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USE OF TRICHODERMA SPP. ISOLATES AS GROWTH PROMOTERS AND MELA DISEASE RESISTANCE INDUCERS FOR COWPEA CULTIVATION

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ABSTRACT

Cowpea has great social and economic importance, but several diseases affect it, causing low production. Thus, the objective of this study was to use Trichoderma spp. Isolates as

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growth promoters in cowpea crops. For this, the seeds were microbiolized with Trichoderma spp. Isolates and planted in the field. The experimental design was in randomized blocks, the treatments consisted of isolates L1 and I1, each one composing a treatment with liquid application and another in powder, a commercial product based on Trichoderma harzianum and the control, thus composing the 6 treatments, which were divided into 4 blocks. Physiological parameters, growth promotion, induction of disease resistance, and productivity were evaluated. The data obtained were submitted to ANOVA and Tukey's test at 5% probability. No statistical differences were observed between the treatments and the control for the parameters evaluated.

Keywords: Microorganisms. Legume. Productivity.

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INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) has great economic and social importance for several Brazilian families because in addition to being used to feed small and medium-sized producers, it is a great generator of employment and income directly and indirectly to its production. In the 2020/21 harvest, the estimated planted area until September 2021 was 1,349,600 ha throughout Brazil, an increase of 3.2% compared to the planted area in the previous harvest (CONAB, 2021). Cowpea obtained 625.2 thousand tons in the 2020/21 harvest (CONAB, 2021). The productivity obtained in this harvest reached 463 kg ha^{-1}, which is a decrease in its productivity compared to the previous harvest, which reached 15% (CONAB, 2021).

This crop also plays a very important role in human nutrition, as its grains are rich in proteins, minerals, and fiber, meeting the food and nutritional needs of its consumers. In addition, it has great value for cooking, as it can be used in various dishes, from baião-de-dois to more elaborate dishes, such as salads and pies.

Its cultivation faces several difficulties that end up reducing productivity, such as the use of unimproved seeds, planting in soils of low fertility, low rainfall, and diseases that appear in the crop (MENDES et al., 2007). Some of the diseases that reduce the productivity of cowpea plants are: damping-off caused by *Rhizoctonia,* stem rot caused by *Pythium*, gray stem rot caused by *Macrophomina phaseoli,* and fusariosis caused by the fungus *Fusarium oxysporum* (CARDOSO, 2000).

The use of *Trichoderma* spp. in fields of cultivation of this legume is very promising since they have scientifically proven efficiency in several crops. These act against phytopathogens and their resistance structures in various ways (MACHADO et al., 2012), causing the harm caused to plants to be inhibited or partially reduced. These fungi are symbiotically related to plants, making them capture minerals from the soil more effectively (VINALE et al., 2008), in addition to increasing the contact surface of plant roots, providing better water absorption (BENÍTEZ et al., 2004).

The use of microorganisms in the biocontrol of phytopathogens brings with it environmental benefits since they reduce the need for agrochemical application in crops. Thus, there are currently some certified products on the market that have fungi of the genus *Trichoderma* in their composition, but more species of this genus must be studied to benefit a wider percentage of crops and increase the number of certified biological products.

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Therefore, the present study aimed to use *Trichoderma* spp. Isolates that are growth promoters and resistance inducers in cowpea cultivation in Vila Nova do Martírios - MA and to contribute to the increase of its production in the region, to ensure environmental preservation and food security.

METHODOLOGY

Two isolates of *Trichoderma* spp., previously selected in the laboratory using *in vitro test* and greenhouse test, were used, as well as a commercial product based on *Trichoderma* (StimuControl®). These fungi come from the Micoteca "Prof. Gilson Soares da Silva" – MGSS, from the Phytopathology Laboratory of the State University of Maranhão – São Luís Campus - MA.

Before the implementation of the field experiment, the soil was collected in a zigzag at a depth of 0 to 20 cm, to form a composite sample that represented the entire area where the experiment would be implemented. The soil was then sent for analysis, and the results are illustrated in Table 1.

Table 1. *Results of the soil analysis of the area where the field experiment was carried out in Vila Nova dos Martírios, a village in Curvelândia, MA, Brazil.*

ph	M.O.	P	K	Ca	Mg	Al	H+Al	SB	CTC	V
CaCl2	g/kg	mg/dm3	----------cmol/dm^3----------							%
4,40	11,30	3,20	0,05	1,45	0,41	0,11	2,50	1,92	4,42	43,40

The soil was characterized as high acidity, with low amount of organic matter and phosphorus. Regarding the amount of calcium and magnesium, the soil was characterized by having low and medium content, respectively. Aluminum saturation was low, even though the soil was highly acidic. Regarding texture, the soil presented 26% of clay in its composition and was therefore classified as medium texture soil.

To overcome the soil deficiency about some nutrients, fertilization was carried out in pits at the time of planting. NPK 4-30-10 mineral fertilizer was used, in a proportion of 350 kg $^{ha-1}$, so each pit received 9.53 g of fertilizer.

- Production and application of *Trichoderma* spp

The *Trichoderma* spp. isolates for inoculation were prepared in two ways:

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1) powder obtained by milling rice grains colonized by *Trichoderma* spp. [6 × 108 conidia $^{g-1}$];
2) conidia suspension - the fungus culture was cultivated in BDA culture medium at ± 25 °C, under photoperiod for seven days and the standard concentration adjusted to 6 × 108 conidia $^{mL-1}$, with the aid of a Neubauer chamber.

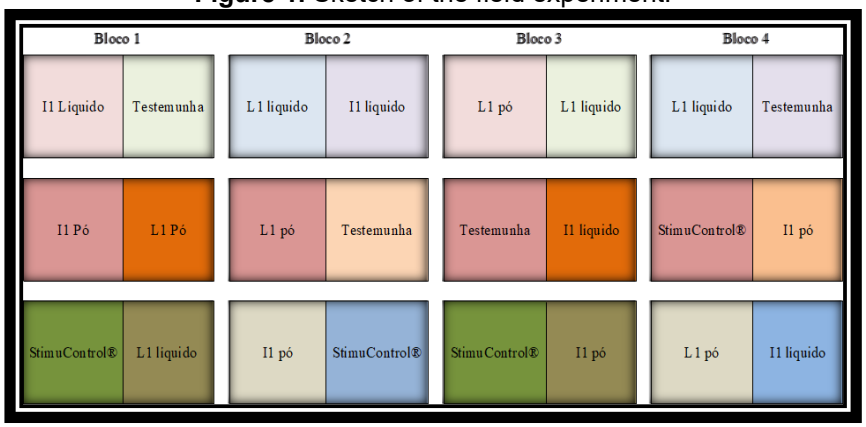
For the fungi to be homogeneously inoculated into the seeds, the formulations were applied to the seeds using 20 mL of the liquid suspension and 20 g of the powder formulation, according to the treatments, and placed on a shaker table for one hour under 1500 revolutions per minute (RPM), so that they could be sown in the field. After sowing, the liquid suspension treatments were named "liquid" and the powder formulation treatments were named "powder".

- Experiment deployment

The experiment, conducted in the field, was carried out in a productive area in the village of Curvelândia, municipality of Vila Nova dos Martírios, Maranhão, 90 km from the city of Imperatriz. The experimental design was randomized blocks, where 4 blocks and 6 treatments were used (L1 net; L1 powder; I1 liquid; I1 powder; StimuControl®; and the control), where each block obtained an experimental unit of each treatment.

Before planting, the seeds were inoculated with fungal species of the genus *Trichoderma* sp. Sowing was carried out manually, adopting a depth of 3 cm and five seeds inoculated per hole, at a spacing of 0.6 m between pits and 0.6 m between rows. Each experimental unit had 25 pits, where the 9 central ones were determined as the useful area. Manual weeding was carried out to keep the crop free of weeds.

Figure 1. Sketch of the field experiment.



Source: Author, 2025.

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- Evaluation of the experiment

In the plants cultivated in the field test, only the incidence of Mela disease occurred, which was evaluated at 63 days after planting, caused by the fungus *Rhizoctonia solani*. This evaluation was carried out by observing the plants accommodated in the useful area of each treatment, according to the scale of grades described by Schoonhoven and Pastor-Corrrales (1987), where scores ranging from 1 to 9 were assigned according to the degree of severity of the disease: 1- plants without symptoms, 3- plants whose attack compromised from 1 to 30% of the leaf area, 5- plants with 31 to 60% of the evaluated leaf area necrotic; 7- for plants with 61 to 90% of the leaf area compromised and 9- when the necrosis of the leaf area was above 90%.

The determination of the leaf area index (LAI) was performed at 37 days after planting. For this, three leaves were selected on each plant, where the length and width of the leaflets of the leaves of the middle and upper third were measured, and then the average area of the leaves between the three plants was calculated. To calculate the leaf area, the circumscribed rectangle method, described by Reis, Reis and Barros (2000), was used by the following formula S = 0.625 (C.L), where S is the leaf area index; 0.625 is the correction factor; C, the length and L the width of the sheet.

The chlorophyll index was measured with the SPAD (*Soil Plant Analysis Development*) – 502 Plus device at 37 days after planting, where the plants were at their maximum vegetative growth. For this, five plants were randomly selected in the useful area of each treatment, and the leaflets of the leaves of the middle third of the plants were read with the SPAD device at five points and the arithmetic mean was removed.

After 37 days of planting, 9 plants were also removed from each replication and, subsequently, the dry and fresh mass of the aerial part of the plants was evaluated, due to the difficulty in completely removing the roots of the plants in the field experiment. Along with this evaluation, the trifoloyle count and the measurement of the length of the plants were carried out.

The experiment was harvested manually in the useful area of each experimental unit after 58 days. After harvest, the number of pods, grains per pods and the weight of grains produced per treatment were evaluated.

The data were statistically analyzed with the aid of the SISVAR program and submitted to analysis of variance, with comparison of means by Tukey's test at 5% probability.

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RESULTS

According to the data analyzed about the leaf area of the upper third of the plants, only two treatments diverged from each other, where the I1 powder treatment obtained greater leaf area when compared to the liquid L1 treatment, the rest of the treatments were statistically equal resembling the control. About the leaf area of the middle third of the plants, there was no significant difference at the level of 5 % probability by the Tukey test between the treatments (Table 2).

Table 2. *Evaluation of the leaf area of the upper and middle third of the plants submitted to treatments in the field experiment in Vila Nova dos Martírios, Curvelândia village, MA.*

Treatments	*Upper third leaf area (cm²)[1]*	*Middle third leaf area (cm2)[1]*
L1 net	*129.74 to*	*140.23 to*
StimuControl®	*138.92 abs*	*134.06 to*
L1 powder	*145.18 abs*	*156.75 to*
I1 Liquid	*154.16 abs*	*165.63 to*
Witness	*157.50 abs*	*153.77 to*
I1 powder	*157.50 b*	*161.03 to*
CV (%)[2]	*9,99*	*9,35*

[1] Consecutive averages of the same letter in the column do not differ statistically from each other, according to Tukey, at 5% probability. [2] Coefficient of variation.

Regarding the chlorophyll index and the evaluation of the severity of mela disease in the plants, no significant difference was observed at the level of 5 % probability by the Tukey test between the treatments for both parameters (Table 3).

Table 3. *Evaluation of the chlorophyll index and the severity of mela disease in plants submitted to treatments in a field experiment in Vila Nova dos Martírios, Curvelândia village, MA.*

Treatments	Chlorophyll index[1]	Mela 1 Review
StimuControl®	44.09 to	3.0 to
I1 powder	45.44 to	2.5 to
L1 powder	47.69 to	3.0 to
I1 Liquid	48.19 to	3.0 to
L1 net	48.80 to	3.0 to
Witness	49.36 to	3.5 to
CV (%)[2]	5,35	18,59

[1] Consecutive averages of the same letter in the column do not differ statistically from each other, according to Tukey, at 5% probability. [2] Coefficient of variation.

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For fresh mass, dry mass, number of trifoletles and shoot length of the plants, no significant difference was observed at the level of 5 % probability by the Tukey test between the treatments analyzed (Table 4).

Table 4. *Evaluation of the fresh and dry mass of the aerial part, number of trifolioles and length of the aerial part of the plants submitted to the treatments in the field experiment.*

Treatments	Fresh mass of plants shoot (g)	Dry mass of the aerial part of plants (g)	Number of trifoletles	Length of Aerial Portion (cm)
I1 powder	0.05 to	5.45 to	17.68 to	29.16 to
L1 net	0.05 to	5.93 to	12.20 to	31.90 to
Commercial Product	0.06 to	6.29 to	11.92 to	34.66 to
I1 Liquid	0.07 to	7.02 to	14.47 to	37.29 to
Witness	0.08 to	7.56 to	14.42 to	38.69 to
L1 powder	0.10 to	6.24 to	12.20 to	35.07 to
CV (%)[2]	55,2	44,32	43,72	16,5

[1] Consecutive averages of the same letter in the column do not differ statistically from each other, according to Tukey, at 5% probability. [2] Coefficient of variation.

Regarding the number of grains, grain weight and number of pods, no significant difference was observed at the level of 5 % probability by the Tukey test between the treatments (Table 5).

Table 5. *Evaluation of the number of pods, amount of grains and total weight of grains produced by the plants submitted to the treatments in the field experiment.*

Treatments	Number of pods[1]	Amount of grains per pod[1]	Total weight of grains (g)[1]
I1 powder	6.67 to	69.52 to	104.00 to
L1 net	7.50 to	81.68 to	143.25 to
I1 Liquid	7.98 to	92.21 to	146.50 to
L1 powder	7.24 to	79.83 to	148.75 to
StimuControl®	7.58 to	84.14 to	149.75 to
Witness	8.13 to	89.92 to	152.00 to
CV (%)[2]	23,75	22,59	24,7

[1] Consecutive averages of the same letter in the column do not differ statistically from each other, according to Tukey, at 5% probability. [2] Coefficient of variation.

DISCUSSION

The results obtained in the evaluation of chlorophyll and shoot height of the plants in the present work showed that the application of these *Trichoderma isolates* did not promote significant increases for these two parameters evaluated, thus resembling the

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data obtained by Aguiar, Bonaldo and Moraes (2014), who when working on the development of the bean crop and the control of anthracnose and mela diseases, in addition to the control of nematodes of the genus *Meloidogyne*, using two commercial formulations with *T. asperellum* and *T. harzianum*, observed that none of the formulations used promoted changes in the amount of chlorophyll or the height of the plants.

Gravel, Antoun and Tweddell (2007), when working on the determination of the growth-promoting effect of tomato plants using five species of bacteria and three species of fungi, obtained promising results in promoting growth with the use of *Trichoderma* in tomato plants, due to the production of indoleacetic acid (IAI) provided by the fungus, which caused the plants treated with this fungus to obtain an increase in the fresh mass of the aerial part, different from what occurred in the present study, where there was no increase in this parameter.

For leaf area, the results of the field experiment of the present work were similar to the results of Gava and Menezes (2012), who, when working with commercial formulations of *Trichoderma* spp. in yellow melon, did not observe significant differences in the leaf area of the treated plants about the untreated ones.

Regarding the length of the aerial part, the result obtained in the present study differed from that obtained by Carvalho et al. (2011) in their research related to the promotion of initial growth of common bean by *Trichoderma harzianum*, as the authors observed that some treatments obtained a significant increase about this parameter. One of the reasons why this difference may have occurred is the difficulty in colonizing plant roots when *Trichoderma* spp. isolates are used in seed treatment as described by Papavizas (1985).

Another factor that may have caused the *Trichoderma* spp. isolates not to reach their potential was the physicochemical characteristics of the soil, as high acidity combined with the scarcity of minerals was observed, causing these organisms to be out of their comfort zone.

Regarding the number of leaves, the results differed from those found by Romero et al. (2021), who worked on the development of forest species using two species of *Trichoderma*, where, for acacia, cypress and eucalyptus plants, a significant difference was observed when treated with fungi. However, the same author did not observe this difference when working with alder plants, a result similar to that observed in this work. The work of this author reaffirms the point that *Trichoderma* spp. has greater efficiency when

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applied in a way other than by seed treatment, since by treating the substrate directly the author obtained promising results about growth promotion.

According to Stewart and Hill (2014), the use of this fungal genus in growth promotion should be carried out with caution due to the variations that can occur because of the various limiting factors, such as the type of crop, type of formulation, development conditions, among others.

Even though the treatments using *Trichoderma* spp. did not differ statistically about the control of the mela disease, the fact that this was the only disease to emerge in the production field is quite encouraging, considering the number of diseases that affect cowpea. The emergence of just one disease greatly reduces production expenses and the emission of agrochemicals in crops, in addition to being, in a way, easier to control or mitigate the damage generated.

CONCLUSION

In the field, only the Mela disease manifested itself, which generates good expectations for cowpea production in the state of Maranhão.

There is no effect of the commercial product based on *Trichoderma harzianum* on the promotion of bean growth in the field in the village of Curvelândia, municipality of Vila Nova dos Martírios - MA.

More research is needed using different species of *Trichoderma*.

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