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Evaluation under Semi-Controlled Conditions of the Pathogenicity of Three Isolates of *Phaeoisariopsis personata* (Berk. & M.A Curt.)

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Abstract

Peanut (*Arachis hypogaea* L.) late leaf spot is an important disease caused by *Phaeoisariopsis personata* (Berk. Et M. A Curt.). This fungus is responsible for the most damaging leaf spots in peanut production. The present experiment was undertaken to evaluate the pathogenic variability of *Phaeoisariopsis personata* in Burkina Faso. To this end, detached leaves and healthy plants of three peanut varieties were inoculated. Isolates I3TF, I2TG and I1TK of the pathogen (10^5 conidia/ml), collected respectively in the western, central and eastern agroecological zones of country, were used. The inoculated leaves were kept in Petri dishes on moist blotting paper and stored in the laboratory during the experimental period. The inoculated plants were grown under glass in pots containing a mixture of sterilized sand and clay. The development of disease was monitored and severity was scored every 15 days using rating scale. The results obtained in the laboratory and in the greenhouse revealed that there is pathogenic variability in the isolates tested. Indeed, for each variety, the highest severity score was recorded in plants inoculated with isolate I3TF and the lowest severity score with isolate I1TG. In the laboratory the severity scores ranged from 6.76 to 8.80 in TS32-1, 6.18 to 8.29 in SH70P and 5.98 to 7.92 in PC79-79. In the greenhouse, the average severity scores ranged from 5.61 to 8.33 in TS32-1, from 5.19 to 8.00 in SH70P, from 4.90 to 7.50 in PC79-79. Thus, the variety TS32-1 was the most susceptible to all three isolates of the pathogen.

Keywords

Peanut, Late Leaf Spot, Pathogenic Variability, Severity Score, Isolate

1. Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop in a tropical and subtropical region of the World [1] [2]. It plays an important role in human and animal nutrition [3]. In almost all African producing countries, groundnut production is dominated by local producers, which accounts for more than 85% of the gross harvest in these countries [4]. In Burkina Faso, national production has been fluctuating for several years [5]. Groundnut is a food and cash crop. It is used in confectionery and is consumed in various forms [6] [7]. Groundnuts thus contribute to agro-pastoral integration which, in regions with low rainfall, is one of the possibilities for the country to maintain its agricultural activity at an economically viable level [8]. In Burkina Faso, in addition to being an important food crop, groundnuts are a source of income for farmers. The price of groundnuts is on the rise due to the high demand on the markets. Leaf spots are among the most destructive diseases of groundnut (*Arachis hypogaea* L.) worldwide [9]. Late leaf spot (LLS) is the most damaging foliar diseases of groundnut (*Arachis hypogaea* L.) which considerably limits its production worldwide compared to the early leaf spot. The effect of late leaf spot and early leaf spot can lead to up to 70% yield loss under severe conditions [10]. Late leaf spot can occur in all groundnut growing regions [11] [12]. This fungal disease caused by *Phaeoisariopsis personata* (Berk. Et M.A Curt.) is found throughout Burkina Faso where peanut is grown, although it is more prevalent in certain agroecological zones. Indeed as [13] have shown that the incidence and severity of the disease varies from one agroecological zone to another. This variation leads to varying responses of genotypes as [14] and control methods in different agroecological zones. In fact, genetic studies on late leaf spot resistance suggest that resistance to this fungal disease is complexe and polygenic in nature and sensitive to environment [15]. It is also evidence that environment factors, such as temperature and humidity, are important affecting genotypes resistance as [14] and infection and development of *Phaeoisariopsis personata* [16]. A pathogen variability is one of the main causes of failure of the crop variety [17]. As a result, a susceptible variety in one area may be resistant in another. Similarly, a control method that is effective in one area may not necessarily be effective in another. Groundnut remains an important oil, food and feed crop of the world [3]. Groundnut is consumed all over the world most of which are traditional cuisine [18]. In view of the importance of peanut grown generally in more than 100 countries as [19] and particularly in sub–Saharan Africa as [8]; the losses caused by late leaf spot in general, with up to 70% in case of heavy attacks [20]. Various control methods have been developed to improve production [21]. Traditionally, LLS disease is controlled by fungicides, which are costly and toxic to the environment [2] [22]. Globally current methods to treat leaf spot include cultural control, chemical control, the use of antagonistic organisms, and host plant resistance. These areas unabatedly continue to be an active research area, and current information on their efficacy will continuously be available [10]. Accurate spatio-temporal weather information is crucial as [23] for disease management. However, a better knowledge of *Phaeoisariopsis personata*, the most destructive leaf spot as [24] pathogen, is needed for an efficient use of these control methods. Therefore, the evaluation of the pathogenicity of *P. personata* isolates collected in different agroecological zones and of the level of resistance of some peanut varieties in these zones is essential. Would there be a difference in the virulence of isolates from different agroclimatic zones? Would the response of peanut genotypes be related to the nature of isolates from each agroclimatic zone? It is in order to answer these concerns that this study was conducted on *P. personata*. This will enable us to assess their virulence under the influence of climatic variability, which is increasingly exacerbated by climate change, and to recommend varieties that are adapted to it as well as effective control methods. The general objective of this work is to study the pathogenicity of isolates of *P. personata* (Berk. et Curt.) collected in the three regions of Burkina Faso; more specifically, it is to assess the pathogenic variability of the different isolates from the three agroecological zones of the country on the one hand, and to compare the reaction of some peanut varieties to these isolates on the other.

2. Materials and Methods

2.1. Collection of the Samples

The collection of peanut leaves naturally infected by late leaf spot was carried out in three agroclimatic zones of the country. These were the experimental station of the Nazi BONI University located in Gampela, in the centre of the country in the North Sudanese zone, the experimental station of the National Center of Scientific Research and Technology (CNRST) located in Farakoba, in the West of the country in the South Sudanese zone and the one located in Fada in the East region in the Sub-Sahelien zone (**Figure 1**). The characteristics of these agroclimatic zones are given in **Table 1**. The various samples collected were

Table 1. Characteristics of the climatic zones of Burkina Faso.

Characteristics of the climatic zones	climatic zones		
	South Sudanese	Nortth Sudanese	Sub-Sahelian
Annual rainfall	900 à 1200 mm	600 à 900 mm	300 à 600 mm
Duration of rainy season (days)	180 - 200	150	110
Number of rainy days	85 - 100	50-70	<45
Average annual temperature	27˚C	28˚C	29˚C
Seasonal amplitude	5˚C	8˚C	11˚C
Average air humidity			
- Dry season	25%	23%	20%
- Wet season	85%	75%	70%
Annual evaporation (class A tank)	1800 - 2000 mm	2600 - 2900 mm	3200 - 3500 mm

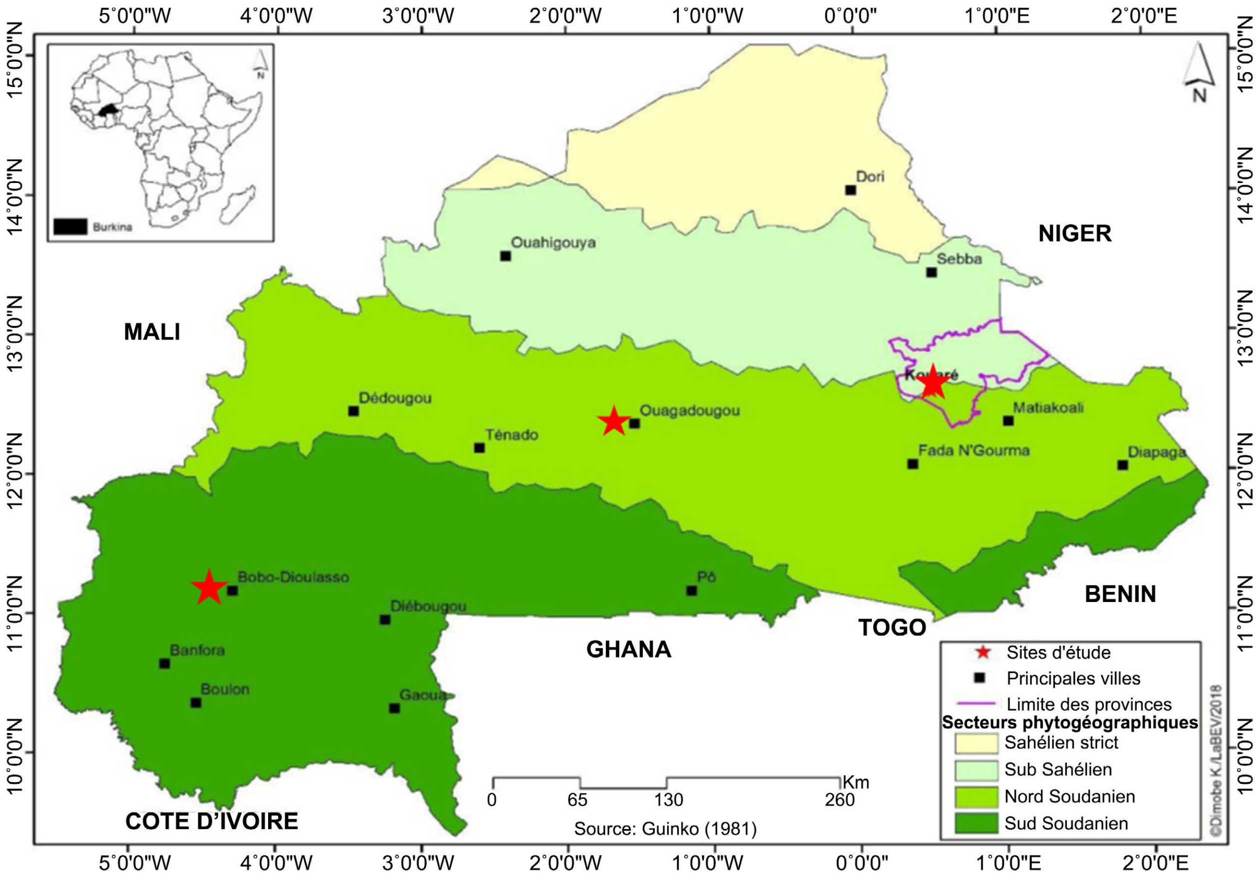

Figure 1. Sample collection sites.

immediately placed in transparent plastic bags and stapled with the date and place of collection. All the bags containing the samples were placed in a cooler containing ice cubes, for good preservation. The collected leaves were incubated in Petri dishes on wet blotting paper at 25˚C - 30˚C under a 12-hour light/12-hour dark photoperiod for 120 hours, allowing good sporulation of conidia on our collected samples.

2.2. Plant Material

The plant material used in this study is composed of three peanut lines. These are TS32-1 and SH470P which are lines from Institute of Environment and Agricultural Research (INERA) Burkina Faso, with a vegetative cycle of three months. They are susceptible to the disease. PC79-79 which is a line from Senegalese institute of agricultural research (ISRA) in Senegal, it has a vegetative cycle of 4 months and is resistant to the disease.

2.3. Preparation of the Inoculum of Isolates

A suspension of *P. personata* conidia was prepared by scraping leaf spots showing good sporulation with a scalpel after immersing the leaves in distilled water [25]. The characteristics of these preparations are given in **Table 2**. The conidial

Table 2. Overview of isolates used in the experiment.

Identity of the Isolates	Relevance	Origin of the isolate	Area of origin	Agroclimatic zone
I1TK	**Isolate 1** of *Phaeoisariopsis personata* from Kouaré	Fada N'gourma	Eastern (Burkina faso)	Sub-Sahelian zone
I2TG	**Isolate 2** of *Phaeoisariopsis personata* from Gampela	Gampela	Central (Burkina faso)	North Sudanese zone
I3TF	**Isolate 3** of *Phaeoisariopsis personata* from Farakoba	Farakoba	Upper Basins (Burkina faso)	South Sudanese zone

concentration of the suspensions was determined using a Mallassez cell and adjusted to 10^5conidia/ml for the contamination of healthy peanut leaves in the laboratory and healthy peanut plants in the greenhouse. Thus, the inoculum prepared from *P. personata* conidia collected at a given site is called an isolate.

2.4. Assessment of Late Leaf Spot Severity in the Laboratory

For in vitro evaluation of the pathogenicity of *P. personata* isolates, healthy leaves of the three peanut lines were placed in Petri dishes on wet blotting paper. The underside of the leaves was sprayed with 10 ml of *P. personata* conidial suspension (10^5 conidia/ml). The control leaves were sprayed with 10 ml of distilled water. The inoculated leaves were kept at room temperature in the laboratory (25˚C - 30˚C) under 12/12h light/dark photoperiod and sprayed daily with distilled water. During the experiment, scoring of late leaf spot severity was carried out every 5 days from the appearance of the first symptom using the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) 9-point scale as [26] adapted by [27].

2.5. Assessment of Late Leaf Spot Severity in the Greenhouse

For this experiment, plastic buckets (24 cm diameter and 28 cm deep) were filled to 2/3 of the depth with a mixture of sterilized clay soil and sand in a 1:1 ratio. Thus, for each variety, two uninjured peanut seeds were sown at a depth of 5 cm per pot. The potted plants were watered as needed. Thirty (30) days after sowing, each plant was sprayed with 10 ml of *P. personata* suspension (10^5 conidia/ml) using a plastic hand sprayer (400 ml). Control plants were sprayed with 10 ml of distilled water. The potted plants were grown in a greenhouse at a temperature of (25˚C - 26˚C) with a relative humidity of 63% to 74%. A fertilizer application at the rate of 100 Kg/ha or 0.45 g/pot was made on the 20th day after sowing. From the 46th day after sowing, the severity of late leaf spot was assessed every 15 days using the rating scale proposed by [26]. This is a nine (9) point scale. It ranges from score 1 which corresponds to the absence of the disease on the plant to score 9 where the plant is fully affected with maximum leaf loss.

2.6. Statistical Analysis

The data on the severity induced by the pathogenicity of the different isolates according to the lines were collected in laboratory and greenhouse, then subjected to an analysis of variance and a multiple comparison of means according to the Tukey test at the threshold of 5% using the MINITAB 18 software. The standard deviation had been calculated to access the dispersion of value around the average.

3. Result

3.1. *In Vitro* Test

Table 3 below presents the results of the evaluation of the pathogenicity of *P. personnata* isolates on detached leaves at 15 days after inoculation. From the analysis of variance of the severity scores of the isolates, it was found that there was a very highly significant difference between isolates (P = 0.0001), variety (P = 0.0001) and a very highly significant Isolates * Varieties interaction (P = 0.0001). At day 90 after sowing, the highest severity score was recorded on the leaves of the three varieties inoculated with isolate I3TF (TS32-1, SH470P and PC79-79). It is followed by isolate I1TK. Isolate I2TG was the least severe on the varieties tested. No symptoms of late leaf spot were observed on the control leaves. These results also indicate that the varieties TS32-1 and SH470P were the most susceptible to the three isolates tested.

Table 3. Average severity scores of *Phaeoisariopsis personnata* (Berk.Et M.A. Curt) isolates in the laboratory.

Isolates	Varieties	Average severity	Ratings * Group	Standard deviation
I3TF	TS32-1	8.80	A	0.577
	SH470P	8.29	B	0.001
	PC79-79	7.72	C	0.001
11TK	TS32-1	7.48	D	0.067
	SH470P	7.31	E	0.045
	PC79-79	6.89	F	0.057
I2TG	TS32-1	6.76	G	0.028
	SH470P	6.18	H	0.005
	PC79-79	5.98	I	0.001
ITE	TS32-1	1.00	J	0.000
	SH470P	1.00	J	0.000
	PC79-79	1.00	J	0.000

*Averages not sharing any letters are significantly different. P-value varieties: 0.0001; P-value Isolates: 0.0001; P-value Varieties * Isolates: 0.0001.

3.2. Greenhouse Trial

The average severity scores of *P. personata* isolates on potted peanut plants are given in Table 4 below. The analysis of variance performed on the severity scores indicated a very highly significant difference between isolates and between varieties (P = 0.0001). It also showed a highly significant difference in the Varieties * Isolate interaction (P = 0.0011). Isolate I3TF was the most severe on each of the three varieties used. Indeed, the highest mean severity scores were found on plants inoculated with this isolate. This was followed by isolate I1TK, which also had high mean severity scores. Isolate I2TG was the least severe on the varieties tested. The control plants did not show any disease, hence the average severity score of 1, regardless of variety. These results revealed that the varieties TS32-1 and SH470P were the most susceptible to the three isolates tested.

4. Discussion

The study of the pathogenic variability of *Phaeoisariopsis personata* (Berk. Et M.A Curt.), the pathogen of late leaf spot, was carried out both in the laboratory and in the greenhouse. It was found that isolates from the different agroclimatic zones were all virulent both in the laboratory on detached young leaves and on peanut plants in the greenhouse for all variety used.

In the laboratory, the results obtained from the leaves of the three peanut varieties that showed the characteristic symptoms of late leaf spot with the different isolates would indicate the existence of variability in the pathogenicity of these isolates. Indeed, isolate I3TF from the Upper-Bassin region was found to

Table 4. Average severity of *Phaeoisariopsis personata* (Berk. Et M.A Curt.) isolates in greenhouse.

Isolates	Varieties	Average severity	Ratings* Group	Standard deviation
I3TF	TS32-1	8.33	A	0.001
	SH470P	8.00	AB	0.001
	PC7-79	7.50	BC	0.577
I1TK	TS32-1	7.16	C	0.064
	SH470P	6.53	D	0.057
	PC7-79	6.06	DE	0.042
I2TG	TS32-1	5.61	EF	0.100
	SH470P	5.19	FG	0.005
	PC7-79	4.90	G	0.016
ITE	TS32-1	1.00	H	0.000
	SH470P	1.00	H	0.000
	PC7-79	1.00	H	0.000

*Averages not sharing any letters are significantly different. P-value Varieties: 0.0001; P-value Isolates: 0.0001; P-value Varieties * Isolates: 0.0011.

be the most virulent with the three peanut varieties used, followed by isolates I1TK and I2TG, respectively. While the location as [17] and climatic conditions as [15] [23] are often cited as an explanation for the variation in leaf spots incidence between climatic zones [28]; using the three isolates under the same laboratory and greenhouse treatment conditions and observing the pathogenic difference, would indicate the existence of variable virulence of strains from each climatic zone. This supports the results as [6] who found that the difference in agronomic performance of peanut species could be related to the fact that the pathogen has different strains and physiological races. It should also be noted that the virulence of late leaf spot fungi at the sites would also be due to certain genetic factors of the isolate, meaning different genetic determinants that are regulated by the genes essential for pathogenesis [13].

The results from the greenhouse were almost identical to those obtained in the laboratory and would further confirm the latter with respect to the pathogenic variability of the three isolates and the difference in the response of these varieties to these isolates. Observations in the greenhouse showed that high humidity of 63% to 74% for 11 hours with temperatures of 25˚C to 26.5˚C favored infestation and disease development. These observations of the study are similar to what was described by [29] [30]. The existence of variability in the disease progression depending on the isolates on the one hand and on the lines used resistance to disease on the other hand [24]; with a significant isolate * variety interaction would reveal the existence of a difference between the isolates which would be a function of the collection site. The Farakoba site in the Upper-Bassins region had been recognized as being very favorable to the development of leaf spot [30] in particular late leaf spot [22]. The disease is more destructive as [24] and most harmful groundnut diseases in the late growth stage [12] [19] [31]; causing considerable losses compared to early leaf spot in this site. The behavior of our varieties in relation to the isolates would indicate that some varieties would be much better able to develop on certain site or environments where they are less attacked. Indeed, this supports the fact, that the choice of a peanut variety depends on the environment and the purpose of production [32]. All this could explain the variable response in terms of resistance of peanut varieties in different environments to the same pathogen, on the one hand because of the impact of the environment as [14] [20] and on the other hand because of the nature of the isolate and then the interaction between the pathogen and the environment [6]. In this study, the isolate (I3TF) of *P. personata* (Berk. Et M.A Curt.) collected in the Farakoba site in the Upper-Basins region was found to be more severe with an average score of 8 on the TS32-1 variety in the greenhouse, which supports the observations made in the field [30]. Reference to [30], the strong development of late leaf spot in Farakoba is linked to the fact that this site is located in an area at high risk of late leaf spot infection due to favorable climatic conditions [28]. However, climatic conditions alone would not explain the severity or virulence of the pathogen *P. personata* (Berk. Et M.A Curt.) [23]. The isolates from the Kouaré (I1TK) and Gampela (I2TG) sites that recorded high severity ratings were equally virulent compared to the water control (ITE) that recorded a rating of 1 with almost no spotting on all varieties. The Varieties * Isolates interaction revealed a different reaction from one variety to another to the different isolates. While the I3TF isolate was more destructive, the Gampela I2TG and Kouaré I1TK isolates were less damaging to our lines. In view of the behavior of the varieties, PC79-79 would be much more suitable for production in the agroclimatic zone of Farakoba where the season is much longer and the disease more virulent. The lines TS32-1 and SH470P, could be recommended in the agroclimatic zones of Gampela and Kouaré.

5. Conclusion

The results of the present pathogenicity evaluation revealed that the three isolates tested have different pathogenicity in all the peanut varieties considered. It was found that the I3TF isolate from the Upper-Basins region in the South Sudanese climatic zone is the most pathogenic. It was followed by the Kouaré isolate I1TK from the eastern region in the Sub-Sahelian climatic zone and finally the Gampela isolate I2TG from the central region in the Northern Sudanian climatic zone. The interaction Varieties * Isolates indicated that the response of each variety is different from the other to the isolates. The variety TS32-1 was the most susceptible to all three isolates while the variety PC79-79 recorded the lowest severity scores. PC79-79 would be much more likely to produce in the Hauts-Bassins region and TS32-1 and SH470P in the Eastern and Central regions. Differences between agroclimatic zones can't be the only factors explaining the diversity of pathogenicity of isolates. The study should be further developed with a morphological and molecular characterization of the pathogen *P. personata* (Berk. Et M.A Curt.) to verify the existence of possible pathotypes.

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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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