure 14. *Cercospora sp; Pure culture (A); morphology of the mycelium under the microscope (B).*

3.4.6. *Aspergillus Flavus*

In pure culture aged 14 days *Aspergillus flavus* presents a brown colored mycelium, with a cottony appearance; under the microscope, the aspergillus heads are biseriate with phialid emuls all around a round vesicle. The conidia are brown, spherical, sometimes ribbed (Figure 15).

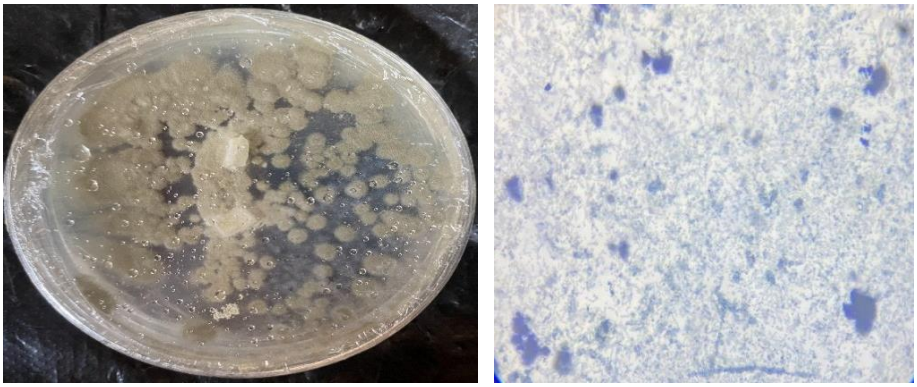

Figure 15. *Aspergillus flavus; Pure Culture (A); Microscopic appearance (B).*

3.4.7. *Rhizoctonia* Sp

This species is characterized by mycelial colonies radiating under a magnifying glass, dense and grayish in color (Figure 16A). Radial growth is very rapid on PDA medium. The mycelium is light brown, septate and has a slight constriction at the level of the septa; the mycelium is devoid of spores (Figure 16B).

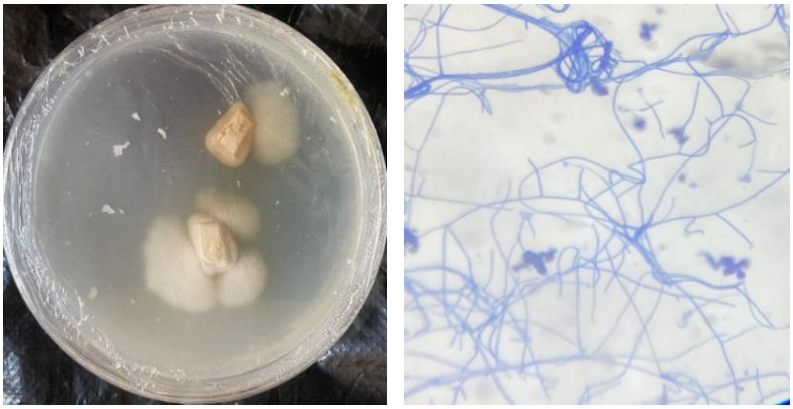

Figure 16. *Rhizoctonia sp; Macroscopic appearance (A); Microscopic appearance (B).*

3.5. Morphological Characteristic of the Causal Agent of Red Pepper Rust

3.5.1. Macroscopic Characteristic of the Causative *Agent of Red Pepper Rust*

Red rust symptoms were observed on the upper surfaces at both sites. In the Njombe site, symptomatic leaves presented numerous circular lesions varying between 43 and 84 with an average of 59.66. The coefficient of variation was 4.02% and the diameters of the lesions on the leaves varied between 1.12 and 3.57 mm with a mean of 2.32 mm. The coefficient of variation of the diameters of the lesions was 25.94%. In the Penja site, the number of lesions per leaf varied between 48 and 146 with an average of 105.33 and a coefficient of variation of 6.18%. The diameters of the lesions in Penja varied between 1.17 and 2.9 mm with an average of 2.36 mm and coefficient of variation of 12.91%. Leaf spots were rusty-orange-brown in color and generally appeared on the upper surface of the leaves (Figure 17A).

Figure 17. *Red rust, (A) macroscopic appearance, (B) microscopic appearance.*

Figure 18. *Necrosis on detached leaves after inoculation; Colletotrichum sp strain (A); Cercospora sp strain (B).*

3.5.2. Microscopic Characteristic of the Causative Agent of Red Pepper Rust

Conidiospores of *C. virescens* are cylindrical, erect, uniquely shaped, septate (1 to 6 septa per conidiophore), branched at the terminal end and long. Conidia have distinctive orange, fuzzy spots or blotches. The "fuzziness" is caused by algae spores and their supporting structures. It is sometimes called green spot because the spots can have a crusty or flaky appearance. Sporangia are produced at the ends of conidiospores in groups of 2 to 7. Conidia are globose, unicellular and white to orange in color.

3.5.3. Bacterial and Viral Diseases

As for bacterial and viral diseases, no characteristic symptoms (dwarfism, stunting, etc.) were observed in the two study sites.

3.6. Pathogenicity Test Results

Inoculation on Leaves

For leaf inoculation, pure cultures of *Colletotrichum* sp., *Cercospora* sp and *Fusarium* sp were used. Characteristic necroses were observed from the 8th day after incubation at

25 ℃ on the leaves inoculated with the pure isolates of *colletotrichum* sp and *Cercospora* sp (Figure 18A and 18B), however no symptoms were observed on the inoculated plants. With strains of Fusarum sp.

4. Discussion

From the different symptoms observed in the field, six (06) diseases were identified in the pepper fields of Njombe and Penja. These include gall, rust, Sigatoka, downy mildew, anthracnose and leaf yellowing. The characteristic symptoms were generally necrosis, burns, yellowing, drying, diffuse white spots and scattered red spots. This number of diseases present in fields may be due to climate, cultivar type and survival on farms….. Furthermore, [12] in the localities of Loum-Manjo, Njombe-Penja (Littoral Region) and Tombel (South-West Region) when working on the identification of the main phytopathogens of pepper (*Piper nigrum* L.) from Penja and control of slow dieback by *Trichoderma asperellum* and the hydroethanolic extract of *Chromoleana odorata* had recorded the main ones are gall, white root rot, slow dieback, crown and root rot of the pepper tree and stem borers.

The average incidence and severity varied from one locality to another. Average disease incidences ranged from 100% for gall, 95 to 100% for rust, and 92.5 to 97.5% for Sigatoka, 65 to 95% for anthracnose, 95% for downy mildew and 80%. At 82.5% for yellowing. Likewise, the severities varied from 50 to 75% for gall, 34.25 to 39.50% for rust, 31.75 to 46.75% for Cercosporiosis, 16.25 to 23.50% for anthracnose, 28 to 48.75% for downy mildew and 20 to 35% for leaf yellowing. Variations in incidence and severity between the different diseases studied may be due to the type of cultivar used, agricultural practices, soil and climate as explained [18]. Or they state that variation in disease incidence in fields may be due to differences in soil moisture, rainfall intensity for disease development during the season. Indeed, rainfall and relative humidity create an environment conducive to the growth and development of the pathogen. Agricultural practices such as excessive use of chemical fertilizers, poor management of crop residues and use of infected seeds can promote the spread of diseases in plantations [19]. These incidence data are different from those obtained in Kodagu district by [18] and [20] in India. The incidence and severity of the disease were high in both areas. This result seems correct to the extent that, in these areas, pepper cultivation is most intensified. In fact, these localities have the largest number of pepper producers and the largest planting areas. Due to the proximity between pepper plantations, parasitic pressure was accentuated in this locality [21].

Fungi are responsible for many pepper plant diseases. In this study, the most encountered fungi were *Cercospora* sp, *Colletotrichum gloeosporioides*, *Colletotrichum necator* and *Fusarium solani*. This result agrees with that of [22] in Asia where it was demonstrated that these pathogenic fungi are the most encountered and the most important. Furthermore, [23] working on the distribution of pathogens responsible for Fusarium wilt of bananas, found certain species of these fungi (*Fusarium* sp, *Colletotrichum* sp) and demonstrated that they were the most invasive.

The results of our disease inventory study show that red rust, caused by *Cephaleurus virescens*, is an emerging disease on pepper plants. The incidence and severity of this disease were very high in both sites. These high incidences and severity can be explained by the fact that red rust being an emerging disease on the pepper plant, no control method has yet been considered. Similar results were obtained by [24] who identified red rust on mango in Brazil, as well as the results of [25] who all identified red rust on cashew.

The attacked leaves had an average of 60 lesions in Njombe, which can be considered a low value compared to Mycosphaerella citri which had an average of 131 lesions per leaf [26]. On the other hand, in Penja, the attacked leaves presented an average of 105 lesions, which corroborates with the results obtained by [27] who obtained an average of 100 lesions on the cashew nut. In both sites, a high percentage of lesions (65% in Njombe and 87% in Penja) had a diameter less than 2.36 mm, which demonstrates a greater capacity of the pathogen to colonize the entire leaf surface. Similar results were obtained by [27] who obtained 98% of lesions with a diameter less than 3 mm.

Observations relating to the pathogenicity test of the different fungi revealed the appearance of symptoms which were diffuse brown necroses with a yellow halo or dark spots and the progressive yellowing of the oldest leaves towards the less old leaves on young plants of pepper plant. These symptoms appeared 8 to 10 days on detached leaves and 3 to 4 weeks on young plants. Similar observations were also recorded by [28] when working on the morphological characterization *of Alternaria alternata* responsible for the deterioration of Solanaceae in Algeria had recorded symptoms 7 to 10 days after inoculation. This result also agrees with that of [29] in Germany who, working on the pathogenicity of Alternaria species on potato plants, reported the same results as those above.

Abbreviations

I	Incidence
Xi	Number of Infected Trees Per Quadrat
Xt	Total Number of Trees Sampled
ISM	Desease Severy Index
PDA	Potato Dextrose Agar
μ	Mean
x	Number of Lesions Per Leaf
N	Total Number of Leaves
CV	Coefficient of Variation

Conflicts of Interest

The authors declare no conflicts of interest.

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