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Effect of compaction of soil on the development and production of cowpea inoculated with rhizobium

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Cowpea is considered as one of the most important species in Brazil, for presenting essential components to the feeding process, which makes it relevant to identify the physical, chemical and biological conditions that benefit or cause damage to its development. Thus, the objective of this study was to evaluate the effect of inoculation with rhizobia strains in compacted soil on the development and production of cowpea. The experiment was conducted in a greenhouse, in a completely randomized design with six replications, in a factorial 3 × 2, with two inoculation treatments (BR3267 - *Bradyrhizobium* sp. and the combination of the strains MT8 - *Rhizobium tropici* and MT15 - *R. tropici*) and a control with nitrogen fertilization - 150 mg dm^{-3}, using urea as a source, in compacted and uncompacted soil. The evaluations were carried out during the crop cycle (35, 45, 52, 58 and 90 days) after the emergence of plants, determining the Falker chlorophyll content, dry mass of grains, nitrogen content, nitrogen accumulation and crude protein in shoots and grains. All data were subjected to statistical analyses using the SISVAR program, performing the analysis of variance and the Tukey test at the level of 5% probability. The results showed positive effect on plants inoculated with the combination of the strains MT8 + MT15 in compacted soil, in all parameters evaluated, being observed, values similar to or greater than in plants grown in uncompacted soil. Therefore, the use of the combination of strains becomes a promising alternative for the cowpea development in soil with a density of 1.6 Mg m^{-3}.

Key words: Biological nitrogen fixation, cerrado, soil density.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) has several favorable characteristics for cultivation in the Midwest of the country, especially the existence of genotypes with increased tolerance to drought, lower requirement

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Table 1. Chemical and granulometric characterization of the 0-0.2 m layer of Dystrophic Red Latosol (Rondonópolis-MT, 2015).

pH	P	K	Ca	Mg	H	Al	SB	CTC	V	O.M.	Sand	Silt	Clay
$CaCl_2$	mg dm^{-3}		----------------cmolcdm^{-3}---------------						%	g dm^{-3}		g kg^{-1}	
4.0	1.4	23	0.4	0.2	5.4	0.8	0.7	6.8	9.7	27.1	423	133	444

pH = Hydrogen ionic potential; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; H = hydrogen; Al = aluminum; SB = sum of bases; CTC = cation exchange capacity; V = base saturation; O.M. = organic matter.

increased tolerance to drought, lower requirement of nutrients and the possibility of use of agricultural machinery in all production processes (Odutayo et al., 2005; Locatelli et al., 2014).

However, the use of agricultural machinery in soil above the optimal humidity may cause changes in the physical properties of the soil and consequently promote compaction (Horn et al., 2003). When there is soil compaction, the plant growth is hindered due to the formation of a cohesive layer, which causes increased resistance to penetration of roots, reduced macroporosity and consequently, there is a decrease in the absorption of water and nutrients (Shittu and Amusan 2015).

Despite the negative effects to the soil when used in inadequate conditions, agricultural machinery has become indispensable because of the large areas, the exploitation of several annual crops and the agricultural modernization (Collares et al., 2011; Streck et al., 2004).
In addition to the physical alterations of the soil, which affect the formation of roots and the grain production, increased density can reduce the activity of microorganisms. In a study by Farias et al. (2013) with dwarf pigeonpea grown in compacted soil, the formation of nodules showed a reduction of up to 76.82%, and this effect is explained by the change in root mass due to the reduction of the pore spaces, which increases the resistance to the penetration of roots in the soil profile (Hamza and Anderson, 2005).

Nonetheless, microorganisms play an important role in the physical characteristics of the soil, due to the ability to unite the mineral fraction of the soil in stable aggregates, favoring the establishment of plants against adverse conditions in the long term (Harris et al., 1966); regarding the short term, bacteria provide the supply of nitrogen and growth hormones, a process that would stimulate the growth of secondary roots, resulting in greater absorption of nutrients and water (Glick, 2012)

This way, tests carried out under controlled conditions may be used for an indication of the responses of crops to stressful conditions, such as inadequate management leading to soil compaction. In this context, the objective was to evaluate the effect of inoculation with rhizobia strains in compacted soil on the development and production of cowpea.

MATERIALS AND METHODS

Study site

The experiment was conducted in a greenhouse at the Institute of Agricultural Sciences and Technology of the Federal University of Mato Grosso, Rondonópolis Campus, Brazil, located at Latitude 16°27'49.62" S, Longitude 54°34'47.78" E, with an average altitude of 227 m.

Experimental design

The design was completely randomized with six replications, in a factorial 3 x 2, with two inoculation treatments (BR3267 - (*Bradyrhizobium* sp.) and the combination of the strains MT8 - *Rhizobium tropici* and MT15 - *R. tropici*), in addition to a control with nitrogen fertilization - 150 mg dm^{-3}, using urea as source, in two soil conditions (compacted and uncompacted soil), totaling 36 experimental plots.

The soil used was collected in a fragment of Cerrado and classified as Dystrophic Red Latosol (Embrapa, 2013). Later, it was sieved on a 4 mm sieve and then, based on chemical analysis, liming with dolomitic limestone (PRNT 80%) was performed, raising the base saturation to 60% for 30 days.
The results of the physical and chemical analysis of the soil shown in the experimental area can be found in Table 1.

Experimental plot

Each experimental plot consisted of three rigid polyvinyl chloride (PVC) rings with a diameter of 0.15 cm and 10 cm in height (Figure 1). At the bottom of the experimental unit, it was inserted, a polyethylene screen with a 1 mm mesh, fixed with a rubber ring obtained through the cross-section of the air chamber. The assembly of the experimental plot was performed by fitting the rings, using an adhesive tape (Silver Tape) to ensure resistance to the transport and the experimental evaluation procedures. Plastic dishes of 100 mm diameter were used, aiming to provide support for the experimental plots and to facilitate the irrigation.

The upper and lower rings referring to layers A and C, respectively, were filled with soil mass without adding compaction, while the central ring (Layer B) was compacted, increasing the density to 1.6 Mg m^{-3}, according to the methodology described by Fagundes et al. (2014).

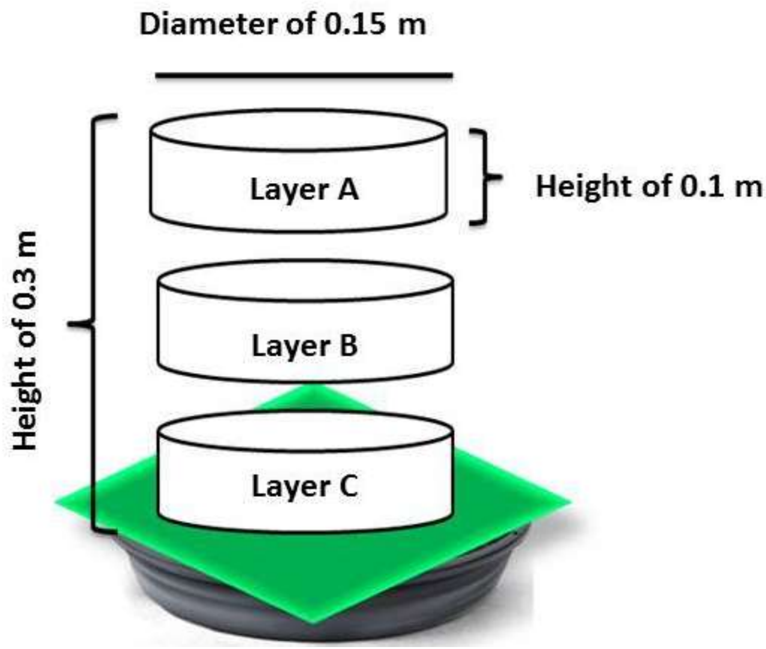

Figure 1. The experimental plot comprising three PVC rings cut with a height of 0.1 m and outer diameter of 0.15 m.

Soil compaction

The ideal moisture for compaction was determined in a laboratory test (Proctor normal test), in accordance with NBR 7182 (ABNT, 1986), establishing the value of 16% on the basis of mass and the weight of the dry soil to be compacted was determined according to the Equation 1:

$$Ds = \frac{\mathrm{MSS}}{\mathrm{VT}}$$

In which Ds- soil density, (kg dm^{-3}); MSS (DSM)- dry soil mass (g); VT- total layer volume (dm^{-3}). The calculation of the mass of the wet soil to be used in the compacted layer was determined according to Equation 2:

$$MSU = MSS + (1 - \theta m)$$

In which: MSU (MSM)- Moist soil mass (g); MSS (DSM)- dry soil mass (g); θm- mass-based moisture content (%).

Fertizer application and seed sowing

Fertilizer was applied at the time of compaction, with the application of 240 mg dm^{3} phosphorus (P_2O_5) and 200 mg dm^{3} potassium (K_2O) in all plots. The nitrogen dose was 150 mg dm^{3}, applied only to the control. Urea, simple superphosphate and potassium chloride were used as source.

Five seeds of the cultivar, BRS Tumucumaque were sown directly in the vessels, and after seven days, thinning was held, leaving only two plants per experimental plot. Soil moisture was maintained by the addition of water on the surface and by capillarity when water was added at the bottom of the experimental plot.

Inoculation

To prepare the inoculum, strains of rhizobia isolated from cowpea (MT8 - *Rhizobium tropici* and MT15 - *R. tropici*) and BR3267 from *Bradyrhizobium japonicum*, a strain recommended by the Laboratory Network to Recommendation, Standardization and Diffusion of the Technology of Microbiological Inoculants of Agricultural Interest - RELARE for cowpea inoculation (Martins et al., 2003) were used.

The bacteria were grown in YM liquid culture medium according to the methodology of Fred and Waksman (1928). Incubation was continued for about 96 h at 28°C. Thereafter, the inoculation with rhizobia was performed 7 days after plant emergence, with the application of 10 mL of the inoculant in each experimental plot.

Collection of samples for analysis

At 35, 45, 52 and 58 days after sowing, assessments of the Falker chlorophyll content were carried out in the leaves collected in the middle third of the plants, through the portable meter Clorofilog 1030.

At 90 days, the cutting of plants was performed at ground level, to evaluate the variables: dry mass of grains, nitrogen and crude protein content in shoots and grains. Initially, the plant material was packed in paper bags and identified, and shortly thereafter, dried in a forced-air oven at 65°C for 72 h or until constant weight, subsequently, weighing was performed on a precision scale.

Table 2. Effect of inoculation with rhizobia strains on the Falker chlorophyll content in cowpea grown in compacted and uncompacted soil.

Treatments	Compacted soil	Uncompacted soil
Falker chlorophyll content at 35 days		
BR3267	65.0^{Aa}	66.2^{Aa}
MT8+MT15	63.7^{Aa}	64.8^{Aa}
Control	66.8^{Aa}	69.8^{Aa}
CV(%) = 9.04		
Falker chlorophyll content at 45 days		
BR3267	68.4^{Aa}	65.4^{Aa}
MT8+MT15	71.2^{Aa}	68.4^{Aa}
Control	63.2^{Aa}	65.1^{Aa}
CV(%) = 9.04		
Falker chlorophyll content at 52 days		
BR3267	66.9^{Ab}	73.0^{Aa}
MT8+MT15	73.8^{Aa}	73.6^{Aa}
Control	72.5^{Aa}	72.7^{Aa}
CV(%) =6.50		
Falker chlorophyll content at 58 days		
BR3267	68.4^{Aa}	67.1^{Aa}
MT8+MT15	71.2^{Aa}	68.0^{Aa}
Control	64.3^{Aa}	66.4^{Aa}
CV(%) = 10.47		

Means followed by the same letter - uppercase, vertically, and lowercase, horizontally - do not differ by Tukey test at 0.05 significance level.

The determination of the total nitrogen content in leaves and grains followed the micro-Kjeldahl method described by Malavolta et al. (1997). The content of crude protein (CP) was calculated using the factor 6.25 for the conversion of total nitrogen to crude protein and the nitrogen accumulation was determined according to the following equation:

$$QNTA = \frac{\text{RMS} * \text{N}}{1000} \; X\, 1000$$

Where, QNTA (TAN)- Total accumulated nitrogen (mg $plant^{-1}$); RMS (DMY)- dry mass yield (g $plant^{-1}$); N- plant nitrogen content (g kg^{-1}).
All results were subjected to statistical analyses using the SISVAR program (FERREIRA, 2011), performing the analysis of variance and the Tukey test at the level of 5% probability.

RESULTS AND DISCUSSION

Soil compaction resulted in changes in the following variables: Falker chlorophyll index, after 52 days; dry mass of grains and nitrogen accumulation in the grains. Notwithstanding, regarding the Falker chlorophyll index at 35, 45 and 58 days, the nitrogen content in shoots and grains and the crude protein in shoots and grains, no statistical difference was observed between the treatments.

The highest averages for the Falker chlorophyll index in the evaluation performed at 35 days after sowing were observed in control plants, which received nitrogen fertilization, but in the assessments made at 45, 52 and 58 days, the combination of the strains MT8 + MT15 provided the best values, regardless of soil compaction, being observed, an increase of up to 11.23% in relation to nitrogen fertilization.

The plants inoculated with the strain BR 3267 showed a significant difference in relation to soil compaction. It was possible to observe a reduction of 8.35% in the Falker chlorophyll content when plants were grown in compacted soil (Table 2).

The results may have the same hypothesis described by Artursson et al. (2006), Smith and Read (2008) and Bashan (1998), in which the authors emphasized that the combination of microorganisms in inoculants may be

Table 3. Effect of inoculation with rhizobia strains on the dry mass of grains of cowpea grown in compacted and uncompacted soil.

Treatments	Compacted soil (g plots $^{-1}$)	Uncompacted soil (g plots^{-1})
BR3267	12.5^{Ab}	16.2^{Aa}
MT8+MT15	16.1^{Aa}	14.5^{Aa}
Control	12.4^{Ab}	15.7^{Aa}
CV(%) = 17.5		

Means followed by the same letter - uppercase, vertically, and lowercase, horizontally - do not differ by Tukey test at 0.05 significance level.

better at promoting greater uptake of nutrients by plants because of the ability of microorganisms to improve some beneficial aspects of the physiology of both, such as, for example, the greater affinity with the host plant, thus increasing the efficiency of the inoculation, besides promoting a greater development of the plants.

The averages achieved by the plants grown in compacted soil were due to the lack of soil densification in layers A and C, which would facilitate the growth and development of roots in this region, not changing the Falker chlorophyll index.

According to the authors, Hamza and Anderson (2005), the sowing of plants that have a deep and aggressive root system, which can grow even with the soil structure changed by increasing density, which is one of the main characteristics required for the management of compacted soil. Furthermore, Reinert et al. (2008) emphasized that the root system develops in the soil zone that has lower resistance to penetration, which favors the absorption of nutrients and water and thus provides increased nodulation.

For dry mass of grains, it was observed that the soil compaction provided a reduction in grain yield of 22.83 and 21.01% for treatments BR3267 and nitrogen fertilization, respectively, when compared with treatments without compaction. However, the plants inoculated with the combination of the strains MT8 + MT15 showed better performance in compacted soil, being observed, an increase of 9.93% as compared to uncompacted soil (Table 3). In uncompacted soil, the strain BR3267 provided the highest grain yield, with up to 10.49% increase as compared to the other treatments (Table 3). These results can be explained by the change in root growth, affecting the absorption of water and nutrients, and by the decrease in macropores, which consequently interferes with soil aeration (Grath and Hakansson, 1992). The soil conditions created by increased density have a direct and indirect effect on the microbial population. A smaller development of the root system results in a smaller amount of substrate for biological activity and, moreover, the remaining roots decrease the release of substances that attract the bacteria to start the infection and, subsequently, the formation of nodules (Flores et al., 1999).

Nevertheless, the strains exhibit different levels of competitiveness and adaptability in compacted soil conditions, where there is excess CO_2, O_2 deficiency and the presence of toxic elements produced by the environment (Miransari et al., 2007). This information is a hypothesis for the result of the treatment with the combination of the strains MT8 + MT15 and the performance of the strain BR3267 in uncompacted soil.

The results agree with Carvalho et al. (2005), who found that soybeans inoculated with the strain SEMIA 5019, along with three variants generated through spontaneous mutations (1A, 2A and 3A), showed better symbiotic efficiency in a test conducted in a greenhouse, using sand and vermiculite as substrate, in the ratio of 3:1.

The authors Cregan and Berkum (1984) pointed out that the plant nitrogen uptake increases in the vegetative growth stage and in the reproductive stage of the crop, decreasing in the grain filling stage, this information leads to assume that the inoculation with the combination of the strains MT8 + MT15 in plants grown in compacted soil provided nitrogen in an adequate quantity to meet a grain production similar to that of plants grown in soil without densification.

Similar to the results presented by plants inoculated with the strain BR3267 and fertilized with nitrogen, Collares et al. (2008) found in a study that the beans grown under field conditions, in soil with different densities (continued no-till- NT; no-till with additional compaction – NTc; and scarification - Sca), had their productivity decreased by 17%, as compared to the planting without compaction. According to these authors, the compaction altered the root system of plants leading to restriction in the absorption of nutrients and water.

For the variable nitrogen content in shoots and grains, the results were similar to the Falker chlorophyll index. The treatments that stood out in compacted soil were those that received nitrogen fertilization and those inoculated with the combination of the strains MT8 + MT15, respectively. It was observed that the combination

Table 4. Effect of inoculation with rhizobia strains on the nitrogen content in shoots and grains of cowpea grown in compacted and uncompacted soil.

Treatments	Compacted soil	Uncompacted soil
Nitrogen content in the shoots		
BR3267	21.6^{Aa}	21.7^{Aa}
MT8+MT15	22.0^{Aa}	22.8^{Aa}
Control	22.5^{Aa}	21.3^{Aa}
CV(%) = 16.8		
Nitrogen content in the grains		
BR3267	45.1^{Aa}	45.0^{Aa}
MT8+MT15	46.3^{Aa}	43.4^{Aa}
Control	43.1^{Aa}	43.1^{Aa}
CV(%) = 9.5		

Means followed by the same letter - uppercase, vertically, and lowercase, horizontally - do not differ by Tukey test at 0.05 significance level.

Table 5. Effect of inoculation with rhizobia strains on the nitrogen accumulation in shoots and grains of cowpea grown in compacted and uncompacted soil.

Treatments	Compacted soil	Uncompacted soil
Nitrogen accumulation in the shoots		
BR3267	245.3^{Aa}	288.7^{Aa}
MT8+MT15	252.1^{Aa}	303.9^{Aa}
Control	257.3^{Aa}	295.7^{Aa}
CV(%) = 19.3		
Nitrogen accumulation in the grains		
BR3267	281.0^{ABb}	367.9^{Aa}
MT8+MT15	377.0^{Aa}	315.3^{Aa}
Control	270.6^{Ba}	338.6^{Aa}
CV(%) = 9.5		

Means followed by the same letter - uppercase, vertically, and lowercase, horizontally - do not differ by Tukey test at 0.05 significance level.

of the strains MT8 + MT15 provided a 6.91% increase in the nitrogen content in grains as compared to the treatment with nitrogen fertilization in compacted soil (Table 4).

In uncompacted soil, the highest contents observed were obtained with the treatments with inoculation, being found, an increase of 6.57 and 4.22% in the nitrogen content in shoots and grains of the treatments MT8 + MT15 and BR3267, respectively. The results indicate that the rhizobia and cowpea symbiosis may have provided nitrogen in similar amounts as compared to nitrogen fertilization, benefiting the crop development.

Alves et al. (2003) conducted a study using three soil types (Dystrophic Red-Yellow Latosol of medium texture, Dystrophic Red-Yellow Latosol, clayey, and Typical Distroferric Red Latosol) and five compaction levels (50, 62.5, 75, 87.5 and 100% of maximum density) and found that the compaction did not alter the nitrogen uptake and the nitrogen content in bean plants, the best values being found at the density of 2.12 Mg m^{-3} in the Dystrophic Red-Yellow Latosol of medium texture.

Mandal et al. (1990), using three compaction levels (moderate - 1.79 kg dm^{-3}, highly compacted - 1.85 kg dm^{-3} and uncompacted - 1.52 kg dm^{-3}) in a sandy loam soil (680 g kg^{-1} sand, 240 g kg^{-1} silt and 80 g kg^{-1} clay), found that the loss of nitrate through leaching was three to four times lower in the highly compacted soil, as compared to the uncompacted soil.

Ferreira et al. (2000) proved the symbiosis efficiency in a experiment carried out with beans under field conditions, with Dystrophic Red Latosol. The results showed foliar nitrogen contents of inoculated plants similar to those found in nitrogen fertilization, which can be an indication that there was biological nitrogen fixation and that it was enough to meet the demand of plants for this nutrient (Martins et al., 2003).

For Figueiredo et al. (2008) and Meghvanshi et al. (2010), the success of the symbiosis depends on the compatibility with the cultivar analyzed, which enables the root invasion and thus provides nitrogen in a sufficient time to meet the plants' needs, a characteristic that was presented by the strains MT8 + MT15 in all variables analyzed.

The variable nitrogen accumulation in shoots of cowpea plants showed no difference between treatments, but the plants inoculated with the combination of the strains MT8 + MT15 provided increments of up to 5% when grown in uncompacted soil. Regarding the nitrogen accumulation in the grains, there was statistical difference between treatments. It was found that the inoculation with the combination of the strains MT8 + MT15 provided the best result in compacted soil, being verified the increase of 28.22% as compared to the control (Table 5).

The soil compaction negatively influenced the plants that received inoculation of the strain BR 3267, a reduction of 23.62% was observed in relation to the plants grown in uncompacted soil. Even with no significant difference, the treatment MT8 + MT15 showed higher nitrogen accumulation in compacted soil, where it was found, an increase of 16.36% as compared to the uncompacted soil.

Work conducted by Brito et al. (2011) showed that the biological nitrogen fixation provided most of the accumulated nitrogen in bean and cowpea plants, followed in descending order by the soil and the nitrogen

Table 6. Effect of inoculation with rhizobia strains on the crude protein in shoots and grains of cowpea grown in compacted and uncompacted soil.

Treatments	Compacted soil	Uncompacted soil
Crude protein in the shoots (mg. plant^{-1})		
BR3267	135.0^{Aa}	135.6^{Aa}
MT8+MT15	137.6^{Aa}	142.5^{Aa}
Control	140.9^{Aa}	133.6^{Aa}
CV(%) = 19.3		
Crude protein in the grains (mg. plant-1)		
BR3267	282.3^{Aa}	281.0^{Aa}
MT8+MT15	289.5^{Aa}	271.4^{Aa}
Control	269.9^{Aa}	269.8^{Aa}
CV(%) = 21.0		

Means followed by the same letter - uppercase, vertically, and lowercase, horizontally - do not differ by Tukey test at 0.05 significance level.

fertilization in an experiment carried out in a greenhouse, using the analysis of the isotopic content as a method. In an experiment carried out by Soares et al. (2006), the authors also found that the rhizobia strain INPA 03-11b provided the highest value for nitrogen accumulation in grains, in a field study, with a Typical Dystrophic Red Argisol. The results observed for the analysis of crude protein in the shoots and grains of cowpea were similar to the nitrogen content. The averages showed no significant difference, but the plants that received nitrogen fertilization and the combined inoculation of the strains MT8 + MT15 reached the highest values for these variables in compacted soil respectively (Table 6). In uncompacted soil, the strains MT8 + MT15 and BR3267 provided higher crude protein in shoots and grains, respectively, being found, a value 6.24 to 3.98% higher than those found in plants fertilized with nitrogen. This result confirms the data related to nitrogen content and yield found in studies by Ferreira et al. (2000), where the authors demonstrated that the bean does not depend on nitrogen fertilization when subjected to inoculation.

Thus, the strains studied in this test have demonstrated the ability to fix nitrogen, by promoting a result similar to that of plants fertilized with nitrogen (150 mg dm^{-3}).

Conclusion

The inoculation of the BR3267 strain contributed positively to the development and production of cowpea when grown on uncompacted soil, becoming more efficient in a management system that has the concern of preserving macropores of the soil. Inoculation with the combination of the MT8 + MT15 strains provided satisfactory results in both compacted soil and non-compacted soil favoring the production of cowpea in soil with a higher density.

Conflict of interest

The authors have not declared any conflict of interest.

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