

Impact of Three Isolates of *Cercosporidium personatum* (Berk. Et M.A Curt.), a Pathogen of Late Leaf Spot, on Defoliation and Yield in Groundnut (*Arachis hypogaea* L.) under Controlled Conditions

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Abstract

Late leaf spot caused by *Cercosporidium personatum* is one of the most widespread groundnut leaf diseases. Along with early leaf spot and rust, it is one of the main fungal diseases hampering groundnut production worldwide. Late leaf spot accounts for significant yield losses throughout the world where groundnuts are grown. This reduction in yield caused by the disease could result in yield losses of between 50% and 70%. In Burkina Faso, the disease is present throughout the country, with incidence varying according to region and season. Could the variability in the incidence of the disease be linked to the nature of the isolates or to the conditions from each agro-ecological zone? In this study, the aim was to assess the capacity of three isolates from three agroclimatic zones of the country to defoliate and reduce groundnut yield. To this end, three isolates of *Cercosporidium personatum* (Berk. Et M.A Curt.) were collected in these zones and evaluated on three contrasting groundnut varieties. A split-plot design was used for the experiment. Isolates were prepared from samples collected in farmers' fields. After incubation in the laboratory, leaf spots showing good sporulation were scraped off with a scalpel after immersing the leaves in distilled water. Inoculations were carried out under controlled environment. After inoculation with the isolate, the percentage

of defoliation and the reduction in yield of these varieties were then evaluated. Inoculation was carried out from the 30th day after sowing with *Cercosporidium personatum* spore suspensions at 10^5 spores/ml. The study showed that the percentage of defoliation ($P = 0.0001$) and the reduction in yield ($P = 0.0001$) were significant. The study revealed that, whatever the variety, isolate I3TF from the Upper Basins region in the South Sudanese zone caused the greatest defoliation and the greatest reduction in yield. The variety TS32-1, regardless of the isolate used for the treatment, recorded the best yield. The variety PC79-79, regardless of the isolate used for the treatment, recorded the lowest percentage of defoliation. The highest defoliation recorded under the effect of the isolates was of the order of 72.20%; the highest yield reduction was of the order of 87.20% compared with the water control.

Keywords

Arachis hypogaea, Isolate, Late Leaf Spot, *Cercosporidium personatum*, Yield, Defoliation

1. Introduction

Groundnut (*Arachis hypogaea* L.) is an annual legume grown worldwide [1] [2]. For a food and cash crop by excellence, it provides a variety of products for both human [3] and animal consumption. Indeed, it is a fodder crop of prime importance in semi-arid zones because of the excellent nutritional value of its seeds [4]. In Burkina Faso, groundnuts play a key role in the diet and are also a source of income for farmers in rural areas, where they are mainly grown by women. Over the last five years (2017-2021), the average groundnut yield in Burkina Faso is estimated at 7513.6 kg/ha, and the average annual area sown to groundnuts over the same period is 515072.8 ha [5]. It is clear that groundnut production in Burkina Faso is below the plant's real potential compared with the average for West Africa. This situation is attributable to various constraints on groundnut cultivation. In addition to the challenge of soil fertility, good rainfall and seed quality, we should mention the importance of leaf diseases, especially fungal diseases, which are detrimental to the revival of the groundnut crop. These include groundnut rust and late leaf spot (caused by *Cercosporidium personatum* (Berk. Et M.A Curt.)) is both the most destructive [6] and the most important economic disease [7] of groundnut. Present where groundnuts are grown [8] [9] and throughout the country [10], late leaf spot causes yield and fodder losses [11]. Several control methods in various forms have been developed [7] [12] against the disease to improve groundnut productivity. Initial leaf spot symptoms usually develop during the vegetative growth stage (4 - 8 weeks after planting) when plants are gaining the canopy that supports pod formation and filling [13]. Severe plant damage occurs after flowering during pod and grain formation, hence leading high yield losses [13]. In fact, early or late leaf spot represents surface areas that considerably reduce the total photosynthetic surface

area of the leaves. These damaged areas are therefore unable to carry out photosynthesis and, if there are a large number of them on the leaflet, the leaves will have a low photosynthetic yield, hindering the overall development of the plant. Given the wide and heterogeneous spatial distribution of this parasite (*Cercosporidium personatum*) across agro-climatic zones that are strongly influenced by climatic variability and increasingly exacerbated by climate change, would the different isolates of this plant pathogenic fungus from these areas have a different impact on reducing groundnut yields and defoliation rate? This study aimed to evaluate the impact of three isolates of *Cercosporidium personatum* (Berk. Et M. A. Curt.) collected in three agro-climatic zones of Burkina Faso on the yield reduction and defoliation percentage of three groundnut varieties resulting from scientific research. The general objective is to assess their capacity to reduce yield and cause defoliation in groundnut. This will involve: 1) determining the isolate that causes the greatest reduction in yield, 2) determining the isolate that causes the highest percentage of defoliation, 3) determining the variety that produces best despite the impact of the isolates, 4) determining the variety that records the lowest percentage of defoliation. The results could lay the foundations for possible research into the pathotype of this fungus. They will also be able to guide us in the choice of varieties recommended for each zone and according to the seasons.

2. Materials and Methods

2.1. Plant Material

The plant material used in this study composed of three groundnut lines. These were TS32-1 and SH470P, lines from INERA Burkina Faso, with a vegetative cycle of three months. They are susceptible to the disease. PC79-79, a line from ISRA in Senegal, has a vegetative cycle of about four months and is resistant to the disease.

2.2. Methods

2.2.1. Collection of Samples

Surveys and collection of groundnut leaf samples were carried out at three experimental stations located in the three agro-climatic zones of Burkina Faso. These were an experimental station at the Nazi BONI University in Gampela (12°22 West longitude and 12°25 North latitude) in the North Sudanese zone (Ouagadoudou) and two experimental stations at the National Center of Scientific and Technologic Research (CNRST) in Farakoba (04°20 West longitude and 11°06 North latitude) in the South Sudanese zone (Bobo dioulasso) and Kouaré (11°95 North latitude and 0°30 East longitude) in the Sub-Sahelien zone (**Figure 1**). The samples collected showed typical symptoms of *Cercosporidium personatum* infection. The various samples collected were immediately placed in transparent plastic bags and stapled together with the date and place of collection. The samples were placed in a cooler containing ice cubes and transported to the laboratory for preparation of the inoculum described below.

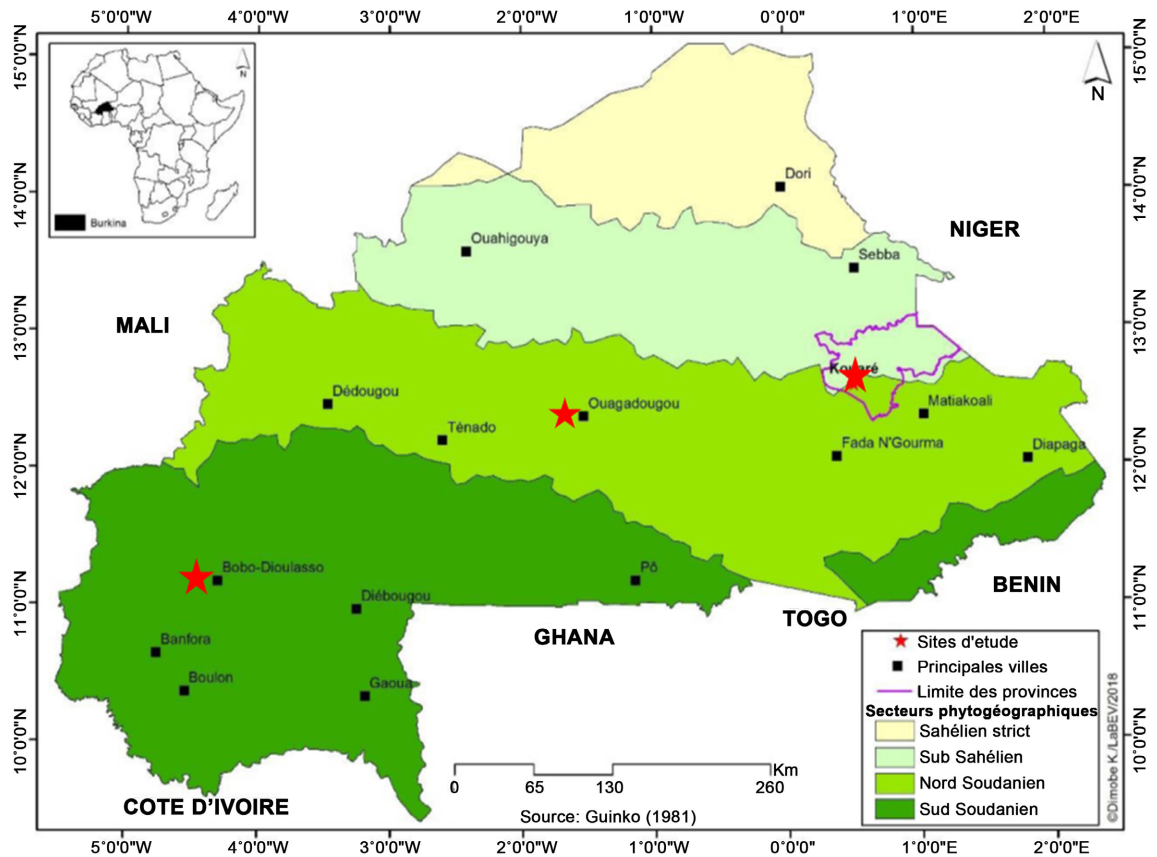


Figure 1. Sample collection sites.

2.2.2. Preparation of Isolates

The collected leaves were incubated in Petri dishes on moist blotting paper at 25°C - 30°C under a 12-hour light/12-hour dark photoperiod for 120 hours to ensure good sporulation of the conidia. For each of the three collection sites, a suspension of *Cercosporidium personatum* conidia was prepared by scraping leaf spots showing good sporulation with a scalpel after immersing the leaves in distilled water [14] [15]. Three isolates were prepared: Kouaré isolate with code **I1TK**, Gampela isolate with code **I2TG** and Farakoba isolate with code **I3TF**. The conidial concentration of the suspensions was determined using a Mallassez cell and adjusted to 10^5 conidia/ml for contamination of healthy groundnut leaves in the greenhouse. The control was distilled water.

2.2.3. Experimental Set-Up

The set-up used in the greenhouse is a split-plot with two factors: isolate and variety, in a set-up with three replicates. Each replication is made up of four sub-blocks, each corresponding to a treatment. For this experiment, plastic buckets measuring 24 cm in diameter and 28 cm deep were filled to 2/3 of their depth with a mixture of sterilised clay soil and sand in a 1:1 ratio. For each variety, two pest-free peanut seeds were sown at a depth of 5 cm per pot. The potted plants were irrigated as required. Fertilizer at a rate of 100 kg/ha was applied on the 20th day after sowing.

2.2.4. Inoculation of Groundnut Plants

Thirty (30) days after sowing, each plant was sprayed with 10 ml of *Cercosporidium personnata* suspension (10^5 conidia/ml) using a plastic hand sprayer (400 ml). Control plants were sprayed with 10 ml of distilled water. For each isolate and for the control, a different hand plastic sprayer was used.

2.2.5. Assessment of Percentage Defoliation

The percentage of defoliation P (%) was observed at the stage of maximum leaf loss. In each pot, the main stems of both plants were observed. On each stem, the number of fallen leaves and the total number of leaves (absent and present) were counted. The percentage of defoliation is the ratio of the number of fallen leaves to the total number of leaves, expressed as 100.

$$P (\%) = \text{NFT} / \text{NTF} \times 100$$

P (%): Percentage of defoliation;

NFT: Number of Fallen Leaves;

NTF: Total number of leaves.

2.2.6. Evaluation of Yield

At the end of the cycle for each variety, the plants were uprooted, and the pods were removed. The resulting pods were placed in small cloth bags in the open air of the laboratory to dry for about a month. The weights of pods corresponding to each variety per isolate were determined using a Scout pro SP202 electronic balance.

2.2.7. Analysis and Expression of Results

Data on the percentage of defoliation and yield reduction induced by the impact of the treatment on the three groundnut varieties by the different isolates from the three agroclimatic zones were collected in the greenhouse, then subjected to an analysis of variance and a multiple comparison of means at the 5% threshold using R software. The coefficient of variation was calculated to show the dispersion of the values around the mean. The results were mainly expressed in the form of tables and figures.

3. Results and Discussion

3.1. Results

3.1.1. Effect of *Cercosporidium personatum* Isolates on Defoliation of the Groundnut

The results of the analysis of variance of the impact of the three *Cercosporidium personatum* isolates on the defoliation of groundnut varieties are given in **Table 1** below. The results show a significant difference between isolates ($P = 0.0001$) in terms of their impact on defoliation percentage. A significant difference between the varieties used in the experiment was also revealed ($P = 0.0001$). Finally, the results show a significant isolate*variety interaction ($P = 0.0001$).

The results on the average percentages of defoliation caused by the different isolates showed that isolate 13TF recorded the highest percentage of defoliation

Table 1. Analysis of variance of defoliation as a function of isolates.

Sources de variation	Dl	Sum sq	Mean Sq	F value	P
Isolat	3	3876.3	1292.09	17085.76	0.0001
Variété	2	304.6	152.3	1116.61	0.0001
Isolat*Variété	6	44.3	7.38	54.13	0.0001

Dl: level of freedom, **Sum sq:** Sum of squares **Mean Sq:** Mean squares; **F-Value;** **P:** P-Value.

with 72.20%. Average defoliation was 57.31%, slightly lower than the water control ITE with 43.48%. Isolate I2TG recorded a defoliation percentage of 52.88%. A multiple comparison of the means at the 5% threshold showed that the isolates were significantly different. The spread of values around the mean remained low, with a coefficient of variation of 0.47. The results obtained with the different varieties show that the TS32-1 variety recorded the highest leaf loss, with over 60% defoliation (**Figure 2**).

The variety PC79-79, recorded the lowest percentage of defoliation with 54.09%. The histograms (**Figure 3**) of the defoliation of each variety according to the isolate used showed that the interaction was quantitative, *i.e.*, the classification of the varieties was the same but the difference in the percentage of defoliation between the varieties was not the same for the different isolates. The results indicate that whatever the isolate used for the treatment, the variety TS 32-1 recorded the highest percentage of defoliation. Treatments with I3TF also resulted in the highest defoliation, irrespective of the variety.

3.1.2. Effect of *Cercosporidium personatum* Isolates on Yield Reduction of the Groundnut

The results of the analysis of variance of the impact of the three *Cercosporidium personatum* isolates on the yield reduction of the varieties used in the experiment are given in **Table 2** below. The results show a significant difference ($P = 0.0001$) between the isolates in terms of their impact on yield reduction compared with the water control. The analysis of variance also revealed significant difference ($P = 0.0001$) between the varieties used in the experiment. Finally, the results of the analysis indicated the existence of a significant ($P = 0.0001$) isolate*variety interaction.

The average reduction in yields caused by the different isolates showed that the inoculation with isolate I3TF recorded the lowest yields, irrespective of the variety, with production often very inadequate (195.02 kg/ha). The average yield was 601.6 kg/ha. The dispersion of values around the average expressed by the coefficient of variation is slightly high with a value of 37.15. The treatments carried out with the ITE water control recorded the highest yields, *i.e.* 1523.93 kg/ha. Multiple comparison of the means at the 5% threshold reveals that the yields recorded with the I1TK and I3TF isolate are not significantly different, although they do differ from those of I2TG and the ITE water control (**Figure 3**). The yield reductions caused by these two isolates were therefore not significantly different.

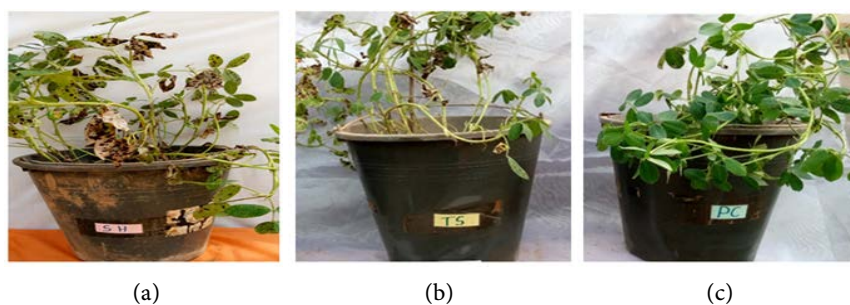


Figure 2. Defoliation of varieties (a) (SH470P), (b) (TS32-1), (c) (PC79-79) towards the end of the vegetative cycle.

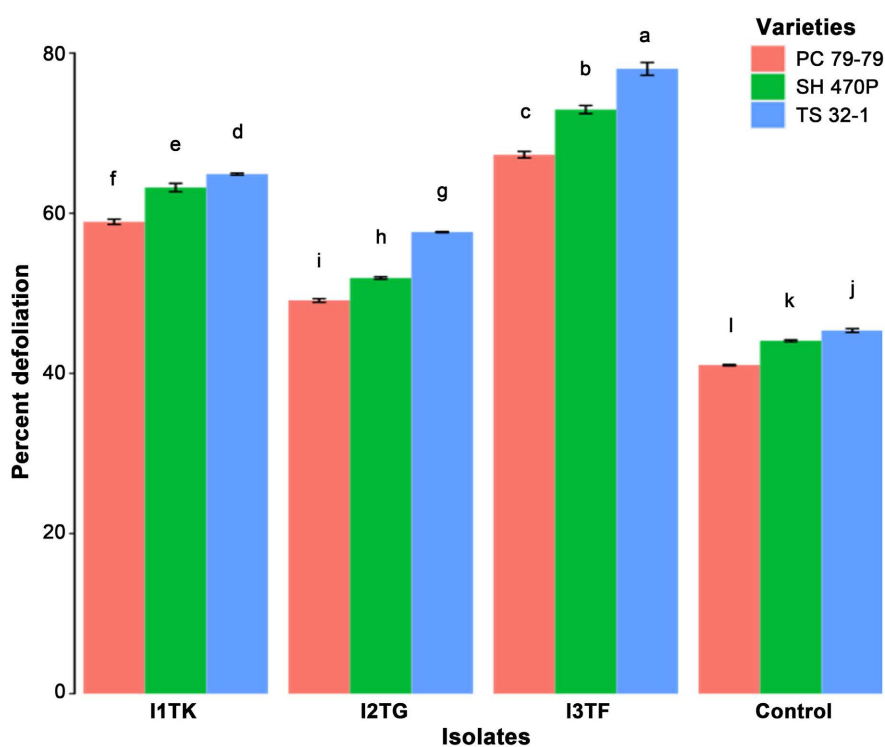


Figure 3. Histogram of the percentage defoliation of each variety by isolate.

Table 2. Analysis of variance of yield reduction as a function of isolates.

Sources de variation	Dl	Sum sq	Mean Sq	F value	P
Isolat	3	1,419,335	473,112	27,616	0.0001
Variété	2	549,647	274,824	16,042	0.0001
Isolat*Variété	6	355,887	59,315	3462	0.0001

Dl: level of freedom, **Sum sq:** Sum of squares **Mean Sq:** Mean squares; **F-Value;** **P:** P-Value.

The average yield results for each variety under treatment with the isolates showed that TS32-1 recorded the highest yield under treatment at 738.03 kg/ha. The variety PC79-79 recorded the lowest yield at 389.30 kg/ha. The dispersion of values around the average is not very high at 7.07. Although TS32-1 recorded the

highest yield compared with PC79-79 and SH47 it suffered the greatest reduction in yield compared with the water control (**Figure 3**). PC79-79 showed the smallest reduction in yield compared with the water control. The histogram of yields (**Figure 4**) for each variety as a function of the treatment used shows that the interaction is quantitative, *i.e.*, the ranking of the varieties is the same but the difference between the varieties is not the same for the different isolates. The results indicated that irrespective of the isolate used for the treatment, the TS32-1 variety always gave the best yield (738.03 kg/ha). It was also noted that treatments with I3TF resulted in the greatest reduction in yield. The differences in yield reduction differed from one isolate to another and from one variety to another; it should be noted that these differences, compared with the water control, were fairly large. The histograms of yield reduction (**Figure 3**) were significantly different, with the exception of TS2-1 subjected to isolate I3TF, which was not significantly different ($P = 0.6028$) from PC79-79 subjected to isolate I1TK. The yield of TS32-1 subjected to isolate I1TK was not significantly different ($P = 0.7033$) from that of PC79-79 subjected to isolate I2TG.

3.2. Discussion

Late leaf spot on groundnut growing is damaging through its capacity of defoliation and yield limitation [16]. The percentages of defoliation obtained with the water control were well below (less than 50%) those obtained under the treatment with the three isolates; this would indicate that the defoliation obtained with the water control was essentially the result of the senescence of groundnut plants, which seems to be a normal process in the growth and natural aging of the plant. Defoliation is also a normal physiological reaction of the plant to reduce

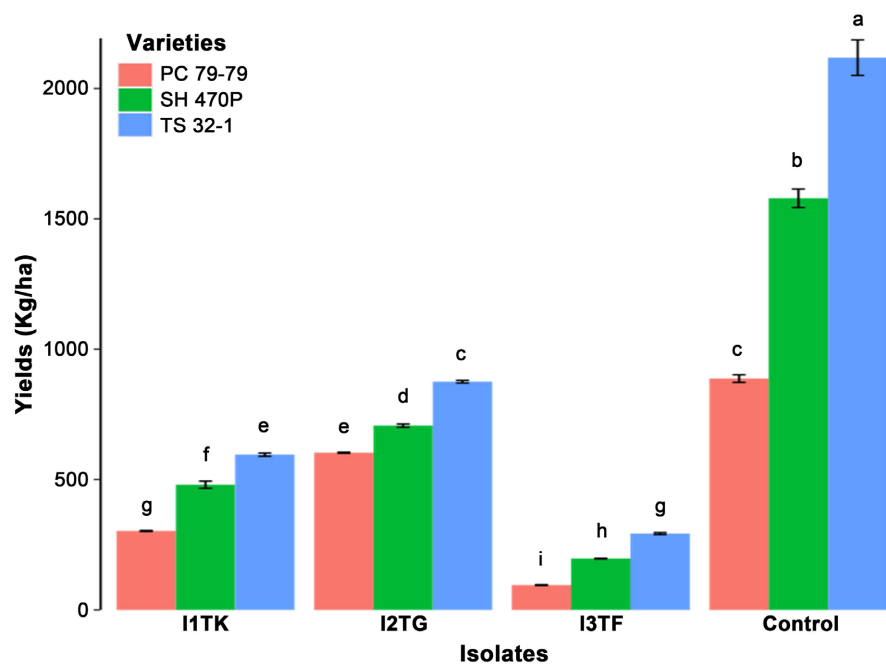


Figure 4. Histogram of the average yield of each variety according to isolate.

transpiration from the leaves [17]. However, apart from this natural aging, defoliation may be due to a number of environmental phenomena, including an attack by pathogens. Groundnut plants infected by this disease defoliated [18]. The percentages of defoliation recorded with each isolate were higher than those of the water control under the same experimental conditions; this shows that the isolates used to treat the varieties were indeed responsible for the defoliation of the groundnut plants. In fact, the lesions caused by the disease after infection by *Cercosporidium personatum* isolates constituted a stimulus for the abscission zone of the leaflet, leading to leaf drop [11] [18]. Attack is greater when there is no fungicide treatment leading to more pronounced defoliation [16] [19]. The high defoliation could also be explained by a hypersensitive reaction in the varieties that does not allow the fungi to sporulate and leads to early leaf drop; this would explain the high defoliation recorded in TS32-1 and even in PC79-79, a variety recognised as resistant. All this goes to show the importance of the effect of late leaf spot on groundnut cultivation in general and defoliation in particular. According to [20], late leaf cause damage to plants by reducing the photosynthetic surface through the formation of lesions, increasing the number and surface area of lesions and causing affected leaflets to fall off [15]. The separation of the means of the percentage of defoliation at the 5% threshold revealed differences between the isolates and the control. This phenomenon would indicate on the one hand that each of these isolates was virulent and on the other hand that this virulence was expressed with variability within isolates I3TF (72.20%); I1TK (62.33%) and I2TG (52.88%). According to [21], in the absence of controls, defoliation rates of late leaf spot infected groundnut can reach 100%. This is because the removal of infected leaves as the disease progresses can lead to complete defoliation in susceptible genotypes [22]. The highest defoliation was obtained with I3TF Isolate from the Upper Basins region, the second wettest region in the country, studying the action of isolates on the same groundnut variety under the same controlled treatment conditions and observing the different results on defoliation and reduction would indicate the existence of variable effects of isolates from each climatic zone. These results corroborate those of [10], who found that the isolate from the Upper Basins region was much more severe than those from the Central and Eastern regions because of the very high late leaf spot severity scores it caused on groundnuts with high incidence of the disease. The percentage of defoliation due to late leaf spot being correlated with the severity of the disease [23] would then explain the difference in the impact of the isolates on defoliation.

The difference in defoliation between the varieties TS32-1, SH470P and PC79-79 would be linked to the intrinsic value or genetic variation within each variety [24], although it is difficult to assess this intrinsic value due to the interactions of the varieties with the environment [25]. The varieties TS32-1 and SH470P are therefore recognised as susceptible and PC79-79 as resistant. TS32-1 recorded the highest defoliation, thus confirming its susceptibility to late leaf spot or developing a hypersensitive reaction to the infection. PC79-79, which recorded the

lowest defoliation, is a long-cycle resistant variety that could be recommended in the Upper Basins and Cascades region, where rainfall is higher and the disease more widespread. The drastic drop in yields recorded under the treatment of the different isolates would indicate, on the one hand, the harmful impact of late-stage of late leaf spot on groundnut yield and, on the other hand, the reduction in yield that would be a function of the virulence of the type of isolate used for the treatments. The yield reductions by the isolates are so high, which corroborates the statements by [2] [26] [27] that late leaf spot is one of the most serious foliar diseases affecting groundnut worldwide, causing huge yield losses. The respective reductions in isolates I3TF (87.20%) and I1TK (70.13) would indicate that in the event of a severe attack or combined with other fungal diseases such as rust and early leaf spot, this would lead to a total loss of yield. Some authors, such as [21], who had worked in greenhouses, had indicated that the disease could lead to yield losses of the order of 70%; for [9] [27]; groundnut plants infected by this disease lead to a yield loss of more than 50%. [18] [28] state that defoliation can cause yield losses of up to 70%.

It should be noted that the virulence of the fungi responsible for late leaf spot at the sites would also be due to certain genetic factors of the isolate, *i.e.* different genetic determinants that are regulated by the genes essential for pathogenesis [29]. The result is a reduction in pod yield, lower seed weight [28] and lower seed oil content.

TS32-1 recorded the highest yield, followed by SH470P and PC79-79. Although the first two varieties recorded the highest defoliation under the effect of the isolates, they obtained higher yields than the PC79-79 variety. This can be explained firstly by their susceptibility, secondly by their short vegetative cycle (90 days) compared with PC79-79 (120 days), and thirdly by the fact that they developed hypersensitivity to late leaf spot, resulting in very high defoliation without much impact on yield as compared to PC79-79, which is a resistant variety with a longer cycle. [24] found that defoliation was correlated with plant height, leaflet length and scores at 100 days after sowing, *i.e.* at the end of the cycle. In addition, [30] found that the difference in the agronomic performance of groundnut varieties could be linked to the fact that the pathogen presents different strains and physiological races.

The defoliation histograms, which clearly showed that TS32-1 suffered the greatest defoliation regardless of the isolate used, can be explained by a hypersensitivity reaction, as mentioned above, which allows the plant to partially avoid the impact of the disease on its yield. This is why the yield histograms showed that irrespective of the isolate used for the treatment, the TS32-1 variety gave the best yield. On the other hand, I3TF was the most virulent isolate, causing the most defoliation, regardless of the variety used. This is in line with the results of [31], who found that the site from which the isolate originated was favorable to late-leaf spot. [10] found that isolates from this site were more virulent than those from the eastern and central regions of the country, and consequently caused greater defoliation and a significant reduction in groundnut

yield. This would explain why treatments with I3TF cause the greatest reduction in yield, regardless of the variety.

4. Conclusion

The study on the effect of the isolates of *Cercosporidium personatum*, a pathogen of late leaf spot under controlled conditions, to defoliate and reduce groundnut (*Arachis hypogaea* L.) yield revealed a significant impact on percent defoliation and on yield reduction. This study showed that the isolate*variety interaction is quantitative, *i.e.* the classification of varieties is the same but the difference between varieties differs from one isolate to another. Regardless of the isolate used for the treatment, the TS 32-1 variety produced a better yield despite the effect of the isolates. It was also noted that treatments with isolate I3TF resulted in the greatest reduction in yield for all varieties. In terms of defoliation percentage, the ranking of varieties was the same, but the difference in defoliation percentage differed from one variety to another for the same isolate. Regardless of the isolate used for the treatment, TS 32-1 recorded the highest percentage of defoliation. Treatments with I3TF resulted in the greatest defoliation, irrespective of the variety. This study opens up prospects for a more in-depth study of isolates I3TF, I1TK and I2TG, which come from three different agro-ecological and climatic zones. This is probably the possible search for pathotypes or the establishment of a clear link between the impact of isolates and climatic conditions. It would also be essential to investigate the mechanism of resistance of TS32-1, PC79-79 and SH470P to late leaf spot.

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Conflicts of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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