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Interaction between thidiazuron and *Azospirillum brasilense* on yield characteristics and productivity of rice

Fernando de S. Buzo[1], Lucas M. Garé[1], Orivaldo Arf[1], José R. Portugal[1], Flavia C. Meirelles[2] & Nayara F. S. Garcia[1]

[1] Universidade Estadual Paulista "Júlio de Mesquita Filho"/Faculdade de Engenharia. Ilha Solteira, SP. E-mail: fsbuzo@gmail.com (Corresponding author) - ORCID: 0000-0003-4103-8171; lucasmgare@gmail.com - ORCID: 0000-0001-6331-2499; arf@agr.feis.unesp.br - ORCID: 0000-0002-8468-0773; jrp.agrunesp@gmail.com - ORCID: 0000-0002-7767-4455; nayaragarcia.agro@gmail.com - ORCID: 0000-0003-3806-9698

[2] Universidade Estadual Paulista "Júlio de Mesquita Filho"/Faculdade de Ciências Agrárias e Veterinárias. Jaboticabal, SP. E-mail: flavia.meirelles1905@gmail.com - ORCID: 0000-0003-3923-8449

ABSTRACT: In rice, the use of the bacterium *Azospirillum brasilense* has shown to be very useful for the crop, being able to increase the final productivity of the same or reduce the need for nitrogen in topdressing. It has recently been noted that there are other regulators that perform other functions, such as thidiazuron (TDZ), a cytokinin-like substance that has been shown to significantly increase upland rice productivity. Thus, the present work aimed to verify the effect of the interaction between the foliar application of *A. brasilense* and thidiazuron doses applied at the occasion of the tillering of the plants on the productive aspects and crop productivity. The experiment was carried out in the agricultural years of 2015/16 and 2016/17, at the UNESP Teaching, Research and Extension Farm belonging to the Engineering Faculty of Ilha Solteira - UNESP, located in the municipality of Selvíria, MS. The experimental design was a randomized complete block design with a 2 x 4 factorial scheme with four replicates. The treatments were the combination of the presence or absence of *A. brasilense* foliar application with doses of TDZ (0, 0.5, 1.0 and 1.5 g ha^{-1}) applied to upland rice cultivar BRS Esmeralda. Based on the results obtained for plant height, number of panicles per square meter, total number of grains and fertility of spikelets, mass of 100 grains and grain yield, it was concluded that, in the two agricultural years observed, there were no interaction and individual effects of the foliar application of the bacteria, and of the TDZ doses used at the time of tillering in the BRS Esmeralda cultivar.

Key words: BRS esmeralda, cytokinin regulator, diazotrophic bacteria

Interação entre thidiazuron e *Azospirillum brasilense* nas características produtivas e produtividade do arroz

RESUMO: No arroz, o uso da bactéria *Azospirillum brasilense* tem se mostrado de grande utilidade para a cultura, podendo incrementar a produtividade final da mesma ou ainda, reduzir a necessidade de nitrogênio em cobertura. Recentemente notou-se que há outros reguladores que desempenham outras funções, como o thidiazuron (TDZ), uma substância de efeito citocinínico que se mostrou capaz de aumentar significativamente a produtividade do arroz de terras altas. Desse modo, o presente trabalho objetivou verificar o efeito da interação entre a aplicação foliar de *A. brasilense* e de doses do thidiazuron aplicadas por ocasião do perfilhamento das plantas sobre os aspectos produtivos e produtividade da cultura. O experimento foi realizado nos anos agrícolas de 2015/16 e 2016/17, na Fazenda de Ensino, Pesquisa e Extensão pertencente à Faculdade de Engenharia de Ilha Solteira - UNESP, situada no município de Selvíria-MS. Utilizou-se o delineamento experimental de blocos ao acaso, com esquema fatorial 2 x 4 com quatro repetições, cujos tratamentos foram a combinação entre a presença ou ausência da aplicação foliar de *A. brasilense* com doses de TDZ (0; 0,5; 1,0 e 1,5 g ha^{-1}) aplicadas no arroz de terras altas cultivar BRS Esmeralda. Com base nos resultados obtidos para altura de plantas, número de panículas por metro quadrado, número total de grãos e fertilidade de espiguetas, massa de 100 grãos e produtividade de grãos, chegou-se à conclusão de que, nos dois anos agrícolas observados, não houve interação e efeitos individuais da aplicação da bactéria via foliar, e das doses de TDZ utilizadas por ocasião do perfilhamento na cultivar BRS Esmeralda.

Palavras-chave: BRS esmeralda, regulador citocinínico, bactérias diazotróficas

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Introduction

Rice originated in Asia and is one of the most cultivated cereals in the world, making up the diet of half the world's population (Kumar & Ladha, 2011). In Brazil, there is predominance of rice cultivation in the Southern region with flood irrigation system. This mode of cultivation is limited due to certain environmental impact and high water demand (Heinemann & Stone, 2009). Thus, upland cultivation in the Midwest region has become a viable alternative for crop expansion (Kluthcouski et al., 1991; EMBRAPA, 2006). However, there is the disadvantage of having a lower productivity than the Southern region (Mendes et al., 2014).

At the time of tillering, the application of thidiazuron (TDZ) at a dose of 0.9 g ha^{-1} on rice crop was beneficial in the sense that it increased the productivity of cv. BRS Esmeralda by 23.5% (Alves et al., 2015). This is phenyl urea with a cytokine-like action (Henny & Fooshee, 1991). Taiz et al. (2017) explained that cytokinins promote increased productivity by stimulating nutrient mobilization and establishing strong drains that are more favored in competition for nutrients, confirming that TDZ can increase crop productivity.

Biological nitrogen fixation (BNF) can reduce the use of nitrogen fertilizers and thus the cost of crop production, since nitrogen fertilization accounts for the highest cost among the fertilizers used (Campos et al., 2003). Bacteria of the genus *Azospirillum* can perform biological nitrogen fixation in association with grasses, in addition to producing substances that promote plant growth (Hungria, 2011).

Thus, in view of the significant contribution of inoculation with *A. brasilense* and the application of thidiazuron as a growth regulator, this study aimed to verify the effect of the foliar application of *A. brasilense* and doses of thidiazuron applied at the occasion of tillering and the interaction of both on the productive aspects and productivity of upland rice.

Material and Methods

The experiment was conducted in the agricultural years of 2015/16 and 2016/17, in an experimental area of the Engineering Faculty of Ilha Solteira - UNESP, located in the municipality of Selvíria, MS, (approximately 51º 22' O and 20º 22' S), with altitude of 335 m. In the experimental area, there is a typical Red Clay Latosol (Santos et al., 2013), originally occupied by Cerrado vegetation. The soil chemical analysis of the area in the 0-20 cm layer, performed according to Raij et al. (1996), resulted in the following characteristics: P_{resin} = 33 mg dm^{-3}; MO = 21 g dm^{-3}; K, Ca, Mg and SB = 3.4, 20, 13, 36.4 $mmol_c$ dm^{-3}, respectively; pH, Al and H+Al equal to 5.3, 0 and 34 $mmol_c$ dm^{-3}, respectively; S-SO_4 = 2 mg dm^{-3}; CTC = 70.4 $mmol_c$ dm^{-3} and V = 52%.

The climate of the region is the Aw type, according to the Köppen classification, with a maximum annual temperature of 31 °C and a minimum annual temperature of 19 °C, with an average annual rainfall of 1.313 mm, annual relative humidity of 70 to 80% (Centurion, 1982). In Figure 1 it is possible to observe data of rainfall, minimum and maximum air temperatures in the first year of the trial, referring to the harvest of 2015/16 (Figure 1A) and in the second year of the trial, referring to the 2016/17 harvest (Figure 1B).

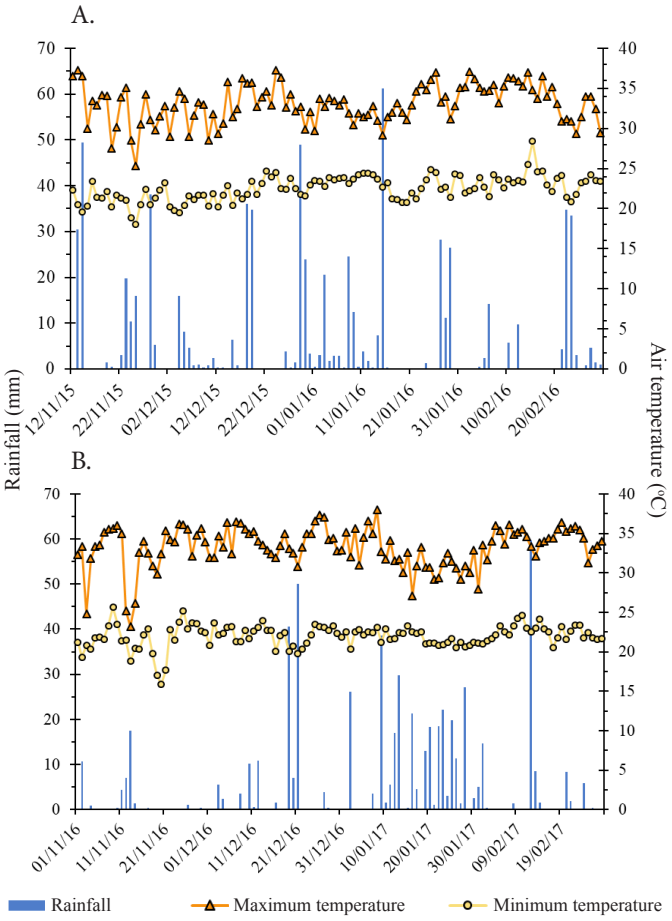

Figure 1. Climatic data for the two rice growing seasons 2015/16 (A) and 2016/17 (B)

A randomized complete block design was used for the experiment, with a 2 x 4 factorial arrangement, with four replications. The treatments consisted in the application or not (presence or absence) of an inoculant containing 2 x 10^8 viable cells of the Ab-V_5 and Ab-V_6 strains of *A. brasilense* bacteria per mL of the commercial product Masterfix gramíneas® at a fixed dose of 200 mL ha^{-1} (the dose is based on work commonly performed in the region and following the recommendations of the inoculant), combined with four doses of the plant regulator thidiazuron (0, 0.5, 1.0 and 1.5 g ha^{-1}), which were defined after analyzing the study of Alves et al. (2015). Each plot had five rows of 5.0 m, spaced 0.35 m between rows, and only three central rows of each plot were considered observation area.

The foliar application of *A. brasilense* was carried out by direct spraying using a knapsack sprayer with manual TX-conical beak and VS2 approximate spray volume of 300 L ha^{-1}. In the first year, this operation was carried out at 27 days after emergence (DAE) and in the second year at 20 DAE. On the occasion of tillering, the doses of thidiazuron (TDZ), with the same previous conditions, were applied at 29 and 22 DAE in the first and second years, respectively. All these applications were made at the end of the day, with weather conditions favorable to the application, without strong winds that caused drift.

In the two-year experiment, the soil was mechanically prepared using a plow and two harrows. Seeding was performed mechanically, with regulation for density of 70 kg ha^{-1} of

seeds on November 12, 2015 (first year) and November 5, 2016 (second year). Before sowing, the seeds were treated with imidacloprid + thiodicarb (45 g + 135 g of a. i. 100kg^{-1} of seeds) for the control of termites and borer caterpillar. Fertilization in the sowing furrow was 150 kg ha^{-1} of NPK 08-28-16 formulation in the two-year trial. Immediately after sowing, the pre-emergence herbicide pendimetalin (1,400 g ha^{-1} a. i.) was administered. In the first and second years, emergence occurred five days after sowing, on November 17, 2015 and November 10, 2016 respectively.

The cultivar used was BRS Esmeralda, which is characterized by high productivity and quality of grain, good tolerance to water stress, high rusticity and good ability to "stay green". Its productive potential is higher than 7,000 kg ha^{-1} and its average productivity is 4,050 kg ha^{-1}, with an approximate cycle of 105-110 days (Castro et al., 2014).

As required, the irrigation was performed by a sprinkler system fixed with a mean precipitation of 3.3 mm h^{-1}. Topdressing fertilization was performed using 70 kg ha^{-1} of N whose source was ammonium sulfate, between 30 and 40 days after emergence of the plants in the two years of cultivation. Soon after fertilization irrigation was carried out, providing a water deth of approximately 15 mm.

For the control of weeds after emergence of the crop, 2.2 g ha^{-1} methyl methsulfuron was applied at 21 days after emergence (DAE) of the crop in the first year and at 22 DAE in the second year. Nevertheless, until the end of the crop cycle, in the first year it was necessary to perform two manual weeding after the application of the post-emergence herbicide. On the occasion of the flowering (02/02/2016 and 23/01/2017, in the first and second harvests, respectively), an insecticide (thiamethoxan, 25 g ha^{-1}) was applied to control the stink bug and fungicide (trifloxystrobin + tebuconazole, 100 + 200 g ha^{-1}) as a preventive measure for blast.

At the end of the cycle, at 100 DAE in the first year and at 102 DAE in the second year, the manual harvesting of two central lines of each experimental plot was carried out; thereafter, the grains were placed in paper trays for natural drying in the shade, in order to reach a moisture content closer to 13% (this natural drying was accompanied by periodic humidity measurements of some samples using an electronic and portable grain moisture meter Gehaka G650i).

During the conduction of the experiment, the evaluations were: height of plants (m), measuring the distance from the soil surface to the upper end of the highest panicle, three points randomly within the observation area of each plot and calculating the average height for each plot; number of panicles per square meter, counting the amount of panicles present in a meter of a line in the observation area of each plot and extrapolating this value to the quantity per m^2; total number of spikelet and spikelet fertility (%), through the collection of 20 panicles per plot at time of harvest and counting of filled and unfilled grains; mass of 100 grains (g), obtained by counting and weighing one hundred grains from each plot, converting the value to moisture content of 13% wet basis (w.b.); grain yield (kg ha^{-1}), estimated by weighing the harvested grains from each plot, extrapolating the values to 1 ha and converting to a moisture content of 13% (w.b.); mass of the hectoliter (kg hL^{-1}), obtained by means of an appropriate scale and correction of the values for moisture content of 13% (w.b.).

The data were submitted to analysis of variance to verify if there was a significant difference between the treatments and interaction between the factors and when it happened, polynomial regression was performed for TDZ doses.

Results and Discussion

Plant height was not affected by the foliar application of *A. brasilense* nor by the doses of thidiazuron used in the two agricultural years (Table 1). It is possible to observe that, in the second year, plant height was slightly higher than in the first year, and in both cases was less than 1.15 m. In the two harvests there was also no interaction between the two factors for plant height.

The absence of significant results in this case is understandable, since the literature indicates that the action of *A. brasilense* on the rice plants is variable, and whether or not there is influence, depends on the cultivar used, the mode of

Table 1. Mean values for plant height, panicles m^{-2}, total number of spikelets and fertility of spikelets of cv. BRS Esmeralda due to the foliar application of *A. brasilense* and doses of thidiazuron (2015/16 and 2016/17)

Treatments	Plant height (m)		Panicles m^{-2}		Total spikelets		Spikelet fertility (%)	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
				A. brasilense (A)				
With	1.12	1.12	223	251	165	140	81.44	79.24
Without	1.10	1.15	206	257	166	152	81.26	80.77
				Doses of thidiazuron (D)				
(g ha^{-1})								
0	1.10	1.14	219	264	162	145	83.07	83.26
0.5	1.11	1.14	214	246	170	143	80.86	78.78
1.0	1.12	1.12	214	272	167	151	81.30	78.66
1.5	1.10	1.14	211	236	166	145	80.11	79.32
				F test				
A. brasilense (A)	2.12^{ns}	2.94^{ns}	2.27^{ns}	0.21^{ns}	0.26^{ns}	0.35^{ns}	0.02^{ns}	0.81^{ns}
Dose (D)	0.35^{ns}	0.25^{ns}	0.09^{ns}	1.51^{ns}	0.30^{ns}	3.91^{ns}	0.73^{ns}	1.65^{ns}
A x D	0.76^{ns}	1.87^{ns}	0.37^{ns}	0.84^{ns}	0.95^{ns}	0.13^{ns}	0.47^{ns}	0.82^{ns}
MSD	0.03	0.04	22.95	27.56	12.10	12.29	3.01	3.54
CV	3.63	5.18	14.55	14.74	9.91	11.44	5.04	6.02
General mean	1.11	1.13	215.00	254.29	166.00	146.09	81.35	80.00

^{ns} Not significant by the F- test; MSD – Minimum significant difference; CV – Coefficient of variation

application or even the environmental conditions in which the cultivation was carried out. In the works of Gitti et al. (2012), Garcia (2017) and Garé et al. (2017), there was no effect of inoculating seeds with this bacterium, as in the present work. Rodrigues et al. (2015) observed that there was interaction between the factors cultivar and inoculation, so that only cv. IAC 202 showed higher inoculated plants in relation to the uninoculated treatment, proving that the results of the inoculation depend on factors such as inoculated cultivar.

As for TDZ the literature for its use in cereals is still scarce. In the work of Alves et al. (2015), for example, TDZ doses had no effect on the plant height of the two cultivars used (BRS Esmeralda and IAC 202). On the other hand, Garé et al. (2017) demonstrated that the height of cv. ANa 5015 was adjusted to a quadratic equation as a function of TDZ doses, whose estimated maximum value was 1.11 m with a dose of 0.41 g ha^{-1}.

It is a fact that plant height is stimulated by the presence of cytokinin regulators, since they promote cell division in the apical meristem of the stem and the subsequent cellular elongation (Taiz et al., 2017). However, it is important to note that when endogenous levels of cytokinin in the plant have been adequately supplied, it probably will not respond to the exogenous application of a similar plant regulator.

The number of panicles per m^2 was not affected by the foliar application of *A. brasilense* nor by the doses of thidiazuron applied at the time of tillering and, likewise, there was no interaction between the factors (Table 1).

The literature indicates that inoculation with *A. brasilense* does not generally affect the number of panicles per m^2 of upland rice, as observed in the present work. This is due to the fact that this characteristic is defined mainly by genetic factors (Rodrigues et al., 2015; Chaves et al., 2016; Garcia, 2017).

Like the results of this study, the number of panicles per m^2 for the two cultivars used was not affected by TDZ in the studies of Alves et al. (2015) and Garé et al. (2017). However, cytokinins may stimulate the development of tiller buds in rice plants by promoting the proliferation of lateral meristems and breaking dormancy, facilitating lateral sprouting in certain crops (Yookongkaew et al., 2007; Liu et al., 2011).

Possible explanations for obtaining results which differ from expectation regarding the effects of TDZ would be that the endogenous level of cytokinins in the rice plants in that assay was already in adequate quantity or that TDZ application was performed after the definition of the number of buds that gave rise to the rice plant, causing the external application not to have effect (Salisbury & Ross, 2013).

The total amount of spikelets per rice panicle and the fertility of the spikelets were unaffected by either the bacteria or the plant regulator applied in the two agricultural years (Table 1). These characteristics depend on genetic factors and the external conditions to which the plant is subjected, mainly during the reproductive phase, from its beginning to about five days before flowering, such as temperature, solar radiation and available nutrients (Buzetti et al., 2006; Fornasieri Filho & Fornasieri, 2006).

Garcia (2017) also did not detect the influence of the inoculation with *A. brasilense* on the number of spikelets per panicle nor on the fertility of the spikelets. This result is corroborated by Gitti et al. (2012) that when inoculating rice seeds with the same bacterium and combining N doses in topdressing, differences were not obtained in the values of spikelets and pines, nor interaction of the inoculation with the doses of nitrogen fertilizer used.

As for the application of thidiazuron, Garé et al. (2017) also did not notice response to the doses of this regulator for the total amount of spikelets nor for the quantity of filled and unfilled grains per panicle. On the other hand, Alves et al. (2015) demonstrated that the dose of 0.9 g ha^{-1} of TDZ resulted in a 13.4% increase in the amount of spikelets per panicle in relation to the control treatment.

It is known that TDZ, as a cytokinin agent, can delay foliar senescence and extend the photosynthetic production of plants, besides promoting the mobilization of nutrients to the treated regions by forming a new source-drain relation, improving the quality of fruits (Vieira et al., 2008; Taiz et al., 2017). Thus, the application of TDZ could increase the total amount of spikelets and their fertility, as verified in the work of Alves et al. (2015).

Unfavorable environmental conditions promote imbalance of phytohormones during the filling of rice grains, reducing the endogenous levels of cytokinins, for example, and damaging the grain filling (Xu et al., 2007). Again, resorting to the idea that if endogenous cytokinin levels are sufficient for the plant, it will not respond to the exogenous application of a plant regulator, it is possible to understand the absence of results for the total amount of spikelets and their fertility in the TDZ doses used in the present study.

The hectoliter mass, the mass of 100 grains and the yield of rice grains were unaffected by inoculation with *A. brasilense* in the two harvests studied (Table 2). The same was observed by Gitti et al. (2012) and Garé et al. (2017), corroborating the results of the present study. Considering this, the idea that the mode of inoculation used (foliar route) is not very effective could be conceived and, therefore, was the reason for not obtaining significant results in the analyzed variables. However, Goes et al. (2017) inoculated the rice seeds with *A. brasilense* instead of foliar application but did not verify significant differences for the hectoliter mass and productivity of the crop. Garcia et al. (2016) also found no change in the hectoliter mass at any of the doses or forms of application of the bacterium in the rice, but observed the effect of the rates of application of the bacteria on the one hundred grain mass and crop productivity independently of the mode of application. Thus, it is possible to affirm that the mode of application of the bacterium is not a preponderant factor in obtaining results from the crop.

The doses of thidiazuron applied at the tillering of the plants did not affect the hectoliter mass, the mass of one hundred grains and neither the yield of the rice grains in the two harvests analyzed (Table 2). The work of Garé et al. (2017) shows a similar result, differing from the present study only because it used a different cultivar (ANa 5015).

Alves et al. (2015) showed an opposite result, in which the TDZ affected the mass of one hundred grains of the cultivars BRS Esmeralda and IAC 202, caused a drop in the hectoliter mass of cv. BRS Esmeralda and increment in the same variable when applied to cv. IAC 202. In their work, the productivity of both cultivars were adjusted to positive linear equations as a function of the TDZ doses used, resulting in a 23.5% increase

Table 2. Mean values for hectoliter mass, mass of one hundred grains and productivity of cv. BRS Esmeralda due to the foliar application of *A. brasilense* and doses of thidiazuron (2015/16 and 2016/17)

Treatments	Hectoliter mass (kg hL^{-1})		Mass of one hundred grains (g)		Productivity (kg ha^{-1})	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
A. brasilense (A)						
With	55.69	48.16	2.72	2.64	5,491	5,086
Without	54.69	48.78	2.73	2.70	5,372	5,056
Doses of thidiazuron (D)						
(g ha^{-1})						
0	55.56	48.92	2.81	2.63	5,397	4,951
0.5	55.15	48.23	2.71	2.68	5,519	5,033
1.0	55.34	47.89	2.73	2.68	5,542	5,191
1.5	54.61	48.86	2.68	2.68	5,270	5,109
F Test						
A. brasilense (A)	1.78^{ns}	1.35^{ns}	0.02^{ns}	1.66^{ns}	0.34^{ns}	0.04^{ns}
Dose (D)	0.34^{ns}	0.88^{ns}	2.60^{ns}	0.26^{ns}	0.38^{ns}	0.48^{ns}
A x D	0.34^{ns}	0.42^{ns}	1.45^{ns}	1.84^{ns}	2.03^{ns}	0.05^{ns}
MSD	1.56	1.11	0.07	0.10	422.00	307.88
C.V.	3.85	3.12	3.43	5.14	10.57	8.26
General mean	55.19	48.47	2.73	2.67	5,432.00	5,071.16

^{ns} Not significant by the F test; MSD - Minimum significant difference; CV - Coefficient of variation

in cv. BRS Esmeralda and 6.5% of cv. IAC 202 at the dose of 0.9 g ha^{-1} compared to the control.

The physiological effects of cytokinin regulators on plants, such as extending the photosynthetic production of plants by delaying leaf senescence, promoting nutrient mobilization by forming a new source-drain relationship, and stimulating cell division and cell stretching justify the use of TDZ in the rice crop, with the objective of increasing productivity. The absence of these beneficial effects in the results of the present study can be explained by the idea already mentioned that in the two harvests discussed here, the plants were submitted to the environmental conditions that allowed the endogenous production of cytokinins sufficient to meet the physiological needs of the plants, precluding the beneficial effects of TDZ application as an exogenous cytokinin.

The existing and above results show that further studies are needed on the subject in order to understand how and in which situations the exogenous application of cytokinin may benefit sprinkler irrigated upland rice cultivation.

Conclusions

1. Plant height was not affected by the foliar application of *A. brasilense* nor by the doses of thidiazuron.

2. The foliar application of *Azospirillum brasilense* did not increase the yield of grains and the productive characteristics of upland rice.

3. The doses of thidiazuron applied at the tillering of rice plants did not influence the various production components and productivity of the upland rice cv. BRS Esmeralda.

4. There was no interaction between the two factors analyzed in terms of the productive characteristics and productivity of upland rice irrigated by sprinkler.

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