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Effects of wood ash and soil water potential on vegetative development of mung bean (*Vigna radiata* L.)

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Abstract

This research aimed to evaluate the amending potential of eucalyptus's wood ash on soil chemical properties and soil-water potential. The experiment was conducted in a greenhouse at the Federal University of Mato Grosso, campus in Rondonópolis. The experimental design was composed of randomized blocks in a 5x5 factorial scheme, including five soil-water potentials (−4, −8, −16, −32 and −64 kPa), and five wood ash doses (0; 8; 16; 24 and 32 g dm^{-3}). The soil samples were collected from the top layer of an Oxisol under natural Cerrado vegetation. Mung bean (*Vigna radiata* L.) growth variables (plant height, numbers of leaves, stem diameter, and SPAD index) were analyzed at three different phenological periods. In general, the wood ash doses increased soil pH, eliminated the exchangeable aluminum, and improved soil essential nutrients availability. As a result, mung bean plants responded positively to wood ash, achieving superior results at doses ranging from 24 to 26 g dm^{-3}. The interaction between wood ash doses and soil water potential was not significant. However, drier soil conditions constrained plant growth severely. According to our experimental conditions, plant growth variables achieved higher performance at soil water potential of -4 kPa.

Keywords: Biomass ash, Brazilian Cerrado, drought stress, matric potential plant nutrition.
Abbreviations: DAE_ days after the emergency; DAS_ day after sowing.

Introduction

The recent population growth of the last decades indicates that by the end of this century the number of inhabitants will be approximately 11 billion. Consequently, this population growth will demand higher food production, pressuring the current agricultural systems to increase yield in a relatively reduced time window (Ray et al., 2013). Besides the global limitations related to the expansion of agricultural frontiers, climate change is expected to reduce the availability of natural resources, while intensifies abiotic and biotic stress on cropping systems (Challinor et al., 2016; Zhao et al., 2017; Pandey et al., 2017). Thus, it is necessary to align higher agricultural yields with the optimization of natural resources demand (e.g., adequate supply of water and nutrients during the phenological cycles of crops).

Brazil is one of the world's largest producers of agricultural commodities (Strassburg et al., 2014), contributing significantly to the food demand from several countries. The importance of Brazilian agriculture becomes even more relevant when it comes to grain production, where the Cerrado region stands out. Although this region is characterized by irregularities in rainfall patterns and for soils with high acidity (and reduced nutrient availability), but it is one of the largest world's grain producers due to the adoption of great quantities of agricultural inputs (Rada, 2013). The low pH reduces the availability of several essential nutrients in the soil, such as phosphorus, potassium, calcium, and magnesium, which become partially available when acidity is amended (Bonfim-Silva et al., 2018; Pantano et al., 2016).

Given the need for this large amount of agricultural inputs (correctives and nutrients) in the soils of the Brazilian Cerrado, a sustainable proposal might be consisted of the adoption of agroindustrial residues as potential sources of corrective and nutrients, especially for those small-scale cropping systems. Potential agro-industrial residues include the wood ash (Zimmermann, 2002; Santos et al., 2014), which is a material with high neutralizing capacity and appreciable amounts of essential nutrients. In addition to the positive aspects related to soil chemistry, wood ash may also improve soil hydraulic properties (Ram and Masto, 2004; Stoof et al., 2010), especially in retention and hydraulic conductivity, which are closely related to soil water availability to plant transpiration (Hansen et al., 2018; Maresca et al., 2017; Symanowicz et al., 2018; Pereira et al., 2016).

Among the many cropping systems in the Cerrado region that could benefit from wood ash, the green mung bean (*Vigna radiata* L.) is one of the most important one. Due to its short growing cycle, mung bean is an alternative crop for the Autumn-Winter growing season (off-season) and as the main crop during the regular growing season for small producers. Mung bean is an excellent N_2 fixer, allowing nutrient cycling, nitrogen addition, and the increasing of soil organic matter content. In addition to the benefits to the following crop (when cropped off-season), the mung bean grain is nutritionally rich in digestive protein (25-28%), making this plant species extensively cultivated in tropical and subtropical regions (Kumar et al., 2013).

The wood ash has shown potential to amend soil pH, and consequently increase in availability of nutrients in the soil. This research aimed to evaluate this agro-industrial residue to alter the soil chemical properties under different values of soil water potential. The experimental setup comprised of mung bean plants grown on designed pots under different doses of wood ash and controlled ranges of soil water content. Mung bean was selected due to its relevant presence among small-scale grain producers, representing a plausible cultivation system suitable for future adoptions of wood ash as an alternative material for soil chemical amendment.

Results and discussion

Soil chemical analysis at the end of the experiment

The chemical analysis of composite soil samples at the end of the experimental period showed that wood ash doses promoted soil pH by increasing the total elimination of exchangeable Al at the dose of 24 g dm^{-3}. Soil organic matter together with P, K, Ca and Mg availability, CEC, and base saturation responded positively to the wood ash doses as well (Table 1).

The increase in soil pH can be mainly attributed to the alkaline property of the wood ash, thus neutralizing soil acidity by the reactions of calcium carbonates (main wood ash component), potassium and magnesium (Etiégni and Campbell, 1991; Ohno, 1992; Erich and Ohno, 1992; Foletto et al., 2005; Ingerslev et al., 2014; Freire et al., 2015). Oxides and hydroxides are also components of wood ash (Hansen et al., 2017; Cruz-Paredes et al., 2017).

Higher soil phosphorus content upon increase in wood ash doses is partly explained by the high content of this element in the wood ash material (Table 6). Furthermore, increase in pH may enhance the natural availability of phosphorus (Donega, 2011; Prado et al., 2002). Likewise, the increased availability of potassium, calcium, and magnesium was also promoted by the appreciable concentration of these elements in the wood ash, and the higher pH values may also have contributed to the greater availability of these nutrients in the soil (Donega, 2011; Prado et al., 2002; Park et al., 2012).

Effect of wood ash on growth variables

The wood ash and soil-water potential significantly affected the analyzed growth variables ($p<0.001$). However, the effects were isolated. There was no interaction between wood ash doses and soil-water potentials. Therefore, the results regarding these treatment groups will be presented and discussed separately.

Response of plant height, number of leaves and stem diameter of mung bean plants to wood ash doses was fitted to the quadratic regression model (Table 2) for the three evaluation periods (15, 30 and 45 DAE) (Figures 1A, 1B and 1C). The maximum plant heights (11.3, 17.3, and 20.8 cm) were observed at wood ash doses of 27, 24.8, and 25.3 g dm^{-3} for the three evaluation periods, respectively. The number of leaves significantly responded to wood ash doses, with the highest values at doses ranged from 24 to 26 g dm^{-3}. The highest stem diameter was observed at doses of 23, 22, and 24 g dm^{-3} for the three evaluation periods, respectively.

Regarding the leaf chlorophyll index, the SPAD index behaved linearly with the wood ash doses for the first evaluation period (15 DAE) and quadratic for the other evaluation periods (30 and 45 DAE) (Table 2). At 15 DAE, the SPAD index was increased by 13.3% at the highest wood ash dose (32 g dm^{-3}) as compared to the control treatment. For the second and third evaluation period, the highest chlorophyll index (53.5 and 53.4) was occurred at doses 28 and 25 g dm^{-3}, respectively (Figure 1D).

According to Table 1, the wood ash used in this research showed the appreciable amount of macronutrients in its composition (Ca, Mg, P and K), thus acting as a promoter of plant growth. Symanowicz et al. (2018) showed that wood ash residue can be undoubtedly considered a source of macronutrients to plants, therefore, prone to adoption in cropping systems. Dallago (2000) observed that wood ash influences the vegetative growth and development of plants, whereas either the lack or the excess of wood ash significantly reduce the plant height. This indicates the need for studies to find the correct balance between wood ash dose and plant metabolism. Bonfim-Silva et al. (2017), evaluated the potential of ash on leguminous plants. They found that vegetative growth may be limited when doses are not within the optimal range for plant phenological development.

The response of the number of leaves to wood ash doses indicates the positive effect of this residue on the initial formation and expansion of leaves, stimulated by the increase of phosphorus and calcium availability in the soil solution. In addition to the aboveground variables, the wood ash also triggered the root system development (Figure 2).

In general, phosphorus has a diverse effect on metabolism of plant tissues. It plays a paramount role in chemical reactions related to energy transfer between cells, respiration and photosynthesis. Restricted phosphorus availability in the early phenological stages of the crop may result in severe plant growth reduction (Malavolta, 1989; Grant et al., 2001). According to Arf (1994), although phosphorus is added to the soil in appreciable quantities, it is still the most limiting nutrient in common bean production in most of the Brazilian soils, mainly due to its immobilization by the adsorption processes, precipitation or conversion into organic forms (Holford, 1997). According to Fageria et al. (2003), the influence of phosphorus on the bean crop is notorious, acting on the increase of shoot biomass production, with a positive effect on the number of pods and grain yield. Besides the element P, other elements such as S, Ca and Mg are essential for the constitution of structural elements of the plant (e.g. cell walls), promoting a balanced plant growth (Figure 2), allowing above and belowground development traits related to lodging resistance, facilitating the harvesting process (Carneiro, 1995; Bonfim-Silva et al., 2016).

The SPAD index indirectly represents the leaf chlorophyll content, which in turn, is highly correlated to plant nitrogen nutritional status (Costa, et al., 2008; Schlichting et al., 2015). In addition to the relationship between the SPAD index and the plant nutritional status regarding the nitrogen, it also allows inferring on chlorophyll-related metabolic processes (Markwell et al., 1995; Guimarães et al., 1999).

Table 1. Average values of soil chemical composition subjected to wood ash doses.

Dose	pH	P	K	Ca	Mg	Al	CEC	O.M.	V
g dm^{-3}	($CaCl_2$)	___mg dm^{3}___		_____cmolc dm^{-3}_____				g dm^{-3}	%
0	3.98	3.78	79.91	0.48	0.24	0.86	7.26	26.46	12.79
8	4.40	7.98	116.65	1.19	0.49	0.54	7.58	28.43	26.16
16	4.72	19.27	170.59	1.63	0.65	0.18	7.52	28.59	36.30
24	5.20	37.43	372.18	2.47	0.97	0.00	8.74	32.77	50.00
32	5.39	44.89	300.53	2.79	1.07	0.00	8.43	32.62	54.79

Dose = wood ash; P = Phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; Al = Aluminum; OM = Organic matter; CEC = Cations Exchange Capacity at pH 7.0; V = Base saturation.

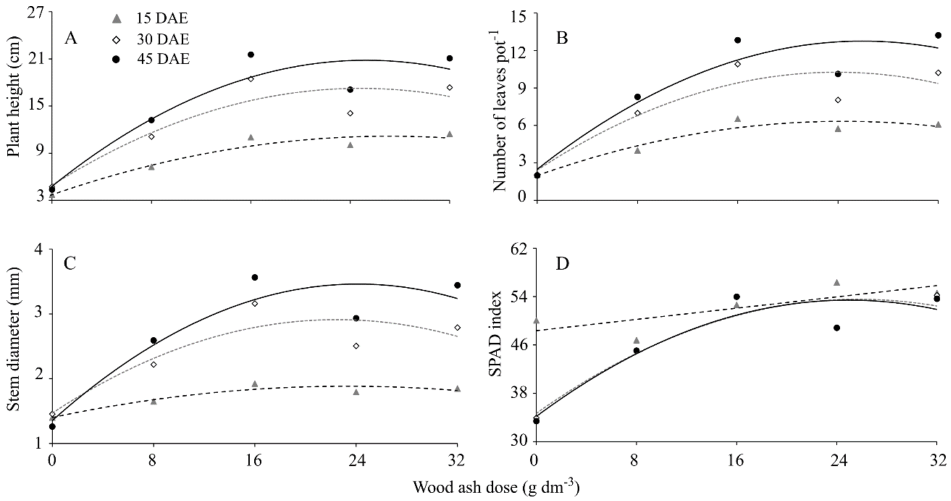

Figure 1. Plant height (A), number of leaves (B), stem diameter (C), and SPAD index (D) of mung bean plants at 15, 30, and 45 DAE subjected to wood ash doses ($p < 0.001$).

Table 2. Fitted regression equations describing mung bean growth variables response to wood ash doses (g dm^{-3})

Variable	Fitted equation		
	15 DAE	30 DAE	45 DAE
Plant height [cm]	$-0.01\omega^2+0.55\omega+3.73$ $R^2=0.94$	$-0.02\omega^2+0.99\omega+4.92$ $R^2=0.84$	$-0.03\omega^2+1.27\omega+4.78$ $R^2=0.88$
Number of leaves [-]	$-0.007\omega^2+0.36\omega+2.0$ $R^2=0.93$	$-0.01\omega^2+0.66\omega+2.43$ $R^2=0.83$	$-0.02\omega^2+0.79\omega+2.49$ $R^2=0.87$
Stem diameter [mm]	$-0.001\omega^2+0.04\omega+1.4$ $R^2=0.90$	$-0.003\omega^2+0.13\omega+1.47$ $R^2=0.81$	$-0.004\omega^2+0.17\omega+1.35$ $R^2=0.87$
SPAD index [-]	$0.23\omega^2+48.37$ $R^2=0.61$	$-0.03\omega^2+1.47\omega+34.7$ $R^2=0.87$	$-0.03\omega^2+1.54\omega+34.2$ $R^2=0.88$

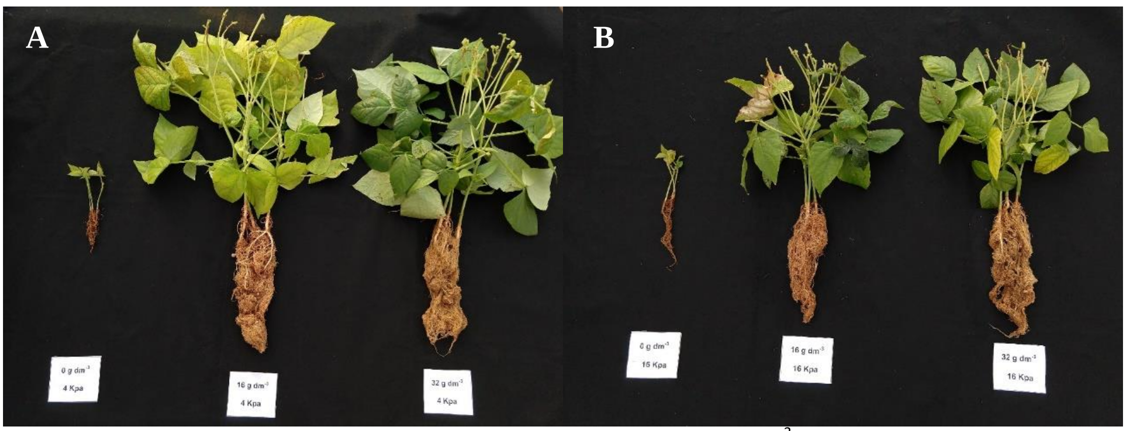

Figure 2. Development of mung bean plants under wood ash doses (0, 16, and 32 g dm^{-3}) under two values of soil-water potential, −4 kPa (A) and −16 kPa (B).

Table 3. Fitted regression equations describing the mung bean growth variables response to soil-water potential |h|.

Variable	Fitted equation		
	15 DAE	30 DAE	45 DAE
Plant height [cm]	$-0.09\|h\|+11$ $R^2=0.84$	$-0.20\|h\|+18.2$ $R^2=0.76$	$-0.29\|h\|+22.61$ $R^2=0.81$
Number of leaves [-]	$-0.05\|h\|+6.23$ $R^2=0.70$	$-0.12\|h\|+10.72$ $R^2=0.71$	$-0.16\|h\|+13.23$ $R^2=0.76$
Stem diameter [mm]	$-0.01\|h\|+1.91$ $R^2=0.85$	$-0.03\|h\|+3.16$ $R^2=0.78$	$-0.04\|h\|+3.87$ $R^2=0.81$
SPAD index [-]	*NS**	$-0.37\|h\|+56.47$ $R^2=0.70$	$-0.36\|h\|+56.01$ $R^2=0.68$

**NS*: not significant.

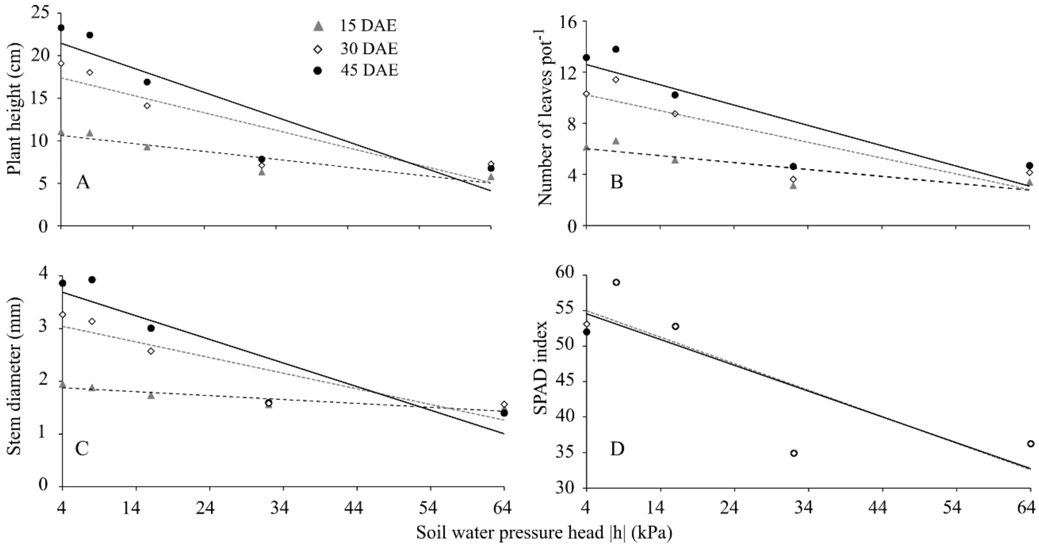

Figure 3. Plant height (A), number of leaves (B), stem diameter (C), and Chlorophyll Index (SPAD) (D) of mung bean plants for decreasing values of soil-water potential at 15, 30 and 45 DAE ($p < 0.001$).

Table 4. Treatments consisting of the 13 combinations of wood ash doses and soil-water potential according to the experimental design 'modified central composite' of Littell and Mott (1975).

Wood ash dose (g dm^{-3})	Soil-water potential(kPa)
0	−4; −16; −64
8	−8; −32
16	−4; −16; −64
24	−8; −32
32	−4; −16; −64

Figure 4. Mung bean plants under the wood ash doses of 16 g dm^{-3} (A) and 32 g dm^{-3} (B) and soil-water potential ranging from −4, –16, and −64 kPa, respectively.

Table 5. Chemical and particle size analysis for the top layer of the sampled Oxisol after collection.

pH	P	K	Ca	Mg	Al	H	CEC	OM	V	Sand	Silt	Clay
$CaCl_2$	mg dm^{-3}			$cmol_c$ dm^{-3}				g kg^{-1}	%	g kg^{-1}		
4.0	1	43	0.5	0.3	1.2	4.7	8.3	29	11	507	116	377

P = Phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; Al = Aluminum; H = Hydrogen; CEC = Cation Exchange Capacity at pH 7.0; OM = Organic matter; V = Base saturation.

Figure 5. Experimental units coupled with a horizontal tube to allow soil water content measurements by a Diviner capacitance probe during the experimental period.

Table 6. Chemical composition of the eucalyptus wood ash used in this research.

pH	N	P_2O_5	K_2O	Ca	Mg	Na	SO_4	Si	Fe	Cu	Mn	B	Zn
$CaCl_2$					g kg^{-1}								
10.7	3.1	9.6	34.7	33.0	21.0	0.12	2.0	274.4	10.3	0.0	0.4	0.1	0.1

N = Nitrogen; P_2O_5 = Phosphorus in neutral ammonium citrate and water (CNA+water); K_2O = Potassium; Ca = Calcium; Mg = Magnesium; Na = Sodium; SO_4 = Sulfur; Si = Silicon; Fe= Iron; Cu = Cupper total; Mn= Manganese Total; B = Boron Total; Zn = Zinc Total.

As no nitrogen fertilizer was applied in this experiment, it can be presumed that plant nitrogen nutrition occurred essentially from the biological nitrogen fixation process, which was probably positively influenced by the wood ash doses (Figure 1D). In acidic soils, the biological fixation of N_2, and consequently the growth and development of leguminous plants are affected by several factors such as the low availability of phosphorus and calcium in the soil and the toxicity of aluminum that acts restraining the nodulation processes (Andrew, 1976; Murphy et al., 1984). Silva et al. (2010) verified positive correlations between nodulation and chlorophyll index in cowpea leaf, indicating that biological nitrogen fixation through nodulation may significantly influence the relationship between chlorophyll index and leaf N concentration. As the process of biological fixation of N_2 requires a considerable amount of energy, a healthy host plant is essential, demanding phosphorus for the cellular energy exchange processes (Chaudhary and Fujita, 1998; Sa and Israel, 1991; Campo and Hungria, 2002).

Effect of soil-water tension on mung bean growth variables

The analyzed variables (plant height, number of leaves, stem diameter, and SPAD index) behaved linearly to soil-water potential for the three evaluation periods (15, 39, and 45 DAE) (Figure 3 and Table 3). The highest values of these variables were occurred at soil-water potential of -4 kPa, with considerable reduction as the soil becomes drier. Regarding the effect of the soil-water pressure on the SPAD index, we observed that the numerical differences were not significant ($p > 0.05$) for the first evaluation period at 15 DAE. On the other hand, the chlorophyll index fitted to linear regression models at 30 and 45 DAE (Figure 3D), being significantly decreased at drier soil conditions.

According to Freitas et al. (2011) and Nezami et al. (2008), the reduction in plant growth is one of the main stress symptoms of water deficit, reducing cell turgidity and negatively affecting cell differentiation, expansion, and elongation (Cairo, 1995; Nogueira et al., 2005). These effects were observed for the treatments under the condition of more negative values of soil-water potential (lower plant heights, reduced number of leaves, thinner stems and lower SPAD index), which probably negatively affect the production and translocation of carbohydrates in the plant (Ludlow and Muchow, 1990; Larcher, 2004).

Soil water availability favors the root extraction process, promoting nutrient translocation as well as cell growth (Pereira et al., 2016). According to Figure 4, as the drier soil condition persists, root and aboveground biomass are greatly reduced.

The observed lower number of leaves in plants subjected to lower soil moisture may occur due to a physiological response mechanism to water deficit to reduce water loss by transpiration (Floss, 2004; Mahajan and Tuteja, 2005; Inman-Bamber et al., 2008). Similar to the results of this research, other studies have observed better performance of growth and yield variables under wetter soil conditions (Sangakkara, 1999; Sengupta, 2013; Das, 2017).

Usually, the soil-water potential of -4 kPa is relatively high for a tropical Oxisol. This indicates possible stress due to excess water in this particular Oxisol coarse-textured with sand content of > 50% (Table 5). Therefore, there is sufficient aeration porosity to meet the respiratory needs of the crop at -4 kPa. The Chlorophyll index tends to be lower in plants under water excess due to the increased concentration of free radicals that disintegrate the chlorophyll molecule (Drew, 1997). However, in the present study, the SPAD index was higher at -4 kPa, which is another indication that stress due to water excess did not occur at this particular soil-water pressure head. Similar to the results of the present study, Sangakkara (1999) showed a reduction in the chlorophyll index of mung bean and cowpea at drier soil conditions.

As shown by this research and other studies cited throughout this manuscript, wood ash has the potential to alter the chemical properties of the soil, and thereby promote plant growth. On the other hand, the hypothesis of its positive effect on soil physical properties is based on static indices, e.g. organic matter content, soil porosity and water retention in some potentials values (Karmakar et al., 2009; Conceição et al., 2005; Islabão et al., 2016). Conversely, to verify this hypothesis a mechanistic evaluation tool is required that allows dynamic analysis of the main variables that control the physical processes of water transfer in the vadose zone (Bonfante et al., 2019; Pinheiro et al., 2019). In this study, there was no interaction between wood ash doses and soil-water potentials, which is an expected result, since changes on soil hydraulic properties may require a much longer time period than the one experienced in this research. Possibly, such analysis would be more conclusive in an experiment under a condition where the natural soil structure is preserved.

Materials and methods

Geographic location and experimental characterization

The experiment was carried out from February to June 2019 in a greenhouse at the Federal University of Mato Grosso, campus in Rondonópolis, located at latitude 16º 28’ S, longitude 54º 35’ W, and 284 m above sea level. The regional climate type according to Köppen classification is Aw, tropical zone with dry winter and wet summer. The average temperature and relative humidity during the experiment were 27.1°C and 53.8%, respectively.

The statistical design used in this research was composed of randomized blocks in a 5^2 fractional factorial design, consisting of five doses of wood ash (0, 8, 16, 24 and 32 g dm^{-3}) and five potential (h) values (−4, −8, −16, −32 and −64 kPa) with four replicates. The number of experimental units (52) was established according to the experimental design based on the modified central composite (Littell and Mott, 1975), consisting of 13 treatments (combinations of wood ash doses, g dm^{-3}, and soil-water potential, kPa) (Table 4).

Experimental units were composed of adapted pots, especially built for this research. Three individual PVC rings (10 cm high and 20 cm diameter each) were assembled on the top of each other, totaling a 30 cm height pot. In the middle PVC ring, a horizontal 5-cm diameter perforation allowed coupling four experimental units by inserting a tube 150-cm long (Figure 5). Through this horizontal tube, soil water content was periodically monitored by a capacitance probe (Diviner 2000®). Discounting the volume occupied by this horizontal tube, each experimental unit presented a volume of 8.7 dm^3.

Soil collection, analysis and correction

The soil material used in this experiment was collected from the top layer (0.0-0.2 m) of an Oxisol (Soil Taxonomy) under natural Cerrado vegetation. The material used to fill the pots passed through a 4 mm sieve. Additional soil samples passed through a 2 mm sieve for chemical and particle size characterization (Table 5) according to the methodology proposed by Embrapa (2017).

Wood ash doses (0, 8, 16, 24, and 32 g dm^{-3}) were used as soil pH amendment. Soil material and the respective wood ash doses were mixed in proper plastic bags. After the homogenization (soil material and wood ash), the mixture material was used to fill the experimental units (pots). The soil-water potential of the accommodated material in each experimental unit was maintained according to each treatment (−4, −8, −16, −32, and −64 kPa). To allow soil acidity reduction by chemical reactions, experimental units with the mixed material remained in repose for 30 days under the respective water tension in the soil, according to each treatment (Table 4).

The wood ash used in this research prepared from the combustion of wood materials in local industrial plants for power generation. The chemical composition of the used ash is shown in Table 6.

Fertilization and irrigation management

After incubation period of 30 days, ten seeds of mung bean (*Vigna radiata* L.) were sown four centimeters deep in each experimental unit. Seedling thinning was performed on the sixth and the twelfth day after sowing (DAS). The final plant population was established in three vigorous plants per each pot unit.

Irrigation was controlled by measuring soil water content using a calibrated capacitance probe (Diviner 2000®). The functional relationship between soil-water potential and soil water content (h-□) of the accommodated soil material was previously established to similar pots designs. Water replenishment was performed manually using a graduated container to maintain the soil-water potential according to the values established for each treatment.

The response variables analysis were performed at 15, 30 and 45 DAE: Plant height [cm]; the number of leaves [-] per experimental unit; average stem diameter [mm] per experimental unit at 2 cm from the soil surface; and chlorophyll index by SPAD meter-502, obtained from the average of five readings taken on random leaves in the middle third of the plants. After the experiment completion, a composite soil sample corresponding to each wood ash dose was subjected to chemical analysis.

Statistical analysis

The response variables were analyzed by the statistical software SISVAR (Ferreira, 2011), testing the interaction between the treatments (wood ash doses x soil-water potentials) followed by the analysis of variance and regression test at $p < 0.05$.

Conclusions

The main findings of our experimental research on evaluating the combined effects of wood ash dose and soil-water potentials on growth variables of mung bean allowed us to conclude that: Plant height, the number of leaves, stem diameter and chlorophyll index are significantly and independently influenced by wood ash doses and soil water stress. Fertilization with wood ash with doses between 24 and 26 g dm^{-3} allowed the best responses for the analyzed variables related to the development of mung bean crop. The analyzed growth variables achieved higher performance at a soil-water potential of –4 kPa, indicating that although being a rustic crop, the mung bean grows more efficiently under higher soil water availability.

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