MACROPHOMINA PHASEOLINA INFECTION ON STOMATAL FEATURES OF LEAVES OF *IPOMOEA BATATAS*

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ABSTRACT

The effect of *Macrophomina phaseolina* causing leaf spot disease on *Ipomoea batatas* was found to affect the chlorophyll content and yield of *Ipomoea batatas*. Koch's postulate was employed to ascertain the pathogenicity of the test fungus [1× 10^6 spores per ml, 1× 10^7 spores per ml and 1× 10^8 spores per ml] to cause leaf spot disease on *Ipomoea batatas.* The effect of leaf spot diseases on anatomical features of the test plants were assessed through stomatal size, density and index was assessed. Results revealed the reduction in stomatal number and distribution thereby resulting in significant reduction in the stomatal size, density and index on adaxial and abaxial surfaces of affected plant leaves compared to healthy plant leaves. The plants leaves treated with test fungus spore suspension concentration of 1× 10^8 spores per ml showed the smallest stomatal size value of 1.08 µm at both adaxial and abaxial surfaces compared to the control plant leaves [healthy plant leaves] with 1.17 µm at both surfaces. Stomatal index value was 8.33 mm^{-2} and 9.63 mm^{-2} at adaxial and abaxial surfaces compared to the control plant leaves with 11.28 mm^{-2} and 11.96 mm^{-2} respectively. The stomatal density of 3.6 mm^{-2} and 4.5 mm^{-2} at adaxial and abaxial surfaces compared to the control plant leaves with 5.0 mm^{-2} and 6.0 mm^{-2} respectively. This study showed that the degree of severity of the leaf spot diseases varied with fungal spore suspension concentration.

Keywords: Fungal spores, *Ipomoea batatas,* leaf spot disease, stomata.
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INTRODUCTION

Ipomoea batatas [L.] *Lam.* [Sweet potato] is a dicotyledonous plant that belongs to the family Convolvulaceae [1]. It is extensively grown in Nigeria and rated the fourth position among root crops in Nigeria [2]. Among food crops in Nigeria that are enriched high carbohydrates content, *Ipomoea batatas* is a good source of vitamins A, B and C, minerals and energy. The demand for the crop increased in urban areas as a substitute for other carbohydrate source (rice, yam etc.) which have become unaffordable to most household in Nigeria.

Ipomoea batatas is susceptible to a number of diseases such as fungal diseases which affect both the roots and leaves in the field [3]. Extensive damage of leaves of *I. batatas* and etiological agents of leaf spot disease of the plants in some farms in South-South Nigeria has been reported [2]. According to Samuel and Adekunle [4], *Macrophomina phaseolina* has been established to be responsible for the recent outbreak of the brownish leaf spot disease of *I. batatas* in the South West of Nigeria and there were correlations between the leaf spot incidence and loss in yield of the plant. The study also reported the effect of the infection on the chlorophyll content and the plant yield. This study therefore, aims to evaluate the effect of *M. phaseolina* infection on stomata features of *I. batatas* leaves.

MATERIALS AND METHODS

Pathogen source

Stock culture of *M. phaseolina used* in this study was collected from the Plant Pathology Laboratory of Department of Botany, University of Lagos. This test organism used has been previously isolated, identified and its pathogenicity as *I. batatas* leafspot disease pathogen confirmed [4].

Pathogen inocula

Three concentrations of *M. phaseolina* inocula were made from a stock culture of the fungus. Spore suspensions of the 21 days old fungus dislodged with a soft artist's brush into a solution of 0.01% Tween 20 in distil water. With the aid of a Fuchs-Rosenthal hemacytometer, three concentrations were achieved; 1 × 10^6 spores per ml, 1 × 10^7 spores per ml and 1 × 10^8 spores per ml.

Pathogenicity test

Pathogenicity test using Koch's postulate was carried out on three set of two rows of planted *I. batatas* in a greenhouse. Each set (consist of 50 running vines of *I. batatas*) were treated through spraying with different concentrations of *M. phaseolina* spore suspensions of the three prepared concentration respectively. The spore suspension was sprayed on three months old healthy *I. batatas* running vines grown in an experimental plot with an atomizer. The fourth untreated set of *I. batatas* served as the control. Test plant leaves were covered with sterile polythene bags for 24 hours, to allow the spores to germinate on the leaves. Inoculated plants were assessed daily for any leafspot disease symptoms.

Anatomical analysis

Anatomical analysis of both control (untreated) plants and treated plants were carried out using the Acid soaking method according to Duan *et al.* [5] and, Kadiri and Olowokudejo [6]. Three to five cm^2 of 4 leaf samples of *Ipomoea batatas* representing treated plant and the other control plant were collected. These leaves samples were soaked in 70% Nitric acid for 40 minutes.

The epidermis was separated, cleaned and stained appropriately. These were then mounted onto the microscope and examined. The features were captured using 'MOTIC' micro-imaging camera attached to Olympus microscope.

RESULTS

This study showed the emergence of leaf spot on the leaf surfaces of treated plants with 10^6 spores per ml concentration three weeks after spraying the plants with the fungal spore suspension. A few days later same leaf spot disease symptoms were noted on other treated plants treated: 1 x 10^7 and 1 x 10^8 spores per ml concentration respectively. There was absent of the leaf spot diseases on the leaves of the control [unsprayed] plants [Plate 1a].

It was also noted that the number of leaf spot on each set of treated plant varied. There was severe leaf spot on the leaves of sprayed set of plants treated with 1 × 10^6 spores per ml concentration suspension. These leaf spots range from 8 to 14 spots [Plate 1d]. The least numbers of leaf spot were observed on treated plants with a fungal spore suspension of 1× 10^6 spores per ml concentration which ranges from 4 to 6 spots [Plate 1c].

Observing under the microscope, the same type of stomata was seen in all treated and untreated plant leaves and it was the paracytic type of stomata observed. Also, it was noted that trichrome is generally absent in all treated and untreated plant leaves. It was observed that the stomatal number also differ when those present at the adaxial section was compared to those of the abaxial axis. Stomata tend to be more in number at the abaxial axis. Same phenomenon was noticed in other stomatal features like, stomatal size, density and index considered [Table 1 a and b]

Though, the stomatal size at both abaxial and adaxial axes of treated leaves with fungal spore suspension of 1× 10^6 spores per ml concentration were the same with little or no difference from the control leaves [Table 1 a and b]. But when the stomata densities were compared, the adaxial axis of the plants treated with 1 × 10^6 spores per ml concentration has the lowest density. This was observed with the stomata index of the adaxial axis of the plants treated with 1 × 10^6 spores per ml.

Another notable observation was the rough striation at the adaxial section of the treated leaves of those sprayed with 1× 10^6 spores per ml and 1 × 10^8 spores per ml concentration [Plate 2 e and f]. Differences were also noted on the guard cells of the treated plant leaves stomata and those of the control (untreated) plant leaves stomata. The guard cells in all treated plant leaves tend to shrink and not illuminated, while these cells are robust and illuminated in the control plant leaves.

Plate Ia: Unsprayed leaves,
Plate **I**b: Leaves sprayed with 1 × 10^8 spores per ml concentration,
Plate **I**c: Leaves sprayed with 1 × 10^7 spores per ml concentration,
Plate **I**d: Leaves sprayed with 1 × 10^6 spores per ml concentration.

Table 1a: Stomatal characters of both experimental and control plants leaves

Adaxial surface (per15 views)	**1 × 10^8**	**1 × 10^7**	**1 × 10^6**	**Control**
Stomata Size (µm)	1.17 ± 0.01	1.12 ± 0.01	1.08 ± 0.01	1.17 ± 0.01
Stomata Index (mm^{-2})	10.28 ± 0.3	10.01 ± 0.3	8.33 ± 0.3	11.28 ± 0.3
Stomata Density (mm^{-2})	4.4 ± 0.1	4.15 ± 0.1	3.6 ± 0.1	5.0 ± 0.1
Stomatal complex type	Paracytic	Paracytic	Paracytic	Paracytic
Trichome	Absent	Absent	Absent	Absent

Values are expressed as (mean ± se)

Table 1b: Stomatal characters of both experimental and control plants leaves

Abaxial surface (per15 views)	**1 × 10^8**	**1 × 10^7**	**1 × 10^6**	**Control**
Stomata Size (µm)	1.17 ± 0.01	1.13 ± 0.01	1.08 ± 0.01	1.17 ± 0.01
Stomata Index (mm^{-2})	10.63 ± 0.2	10.08 ± 0.2	9.63 ± 0.2	11.96 ± 0.2
Stomata Density (mm^{-2})	5.30 ± 0.1	5.06 ± 0.1	4.50 ± 0.1	6.0 ± 0.1
Stomatal complex type	Paracytic	Paracytic	Paracytic	Paracytic
Trichome	Absent	Absent	Absent	Absent

Values are expressed as (Mean ± SE)

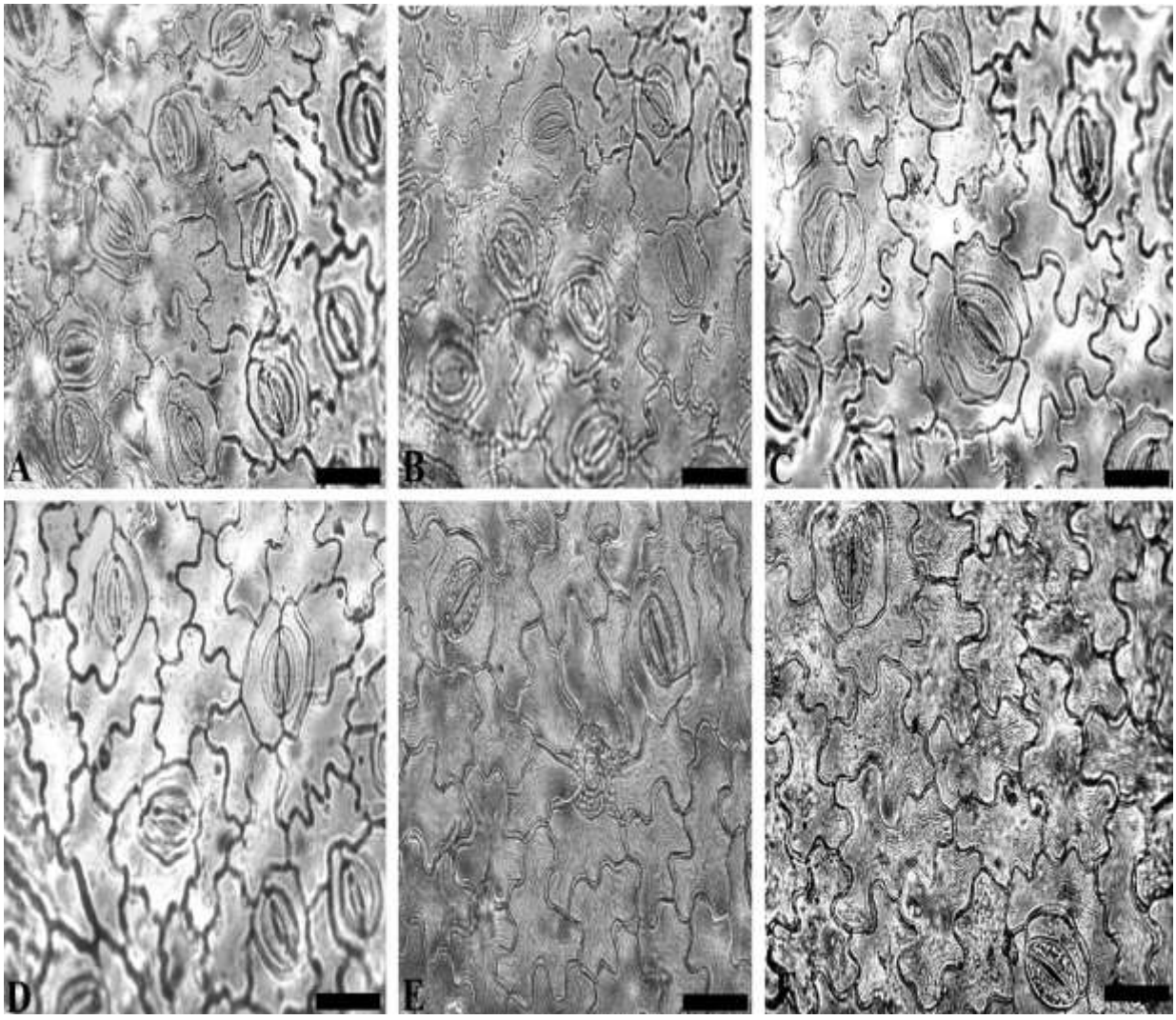

Plate II: Photography of abaxial and adaxial leaves surfaces of both experimental and control leaves
(Mag. X 100) (a – abaxial control leaf , b&c – abaxial experimental leaves); (d - adaxial control leaf, e&f - adaxial experimental leaves).

DISCUSSION AND CONCLUSION

Distinct differences were noticed between *I. batatas* leaves treated with *Macrophomina phasolina* spores and untreated. *I. batatas* leaves used as experimental control after three weeks of inoculation. Though, the leafspot disease symptoms were first noticed on *I. batatas* treated with 1×10^6 spores per ml concentration, while these leaf spots were completely absent in the unsprayed *I. batatas.* This agreed with the report of Abdulrahamen *et al.* [7] and that of Samuel and Adekunle [4]. It was also noted that the symptoms of leafspot diseases which were initially noticed on the adaxial surface of the plant leaves extended to the abaxial surface of the leaves with time. Results from the study revealed that the degree of severity of the leaf spot disease varied with the fungal spore suspension concentration. This agreed with Backman and Crawford [8], who in their report stated there is a relationship between yield loss and severity of leaf spot disease of *Arachis hypogaea*. Fusicoccin [9] and, Ganeva and Uzunova [10] in their report also stated that reported increase in number leaf spots reduced the surface area necessary for photosynthesis due to the fact that leaves cell towards shrinkage. This is in agreement with the rough striation present at the adaxial of the treated leaves compared to that of the control leaves in the present study. Though, the stomatal type seen is the same [paracytic] and trichrome is generally absent. It was concluded from this study that there is a significant difference in stomatal appearance, numbers, size, density and index on adaxial and abaxial of the infected plant leaves as the concentration of the fungal spore's increases. In conclusion, it was deduced from this study that the more concentrated the fungal spores, the more disruption and damages in on the stomata features. These damages will definitely translate into low output/yield of the plant host.

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