Research Article

Effect of fungicides on the symbiosis between *Bradyrhizobium* strains and peanut[1]

Allef Barbosa dos Santos[2], Tarcísio Marcos de Souza Gondim[3], Paulo Ivan Fernandes Júnior[4], Liziane Maria de Lima[3]

ABSTRACT

Seed treatment with fungicides is an important practice for the control of phytopathogens in peanut crops. However, these products can harm rhizobacteria (*Bradyrhizobium*) and inhibit processes such as biological nitrogen fixation. This study aimed to verify the effects of the treatment of peanut seeds cv. BR1 inoculated with *Bradyrhizobium* spp. with fungicides. The experiment was conducted in a greenhouse, using two combinations of fungicides [C1: carboxin (200 g L^{-1}) + tiram (200 g L^{-1}); C2: pyraclostrobin (25 g L^{-1}) + thiophanate methyl (225 g L^{-1}) + fipronil (250 g L^{-1})] and one control without fungicide; two inoculants based on *Bradyrhizobium* spp. (SEMIA 6144 and ESA 123) and one control with a nitrogen chemical source (ammonium nitrate). The experimental design was completely randomized, in a 3 (2 with fungicide and 1 without fungicide) x 4 (2 inoculations based on rhizobia, 1 N mineral source and 1 without N) x 2 (1 or 2 inoculants applications) factorial scheme, with 5 replications. The root and shoot dry mass, plant height, nodulation and leaf nitrogen accumulation were evaluated. The application of both combinations reduced the number of nodules on plant roots, mainly for C1. The vegetative growth and nitrogen in the leaves were affected by C1, whereas, for C2, there were increments higher than for the control without fungicides. ESA 123 was more tolerant to the effects of fungicides on nodulation. Regarding the number of inoculations, there was an increase in the nitrogen rate with two inoculations. C2, despite affecting the nodulation, was less toxic to the inoculants SEMIA 6144 and ESA 123.

KEYWORDS: *Arachis hypogaea* L., inoculants, biological nitrogen fixation, phytopathogens.

RESUMO

Efeito de fungicidas na simbiose entre estirpes de *Bradyrhizobium* e amendoim

O tratamento de sementes com fungicidas é uma prática importante para o controle de fitopatógenos na cultura do amendoim. Todavia, esses produtos podem prejudicar rizobactérias (*Bradyrhizobium*) e inibir processos como a fixação biológica de nitrogênio. Objetivou-se verificar os efeitos do tratamento de sementes de amendoim cv. BR1 inoculadas com estirpes de *Bradyrhizobium* spp. com fungicidas. O experimento foi conduzido em casa-de-vegetação, utilizando-se duas combinações de fungicidas [C1: carboxin (200 g L^{-1}) + tiram (200 g L^{-1}); C2: pyraclostrobin (25 g L^{-1}) + thiophanate methyl (225 g L^{-1}) + fipronil (250 g L^{-1})] e uma testemunha sem fungicida; dois inoculantes à base de *Bradyrhizobium* spp. (SEMIA 6144 e ESA 123) e uma testemunha com fonte química nitrogenada (nitrato de amônio). O delineamento experimental foi inteiramente casualizado, em esquema fatorial 3 (2 com fungicida e 1 sem fungicida) x 4 (2 inoculações à base de rizóbios, 1 fonte mineral de N e 1 sem N) x 2 (1 ou 2 aplicações de inoculantes), com 5 repetições. Foram avaliados a massa seca da raiz e da parte aérea, altura de plantas, nodulação e acúmulo de nitrogênio nas folhas. A aplicação de ambas as combinações reduziu o número de nódulos nas raízes das plantas, principalmente para C1. O crescimento vegetativo e o nitrogênio nas folhas foram afetados por C1, enquanto, para C2, houve incrementos superiores aos da testemunha sem fungicidas. ESA 123 foi mais tolerante aos efeitos dos fungicidas na nodulação. Em relação ao número de inoculações, observou-se incremento na taxa de N com duas inoculações. C2, apesar de afetar a nodulação, foi menos tóxica aos inoculantes SEMIA 6144 e ESA 123.

PALAVRAS-CHAVE: *Arachis hypogaea* L., inoculantes, fixação biológica de nitrogênio, fitopatógenos.

INTRODUCTION

Worldwide, 40-66 million tons of nitrogen (N_2) are fixed annually by plants of the Fabaceae family (legumes), which constitute almost half of the N used in agriculture (Egamberdieva et al. 2017). The biological nitrogen fixation brings economic and environmental benefits to nitrogen deficient soils. This process makes it possible to replace nitrogen fertilizers, which have high prices

[1] Received: June 15, 2021. Accepted: Sep. 01, 2021. Published: Oct. 06, 2021. DOI: 10.1590/1983-40632021v5169089.
[2] Universidade Estadual da Paraíba, Departamento de Ciências Biológicas, Campina Grande, PB, Brasil.
Email/ORCID: allefbiologia@gmail.com/0000-0002-3552-2154.
[3] Empresa Brasileira de Pesquisa Agropecuária (Embrapa Algodão), Campina Grande, PB, Brasil.
Email/ORCID: tarcisio.gondim@embrapa.br/0000-0002-7164-1142; liziane.lima@embrapa.br/0000-0003-2314-6881.
[4] Empresa Brasileira de Pesquisa Agropecuária (Embrapa Semiárido), Petrolina, PE, Brasil.
Email/ORCID: paulo.ivan@embrapa.br/0000-0002-6390-3720.

and elevated production costs (Coba de la Peña & Pueyo 2012).

Rhizobia have the nitrogenase enzyme, capable of breaking the triple bond of nitrogen (N≡N) in the atmosphere, making it assimilable to the plant as ammonia nitrogen. In exchange, the plant offers protection to microorganisms within its roots and organic compounds from photosynthesis (Fernandes Junior & Reis 2008, Geurts et al. 2016).

Peanut (*Arachis hypogaea* L.) can efficiently associate with rhizobia lineages, but is more efficient with the *Bradyrhizobium* genus (Valetti et al. 2016, Barbosa et al. 2018, Brito et al. 2019). Crop yield can be maximized by using inoculants containing competent strains, favoring the crop management by small producers (Sizenando et al. 2016, Valetti et al. 2016).

For the satisfactory development of this and other crops, seed germination is among the most important factors. One of the tools used to ensure a good germination is seed treatment with fungicides (Ayesha et al. 2021). This practice is used worldwide, as many fungicides provide a broad spectrum and control of major diseases, with the potential to reduce the overall use of pesticides, thus decreasing environmental impacts (Lamichhane et al. 2020). Although the effectiveness of fungicides applied to seeds is notorious for crops, many of them may have negative effects on beneficial microorganisms and microbiological processes such as biological nitrogen fixation (Pereira et al. 2009, Silva Neto et al. 2013).

The treatment of soybean seeds with various active ingredients has shown that, under certain circumstances, fungicides may reduce plant nodulation by more than 50 % and grain yield by more than 20 % (Campo et al. 2009). On the other hand, there may be compatible interactions between fungicides and bacteria (Silva Neto et al. 2013, Shahid & Khan 2019). This combination was observed by Silva Neto et al. (2013), who concluded that the treatment of cowpea seeds with fungicides, combined with inoculation with *Bradyrhizobium* spp. strains, does not affect the rhizobia survival, nodulation and grain yield. Another study by Shahid & Khan (2019) confirmed that certain *Bradyrhizobium* strains can tolerate the toxic effects of the active principles of fungicides, improving plant characteristics and increasing profitability and yield.

This study aimed to evaluate the compatibility and effects resulting from the treatment of peanut (*A. hypogaea* L.) seeds with fungicides on nodulation with bacteria of the *Bradyrhizobium* spp. genus on plant development and leaf nitrogen content.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse, in Campina Grande, Paraíba state, Brazil (07º13'S; 53º31'W), in February 2019. Pots measuring 30 x 15 cm (height x diameter) filled with sandy loam soil were used. The soil chemical and fertility analysis presented the following values: pH (H_2O) = 5.5; Ca^{2+} = 12.3 $mmol_c$ dm^{-3}; Mg^{2+} = 5.6 mmol dm^{-3}; Na^+ = 2.7 mmol dm^{-3}; K^+ = 2.3 mmol dm^{-3}; sum of exchangeable bases = 22.9 $mmol_c$ dm^{-3}; H + Al = 8.3 mmol dm^{-3}; cation exchange capacity = 31.5 $mmol_c$ dm^{-3}; base saturation = 73.5 %; Al^{+3} = 0.0 mmol dm^{-3}; P = 10.3 mg dm^{-3} ($Mehlich^{-1}$); organic matter = 7.0 g kg^{-1}. The soil was autoclaved, corrected with limestone and fertilized before sowing with 3 mg pot^{-1} of simple superphosphate.

Two inoculants based on *Bradyrhizobium* spp. (ESA 123 and SEMIA 6144) and two combinations of fungicides of the systemic and contact chemical defensive class [C1: carboxin (200 g L^{-1}) + tiram (200 g L^{-1}); C2: pyraclostrobin (25 g L^{-1}) + thiophanate methyl (225 g L^{-1}) + fipronil (250 g L^{-1})] were used at the dose recommended by the manufacturer (300 mL of commercial product/100 kg of seeds). These products were applied to the seeds in a single dose, at 24 hours before the beginning of the experiment.

The experimental design was completely randomized, in a 3 (2 with fungicide and 1 without fungicide) x 4 (2 inoculations based on rhizobia, 1 mineral N source and 1 without N) x 2 (1 or 2 applications of inoculants, with interval of 15 days) factorial scheme, with 5 replications. For the treatment with mineral N source, 1 g pot^{-1} of ammonium nitrate was applied, and, for the treatments with inoculants, 1 mL $seed^{-1}$ $seedling^{-1}$. With the aid of a micropipette, 1 mL of the inoculant was applied to each seed inside the pit. In the second inoculation, 15 days after sowing, 1 mL of the inoculant was applied to the base of the seedling for treatments with 2 inoculations.

Bacteria were cultivated in Petri dishes, in a solid medium containing Yeast Extract Malt Agar (YMA; 1 % of glucose, 2 % of agar, 0.5 % of peptone, 0.3 % of malt and 0.3 % of yeast extract), and incubated for five days at 28 ºC in a BOD. Then, they were subcultured in a liquid YMA medium and incubated at 28 ºC, under agitation at 150 rpm, for approximately 7 days, at the end of the exponential phase of bacterial growth, when it had 1.0 x 10^9 CFU mL^{-1} (Vincent 1970). The medium with the bacteria was used directly in the inoculation.

In the treatment without fungicide, the seeds were disinfested with 30 % hydrogen peroxide for 20 min and washed 10 times with distilled water (Drechsel & Baldani 2006). Only one plant was kept in each pot. Irrigation was done daily, trying to keep the soil moisture close to the field capacity.

At 30 days after emergence, the following agronomic variables were analyzed: plant height (cm), measured from the base to the apex of the main stem; shoot and root dry mass (g), with the dry matter obtained by drying in an oven with forced air circulation at 64 ºC, for 72 hours, and weighed on a precision scale; number of nodules; nodules dry mass (g), with nodules excised, dried in an oven with forced air circulation at 64 ºC, for 72 hours, and weighed on a precision scale; and total leaf nitrogen.

The total leaf nitrogen analysis was based on the Kjeldahl method, adapted by Bezerra Neto & Barreto (2011). The shoots were dried in a forced air circulation oven at 64 ºC, for 72 hours, and then ground in a mill. An aliquot of dry plant material (1 mg) was mixed with 50 mg of sodium sulphate, 0.5 mg of copper sulphate (5 %) and 5 mL of sulfuric acid for cold pre-digestion, at room temperature, for 12 hours. Then, the solution was incubated at 350 ºC, in a digester block, until all organic matter had dissolved and the solution became clear.

For the N quantification, 1 mL of the extract digested in a volumetric flask (50 mL) containing 40 mL of deionized water, 1 mL of sodium hydroxide (10 %), 1 mL of sodium silicate (10 %) and 2 mL of Nessler's reagent was used, with the volume made up of deionized water to 50 mL. The reading was performed in a spectrophotometer at 410 nm and the nitrogen content calculated according to Alcantara et al. (2014).

The data were subjected to analysis of variance and averages compared by the Tukey test at 5 % of probability, using the statistical software Sisvar, version 5.6 (Ferreira 2011).

RESULTS AND DISCUSSION

There was a difference in root dry mass and shoot dry mass in the treatments with the strains, for the interaction with the tested fungicides (Table 1). For the root dry mass production, C1 promoted reductions of 19 and 23 % for ESA 123 and SEMIA 6144, respectively, when compared to the control without fungicide; while, for shoot dry mass, there was a decrease of 37 % for SEMIA 6144. For ESA 123, although no statistical difference was detected, a 13 % reduction was observed when treated with C1.

A lower plant growth from seeds treated with fungicide may be due to the reduction of nodules, which, therefore, reduced the nitrogen supply to the plant. Nitrogen is an essential element in plant nutrition, being a constituent element of enzymes and a structural component of macromolecules such as proteins and nucleic acids; thus, the deficiency of this element in plant tissues results in less accumulation of dry matter in the plant (Marschner 1995).

No differences were observed for C1, in relation to the control without fungicide, for root dry mass, shoot dry mass and plant height, although a restriction in shoot dry mass was noted (11 % in relation to the control without fungicide). Increments were verified in treatments with C2 in plants inoculated with ESA 123 (7 % for root dry mass and plant height) (Table 1), while, with SEMIA 6144, root dry mass and plant height were similar

Table 1. Mean values for root dry mass, shoot dry mass and plant height of peanut generated from seeds treated with fungicides and inoculated with *Bradyrhizobium* strains, in a greenhouse.

Treatment	C1[(a)]	C2[(b)]	Without fungicide
Root dry mass			
SEMIA 6144	0.27 Bab[(c)]	0.31 ABa	0.35 Aa
ESA 123	0.22 Bb	0.29 Aa	0.27 ABb
With nitrogen	0.21 Ab	0.27 Aa	0.20 Ab
Without nitrogen	0.32 Aa	0.28 ABa	0.23 Bb
Shoot dry mass			
SEMIA 6144	2.13 Ba	3.07 Aa	3.40 Aa
ESA 123	2.53 Aa	2.92 Aa	2.94 Aab
With nitrogen	2.15 Aa	1.97 Ab	2.43 Ab
Without nitrogen	2.56 Ba	3.38 Aa	2.69 Bb
Plant height			
SEMIA 6144	32.50 Ba	33.75 Aba	35.53 Aa
ESA 123	30.11 Aa	32.58 Aab	30.30 Ab
With nitrogen	26.33 ABb	29.66 Ab	25.33 Bc
Without nitrogen	23.83 Cb	35.16 Aa	30.50 Bb

[(a)] carboxin (200 g L^{-1}) + tiram (200 g L^{-1}); [(b)] pyraclostrobin (25 g L^{-1}) + thiophanate methyl (225 g L^{-1}) + fipronil (250 g L^{-1}). [(c)] Means followed by the same letter, uppercase in the row and lowercase in the column, for each variable, do not differ by the Tukey test, at 5 % of probability.

to the control without fungicide. These effects may be related to the presence of pyraclostrobin in the product composition, which is reported to be involved in plant growth improvement. The results found in this study corroborate Balardin et al. (2011), who recorded increases in the production of root dry mass and plant height when soybean seeds were treated with pyraclostrobin-based fungicides.

In this context, Fagan et al. (2010) mentioned that the increase in phytomass in plants treated with pyraclostrobin-based products is due to their increased physiological activity. These authors state that the possible cause of the increase in phytomass is associated with increased net photosynthesis, decreased respiration, greater CO_2 assimilation and increased nitrogen assimilation due to a greater activity of the nitrate reductase enzyme. On the other hand, Castro et al. (2007) mentioned that insecticides and fungicides are developed aiming at efficiency in the control of pests and diseases, but some may cause effects that are still little known, capable of modifying the plant metabolism and morphology. In an experiment with peanut inoculated with *Bradyrhizobium*, Hashem et al. (1997) found a significant reduction in shoot dry weight, when compared to the controls, when seeds were treated with C1. These studies emphasize the importance of agronomic research on the fungicide-inoculant interaction.

There were differences in the number of nodules and nodules dry mass produced by the evaluated strains, and the fungicides negatively affected ($p < 0.01$) these variables, regardless of the number of inoculations, although the second inoculation favored a better nodulation of the plants, even in the presence of fungicides (Table 2).

Among the chemical treatments, C1 was the most harmful, reducing by up to 70 % the number and mass of nodules for both strains, in both inoculations (Table 2). These results corroborate those obtained by Hashem et al. (1997), in greenhouse and field experiments with peanut inoculated with *Bradyrhizobium*, in which all tested fungicides, including C1, reduced nodulation. Pereira et al. (2009) reported that fungicides, in addition to reducing the number of nodules, also compromise their growth. Similar results were found by Mercante et al. (2010), in which the number and dry mass of nodules were reduced in 77 and 96 %, respectively, with the application of the fungicides captan, thiram, carbendazim + thiram and carboxin + thiram.

It was verified that the use of C2, associated with bacteria inoculation, promoted negative effects between 14 and 23 %, in relation to the control without fungicide (Table 2). Although there was an increase in nodulation with the second inoculation, the increase did not cancel out the toxicity of the products, since the decreases in the number and mass of nodules varied between 5 and 54 % with two inoculations, when compared to the control without fungicide (Table 2). The interaction of C2 with SEMIA 6144 and ESA 123 strains, if compared to C1, although negative, was better. Negative effects were also found by Zilli et al. (2010), working with soybean, even with the increase of the inoculant dose, when using carbendazim + thiram.

For Pereira et al. (2009), the application of fungicides on seeds can negatively affect the survival of *Bradyrhizobium* spp. due to its active ingredients, pH and/or solvents used in the formulations. It should be noted that contact fungicides, when applied to the

Table 2. Mean values for the number of nodules and nodules dry mass and percentage of interaction among the treatments with fungicides, nitrogen sources and number of inoculations.

Treatment	Number of nodules				Nodules dry mass			
	SEMIA 6144	(%)[(a)]	ESA 123	(%)	SEMIA 6144	(%)	ESA 123	(%)
			One inoculation					
Without fungicide	30.00 Aa[(b)]		25.66 Aa		0.028 Aa		0.034 Aa	
C1[(c)]	8.33 Ab	-72	8.00 Ab	-68	0.008 Ab	-71	0.009 Ab	-73
C2[(d)]	23.00 Aa	-23	27.00 Aa	8	0.031 Aa	10	0.029 Aa	-14
			Two inoculations					
Without fungicide	46.00 Aa		20.00 Ba		0.039 Aa		0.027 Ba	
C1	12.00 Ab	-74	11.33 Aa	- 43	0.028 Aa	- 28	0.018 Ba	-33
C2	21.00 Ab	-54	19.00 Aa	- 5	0.030 Aa	-23	0.023 Aa	-14

[(a)] Based on the mean values for number of nodules and nodules dry mass, with the respective percentages, in relation to the treatment without fungicide, being calculated; [(b)] averages followed by the same letter, uppercase in the row and lowercase in the column, for each variable, do not differ by the Tukey test, at 5 % of probability; [(c)] carboxin (200 g L^{-1}) + tiram (200 g L^{-1}); [(d)] pyraclostrobin (25 g L^{-1}) + thiophanate methyl (225 g L^{-1}) + fipronil (250 g L^{-1}).

seed surface, act as a toxic barrier, preventing the entry of any microorganisms. Furthermore, due to their non-specific mode of action, they can penetrate the interior of fungi during the germination of spores and provoke non-specific chemical reactions with chemical groups present in proteins, nucleic acids and their precursors (Garcia 1999). In this context, as they do not easily decompose in the environment and remain in the rhizosphere for longer without leaching, their effects can be prolonged (Garcia 1999), what explains the decrease in plant nodulation, even with a second inoculation of the strains (Table 2).

According to Martensson (1992), the presence of these chemicals in the rhizosphere alters root exudates and, consequently, inhibits the emission of molecular signals and the initial stages necessary for the nodulation process. In addition, fungicides based on thiram and captan are not very compatible with inoculation (Campos & Hungria 2000). In the present study, the seed treatment with thiram-based fungicide was possibly harmful to the inoculated bacteria and allowed a lower percentage of nodulation in the treatments.

C2, although having negative effects on nodulation, was less phytotoxic than C1, with increases of 8 % for number of nodules with ESA 123 and 10 % for nodules dry mass with SEMIA 6144 (Table 2). Kintschev et al. (2014) tested the effect of various fungicides on the survival of strains recommended for common bean and found that fipronil + thiophanate methyl + pyraclostrobin-based products do not inhibit the rhizobia growth, what may be correlated with the less toxic effects of active principles.

Regarding nitrogen, more negative effects of C1 on SEMIA 6144 were observed, in relation to the other treatments, although for ESA 123 there was no difference between this fungicide and the control without fungicide (Figure 1). Similar results were found by Zilli et al. (2010) in the field, who observed a negative effect on the accumulation of N in the shoot of soybean plants inoculated and treated with carbendazim + thiram.

The use of C2 had no negative effect on the accumulation of nitrogen in the shoots. Considering the positive increments of this fungicide in other variables already evaluated in this study, C2 also provided positive increments in the concentration of N in all evaluated treatments, except for the uninoculated and non-nitrogen control (Figure 1).

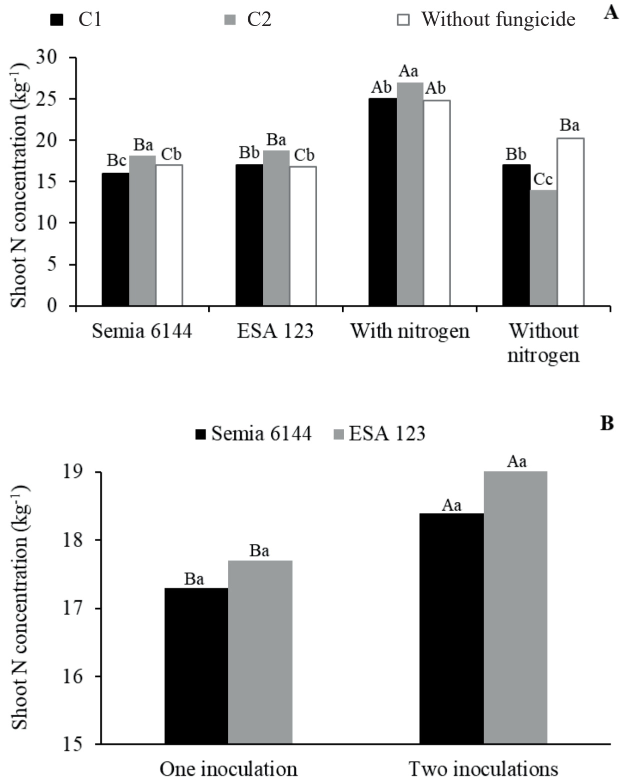

Figure 1. A) Interaction 'nitrogen source x fungicide treatment' for N accumulation in plant shoots. Uppercase letters compare the means between the N sources and lowercase letters the fungicides and the control (Tukey test at 5 % of probability); B) Interaction for 'number of inoculations x treatments' with stems in the presence of fungicides for N accumulation in the shoots. Uppercase letters compare the means between the number of inoculations and lowercase letters between strains (Tukey test at 5 % of probability). C1: carboxin (200 g L^{-1}) + tiram (200 g L^{-1}); C2: pyraclostrobin (25 g L^{-1}) + thiophanate methyl (225 g L^{-1}) + fipronil (250 g L^{-1}).

Fagan et al. (2010), while evaluating the effect of the application of pyraclostrobin on physiological variables, observed an increase in the photosynthetic rate and, consequently, a greater phytomass production. However, the authors emphasize that the increase in phytomass in plants does not only require carbon assimilation through increased photosynthesis, but also nitrogen availability.

Soares et al. (2011) found an increase in the activity of the nitrate reductase enzyme with the application of a pyraclostobin-based fungicide in soybean. For these authors, the increase in nitrogen absorption due to the application of pyraclostrobin may be correlated with physiological changes, such as the increase of chlorophyll content and in nitrogen assimilation, via nitrate reductase, reflecting on the increase in crop yield. Nitrogen assimilation is a vital process that controls plant development and growth and has marked effects on phytomass production and grain yield (Reis 2007).

Regarding the number of inoculations, there was an increase in the nitrogen rate in treatments that received two inoculations for both strains, although the best responses were verified for ESA 123 (Figure 1). It should be noted that the greater the number of viable bacteria in the rhizosphere, the greater the probability of nodulation and N fixation. If there is toxicity caused by any component of the fungicide used, there may be a reduction in root nodulation and, consequently, a lower efficiency of the biological nitrogen fixation process.

In this sense, to minimize the damage caused by certain fungicides on rhizobia and, consequently, grain yield losses in crops, due to the unavailability of N in sufficient quantities to obtain high yields, it has been suggested to use higher doses of microbial inoculant (Costa et al. 2013).

The results of the present study show negative effects of fungicides on the nodulation and growth of plants; however, it should be noted that their effects varied according to the chemical properties of the products and tolerance characteristics of *Bradyrhizobium* spp. In this sense, studies that focus on the effect of fungicides on *Bradyrhizobium* inoculants in peanut are important to understand the effects of these products on nodulation and biological nitrogen fixation, and provide data that can support, in the future, the development of chemical pesticides more compatible with rhizobia or provide the selection of tolerant strains to certain agrochemicals.

CONCLUSIONS

1. Pyraclostrobin (25 g L^{-1}) + thiophanate methyl (225 g L^{-1}) + fipronil (250 g L^{-1}), despite affecting nodulation, is less toxic to *Bradyrhizobium* spp. (SEMIA 6144 and ESA 123); therefore, the plants have a greater growth and increase in the shoot dry mass and nitrogen accumulation;
2. It is viable to use the ESA 123 inoculant in seeds treated with carboxin (200 g L^{-1}) + tiram (200 g L^{-1}) and pyraclostrobin (25 g L^{-1}) + thiophanate methyl (225 g L^{-1}) + fipronil (250 g L^{-1}).

ACKNOWLEDGMENTS

To the Empresa Brasileira de Pesquisa Agropecuária (Embrapa), for the financial support; and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for the scholarship granted.

REFERENCES

ALCANTARA, R. M. C. M.; XAVIER, G. R.; RUMJANEK, N. G.; ROCHA, M. M.; CARVALHO, J. S. Eficiência simbiótica de progenitores de cultivares brasileiras de feijão-caupi. *Revista Ciência Agronômica*, v. 45, n. 1, p. 1-9, 2014.

AYESHA, M. S.; SURYANARAYANAN, T. S.; NATARAJA, K. N.; PRASAD, S. R.; SHAANKER, R. U. Seed treatment with systemic fungicides: time for review. *Frontiers in Plant Science*, v. 12, e1581, 2021.

BALARDIN, R. S.; SILVA, F. D. L.; DEBONA, D.; CORTE, G. D.; FAVERA, D. D.; TORMEN, N. R. Tratamento de sementes com fungicidas e inseticidas como redutores dos efeitos do estresse hídrico em plantas de soja. *Ciência Rural*, v. 41, n. 7, p. 1120-1126, 2011.

BARBOSA, D. D.; BRITO, S. L.; FERNANDES JUNIOR, P. I.; FERNANDES, P. D.; LIMA, L. M. Can *Bradyrhizobium* strains inoculation reduce water deficit effects on peanuts? *World Journal of Microbiology & Biotechnology*, v. 34, e87, 2018.

BEZERRA NETO, E.; BARRETO, L. P. *Análises químicas e bioquímicas em plantas*. Recife: Ed. UFRPE, 2011.

BRITO, S. L.; SANTOS, A. B.; BARBOSA, D. D.; FERNANDES, P. D.; FERNANDES JUNIOR, P. I.; LIMA, L. M. *Bradyrhizobium* spp. as attenuators of water deficit stress in runner peanut genotypes based on physiological and gene expression responses. *Genetics and Molecular Research*, v. 18, n. 4, p. 1-12, 2019.

CAMPO, R. J.; ARAUJO, R. S.; HUNGRIA, M. Nitrogen fixation with the soybean crop in Brazil: compatibility between seed treatment with fungicides and bradyrhizobial inoculants. *Symbiosis*, v. 48, n. 1,p. 154-163, 2009.

CAMPOS, R. J.; HUNGRIA, M. *Compatibilidade de uso de inoculante e fungicidas no tratamento de sementes de soja*. Londrina: Embrapa Soja, 2000.

CASTRO, P. R. C.; PITELLI, A. M. C. M.; PERES, L. E.; ARAMAKI, P. H. Análise da atividade reguladora de crescimento vegetal de tiametoxam através de biotestes. *Publicatio*, v. 13, n. 3, p. 25-29, 2007.

COBA DE LA PEÑA, T.; PUEYO, J. J. Legumes in the reclamation of marginal soils, from cultivar and inoculant selection to transgenic approaches. *Agronomy for Sustainable Development*, v. 32, n. 1, p. 65-91, 2012.

COSTA, M. R.; CAVALHEIRO, J. C. T.; GOULART, A. C. P.; MERCANTE, F. M. Sobrevivência de *Bradyrhizobium japonicum* em sementes de soja tratadas com fungicidas e os efeitos sobre a nodulação e a produtividade da cultura. *Summa Phytopathologica*, v. 39, n. 3, p. 186-192, 2013.

DRECHSEL, M. M.; BALDANI, V. L. D. *Método de desinfestação superficial para obtenção de sementes de milho livres de microrganismos*. Serópedica: Embrapa Agrobiologia, 2006.

EGAMBERDIEVA, D.; RECKLING, M.; WIRTH, S. Biochar-based *Bradyrhizobium* inoculum improves growth of lupin (*Lupinus angustifolius* L.) under drought stress. *European Journal of Soil Biology*, v. 78, n. 1, p. 38-42, 2017.

FAGAN, E. B.; DOURADO NETO, D.; VIVIAN, R.; FRANCO, R. B.; YEDA, M. P.; MASSIGNAM, L. F.; OLIVEIRA, R. F.; MARTINS, K. V. Efeito da aplicação de piraclostrobina na taxa fotossintética, respiração, atividade da enzima nitrato redutase e produtividade de grãos de soja. *Bragantia*, v. 69, n. 4, p. 771-777, 2010.

FERNANDES JUNIOR, P. I.; REIS, V. M. *Algumas limitações à fixação biológica de nitrogênio em leguminosa*. Serópedica: Embrapa Agrobiologia, 2008.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia*, v. 35, n. 6, p. 1039-1042, 2011.

GARCIA, A. *Fungicidas I*: utilização no controle químico de doenças e sua ação contra os fitopatógenos. Porto Velho: Embrapa Rondônia, 1999.

GEURTS, R.; XIÃO, T. T.; HUREK, B. R. What does it take to evolve a nitrogen-fixing endosymbiosis? *Trends in Plant Science*, v. 21, n. 3, p. 199-208, 2016.

HASHEM, F. M.; SALEH, S. A.; BERKUM, P. V.; VOLL, M. Survival of *Bradyrhizobium* sp. (*Arachis*) on fungicide-treated peanut seed in relationship to plant growth and yield. *World Journal of Microbiology and Biotechnology*, v. 13, n. 3, p. 335-340, 1997.

KINTSCHEV, M. R.; GOULART, A. C. P.; MERCANTE, F. M. Compatibility between rhizobium inoculation and fungicide application in seeds of common beans. *Summa Phytopathologica*, v. 40, n. 4, p. 338-346, 2014.

LAMICHHANE, J. R.; YOU, M. P.; LAUDINOT, V.; BARBETTI, M. J.; JEAN-NOËL AUBERTOT, J. N. Revisiting sustainability of fungicide seed treatments for field crops. *Plant Disease*, v. 104, n. 3, p. 610-623, 2020.

MARSCHNER, H. *Mineral nutrition of higher plants*. San Diego: Academic Press, 1995.

MARTENSSON, A. M. Effects of agrochemicals and heavy metals on fast-growing rhizobia and their symbiosis with small-seeded legumes. *Soil Biology and Biochemistry*, v. 24, n. 5, p. 435-445, 1992.

MERCANTE, F. M.; MORETTO, H. J. N.; TARASIUK, V. A.; GOULART, A. C. P. Efeitos de fungicidas na nodulação de feijoeiros inoculados com *Rhizobium tropici*. *In*: REUNIÃO DA REDE DE LABORATÓRIOS PARA RECOMENDAÇÃO, PADRONIZAÇÃO E DIFUSÃO DE TECNOLOGIA DE INOCULANTES MICROBIOLÓGICOS DE INTERESSE AGRÍCOLA, 14., 2010, Bonito. *Anais*... Dourados: Embrapa Agropecuária Oeste, 2010. p. 50-51.

PEREIRA, C. E.; OLIVEIRA, J. A.; OLIVEIRA, G. E.; ROSA, M. C. M.; COSTA NETO, J. Fungicide treatment by film coating and soybean seed inoculation with *Bradyrhizobium*. *Revista Ciência Agronômica*, v. 40, n. 3, p. 433-440, 2009.

REIS, A. R. *Metabolismo do nitrogênio e estado nutricional do cafeeiro (Coffea arabica)*. 2007. Dissertação (Mestrado em Agronomia) - Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, 2007.

SHAHID, M.; KHAN, M. S. Fungicide tolerant *Bradyrhizobium japonicum* mitigate toxicity and enhance greengram production under hexaconazole stress. *Journal of Environmental Sciences*, v. 78, n. 1, p. 92-108, 2019.

SILVA NETO, M. L.; SMIDERLE, O. J.; SILVA, K.; FERNANDES JUNIOR, P. I.; XAVIER, G. R.; ZILL, J. E. Compatibilidade do tratamento de sementes de feijao-caupi com fungicidas e inoculação com estirpes de *Bradyrhizobium*. *Pesquisa Agropecuária Brasileira*, v. 48, n. 1, p. 80-87, 2013.

SIZENANDO, C. I. T.; RAMOS, J. P. C.; FERNANDES JUNIOR, P. I.; LIMA, L. M.; FREIRE, R. M. M.; SANTOS, R. C. Agronomic efficiency of *Bradyrhizobium* in peanut under different environments in Brazilian Northeast. *African Journal of Agricultural Research*, v. 11, n. 37, p. 3482-3487, 2016.

SOARES, L. H.; FAGAN, E. B.; CASAROLI, D.; ANDRADE, D. D.; SOARES, A. L.; MARTINS, K. L.; ROCHA, F. J. Application of different strobilurin on the soybean crop. *Revista da FZVA*, v. 18, n. 1, p. 78-97, 2011.

VALETTI, L.; ANGELINI, J. G.; TAURIAN, T.; ANZUAY, M. S.; FABRA, A.; CERIONI, G. A. Development and field evaluation of liquid inoculants with native bradyrhizobial strains for peanut production. *African Crop Science Journal*, v. 24, n. 1, p. 1-13, 2016.

VINCENT, J. M. *A manual for the practical study of root nodule bacteria*. Oxford: Blackkwell Science, 1970.

ZILLI, J. E.; GIANLUPPI, V.; CAMPOS, R. J.; ROUWS, J. R. C.; HUNGRIA, M. Inoculação da soja com *Bradyrhizobium* no sulco de semeadura alternativamente à inoculação no sulco de semeadura. *Revista Brasileira de Ciências do Solo*, v. 34, n. 6, p. 1875-1881, 2010.