

Memories of the Agricultural and Livestock Area of the VI CCIUTM, Ecuador

Charcoal Rot on Cotton in Ecuador: Symptoms, Etiology and Management with Seaweeds

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Abstract | In this work, the effect of commercial algal extracts was examined based on *Ascophyllum nodosum*, *Sargassum* spp., and *Spirulina* spp. on the incidence of dead plants (%), Area Under the Disease Progress Curve (AUDPC) concerning that variable, severity (%) of rot in roots and hypocotyls, and vascular necrosis in hypocotyls (%) associated with charcoal rot, and the chlorophyll index (SPAD) in cotton plants (*Gossypium hirsutum* L.) varieties DP ALCALA 90 and COCKER 310 established under field conditions. Under semi-controlled conditions, emergence (%) and damping-off (%) were evaluated in seedlings of the two genotypes grown in soil where the field experiment was carried out and another obtained from a distant place where no agricultural activity was carried out. Under the same conditions, the size of microsclerotia (n=20) and pathogenicity of two *Macrophomina* isolates on COCKER seedlings were determined. After the data were subjected to a two-way ANOVA, the Tukey test separated the means ($P \leq 0.05$). All the plants analyzed presented charcoal rot and presence of microsclerotia, mainly in hypocotyls. Only vascular damage in hypocotyls was higher in COCKER 310 plants compared to those of the other genotype. A significant interaction between varieties and algal extracts was found for dead plants incidence ($P \leq 0.0305$). Seedling emergence (%) was higher in COCKER 310 seedlings grown in infested soil compared to those of the other genotype ($P \leq 0.0276$). To our knowledge, this is the first report of *Macrophomina phaseolina* on cotton in Ecuador.

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Introduction

Cotton (*Gossypium* spp.) represents a significant agricultural resource worldwide due to its natural

textile fiber (Wang *et al.*, 2019). Its seeds are used as a source of protein and oil (Cañarte-Bermúdez *et al.*, 2020), and its fiber is made up of complex structures composed almost exclusively of cellulose

molecules and D-glucose residues (French and Kim, 2018). Although the cultivable species worldwide are *Gossypium hirsutum*, *G. barbadense*, *G. herbaceum*, and *G. arboreum* (Cai *et al.*, 2015), the first one stands out as the most cultivated due to its high boll yield (Wang *et al.*, 2019).

The global cotton area is 31,840,226 ha, with a production of 83,112,924 million t of fiber, distributed in America (22.5%), Africa (5.8%), Europe (1.3%) and Oceania (0.40%) (FAO, 2020). India, China, the United States, Pakistan, and Brazil are the leading cotton fiber-producing countries (Cañarte-Bermúdez *et al.*, 2020). In agricultural regions such as South America, the economy of many of its countries is influenced by cotton cultivation (INIAP, 2020). In Ecuador, cotton cultivation has been gaining importance since the execution of the +Cotton Project, especially in the provinces of Manabí and Guayas (Zambrano-Gavilanes *et al.*, 2022).

Main problems affecting cotton cultivation in Ecuador are insect pests and viral, bacterial, and fungal diseases. Among the pathogenic fungi of cotton are *Cercospora gossypina* Cooke (Cercospora leaf spot), *Rhizoctonia solani* J.G. Kühn (damping off), *Colletotrichum gossypii* Southw (anthracnose), *Diplodia gossypina* Cooke (boll rot), and *Ramulariopsis pseudoglycines* (Ramularia leaf spot) (Sión *et al.*, 1992; Palma-Zambrano *et al.*, 2022; Parrales-Rodríguez *et al.*, 2024). Although many of these pathogens and their diseases that infect aerial tissues negatively affect cotton leaf area and fiber yield (Ascari *et al.*, 2016), others, such as *Macrophomina phaseolina* (charcoal rot), can compromise water and nutrient uptake in plant roots (Siddique *et al.*, 2020; Marquez *et al.*, 2021).

The fungus *M. phaseolina* is a soil-borne phytopathogen that affects approximately 500 plant species (including more than 100 families), causing rot in seed, root, stem, and leaf tissues (Marquez *et al.*, 2021; Haider and Javaid, 2023; Kumar *et al.*, 2023). The pathogen can cause death of seedlings and adult plants, and spindle-shaped lesions with a light gray center and dark edge, covered with small microsclerotia, and pycnidia in some instances, can be observed in root and crown tissues (Kaur *et al.*, 2012). The pathogen reduces the absorption and transport of water and nutrients from underground to aboveground tissues (Siddique *et al.*, 2020; Marquez *et al.*, 2021). This causes plants infected by *M. phaseolina* to wilt, and

canker-like lesions can even be observed on old branches, which then elongate and form pycnidia and sclerotia of the fungus on their surface (Garrido *et al.*, 2016; Marquez *et al.*, 2021). Microsclerotia present in the soil from previous harvests are the primary source of inoculum (Luna *et al.*, 2017; Marquez *et al.*, 2021; Kumar *et al.*, 2023) and could be associated with epidemics in subsequent cotton crop cycles.

Although synthetic fungicides are one of the most widely used methods for managing charcoal rot, it has been known for some years that many of the molecules are losing their effectiveness (Tonin *et al.*, 2013; Dahikar and Nagarka, 2024). Thus, using seaweed could be a more effective and ecological management method. In fact, an increase in the use and application of marine resources such as seaweed extracts and derivatives has been observed worldwide (Garcés-Fiallos *et al.*, 2021). Seaweeds induce tolerance to abiotic and biotic stresses such as diseases (Garcés-Fiallos *et al.*, 2020; Tajdinian *et al.*, 2022; Dangariya *et al.*, 2024). In general, plants infected with *M. phaseolina* and treated with algae improve significantly, possibly due to the increase in photosynthetic pigments, total phenol content, flavonoids, and antioxidant activity such as the enzymes phenylalanine ammonia-lyase and polyphenol oxidase, and to the reduction of electrolyte loss in the cell membrane and accumulation of ROS, enhancing phytopathogen tolerance and crop productivity (Garcés-Fiallos *et al.*, 2021; Tajdinian *et al.*, 2022; Dangariya *et al.*, 2024).

There is a growing interest in using marine macroalgae as fertilizers, biostimulants, soil conditioners, and biotic protectors in plants. For instance, *Sargassum* seaweed is a biostimulant in agriculture due to its rich bioactive compounds, which promote plant growth, nutrient absorption, and tolerance to biotic and abiotic stress in agricultural and horticultural crops (Bosmaia *et al.*, 2023; Dangariya *et al.*, 2024; Senthilkumar *et al.*, 2024). The biotic stress caused by *M. phaseolina* in cotton crops, *i.e.*, reduced water and nutrient uptake leading to mortality of seedlings and adult plants (Kaur *et al.*, 2012; Siddique *et al.*, 2020; Marquez *et al.*, 2021), could be alleviated by the application of seaweed. To date, there are few studies on the effect of seaweed on the management of charcoal rot in cotton. Knowing that the application of some seaweeds shows a positive effect on growth and induction of defense against *M. phaseolina* in plants (Tajdinian *et al.*, 2022; Dangariya *et al.*, 2024), we aimed to

evaluate the effect of the application of commercial seaweeds on charcoal rot in cotton cultivation under field conditions. We also determined morphologically and pathogenetically the possible etiological agent associated with the disease on cotton in Ecuador.

Materials and Methods

Location of the experiments

The present research was carried out at the Faculty of Agricultural Engineering (FAE) of the Technical University of Manabí, located at an altitude of 47 m above sea level, in Lodana parish, Santa Ana, Manabí province (01 ° 10'25" S and 80 ° 23'14" W). Under field conditions during the dry season of 2022, we evaluated the effect of commercial seaweed extracts on charcoal rot in cotton plants. Also, in semi controlled conditions, we quantified the emergence and damping-off and morpho-pathogenically the possible etiological agent associated with charcoal rot in cotton seedlings.

Field experiment management

Prior to the research, three consecutive cotton cycles were established. The soil was mechanically prepared employing harrow, romero plow, and rotavator. Subsequently, cotton seeds of the varieties DP ALCALA 90 and COCKER 310 were sown at a depth of approximately 5 cm in holes previously prepared manually, at 0.40 and 0.75 m between plants and rows, respectively. Although four seeds were placed per hole, only one plant was finally left per site. The total area of each plot and the entire experiment was 15 and 936 m², respectively, leaving the plants from the three central rows of each plot for sanitary and physiological analysis.

Cotton plants were organically fertilized based on previous soil analysis, which consisted of 100 kg N ha⁻¹ of empty manure. Pest insects were managed by applying the insecticides Tiametoxam + Lambdaihalotriona (200 mL ha⁻¹) and Lufenuron (200 mL ha⁻¹) 15 days after emergence and 20 days after the first application. Plants were irrigated using a drip irrigation system once before sowing and subsequent irrigations during crop development based on their water needs. Commercial seaweed extracts based on *Ascophyllum nodosum*, *Sargassum* spp., and *Spirulina* spp. were applied to leaf area of cotton plants at 15, 30, and 45 days after sowing, at a dose of 2 L ha⁻¹ in each application, using a manual sprayer. Plants

treated with irrigation water constituted the controls.

Evaluation of symptoms and microsclerotia in plants

We identified and evaluated the symptoms from their initial appearance (mostly in lower leaves) between September 28 and November 16, 2022. In this last period, some plants with different degrees of affection were uprooted, and their roots and crowns were scraped with a knife until the epidermis was removed. Both tissues were taken to the Laboratory, where we analyzed the presence of microsclerotia. From there, we observed and measured these assimilative structures under the microscope (Nikon, Eclipse E200) with a coupled camera [MF-BG(U) Led] and imaging system (Mshot). Finally, samples of symptomatic roots (~0.5 mm) were disinfested by successive immersions in ethanol (70%) per 45 seg, sodium hypochlorite (2%) per three min, and sterile distilled water and immediately sown on Petri dishes containing potato dextrose agar (PDA) culture medium.

Quantification of the incidence of dead plants

The incidence of dead plants was assessed weekly between September 28 and November 16, 2022, totaling eight assessments. For this, the plants that presented death and drying were counted in each of the three central rows of each plot, and they were averaged for the total number of plants present in the same helpful plot. With this information, the Area Under the Disease Progress Curve (AUDPC) was analyzed concerning the incidence of dead plants caused by charcoal rot.

Analysis of root and hypocotyl tissues

This evaluation was carried out at the end of the experiment on November 16, 2022, on primary roots and hypocotyls of eight cotton plants randomly chosen in each central plot. The primary root and the hypocotyl were carefully separated from each plant, and each tissue was cut in a basipetal and acropetal direction from the base of the soil into 10 cm pieces, respectively. First, the incidence of microsclerotia in hypocotyls was quantified, and the epidermis was carefully removed to observe these structures using a stereomicroscope. Subsequently, hypocotyls and roots were analyzed for the severity (%) of rot or discoloration induced by the pathogen, measuring the damage length in each tissue with a digital caliper. Finally, vascular and medullary damage (%) was evaluated in the longitudinally cut hypocotyls.

Chlorophyll index estimation

On October 26, 2022, the chlorophyll index (SPAD-502Plus, Konica Minolta) was estimated on the leaf located at the fourth node, in the apex-base direction of ten plants, using the SPAD meter. Four readings were taken on each evaluated leaf at four different positions, finally obtaining the average (SPAD units).

Evaluation of emergence and damping-off in seedlings

Cotton seeds of the varieties DP ALCALA 90 and COCKER 310 were sown at a depth of approximately 3 cm in 50-cell germination trays (~40 cm³ each cell) containing two types of soil, one obtained in the same place where the field experiment was carried out to take advantage of the source of pathogen inoculum, and another obtained in a distant place where no agricultural activity is carried out. The trays remained under shade (25±4 °C, relative humidity ≥75%, and 12 h of light) 15 days after sowing (DAS). After that, we evaluated the seedlings' emergence (%) and damping-off (%) percentages.

Inoculation and symptom assessment in cotton seedlings

Seedlings (15-day-old) grown in the previously sterilized substrate (Flora gard®) were inoculated with *Macrophomina* sp. isolates MP2 and MP3 using the toothpick inoculation method. Initially, four monohyphal strains of the pathogen (MP1, MP2, MP3, and MP4) were isolated, purified, and obtained from symptomatic cotton roots from the field experiment. From there, discs (Ø 5mm) of the MP2 and MP3 isolates previously grown in Petri dishes containing PDA culture medium were placed in the center of new dishes containing the same medium and previously sterilized toothpicks.

After seven DAS, infested toothpicks were embedded at the cotton seedlings' stem base (~2 cm from the soil surface). Plants inoculated with disinfested toothpicks were the controls. These seedlings were maintained in a screen house at room temperature (26±3 °C, relative humidity ≥80%, and 12 h of light) and irrigated twice. After 20 days post-inoculation, we assessed the incidence of death (%) and vascular necrosis (%) in cotton seedlings.

Experimental design and statistical analysis

Under field conditions, a completely randomized block experimental design was used with a factorial arrangement of 2 (varieties DP Alcala 90 and Cocker 31) x 4 (marine algae extracts *A. nodosum*, *Sargassum*

spp., *Spirulina* spp., and the control), in four blocks. Under semi-controlled conditions, we evaluated the emergence and damping-off in seedlings on infested soil and the effect of pathogen isolates on cotton seedlings.

In general, the homogeneity of variances and normality of residuals was verified by applying the Bartlett and Shapiro-Wilks tests, respectively. The AUDPC data were transformed to Log (x). After satisfying the assumptions, the data were subjected to two-way ANOVA. Subsequently, the Tukey test was used to separate means ($P \leq 0.05$). All analyses were performed using the statistical software GraphPad Software version 5.

Results and Discussion

Symptoms and microsclerotia in adult plants

Cotton plants initially showed leaf yellowing (data not shown). As the disease progressed (Figure 1), the plants began to dry out and die, leaving their leaves attached (Figure 1A, B). At the end of the crop, the roots and part of the crown of the plants showed small, dark brown lesions (Figure 1B). The plants and stems of symptomatic plants showed a reduction in height and diameter, respectively (Figure 1B, C). In internal tissues of symptomatic hypocotyls, we observed necrosis in the epidermis, cortex, vascular bundles, and pith (Figure 1C). Many symptomatic plants' hypocotyls showed large microsclerotia production (Figure 1D, F).

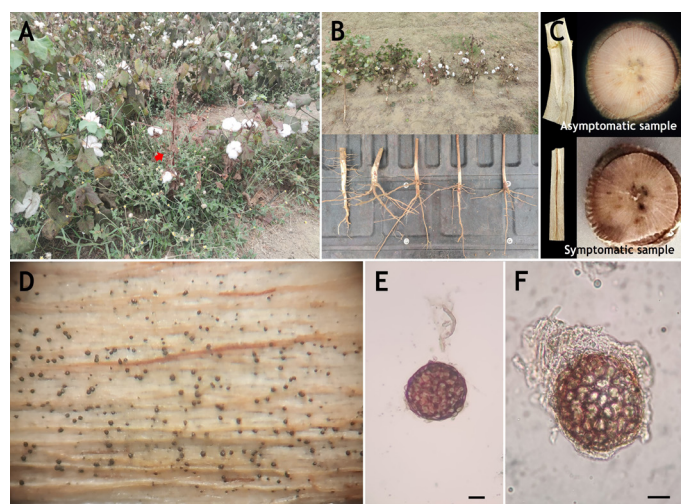


Figure 1: Symptoms and signs associated with charcoal rot in cotton plants. A) Drying and death of plants. B) Root and crown rot and wilting on plants. C) Asymptomatic and symptomatic hypocotyls showing healthy and damaged internal tissues (necrosis in the epidermis, cortex, vascular bundles, and pith), respectively. D-F) Presence of microsclerotia in hypocotyls of symptomatic plants (E), and observation of these assimilative structures under a microscope (F-G). Scale bars = 20 µm.

Table 1: Incidence of dead plants (%), Area Under the Disease Progress Curve (AUDPC) concerning that variable, severity (%) of root and hypocotyl rot, vascular necrosis in hypocotyls (%), and chlorophyll index (SPAD units) in DP ALCALA 90 and COCKER 310 cotton plants treated with seaweed extracts of *Ascophyllum nodosum*, *Sargassum* spp., *Spirulina* spp. or water. Lodana, Manabí, Ecuador.

Factors	Dead plants (%)	AUDPC	Severity (%)		Vascular necrosis in hypocotyls (%)	Chlorophyll index (SPAD units)
			Roots	Hypocotyls		
Varieties	ns.	ns.	ns.	ns.	0,0287	ns.
DP ALCALA 90	19.2	338.0	24.5	28.6	52.3 b*	32.8
COCKER 310	22.8	309.0	33.1	31.7	74.2 a	31.7
Seaweed extracts	ns.	ns.	ns.	ns.		ns.
<i>Ascophyllum nodosum</i>	25.1	366.1	33.5	30.3	64.0	31.6
<i>Sargassum</i> spp.	17.2	329.5	28.6	33.0	64.1	35.0
<i>Spirulina</i> spp.	21.4	312.2	25.4	32.1	64.1	31.4
Control (water)	20.4	286.2	27.7	25.2	60.9	31.7
Interaction	0.0305	ns.	ns.	ns.	ns.	ns.
CV (%):	33.4	40.1	40.72	20.89	13,52	9.97

ns: non-significant analysis. * Means followed by different letters within the column show significant differences (Tukey test; $P \leq 0.05$) between the two cotton varieties.

Table 2: Significant interaction between varieties (factor A) and seaweed extracts (factor B) regarding the incidence of dead plants in cotton plants (%). Lodana, Manabí, Ecuador.

Factors	<i>Ascophyllum nodosum</i>	<i>Sargassum</i> spp.	<i>Spirulina</i> spp.	Control (water)
DP ALCALA 90	19.80 bA	17.93 aA	24.98 aA	14.18 bA
COCKER 310	30.38 aA*	16.38 aB	17.90 aB	26.53 aAB

* Means followed by the identical lowercase and uppercase letters in the row and column, respectively, do not differ statistically (Tukey test; $P \leq 0.05$).

Symptoms and chlorophyll index in adult plants treated with seaweed extracts

Under field conditions, we did not find any significant difference for the last evaluation of the incidence of dead plants and the AUDPC for that same variable (Table 1). The incidence of dead plants was similar between the varieties DP ALCALA 90 and COCKER 310 and between the plants treated or not with the seaweed extracts, averaging 21.0 %. The interaction between the varieties and the seaweed extracts was significant ($P \leq 0.0305$). Something similar happened with the AUDPC, where no significant difference was found between the varieties or between the treatment or not with seaweed extracts, presenting an average of 323.5 %.

The severity of the disease in both roots and stems was similar when the varieties and seaweed extracts were compared separately, presenting averages of 28.8 and 12.06, respectively (Table 1). Vascular necrosis in hypocotyls was 1.4 times higher in COCKER 310 plants when compared to those DP ALCALA 90 ($P \leq 0.0287$). However, when plants treated or not with

seaweed were compared, an average vascular damage of 63.2 % was found. A significant interaction between factors was not found in the abovementioned three variables.

The chlorophyll index was similar between both varieties evaluated (32.3 SPAD units on average) and between seaweed extracts (32.4 SPAD units on average). Also, the interaction between factors was not significant (Table 1).

The interaction between factors was significant between varieties and the application or not of seaweed extracts (Table 2). The incidence of dead plants was lower only in DP ALCALA 90 plants treated with *A. nodosum* (1.5 times) and untreated (1.9 times), compared to COCKER 310. Likewise, when plants treated with seaweed extracts were not compared, a higher incidence of dead plants was found in var. COCKER 310 treated with *A. nodosum* and untreated, compared to those treated with the other seaweed extracts.

Emergence and damping-off in seedlings

Emergence (%) and damping-off (%) are presented in Figure 2. Emergence (Figure 2A, B) was almost two times lower (41 %) in COCKER 310 seedlings grown in infested soil ($P \leq 0.0276$) compared to those of the same genotype grown in uninfested soil and to those of the var. DP ALCALA 90 grown in both soils (80.7 % on average). No significant difference was found regarding damping-off, with an average of 7 % in plants of both varieties grown in both soils (Figure 2C). The symptoms found were yellowing, growth reduction, and premature death of seedlings.

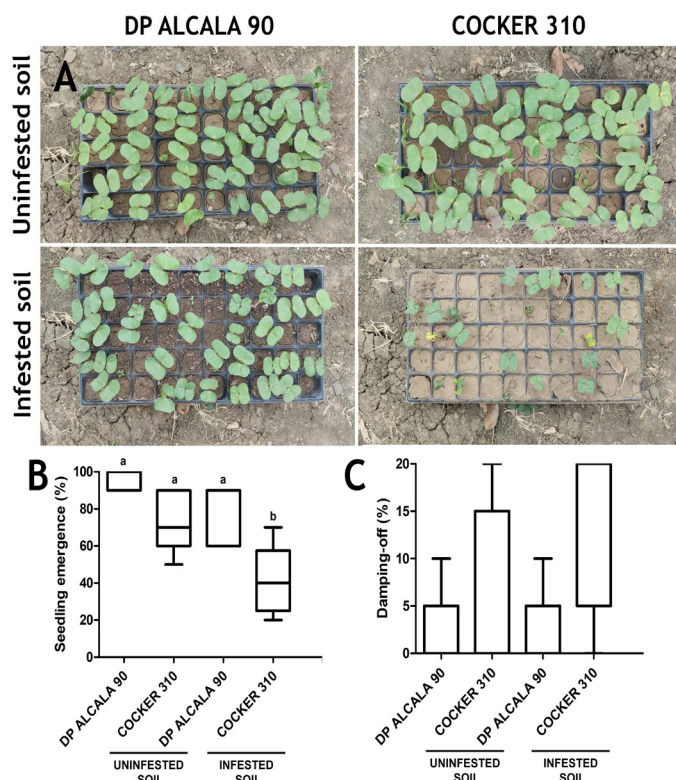


Figure 2: Emergence (%; A-B) and damping-off (%; C) in seedlings of the varieties COCKER 310 and DP ALCALA 90 grown in infested and uninfested soil. Lodana, Manabí, Ecuador.

Symptoms in cotton-inoculated seedlings

COCKER 310 seedlings inoculated with infested toothpicks with isolates MP2 and MP3 (Figure 3A-B) began to show wilting at 7 DAI. As the infection progressed, the plants showed a reduction in height and death (20 DAI; Figure 3C). Necrosis was also observed inside the stems of the inoculated seedlings. Control seedlings did not show symptoms. The microsclerotia of isolates MP2 (Figure 3B) and MP3 measured an average of $103.5 \pm 20.0 \mu\text{m}$ ($71.9 \times 135.8 \mu\text{m}$) and $109.5 \pm 18.3 \mu\text{m}$ ($67.6 \times 143.3 \mu\text{m}$), respectively.

Although charcoal rot has been reported and

quantified in Ecuador in crops such as beans under field conditions (Garcés-Fiallos, 2013; Garcés-Fiallos and Vera-Alcívar, 2014; Garcés-Fiallos and Gamarra-Yáñez, 2014; Garcés-Fiallos *et al.*, 2015), it has not yet been reported or evaluated in cotton. Its causal agent, *M. phaseolina*, is a polyphagous phytopathogen that affects several plant species (Siddique *et al.*, 2020; Kumar *et al.* 2023). Thus, in this research, for the first time in the country, we present the symptoms and etiology associated with charcoal rot and its management with seaweeds.

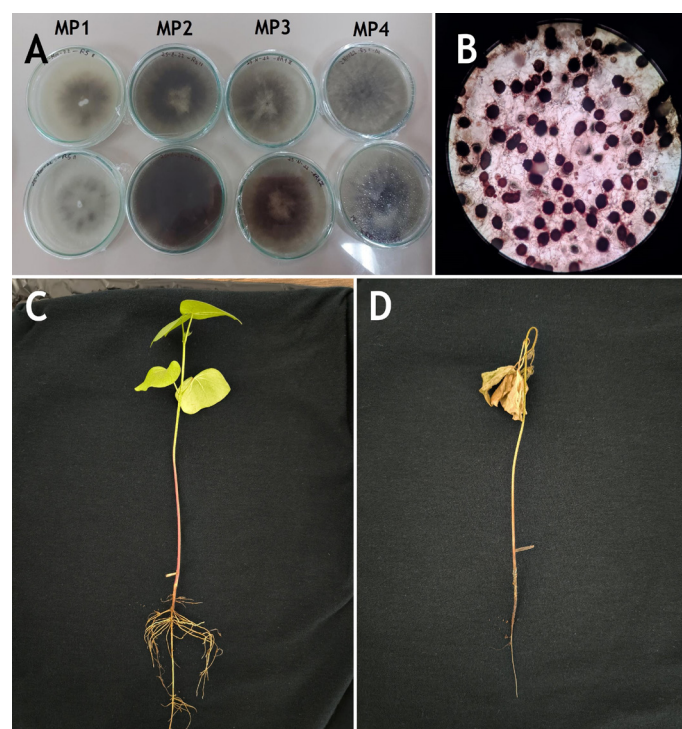


Figure 3: Isolates (MP1, MP2, MP3, and MP4) of *Macrophomina* sp. grown for seven days on Petri dishes containing potato dextrose agar culture medium (A), presence of microsclerotia (B) and COCKER 310 seedlings not inoculated (C) and inoculated with isolate MP2 showing symptoms of death and height reduction (D).

Regardless of the treatment evaluated in the field experiment, cotton plants initially exhibited leaf yellowing. Symptoms develop in the later stages of growth, resulting in wilting and death (Degani *et al.*, 2023). As the disease advanced, the plants started to wither die, with their leaves sticking together. Cotton plants may exhibit chlorosis and wilting (Siddique *et al.*, 2020; Cohen *et al.*, 2022). Symptoms observed on plants are less noticeable at early stages of development and under temperate conditions than on plants at flowering and fruit setting stages and grown under warm conditions (Degani *et al.*, 2023). Also, plants and stems of symptomatic plants showed a reduction in height and diameter, respectively. The pathogen causes a reduction in plant height and dry

weight in cotton seedlings (Omar *et al.*, 2007).

In the last evaluation, we confirmed that both root and crown tissues presented small lesions of dark brown coloration and vascular damage in the pith and vascular bundles of hypocotyls. Plants with hard and compact tissues, such as cotton, succumb to the toxins produced by *M. phaseolina*, causing plant wilting (Cohen *et al.*, 2022). Later foliar symptoms include premature senescence, wilting, and premature plant death (Luna *et al.*, 2017). Vascular bundles of roots and stems show discoloration, possibly due to the interaction of toxins and the sesquiterpene phytoalexin gossypol (Cohen *et al.*, 2022). Mycelium of the pathogen can be found in the cortical, medullary, and vascular regions (Siddique *et al.*, 2020), similar to our findings. Finally, we confirmed that most hypocotyls of symptomatic plants showed high production of microsclerotia. Indeed, *M. phaseolina* produces microsclerotia or pycnidia inside roots and stems in the host, allowing it to subsequently persist in crop residues and soil for a prolonged period (Luna *et al.*, 2017; Marquez *et al.*, 2021).

The incidence in plants of both genotypes was 100 %. The incidence of charcoal rot in bean genotypes under field conditions in Ecuador is between 1.8 and 47 % (Garcés-Fiallos, 2013; Garcés-Fiallos and Vera-Alcívar, 2014; Garcés-Fiallos and Gamarra-Yáñez, 2014; Garcés-Fiallos *et al.*, 2015). If we compare the results obtained in cotton with those of beans, we can see that the incidence of the disease is very high in cotton. Therefore, we could say that the severity of charcoal rot of 28.8 and 30.15 % found in roots and stems, respectively, would be associated with the high source of inoculum accumulated in previous harvests (Luna *et al.*, 2017; Kumar *et al.*, 2023). Microsclerotia present in the soil are the primary source of inoculum and can survive up to 15 years (Marquez *et al.*, 2021).

Under field conditions, vascular necrosis in hypocotyls (%) was higher in var. COCKER 310 compared to genotype DP ALCALA 90. Under semi-controlled conditions, emergence (%) was lower in COCKER 310 seedlings grown in infested soil compared to those of the same genotype grown in uninfested soil and those of var. DP ALCALA 90 grown in both soils. The source of inoculum (possibly composed of microsclerotia) present in the soil allowed us to observe the effect of the pathogen on adult plants and seedlings (Luna *et al.*, 2017; Kumar *et al.*, 2023). *M. phaseolina* causes pre- and post-emergence damping-

off and reduces plant height and dry weight in cotton seedlings (Omar *et al.*, 2007; Garcés-Fiallos, 2013). Plants can respond differently to *M. phaseolina* infection (Omar *et al.*, 2007; Claudino and Soares, 2014). Thus, we could infer that the response of DP ALCALA 90 seedlings and adult plants to the pathogen would be associated with some defense mechanism on the part of the host (Yan *et al.*, 2021).

Charcoal rot and chlorophyll index were similar in plants treated or not with seaweed extracts. However, the interaction between factors (varieties and seaweed extracts) was significant for the incidence of dead plants. This variable was lower in DP ALCALA 90 plants treated with *Sargassum* spp. and *Spirulina* spp., with the weak response of the COCKER 310 genotype also being observed. In general, seaweed extracts can reduce the intensity of diseases or completely suppress infection by *M. phaseolina* in plants, also improving their growth, root length and shoot weight, and microbial activity in the soil, especially of *Pseudomonas fluorescens* (Ehteshamul-Haque *et al.*, 2013; Garcés-Fiallos *et al.*, 2020, 2021; Rahman *et al.*, 2021). Seaweeds possess antioxidants, phytohormones, glucosinoids, bioactive compounds, and enzymes, which activate molecular defense mechanisms in plants against *M. phaseolina* (Garcés-Fiallos *et al.*, 2021; Bosmaia *et al.*, 2023; Senthilkumar *et al.*, 2024). The poor management of charcoal rot in cotton with seaweeds in our research would be associated with the disease's high incidence (100%) and severity (between 28.8 and 30.15%) due to the high inoculum pressure. It should also be considered that managing a disease with seaweeds depends on the biological interaction between the genotype and the environment (Delgado *et al.*, 2013; Garcés-Fiallos *et al.*, 2020).

Macrophomina phaseolina MP2 and MP3 isolates induced wilting and subsequent height reduction and death in cotton seedlings var. COCKER 310. Charcoal rot caused by *M. phaseolina* has been reported in cotton (Baird and Brock, 1999). The phytopathogen can cause the death of all infected plants under controlled conditions (Claudino and Soares, 2014). Infected plants show dark brown to black lesions on primary and secondary roots in infected plants under natural and artificial conditions (Baird and Brock, 1999). On the other hand, the microsclerotia (n = 25) of isolates MP2 and MP3 measured an average of 103.5±10.0 µm (71.9 x 135.8 µm) and 109.5±9.3 µm (67.6 x 143.3 µm). These

measurements are within the range found in other investigations. For instance, Siddique *et al.* (2020) found microsclerotia with a range of 80 x 120 µm in sunflower tissues affected by *M. phaseolina*. There are only reports of *M. phaseolina* affecting cotton plants (Baird and Brock, 1999; Omar *et al.*, 2007; Degani *et al.*, 2023). Considering this assertion, as well as the measurements found of the microsclerotia and the pathogenicity results in seedlings, we can infer that the causal agent of charcoal rot in cotton in Ecuador is *M. phaseolina*. In any case, conducting a molecular analysis or another more detailed study would be essential to refute or confirm these results.

Conclusions and Recommendations

Charcoal rot in cotton plants established in field conditions initially induces leaf yellowing. As it progresses, the disease reduces height and drying until the plant dies, with its leaves remaining attached. At the end of the crop cycle, the surface of plant, roots and crowns show small dark brown lesions and necrosis in the epidermal, cortical, and mainly in the vascular and medullary areas. Hypocotyls of symptomatic plants show a large production of microsclerotia. Charcoal rot is present in all plants of the DP ALCALA 90, and COCKER 310 varieties, but only vascular damage in hypocotyls is greater in plants of the COCKER 310 variety compared to those of the other genotype. Despite the positive effects of marine algal extracts in agriculture, in our field experiment, most of the variables associated with charcoal rot and chlorophyll index are not affected in cotton plants. A significant interaction between varieties and algal extracts is observed for dead plants, highlighting the DP ALCALA 90 plants treated with *A. nodosum* and water compared to those of COCKER 310. Seedling emergence (%) is higher in COCKER 310 seedlings grown in infested soil than in the other genotype. Symptoms associated with damping-off were yellowing, growth reduction, and premature death in seedlings. The artificial infection of COCKER 310 seedlings using infested toothpicks with isolates MP2 and MP3 and the morphological characterization of both strains (microsclerotia) allows reporting of the presence of *Macrophomina phaseolina* in cotton on Ecuador for the first time.

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Novelty Statement

This study provides the first report of *Macrophomina phaseolina* infecting cotton crops (*Gossypium hirsutum* L.) in Ecuador, highlighting its association with charcoal rot disease. Furthermore, the work demonstrates the effects of commercial algal extracts on disease incidence and severity across DP ALCALA 90 and COCKER 310 cotton genotypes. The findings reveal genotype-specific responses to algal extracts and infested soils, contributing novel insights into charcoal rot management in cotton crops.

Author's Contribution

Frank G. Cedeño-Lozano and Felipe R. Garcés-Fiallos: Material preparation, data collection and analysis.

Freddy Zambrano-Gavilanes and Felipe R. Garcés-Fiallos: Conceptualization and research work.

Felipe R. Garcés-Fiallos: Written the first draft of the manuscript.

All authors contributed to the study conception and design, reviewed previous versions of the manuscript, read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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