REVISTA DE CIÊNCIASAGRÁRIAS
Amazonian Journal
of Agricultural and Environmental Sciences
www.ajaes.ufra.edu.br

http://dx.doi.org/10.4322/rca.2012.078

ORIGINAL ARTICLE

Rice sheath blight biocontrol and growth promotion by *Trichoderma* isolates from the Amazon

Biocontrole da queima da bainha em arroz e promoção do crescimento por isolados de Trichoderma da Amazônia

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KEYWORDS
Growth stimulation
Integrated disease management
Oryza sativa L.
Thanatephorus cucumeris

PALAVRAS-CHAVE
Estimulante do crescimento
Manejo integrado de doenças
Oryza sativa L.
Thanatephorus cucumeris

ABSTRACT: This study was carried out to select *Trichoderma* isolates from Amazon forest soil samples, identify their potential for sheath blight (*Rhizoctonia solani*) suppression in rice, and promote plant growth. Four out of the 13 isolates (T.06, T.09, T.12, T.52) which showed *in vitro* potential were evaluated through assays under greenhouse conditions utilizing four application methods: 1) preventive and curative sprays; 2) seed treatment + curative sprays; 3) seed treatment; 4) substrate treatment. These four isolates also showed reduced *R. solani* mycelial growth and sclerotial viability (>50%) and were positive for phosphate solubilization and cellulose degradation. They significantly reduced sheath blight severity when applied as seed treatment, substrate incorporation or foliar spray. However, the preventive and curative sprays were the most efficient method, reducing sheath blight severity by 43% and the area under the disease progress curve by 45%. Isolates T.12 and T.52 applied in substrate treatment increased aerial and root dry weight by 61.2 and 32.9%, respectively. These two isolates showed potential as growth stimulants and can be used as novel biological products and bioinoculants in agriculture for increasing grain yield.

RESUMO: *Foi realizada a seleção de isolados de Trichoderma spp. provenientes de amostras de solo da Floresta Amazônica e identificado o seu potencial para suprimir a queima da bainha (QB) (Rhizoctonia solani) e para promover o crescimento de plantas de arroz. Quatro de 13 isolados (T.06, T.09, T.12, T.52) que mostraram potencial in vitro foram avaliados em experimentos em casa de vegetação, utilizando-se quatro métodos de aplicação: 1) pulverização preventiva e curativa; 2) tratamento de semente + pulverização curativa; 3) tratamento de semente; 4) tratamento do substrato. Esses quatro isolados, in vitro, reduziram o crescimento micelial de Rhizoctonia solani e a viabilidade do escleródio (>50%), sendo positivos para solubilização de fósforo e degradação da celulose. Em casa de vegetação, os quatro isolados reduziram significativamente a severidade da QB quando aplicados via tratamento de semente, incorporados ao substrato e por pulverização foliar. Entretanto, a pulverização preventiva + curativa foi mais eficiente, reduzindo em 43% a severidade da QB e em 45% a área sob curva de progresso da doença. Os T.12 e T.52 aplicados via substrato promoveram o incremento em massa seca da parte aérea e radicular em 61,2 e 32,9%, respectivamente. Esses dois isolados mostraram potencial como promotores do crescimento e poderão ser usados como novo produto biológico e bioinoculantes na agricultura para aumento de produtividade.*

Recebido: 13/09/2012
Aceito: 19/10/2012

1 Introduction

Rice (*Oryza sativa* L.) is one of the most important cultivated cereals consumed in the world, mainly in Asian and developing countries such as Brazil. The continuous growth in world rice consumption has imposed pressure on the production sector for developing sustainable crop cultivation techniques. In the State of Pará, rice is grown on the river banks composing Amazon basin. One of the limiting factors to a sustainable rice production is sheath blight, disease caused by fungus *Rhizoctonia solani* Kühn, which has been reported to cause economic loss as high as 32% in grain weight/panicle (ARAÚJO; PRABHU; SILVA, 2006).

The difficulty of controlling the disease lies in the ability of the pathogen to form sclerotia, its broad host range, and lack of resistant rice. The disease control requires foliar application of fungicides which incurs production cost, besides causing environmental concern.

Biological control through the use of antagonistic fungi such as *Trichoderma* is a viable disease management. The main modes of action of the biocontrol agent include competition for nutrients, competition for space (DJONOVIC et al., 2007) production of cell wall degrading enzymes, production of antifungal diffusible and volatile metabolites and mycoparasitism. *Trichoderma* spp. are considered to be antagonistic to many plant pathogenic fungi including *Rhizoctonia solani*, *Sclerotinia* spp. and *Fusarium* spp. (SULEMAN; ABDULLAH; ALI, 2008). *Trichoderma* ranks first in order of importance (LORITO et al., 2010), because it is one of the predominant members of the rhizosphere, very effective against plant pathogens, relatively easy to isolate, grow quickly on many substrates and produces metabolites with antibiotic activity, besides its positive influence on growth promotion and increased tolerance to various abiotic stresses (GRAVEL; ANTOUN; TWEDDELL, 2007). Growth promoting characteristics have been related to *Trichoderma* spp. use in cultivated crops such as common bean, corn and cotton, and several ornamental plants (HARMAN et al., 2004; GRAVEL; ANTOUN; TWEDDELL, 2007). Investigations on common bean have shown that increments of growth promotion are related to rhizosphere composition, increased nutrient availability, changes in plant metabolism such as indole acetic acid pathway and endophytic capacity of individual isolates (HOYOS-CARVAJAL; ORDUZ; BISSETT, 2009a). Nevertheless, *Trichoderma* efficiency depends on pathosystem, isolate, mode and number of antagonist applications, and environmental conditions (LORITO et al., 2010). Strains of *T. viride* and *T. harzianum* have been reported to efficiently control *R. solani* in rice (KRISHNAMURTHY et al., 1999), although under high humidity and temperature conditions, prevailing in the tropics. Alterations in composition and activity of rhizosphere microorganisms often occur, which may affect their efficiency as biological control agents (SHORESH; HARMAN; MASTOURI, 2010). Previous studies on soils of Amazon forest have shown that they constitute the most diverse ecosystems in the world (FERNÁNDEZ et al., 2008). However, there is no information on how the microbial diversity niche could support a sustainable rice cultivation on the river banks in the Amazon region.

The main goal of the present investigation was to select *Trichoderma* spp. isolates from Amazon forest soil samples, which exhibit potential to reduce sheath blight severity and promote rice plant growth.

2 Material and Methods

Soil samples were collected from reforested and native areas in Amazon forest, Urucu, - Amazonas (geographic coordinates: 4° 51' 18" S and 65° 17' 58" W), and processed at the Plant Protection laboratory/UFRA, Belém, PA, Brazil). Twelve *Trichoderma* spp. isolates were obtained by a serial dilution method (ZAMBOLIM; PEREIRA, 2012), conserved at potato dextrose agar (PDA) medium for posterior utilization in *in vitro* and *in vivo*. The isolate *T. asperellum*, donated by the Federal University of Viçosa, MG, Brazil, was used as a standard control.

The *Trichoderma* isolates for inoculation were prepared in two different forms: 1) powder obtained by grinding grain rice colonized by the *Trichoderma* spp. [6×10^8 conidia g^{-1}]; and 2) conidial suspension – the fungal culture was grown on PDA culture medium at ±25 °C, under continuous light for five days, and the standard concentration adjusted 6×10^8 conidia mL^{-1}.

Rhizoctonia solani isolate 4F was obtained from the culture collection of Embrapa Arroz e Feijão (Santo Antônio de Goiás, GO, Brazil) and the inoculum was produced in rice husk and grain culture medium RHG (three parts of rice husk: one part of rice grain) according to Sharma, Teng and Olivares (1990).

Six different *in vitro* bioassays were carried out with 13 *Trichoderma* isolates: T.06, T.07, T.09, T.12, T.13, T.20, T.21, T.34, T.42, T.46, T.47, T.52, and *T. asperellum* (a control isolate) in order to identify the most efficient bioagent against *R. solani*.

Antagonism: The experiment includes 13 treatments (12 *Trichoderma* spp. isolate and one control with *R. solani* only) and five replications. The mycelia discs (5 cm ∅) of the isolates of *R. solani* and *Trichoderma* spp. were transferred to the Petri dish (9 cm ∅) containing PDA. The discs were placed equidistant, 1.0 cm from edge of the plate, and incubated at 25 °C, during 72 hours. The diameter of *R. solani* colony was measured (cm), horizontally and vertically, and the data were statistically analyzed.

Hyphae interaction: The 13 isolates were evaluated using the procedure described above in Petri plates containing PDA. To analyze the interaction between *Trichoderma* spp. and *R. solani* hyphae, a slide was placed in the middle of the plate. After 48 hours the slides were observed using bromophenol blue stain [0,1 g L^{-1}], under optical microscope (40×).

Volatile toxic compounds: The lids of two Petri dishes containing PDA were used for this assay. Five cm diameter discs of *Trichoderma* spp. isolate was transferred to one lid of the dish and five cm diameter discs of *R. solani* were transfer to the other lid of the dish. Both lids were placed face to face, taped together and incubated at 25 °C, during three days. The assay was composed of 13 treatments, 12 *Trichoderma* spp. isolates and one control (containing five cm diameter discs of *R. solani* only) in five replications. The size of *R. solani* colonies was measured and data were statistically analyzed.

Phosphate solubilization: *Trichoderma* isolates were transferred to Petri dishes containing PDA culture medium, where tribasic calcium phosphate was used as the phosphate source, and incubated during 48 hours. The bromocresol purple (0,1 g L^{-1}) stain included in the media for acidification was the pH indicator (VÁZQUEZ et al., 2000). The positives for phosphate solubilization were identified when the purple medium became yellow. There were five treatments and three replications, five plates per replication and for each procedure plates were inoculated with 100 µL of a suspension of 1×10^6 conidia mL^{-1} and incubated at the temperature of 25 °C.

Indole acetic acid (AIA) or analogues production: The method described by Brito and Gagné (1995) was adopted. All the isolates were tested with and without the addition of five mM tryptophan. Water agar medium were inoculated with 100 µL of a suspension of 1×10^6 conidia mL^{-1} of *Trichoderma* spp. and covered with an 82-mm nitrocellulose membrane disk (RPN82D, Amersham, Buckinghamshire, UK). After seven days incubation at 25 °C, the membrane disks were removed and overlaid on Whatman Nº 2 (90 mm Ø) filter paper saturated with Salkowski reagent at room temperature. After five minutes strains producing IAA or analogues were identified by a characteristic pink or red colored halo in the membrane, and strains producing other indoles produced a yellow to yellow–brown pigment. The experiment in a completely randomized design consisted of four treatments (four *Trichoderma* spp. isolates and one control) with three replications. *Pseudomonas fluorescens* (Rizo-55) was used as a positive control.

Viability of *Rhizoctonia solani* sclerotia after *Trichoderma* spp. Treatment: The experiment was conducted in a completely randomized design with four replications. Six *R. solani* sclerotia were transferred to Petri dish containing 11- day old *Trichoderma* spp. colony grown on PDA medium. The treatments were composed with the best four isolates previously identified and one control, a PDA plate containing only *R. solani*. Treatments were incubated in the dark at 25 ± 2 °C, during 15 and 30 days. After sclerotia were transferred to water agar medium and incubated again during 24 hours to induce germinations and growth. The viability was determined by the number of germinated sclerotia and the size of formed colony.

The experiments on sheath blight suppression conducted under greenhouse conditions were all repeated twice. *Rhizoctonia solani* challenge inoculation: Seeds of rice cultivar BRS Jaburu were sown in pots containing 2.0 kg of the substrate Plantmax® (Eucatex Agro) fertilized with 5.0 g NPK (10-10-10) and, subsequently, three plants were maintained per pot under greenhouse conditions. Forty-five-days-old rice plants were challenge-inoculated with *R. solani* by adding 5.0 g of RHG inoculums around each plant, and incubated for 48 hours at temperatures ranging from 25 to 30 °C and 90% humidity.

Timing of application of *Trichoderma* spp. in suppressing sheath blight: The experiment was performed using a completely randomized block design in a factorial scheme in 4 × 4 + 1 [four *Trichoderma* spp. isolates (T.06, T.09, T.12, and T.52) × four application timings + one control] with four replications and three rice plants per pot. *R. solani* was challenge-inoculated 49 days after planting

The *Trichoderma* spp. treatments consisted of spraying 10 mL of a conidial suspension [6×10^8 conidia mL^{-1}] in 3 different moments: P1 – spraying *Trichoderma* spp. conidial standard suspension five days before challenge-inoculation with *R. solani*; P2 – simultaneously spraying *Trichoderma* spp. conidial suspension and challenge-inoculation with *R. solani;* P3 – spraying *Trichoderma* spp. conidial suspension six days after challenge-inoculation with *R. solani*; P4 – spraying *Trichoderma* spp. conidial suspension 12 days after challenge-inoculation with *R. solani*. The plants were incubated after challenge as described before. Disease severity was assessed based on lesion length measured five times beginning four days after inoculation every two days period. The area under the disease progress curve (ASCPD) was calculated considering all the five observations of disease severity according to Shaner and Finney (1977).

Seed treatment combined with curative sprays of *Trichoderma* spp. for sheath blight suppression: The experiment was performed used a completely randomized block design in a factorial scheme 4 × 3 + 1 [four *Trichoderma* spp. isolates (T.06, T.09, T.12, and T.52) × three application timings + one control] with four replications and three rice plants per pot. Rice seeds were treated with *Trichoderma* spp. powder (5.0 g kg^{-1} of seeds). *Rhizoctonia solani* was challenge-inoculated 47 days after planting. Later, 10 mL of a conidial suspension [6×10^8 conidia mL^{-1}] of *Trichoderma* spp. was sprayed at three different periods: E1 – seven days; E2 – 11 days; E3 – 15 days, after challenge-inoculation with *R. solani*. The plants were incubated after challenge as described before. Disease severity was assessed based on lesion length measured five times starting four days after inoculation, at every two days period. The AUDCP was evaluated for each treatment.

Seed treatment combined with dusting substrate with *Trichoderma* spp. for sheath blight suppression: The experiment was carried out using a completely randomized block design in a factorial scheme 4 × 3 + 1 [four *Trichoderma* spp. isolates (T.06, T.09, T.12, and T.52) × three methods of treatment with *Trichoderma* spp. + one control] with four replications and three rice plants. The efficiency of *Trichoderma* spp. isolates was evaluated using three methods: M1 – dry seed treatment with *Trichoderma* spp. in powder form; M2 – wet seed treatment with *Trichoderma* spp. using conidial standard suspension; M3 – dusting substrate with *Trichoderma* spp. (5.0 g 2 kg^{-1} of substrate). *R. solani* was challenge-inoculated 45 days after planting. The plants were incubated after challenge as described before. Disease severity was assessed based on lesion length measured five times starting four days after inoculation, at every two days period. The AUDCP was evaluated for each treatment.

Seed treatment combined with foliar spray of *Trichoderma* spp. for rice growth promotion: This experiment was performed using a completely randomized design with four replications, each one composed by a pot containing three plants. Rice seeds were treated with four *Trichoderma* spp. isolates (T.06, T.09, T.12, and T.52) powder (5.0 g kg^{-1} of seeds), 45-day old plants were sprayed with aqueous conidial standard suspension, and the control was sprayed only with water. The length of aerial part (shoot length) and roots was measured using digital paquimeter 45 days after treating the

plants with *Trichoderma* spp. and dry weight was determined after drying plants in an oven with forced air circulation at 70 °C for 72 hours.

The analyses of variance of all variables were performed and the means compared by the Scott-Knott test ($p < 0.05$).

3 Results and Discussion

Antagonism and production of volatile toxic compounds: All 13 *Trichoderma* spp. isolates evaluated were significantly different from the control in relation to antagonism to *R. solani* (Table 1). However, four isolates, T.06, T.52, T.12, T.09 were highly antagonistic compared to the others. The isolate T.06 performed as the best antagonist to *R. solani*, whereas T.52 was found to be the best isolate producing volatile compounds, compared to control (Table 1). Also, isolates T.12, T.09, and *T. asperellum* were significantly different from the control in relation to both antagonism as well as volatile compound producers (Table 1).

All *Trichoderma* spp. isolates were positive for phosphate solubilization. Isolates T.06, T.12, and T.52, in the absence of staining, presented higher growth rates (≥6.7 cm), and were statistically different from T.09 (2.2 cm) (Table 2). The isolates, T.12 and T.52 were positive for IAA production in the presence of tryptophan (Table 2).

Viability of *Rhizoctonia solani* sclerotia after *Trichoderma* spp. Treatment: Although, the isolates T.06, T.09, T.12, and T.52, after 15 and 30 days of incubation significantly reduced the mycelial growth of *R. solani* sclerotia (Figure 1), did not inhibit sclerotial germination.

In Greenhouse trials the timing of application of *Trichoderma* spp. in suppressing sheath blight. All *Trichoderma* spp. isolates tested, regardless of application timing, reduced sheath blight severity and ASCPD compared to the control. Spraying five days before challenge-inoculation (P1) proved was the most efficient treatment in reducing disease severity in rice plants. The lesion length on sheath was reduced to 4.88 cm compared to 13.75 cm

Table 1. Effect of *Trichoderma* spp. on the diameter of *Rhizoctonia solani* colonies by direct antagonism and production of volatile compounds.

Trichoderma spp. isolates	Diameter of *Rhizoctonia solani* colonies (cm)[2]	
	Direct antagonism	Volatile compounds
T.06	1.97a	8.00b
T.13	2.87b	7.93b
T.52	2.90b	1.40a
T.34	2.97b	8.00b
T.09	3.00b	5.45a
T.12	3.47c	5.17a
T.20	3.47c	8.00b
T.21	3.47c	8.00b
T.42	3.63d	8.00b
T.46	3.63d	8.00b
T.47	3.87d	7.20b
T.07	3.87d	8.00b
T. asperellum	4.07e	5.63a
Control	8.00f	8.00b
CV (%)	6.80	4.65

[1]Control: *R. solani*. [2]Means followed by the same letter are not significantly different ($p < 0.05$) according to the Scott-Knott's test. [3]Phosphate solubilization and Production of indole acetic acid (IAA) or analogues.

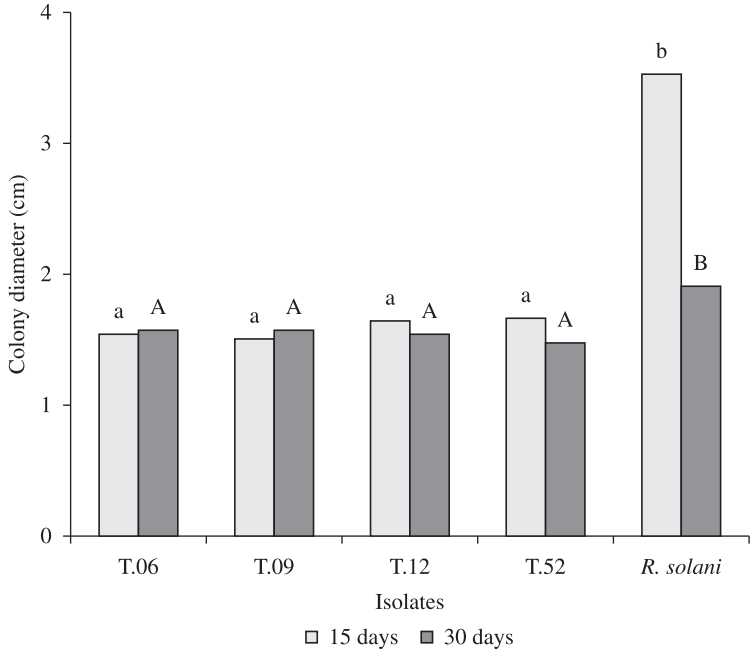

Figure 1. Effect of *Trichoderma* spp. on the colony diameter of *Rhizoctonia solani* sclerotia 15 and 30 days after incubation. Treatments: isolates of *Trichoderma* spp.: T.06, T.09, T.12, and T.52; control: *R. solani*. Barrs followed by the same letter are not significantly different ($p < 0.05$) according to the Scott-Knott's test.

Table 2. Production of metabolites and growth of isolates of *Trichoderma* spp. in culture medium.

Trichoderma spp. isolates	Phosphate solublization[1] (cm)		Auxin production[2]
	With stain	Without stain	
T.06	6.2a[3]	8.0a	–
T.09	3.4a	2.2b	–
T.12	3.7a	6.7a	+
T.52	3.3a	8.0a	+
CV (%)	22.28	19.26	–

[1]Phosphate solubilization activity by acid production, PDA culture medium, where tribasic calcium phosphate. The bromocresol purple (0,1 g^{-1} L) stain included in the media for acidification was the pH indicator (VÁSQUEZ et al., 2000). Were evaluated the diameter of the halo. [2] Production of auxins and/or analogues, + positive for AIA production; – negative for AIA production (BRITO; GAGNÉ, 1995). [3]Means followed by the same letter are not significantly different ($p < 0.05$) according to the Scott-Knott's test.

in the control (Figure 2a). In general the mean ASCPD of all *Trichoderma* spp. treatments was 150 against 235 of the control. However, no statistically significant differences were found between treatments with isolates T.06 and T.09 applied either as preventive spray (P1) or as foliar spray using conidial suspension applied simultaneously with *R. solani* challenge-inoculation (P2) (Figure 2b).

Seed treatment combined with curative sprays of *Trichoderma* spp. for sheath blight suppression: Seed treatment combined with curative sprays of *Trichoderma* spp. reduced disease severity compared to the control, regardless of isolate and application timing (Figure 2c). A lesion length of 5.5 cm was obtained in treated plants compared to 10.7 cm in control treatment. The ASCPD of E1 and E2, for isolates T.06, T.09 and T.12 were significantly lower than for T 52 and control (Figure 2d). However, the ASCPD of E3, only o the isolate T.12 was not differing of control treatment.

Seed treatment combined with dusting substrate with *Trichoderma* spp. for sheath blight suppression: All treatments (isolates × mode of application) significantly reduced lesion length on sheath blight. The lesion length ranged from 2.6 cm to 6 cm (Figure 2e). Of the 12 treatments, eight significantly reduced ASCPD. Treatments M2 (wet seed) and M3 (dusting substrate) were significantly lower than M1 for all tested isolates and control treatment considered the ASCPD (Figure 2f).

Seed treatment combined with foliar spray of *Trichoderma* spp. for rice growth promotion: The growth of the aerial parts was stimulated by isolates T.52, T.06, and T.09 and significantly differed from T.12 and the control . There was no increase in root length for any of the treatments (Table 3). Although three of four tested isolates stimulated biomass increase, T12 was the best one. Also, T12 followed by T.52 stimulated a root length increase statistically different from control (Table 3).

The i*n vitro* assays showed that more than one mechanism of action are simultaneously involved in the antagonistic action of *Trichoderma* against *R. solani*. *Trichoderma* isolate T.06 was able to effectively control the growth of pathogen and reduce *R. solani* colony diameter by 84% compared with control. The direct parasitism of *R. solani*, *Trichoderma* spp. cultivated in the presence of *R. solani*, may have led to the production and/or release of elicitor molecules that stimulate the biological control agent to secrete compounds with deleterious effects on the challenging pathogen (WIJESINGHE et al., 2010). In the present study, volatile toxic compounds produced by isolates T.52, T.12, T.09, and *T. asperellum* reduced *R. solani* mycelial growth by 83%, 35%, 33%, and 30%, respectively. Based on the results of direct antagonism and production of volatile toxic compounds, the isolates T.06, T.09, T.12 and T.52 were selected for subsequent trials. The sclerotial germination of *R. solani* was not affected by any of the four isolates selected. However, the results showed mycelial growth reduction of sclerotia by 57% after 15 days of incubation.

Greenhouse trials: The time and mode of application of the antagonist is an important factor in determining the efficacy of biocontrol active (WIJESINGHE et al., 2010). The results of greenhouse trials, in the present study showed that four of the 13 isolates selected reduced sheath blight severity when applied as seed treatment, substrate incorporation or as foliar spray. The preventive sprays (P1) was efficient in reducing lesion length on sheath blight by 43% and area under the disease progress curve by 65.5% and was found to be the best method. The possible explanation is that *Trichoderma* spp. colonizes the cut end of the culm before *R. solani* and reduce the chance of penetration by the pathogen. In order, to maintain high amount of propagules of antagonist in the soil, Caihong and Qian (2007) used weekly sprays of *T. harzianum* to control *Botrytis cinerea* with the combination of preventive and curative sprays. The seed treatment combined with the curative sprays of *Trichoderma* spp. and seed treatment combined with the application of *Trichoderma* spp. to substrate reduced lesion length on sheath blight by more than 30% in relation to control. The seed treatment with *Trichoderma* spp. formulated as suspension or as dust in two trials and the substrate treatment delayed the onset of epidemic (Figure 2). These results demonstrated the efficiency of Amazonian *Trichoderma* spp. isolates in reducing the initial inoculum in the rice × *R. solani* pathosystem. The addition of the antagonist to the substrate for growing is also important for controlling soil plant pathogens because this strategy permits colonization and establishment of these agents much before the plant is exposed to the inoculum present in the field. In the present work, the use of wet seed and dusting substrate with *Trichoderma* spp. indicated that the interact in more efficient way against *R. solani* than when applied as dry seed.

In the present investigation for growth promotion by *Trichoderma* spp. isolates, significant increase in biomass in terms of dry weight of aerial parts and roots by some isolates was evident. These results are in accord with those observed in other agricultural crops inoculated with specific strains of *Trichoderma* spp. by Harman et al. (2004). According to Hoyos-Carvajal, Orduz and Bissett (2009b) the increment in biomass related to production of plant growth hormones or analogues is another mechanism by which strains of *Trichoderma* spp. can enhance plant growth. Various species of fungi have been reported to produce auxins, which are key hormones effecting plant growth and development that can be produced by fungi in symbiotic interactions with plants (GRAVEL; ANTOUN; TWEDDELL, 2007). The isolates

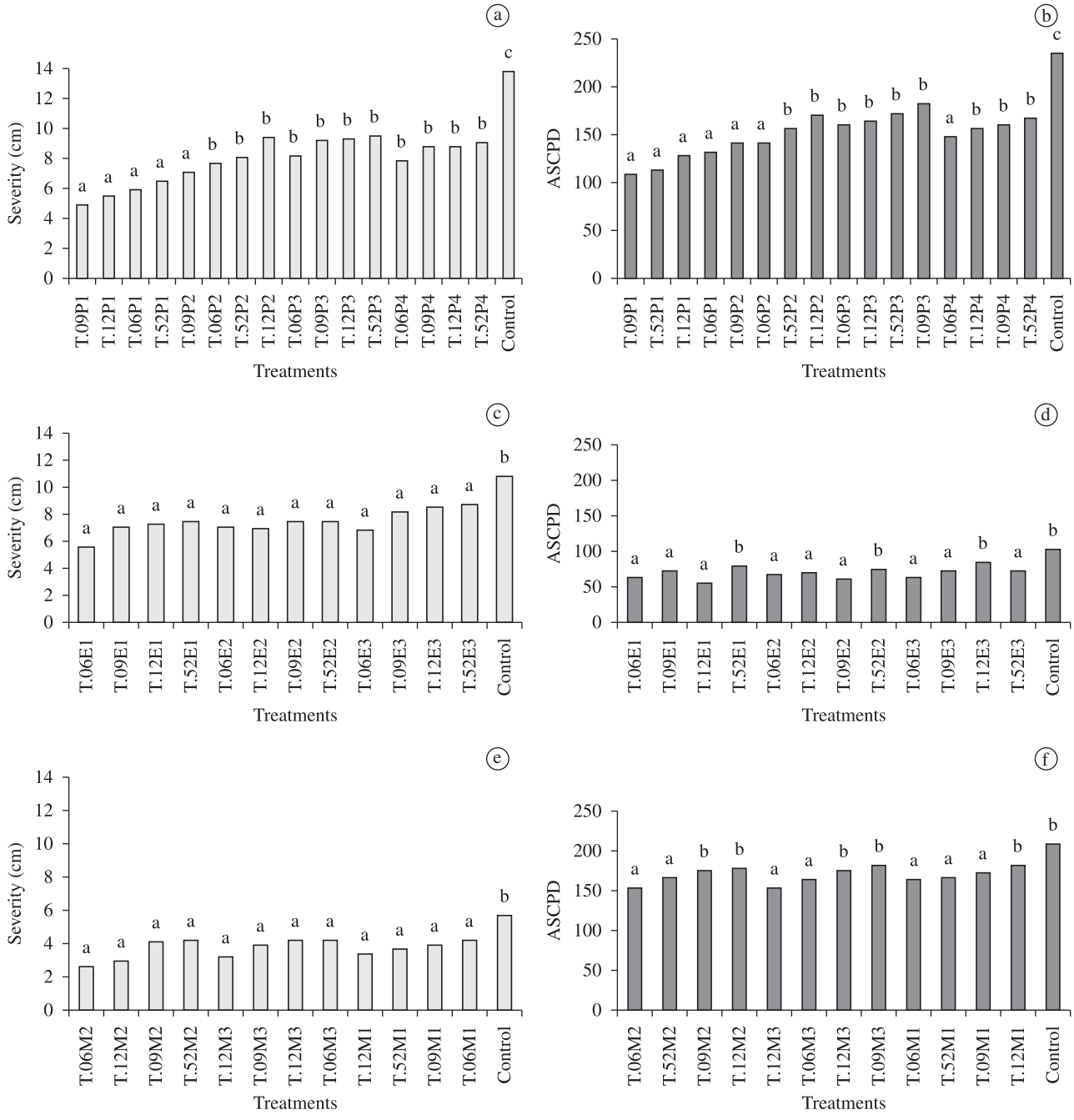

Figure 2. Sheath blight (*Rhizoctonia solani*) severity and area under the disease progress curve (ASCPD) in rice plants sprayed *Trichoderma* at four different timings (P1, P2, P3 and P4) (a and b), seeds were treated with *Trichoderma* combined with three curative spray application timings (E1, E2 and E3) (c and d) and three methods of formulation of *Trichoderma*, in powder and conidial suspension (M1, M2 and M3) (e and f). For the controls were plants use only with water and inoculated with the *R. solani*. Means followed by the same letter do not significantly differ according to the Scott-Knott test ($p < 0.05$).

T.12 and T.52 which were positive to produced IAA also promoted increased biomass (aerial and root parts) in rice plants, indicating the relation between hormone production and biomass. Metabolic factors that were considered beside auxin production, for example is the capacity for phosphate solubilization. Phosphorus frequently is the least accessible macronutrient in many ecosystems and its low availability is often limiting to plant growth (RAGHOTHAMA, 1999). Altomare et al. (1999) reported three possible mechanisms by which *Trichoderma* spp. might convert phosphate to a soluble form (i) acidification, (ii) production of chelating metabolites, and (iii) redox activity, concluding that chelation was the more likely mechanism for P solubilization by *Trichoderma* spp. In the present study, all *Trichoderma* spp. isolates showed sufficient acid production to lower the pH of media, but T.06, T.12, and T.52 presented higher growth rates in the absence

Table 3. Effect of seed treatment with isolates of *Trichoderma* spp. on growth promotion of rice.

Treatment	Length (cm)		Dry Biomass (mg)	
	Shoot (SL)	Root (LR)	Shoot (SL)	Root (LR)
T.52	79.10a[1]	21.80a	2.10b	0.65b
T.06	75.30b	20.40a	1.70c	0.20d
T.09	74.30b	20.20a	1.50d	0.40c
T.12	71.80c	19.00a	3.40a	0.70a
Control	72.70c	21.70a	1.32d	0.47c
CV (%)	2.34	8.30	8.61	19.74

[1]Means followed by the same letter are not significantly different ($p < 0.05$) according to the Scott-Knott's test.

of staining, indicating that acidification of the media com bromocresol, indicated by production of a yellow pigment was not necessarily an effective indicator of phosphate solubilization (HOYOS-CARVAJAL; ORDUZ; BISSETT, 2009b). According to Fernández et al. (2008) the tropical soils are typically acidic (pH 4–5) and show low availability of labile phosphorus, *Trichoderma* spp. isolates may be used for reliable phosphate solubilization in tropical soils.

4 Conclusions

The findings of this investigation showed that selected *Trichoderma* spp. isolates from soils of Amazon forest, reduced sheath blight severity on rice, mainly when used in a preventive spray method, in greenhouse conditions. Furthermore, three of them improved biomass of rice plants showing also the potential as growth promoters.

Acknowledgements

We are grateful to Fundação de Amparo à Pesquisa do Estado do Pará (FAPESPA) for providing financial support his research and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for granting a research fellowship to the first author.

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