academicJournals

Vol. 13(49), pp. 4501-4507, 3 December, 2014
DOI: 10.5897/AJB2014.14059
Article Number: 0C75A1348918
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Synergistic action of *Azospirillum brasilense* combined with thiamethoxam on the physiological quality of maize seedlings

Andre Gustavo Battistus[1*], Tiago Luan Hachmann[1], Thaísa Muriel Mioranza[1], Mônica Anghinoni Müller[1], Tiago Madalosso[1], Patricia Aparecida Favorito[1], Vandeir Francisco Guimarães[2], Jeferson Klein[2], Débora Kestring[1], Adriano Mitio Inagaki[1] and Lucas Guilherme Bulegon[1]

[1]State University of West Paraná, Pernambuco street, Number 1777, Zip Code: 85960-000, Box: 91, Marechal Cândido Rondon, Paraná, Brasil.
[2]Pontifical Catholic University of Paraná, Avenue of Union, Number 500, Zip Code: 85902-532, Jardim Coopagro.Toledo, Paraná, Brasil.

Received 20 July, 2014; Accepted 25 November, 2014

Plant growth promoting substances, either from biological or chemical origin, are widely used in modern agriculture. In this context, this study evaluated the physiological quality of maize seed treated with thiamethoxam and inoculated with *Azospirillum brasilense*. Initially, we tested bacterial growth on medium with increasing concentrations of thiamethoxam. We also evaluated the physiological quality of seeds through morphometric measurements, emergence speed index and chlorophyll content by SPAD through a 2x3 factorial arrangement with seed inoculation with *A. brasilense* (100 mL per 25 kg of seed) and three doses of thiamethoxam (0, 80 and 120 ml per 60,000 seeds). Bacterial population showed a linear reduction according to increasing doses of thiamethoxam. The dose of 120 ml thiamethoxam (42 mg active ingredient) caused a decrease in several variables, while, the inoculation proved a positive activity in seedlings physiological quality. Thus, the inoculation combined with 80 ml thiamethoxam (28 mg active ingredient) show synergistic action in early development in maize seedlings.

Key words: Thiamethoxam, inoculant, plant growth promoting bacteria.

INTRODUCTION

Maize (*Zea mays* L.) is a cereal grown on all continents, with high economic relevance due to the variety of forms of use (Fancelli and Dourado Neto, 2003). This is possible because this cereal is used in numerous products, ranging from food and feed to the high-tech industry (Paes, 2008). Recent data indicate the high importance of this crop in Brazil, because only in the 2013/2014 season the country produced about 75.18 million tons of this cereal in a planted area of 15.12 million hectares (National Supply Company, 2014). These numbers are the result of years

*Corresponding author. E-mail: andre_battistus@hotmail.com. Tel: +55(45) 32847878. Fax: +55(45) 32847879.

of research, producing improved cultivars, inputs and appropriate farming techniques, coupled with expansion of the planted area (Paes, 2008). Plant growth promoting substances have propelled the recent increases in productivity. These substances have been widely used in modern agriculture, and have biological or chemical origin (Busato et al., 2010). One of the most frequently growth promoting substances used today is the molecule thiamethoxam, a synthetic insecticide with effect of plant bioactivation by triggering several physiological reactions. It promotes the activation of membrane transport proteins, and enzymatic activation, which increases plant metabolism through the synthesis of amino acids, precur-sors of proteins, and endogenous synthesis of plant hormones (Carvalho et al., 2011), but without exhibit activity of plant regulation (Castro et al., 2007).

Biochemically, this insecticide stimulates the activity of peroxidases, whose activity focuses on combating reactive forms of oxygen, before they damage biomolecules, preventing plant cell death, being indirect effects of their mechanisms of action observed by increased biomass, increased photosynthetic rate and formation of deeper roots (Almeida et al., 2012). Likewise, plant growth promoting substances may also be of biological origin, such as the plant growth promoting bacteria. Microorganisms of the genus *Azospirillum* are capable to survive, both in the rhizosphere and inside plants belonging to the family Poaceae (Huergo et al., 2008). These microorganisms stimulate plant development especially due to biological nitrogen fixation (Hungria, 2011), phosphate solubilization (Rodriguez et al., 2004), hormone production (Perrig et al., 2007) and reduction of ethylene activity through the action of the enzyme ACC deaminase (Blaha et al., 2006).

However, many researchers combine the effects of *Azospirillum* mostly to production or stimulation of hormones, promoting an increase in the plant size and root development verified by Rampim et al. (2012) and Dartora et al. (2013), being results mainly associated with indole-3-acetic acid (IAA) and gibberellins (Bashan et al., 2004; Perrig et al., 2007; Radwan et al., 2004). According to Hungria et al. (2010), the greater root development can also provide better absorption of macro and micronutrients by the plant.

In this context, considering the use of chemical and biological products to promote an increase in plant development, it is necessary to check if the effects are synergistic when applied together. Thus, this study aimed to evaluate the physiological quality of maize seedlings in the association of thiamethoxam seed treatment and inoculation with *Azospirillum brasilense* strain AbV5, as well as the effect of the insecticide on the bacterial population.

MATERIALS AND METHODS

The work was developed in the Laboratory of Plant Physiology and Laboratory of Phytopathology, State University of West Paraná – Unioeste, Campus Marechal Cândido Rondon, Paraná State, in October 2013. Strain of *A. brasilense* AbV5 used in the study was obtained from the collection of growth promoting bacteria of Federal University of Paraná - Campus Curitiba, Paraná, Brazil. Bacterial cells were grown in enlermeyers flask with 250 mL containing 100 mL of NFb medium (Dobereiner et al., 1976) at 30°C under constant agitation at 80 rpm, until exponential growth phase at optical density ($OD_{450\ nm}$) of 0.5, corresponding to population of 6.67 x 10^7colony-forming units (CFU) ml^{-1} in NFb-agar. Initially, we tested the toxicity of the insecticide thiamethoxam on the bacterial population. To this end, solid medium DYGS (Baldani, 1996) were added with doses of Cruiser® (350 mg thiamethoxam ml-1) at 0; 40; 80; 120; 160 and 200 ml of commercial product (c.p.), corresponding to 0; 14; 28; 42; 56 and 70 mg of thiamthoxam per 60,000 seeds, while the medium was at 45 to 55°C, so that the active ingredient was not inactivated. Thiamethoxam was sterilized by filtration through Millipore filter (0.25 µm) and homogenized with the medium in a continuous air flow chamber, and distributed in 20 ml Petri dishes.

The inoculant containing *A. brasilense* strain AbV5 was serially diluted by adding 100 ml of inoculant to 900 µl of saline solution (0.85% NaCl), and so forth, comprising dilutions from 10^{-1} to 10^{-8}. Then, dilutions were distributed in Petri dishes using the microdrop technique with a volume of 10 µl per drop, with three replicates per dilution on each plate. Through mathematical relationships, each microdrop of the dilutions occupied an area upon the culture media corresponding to the area of a seed where bacteria were grown in the same *Azospirillum*/thiamethoxam ratio. We used four replications in a completely randomized design, in a total of 24 plates. Subsequently, they were incubated at 30°C for 48 h, when we counted the colonies, and were expressed as CFU ml^{-1} inoculum. To evaluate the influence of the association of thiamethoxam with the inoculation with *A. brasiliense* on seed germination, we adopted a completely randomized design in a 2x3 factorial arrangement. In the first factor, we assigned the seed inoculation with *A. brasiliense* (with and without inoculation) at 100 ml per 25 kg seed. In the second factor, we assigned the doses of thiamethoxam (Cruiser®) with 0; 80 and 120 ml c.p. per 60,000 seeds, as recommended by the manufacturer. Treatments were added to seeds by hand shaking in polyethylene bags until visual homogeneity. The inoculants had an initial bacterial population of 6.67 × 10^7 CFU ml^{-1} *A. brasilense* AbV5. We used commercial seeds of the cultivar Dekalb DKB 240 VT PRO as the standard for the test.

On germitest paper moistened with distilled water at a volume equivalent to 2.5 times its mass, four experimental replicates were performed with 50 seeds each, placed in a BOD germination chamber at 25 ± 1°C, with photoperiod of 12:12. Four days later, we determined the number of seeds that had produced radicle longer than two millimeters and straight and well developed coleoptile, composing the variable vigor. At day seven, we held the same procedure, regarding the variable viability, according to the Rules for Seed Analysis (Brasil, 2009), both expressed in percentage. Alongside, 25 seeds were sown in polyethylene trays, with four replicates per treatment; the substrate was sand sterilized by autoclaving for 20 min at 121±1°C. The trays were placed in a BOD germination chamber at 25 ±1°C, 12:12 (light: dark) photoperiod. Trays were weighed daily and watered until reaching field capacity. We counted the seedlings emerged for seven days, according to the Rules for Seed Analysis (Brazil, 2009), determining the Emergence Speed Index (ESI) as described by Maguire (1962). At the end of seven days, we determined the chlorophyll content through digital chlorophyll meter SPAD-502-Plus based on sampling ten seedlings per plot, obtained using the arithmetic mean of three measurements per plant. The same seedlings were selected for determination of morphometric characteristics. We counted the number of roots. The root length and shoot length were measured with a graduated scale. The steam diameter was measured with a digital caliper. We calculated the shoot

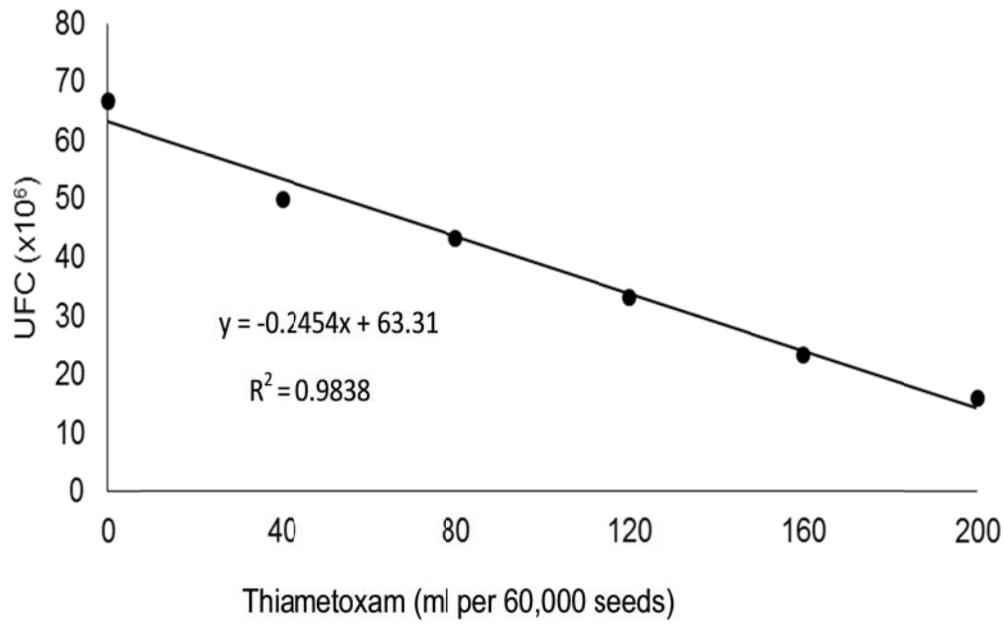

Figure 1. Colony forming units (CFU) based in the dose of thiametoxam.

length/stem diameter ratio, by dividing the length by stem diameter, expressing the robustness of the seedling. Finally, with the aid of a graduated cylinder, we identified the volume of the root system using the water column displacement technique, based in difference of water volume before and after the immersion of the roots.

After the morphometric measurements, plants were separated into shoot and roots and oven dried at 65 ± 2°C for 72 h until constant weight, when they were weighed on an accurate analytical balance. Each value of shoot dry weight and roots was obtained by dividing the total weight of the organ by the number of seedlings used in the test; the results were expressed in $g.seedling^{-1}$. The sum of the shoot dry weight and roots represented total dry matter. The root/shoot ratio was obtained by dividing the root dry weight by the shoot dry weight. Data were tested for normality (Shapiro-Wilk), and then subjected to analysis of variance with the software Sisvar 5.1 Build 72 (Ferreira, 2011). In the case of significant effect at 95% significance, mean values were compared by Tukey's test at 5% probability of error.

RESULTS AND DISCUSSION

The mortality caused by thiamethoxam to bacterial population of *A. brasilense* (Figure 1) showed a linear decrease according to the increase in the dose of insecticide, with mortality of 35 and 50% for doses of 80 and 120 ml c.p. per 60,000 seeds (doses recorded in maize, by the manufacturer), respectively. In agreement with Andaló et al. (2004), thiamethoxam has low toxicity to microorganisms, with no effect on germination, growth and production of conidia of *Beauveria bassiana*. Also, for phylogenetically close organisms such as the bacterium *Herbaspirillum seropedicae*, the same insecticide showed no effect on generation time and lag phase of growth, exhibiting a behavior similar to the control (Fernandes et al., 2012). Nevertheless, Gomez et al. (1998) found susceptibility of *A. brasilense* to chemicals as bromopropilate and methidathion, showing the same linear decreasing behavior observed in this study. However, Dartora et al. (2013) observed compatibility between inoculation and use of chemicals in seed treatments, evaluating the early development of maize seedlings. Thus, it is shown that the sensitivity of *A. brasilense* depends on the interaction with the chemical compound to which it is subjected.

ANOVA detected interaction between thiamethoxam doses and inoculation (Table 1) for ESI, stem diameter, root volume, shoot length, shoot/stem ratio, SPAD content, and root/shoot ratio. Vigor, viability and number of roots were not affected by the treatments with thiamethoxam and by the inoculation with *A. brasilense*. As to ESI (Table 2), the lowest value was found when the inoculation was conducted with the highest dose of thiamethoxam. When maize seeds were inoculated with *A. brasiliense*, higher emergence speed index were obtained without the use of the insecticide, suggesting its effect on the population and action of bacteria. When there was no inoculation, the highest emergence speed for the higher doses of thiamethoxam was registered. These results corroborate those obtained by Lauxen et al. (2010), who found increased ESI in cotton seeds treated with this insecticide.

In the same way, Almeida et al. (2012) evaluated the influence of thiamethoxam on physiological performance in oat and observed that the product is capable of stimulating the performance of the crop, especially at a dose of 300 ml of commercial product per 100 pounds of seeds. Comparing the effect of inoculation within each dose, even with no statistical difference, there was a

Table 1. Analysis of variance and F-test for analyzed variables.

Parameter	Mean square (QM)				
	Tiamethoxam	Inoculation	Interaction	Error	CV (%)
Vigor	1.166667	16.666667	7.166667	6.111111	2.57
Viability	2.666667	20.166667	2.666667	6.277778	2.60
ESI	0.162554	0.005400	1.127187**	0.820720	5.79
Indíce SPAD	11.616867**	9.151350**	21.497550**	1.042208	3.10
Shootlength	1.497088*	0.018150	7.960363**	0.316364	5.02
Stemdiameter	0.041679**	0.025350*	0.028213**	0.003492	2.28
Numberof root	0.016250	0.060000	0.008750	0.048611	5.62
Root length	5.012904*	0.297038	2.416512	0.911635	5.03
Root volume	0.011629	0.006667	0.016529*	0.004442	13.51
Shoot/stemratio	0.469029**	0.108004	0.687079**	0.054938	5.41
Shootdrymass	0.000015	0.000012	0.000076**	0.000006	6.22
Root drymass	0.000994**	0.000536**	0.000118	0.000057	12.56
Total drymass	0.001049**	0.000710**	0.000115	0.000073	8.66
Root/shootratio	0.642141**	0.251843*	0.316697**	0.034789	11.73

**significant at 1% by F-test; *significant at 5% by F-test; ^{ns} non-significant by F-test; CV, coefficient of variation.

Table 2. Emergence speed index and SPAD index of maize seedlings derived from seeds treated with thiamethoxam (0; 80 and 120 mL per 60,000 seeds) and inoculated with *A. brasilense* (0 and 100 mL per 25 kg seeds).

Thiametoxam dosage (ml)	Emergence speed index			SPAD Index		
	NI	I	Mean	NI	I	Mean
0	4.77Ab	5.41Aa	5.09	31.41Ab	35.34Aa	33.38
80	4.82Aa	5.07Aa	4.94	32.68Ab	34.87Aa	33.78
120	5.21Aa	4.40Bb	4.81	32.73Aa	30.31Bb	31.52
Mean	4.93	4.96	4.95	32.27	33.51	32.89

Means followed by different letters, uppercase in the column and lowercase in the row, are significantly different by Tukey's test at 5%; NI, Non-Inoculated; I, Inoculated.

positive increase in ESI when seeds were inoculated with bacteria at 0 and 80 ml of the insecticide. Araújo et al. (2010) stated that the inoculation of five out of six strains of diazotrophic bacteria promotes higher speed of germination in seeds of two rice cultivars. Similarly, Cassán et al. (2009) argued that the inoculant based on *A. brasilense* strain Az39 is capable of promoting faster seed germination and the early development of maize plants. In addition, this plant growth promoting bacterium is able to excrete indole-3-acetic acid, gibberellic acid and zeatin, hormones that can contribute to increased emergency speed index. Thus, nitrogen-fixing bacteria may contribute to plant growth through auxin production (Radwan et al., 2004), leading to higher values of emergence speed index. For the chlorophyll content in seedlings (SPAD index) (Table 2), there was a positive increase following the increase in the thiamethoxam dose, without inoculation, indicating a beneficial effect of the insecticide for this variable. Thiamethoxam works physiologically by activating membrane transport proteins and elevating the enzyme activation, increasing metabo-lism, synthesis of amino acids precursors of new proteins and the endogenous synthesis of plant hormones (Carvalho et al., 2011). Therefore, with the increase of its dose, the production of secondary metabolites increased, diverting photosynthates that would be used in growth, particularly nitrogen and consequently reducing the size and color of the plant. When inoculation was performed, the chlorophyll content was higher combined with doses of 0 and 80 ml of thiamethoxam.

Studies have presented conflicting results regarding the relationship between chlorophyll and nitrogen (Wolff and Floss, 2008). The majority found positive correlations between the SPAD index and the nitrogen content (Guimarães et al., 1999; Lima et al., 2001), but is not very accurate for the determination of this element in the initial stages of the maize crop, being reliable only for chlorophyll concentration (Argenta et al., 2001). However, nitrogen is an element directly related to chlorophyll, and is an essential component of the chemical structure of

Table 3. Shoot length, stem diameter, shoot/stem ratio, root length and root volume of maize seedlings derived from seeds treated with thiamethoxam (0; 80 and 120 mL per 60,000 seeds) and inoculated with *A. brasilense* (0 and 100 mL per 25 kg seeds).

Thiametoxam dosage (ml)	Shoot length (cm)			Stem diameter (mm)			Shoot/stem ratio		
	NI	I	Mean	NI	I	Mean	NI	I	Mean
0	10.77 Bb	12.06 Aa	11.41	2.42 Bb	2.60 Aa	2.51	4.46 Aa	4.64 Aa	4.55
80	11.07 ABb	11.96 Aa	11.51	2.60 Aa	2.66 Aa	2.63	4.26 Aa	4.49 Aa	4.37
120	11.89 Aa	9.55 Bb	10.72	2.66 Aa	2.61 Aa	2.63	4.47 Aa	3.66 Bb	4.07
Mean	11.24	11.19	11.22	2.56	2.62	2.59	4.40	4.26	4.33

Thiametoxam dosage (ml)	Root length (cm)			Root volume (cm^3)		
	NI	I	Mean	NI	I	Mean
0	18.29	19.53	18.91AB	0.50Aa	0.49ABa	0.49
80	19.64	20.01	19.82A	0.46Ab	0.60Aa	0.53
120	18.72	17.78	18.25B	0.47Aa	0.44Ba	0.46
Mean	18.88	19.10	18.99	0.48	0.51	0.49

Means followed by different letters; uppercase in the column and lowercase in the row are significantly different by Tukey's test at 5%. NI, Non-Inoculated; I, Inoculated.

chlorophyll *a* and *b* (Streit et al., 2005). Dietrich et al. (2005) suggest that when the plant is subjected to any induction involving high metabolic costs, it is necessary to complement the fertilization with higher nitrogen levels in order to compensate for the extra consumption of the element, thus explaining the lower values of the SPAD index for the application of thiamethoxam at 120 ml per 60,000 seeds associated with inoculation. In relation to shoot length (Table 3), we detected an interaction between doses of thiamethoxam and inoculation with *A. brasiliense*. In the case of non-inoculation, plants showed higher values for the highest dose of thiamethoxam, and an opposite effect was verified when plants were inoculated, where plants were greater in length at a dose of 80 ml and in the absence of the insecticide (0 ml). According to Almeida et al. (2011), increased shoot length with the use of thiamethoxam, depending on the dose applied, can increase the uptake of water and stomata resistance to water loss, favoring the metabolism and enhancing the resistance to environmental stress; in addition, it can also increase efficiency in the uptake, transport and assimilation of nutrients.

Stem diameter increased when inoculated with *A. brasiliense*, only in the absence of thiamethoxam (0 ml). For the other doses, no difference was found for inoculation. Several studies have highlighted the positive effect of thiamethoxam on physiological quality in seed germination of several crops (Almeida et al., 2009; Almeida et al., 2012; Lauxen et al., 2010), demonstrating increased rooting and development of shoots. Thus, higher values of stem diameter are also possible due to the stimulation of seedling growth. Shoot/stem ratio was reduced at the highest dose of thiamethoxam (120 ml) associated with inoculation. This is as a result of the relationship between smaller height in the same treatment, without changing stem diameter. The root length was affected only by thiamethoxam doses. In absolute numbers, the roots were longer at the dose of 80 ml of thiamethoxam, but this dose was not different from the control. The dose of 120 ml reduced the development of roots. Similar results were obtained by Corrêa Junior et al. (2013) when worked with different chemicals on germination of maize seeds. According to these authors, these results show that the application of the insecticide thiamethoxam at high doses produced a phytotoxic effect for root growth in maize seedlings.

There was interaction between the doses of thiamethoxam and inoculation with *Azospirillum* for the root volume. When seeds were not inoculated, there was no effect of thiamethoxam doses. As well as for root length, the phytotoxic effect of the insecticide was observed when applied at high doses. Tavares et al. (2007) examined the effect of thiamethoxam on soybean seed germination, and observed higher root and leaf volume when the product was applied. In accordance with Almeida et al. (2011), this difference between the dose zero and the dose providing the highest response, containing thiamethoxam, can be because this insecticide enables a greater expression of the germination potential, leaf area, root length and seedling length. With regard to the inoculation with *Azospirillum* combined with the dose of 80 ml of thiamethoxam, the inoculation promoted an increase of 30% in root volume. In the other treatments (0 and 120 ml), there was no effect of inoculation. Quadros (2009) registered a root volume of 60 to 80% higher in seedlings whose seeds were inoculated with *Azospirillum* compared with non-inoculated seedlings. For the shoot dry weight (Table 4), the treatments with the lowest doses of thiamethoxam inoculated with *A. brasiliense* were better than the non-inoculated. Likewise, Dartora et

Table 4. Shoot dry weight, root dry weight, total dry weight and root/shoot ratio of maize seedlings derived from seeds treated with thiamethoxam (0; 80 and 120 mL per 60,000 seeds) and inoculated with *A. brasilense* (0 and 100 mL per 25 kg seeds).

Thiametoxam	Shoot dry weight (g seedling^{-1})			Root dry weight (g seedling^{-1})		
Dosage (ml)	NI	I	Mean	NI	I	Mean
0	0.0360Ab	0.0410Aa	0.0384	0.0476	0.0483	0.048B
80	0.0370Ab	0.0420Aa	0.0394	0.0622	0.0772	0.07A
120	0.0400Aa	0.0340Bb	0.0367	0.0568	0.0695	0.063A
Mean	0.0374	0.0389	0.0382	0.056b	0.065a	0.0603
	Total dry weight (g seedling^{-1})			Root/shoot ratio		
	NI	I	Mean	NI	I	Mean
0	0.0832	0.0893	0.0863B	1.35Aa	1.18Ba	1.26
80	0.0992	0.1188	0.1090A	1.68Aa	1.85Aa	1.76
120	0.0963	0.1033	0.0998A	1.44Ab	2.05Aa	1.74
Mean	0.0929b	0.1038a	0.0984	1.49	1.69	1.59

Means followed by different letters; uppercase in the column and lowercase in the row are significantly different by Tukey's test at 5%. NI, Non-Inoculated; I, Inoculated.

al. (2013) observed increased yield of root dry weight for treatments inoculated with *Azospirillum* spp. The high dose of thiamethoxam associated with the inoculation was the treatment with the lowest increase in weight.

Considering the root dry weight, between the three doses of thiamethoxam, the lowest values were found at the dose of 0 ml. The inoculated treatments exhibited the highest mean value of root dry weight than non-inoculated treatments, corroborating the results of Santos et al. (2008) inoculating the growth promoting bacteria in melon plants, and Dawwam et al. (2013) working with seven isolates in potato. For the root/shoot ratio, all treatments showed mean values above 1, indicating that seedlings invested more in root than shoot. At the highest dose of thiamethoxam (120 ml), the non-inoculated treat-ment showed the lowest ratio compared with the inocu-lated treatment. This is because, when inoculated, *Azospirillum* affects the plant cell membrane activity, leading to changes in the morphology of roots, resulting in an increase in the root system (Bashan et al., 2004).

Conclusions

The chemical compound thiamethoxam is toxic to *A. brasilense*, reducing its population. Seed inoculation with *A. brasilense* results in significant gains on the physiological quality of maize seedlings, represented by increases in various morphometric variables along with increased emergence speed. The dose of 80 ml thiamethoxam per 60,000 seeds performs best when combined with inoculation acting synergistically, due to the reduction in bacterial mortality and to the moderate effect of bioactivation, showing better results of growth in roots and shoots.

Conflict of Interest

The author(s) have not declared any conflict of interests.

ACKNOWLEDGMENTS

Authors are grateful to Araucaria Foundation of Support, the Scientific Development and Technological of Paraná, affiliated to the State Secretariat of Science, Technology and Higher Education - SETI, CAPES/PNPD, INCT-FBN for the financial support and to CNPq for researcher on productivity to Vandeir Francisco Guimarães.

REFERENCES

Almeida AS, Carvalho I, Deuner C, Tillmann MAA, Villela FA (2011). The role of bioactivators in the physiological performance of rice seeds. Rev. Bras. Sementes 33(3):501-510.

Almeida AS, Tillmann MAA, Villela FA, Pinho MS (2009). Bioactivator in the physiological performance of carrot seeds. Rev. Bras. Sementes 31(3):87-95.

Almeida AS, Villela FA, Meneghello GE, Lauxen LR, Deuner C (2012). Physiologic performance of oats seeds treated with thiamethoxam. Semina: Ciências Agrárias 33(5):1619-1628.

Andaló V, Moino Junior A, Santa-Cecília LVC, Souza GC (2004). Compatibility of *Beauveria bassiana* with chemical pesticides for the control of the coffee root mealybug *Dysmicoccus texensis* Tinsley (Hemiptera: Pseudococcidae). Neotrop. Entomol.33(4):463-467.

Araújo AES, Rossetto CAV, Baldani VLD, Baldani JI (2010). Rice seed germination and vigour as affected by the inoculation with diazotrophic bacteria. Ciênc. agrotec. 34(4):932-939.

Argenta G, Silva PRF, Bortolini CG, Forsthofer EL, Strieder ML (2001). Relationship of reading of portable chlorophyll meter with contents of extractable chlorophyll and leaf nitrogen in maize. Theor. Exp. Plant Physiol. 13(2):158-167.

Baldani VLD (1996). Effect of inoculation with *Herbaspirillum* spp. the colonization and infection of rice plants and the occurrence and partial characterization of a novel bacterium process diazotrophic. Master's thesis. Federal Rural University of Rio de Janeiro.

Bashan Y, Holguin G, Luz E (2004). *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). Can. J. Microbiol. 50(8):521-577.

Blaha D, Prigent-combaret C, Mirza MS, Moe Y (2006). Phylogeny of the1-aminocyclopropane-1-carboxylic acid deaminase-encoding gene acdS in phytobene ¢ cial and pathogenic Proteobacteria and relation with strain biogeography. FEMS Microbiol. Ecol. 56(3):455-470.

Brazil (2009). Ministry of Agriculture, Livestock and Food Supply. Rules for testing seeds / Ministry of Agriculture, Livestock and Food Supply 399 p. Brasília: Mapa/ACS.

Busato JG, Zandonadi DB, Dobbss LB, Façanha AR, Canellas LP (2010). Humic substances isolated from residues of sugar cane industry as root growth promoter. Sci. Agric. 67(2):206-212.

Carvalho NL, Perlin RS, Costa EC (2011).Thiamethoxam Seed Treatment. REMOA 2(2):158-175.

Cassán F, Perrig D, Sgroy V, Masciarelli O, Penna C, Luna V (2009). *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). Eur. J. Soil Biol.45(1):28-35.

Castro PRC, Pitelle AMCM, Peres LEP, Aramaki PH (2007).Analysis of the regulatory activity of thiametoxan in plant growth by means of biotests. Publ.UEPG Ci. Exatas Terra, Ci. Agr. Eng.13(3):25-29.

Corrêa Junior ES, Hossen DDC, Guimarães S, Lima AM, Galon L, Nunes UR (2013).Physiological responses of maize seeds to chemical treatments. Trop. Ci. Agr. Biol.7(1):58-65.

Dartora J, Guimarães VF, Marini D, Pinto Júnior AS, Cruz LM, Mensh R (2013).Influence of seed treatment on the initial development of maize and wheat seedlings inoculated with *Azospirillum brasilense*. SAP12(3):175-181.

Dawwam GE, Elbeltagy A, Emara HM, Abbas IH, Hassan MM (2013). Beneficial effect of plant growth promoting bacteria isolated from the roots of potato plant. Ann. Agric. Sci. 58(2):195-201.

Dietrich R, Ploss K, Heil M (2005). Growth responses and fitness costs after induction of pathogen resistance depend on environmental conditions. Plant Cell Environ. 28(2):211-222.

Dobereiner J, Marriel IE, Nery M (1976). Ecological distribution of Spirillum lipoferum Beijerinck. Can. J. Microbiol. 22(10):1464-73.

Fancelli AL, Dourado Neto D (2003). Corn: Management strategies for high productivity.1st ed. 208 p. Piracicaba: ESALQ-USP-LPV.

Fernandes MF, Procópio SDO, Teles DA, Sena Filho JG, Cargnelutti Filho A, Machado TN (2012).Toxicity of insecticides used in sugarcane crop to the diazotrophic bacteria *Herbaspirillum seropedicae*. Rev. Ciênc. Agrar. 55(4):318-326.

Ferreira DF (2011). SISVAR : A computer statistical analysis system. Ciênc. agrotec. 35(6):1039-1042.

Gomez F, Salmeron V, Rodelas B, Martinez-Toledo MV, Gonzalez-Lopez J (1998). Response of *Azospirillum brasilense* to the pesticides bromopropylate and methidathion on chemically defined media and dialysed-soil media. Ecotoxicology 7(1):43-47.

Guimarães TG, Fontes PCR, Pereira PRG, Alvarez VH, Monnerat PH (1999).Relations among chlorophyll contents determined by a portable meter and nitrogen forms in leaves of tomatoes cultivated in two soil types. Bragantia 58(1):209-216.

Huergo LF, Monteiro RA, Bonatto AC, Rigo LU, Steffens MBR, Cruz LM, Pedrosa FO (2008). Regulation of nitrogen fixation in *Azospirillum brasilense*. In Cassán FD, Salamone IG (Eds.) *Azospirillum* sp.: cell physiology, plant interactions and agronomic research in Argentina. 1st ed., Buenos Aires: Argentina Association of Microbiology. 276 p.

Hungria M (2011).Inoculation with *Azospirillum brasilense*: Innovation in yield at low cost. Documentos. 1st ed., vol. 325, 36 p. Londrina: Embrapa Soybean.

Hungria M, Campo RJ, Souza EM, Pedrosa FO (2010). Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. Plant Soil 331(1-2):413-425.

Lauxen LR, Villela FA, Soares RC (2010).Physiological performance of cotton seeds treated with thiametoxam. Rev. bras. Sementes 32(3):61-68.

Lima EV, Aragão CA, Morais OM, Tanaka R, Grassi Filho H (2001).Nitrogen and potassium fertilization and the development and macronutrient composition during flowering of common beans. Sci. Agric. 58(1):125-129.

Maguire JD (1962). Speed of Germination-Aid In Selection And Evaluation for Seedling Emergence And Vigor. Crop Sci. 2(2):176-177.

National Supply Company (2014). Monitoring of the Brazilian grain harvest 1:83.

Paes MCD (2008). Physical, chemical and technological aspects of maize grains. In Cruz JC, Karam D, Monteiro MAR, Magalhães PC (Eds.) The Culture of Maize. 1st ed. Sete Lagoas: Embrapa Maize and Sorghum. 517 p.

Perrig D, Boiero ML, Masciarelli O; Penna C, Ruiz O, Cassán FD, Luna MV (2007). Plant-growth-promoting compounds produced by two agronomically important strains of Azospirillum brasilense, and implications for inoculant formulation. Appl. Microbiol. Biotechnol. 75(5):1143-50.

Quadros PD (2009).Inoculation of *Azospirillum* spp. In seeds of maize cultivars cropped on south Brazil. Master's thesis. Federal University of Rio Grande do Sul.

Radwan TESED, Mohamed ZK, Reis VM (2004).Effect of inoculation with *Azospirillum* and *Herbaspirillum* on production of indolic compounds and growth of wheat and rice seedlings. Pesq. agropec. bras. 39(10):987-994.

Rampim L, Rodrigues-Costa ACP, Nacke H, Klein J, Guimarães VF (2012). Qualidade fisiológica de sementes de três cultivares de trigo submetidas à inoculação e diferentes tratamentos. Rev. bras. sementes 34(4):678-685.

Rodriguez H, Gonzalez T, Goire I (2004). Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium Azospirillum spp Naturwissenschaften 91(11):552-555.

Santos MM, França S, Conrado F, Peixoto AR, Domingos C (2008).Evaluation of watermelon (*Citrulluslanatus* L.) inoculated with plant growth-promoting bacteria in greenhouse. Hortic. bras. 26:1550-1553.

Streit NM, Canterle LP, Canto MW, Hecktheuer LHH (2005). The Chlorophylls. Cienc. Rural 35(3):748-755.

Tavares S, Castro PRC, Ribeiro RV, Aramaki PH (2007).Evaluation of phisiological effects of thiametoxan on soybeans seed treatment. Revista de Agricultura 82(1):47-54.

Wolff WM, Floss EL (2008).Correlation among nitrogen and chlorophyll contents of leaves and grain yield of oat. Cienc. Rural 38(6):1510-1515.