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CORN INOCULATED WITH *Azospirillum brasilense* ASSOCIATED WITH POULTRY LITTER FERTILIZATION

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ABSTRACT - The use of organic fertilization can have a great contribution to the physiological and chemical aspects of plant leaves, and can be aided by the use of microorganisms that promote greater use of soil nutrients. In this work, the objective was to study the gas exchange and foliar N, P, and K contents of corn as a function of seed inoculation with *A. brasilense* associated with poultry litter fertilization. The work was carried out in the field in two areas, with a randomized block design, in a 2x6 factorial scheme. The first factor was the inoculation of seeds with *A. brasilense* and control without inoculation. The second factor was six doses of poultry litter, defined as a function of the nutrient content in the soil and in the poultry litter: 0, 50, 100, 150, 200, 250% of the recommended dose for each area. In both areas, gas exchange indexes were evaluated, such as photosynthetic rate (*A*), stomatal conductance (*gs*), leaf transpiration rate (*E*) and water use efficiency (*WUE*), as well as analysis of nitrogen, phosphorus and potassium in the leaves. Seed inoculation with *A. brasilense* decreased the photosynthetic rate of corn leaves and increased the phosphorus content in leaf biomass when compared to the control treatment. The doses of poultry litter increased the phosphorus and potassium contents of the leaves. The maximum foliar P content was reached with the dose of 5830 kg ha^{-1} of poultry litter.
Keywords: *Zea mays* L., organic fertilization, PGPB.

MILHO INOCULADO COM *Azospirillum brasilense* ASSOCIADO À FERTILIZAÇÃO COM CAMA DE FRANGO

RESUMO - O uso da adubação orgânica pode ter grande contribuição para os aspectos fisiológicos e químicos das folhas de plantas, podendo ser auxiliado pelo uso de microrganismos que promovem o maior aproveitamento dos nutrientes do solo. Neste trabalho, objetivou-se estudar as trocas gasosas e teores foliares de N, P e K do milho em função da inoculação de sementes com *A. brasilense* associadas à fertilização com cama de frango. O trabalho foi conduzido a campo em duas áreas, com delineamento em blocos casualizados, em esquema fatorial 2x6. O primeiro fator foi a inoculação das sementes com *A. brasilense* e controle sem inoculação. O segundo fator foram seis doses de cama de frango, definidas em função dos teores de nutrientes no solo e na cama de frango: 0, 50, 100, 150, 200, 250% da dose recomendada para cada área. Nas duas áreas, foram realizadas avaliações dos índices de trocas gasosas, como a taxa fotossintética (*A*), condutância estomática (*gs*), taxa de transpiração foliar (*E*) e eficiência do uso da água (*EUA*), bem como, análise dos teores de nitrogênio, fósforo e o potássio nas folhas. A inoculação das sementes com *A. brasilense* diminuiu a taxa fotossintética das folhas de milho e incrementou o teor de fósforo na biomassa das folhas quando comparada ao tratamento controle. As doses de cama de frango aumentaram os teores de fósforo e potássio das folhas. O teor máximo de P foliar foi alcançado com a dose de 5830 kg ha^{-1} de cama de frango.
Palavras-chave: *Zea mays* L., adubação orgânica, BPCV.

INTRODUCTION

The corn plant requires a high amount of nutrients such as nitrogen, phosphorus, and potassium, extracted from the soil to complete its physiological cycle, the values of each nutrient vary according to the chemical quality of the soil, the technological level used, and the expected productivity. Thus, in the production chain, management needs to be efficient and viable, making it essential to search for technologies and tools that help maintain corn productivity, aiming at its cost-benefit ratio.

Among the tools available to increase the sustainability of the culture, there is the practice of inoculation in seeds using plant growth-promoting bacteria (PGPB), such as bacteria of the genus *Azospirillum* sp., which are widely studied. These microorganisms provide direct and indirect beneficial effects to plants, as they are facultative bacteria and can colonize both the interior and the surfaces of the roots of cultivated grass species (GUIMARÃES et al., 2017).

Inoculation with these microorganisms such as *Azospirillum brasilense* can, in addition to promoting the availability of nutrients such as phosphorus (P), through the extraction or solubilization of insoluble fractions from the soil (PERES et al., 2020), promote biological nitrogen fixation (BNF), making this essential nutrient available to the plant (GONÇALVES et al., 2020). These

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microorganisms may also be related to multiple mechanisms capable of stimulating plant growth, such as hormone production and excretion, in which auxins, gibberellins, and cytokinins can be highlighted (COSTA et al., 2015), resulting in the promotion of plant root growth.

It is worth noting that although *A. brasilense* performs biological nitrogen fixation, it is not able to fully meet the needs of the culture, and yet it can provide savings of up to 50% in the use of nitrogen fertilizers (HUNGRIA et al., 2010). This information is of paramount importance because the use of PGPB can be considered a technology that is in line with the current approach to agriculture, respecting the need to be a sustainable technique, bringing even more benefits to the environment, by dispensing part of nitrogen fertilization in grass cultures.

It is known that growth-promoting rhizobacteria are being widely researched due to their positive effects on grass plants that had the effects of water deficit minimized with the foliar application of *A. brasilense* (BULEGON et al., 2017).

Just as PGPB can be considered a viable alternative for the culture, at the same time, the use of organic fertilizer can also be used as a complementary way to the nutritional requirement of corn plants, due to the high content of nutrients present in its composition (SANTOS et al., 2014). Among the waste from animal production, poultry litter is available on properties at a low cost, and its application in commercial crops is an alternative for sustainable use when properly managed (LIMA NETO et al., 2019).

The prospects for increased poultry activity are concomitant with the generation of organic waste. Thus, there is a need to use poultry litter for other activities, such as its use in agriculture as a source of nutrients (PITTA et al., 2012). When applied to the soil, the poultry litter has a positive effect on its chemical, physical, and biological aspects, being able to become a rich source of nutrients, capable of supplying their needs, which would be provided by the chemical fertilizer. Therefore, the recommended dose for application must take into account the needs of the culture and the physicochemical properties of the soil. However, this residue presents variability in its composition, depending on the creation system, number of lots on the poltry litter, way and time of composting (LIMA NETO et al., 2019).

Pitta et al. (2012) demonstrate that the highest rates of decomposition and release of nutrients from poultry litter occurred in the first 60 days of incubation in the soil, in this period, 40, 34, 91, and 39% of N, P, K, and Ca, respectively. Studies carried out recognize the efficiency of the use of poultry litter in the corn culture (SANTOS et al., 2014).

A better understanding of the relationships between corn plants inoculated with nitrogen-fixing bacteria and associated with organic fertilization is of paramount importance for the agronomic performance of the culture in the current agricultural scenario. Therefore, it is expected that the use of different doses of poultry litter associated with *A. brasilense* in the seed will provide increments on the plant's gas exchange and increase in the nutritional contents of the leaves in the culture, when compared to its use alone. In view of the actions and positive responses on the plant development of *A. brasilense*, the present study aimed to evaluate the performance of gas exchange and the nutritional contents of corn plants inoculated via seed with *Azospirillum brasilense* and fertilized with doses of poultry litter.

MATERIAL AND METHODS

The work was carried out in two areas, under field conditions, during the 2017/2018 agricultural year. The first area was in the Experimental Farm “Professor Antônio Carlos dos Santos Pessoa”, with geographic coordinates of 24º31'57” South, 54º01'11” West, and altitude of 420 m, in the city of Marechal Cândido Rondon (PR), belonging to the Western Paraná State University (Unioeste) - Campus of Marechal Cândido Rondon. The second area was on a private property, with geographic coordinates of 24º38'35” South, 53º54'52” West, and approximate altitude of 450 m, located in the community of Dois Irmãos, in the municipality of Toledo (PR).

Both study areas present Eutroferric RED LATOSOL (SANTOS et al., 2018). The climate of the region is *Cfa* type, subtropical, with rainfall distributed throughout the year and hot summers, with average annual temperatures of 22ºC and average rainfall of 1600 to 1800 mm (APARECIDO et al., 2016). The mean temperature (°C), relative humidity (%), and rainfall (mm) data used in experiment I were provided by the Automatic Weather Station in the municipality of Marechal Cândido Rondon. Data from experiment II were provided by Cooperativa Agropecuária Mouraoense (COAMO), located in Dois Irmãos (Figure 1).

Each experimental unit consisted of ten cultivation lines, spaced 0.50 m apart, 6 m long and with a population of 65,000 plants ha^{-1}, of the hybrid 30F53 VYHR from the company Pionner®. Thus, the 6 central lines of the plot were considered useful, excluding 1 m from each end and 2 lines on the sides.

The experimental design used in both experiments was randomized blocks, in a 2 x 6 factorial scheme, containing four replications, totaling 48 experimental units. The first factor was composed of corn seeds inoculated and not inoculated with *A. brasilense*. The second factor was composed of six doses of poultry litter (0, 50, 100, 150, 200, 250% of the recommended dose), determined according to the results obtained from the chemical analysis of soil/poultry litter.

The two areas had different managements, as the soil of the first area did not contain corn stover as a ground cover, due to the fact that it was used for the production of whole corn plant silage, thus presenting a lower content of organic material on its surface. The area of the second experiment had corn stover from the previous crop on its surface, as a ground cover. Before the implantation of the culture, the chemical characteristics of the soil of the two areas were determined, in the depth of 0.00-0.20 m, and the recommendation of fertilization with poultry litter was based on that.

According to laboratory analyses, the soil of experiment I, in Marechal Cândido Rondon/PR, presented in its chemical constitution, values of: 8.89 g dm^{-3} of organic matter (OM); pH (0.01 mol L^{-1} $CaCl_2$) = 4.82; 40.49 mg dm^{-3} of phosphorus [P(melich^{-1})]; 0.36 cmol$_c$ dm^{-3} of potassium (K^+); 4.47 cmol$_c$ dm^{-3} of calcium (Ca^{2+}); 1.77 cmol$_c$ dm^{-3} of magnesium (Mg^{2+}); 12.39 cmol$_c$ dm^{-3} of Cation Exchange Capacity (CEC); 53.27% of base saturation; and Ca/Mg ratio of 2.52. As for the soil of experiment II, in Toledo/PR, it presented in its chemical constitution the values of: 17.77 g dm^{-3} of OM; pH (0.01 mol L^{-1} $CaCl_2$) = 4.95; 7.51 mg dm^{-3} of P (melich^{-1}); 0.91 cmol$_c$ dm^{-3} of K+; 7.34 cmol$_c$ dm^{-3} of Ca^{2+} ; 1.40 cmol$_c$ dm^{-3} of Mg^{2+}; 14.14 cmol$_c$ dm^{-3} of CEC; 68.18% of base saturation; and Ca/Mg ratio of 5.24.

The poultry litter used for the experiment came from a rural property in the region, which reused sawdust in 9 lots of broilers, each lot lasting approximately 42 days. After the last lot left, the litter was milled and rested for 30 days under black plastic, for the mineralization of its nutrients. Then, a sample was submitted to the analysis of its chemical composition, which presented the composition: 78.49% of dry biomass; 24.35% of total mineral residue; pH (0.01 mol L^{-1} $CaCl_2$) = 8.62; 1.16% of total N; 1.45% of total P; 1.41% of total K; 4.70% of total Ca; 0.31% of total Mg; 41.57 mg kg-1 of total Cu; 239.39 mg kg^{-1} of total Zn; 330.29 mg kg^{-1} of total Mn; 4121.51 mg kg^{-1} of total Fe; and 19.43 mg kg^{-1} of total B. This analysis was done according to organic fertilizer analysis and nutrient data determined based on the dry biomass of the fertilizer.

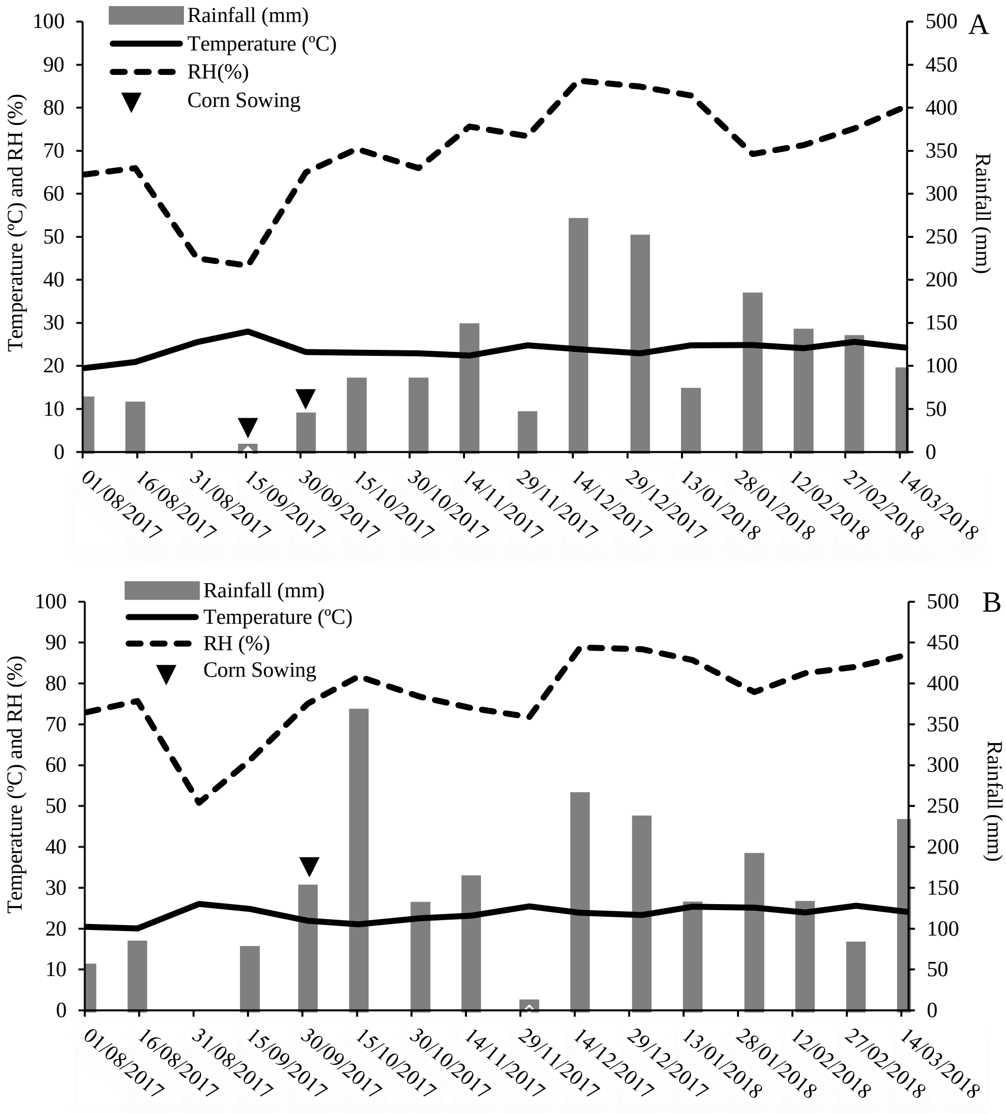


FIGURE 1 - Fortnightly averages of average temperature (°C), relative humidity (RH) (%), and rainfall (mm) during the period of experiment I (A) and experiment II (B).

Thus, calculations were carried out to determine the manure doses, based on the total P content of the residue, since this nutrient was the one with the highest amount in the organic fertilizer, based on the recommendation of the Fertilization and Liming Manual for the state of Paraná/Brazil (PAVINATO et al., 2017).

For experiment I, a recommended dose of 4000 kg ha^{-1} was defined, and for poultry litter doses of 0, 2000, 4000, 6000, 8000, and 10000 kg ha^{-1} were defined. For experiment II, the recommended dose of 6600 kg ha^{-1}, and doses of 0, 3300, 6600, 9900, 13200, and 16500 kg ha^{-1} of poultry litter were defined, all applied manually, 40 days in advance to the crop sowing. The liming in the area, as well as the chemical fertilization in the implantation of the culture and top dressing were not carried out.

The seeds were inoculated with bacteria of the species *A. brasilense*, strains AbV5 and AbV6 (2 x 108 CFU mL^{-1} for 600 seeds or 100 mL ha^{-1}), using the inoculant Gramíneas Nitro1000® as a commercial product. Inoculation was carried out 30 min. before sowing, in plastic bags, adding the corresponding volume of inoculant and homogenizing, by manual agitation, for 3 min. Sowing was carried out mechanically, in the last half of September, in 2017. Cultivation treatments, such as the application of insecticides, fungicides, and herbicides were carried out according to the demand of the culture.

Gas exchange evaluations were performed when the corn plants reached the plant bolting stage, at 90 days after sowing (DAS). Previously, a fully developed leaf, photosynthetically active, and devoid of apparent lesions was identified in the middle third of the plant, located where it was hit by direct sunlight. The IRGA equipment (Infra Red Gas Analyzer), model LI-6400XT (Licor Inc. Lincoln, NE) was used for the readings, in the morning, between 9:00 and 11:00, using CO_2 content in the IRGA chamber of 400 µmol mol^{-1}.

The net CO_2 assimilation rate (*A*), stomatal conductance (*gs*), and leaf transpiration rate (*E*) were then measured, in addition to *WUE = A/E* ratio (ZHANG et al., 2001), which corresponds to the efficiency of water use.

During the flowering stage (R1) of the plants, or after the appearance of the female inflorescence and beginning of pollination, at 100 DAS, the opposite leaf and below the ear of each plant were collected, in the useful area of the plot, in 10 plants. After collection, the leaves were taken to dry in an oven with forced air circulation, at 65°C, until reaching constant biomass. Then, the samples were ground in a knife mill and sent for analysis at the Institution's Soil Fertility Laboratory, where the levels of macronutrients present in the leaf were determined (LANA et al., 2016).

Regarding data analysis, before performing the statistical analysis, the collected variables were submitted to the Shapiro-Wilk normality test and, later, the data obtained underwent analysis of variance using the F test ($p \leq 0.05$). When significant differences were detected, Tukey's test was performed, at 5% error probability, for inoculation and regression analysis for dose studies. Statistical analyzes were performed using the SISVAR software (FERREIRA, 2014).

RESULTS AND DISCUSSION

The mean square values of stomatal conductance (*gs*), leaf transpiration (*E*) and water use efficiency (*WUE*) did not change significantly ($p \leq 0.05$) by the treatments applied. Thus, the different doses of poultry litter applied and inoculation with A. *brasilense* performed on the seeds did not influence these variables in both experiments (Table 1). However, the net assimilation rate of CO_2 (*A*) of the plants of the treatments that were inoculated with *A. brasilense* presented a lower average in relation to the non-inoculated treatment, with a reduction of 10.2% in the photosynthetic rate of the plant.

TABLE 1 - Summary of analysis of variance indicating variation factors, degrees of freedom (DF) and mean squares for net CO_2 assimilation rate (*A*), stomatal conductance (*gs*), leaf transpiration rate (E), and water use efficiency (*WUE*) evaluated in the two experiments in the corn culture.

Variation factor	DF	mean squares			
		A (µmol CO_2 m^{-2} s^{-1})	*gs* (µmol m^{-2} s^{-1})	*E* (mmol H_2O m^{-2} s^{-1})	*WUE* [µmol CO_2 (mmol H2O)$^{-1}$]
		Experiment I			
Inoc.	1	299.78*	0.03^{ns}	3.33^{ns}	0.01^{ns}
Dose	5	71.10^{ns}	0.01^{ns}	0.29^{ns}	1.27^{ns}
Inoc. x dose	5	87.40^{ns}	0.01^{ns}	1.47^{ns}	1.17^{ns}
Error	33	65.53	0.01	1.68	2.30
CV(%)		17.40	38.20	27.94	14.39
Average		46.52	0.28	4.64	10.54
		Experiment II			
Inoc.	1	12.51^{ns}	0.00^{ns}	0.10^{ns}	0.00^{ns}
Dose	5	9.50^{ns}	0.00^{ns}	0.17^{ns}	0.26^{ns}
Inoc. x dose	5	56.75^{ns}	0.01^{ns}	0.94^{ns}	0.30^{ns}
Error	33	29.96	0.01	0.67	0.54
CV(%)		16.22	38.55	21.69	8.06
Average		33.75	0.23	3.77	9.09

*Significant at 5% probability of error by F Test, ns = not significant. Inoc. = treatment with corn seed inoculated with *Azospirillum brasilense*, dose = treatment with doses of poultry litter, CV = coefficient of variation.

Regarding the nutrient contents found in leaves of corn plants inoculated with PGPB and fertilized with poultry litter, it was observed that there was no significant interaction between the inoculation treatments and the doses of poultry litter (Table 2), meaning that the behavior in relation to the doses of organic fertilizer was the same, regardless of the action of the microorganisms applied.

TABLE 2 - Summary of analysis of variance with variation factors, degrees of freedom (DF) and mean squares for nitrogen (N), phosphorus (P), and potassium (K) in leaves evaluated in the two experiments in the corn culture.

Variation factor	DF	Mean squares N ($g\ Kg^{-1}$ of MS)	Mean squares P ($g\ Kg^{-1}$ of MS)	Mean squares K ($g\ Kg^{-1}$ of MS)
Experiment I				
Inoc.	1	0.07^{ns}	2.60*	2.04^{ns}
Dose	5	11.08^{ns}	4.36**	5.22**
Inoc. x dose	5	8.09^{ns}	0.56^{ns}	0.18^{ns}
Error	33	13.59	0.55	1.19
CV(%)		15.04	18.98	5.16
Average		24.50	3.92	20.23
Experiment II				
Inoc.	1	55.9^{ns}	0.48^{ns}	1.02^{ns}
Dose	5	22.61^{ns}	0.20^{ns}	2.89*
Inoc. x dose	5	25.87^{ns}	0.12^{ns}	0.06^{ns}
Error	33	19.32	0.16	1.12
CV(%)		20.94	14.16	4.36
Average		20.98	2.82	24.23

* and **Significant at 5% and 1% error probability by the F Test, respectively, ns = not significant. Inoc. = treatment with corn seed inoculated with *Azospirillum brasilense*, dose = treatment with doses of poultry litter, CV = coefficient of variation.

According to the data from this work, the net assimilation rate of CO_2 (*A*) (Table 3) presented a quadratic effect with maximum point, similar to the effect of phosphorus when there was an increase in the doses of poultry litter (Table 4), leading to believe that the excess of phosphorus absorbed by the plant was what led to the decrease in the rate of foliar photosynthesis in corn plants that received inoculation via seeds with growth-promoting bacteria.

TABLE 3 - CO_2 net assimilation rate (*A*) and stomatal conductance (*gs*) in corn plants inoculated with plant growth-promoting bacteria and fertilized with poultry litter in the two experimental areas.

Experiment I: Doses ($kg\ ha^{-1}$ of poultry litter)	*A* ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) Without azo	With azo	Average	Experiment II: Doses ($kg\ ha^{-1}$ of poultry litter)	*A* ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) Without azo	With azo	Average
0	51.87	37.89	44.88	0	28.98	36.02	32.50
2000	54.23	47.87	51.05	3300	30.79	36.33	33.56
4000	46.68	52.27	49.47	6600	37.38	30.53	33.96
6000	45.93	44.82	45.37	9900	37.33	33.80	35.57
8000	48.20	40.85	44.53	13200	31.51	34.14	32.82
10000	47.26	40.44	43.83	16500	33.48	34.78	34.13
Average	49.02a**	44.02b		Average	33.25a	34.27a	
Calculated F values							
Inoculation	4.58*			Inoculation	0.83^{ns}		
Doses	1.09^{ns}			Doses	0.42^{ns}		
Inoc. x dose	1.33^{ns}			Inoc. x dose	0.32^{ns}		
CV(%)	17.40			CV(%)	16.22		

Continuation of Table 3 - CO_2 net assimilation…

Experiment I				Experiment II			
Doses (kg ha^{-1} of poultry litter)	*gs* (µmol m^{-2} s^{-1})			Doses (kg ha^{-1} of poultry litter)	*gs* (µmol m^{-2} s^{-1})		
	Without azo	With azo	Average		Without azo	With azo	Average
0	0.32	0.23	0.28	0	0.16	0.26	0.21
2000	0.36	0.26	0.31	3300	0.20	0.27	0.24
4000	0.24	0.35	0.30	6600	0.26	0.18	0.22
6000	0.33	0.28	0.30	9900	0.28	0.24	0.26
8000	0.33	0.22	0.28	13200	0.21	0.23	0.22
10000	0.25	0.22	0.24	16500	0.24	0.25	0.25
Average	0.31a	0.26a		Average	0.23a	0.24a	
Calculated F values							
Inoculation	2.19^{ns}			Inoculation	0.24^{ns}		
Doses	0.48^{ns}			Doses	0.38^{ns}		
Innoc. x dose	1.03^{ns}			Innoc. x dose	1.23^{ns}		
CV(%)	38.20			CV(%)	38.55		

*Significant at 5% probability of error by F Test, ns = not significant. Inoc. = treatment with corn seed inoculated with Azospirillum brasilense, dose = treatment with doses of poultry litter, CV = coefficient of variation. **Equal lowercase letters on the line do not differ from each other, by Tukey's test, at 5% error probability. Without Azo = without *Azospirillum brasilense*, With Azo = with *Azospirillum brasilense*.

TABLE 4 - Transpiration (*E*) and water use efficiency (*WUE*) in corn plants inoculated with plant growth-promoting bacteria and fertilized with poultry litter in the two experimental areas.

Experiment I				Experiment II			
Doses (kg ha^{-1} of poultry litter)	*E* (mmol H_2O m^{-2} s^{-1})			Doses (kg ha^{-1} of poultry litter)	*E* (mmol H_2O m^{-2} s^{-1})		
	Without azo	With azo	Average		Without azo	With azo	Average
0	5.14	4.02	4.59	0	3.15	4.05	3.60
2000	5.36	4.35	4.85	3300	3.47	4.25	3.86
4000	4.14	5.27	4.71	6600	4.12	3.23	3.68
6000	5.01	4.42	4.71	9900	4.18	3.76	3.97
8000	5.26	4.16	4.71	13200	3.63	3.71	3.67
10000	4.53	4.07	4.29	16500	3.82	3.91	3.87
Average	4.91a**	4.38a		Average	3.73a	3.82a	
Calculated F values							
Inoculation	1.98^{ns}			Inoculation	0.15^{ns}		
Doses	0.17^{ns}			Doses	0.25^{ns}		
Inoc. x dose	0.87^{ns}			Inoc. x dose	1.40^{ns}		
CV(%)	27.94			CV(%)	21.69		

Experiment I				Experiment II			
Doses (kg ha^{-1} of poultry litter)	*WUE* [µmol CO_2 (mmol H_2O)$^{-1}$]			Doses (kg ha^{-1} of poultry litter)	*WUE* [µmol CO_2 (µmol H_2O)$^{-1}$]		
	Without azo	With azo	Average		Without azo	With azo	Average
0	10.09	9.42	9.75	0	9.21	8.90	9.05
2000	10.13	11.01	10.56	3300	8.87	8.54	8.71
4000	11.27	9.91	10.59	6600	9.08	9.44	9.26
6000	9.16	10.15	9.66	9900	8.93	8.98	8.96
8000	9.16	9.83	9.49	13200	8.68	9.20	8.94
10000	10.42	9.95	10.18	16500	8.76	8.90	8.83
Average	10.04a	10.05a		Average	8.92a	8.99a	

Continuation of Table 4 – Transpiration…

Calculated F values			
Inoculation	0.00^{ns}	Inoculation	0.00^{ns}
Doses	0.55^{ns}	Doses	0.48^{ns}
Inoc. x dose	0.52^{ns}	Inoc. x dose	0.56^{ns}
CV(%)	14.39	CV(%)	8.06

*Significant at 5% probability of error by F Test, ns = not significant. Innoc. = treatment with corn seed inoculated with Azospirillum brasilense, dose = treatment with doses of poultry litter, CV = coefficient of variation. **Equal lowercase letters on the line do not differ from each other, by Tukey's test, at 5% error probability. Without Azo = without *Azospirillum brasilense*, With Azo = with *Azospirillum brasilense*.

No statistical difference ($p \leq 0.05$) was found for the nitrogen contents in the corn plants for the inoculation use data when compared to the control or non-inoculated treatments (Table 5). These results are similar to the results found by Moraes et al. (2017), who reported no N accumulation in corn leaves in treatments inoculated with growth-promoting bacteria, such as *Azospirillum brasilense*.

TABLE 5 - Nitrogen (N) and phosphorus (P) contents present in the leaves of corn plants, inoculated with plant growth-promoting bacteria and fertilized with poultry litter in two experimental areas.

Experiment I				Experiment II			
Doses (kg ha^{-1} of poultry litter)	N (g kg^{-1})			Doses (kg ha^{-1} of poultry litter)	N (g kg^{-1})		
	Without azo	With azo	Average		Without azo	With azo	Average
0	24.51	24.91	24.71	0	19.25	21.74	20.50
2000	23.84	27.27	25.56	3300	25.38	22.28	23.83
4000	24.70	25.25	24.98	6600	20.10	21.88	20.99
6000	23.50	22.80	23.15	9900	21.41	21.88	21.64
8000	27.03	24.33	25.68	13200	17.09	20.56	18.83
10000	23.23	22.73	22.98	16500	16.23	24.07	20.15
Average	24.47a	24.55a		Average	19.91a	22.07a	
Calculated F values							
Inoculation	0.01^{ns}			Inoculation	2.89^{ns}		
Doses	0.82^{ns}			Doses	1.17^{ns}		
Inoc. x dose	0.60^{ns}			Inoc. x dose	1.34^{ns}		
CV(%)	15.04			CV(%)	20.94		
Experiment I				Experiment II			
Doses (kg ha^{-1} of poultry litter)	P (g kg^{-1})			Doses (kg ha^{-1} of poultry litter)	P (g kg^{-1})		
	Without azo	With azo	Average		Without azo	With azo	Average
0	2.14	3.16	2.65	0	2.56	2.73	2.65
2000	3.58	3.79	3.68	3300	2.81	2.82	2.81
4000	4.75	4.36	4.56	6600	2.80	2.78	2.78
6000	4.19	5.14	4.66	9900	2.85	2.97	2.91
8000	4.02	4.35	4.19	13200	2.94	3.26	3.09
10000	3.44	4.11	3.78	16500	2.37	3.01	2.69
Average	3.69b	4.15a		Average	2.72a	2.92a	
Calculated F values							
Inoculation	4.69*			Inoculation	2.98^{ns}		
Doses	7.86**			Doses	1.27^{ns}		
Inoc. x dose	1.01^{ns}			Inoc. x dose	0.74^{ns}		
CV(%)	18.98			CV(%)	14.15		

* and **Significant at 5% and 1% error probability by the F Test, respectively, ns = not significant. Innoc. = treatment with corn seed inoculated with *Azospirillum brasilense*, dose = treatment with doses of poultry litter, CV = coefficient of variation. Equal lowercase letters on the line do not differ from each other, by Tukey's test, at 5% error probability. Without Azo = without *Azospirillum brasilense*, With Azo = with *Azospirillum brasilense*.

The N content in the leaves of the plants did not differ statistically ($p \leq 0.05$) for the use of different doses of organic fertilizer (Table 5). When considering the P content of the leaves, a significant difference was found as a function of the inoculation and control treatments in experiment I, with an increase of approximately 12.5% when using growth-promoting bacteria in corn seeds. These results suggest that inoculation with *A. brasilense* causes increases in the absorption of minerals such as phosphates available in the soil, improving the efficiency of the use of these applied nutrients. The results described are in agreement with authors who researched the effect of PGPB in plants such as corn on fertilizers containing phosphates made available to the plant and rhizobacteria capable of solubilizing phosphates and making them available to the plant (GULNAZ; FATHIMA, 2018).

Authors were able to verify that *A. brasilense* present in the rhizosphere of plants are able to extract or solubilize phosphorus from insoluble fractions in the soil. Furthermore, its solubilization may result from the production of CO_2 and organic acids from the mineralization of carbon present in organic matter, or from the production of enzymes and chelating and complexing compounds by the microbiota, exerting a direct solubilizing action on inorganic phosphates, transforming CO_2 into acids that are able to solubilize calcium and magnesium phosphates present in the soil (GUIMARÃES et al., 2017).

In the evaluation carried out on the levels of foliar phosphorus in relation to the doses of poultry litter applied, significant differences ($p \leq 0.05$) were observed for the different amounts of organic fertilizer applied in the treatments (Figure 2A). The application of doses on the rates of P present in the dry mass of the plant leaves resulted in a quadratic effect, presenting maximum point, whose value means the dose at which the greatest absorption of the nutrient occurs and, consequently, its presence in the leaves of the plant. The value found as the ideal dose was 5830 kg of organic fertilizer per hectare.

This result is in agreement with some authors such as Menezes et al. (2018), who, when working with corn, realized that the increases in P content and absorption by the plant leaf could be attributed to the increase in P availability in the soil due to the application of organic fertilizers such as swine manure. Botega (2019) also revealed in his research that the poultry litter, when going through the composting process, tends to present a higher concentration of available P compared to the beginning of the process, as the mineralization process occurs, in which there is a reduction of solids material, causing the concentration of nutrients important for plant metabolism in the final compost to be higher and more available, as is the case with P.

When referring to the potassium content of the leaves, there were no significant differences between the treatments that received inoculation with the PGPB and the control treatments (Table 6). However, the evaluations resulted in significant effects for the amount of poultry litter applied when compared to the witness (control) (Figure 2B and 2C). As well as increasing the dose, there was also an increase in the amount of K found in the samples of the experiment. I ($p \leq 0.01$) and II ($p \leq 0.05$).

TABLE 6 - Potassium (K) levels in the leaves of corn plants inoculated with plant growth-promoting bacteria and fertilized with poultry litter in two experimental areas.

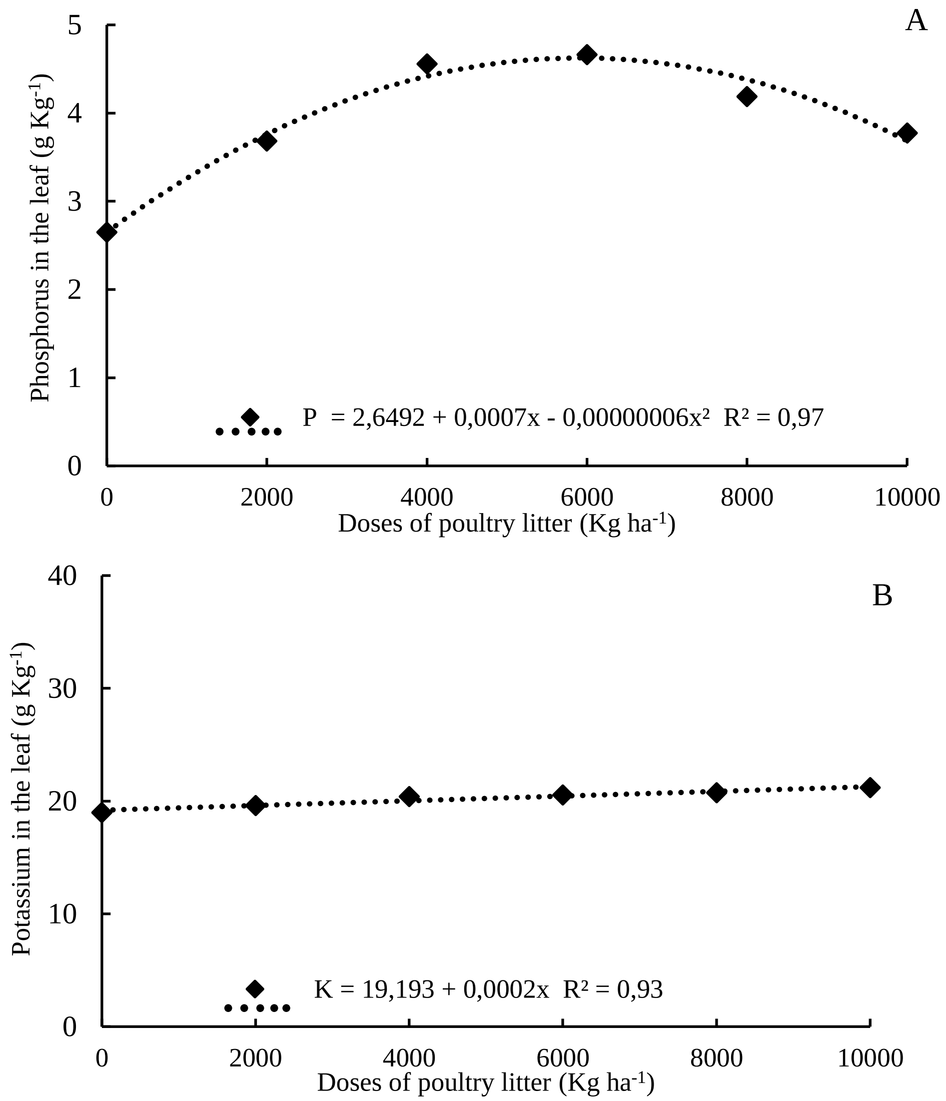
Experiment I				Experiment II			
Doses (kg ha^{-1} of poultry litter)	K (g kg^{-1})			Doses (kg ha^{-1} of poultry litter)	K (g kg^{-1})		
	Without azo	With azo	Average		Without azo	With azo	Average
0	18.94	18.99	18.97	0	23.22	23.34	23.28
2000	19.48	19.74	19.61	3300	23.74	24.13	23.94
4000	20.05	20.73	20.39	6600	24.04	24.18	24.11
6000	20.11	20.97	20.54	9900	24.26	24.73	24.49
8000	20.55	20.93	20.74	13200	24.48	24.64	24.56
10000	21.06	21.30	21.18	16500	24.78	25.26	25.02
Average	20.03a	20.44a		Average	24.09a	24.38a	
Calculated F values							
Inoculation	1.868^{ns}			Inoculation	0.917^{ns}		
Doses	4,781**			Doses	2,589*		
Inoc. x dose	0.166^{ns}			Inoc. x dose	0.054^{ns}		
CV(%)	5.16			CV(%)	4.36		

ns = not significant by the F test, at 5% error probability, ** and *significant at 1% and 5% error probability, respectively, by the F test. Inoc. = treatment with corn seed inoculated with *Azospirillum brasilense*, dose = treatment with doses of poultry litter, CV = coefficient of variation. Equal lowercase letters on the line do not differ from each other, by Tukey's test, at 5% error probability. Without Azo = without *Azospirillum brasilense*, With Azo = with *Azospirillum brasilense*.

According to the data obtained in the work, the results were significantly higher when there was an increase in the doses of poultry litter, with an increasing linear response in the potassium levels in the leaf, reaching values 11.6% (Figure 2B) higher when analyzed experiment I and 7.4% (Figure 2C) higher in experiment II. Other authors such as Malafaia et al. (2016) also noticed an increase in the foliar potassium concentration of plants cultivated with the application of compounds from organic origin, when compared to plants cultivated without the addition of organic fertilizer. The same authors stated that the increase in the K content of corn leaves as a result of the addition of organic matter can also be attributed to the higher concentration of the macronutrient present in the organic fertilizer used, when compared to the initial soil concentration.

Even though other studies show positive responses regarding the inoculation of PGPB such as *A. brasilense* (COSTA et al., 2015; SZILAGYI-ZECCHIN et al., 2017), inconsistencies are still found in the few studies carried out, with a lot of variation in the environment, soil, substrate management, in plants, and microflora components in general, and these factors are considered responsible for the variation between different experiments.

However, organic fertilizers (MALAFAIA et al., 2016; MENEZES et al., 2018; BOTEGA, 2019) can show promising results, becoming an option that allows the reduction of mineral fertilizers, and may even reduce the environmental pollution caused by the fertilizers. The variation of results in the use of organic fertilizers and PGPB makes it increasingly necessary to explore and elucidate the behavior of these sustainable technologies in order to increase the efficiency of their use in relation to the environment and for agricultural production.



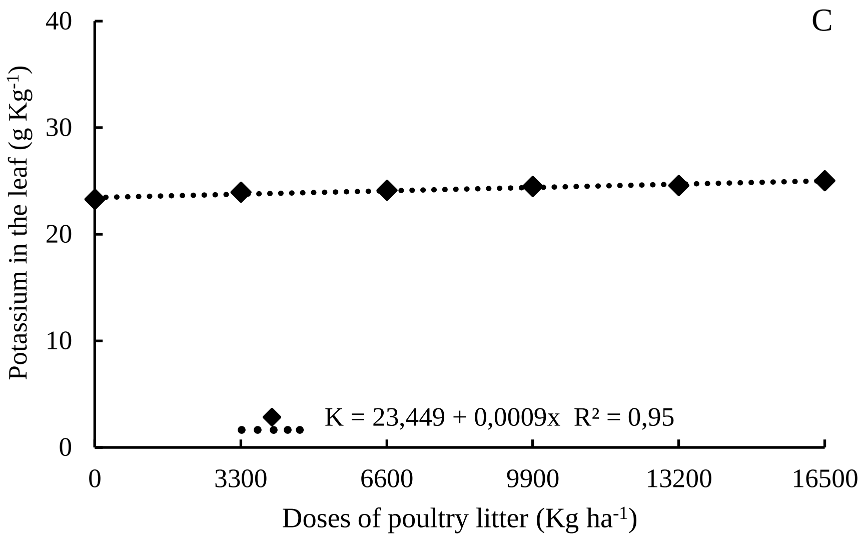


FIGURE 2 - Phosphorus contents in corn leaves in experiment I (A), potassium contents in corn leaves in experiment I (B) and potassium contents in corn leaves in experiment II (C), as a function of doses of poultry litter.

CONCLUSIONS

The use of an inoculant based on *A. brasilense* decreased the photosynthetic rate of corn leaves and increased the phosphorus content in the dry biomass of the leaves when compared to the control treatment.

The doses of poultry litter increased the phosphorus and potassium contents of the plant leaves when compared to the control treatment.

Fertilization with poultry litter provided higher foliar phosphorus content when applied at a dose of 5830 kg ha^{-1}, indicating greater absorption and utilization of the nutrient by corn plants when compared to the control without fertilization.

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