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Application of *Trichoderma harzianum* on the physiological quality of cowpea seeds

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Abstract

The use of fungi in seed treatment can improve the plant's physiological characteristics. The aim of this study was to evaluate the influence of the use of *Trichoderma harzianum* on the physiological quality of bean seeds. The experiment was set up and conducted in the forest seed laboratory of the Federal University of Western Para, in a randomized design, using 4 procedures on the basis of a colony forming unit (CFU): T1 (0 CFU), T2 (4 x 10^9 CFU), T3 (8 x 10^9 CFU) and T4 (12 x 10^9 CFU), with 5 repetitions. Germination percentage (G%), germination speed index (GSI), hypocotyl length (HL) and radicle length (RL) were evaluated as variables. The data were analyzed by variance analysis and the averages were compared using the Tukey test ($p \leq 0.05$), in addition to the regression analysis, using the Minitab© *version* 18 statistical software. There were differences between the germination percentage tests, in which T2 obtained 100% of germinated seeds, for GSI it was observed that T2 and T3 were equal and superior to the other treatments, while for RL T1 and T2 obtained the best results, however, for HL there were no statistical differences between treatments. T2 was more efficient in the physiological quality of seeds to germination percentage and germination speed index.

Keywords: Germination, initial development, physiology, *Vigna unguiculata*, dosages.
Abbreviations: HL_Hypocotyl lenght; RL_Radicle lenght; G%_Germination percentage; GSI_Germination speed index.

Introduction

The cowpea *(Vigna unguiculata*) has its own characteristics and is of great nutritional importance in the Brazilians diet, because it is a rich source of calcium, iron and proteins. In addition to being grown on all continents as a source for human consumption, it can be used as a source of animal protein (Araújo et al., 2018). According to Monteiro et al. (2017), due to the great expansion of planted areas and the consumption of this legume in recent years, beans have become an excellent billing alternative.
It is a culture of great social and economic importance, especially in the North and Northeast regions due to the generation of employment and income, mainly cultivated by small producers (Silva et al., 2016). However, despite having great importance for the North region, this crop has reduced productivity, being related to the techniques in the production process.
The world beans production in 2018 was 30.4 million tons in a harvested area of 34.4 million hectares, with Brazil being one of the largest producers of this legume, contributing with a production for 2018 of 2.9 million tons (Fao, 2020). Nevertheless, despite having great importance for Brazil, productivity is low, and as stated by Matias et al. (2019) the demand for quality food is high and is related to proper management.
As believed by Silva et al. (2018), among the factors that cause the reduction of crop productivity, in addition to the lack of adequate technical assistance, the physiological quality of seeds is a limiting factor. Therefore, the physiological quality of the seeds becomes essential in the development of the crop, especially in areas of family agriculture. In line with Smiderle et al. (2017), cowpea is extremely important for low-income populations due to its high protein value.
One of the factors affecting the physiological and sanitary quality of seeds is the transmission and dissemination by pathogens that cause deteriorating processes, which reduce the germination rate. The use of biological products becomes an alternative control technology, because in addition to combating the action of the pathogen, it reduces the excessive use of chemicals, and promotes the healthy development of the plant through a biocontroller agent (Maciel et al., 2017).

Thus, the use of beneficial microorganisms on plants is a sustainable alternative to conventional agriculture (Rocha et al. 2019). Although, the application of these microorganisms on a large scale in large plantations becomes economically unfeasible (Oliveira et al. 2016). Therefore, Glick (2012) states that it is necessary to use efficient methods in the application of biocontrol agents. Thus, application to seeds becomes a low-cost and high-precision method of inoculation.

The use of biological agents has been obtaining excellent results in the quality of bean seeds, ensuring a uniform stand. Among the microorganisms that make the biocontrol, bacteria and fungi are essential, especially the fungus of the genus *Trichoderma* spp. which assists in the control of plant phytopathogens (Saba et al., 2014), in addition to promoting improvements in growth. Seed treatment reduces the inoculum of root pathogens, inhibiting primary seed infection, increasing the chances of establishment in the field.

Currently, the use of *Trichoderma* spp. in the treatment of seeds for the cultivation of beans in the northern region of Brazil requires more attention with an emphasis on physiological quality mainly in seeds used by family farmers. Observing this need, the objective of this study was to evaluate the influence of the use of *Trichoderma harzianum* on the physiological quality of cowpea seeds in the Monte Alegre region, and specifically to evaluate the effect on germination, germination speed index, length of the hypocotyl and radicle.

Results and discussion

Descriptive analysis

Regarding the descriptive analysis of the experiment data for the variable hypocotyl length (HL), it was observed that the standard deviation presented was lower than the deviation presented by the other variables (G, GSI, RL), thus the reference points are close to the sample mean of 19.63, with maximum and minimum values 25.77 and 14.42 mm, respectively (Table 1). It is worth mentioning that the standard deviation as stated by Silva et al. (2020) is a measure that allows expressing the degree of dispersion of the data set or its uniformity.

It was observed for the variable GSI, that the standard deviation was higher than that observed in the other variables, however the variation coefficient remained acceptable, remaining at 16.25, whereas the average for this variable was 79.90, obtaining maximum and minimum values of 93.05 and 58.49 respectively, in consonance with Table 1.

The data demonstrate that the radicle variable has the highest variation coefficient, whose value is 21.52, anyhow this value is below 30, so it is considered acceptable for proving the data. As claimed by Pimentel-Gomes (1985), the variation coefficient range is considered low when lower than 10%, average between 10 and 20%, and high between 20 to 30%. The germination variable, on the other hand, obtained the lowest variation, reaching a maximum value of 100.00 and a minimum of 84.00, this variable being the only variable that obtained a value of 100 (Table 1).

From the descriptive analysis of quartile values, it was observed that germination was the variable that obtained values closest to the median, in which quartile 1 (Q1) was 93.00 and quartile 3 (Q3) was 100.00. Carvalho et al. (2019) state that quartiles have relevance in comparing a set of data, representing, according to Freitas et al. (2018), the first and second half of distribution from the median.

Dispersion analysis

For the data dispersion graph of the treatment variables, the interquartile range was used, where T2 obtained a reduced data dispersion when compared to T1 for the germination variable, and this value is related to the low coefficient of variation (Fig. 1A). Devore and Berk (2012) classify dispersion analysis as a descriptive measure, the interval between quartiles being fundamental to better describe the data. For treatments referring to germination speed index variable, a minimum acceptable dispersion was found for the experiment, so the data remained close to the sample average (Fig. 1B).

Nevertheless, in the distribution of data referring to treatment 1 for the radicle variable, a high dispersion within the sample set between 24 and 35 mm was observed, however, the data set in relation to the other treatments was homogeneous for this variable (Fig. 1C). For the hypocotyl variable, the data dispersion was relatively low for all treatments studied, obtaining an interquartile range between 15 and 25 mm, being related to the low standard deviation and coefficient of variation. Neto et al. (2017) state that the values must present central tendency, dispersion and symmetry, as identified in Figure 1D.

Averaging analysis

For the average comparison test between the physiological variables of cowpea, it was observed that there were significant differences ($p \leq 0.05$) between treatments, and only for hypocotyl length the use of *Trichoderma harzianum* was not effective (Table 2). In consonance with Machado et al. (2015), the growth of the plant provided by the mechanisms of action of fungi is specific, but tends to vary based on the conditions of the environment, such as interference or availability of nutrients.

Significant differences were found in the use of *Trichoderma harzianum* for the percentage of germination of bean seeds, in which the treatments received applications of the fungus were statistically superior to the control treatment, and T2 obtained 100% germination. Therefore, for the germination percentage, the fungus was considered effective, which may be related to the reduction of attack by pathogens.

Because of its capacity to aggressively occupy the pathogen establishment sites in the seeds, the *Trichoderma* spp. becomes responsible for the biocontrol in bean seeds (Carvalho et al., 2014), which tends to increase the seed germination rate. Sá et al. (2019) observed that the use of *Trichoderma* spp. in the bean germination process, the germination percentage increased to 99%, which corroborates with the data presented. For Dalzotto et al. (2020) seed germination induced by *Trichoderma* spp. is related to hormone production, with Bucio et al. (2015), state that *Trichoderma harzianum* is capable of producing harzianic and isoharzhanic acid, which helps in plant growth.

Similar results were found for the germination speed index, in which T2 and T3 were statistically similar, obtaining 91.24 and 91.21 respectively, differing from the control treatment, which obtained an GSI of 61.56. A study by Rocha et al. (2017) obtained similar results, where the germination speed index was affected by the application of *Trichoderma asperellum*, increasing the values for this variable. The effects of the treatments found for GSI differed from those

Table 1. Descriptive analysis of the variables analyzed for the cowpea experiment.

Variable	Average	Median	Mode	Values		Coefficients			Q1	Q3
				Min	Max	Var.	Desv Pad.	Coef. Var		
G	96.50	98.00	100	84.00	100.00	21.07	4.59	4.76	93.00	100.00
GSI	79.90	82.72	0	58.49	93.05	168.8	12.9	16.25	68.92	91.74
RL	22.11	21.08	0	15.39	35.04	22.64	4.76	21.52	18.99	24.89
HL	19.63	19.65	0	14.42	25.77	9.915	3.14	16.04	17.78	21.02

G: Germination; GSI: Germination speed index; RL: Radicle lenght; HL: Hipocotyl lenght; Min: Minimum; Max: Maximum; Var: Variance; Desv.Pad: standard deviation; Coef.Var: Coefficient of variation; Q1: Quartile 1; Q3: Quartile 3.

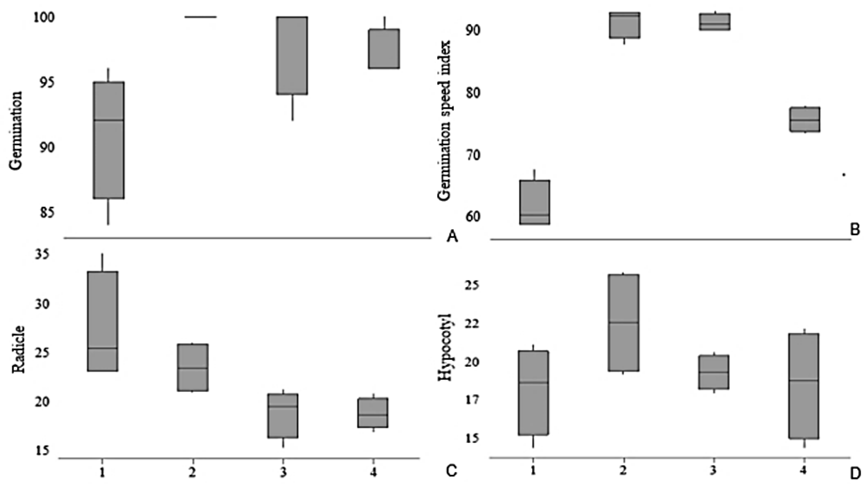

Fig 1. Dispersion analysis according to treatments for the variables germination (A), germination speed index (B), length of the hypocotyl (C) and radicle length (D) from the treatments.

Table 2. Comparison of means for germination (G), germination speed index (GSI), hypocotyl length (HL) and radicle length (RL) in cowpea seeds under the application of *Trichoderma harzianum*.

Treatments	G(%)	GSI	HL	RL
T1	91.00 B	61.56 C	18.18 A	27.24 A
T2	100 A	91.24 A	22.49 A	23.47 A
T3	98.00 AB	91.21 A	19.30 A	18.91 B
T4	97.00 AB	75.58 B	18.53 A	18.78 B
C.V(%)	3.25	3.18	14.25	14.85

Averages followed by the same letter do not differ from each other by the Tukey test (p≤0.05).

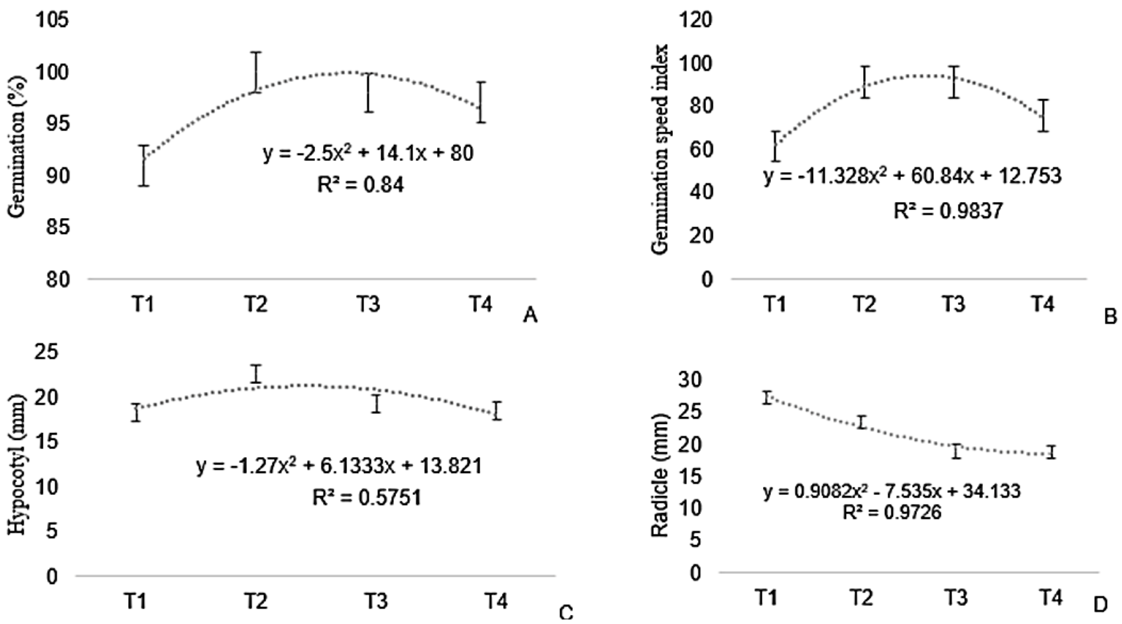

Fig 2. Regression analysis for the germination variable (A), germination speed index (B), length of the hypocotyl (C) and radicle length (D) from the treatments.

Trichoderma was statistically identical to the control treatment for the emergence speed index.

For the variable radicle length, the control treatment was statistically equal to T2, which differed from the others. Notwithstanding, it was observed that from the control treatment, there was a reduction of the radicle with the application of *Trichoderma harzianum*. Hassan et al. (2013) relate the negative effect to the capacity of *Trichoderma* spp. to synthesize secondary metabolites that tend to have a phytotoxic effect on certain concentrations and plant species.

On the other hand, this result may be strictly related to the environment in which the experiment was carried out. Sottero et al. (2006), working with the application of rhizobacteria in vitro, obtained root reduction of lettuce seedlings, which did not occur for *in vivo* tests, probably due to *in vitro* tests obtaining different conditions than natural ones.

Silva et al. (2015) working with *Trichoderma* spp. in lettuce seeds with and without pathogens, they observed that *Trichoderma harzianum* tends to influence the increase of the radicle when it is infested by pathogens, being a response of the plant to the attack of the pathogen, forcing the growth of the roots in search of nutrients.

Regression analysis

Regarding the regression analysis to adjust the behavior of the doses of *Trichoderma*, it was observed that the dosages explained 84% of the results, where the determination coefficient (R^2) was 0.84. Therefore, the polynomial model adjusted the dosages (Fig. 2A). This result demonstrates that the use of the fungus in the 4 x 10^9 CFU dosage increases the percentage of bean germination under controlled conditions. Chagas et al. (2017) state that the fungus *Trichoderma* could positively influence seed germination due to the production of growth-promoting substances.

For the germination speed index, the regression model explained 98% of the results obtained, obtaining a coefficient of variation considered high, being R^2: 0.98 (Fig. 2B). T2 was the treatment that corresponded to the high rate of germination speed, as well as the results presented for the variable germination. Machado et al. (2012) affirm that microorganisms can influence the physiological characteristics of plants, including the speed and germination of plants, in addition to grain growth and productivity.

The germination speed is related to the physiological quality of the seed, therefore, the faster it occurs, the better for seedling development, due to the reduction of deterioration processes. According to Dias et al. (2020), the speed of the germination process has a strong influence on the physiological state of the seeds, that is, the process of seeds deterioration will take longer to complete, under conditions with healthy seeds.

Regarding the length of the hypocotyl, the regression was able to explain 57% of the acquired results, even though, the coefficient of determination was relatively low, when compared to the other variables studied (Fig. 2C). Nonetheless, when compared to the averages test, there were no different results between the treatments evaluated, and the application of *Trichoderma* increased the hypocotyl in T2, but it was not statistically different from the control treatment (T1).

It was observed that for radicle length, the effect of *Trichoderma harzianum* (T2) was similar to the control treatment, and the regression had a coefficient of 0.97, being considered high (Fig. 2D). The radicle reduction from the treatments with the fungus for this study, is related to the increase in the dosage of *Trichoderma harzianum*. Reis et al. (2019) stated that the dosages of *Trichoderma* in cowpea were effective for root development up to 4 x10^8 CFU, with higher doses impairing root development and germination.

Therefore, the application of *Trichoderma harzianum* did not provide a positive effect for the development of the plant's root system, with reduction being observed. Machado et al. (2011) working with black oats, observed that for the variable root volume and root dry weight, the isolated use of *Trichoderma harzianum* did not provide an effect for these variables, being the same as the control treatment. However, the use of the fungus combined with strains of rhizobia tended to increase the root system.

Nevertheless, in results by Hermosa et al. (2013), it was found that species of *Trichoderma* are capable of producing growth-promoting hormones that mainly favor the growth of secondary roots, thus the energy for the growth of the main root is moved to the expansion of these roots.

Materials and methods

Plant material

The experiment was conducted with cowpea (*Vigna unguiculata*), which is the genetic material used by producers of the area, the seed bank was made available by the local producers themselves, and the best materials were selected to conduct the experiment.

Location of experiment

The experiment was installed and conducted at the forest seed laboratory of the Federal University of Western Pará - UFOPA, located in the city of Santarém (PA), in the first semester of 2019, and the seeds were obtained in a small family farmer area under the coordinates 1°58'15 "south latitude and 54°26'47" west longitude, with an altitude of 40 m, in the city of Monte Alegre-PA.

Conducting the experiment and treatments

The design used was completely randomized, with 4 treatments and 5 repetitions, totaling 20 experimental plots. *Trichoderma harzianum* was used as a source for the experiment with the concentration of the dosage performed in colony forming unit (CFU), and the estimated measure for the number of viable fungi. Treatment 1 (T1) corresponds to the control, treatment 2 (T2) corresponds to the use of 4 x 10^9 CFU *Trichoderma harzianum*, whereas treatment 3 (T3) corresponds to the use of 8 x 10^9 CFU, and treatment 4 (T4) corresponds to the use of 12 x 10^9 CFU, being applied by spraying the seeds for each treatment.

For each treatment, the following variables were evaluated: germination (G), germination speed index (GSI), hypocotyl length (HL) and radicle length (RL), as established by the RAS seed analysis rules (Brazil, 2009). Each experimental plot consists of 25 bean seeds, distributed in a colorless and transparent gerbox.

For the germination test, the seeds were placed equidistant, on two sheets of sterile filter paper and moistened with distilled water in an amount equivalent to 2.5 times the weight of the paper and kept at a temperature of 25 ºC in a colorless and transparent gerbox. The evaluations were carried out at 5 and 9 days after sowing, counting the number of germinated seeds, for the final determination in percentage.

The germination speed index was performed in conjunction with germination, with the seed count being performed daily, starting on the first day after the experiment was set up, until the last day of evaluation. The germination speed index was performed conforming to the methodology proposed by Maguire (1962), being:

GSI = (G1/N1) + (G2/N2) + (G3/N3) +...+ (Gn/Nn)

GSI: germination speed index; G1, G2, G3 and Gn: number of seedlings computed in the first, second, third and last count; N1, N2, N3 and Nn: number of days from sowing to the first, second, third and last count.

Subsequently, with the aid of a digital caliper, the measurement of the length of the hypocotyl and the radicle length of the seedlings considered normal were performed, in agreement with the method of Brazil (2009).

Statistical analysis

The data were submitted to analysis of variance and comparison of averages using the Tukey test at 5% probability, and subsequently performed polynomial regression analysis for the different dosages of *Trichoderma harzianum*. Descriptive and normality analyzes (Shapiro-wilk) were performed for all variables, using the statistical program Minitab© *version* 18.

Conclusion

The use of the dosages of *Trichoderma harzianum* were effective in increasing the percentage of germination (100%) and germination speed index (91.24), T2 being the most effective treatment. For length of hypocotyl and radicle, the use of *Trichoderma harzianum* obtained results similar to the control treatment. T2 was the most efficient for the physiological quality of bean seeds.

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