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Volatile organic compounds produced by *Trichoderma* sp. morphophysiologically altered maize growth at initial stages

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Abstract

Fungi of the genus *Trichoderma* are important microorganisms for increasing plant growth. However, few studies have evaluated the potential of volatile compounds produced by the fungus *Trichoderma* spp. and it's potential as promoters and beneficiaries with respect to maize cultivation. Thus, this work aimed to evaluate the efficiency of volatile compounds produced by *Trichoderma* spp. and their potential for morphophysiological improvement in the initial growth of corn crops. The experiment was conducted in a factorial scheme (2x6+1), arranged in a completely randomized design, with two evaluation times (7 and 15 days after emergence (DAE) and five *Trichoderma* spp. isolates (plus one control). The isolates were classified as the following species and identified with the following codes: UFT-25: *Trichoderma harzianum*; UFT-37: *Trichoderma pinnatium*; UFT-57: *Trichoderma virens*; UFT-201: *Trichoderma asperellum* and UFT-204: *Trichoderma longibrachiatum*. The volatile compounds produced by Trichoderma spp. and inoculated in culture medium present in each experimental unit without direct contact with the roots of the plants, promoted an increase mass production and changed morphology and physiology, especially plant height, leaf area, absolute and relative growth rate, Falker chlorophyll index, instantaneous carboxylation efficiency (RuBisCo), and net photosynthesis rate.

Keywords: Biostimulants; Fungi; Physiology; Production; Efficiency.
Abbreviations: *A*_net photosynthesis rate; AGR_absolute growth rate; BDA_potato dextrose-agar; *Ci*_Internal CO_2 concentration; DAE_days after emergence; DWR_dry weight of root; DWS_dry weight of shoot; FCI_ Falker chlorophyll index; *gs*_stomatal conductance; IWUE_intrinsic water-use efficiency; LA_leaf area; NAR_net assimilation rate; PH_Plant height; RGR_relative growth rate, RL_root length; SLA_specific leaf area; TDW_total dry weight; TEF_translation elongation factor; TFW_total fresh weight; UFT-201: *Trichoderma asperellum*; UFT-204_*Trichoderma longibrachiatum*; UFT-25_*Trichoderma harzianum*; UFT-37_*Trichoderma pinnatium*; UFT-57_*Trichoderma virens*; VOCs: Volatile Organic Compounds

Introduction

Soil microorganisms are potential sources of volatile organic compounds and play a huge role in various interactions between biotic and abiotic factors of the ecosystem (Bitas et al., 2013). About 500 species of bacteria and fungi have been exploited to produce different volatile compounds, including alcohols, ketones, mono and sesquiterpenes, esters, thioalcohol, lactones and thioesters (Splivallo et al., 2011; Effmert et al., 2012; Kramer e Abraham, 2012; Lemfack et al., 2018).

The production of volatile and nonvolatile secondary metabolites has been reported in the literature and the *Trichoderma* fungus is considered to play a significant and effective role in plant-pathogen as well as in suppressing and promoting plant growth (Bisen et al., 2016; Singh et al.,2017).

Among the advantages that volatile metabolites have over nonvolatile metabolites are the ability of solubilization between soil particles and diffusion in the interstices, and is not limited by dilution, not subject to absorption and biodegradation (Santos, 2008). Due to their volatility and diffusion through air and liquid spaces, VOCs (Volatile Organic Compounds) have ideal biophysical properties to function as above-ground but also below-ground signaling molecules through pores in the soil matrix (Guo et al., 2019).

Among the compounds produced by *Trichoderma* sp. include 6-pentyl-α-pyrone, antibiotics gliotoxin, viridine, gliovirine, glisoprenin, heptelidic acid, koninginins, anthraquinones, tricodermamides, peptaibols, polyketides, terpenoids, polypeptides, trichothecenes, trichodermaids, amino-alpha-derivative azafilones (Keswani, 2015).

The volatile compounds produced by *Trichoderma* sp. act as antibiotics against pathogenic fungi and may have a plant growth-promoting effect as well as increasing systemic plant resistance (Hung et al., 2013; Lee et al., 2016; Nieto-Jacobo et al., 2017; Siddiquee et al.,2012).

The action of *Trichoderma* as a growth stimulant is complex and performed by interactions with biochemical factors and the production of several beneficial enzymes and compounds (Machado, 2012).

Trichoderma is known to contribute to the growth stimulus for crops, but it is not clear which characteristics the volatile compounds produced by the fungus alter in the plant to generate this growth increase. Studies are still scarce in the literature when it comes to information about the influence of volatile compounds produced by *Trichoderma* on gas exchange and photosynthesis of crops, and so far there is no known work on this with corn, requiring research to obtain results that differentiate the fungus *Trichoderma* as promoters and beneficiaries regarding the physiology of the corn plant.

Thus, this work aimed to evaluate the efficiency of volatile compounds produced by *Trichoderma* spp. and its potential for morphophysiological improvement in the initial growth of corncrop.

Results and Discussion

Results of variance analysis

The use of *Trichoderma* spp. promoted significant effects ($p < 0.05$) on the development of maize crops, with improvement in the initial development of the crop, which directly reflected in the morphophysiology of the plant. The representation of variance analysis for different epochs, *Trichoderma* spp. and their interaction showed that there was no effect on any of the treatments for the variables *E* and *WUE* (Table 2). Thus, with regard to these characteristics, it is verified that regardless of the treatments used and the interaction between them, did not provide a significant effect on the development of maize crop.

In the characteristics *Ci*, *IWUE*, RuBisCO there was no significant effect when comparing the species of *Trichoderma* and RL, SLA, *gs*, *IWUE,* there wasn't any interaction between according the analysis of variance. The characteristics PH, TFW, DWS, DWR, TDW, LA, A, and FCI were the characteristics that, independently of the treatments used and the interaction between them, got a significant effect (Table 2).

In relation to the evaluated times, it should be noted that the volatile compounds produced by *Trichoderma* sp. have a superior effect in the period of 15 DAE. According to Lee et al. (2016), evaluating VOCs emitted by *Trichoderma* sp. mediating the growth of tomato and *Arabidopsis thaliana* plants, observed that the growth of tomato plants depended on the duration of the volatile exposure, the same was observed in this study because the superior effect was observed in the greatest time of exposure.

Growth and morphology

The *Trichoderma* UFT-57 isolate was the only one to present statistical superiority in all evaluated traits (Fig 1), showing no difference with the UFT-204, PH characteristic, with UFT-37 in DWR and with UFT-37 and UFT-204 in the TDW.

In general, the PH was increased by the application of volatile compounds, independently of the species of *Trichoderma* and evaluated times (Fig 1A). The treatments UFT-57 and UFT-204 were the ones that presented the best result, being an average height of (33.11 cm), resulting in plants up to 10.66 cm higher when compared to the control. As for RL, there was no significant difference between the *Trichoderma* having an average growth of 38.57 cm. This result indicates that there was a root growth of corn crop 24.38% higher than the control treatment (31.01 cm) (Fig 1B).

Some species of *Trichoderma sp*. can increase growth and induce salt tolerance in *Arabidopsis thaliana,* the presence of VOCs the plants become taller with more leaf surface area, more side roots (Jalali et al., 2017). The same can be observed in corn crop because there was an increase in plant height in relation to the control. Lee et al. (2016), also observed that tomato seedlings exposed to *T. viride* VOCs significantly increased the weight of roots in (61.2%) and biomass (41.2%), values compared to the standard control without *Trichoderma* VOCs.

The DWS characteristic was where the *Trichoderma* UFT-57 species obtained the highest statistical difference with 0.39 g, with an average of 17.94 and 141.09% higher than the others and the control, respectively (Fig 1C). For the characteristic DWR, it was noted that the treatments UFT-37 and UFT-57 promoted the highest response with an average production of 0.97 g, being on average 10.22% higher than the other treatments and with the improvement of 29.33% when compared to the absence of *Trichoderma* (Fig 1D).

The VOC effect of *Trichoderma* spp. when tested on a model system with *Arabidopsis thaliana*, 14 days of growth with *T. viride*, demonstrated longer root length and root mass when compared to control, the same can be observed in this study (Fig 1B and 1D), in which *Trichoderma* VOCs are efficient in promoting root growth also for maize (Hung et al., 2013).

The production of TFW and TDW of maize crop were influenced differently by volatile compounds as a function of the epochs and different species of *Trichoderma* (Fig 1E and F). In general terms, the best results were from the treatments UFT-37, UFT-57, and UFT-204 that promoted an increase in the masses. In these concentrations, the average production of 1.33 g $vase^{-1}$ of natural matter produced was increased by 44.56% compared to plants that did not receive *Trichoderma*.

When analyzing *Trichoderma* sp. VOC in the biomass of Arabidopsis plants after 7 days of co-cultivation, the compounds emitted by four *Trichoderma* strains *(T. sp*." Atroviride", *T. virens* and *T. asperellum*) showed a significant increase in the shoot, root and total biomass in relation to seedlings control (Nieto-Jacobo et al., 2017). VOCs issued by *T. reesei* had no effect on increased biomass in shoot and roots in relation to control, thus the different strains influence

differently in the addition of corn biomass (Nieto-Jacobo et al., 2017).

Leaf development

The responses of the evaluation of LA and FCI of corn crop show that the *Trichoderma* sp. UFT- 37, 57 and 204, were Statistically higher (p 0.05) With a mean of 99,66 cm^2 for LA being 18.82 and 84.83% higher compared to the others and the control respectively and FCI of 41.28 with 15.08 and 36.75% more when compared with the other *Trichoderma* species evaluated and the control, respectively.

The strains analyzed promoted a significant increase in chlorophyll content, the main constituent of the chlorophyll molecule is nitrogen, 50% of in the leaves is found in chlorophyll and chloroplast compounds, The ICF is a good indication of in plants, as an indirect measure of chlorophyll (Arantes et al., 2017). According to Lee et al. (2016) there noted that exposure to mixtures of volatile organic compounds emitted by strains of *Trichoderma* sp. increased chlorophyll content (82.5 and 89.3%) in *Arabidopsis thaliana* (Figure 2C).

For SLA there was no statistical difference between the treatments evaluated with the fungus (P <0.05), having observed the greater response in the control with a maximum point of 325,39 cm^2. The increase in SLA increases the appetite and fragility of the leaves, increasing the risk of premature tissue loss (Lusk, 2002), while the thicker leaves have high correlations with higher lignification, lower cell size, low moisture content and low N concentration (Castro-Díez et al., 2000).

Morphogenic parameters

The volatile compounds produced by the *Trichoderma* in the maize (*Zea mays*) crop promoted alterations in the initial growth of the plants, as presented by the analysis of variance of the data evaluated (Table 3). It is noted that there was a significant difference by the Duncan test (p <0.05) for the characteristics absolute growth rate (AGR), relative growth rate (RGR). The net assimilation rate (NAR) did not differ significantly between *Trichoderma*.

Regarding the AGR, it was noted that the treatments UFT-37 and UFT-57 promoted the highest response with an average production of 0.225 g^{-1} beings on average 12% higher than the other treatments and with the improvement of 32.35% when compared to the absence of *Trichoderma* (Fig 3A). For the RGR characteristic, there was no significant difference between the *Trichoderma* UFT-37, 57, 204 and 201, having an average growth of 0.133 $g.g^{-1}$ day^{-1} this result indicates that there was a relative growth in maize 20.91% higher than the treatment UFT-25 and 47.78% higher the control plants (0,09 g g^{-1} day^{-1}) (Fig 3B).

Contribution of volatile compounds in plant physiology

The physiological evaluation performed in the plants showed significant differences for the variables analyzed according to the different epochs and *Trichoderma* sp. except for *gs* and *IWUE* (Fig 4). In relation to the C*i*, its content in the plants was reduced with the presence of *Trichoderma* VOCs (Fig 4A). The best result occurred under the control treatment, with an accumulation of 231.2 μmol CO_2 mol^{-1} air, which confers an approximate increase of 44.25 μmol CO_2 mol^{-1} air when compared to other sources.

As for *gs*, there was no difference between treatments, however, a mean increase of 102% was observed in plants when compared to the control with 0.19 mmol H_2O $m^{-2}s^{-1}$ (Fig 4B). There was a difference between the different evaluation periods and the 15 DAE was higher statistically by the Duncan test (p < 0,05), with a difference of 0,124 mmol H_2O $m^{-2}s^{-1}$ more in relation to the 7 DAE. According to Almeida (2017) evaluating the use of *Trichoderma* sp. In the emergence of soursop seedlings evaluating the variable stomatal conductance (*gs*), The seedlings conducted in the clear sky environment presented the highest value, with 0,088 H_2O $m^{-2}s^{-1}$, approximate values to those analyzed in the study also conducted in the clear sky.

The *A* varied according to the interaction between *Trichoderma* and epochs, where the maximum rate of 43,26 μmol CO_2 $m^{-2}s^{-1}$was with the UFT-57 treatment (Fig 4C). This result indicates that there was *A* by the maize crop 22.29% higher than the other treatments and 150.76% higher than the control plants (17.25 μmol CO_2 $m^{-2}s^{-1}$). Dildey (2014) observed that plant growth is primarily conditioned to obtaining energy from solar radiation through interception and use in the photosynthesis process. The liquid photosynthesis reflects on the biomass production, which can be influenced by several factors such as light, temperature, humidity, soil fertility, and the culture management adopted, and therefore, important conditioners (Lopes et al., 2013).

In a specific way, volatile compounds contributed to the changes observed in maize plants as to the *IWUE* e RuBisCo (Fig 4D). At 15 DAE the use of *Trichoderma* sp. was significantly higher, with *IWUE*, approximately 1.31 times higher than 7 DAE, however, it did not differ statistically between treatments with different isolates of *Trichoderma* (Fig 4E).

The development of the plant is closely related to the quantification of gaseous exchanges performed on the leaves comprising the net assimilation of CO_2, as well as perspiration, stomatal conductance, internal CO_2 concentration, in the substomatal chamber, water-use efficiency, among others that were not evaluated in the present study (Taiz and Zeiger, 2017).

Therefore, the volatile compounds produced by the different *Trichoderma* species used in the study and the epochs influenced integrally in the maize variables, improving, in general, biometric, morphological and growth and initial development of the crop. However, we emphasize the need for more research that investigate beyond the aspects observed and correlated in this study, aiming to guide the use of these species of *Trichoderma* in the maize crop, presenting the effects promoted in plants and their advantages for Brazilian agriculture.

Materials and methods

Study site and experimental plots

The experiment was conducted in the experimental area of the Federal University of Tocantins (UFT), Gurupi University

Table 1. Identification of *Trichoderma* sp. isolates.

Isolates	Species identification	GenBank	Reference
UFT-25	*T. harzianum* CIB T131	EU279988	Hoyos-Carvajal *et al*. (2009)
UFT-37	*T. pinnatium* GJS 02-120	JN175572	Druzhinina *et al*. (2012)
UFT-57	*T. virens* CIB T147	EU280060	Hoyos-Carvajal *et al*. (2009)
UFT-204	*T. longibrachiatum* DAOM 167674	EU280046	Hoyos-Carvajal *et al*. (2009)
UFT-201	*T. asperellum* GJS 04-217	DQ381958	Samuels *et al*. (2010)

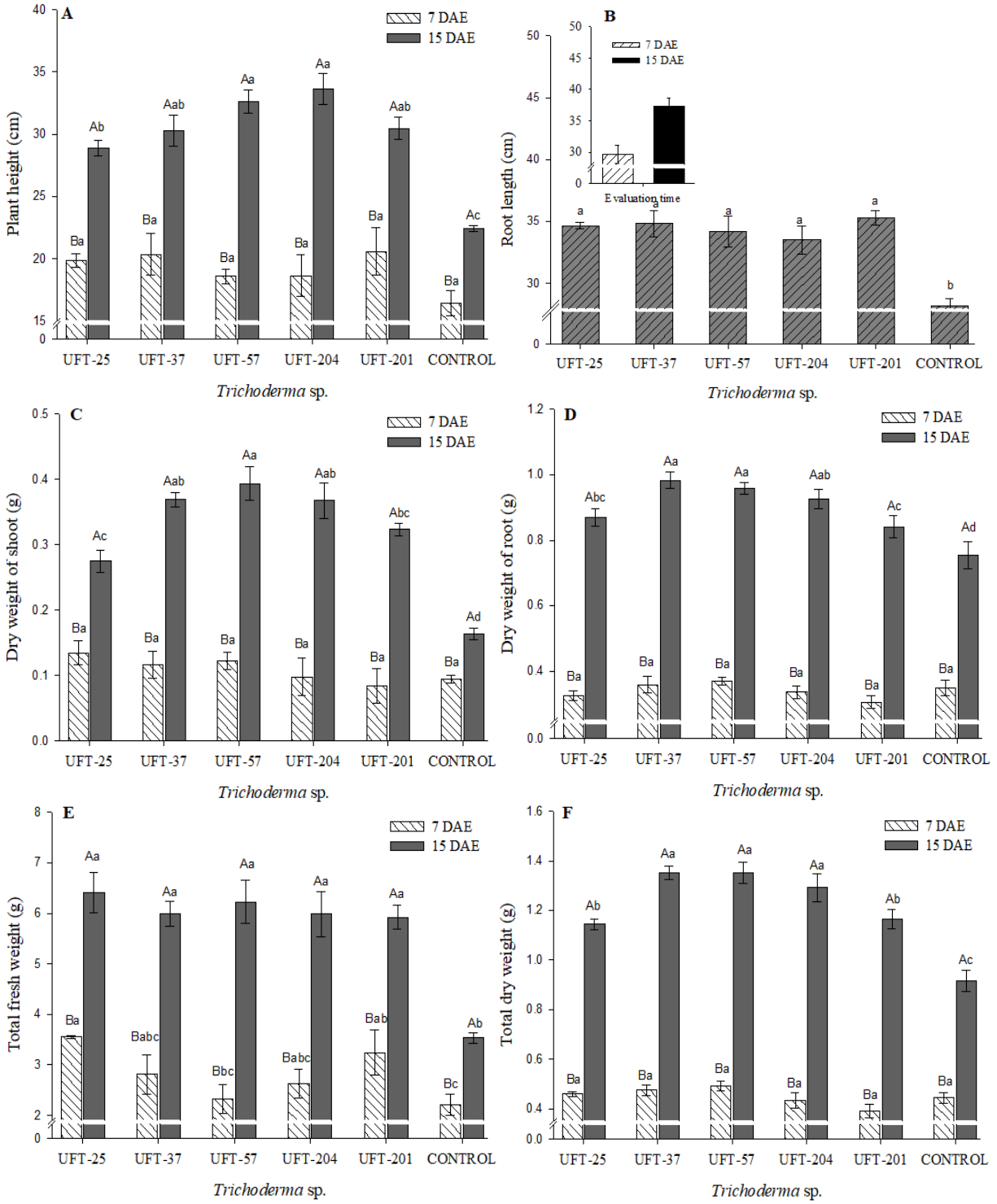

Fig 1. Plant height (A), root length (B), dry weight of shoot (C), dry weight of root (D), total fresh weight (E), total dry weight (F) of corn cultivar at different evaluation times and *Trichoderma* sp. Means followed by the same lowercase letter, comparing the isolates of *Trichoderma* sp., and uppercase, comparing the different evaluation times (Duncan, $p \leq 0.05$).

Table 2. Analysis of variance for evaluations: Plant height (PH), root length (RL), dry weight of shoot (DWS), dry weight of root (DWR), total fresh weight (TFW), total dry weight (TDW), leaf area (LA), specific leaf area (SLA), Falker chlorophyll index, Internal CO_2 concentration (*Ci*), stomatal conductance (*gs*), net photosynthesis rate (*A*), intrinsic water-use efficiency (IWUE) e Instant carboxylation efficiency (RuBisCo), of corn cultivar at different evaluation time and *Trichoderma* sp.

Evaluations	Source of variation				Average	CV (%)
	Time (E)	*Trichoderma*(T)	Int. ExT	Residual		
	Degrees of freedom					
	1	5	5	36		
PH	1358.94**	49.90**	22.10**	5.41	24.40	9.53
RL	711.87**	55.50**	13.21ns	10.14	33.46	9.52
DWS	0.514**	0.017**	0.014**	0.0014	0.21	17.99
DWR	3.602**	0.0186**	0.0119**	0.0024	0.61	7.95
TFW	99.79**	4.105**	1.531**	0.413	4.24	15.18
TDW	6.8479**	0.068**	0.0489**	0.0042	0.83	7.84
LA	34778**	788**	733**	199	59.86	23.54
SLA	6975.7**	2285.1*	1597.1ns	879.5	293.32	10.11
FCI	133.93*	68.79**	71.154**	18.59	35.96	11.99
ci	41260**	398ns	2505*	813	165	17.28
E	16.81ns	8.49ns	2.86ns	5.87	8.03	30.16
gs	0.185**	0.0314**	0.011ns	0.0072	0.29	29.02
A	303.26**	249.06**	121.84*	36.29	31.16	19.33
WUE	0.069ns	1.629ns	0.693ns	1.765	4.08	32.6
IWUE	11144.4**	231.0ns	544.4ns	333.5	112.52	16.23
RuBisCo	0.012*	0.0047ns	0.010**	0.0025	0.20	25.06

**Significant by t-test analysis (p<0.01); *Significant by t-test analysis (p<0.05); ns non-significant by T-test analysis; CV: Coefficient of Variation.

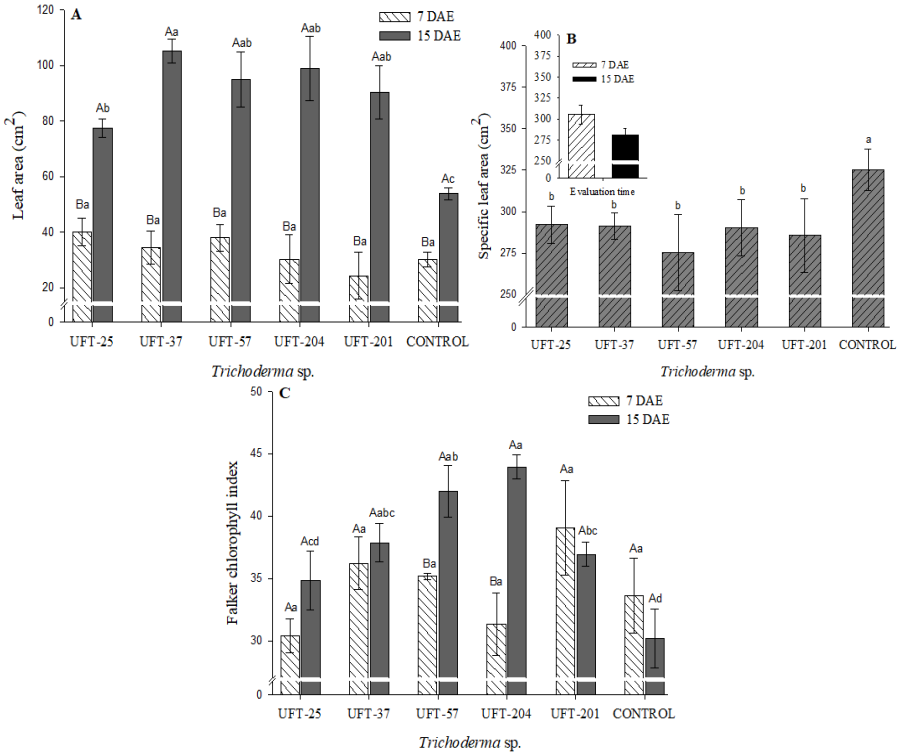

Fig 2. Leaf area (A), specific leaf area (B), Falker chlorophyll index (C) of corn cultivar at different evaluation time and *Trichoderma* sp. Means followed by the same lowercase letter, comparing the isolates of *Trichoderma* sp., and uppercase, comparing the different evaluation times (Duncan, $p \leq 0.05$).

Table 3. Analysis of variance for evaluations: absolute growth rate (AGR), relative growth rate (RGR), net assimilation rate (NAR) of corn cultivar at different evaluation time and *Trichoderma* sp.

Source of variation	Degrees of freedom	Medium square		
		AGR	RGR	NAR
Trichoderma	5	0.00213**	0.00128**	$8.98.10^{-9}$ns
Resídue	18	0.000125	0.000240	$8.48.10^{-9}$
Total	23			
CV (%)		5.41	12.62	40.05

**Significant by T-test analysis (p<0.01); *Significant by T-test analysis (p < 0.05); ns non-significant by T-test analysis; CV: Coefficient of Variation.

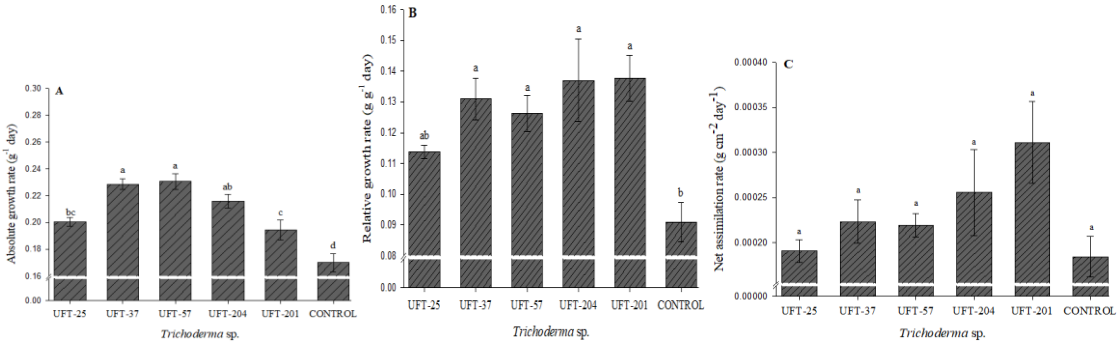

Fig 3. Absolute growth rate (A), relative growth rate (B), net assimilation rate (C) of corn cultivar at different evaluation time and *Trichoderma* sp. Gurupi-TO, 2019. Means followed by the same lowercase letter, comparing the isolates of *Trichoderma* sp. (Duncan, p ≤ 0,05).

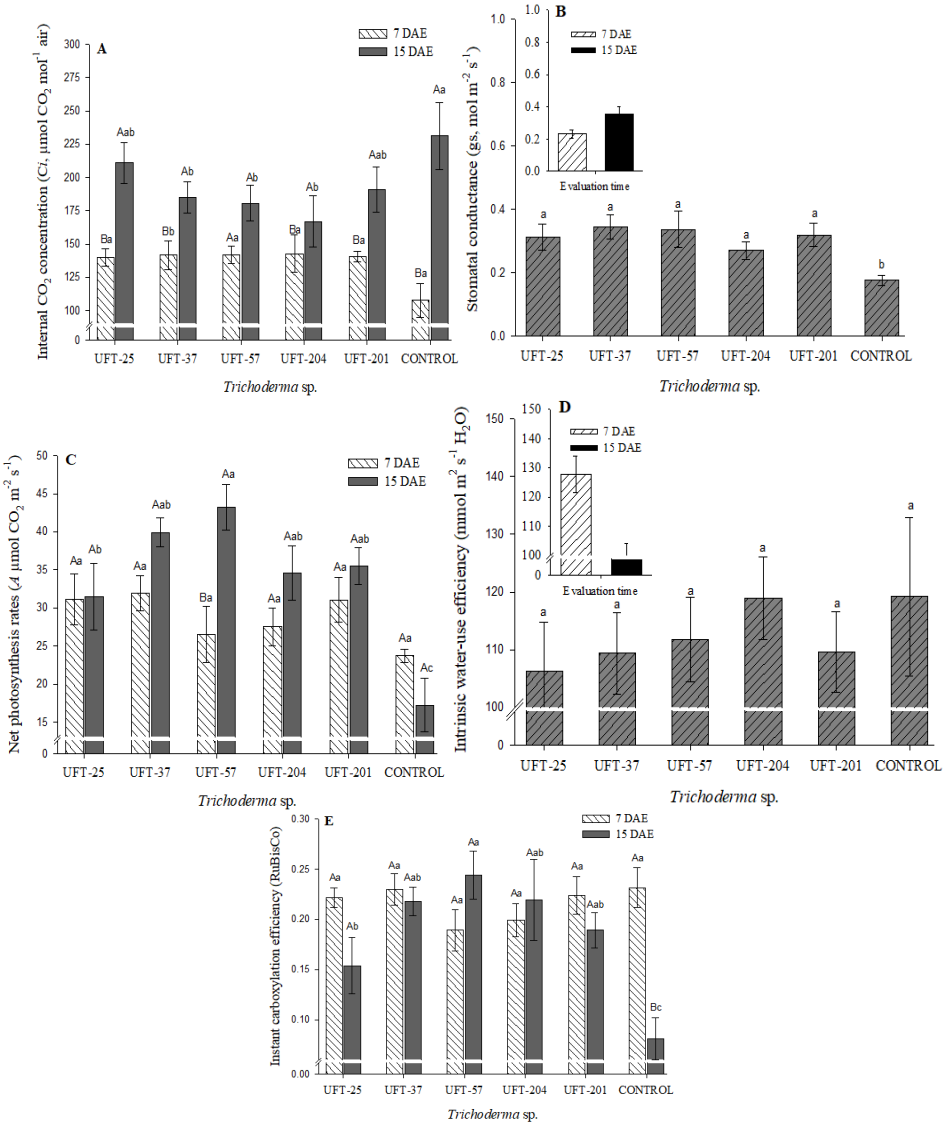

Fig 4. Internal CO_2 concentration (A), stomatal conductance (B), net photosynthesis rate (C), intrinsic water-use efficiency (D) and RuBisCo (E) of corn cultivar at different evaluation time and *Trichoderma* sp.Means followed by the same lowercase letter, comparing the isolates of *Trichoderma* sp., and uppercase, comparing the different evaluation times (Duncan, p ≤ 0.05).

Campus. The area is located at the coordinates of 11° 43'45" S and 49° 04'07" W, at 280 m altitude in the southern state of Tocantins. The regional climate is humid B1wA'a 'with moderate water deficiency (Inmet, 2019).

The research was carried out in a factorial scheme (2x6), where the treatments were arranged in a completely randomized design, being the factor and two evaluation times (7 and 15 days after emergence (DAE) and factor T for five Trichoderma isolates. (UFT-25, UFT-37, UFT-57, UFT-201 and UFT-204) and a control without fungal cultivation.

Obtaining isolations of microorganisms

Trichoderma species were obtained by soil isolation from the experimental area of the UFT, and in floodplain areas of Lagoa to confusão - TO (10°47'37" S and 49°37'25" W, 200 m altitude average). Preliminary soil samples were collected in the mentioned areas and taken to the Agromicrobiology Laboratory of the Federal University of Tocantins. Then, a sample (1g) of each soil was deposited in a petri dish containing BDA (potato dextrose-agar) culture medium modified with oxytetracycline hydrochloride (100 mg L^{-1}) (Terramicina® - Pfizer) to inhibit bacterial growth. Immediately after the plates were incubated in a growth chamber, type B.O.D., at 25 °C with a photoperiod of 12 hours, for seven days.

Subsequently, in the laminar flow hood, small portions of the fungus-containing mycelium medium with more aggressive growth characteristics and green-colored filamentous appearance were transferred to new Petri dishes with BDA medium and incubated again. The subculture procedure was repeated in order to obtain pure Trichoderma colonies. Gender identifications have been previously morphologically identified (Barnett and Hunter, 1998; Zafari et al., 2004), and characterized by sequencing of the TEF (translation elongation factor) region and identified by access codes in GenBank performed by the Biological Institute of São Paulo, being the isolates classified as the following species and identified with the codes. UFT-25: *Trichoderma harzianum*; UFT-37: *Trichoderma pinnatium*; UFT-57: *Trichoderma virens*; UFT-204: *Trichoderma longibrachiatum*; UFT-201: *Trichoderma asperellum* (Table 1). The isolates were kept in a refrigerator with subcultures in BDA medium and kept in water, according to the methodology of (Castellani, 1967).

Corn and fungi cultivation for volatile compounds evaluation

For the cultivation of maize, 350 mL pots were used coated with disposable caps to prevent the substrate from collapsing on the culture medium with the isolates, being made sepsis with 70% alcohol and led to laminar flow for sterilization under ultraviolet light 24 hours.

In the cultivation of the fungus, 350 mL plastic pots were submerged in water with hypochlorite for 24 hours, subsequently washed with autoclaved water to remove the residues. They were then brought to the laminar flow for sterilization in ultraviolet light for 24 hours. After sterilization, the isolates were subcultured by placing an agar disk containing the fungus mycelium in the 40 mL pots of BDA medium (Potato, Dextrose, and Agar), then incubated in a B.O.D chamber at 25 ⁺ 2° C, 12 hours of light.

For planting the seeds of cultivar ANHEMBI – Priorizi seeds autoclaved substrate and sand were used at 120 ºC for 60 minutes. They were then mixed in a 1:1 ratio containing 260 g per pot, 6 treatments and 4 replicates with Trichoderma UFT-25, UFT-37, UFT-57, UFT-201, UFT-204 isolates and the control without the fungus.

The isolates were seeded 3 DAS of the corn, and only 24 hours after the seedling, the pots were superimposed over the pots containing the culture medium with Trichoderma sp. and being fitted and sealed so that no volatile compounds could escape. Then, the pots were placed on a bench inside the laboratory, and for better plant conditioning, an electric extension with 5 incandescent lamps with 12 hours light was used, and a room humidifier turned on at night. The pots were daily watered manually with a spray bottle and 20 ml of autoclaved distilled water per pot was added.

Morphological and biometric parameters

With the morphological and biometric evaluations performed, the following characteristics were determined: Plant height (PH in cm), measured with a ruler graduated in millimeters, measuring the distance between the lap and the apex of the plant Root length (RL in cm), measured with a graduated ruler in millimeters, measuring the distance between the lap and the tip of the root; dry weight of shoot (DWS in g), the leaves were detached from the stem and washed in running water, being packaged in paper bags identified; dry weight of root (DWR in g), the roots were separated of the aerial part washed in running water, with the aid of a sieve and a basin, to avoid the loss of the finer parts and total dry weight (TDW in g): performing the summation of DWS and DWR. the material for determining the DWS, DWR and TDW were subjected to drying in a forced air circulation oven-dried at, 65 °C for 72 hours and then weighed on a semi-analytical scale (0.001 g).

It was also determined the leaf area (LA in cm^2), calculated using the "disc method", that is, leaf blade discs (main leaves) were collected in each repetition. The technique consists of the removal of leaf discs (0.159 cm^2) from a known area of a set of sheets, distributed symmetrically, avoiding the sampling of the central rib, according to studies of (Huerta and Alvim, 1962; Gomide, 1977) specific leaf area (SLA in g cm^2). Determined by the relation between leaf area (cm^2) dry weight of shoot (g); net assimilation rate (NAR), absolute growth rate (AGR) and relative growth rate (RGR) were calculated using formulas according to the (Perez and Fanti, 1999).

Physiological evaluation

Chlorophyll was determined through the ChlorofiLOG equipment using photodiodes emitted in three wavelengths (Falker, 2008): Two emit inside the red band, close to the peaks of each type of chlorophyll (=635 and 660nm) and another in the near-infrared(=880nm). In the same way as SPAD, a lower sensor receives the radiation transmitted through the leaf structure. From this data, the apparatus provides values called Falker Chlorophyll Index (FCI)

proportional to the absorbance of Chlorophyll (Junior et al., 2012).

To evaluate the photosynthetic activity of leaves, gas exchange assessments were performed on leaves at 7 and 15 days. The evaluations were performed using an open-source photosynthesis system with CO_2 Analyzer and water vapor by IRGA (Infra-Red Gas Analyzer, model LI-6400, Li-Cor), In the period from 8 to 10 h, in fully expanded leaves, without signs of senescence and sound.

The gas exchange evaluation carried out were: net photosynthesis rate (*A*, μmol CO_2 $m^{-2}s^{-1}$), transpiration rate (*E*, mmol water vapor $m^{-2}s^{-1}$), stomatal conductance (*gs*, mol $m^{-2}s^{-1}$) e Internal CO_2 concentration on the leaf (C*i*, μmol CO_2 mol^{-1} air).

Water-use efficiency (WUE, μmol CO_2 (mmol H_2O^{-1}) was determined by the relationship between *A/E* intrinsic water-use efficiency (IWUE, (mmol $m^{-2}s^{-1}$ H_2O) was determined by the relation between *A/gs*, instant carboxylation efficiency enzyme Ribulose 1, 5-diphosphate carboxylase (RuBisCo) calculated by the relation *A/Ci*, both as described by (Zhang et al., 2001).

The reference CO_2 concentration used during the evaluation was present in the environment. to homogenize the repetitions, the photosynthetically active photons flux density (DFFFA) Is generated by a led light coupled to the photosynthesis chamber, standardizing the luminosity in each evaluation period, so that all plants are under the same light conditions; to do so, will be used 1500 μmol $m^{-2}s^{-1}$.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and the averages were compared by the Duncan test ($p \leq 0.05$), performing unfolding when the interaction was significant, using the software R, version 3.5 (Team, 2013). The graphs were plotted using the software SigmaPlot® version 10 (Sistat, 2014).

Conclusion

The volatile compounds produced by the *Trichoderma* species in the study increase the mass production and alter the morphology and physiology, mainly, plant height, leaf area, absolute and relative growth rate, Falker chlorophyll index, instant carboxylation efficiency (RuBisCo) and net photosynthesis rate. The species *Trichoderma virens* (UFT-57) was the one that had, in general, the best result among the main characteristics evaluated, being the one that made available the most efficient volatile compounds for culture, increasing the initial growth of maize. At 15 DAE was the most efficient time for the growth of *Trichoderma* species and production of volatile compounds, and as a consequence was where the best results were obtained for the corn crop.

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