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Colletotrichum truncatum TRANSMISSION VIA LIMA BEAN SEEDS

Rommel dos Santos Siqueira GOMES[1], Hilderlande Florêncio da SILVA[1], José George Ferreira MEDEIROS[2], Walter Esfrain PEREIRA[1], Luciana Cordeiro do NASCIMENTO[1]

[1] Postgraduate Program in Agronomy, Universidade Federal da Paraíba, Areia, Paraíba, Brazil.
[2] Academic Unit of Development Technology, Universidade Federal de Campina Grande, Sumé, Paraíba, Brazil.

Corresponding author:
Rommel dos Santos Siqueira Gomes
rommelssgomes@gmail.com

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Abstract

Anthracnose is the primary disease in *Phaseolus lunatus* cultures, causing severe losses. Inoculation techniques are vital for assessing genotype resistance and control methods at the early stages of seedling development. This study aimed to compare inoculation methods and exposure times of a lima bean seed variety to *Colletotrichum truncatum* using a completely randomized 4x5 factorial design with five replications. Seed inoculation methods by direct contact with mycelium, mannitol water restrictor, and sucrose water restrictor and immersion in conidia suspension were compared and submitted to substrates containing the developed pathogen or not at exposure times of 0, 36, 60, 84, and 108 hours. Evaluations were made by analyzing the severity, incidence, and disease index for anthracnose and seedling physiological quality under greenhouse conditions. The inoculation method by direct contact with sucrose solute for 36 hours was the most suitable for *C. truncatum* inoculation in lima bean seeds, providing a higher transmission rate but slightly affecting their physiological parameters. *C. truncatum* damage to lima bean seed performance increased with longer exposure times, regardless of the inoculation method.

Keywords: Anthracnose. *Phaseolus lunatus.* Physiological quality. Water restriction. Seed health.

1. Introduction

The *Colletotrichum truncatum* (Schwein) Andrus & Moore fungus is the causal agent of anthracnose and among the most significant diseases of lima beans (*Phaseolus lunatus* L.). It is found in production fields, potentially decreasing yield (Gomes and Nascimento 2018). This fungus can survive in crop residues, but its subsistence and dissemination occur mainly through infected seeds, which may be an inoculum source for primary infections under field conditions (Yang and Hartman 2016).

Currently, two species of *Colletotrichum* spp. reportedly infect lima beans: *C. truncatum* and *C. sichuanensis*, commonly found in production fields in Brazil, especially in the northeast region (Cavalcante et al. 2019).

Lima bean anthracnose symptoms show depressed necrotic lesion spots on leaves, petioles, and pods. The lesions eventually become reddish, and fungus acervuli are formed on them with a whitish color and numerous pathogen setae (Carvalho et al. 2015).

One of the main transmission methods of this pathogen is through infected seeds, which may carry pathogen inoculum associated with their surface, inside, or mixed with them and start new epidemics in

crop fields. The inoculum source might be present in different forms, such as resistance structures or other specific formations of fungi, bacteria, nematodes, and viruses (Santos et al. 2011).

Seed immersion in conidia suspension is a simple and effective inoculation method (Ahn et al. 2023). Osmotic conditioning (water restriction), also known as physiological conditioning, is another technique used in seed technology to potentiate the germination process of some species and in seed inoculation tests with fungi and bacteria (Pedroso et al. 2010; Menezes et al. 2011). However, these techniques may not provide conditions for the pathogen to infect the seeds, which requires adjustments such as the time of contact between them and the pathogen (Rodrigues et al. 2016). Therefore, methodologies that allow pathogen transmission and infection via seeds to artificially inoculated seedlings must be standardized.

Maintaining the sanitary quality of seeds is one of the most critical aspects for higher yield due to the numerous potentially associated pathogens. Seeds of good physiological quality allow for a rapid and uniform primary root emission in the germination process, high growth rate, seedlings with larger initial size, and high yield (Munizzi et al. 2010; Piveta et al. 2010).

This study aimed to compare different inoculation methods and exposure times of lima bean seeds to *C. truncatum.*

2. Material and Methods

The tests were conducted between May and December 2018 in a greenhouse environment at the Phytopathology Laboratory of the Federal University of Paraíba, Center of Agrarian Sciences, in Areia, PB, Brazil.

The study used lima bean seeds (accession number UFPB04) with a determinate growth habit (Gomes and Nascimento 2018). The seeds were obtained from a field in Remígio, PB, Brazil. Before the test, they were submitted to a germination trial for viability analysis (Brasil 2009), obtaining a mean of 82% of germinated seeds.

Seed samples were disinfested in a 70% alcohol solution for 30 seconds, 1% sodium hypochlorite for one minute, triple-washed in sterile distilled water (SDW) and dried at room temperature (25±2°C).

The *C. truncatum* isolate was obtained through direct isolation by collecting the mycelium structure and spores on the seed surface. The fragments were plated on a potato dextrose agar (PDA) medium and incubated in BOD (Biochemical Oxygen Demand) at 28±1°C for seven days. After the incubation period, microscopic structures were visualized, from which slides were made with the fungal propagules and observed under an optical microscope to confirm pathogen isolation.

Essay by direct contact with mycelium

The study used a complete randomized experimental design in a 4x5 factorial scheme (inoculation methods x exposure times) with five replications.

In the direct contact exposure, 5-mm-diameter discs of the pathogen colony were transferred to Petri dishes (9 cm) containing PDA medium. These dishes were incubated at 28±1°C for a 12-hour photoperiod for 14 days. Thirty previously disinfested seeds were placed on each plate, distributed in rows. These plates were sealed and manually shaken for 30 seconds to mix the seeds with the pathogen infection structures. Then, the inoculated seeds remained incubated in a BOD chamber for 0, 36, 60, 84, and 108 hours.

Essay by direct contact with the mannitol water restrictor

Seed inoculation in direct contact with the mannitol water restrictor used a PDA growth medium modified with mannitol ($C_6H_{14}O_6$) at the osmotic potential of -1.0 MPa. A dose of 46.3 g of the solute was added to 1000 mL^{-1} of the medium to reach the desired potential (-1.0 MPa) (Araújo et al., 2016). Thirty seeds were distributed in 9-cm Petri dishes containing the 14-day fungal colony, incubated at 28±1°C for a

12-hour photoperiod in a BOD chamber. The Petri dishes containing seeds were manually shaken for 30 seconds and incubated for 0, 36, 60, 84, and 108 hours as described.

Essay by direct contact with the sucrose water restrictor

Seed inoculation by direct contact with the sucrose water restrictor was similar to the mannitol method, using a modified PDA medium (water restriction) with a sucrose solute ($C_{12}H_{22}O_{11}$) at the osmotic potential of -1,0 MPa. Then, 16.86 g of the solute was added to 1000 mL^{-1} of the medium (Rey et al., 2008) with the 14-day fungal colony under the same conditions described. Thirty seeds were placed on the fungal colony, manually shaken, and incubated in a BOD chamber at 28±1°C for 0, 36, 60, 84, and 108 hours.

Essay by immersion in conidia suspension

Previously disinfested seeds were immersed in the *C. truncatum* conidia suspension for five minutes and incubated at 0, 36, 60, 84, and 108 hours. After inoculation, the seeds were placed on Petri dishes containing a double layer of filter paper. As for the conidia suspension, *C. truncatum* colonies were grown in PDA medium for 14 days at 28±1°C for a 12-hour photoperiod in a BOD chamber. After incubation, 10 mL of SDW was added to the fungal colony and scraped with a sterilized brush to release the conidia. Subsequently, 25 mL of the suspension was adjusted to $1x10^4$ conidia mL^{-1} aided by a Neubauer chamber, followed by the immersion of 30 seeds, which remained immersed in the suspension for five minutes. Then, they were placed on Petri dishes (9 cm) containing a double layer of filter paper, sealed, and incubated in the BOD chamber.

Disinfested and non-inoculated seeds were used in all inoculation methods tested in the control period (0-hour). All fungal colonies used in the inoculation methods were incubated at 28±1°C for a 12-hour photoperiod for 14 days. The seeds were removed from the Petri dishes after inoculation and sown 1-cm-deep in 1.5L pots containing peat substrate, vermiculite, and rice straw (2:1:1). The 60% water capacity of the substrate was calculated for the first irrigation before sowing following irrigation shifts at every 72 hours. Five replications (five pots) with five seeds each were distributed in the greenhouse, and the evaluations started three to 15 days after sowing (DAS).

Anthracnose severity in stems

Anthracnose severity in stems from inoculated lima bean seeds was evaluated three, six, nine, 12, and 15 DAS. A scale by Raoof and Rao (1996) with scores from one to three for infected seedlings was used, where 1 = the absence of symptoms; 2 = chlorosis or necrosis symptoms with and without growth impairment; 3 = dead seedlings.

Anthracnose severity in seedlings

The same described period was used to assess anthracnose severity in seedlings, with a scale adapted from Souza et al. (2014). The scores ranged from zero to six for infected seedlings, where 0 = no symptoms; 1 = collar lesion of up to 1 cm (superficial); 2 = collar lesion larger than 1 cm (depressed); 3 = scores, necrotic spots, petiole lesions; 4 = holes in the leaves; 5 = death of the apical meristem; 6 = seedling in partial and/or total collapse (death).

The area under the disease progress curve, disease index, and transmission rate

Anthracnose severity in seedlings was evaluated three, six, nine, 12, and 15 DAS using a scale adapted from Souza et al. (2014). Severity values were used to calculate the area under the disease progress curve, according to Campbell and Madden (1990). The disease index was evaluated at the end of the experiment (15 DAS) with the scale adapted from Souza et al. (2014). The data were adjusted using the

formula by Erkilic et al. (2006). The transmission rate (TR) was determined according to the equation by Teixeira and Machado (2003) at the end of the evaluation period (15 DAS).

Disease symptoms on leaves and stems and damping-off

Disease incidence was determined at the end of the experiment by visually observing the symptoms on leaves and stems and seedling damping-off. The results were expressed in percentages of diseased plants.

Seedling height

Seedling height was based on their length at the end of the test by measuring normal seedlings, which did not experience damage to the root and hypocotyl. A graded ruler was used, and the results were expressed in cm·seedlings^{-1}.

Shoot, root, and total dry mass

Shoot, root, and total dry mass weights were obtained by cutting the seedlings at the base of their collar, aided by a scalpel, and taking them to a forced air circulation oven at 70±2°C for 48 hours. The samples cooled off in a desiccator, their mass was determined on a precision scale (0.001 g), and the results were expressed in g seedling^{-1}.

Percentage of hard and dead seeds

The percentage of hard and dead seeds was also recorded in the final evaluation. Hard seeds did not emerge and resisted pressure with tweezers, and disintegrated ones were considered dead (Brazil, 2009). The results were expressed as percentages.

Statistical analysis

The data were submitted to the analysis of variance (ANOVA) and, according to the F test significance, the means of the inoculation methods at each exposure time and the exposure times for each inoculation method were compared with Tukey and F tests ($p \leq 0.05$), respectively.

Polynomial regression analysis was applied to investigate the effect of evaluation days, considering models up to the 2nd degree and $R^2 \geq 60\%$. The R® statistical software (Core Team 2018) was used.

3. Results

***Colletotrichum truncatum* transmission**

The independent variables, inoculation methods, and hours after infection in the days after sowing (DAS) showed a significant interaction ($p \leq 0.01$) for the scores attributed to the severity in stems and seedlings (Table 1), confirming the transmission of *C. truncatum* via lima bean seeds to seedlings.

Typical chlorosis and necrosis symptoms appeared six DAS in all treatments and the control (0-hour), evaluated through the severity in stems (Table 1). The first anthracnose symptoms on leaves emerged after the 3rd DAS, ranging from 1% to 50% of the infected leaf area. Only inoculation by contact with the mannitol water restrictor for 84 hours did not statistically differ from the control and produced asymptomatic seedlings (Table 1).

Seeds exposed to the pathogen in direct contact with mycelium for 36 hours showed the highest mean severity in stems (Table 1). The symptoms varied with chlorotic or necrotic lesions until the seedling's death.

Anthracnose severity in seedlings from seeds inoculated by direct contact with the sucrose water restrictor for 60 hours, followed by direct contact with mycelium and immersion in conidia suspension for 36 and 84 hours, respectively, showed the highest severity 15 DAS and were the most aggressive methods (Table 1).

A significant interaction ($p \leq 0.01$) occurred between inoculation methods and exposure times, and the quadratic regression model was fitted according to the variables, the area under the disease progress curve, disease index, and transmission rate (Figure 1).

Table 1. Anthracnose severity caused by *Colletotrichum truncatum* in lima bean (variety UFPB04; *Phaseolus lunatus*) stems and seedlings three, six, nine, 12, and 15 days after sowing (DAS).

		Stems					Seedlings				
MI	h	3	6	9	12	15	3	6	9	12	15
1	0	1bA	1 cA	1 cA	1 bA	1 bA	0 cA	0 cA	0 cA	0 cA	0 cA
1	36	1.8 aB	2.2 aAB	2.2 aAB	2.6 aA	2.8 aA	3.4 aA	3.4 aA	5 aA	5 aA	5 aA
1	60	1.8 aA	2 abA	2.2 aA	2.2 bA	2.4 aA	2.6 abA	2.6 abA	2.6 bA	3.4 abA	3.4 aA
1	84	1 bA	1.2 cA	1.2 bcA	1.2 bA	1.8bA	1.4abcA	1.4 abcA	1.4 bcA	1.4bcA	1.4 bcA
1	108	1 bC	1.4 bcBC	1.8 abB	2.8 aA	2.8 aA	1.2 bcB	1.2 bcB	3.4 abA	3.4 abA	3.8 aA
2	0	1 bA	1bA	1 bA	1 cA	1 cA	0 bA	0 bA	0 bA	0 cA	0 cA
2	36	2.2 aA	2.2 aA	2.2 aA	2.2abA	2.2abA	3.8 aA	3.8 aA	3.8Aa	4.2 aA	4.2 aA
2	60	2 aA	2 aA	2 aA	2abA	2.2abA	1.2bA	1.2 bA	1.2 bA	2.2 abA	2.6 abA
2	84	1 bB	1 bB	1 bB	1.8 bA	1.8 bA	0bB	0bB	0bA	0.4 bcA	0.4 bcA
2	108	1.8aB	1.8 aB	2 aAB	2.6 aA	2.6 aA	1 bB	3.8aA	3.8aA	3.8aA	3.8aA
3	0	1 bA	1 bA	1 bA	1bA	1 bA	0 bA	0 bA	0 cA	0 cA	0 cA
3	36	1 bB	1 bB	2 aA	2aA	2aA	0bB	2.2aA	2.2 bcA	2.2 bA	2.2 bA
3	60	1.6 abA	1.8aA	2 aA	2aA	2.6 aA	3.2 aB	3.2 aB	5.4aA	5.4 aA	5.8aA
3	84	1.8 aB	1.8 aB	2.6 aA	2.6 aA	2.6 aA	2.4 aA	2.4 aA	3.8 abA	3.8 abA	3.8 abA
3	108	1.6 abA	1.8 aA	2.2 aA	2.2aA	2.2aA	2.8 aA	2.8 aA	3.0bA	3.4 abA	3.4 bA
4	0	1 aA	1 bA	1 bA	1 cA	1 cA	0 bA	0 cA	0 cA	0 cA	0 cA
4	36	1 aB	1.8 aA	2 aA	2abA	2abA	1.8abA	1.8abcA	3.4 abA	3.4 abA	3.8 abA
4	60	1aB	2 aA	2 aA	2abA	2abA	2.2 aA	2.2 abA	2.2 abA	2.2bA	2.2bA
4	84	1 aB	2 aA	2 aA	2.2aA	2.4 aA	2.4aB	3.4 aAB	3.8 aAB	5.4 aA	5.4 aA
4	108	1.4 aA	1.4 abA	1.4abA	1.4bcA	1.4bcA	0.4 abC	0.6 bcC	1.4 bcAB	3.4 abA	3.4 abA
CV				26.4%					56.03%		

*Means followed by different uppercase letters in the row and lowercase letters in the column statistically differ by Tukey's test ($p \leq 0.05$). MI= inoculation method by direct contact with mycelium (1), direct contact with the mannitol water restrictor (2), direct contact with the sucrose water restrictor (3), and immersion in conidia suspension (4); h= exposure time (hours).

The area under the disease progress curve showed a significant interaction ($p \leq 0.01$) between inoculation methods and exposure times, with the data adjusted to the quadratic regression model (Figure 1a), except for the treatment by direct contact with the mannitol water restrictor.

Direct contact in the sucrose water restrictor for 60 hours promoted a larger area under the disease progress curve between treatments, which statistically differed (Figure 1a). Seeds in direct contact with the mannitol water restrictor for 36 hours showed a larger area under the disease progress curve.

The disease index presented a significant effect ($p \leq 0.01$) between exposure times and inoculation methods (Figure 1b). The data of direct contact with the sucrose water restrictor and immersion in conidia suspension were fitted to the quadratic regression model. The seeds exposed to the pathogen by direct contact with mycelium and the mannitol water restrictor for 36 hours, followed by direct contact with the sucrose water restrictor and immersion in conidia suspension for 84 hours, showed a higher disease index than the other treatments (Figure 1b). The method with suspension for 108 hours had the lowest disease index and did not statistically differ from the control (Figure 1b).

The transmission rate of *C. truncatum* through seeds demonstrated a significant effect ($p \leq 0.01$) on the interaction between exposure times and inoculation methods. The data of direct contact with the mannitol water restrictor and immersion in conidia suspension were fitted to the quadratic regression model (Figure 1c).

Inoculation by direct contact with the mannitol water restrictor for 36 and 60 hours promoted the highest transmission percentages of 64% and 44%, respectively, statistically differing from the other

treatments and the control (Figure 1c). Anthracnose transmission was verified in exposure times (36, 84, and 108 hours) by direct contact with the sucrose water restrictor, except for the exposure time of 60 hours (Figure 1c). Seeds inoculated by direct contact with mycelium for 108 hours, the mannitol water restrictor for 84 hours, and sucrose for 60 hours resulted in low transmission values of 0% and 4%, respectively, not differing from the control (Figure 1c).

The disease incidence in the stems significantly affected ($p \leq 0.01$) the interaction between exposure times and inoculation methods, and the data of direct contact with the mannitol water restrictor and immersion in conidia suspension were fitted to the quadratic regression model (Figure 2a).

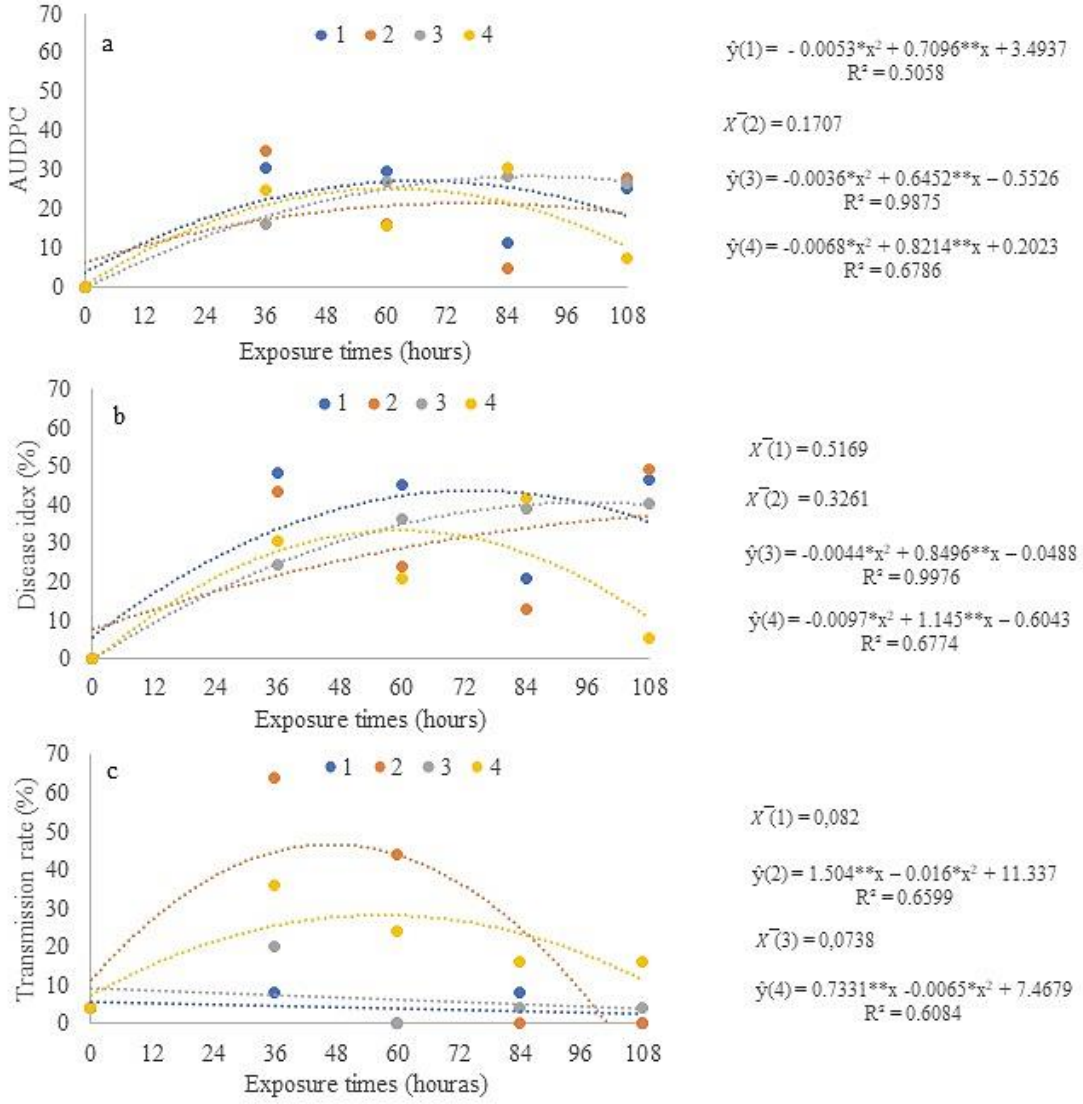

Figure 1. Area under the disease progress curve (AUDPC) (a), disease index (b), and transmission rate (c) in lima beans, variety UFPB04 (*Phaseolus lunatus*), inoculated with *Colletotrichum truncatum* by the direct contact with mycelium (1), direct contact with the mannitol water restrictor (2), direct contact with the sucrose water restrictor (3), and immersion in conidia suspension (4) for different exposure times 15 days after sowing. * and ** significant at 5% and 1% by the F test, respectively.

The maximum incidence of diseased stems occurred in the treatment by direct contact with the mannitol water restrictor for 60 hours, followed by immersion in conidia suspension for 36 hours (Figure 2a). However, such incidence decreased after 84 hours, regardless of the inoculation method, not statistically differing from the control (0-hour).

The incidence of diseased leaves also presented a significant effect ($p \leq 0.01$) among exposure periods, and only the data of direct contact with the mannitol water restrictor was fitted to the quadratic regression model (Figure 2b).

All inoculation methods with 36 hours of exposure favored the highest incidence rate in the leaves and statistically differed from the control ($p \leq 0.01$) (Figure 2b). Inoculation by immersion in conidia

suspension and direct contact with the mannitol water restrictor for 36 hours provided the highest percentages of infected leaves, reaching approximately 24% of incidence, statistically differing from the other exposure times (Figure 2b). The seeds inoculated by the direct contact with mycelium, the mannitol water restrictor, and sucrose for 108 hours promoted lower percentages of diseased leaves, similar to the control (Figure 2b).

Seedling damping-off demonstrated a significant effect ($p \leq 0.01$) between inoculation methods and exposure times. The data of the direct contact with the mannitol hydrochloride restrictor were fitted to the quadratic regression model (Figure 2c).

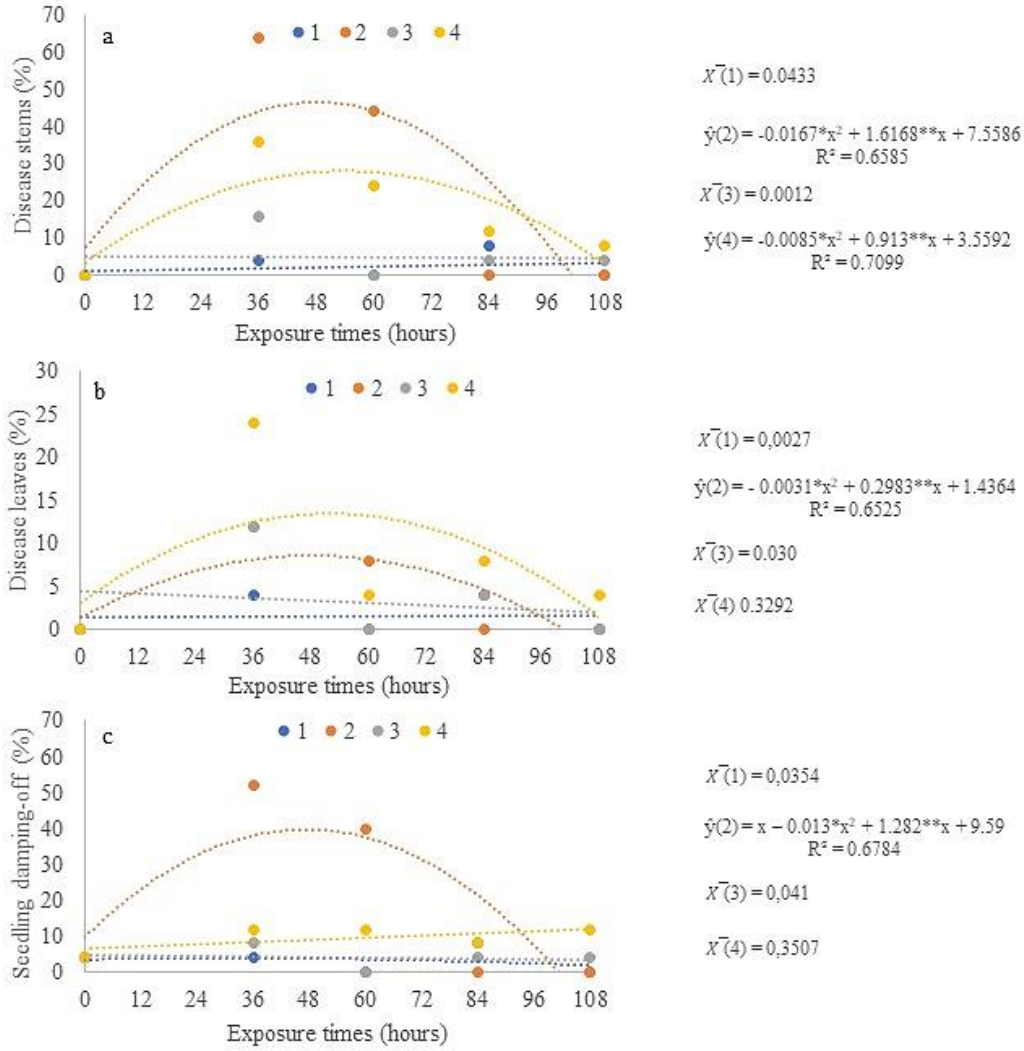

Figure 2. Percentages of diseased stems (a), diseased leaves (b), and seedling damping-off (c) of lima beans, variety UFPB04 (*Phaseolus lunatus*), inoculated with *Colletotrichum truncatum* by direct contact with mycelium (1), direct contact with the mannitol water restrictor (2), direct contact with the sucrose water restrictor (3), and immersion in conidia suspension (4) for different exposure times 15 days after sowing. * and ** significant at 5% and 1% by the F test, respectively.

The direct contact with the mannitol water restrictor for 36 and 60 hours resulted in higher percentages of seedling damping-off ($p \leq 0.01$) than the other treatments and the control (Figure 2c). However, lima bean seedling damping-off ceased only after 60 hours of exposure.

Physiological quality

Seedling height showed a significant effect ($p \leq 0.01$) on the interaction between exposure times and inoculation methods 15 DAS (Figure 3a). The treatments were fitted to the quadratic regression model regardless of the exposure period. The methods of direct contact with mycelium and the mannitol water restrictor were fitted to a linear model (Figure 3a).

The treatments by direct contact with the mannitol water restrictor for 36 and 60 hours and the sucrose water restrictor for 60 hours significantly differed from the control (Figure 3a). The treatment by direct contact with the sucrose water restrictor for 36 hours promoted higher seedling height than the others and did not differ from the control (Figure 3a). Inoculation by direct contact with mycelium completely inhibited shoot development at all exposure times and statistically differed from the control (Figure 3a).

The percentage of hard and dead seeds showed a significant interaction ($p \leq 0.01$) between inoculation methods and exposure times, and the data were fitted to the linear and quadratic regression models in response to seed exposure times (Figures 3b and 3c).

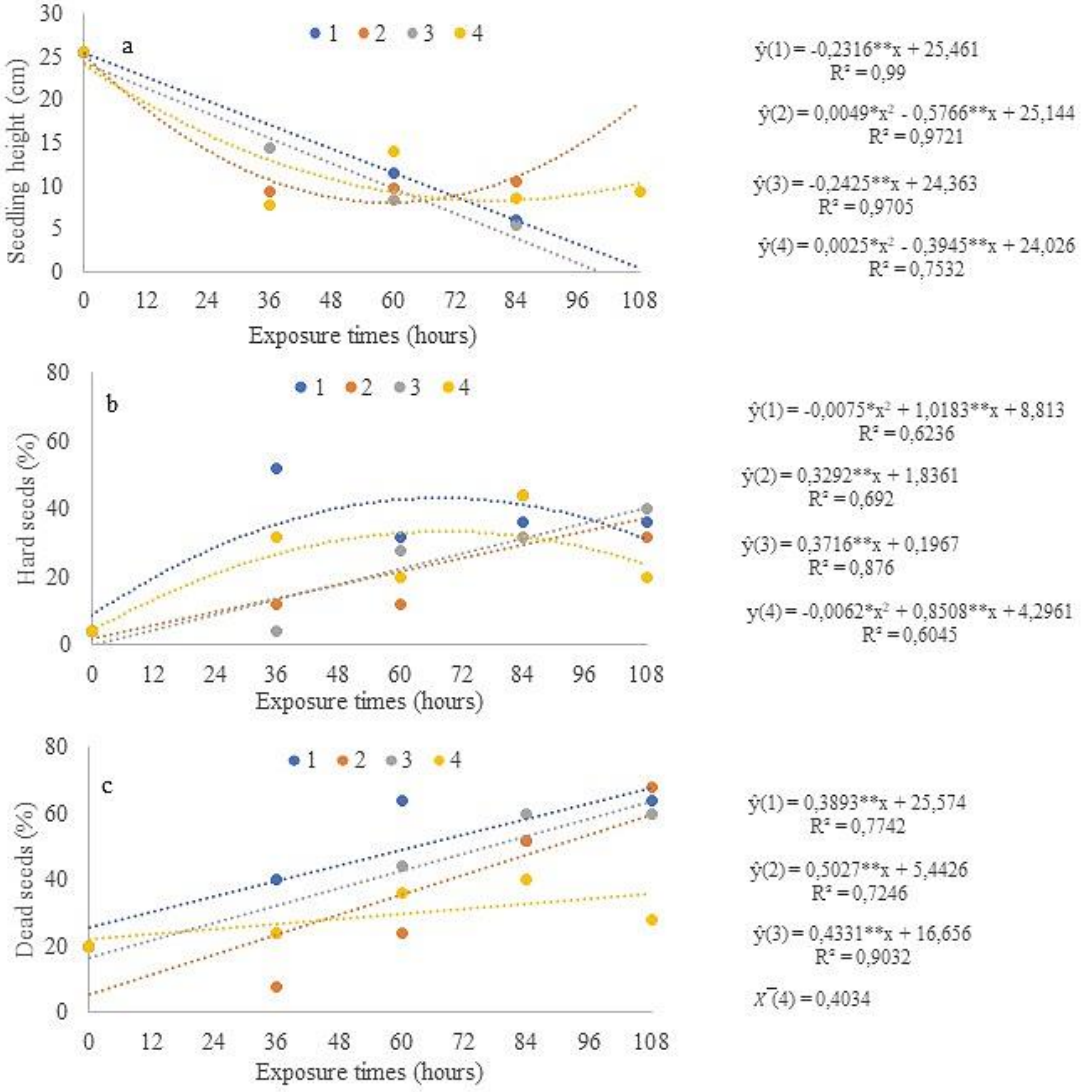

Figure 3. Seedling height (a), percentage of hard seeds (b) and dead seeds (c) of lima beans, variety UFPB04 (*Phaseolus lunatus*), inoculated with *Colletotrichum truncatum* by direct contact with mycelium (1), direct contact with the mannitol water restrictor (2), direct contact with the sucrose water restrictor (3), and immersion in conidia suspension (4) for different exposure times 15 days after sowing. * and ** significant at 5% and 1% by the F test, respectively.

The direct contact with mycelium for 36 hours provided a higher percentage of hard seeds (52%), statistically differing from the other treatments and the control (Figure 3b). The treatments harmed seedling emergence, with higher rates of hard seeds than the control, except for the direct contact with the sucrose water restrictor for 36 hours, which showed a lower rate (4%) and did not differ from the control (Figure 3b).

The inoculation methods harmed seedling emergence, regardless of the exposure time. The percentage of dead seeds was higher than the control for the treatments, except for the direct contact with the mannitol water restrictor for 36 hours, which promoted the lowest rate of dead seeds (8%). After

60 hours of exposure, all inoculation methods showed higher rates of dead seeds, from 28.5% to 68%, and statistically differed from the control (Figure 3c).

Shoot, root, and total dry mass in the final stand (Figure 4) showed a significant interaction (p≤0.01) with inoculation methods and exposure times, and the data were fitted to the linear regression and quadratic models, according to the last dependent variable (Figure 4).

The treatments by direct contact with the mannitol water restrictor for 36 and 60 hours and sucrose for 60 hours showed a slight reduction in shoot, root, and total dry mass, differing only from the control (Figure 4). The treatment by direct contact with the sucrose water restrictor for 36 hours allowed a significant increase of 15.2%, 43.9%, and 42.7% for shoot, root, and total dry mass of normal seedlings, respectively (Figure 4).

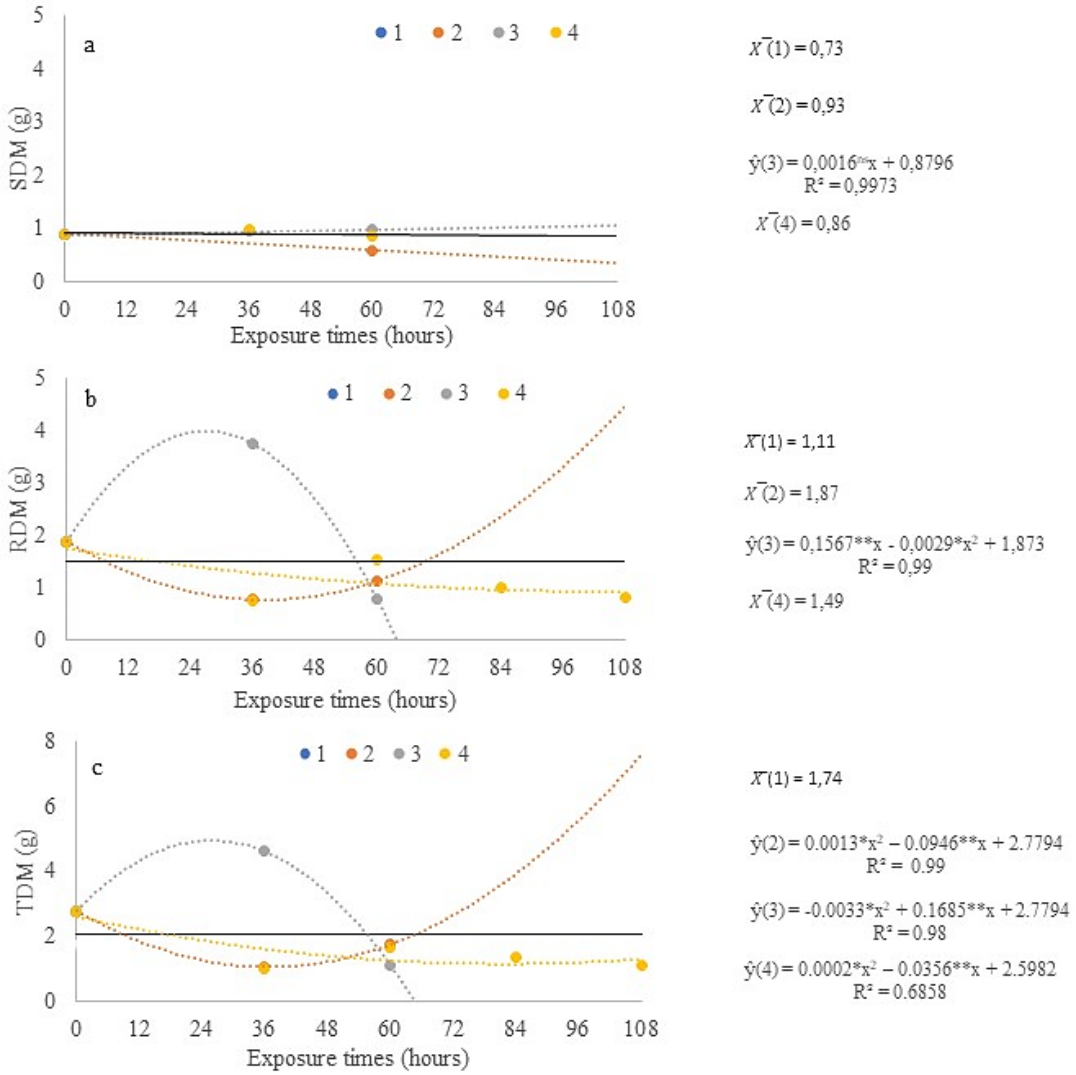

Figure 4. Shoot dry mass (SDM) (a), root dry mass (RDM) (b), and total dry mass (TDM) (c) of lima bean seedlings, variety UFPB04 (*Phaseolus lunatus*), inoculated with *Colletotrichum truncatum* by direct contact with mycelium (1), direct contact with the mannitol water restrictor (2), direct contact with the sucrose water restrictor (3), and immersion in conidia suspension (4) for different exposure times 15 days after the sowing. * and ** significant at 5% and 1% by the F test, respectively.

Similar to the evaluation of normal seedling height, it was impossible to quantify shoot, root, and total dry mass in the treatment by direct contact with mycelium, regardless of exposure times, which showed a total inhibition of shoots and roots. That statistically differed (p≤0.01) from the control, in which the average shoot dry mass was 0.78 g, and root and total dry mass were 1.85 g and 2.63 g, respectively (Figure 4).

The inoculation methods used in this study were based on the literature on the Phaseolus genus, which showed distinct changes in the quality aspect of *P. lunatus* seeds depending on the exposure time in each inoculation method (Figures 3 and 4).

4. Discussion

Mota et al. (2019) evaluated the transmission of *C. truncatum* in lima bean seeds with symptoms in cotyledons, stems, and leaves, finding a maximum of 96.8%, 60%, and 94.2%, respectively, seven days after sowing (DAS). The symptoms became more severe 14 and 21 DAS from seeds inoculated by different osmotic potentials with mannitol. However, the present study verified the anticipation of disease symptoms in stems and seedlings (Table 1), with disease severity increasing over time.

Yesuf and Sangchote (2005) studied the growth stages of two bean varieties under different seed infection levels at three locations. They obtained similar results for the area under the disease progress curve to those in lima beans infected with *C. truncatum*.

Basso et al. (2015) evaluated the area under the disease progress curve in soybean (*Glycine max* (L.) Merrill) leaves, finding similar results when submitting seeds to the same inoculation methods, even though without significant differences, regardless of the time of exposure to *C. truncatum*. The authors assumed it was due to the low anthracnose severity related to the reduced concentration of the initial inoculum from certified seeds, and the inoculum was present in low concentrations or absent.

Anthracnose severity increased in all inoculation methods, as confirmed by the disease index (Fig. 1b), increasing even more as the time of exposure to the pathogen extended. These results corroborate Barrocas et al. (2014), who showed an increase in the disease index of cotton seedlings (*Gossypium hirsutum* L.) inoculated with *Colletotrichum gossypii* var. *cephalosporioides* proportional to the higher initial water stress, resulting in a maximum of 40%, slightly lower than the present study.

Mota et al. (2019) observed 59% to 70% of anthracnose incidence on lima bean stems and leaves in seeds inoculated by direct contact with mycelium and the mannitol water restrictor 14 DAS.

Migliorini et al. (2017) found 33% and 60% of disease symptoms in bean hypocotyl from seeds inoculated with *C. lindemuthianum* by immersion in conidia suspension and direct contact with mycelium. Necrotic lesions appeared along the veins in the lower part of leaves, and incidence varied from 88% to 89% among inoculation methods, differing only from the control with 11% incidence on leaves. For these authors, *C. lindemuthianum* presented a natural incidence of 0.5% before disinfestation, efficiently transmitted to common bean seeds even in low percentages, which was not observed for *C. truncatum* in lima bean seeds (Fig. 1f).

Seedling damping-off of seeds previously inoculated by immersion in conidia suspension at different exposure times was similar to Begum et al. (2010), who verified a mean of 10% of seedling damping-off in soybean post-emergence from seeds inoculated with *C. truncatum*.

Mota et al. (2019) evaluated *C. truncatum* transmission in lima bean seeds, obtaining the maximum infection and transmission rates of 9% and 83.1%, respectively, on seeds inoculated by direct contact with mycelium after 48 hours of exposure. Pereira et al. (2014) showed inoculation efficiency from the mannitol water restrictor with *C. truncatum* in soybean seeds, reaching nearly 40% of transmission.

We may infer that *C. truncatum* transmission to lima bean seeds was efficient and may cause a higher or lower transmission rate depending on the inoculation method and exposure time.

Flavio et al. (2014) mention that seeds are efficient means of survival and dissemination of phytopathogens to new areas, promoting yield loss and a significant increase in production costs.

Inoculations by direct contact with mannitol and sucrose water restrictors and conidia suspension immersion efficiently transmitted *C. truncatum* to lima bean seeds. *C. truncatum* inoculated in a medium containing the sucrose water restrictor for 36 hours and mannitol for 60 hours allowed higher transmission rates, not compromising the seedling's final stand.

Rey et al. (2009) showed that *C. lindemuthianum* also harmed seedling growth of inoculated common bean seeds, with values between 5.65 and 6.87 cm, while the control showed values superior to 10.3 cm, suggesting a harmful effect of the pathogen on seedling development.

Similarly, Teixeira and Machado (2003) observed a decrease of about 40.7% in seedling height with *Acremonium strictum* inoculation by the direct contact with the mannitol water restrictor for 120 hours of exposure of maize seeds (*Zea mays* L.).

Coelho et al. (2010) verified similar results regarding the reduction in shoot and total dry mass (Fig. 4) when evaluating water stress with a mannitol solution under lower osmotic potential (-1.61 MPa) in common bean seeds and differential protein expression during germination. The authors found the opposite effect of this solution under the same potential for root mass, which is related to mitoses in the root cells and could be stimulated by calcium, considering that other solutions presented similar results.

For Galli et al. (2007), *C. truncatum* inoculation in soybean seeds by direct contact with mycelium for 40 hours caused seed death, reducing the number of soybean seedlings in the final stand. For these authors, the longer exposure time of seeds to the pathogen increased the level of inoculum in the seeds, drastically compromising embryo development.

According to Machado et al. (2012), the ideal inoculation method must ensure seed infection and preserve their germination power satisfactorily for later use.

Inoculation methods by direct contact with mannitol and sucrose water restrictors for 36 hours were the most suitable for *C. truncatum* in lima bean seeds because they provided a higher transmission rate and slightly affected the seeds' physiological parameters.

5. Conclusions

The damage from *C. truncatum* to seedling performance increased with longer exposure times, regardless of the inoculation method.

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