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RESEARCH ARTICLE

Cercospora leaf spot management with nitrogenous fertilizers in cotton is dependent on the disease amount in the plant canopies

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Abstract

The aim of this study was to examine the impact of both organic and inorganic nitrogen fertilizers on the intensity and epidemiological components of Cercospora leaf spot across three different canopies of cotton plants, specifically the variety DP ACALA 90, under field conditions. Fertilizers used in the study included bovine manure, *Jatropha curcas* seedcake, poultry manure, and urea (the latter serving as a control). These were applied at 20 days after plant emergence and then during the flowering stage until the total nitrogen (N) dose reached 50, 100, 150, or 200 kg N per hectare. The incidence and severity of the disease were assessed starting at the reproductive stage B1 (the first visible flower bud) across the lower, middle, and upper canopies of four cotton plants, with six evaluations conducted over time. To calculate the initial inoculum (Y_0) and the disease progress rate (r), the Exponential, Gompertz, and Logistic models were employed based on temporal data. The study was designed as a randomized complete block with a 4x4 factorial arrangement (fertilizer type x dose), and mean comparisons were made using Tukey's test ($p \leq 0.05$). It was found that disease intensity within each canopy level varied in response to the type of nitrogen source and the applied dose, with the severity of the disease (including the area under the disease progress curve) being particularly influenced. A significant interaction between the type of fertilizer and the dose regarding the intensity of Cercospora leaf spot was observed. The Exponential model most accurately depicted the disease's temporal progression. Notably, poultry manure and urea were the fertilizers that most adversely influenced the intensity and initial inoculum (Y_0) of Cercospora leaf spot across the various canopies of the cotton plants. The findings suggest that the use of organic fertilizers in cotton cultivation could represent a viable sustainable management strategy.

Keywords: *Gossypium hirsutum* L.; *Cercospora* sp.; Non-linear models progress; Organic and inorganic fertilization; Dose.

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1. Introduction

Cotton (*Gossypium hirsutum* L.) is recognized as a crucial crop globally (Ansell et al., 2009), known for producing a fiber that is rich in cellulose (82%-96%), with varying amounts of hemicellulose (2%-6.4%), lignin (0%-5%), and pectin (<1%-7%). These components are uniquely distributed across different layers within the plant species (Fang, 2018; Salem et al., 2022). Beyond its pivotal role in the textile industry, cotton fiber's seeds emerge as a valuable source of protein, oil, and minerals, heralding potential benefits for human health and livestock nutrition (Bellaloui et al., 2021). While cotton once stood as a cornerstone of Ecuador's agricultural sector, its cultivation area has seen a gradual decline over the years (García et al., 2019).

Essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K), alongside micronutrients like iron (Fe), zinc (Zn), and copper (Cu), are vital for cotton plants. They support the plant's physiological functions and maximize yield potential (Echer et al., 2020). Among these, N is particularly critical, acting as a primary and limiting factor in plant growth and production. Its application profoundly influences physiological parameters, growth patterns, boll development, yield, and the quality of cotton fiber (Bondada & Oosterhuis 2001; Cañarte-Bermúdez et al., 2020).

The need for N in the cotton crop can be met with the application of inorganic and organic sources, primarily derived from ammonia (NH_3) or ammonium (NH_4^+), respectively. However, the use of inorganic or synthetic fertilizers can be detrimental to an agroecosystem because it may lead to air and groundwater pollution, soil acidification, eutrophication, and a reduction of biodiversity, which includes microorganisms (Scudlark et al., 2005; Zheng et al., 2018). As an alternative to this approach in nutritional management for cotton cultivation, more environmentally friendly practices can be adopted, such as the use of organic nitrogen sources (Palma-Zambrano et al., 2022).

Nitrogen fertilizer, regardless of its source (NH_3 or NH_4^+), can not only positively affect root condition, plant growth, boll quality, and fiber production in cotton crops (Chen et al., 2019; 2020) but also suppress various phytopathogens, potentially influencing their interaction with the plant (Mur et al., 2017; Palma-Zambrano et al., 2022). In crops such as oilseed rape (*Brassica napus* L.), increased N availability may lead to the emission of acetic acid (a volatile antifungal component), which can potentially reduce the levels of Alternaria leaf spot [*Alternaria brassicae* (Berk.) Sacc.] in the leaf area (Veromann et al., 2013). In cotton, higher nitrogen doses can enhance root growth (especially in the upper soil layers) and boost physiological and biochemical processes in leaves (Chen et al., 2019) and may also diminish the intensity or epidemiological components of diseases such as boll rot (*Diplodia gossypina* Cooke) and Ramularia leaf spot (*Ramulariopsis pseudoglycines* Videira, Crous & U. Braun) (Palma-Zambrano et al., 2022).

Cotton crops in Ecuador may be affected by diseases such as damping-off (*Rhizoctonia solani* J.G. Kühn), anthracnose (*Colletotrichum gossypii* Southw), boll rot, and ramularia leaf spot (Sión et al., 1992; Palma-Zambrano et al., 2022). Diseases like Ramularia leaf spot can negatively affect cotton plants' leaf area and fiber yield (Ascari et al., 2016). Other diseases identified in cotton crops in other countries such as charcoal rot [*Macrophomina phaseolina* (Tassi) Goid], bacterial blight [*Xanthomonas campestris* pv. *malvacearum* (Smith) Dye], Cercospora leaf spot (*Cercospora gossypina* Cooke), rust [*Phakopsora gossypii* (Lagerheim) Hiratsuka], can impact cotton crops (Sharma & Bambawale, 2008).

Cercospora leaf spot has been gaining prominence in cotton crops in countries such as Brazil due to its high severity (Puia et al., 2021). The disease causes severe output damage and losses if not detected and controlled quickly (Dhawan et al., 2023). Cercospora leaf spot can reach a severity of 11% in susceptible cotton genotypes (Puia et al., 2021). Although the use of fungicides should reduce the disease, this method is not sustainable over time. Despite the importance of Cercospora leaf spot in cotton crops, this has not been studied or reported in Ecuador. Also, very little is known about its response to the application of organic nitrogen fertilizers in cotton production worldwide.

In Ecuador, cotton is produced mainly in the coastal provinces of Guayas and Manabí (Arriel et al., 2023). In recent years, the Food and Agriculture Organization of the United Nations (FAO) has contributed to strengthening the cotton production chain in Ecuador through Project + Cotton. This research hypothesized that at least one of the fertilizers would have an impact on Cercospora leaf spot in at least one canopy layer of cotton plants. Thus, in the present work was evaluated the effect of organic and inorganic nitrogen fertilizers on the intensity and epidemiological components of Cercospora leaf spot in three canopy layers of cotton plants, variety DP ACALA 90, under field conditions.

2. Methodology

2.1. Study area and experimental management

This research was carried out at the experimental campus of La Teodomira, Faculty of Agronomic Engineering, Technical University of Manabí, Ecuador (01° 09 ´51 S, 80° 23' 24'' W, at an altitude of 60 meters), under field conditions on a clay loam soil (Table 1).

Cotton seeds of the variety DP ACALA 90 were planted with spacing of 0.40 meters between plants, 1 meter between rows, and 2 meters between blocks on land that had previously been used for cotton cultivation. The size of the experimental plot was 2688 m^2 (48 m x 56 m), with each plot measuring 36 m^2 (6 m x 6 m) and designating a core area of 20.8 m^2 (5.20 m x 4 m) for focused study. The land was cleared of any crop residues, and its soil boasted an organic matter content of 0.90% (Table 1). The nitrogen fertilizers utilized in this study included bovine manure, *Jatropha curcas* seedcake, poultry manure, and urea (the latter serving as a control). Details on the nutrient composition (N, P, K, Ca, and Mg) of the organic fertilizers can be found in Table 2. Fertilization commenced 20 days post-emergence (DDE), with half of the intended dose administered in each treatment. A second application was administered during the flowering stage (50 DDE), fulfilling the total N requirement (50, 100, 150, 200 kg N per hectare) for each source of nitrogen.

Table 1
Physical (soil type and pH: ionic potential of hydrogen) and chemical (OM: organic matter, N: nitrogen, P: phosphorus, K: potassium, Ca: calcium, Mg: magnesium, H: hydrogen, Mn: manganese, Co: cobalt, and Z: zinc) characteristics of the soil

Suelo	pH	OM	N	P	K	Ca	Mg	H	Mn	Co	Z
		%	mg kg^{-1}			cmol kg^{-1}		mg kg^{-1}			
Clay loam	7.5	0.90	0.04	17.4	1.06	15.25	5.27	26.7	5.55	2.19	< 2.60

Table 2
Concentrations (%) of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) found in the organic fertilizers bovine manure, *Jatropha curcas* seedcake, and poultry manure

Fertilizers	Concentrations (%)*				
	N	P	K	Ca	Mg
Bovine manure	3.9	0.6	1.6	1.4	0.5
Jatropha curcas seedcake	2.1	0.9	2.6	0.9	0.6
Poultry manure	3.0	0.7	2.3	2.7	0.6

* Percentage of concentration for each element analyzed.

2.2. Cercospora leaf spot assessment

Disease intensity was evaluated starting at reproductive stage B1 (first visible flower bud, 76 DAE) in the four cotton plants' lower, middle, and upper canopy (one plant leaf per canopy) in the useful plot for six evaluations over time. Disease incidence was quantified by counting each symptomatic leaf tissue present on the plants and converting the mean for each canopy to a percentage, according to the following equation:

$$\text{Incidence} = \frac{\text{Nº of plants infected}}{\text{Nº of plants observed}} \times 100$$

In the absence of diagrammatic scales for assessing the Cercospora leaf spot, disease severity was determined by estimating of leaf area affected, like the methodology employed by Garcés-Fiallos et al. (2014).

To confirm the presence of the pathogen causing Cercospora leaf spot, leaf samples from the lower canopy of cotton plants were collected and analyzed in the Phytopathology laboratory. By utilizing a stereomicroscope and a bacteriological loop, several Lactophenol cotton blue preparations were prepared. Subsequently, the vegetative and reproductive structures of the fungus potentially associated with Cercospora leaf spot symptoms were examined under a binocular optical microscope.

2.3. Experimental design and statistical analyses

A randomized complete block design with a 4x4 factorial (fertilizer x dose) was used, with a total of 16 treatments and four replications. After confirming the normality and homoscedasticity of the values obtained, an analysis of variance was performed, and the means were compared using Tukey's test ($p \leq 0.05$). Each of the significant interactions obtained was presented separately.

For the analysis of disease progress, the initial inoculum (Y_0) and disease progress rate (r) were estimated using the equations of the Exponential (Eq. 1), Gompertz (Eq. 2), and Logistic (Eq. 3) models (Berger 1981; Zwietering et al., 1990; Tjørve & Tjørve, 2017):

$y = Y_0 \exp rt$ (1)

where Y_0 is the initial value, r is the rate and t is the time

$y = \exp(-B^* \exp(-kt))$ (2)

where B is a position parameter, k is the rate and t is the time

$y = 1(1+\exp(-a+rt))$ (3)

where y = disease proportion ($0 < y < 1$), a = logit (Y_0), r is the rate, and t is the time.

The means of each factor were compared (Tukey test $p \leq 0.05$) for each epidemiological parameter when the probability of all factor values was significant. Cercospora leaf spot progress curves were modeled using the epifitter package (Alves & del Ponte, 2020). All analyses were performed using Rstudio (RStudio Team, 2017).

3. Results and discussion

Symptoms of Cercospora leaf spot were observed on cotton plant leaves (Figure 1). These symptoms included circular spots with a whitish center appearing on both the upper (Figure 1A) and lower (Figure 1B-C) surfaces of the leaves. The conidiophore (Figure 1D) and the elongated, multiseptated conidia (Figure 1E) appeared hyaline. Species within the genus *Cercospora* (Mycosphaerellaceae, Capnodiales) are common causes of leaf spots (Bakhshi et al., 2018) and were first described by Fresenius (1863) as resembling *Passalora*-like species with multiseptated conidia. Dalbelo-Puia et al. (2021) note that Cercospora leaf spot is caused by *C. gossypina*, which produces irregular brown or brownish spots with dark edges and a light center on cotton plant leaves. These symptoms resemble those were observed on cotton leaves. While further research into the disease's etiology is necessary, to our knowledge, this represents the first documentation of Cercospora leaf spot on cotton plants in Ecuador.

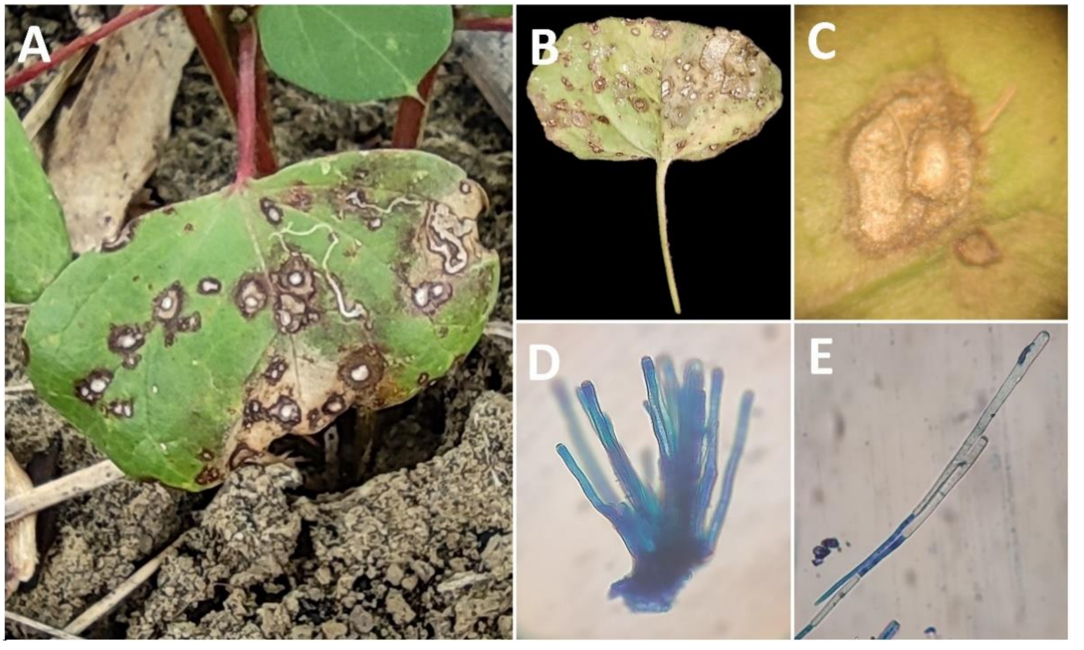

Figure 1. Symptoms of Cercospora leaf spot and disease-associated structures of *Cercospora* sp. on leaves of cotton plants. A-C) Irregular circular spots with a whitish center on the upper (A) and lower (B-C) sides of the leaves. D-E) Conidiophore (D) and elongated multiseptate hyaline conidia (E).

Figure 2. Factorial interaction (fertilizers x dose) for Cercospora leaf spot incidence (%) in the lower canopy of cotton plants under organic (bovine manure, *Jatropha curcas* seedcake, and poultry manure) and inorganic (urea) fertilization, using four doses (50, 100, 150 y 200 kg N ha^{-1}) for each source.

The intensity of Cercospora leaf spot varied based on the type of nitrogen source and the dosage applied (Table 3). A significant interaction between these factors was observed only in the lower canopy regarding disease incidence. Conversely, the severity of Cercospora leaf spot was influenced by the doses of fertilizer, with an interaction between the factors being observed across all layers. However, only in the lower and middle canopies, as well as in the aggregate analysis of all canopies, did the type of fertilizer does not impact the severity of the disease. Similarly, no effect of fertilizers on disease severity was noted in both the lower and upper canopies and when considering the average across all canopies.

Concerning Factor A (fertilizers), poultry manure specifically led to a reduction of 23% and 48% in the AUDPC and the severity of Cercospora leaf spot, respectively, in the upper canopy of cotton plants, when compared to other fertilizers (Table 4). Additionally, in the same canopy, *J. curcas* seedcake and urea were found to moderately reduce disease severity and AUDPC, respectively. Two aspects warrant discussion: the impact of two organic fertilizers and their observed effects in the upper canopy. Specifically, regarding poultry manure and *J. curcas* seedcake, Palma-Zambrano et al. (2022) suggest that these fertilizers might negatively influence the incidence of Ramularia leaf spot in cotton plants, though they did not measure this impact across the canopies. It can be suggested that the use of organic fertilizers influences the intensity of Cercospora leaf spot primarily in the upper canopy, despite potential implications for the lower canopy.

Table 3
Results of two-way analysis of variance for incidence, as well as for severity and its Area Under the Disease Progress Curve (AUDPC) regarding Cercospora leaf spot

Canopy	Factors	GL[1]	F[2]	*P* – valor*
Incidence				
Lower	Fertilizers	3	0.9445	0.3364
	Dose	3	2.7301	0.1056
	Interaction	9	4.3105	0.0437*
Middle	Fertilizers	3	0.1984	0.6582
	Dose	3	0.6075	0.4400
	Interaction	9	0.0335	0.8556
Upper	Fertilizers	3	1.0477	0.3116
	Dose	3	1.7707	0.1902
	Interaction	9	2.2915	0.1372
Mean	Fertilizers	3	0.1594	0.6917
	Dose	3	0.0896	0.7660
	Interaction	9	0.2421	0.6252
Final severity				
Lower	Fertilizers	3	2.3804	0.0697
	Dose	3	5.7030	0.0008**
	Interaction	9	4.1518	0.0005**
Middle	Fertilizers	3	0.3334	0.8012
	Dose	3	2.8335	0.0385*
	Interaction	9	1.9623	0.0433*
Upper	Fertilizers	3	3.6681	0.0127*
	Dose	3	2.7149	0.0450*
	Interaction	9	3.0803	0.0015*
Mean	Fertilizers	3	0.5312	0.6612
	Dose	3	3.1987	0.0237*
	Interaction	9	2.2482	0.0191*
AUDPC respect to the severity				
Lower	Fertilizers	3	0.8752	0.3533
	Dose	3	2.6149	0.1111
	Interaction	9	4.0202	0.0495*
Middle	Fertilizers	3	0.2596	0.6123
	Dose	3	0.0712	0.7905
	Interaction	9	0.0003	0.9856
Upper	Fertilizers	3	6.4771	0.0135*
	Dose	3	7.2773	0.0091**
	Interaction	9	6.0512	0.0168*
Mean	Fertilizers	3	0.0564	0.81314
	Dose	3	0.2420	0.62456
	Interaction	9	0.5608	0.45686

[1] GL: degrees of freedom; [2] F: Fisher's calculated value; * Statistical significance $p \leq 0.05$.

The introduction of different nitrogen forms into the soil, such as NH_3 and NH_4^+, is known to affect plant disease intensity through biochemical, physiological, and molecular changes (Arauz et al., 2020). Moreover, the microorganisms associated with each type of organic fertilizer may impact plant roots, potentially improving conditions in the rhizosphere (Devi et al., 2012). A significant aspect for future research is the observed superiority of poultry manure over other organic and inorganic fertilizers, despite its lower concentrations of N, P, K, and Mg. It is conceivable that the higher levels of Ca in poultry manure, being twice as abundant as in other fertilizers, might contribute uniquely to the health of cotton plants. This suggests that the incidence of diseases in cotton plants may vary significantly with the type of fertilizer used (Palma-Zambrano et al., 2022).

Upon analyzing Factor B (dosage), the severity of Cercospora leaf spot in the lower canopy of cotton plants decreased by 29% with applications of 100 to 200 kg N ha^{-1}, in comparison to lower doses. In the upper canopy, for the same factor, reductions in AUDPC and disease severity of 33% and 61%, respectively, were observed with dosages between 100 and 200 kg N ha^{-1} (Table 4). Specifically, an application of 200 kg N ha^{-1} was required to achieve a 15% reduction in the average severity of Cercospora leaf spot across the canopy, when compared to lower dosages. Generally, higher doses, starting at 100 kg N ha^{-1}, led to decreased severity of Cercospora leaf spot in cotton plants. A dosage of 100 kg N ha^{-1} was effective in reducing the incidence of boll rot (Palma-Zambrano et al., 2022), while dosages exceeding 200 kg ha^{-1} enhanced root growth, as well as the physiological and biochemical functions of the leaves (Chen et al., 2019). In other crops like oilseed rape (*Brassica napus* L.), an increase in N availability has been shown to prompt the release of acetic acid, a volatile antifungal agent, potentially lowering the occurrence of dark spot disease caused by *Alternaria brassicae* (Veromann et al., 2013).

In the interaction (fertilizers x dose) for the incidence (%) of Cercospora leaf spot in the lower canopy, it was noted that the effect of each fertilizer depended on the dose (Figure 2). Specifically, when 50 kg N ha^{-1} of both bovine manure and *J. curcas* seedcake was applied, the disease incidence decreased; however, with the same dose of poultry manure and urea, the incidence of Cercospora leaf spot increased.

The factorial interaction (fertilizers x dosage) for the severity of Cercospora leaf spot (%) revealed a significant relationship between the factors and a varied disease response across different canopies of cotton plants (Figure. 3). In the lower canopy, plants treated with organic fertilizers exhibited an increase in disease severity. In contrast, severity decreased in plants treated with doses of 100 and 200 kg N ha^{-1} of urea (Figure 3A). In the middle canopy, severity was reduced in plants treated with *J. curcas* seedcake and cattle manure at doses of 50 and 200 kg N ha^{-1}, respectively (Figure 3B). Disease severity was generally lower in the upper canopy for plants treated with all types of fertilizers and various doses (Figure 3C). For instance, at this level, applying *J. curcas* seedcake dramatically reduced disease severity at dosages of 100 kg N ha^{-1}and higher. Yet, when considering the average severity across all canopies, plants fertilized with poultry manure and urea at doses starting from 100 kg N ha^{-1} showed reduced severity (Figure 3D).

Table 4
Severity (%) and Area Under the Disease Progress Curve (AUDPC) concerning the severity of Cercospora leaf spot in the lower, middle, upper, and mean canopy of cotton plants under organic fertilization, using four doses (50, 100, 150, and 200 kg N ha^{-1}) for each source

Factors	Severity (%)				AUDPC
	Lower	Middle	Upper	Mean	Upper
Fertilizers (A)	0.0697	0.8012	0.0127	0.6612	0.0135
Bovine manure	4.72	2.60	1.01 a	2.76	675.00 a
J. curcas seedcake	3.96	3.19	0.86 ab	2.67	634.70 a
Poultry manure	3.25	1.72	0.50 b	1.78	481.80 c
Urea	1.60	1.87	1.02 a	1.49	560.00 b
Mean					
Dose (B)	0.0008	0.0385	0.0450	0.0237	0.0091
50 kg N ha^{-1}	3.89 a	1.63	1.57 a	2.35 a	784.40 a
100 kg N ha^{-1}	2.91 ab	2.70	0.59 b	2.05 a	521.80 b
150 kg N ha^{-1}	4.04 a	2.62	0.57 b	2.37 a	506.30 b
200 kg N ha^{-1}	2.69 b	2.44	0.67 b	1.93 b	539.00 b
Mean					
Interaction (A x B)	0.0005	0.0433	0.0015	0.0191	0.0168
CV (%):	12.55	11.69	8.07	9.22	10.34

* An analysis of variance with its respective comparison of means was performed only when the probability for all the factor values was significant ($p \leq 0.05$).
** Lowercase letters indicate a significant difference by Tukey's test ($p \leq 0.05$).

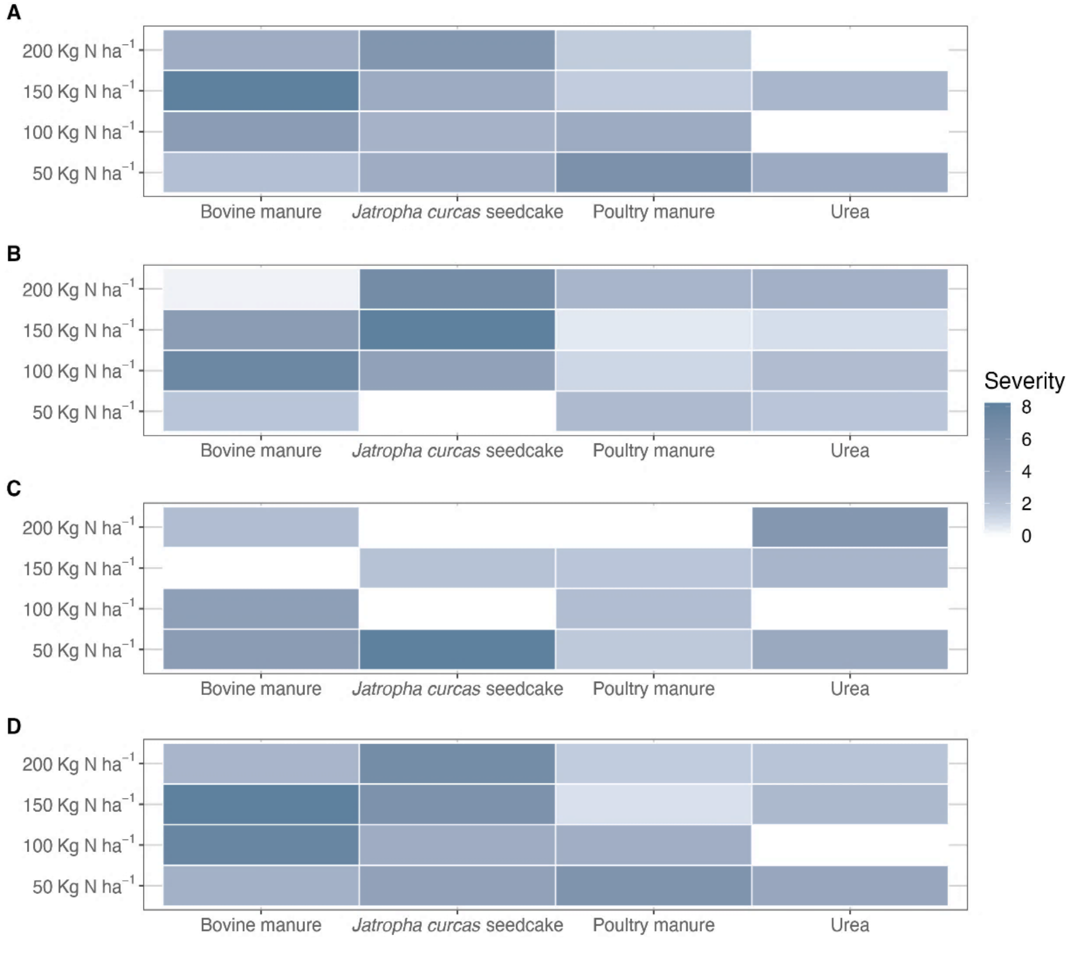

Figure 3. Factorial interaction (fertilizers x dose) for Cercospora leaf spot severity (%) in the lower (A), middle (B), upper (C), and mean (D) canopy of cotton plants under organic (bovine manure, *Jatropha curcas* seedcake, and poultry manure) and inorganic (urea) fertilization, using four doses (50, 100, 150 y 200 kg N ha^{-1}) for each source.

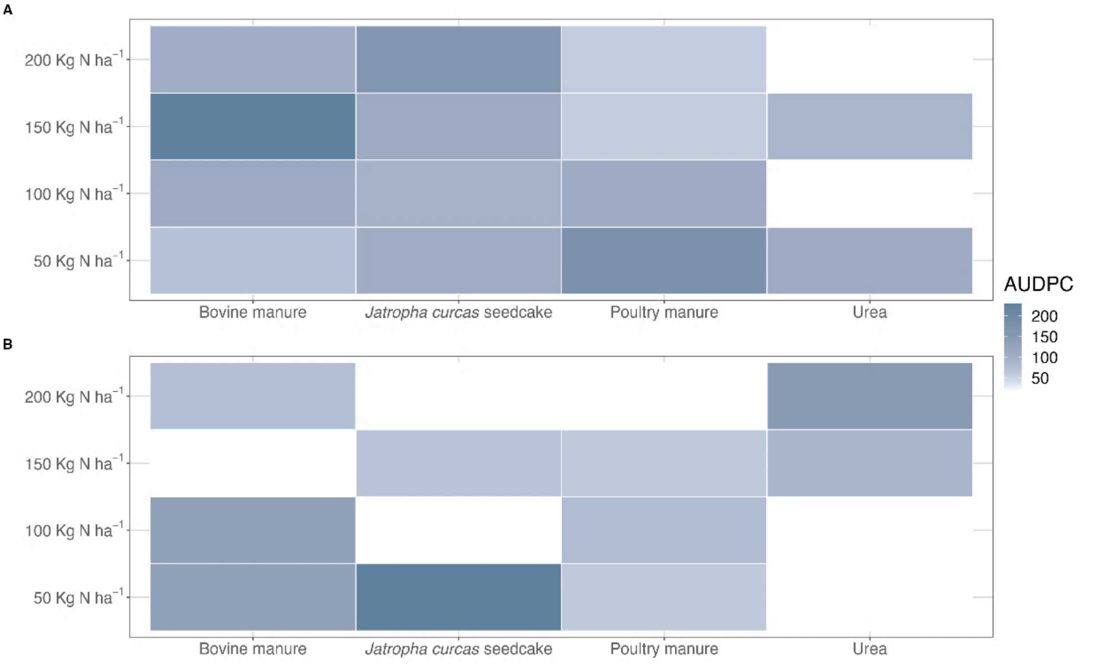

Figure 4. Factorial interaction (fertilizers x dose) for Area Under the Disease Progress Curve (AUDPC) regarding Cercospora leaf spot severity in the lower (A) and upper (B) canopy of cotton plants under organic (bovine manure, *Jatropha curcas* seedcake, and poultry manure) and inorganic (urea) fertilization, using four doses (50, 100, 150 y 200 kg N ha^{-1}) for each source.

The factorial interaction (fertilizers x dosage) regarding AUDPC related to Cercospora leaf spot severity in both the lower and upper canopies of cotton plants was negatively influenced by all fertilizers and dosages (Figure. 4). Plants treated with doses of 100 and 200 kg ha^{-1} of urea exhibited the lowest AUDPC in the lower canopy (Figure 4A). Conversely, AUDPC was reduced in the upper canopy of plants fertilized with any fertilizer or dosage, with notable reductions observed for doses of 50 and 100 kg ha^{-1} of urea (Figure 4B).

Upon examining the significant interactions, distinct responses were observed among the factors concerning the severity of Cercospora leaf spot across different canopies of cotton plants. In the lower canopy, applications of urea at 100 kg N ha^{-1} and poultry manure at 200 kg N ha^{-1} were effective in reducing the disease. Meanwhile, in both the middle and upper canopies, other fertilizers managed to decrease the severity of Cercospora leaf spot at any given dose. It has been established that applying 120 and 180 kg N ha^{-1} to medium and high-density cotton plants, respectively, enhances boll yield (Shah et al., 2021). Yet, the dynamics between the type of fertilizer and its dosage in controlling disease remain elusive. Urea, potentially due to its swift decomposition and absorption by the cotton roots, might have been more effective than organic fertilizers in mitigating the disease, primarily in the lower canopy.

On the other hand, organic fertilizers provide not only nitrogen (N) but also other essential nutrients including P, K, calcium (Ca), and magnesium (Mg), along with a variety of microorganisms. Synergies between elements like N and K have been shown to lead to higher crop yields (Xu et al., 2023). Furthermore, the use of organic amendments is known to foster the growth of beneficial microbes within the rhizosphere and bolster plant development in challenging environments (Das et al., 2022). Grasping how plants react to nutrients like N and their interplay with dosage offers significant insights into pursuing sustainable human development (Krouk & Kiba, 2020).

In the non-linear modeling of Cercospora leaf spot severity (as a proportion), the Exponential and Gompertz models were identified as the most fitting representations of the disease's temporal progression (Table 5). While the Logistic model was utilized, it did not achieve the same level of accuracy, failing to produce significant results across any of the factors or canopies (data not shown). The Gompertz model, however, was effectively significant in modeling the severity of Cercospora leaf spot across all canopies and their average, albeit its significance varied depending on the type of fertilizer or dosage. For instance, significant results were observed in the average canopy level for plants treated with *J. curcas* seedcake ($p \leq 0.0050$) and urea ($p \leq 0.0272$). Conversely, the exponential model demonstrated statistical significance across all canopies and their overall average for plants treated with any type of fertilizer, specifically in the lower canopy with a dosage of 50 kg N per hectare, with *p*-values ranging from 0.0000 to 0.0453.

Among the epidemiological components, initial disease severity (Y_0) and disease progression rate (r), Y_0 was more significantly impacted by the type of fertilizer used (Table 5). Y_0 experienced a reduction of 52% with urea and 37% with *J. curcas* seedcake in the lower and middle canopies, respectively, when compared to the effects of other fertilizers. In the upper canopy, *J. curcas* seedcake and poultry manure notably decreased Y_0 by 37% and 84%, respectively, in contrast to bovine manure and urea. Conversely, poultry manure was the sole fertilizer that did not have a negative impact on r within the same canopy layer. Across all canopy layers, poultry manure and urea collectively reduced Y_0 by an average of 35%, standing out against the other two organic options.

Table 5

Initial disease (Y_0) and disease progression rate (r) obtained from the quantification of Cercospora leaf spot severity (proportion), over time in the lower, middle, upper, and mean canopy of cotton plants under organic fertilization (bovine manure, *Jatropha curcas* seedcake, and poultry manure) and synthetic (urea), using four doses (50, 100, 150 and 200 kg N ha^{-1}) for each source. From the analysis obtained for the models Exponential and Gompertz, the coefficient of determination was obtained (R_2) and the probability (p)

Factors	Exponential				Gompertz			
	Y_0	r	R_2	p-value	Y_0	r	R_2	p-value
Lower canopy								
Fertilizers								
Bovine manure	0.042 a	0.026 ^{ns}	0.20	0.0007	0.412	0.032	0.22	0.1095
J. curcas seedcake	0.039 a	0.024	0.26	0.0001	0.300	0.034	0.29	0.0452
Poultry manure	0.032 a	0.024	0.18	0.0012	0.238	0.035	0.20	0.1399
Urea	0.018 b	0.026	0.17	0.0157	0.095	0.055	0.06	0.6535
Dose								
50 kg N ha^{-1}	0.064	0.014	0.09	0.0171	0.166	0.064	0.12	0.2271
100 kg N ha^{-1}	0.145	0.134	0.04	0.1143	0.866	0.091	0.01	0.6710
150 kg N ha^{-1}	0.207	0.015	0.04	0.0801	0.126	0.050	0.01	0.6475
200 kg N ha^{-1}	0.128	0.011	0.04	0.1581	0.351	0.084	0.01	0.6399
Middle canopy								
Fertilizers								
Bovine manure	0.034 b	0.030 ^{ns}	0.23	0.0005	0.470	0.035	0.24	0.2713
J. curcas seedcake	0.053 a	0.026	0.19	0.0008	0.470	0.034	0.21	0.3092
Poultry manure	0.027 b	0.027	0.09	0.0328	0.228	0.053	0.11	0.4622
Urea	0.039 b	0.021	0.08	0.0381	0.201	0.043	0.09	0.4470
Dose								
50 kg N ha^{-1}	0.054	0.010	0.01	0.3400	0.064	0.000	0.01	0.3300
100 kg N ha^{-1}	0.201	0.008	0.14	0.3066	0.208	0.002	0.01	0.0124
150 kg N ha^{-1}	0.186	0.006	0.01	0.3752	0.154	0.004	0.04	0.1471
200 kg N ha^{-1}	0.091	0.009	0.02	0.2943	0.168	0.022	0.06	0.8614
Upper canopy								
Fertilizers								
Bovine manure	0.034 a	0.0244 b	0.08	0.0453	0.233	0.058	0.10	0.4618
J. curcas seedcake	0.020 b	0.0305 b	0.06	0.0709	0.470	0.034	0.21	0.3092
Poultry manure	0.005 c	0.0467 a	0.11	0.0378	0.239	0.050	0.12	0.6046
Urea	0.030 a	0.0263 b	0.09	0.0402	0.266	0.042	0.08	0.5499
Dose								
50 kg N ha^{-1}	0.176	0.003	0.00	0.7342	0.109	0.004	0.01	0.2634
100 kg N ha^{-1}	0.135	0.023	0.03	0.1806	0.132	0.003	0.03	0.4695
150 kg N ha^{-1}	0.008	0.038	0.06	0.1039	0.004	0.001	0.05	0.8775
200 kg N ha^{-1}	0.001	0.048	0.10	1.0000	0.142	0.009	0.17	0.0321
Mean canopy								
Fertilizers								
Bovine manure	0.056 a	0.0274 ^{ns}	0.27	0.0001	0.587	0.035	0.29	0.2362
J. curcas seedcake	0.058 a	0.0254	0.37	0.0000	0.534	0.031	0.40	0.0050
Poultry manure	0.036 b	0.0239	0.26	0.0001	0.367	0.035	0.28	0.2388
Urea	0.038 b	0.0281	0.17	0.0019	0.228	0.045	0.20	0.0272
Dose								
50 kg N ha^{-1}	0.113	0.009	0.04	0.0743	0.051	0.005	0.06	0.1284
100 kg N ha^{-1}	0.230	0.012	0.03	0.1170	0.188	0.001	0.05	0.4849
150 kg N ha^{-1}	0.236	0.025	0.03	0.1845	0.229	0.001	0.02	0.7517
200 kg N ha^{-1}	0.136	0.000	0.00	0.9641	0.060	0.006	0.03	0.1210

£ An analysis of variance with its respective comparison of means was performed only when the probability for all the factor values was significant ($p \leq 0.05$).

¥ Lowercase letters in the column indicate the difference between means by Tukey's test $p \leq 0.05$).

ns There is no difference between column means by Tukey's test ($p \leq 0.05$).

Epidemiological parameters of leaf diseases can be affected by cultivar genetics, cultural practices such as strip tillage, and other factors (Cantonwine et al., 2007; Fischer et al., 2021; Chávez-García et al., 2022). However, some organic or inorganic fertilizers and doses in cotton plants can also reduce Y_0 and r parameters of diseases, such as Ramularia leaf spot (Palma-Zambrano et al., 2022). Also, in the present work, some fertilizers reduced the Y_0 of Cercospora leaf spot in each canopy of cotton plants, highlighting poultry manure and urea. Two hypotheses could explain this result. The first suggests that each nitrogen source (NH_3, NH_4, etc.) could have a positive impact on cotton plants in some manner, thereby indirectly reducing Y_0 (across all canopies) and r (in the upper canopy). For instance, the application of nitrogen sources might stimulate new leaf growth and temporarily lessen the severity of Cercospora leaf spot in cotton plants (Saude et al., 2014). Secondly, given that a cotton crop was previously cultivated in the same area, any organic fertilizer introduced to the soil before planting would have had ample time to either foster a beneficial environment for biocontrol agents or, conversely, for pathogens. However, the specific mechanisms by which nitrogen sources influence Cercospora leaf spot remain unclear.

The present study demonstrates that organic fertilization with poultry manure and *J. curcas* seedcake could serve as a sustainable practice for cotton cultivation in the future, particularly for mitigating foliar diseases such as Cercospora leaf spot. Organic manures have the potential to improve the rhizosphere by enhancing populations of antagonistic organisms and functional groups, including fungi and actinomycetes (Huang et al., 2006), thereby offering protection to cotton plants against pathogens such as *C. gossypina*. Although additional research is necessary to fully comprehend the disease dynamics in response to nitrogen fertilization, existing studies, including those by Palma-Zambrano et al. (2022) and our own, suggest that organic fertilization presents a viable option in the near term.

4. Conclusions

The study conclusively demonstrated that conidiophores and elongated multiseptated conidia hyalines of *Cercospora* sp. would be associated with Cercospora leaf spot disease. Furthermore, under the experimental conditions, the intensity of Cercospora leaf spot in cotton plants was significantly influenced by both the type of nitrogenous fertilizer applied and its dosage. Specifically, organic fertilizers, especially poultry manure and *Jatropha curcas* seedcake, showed potential in reducing the severity and initial inoculum of the disease across different canopy levels of cotton plants. This suggests a promising avenue for integrating specific organic nitrogen sources into sustainable management strategies for controlling Cercospora leaf spot in cotton crops.

A nuanced response was observed in the disease dynamics based on the interaction between the type of fertilizer and its dosage. Higher doses (100 to 200 kg N per hectare) were effective in reducing the severity of Cercospora leaf spot, particularly in the upper canopy. This highlights the importance of precision in fertilizer application, tailoring both the choice of fertilizer and its dosage to optimize disease management outcomes. The positive impact of poultry manure and *J. curcas* seedcake on reducing the severity of Cercospora leaf spot underscores the potential of organic fertilization in fostering more sustainable and environmentally friendly agricultural practices.

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Author Contributions Statement

VFP-R: raw data organization, validation, visualization, and writing-original draft. FZ-G: supervision, writing-review, and editing. DP: conceptualization, data curation, methodology, and writing-review and editing. FRG-F: conceptualization, supervision, and writing-review and editing. All authors have read and agreed to the published version of the manuscript.

Declarations

The authors declare no conflict of interest.

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