Original Research

Effect of Physic Nut Seed Cake on Common Bean Development and Clay Dispersion of Soil

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Abstract

Jatropha cake is a residue rich in nutrients that when used as organic fertilizer can contribute to plant development and soil properties improvement. Thus, this study aimed to evaluate *Jatropha* cake, digested and non-digested, on common bean plant (IPR-Colibri) growth and in soil properties. A greenhouse experiment was carried out in a sand-clay-loam soil to assess the effect of *Jatropha* cake (non-digested and digested) on soil Ca^{2+}, Mg^{2+}, Al^{3+}, pH, organic carbon and clay dispersion in water and on plant leaf area, height, stem diameter, number of nodules, dry mass of root and shoot and, phosphorus (P) uptake in the shoot. The amount of *Jatropha* cake applied was calculated based on the equivalent to P content (50, 100, 200 and 300 kg P ha^{-1}). It included two treatments: control and mineral fertilization. The *Jatropha* non-digested cake in doses of 200 and 300 kg P ha^{-1} inhibited seedling emergence, while a dose of 50 kg P ha^{-1} had positive influence on the development of the plants and in the P uptake in the shoot. There were water-dispersed clay values reduction and an increase in organic carbon with applications of *Jatropha* non-digested cake into the soil.

Introduction

The physic nut (*Jatropha curcas* L.) is a plant resistant to drought [1]. It is an important oleaginous crop for bioenergy generation and relevant for biodiesel production due to the high oil concentration in its seeds [2, 3], ranging from 40 to 60%. When the oil extraction process generates a cake, despite having high nutritional content, it is not used in feed due to the presence of such toxic components as curcin and phorbol esters [4].

In India, it is estimated that *Jatropha* will be cultivated on over 20 million hectares in upcoming years and production of about 20 million tons of cake per year is expected, generating an organic waste proportion that demands a safe destination [5].

Studies have demonstrated the potential use of the cake due to its macro chemical composition and usable micronutrients in agriculture as organic fertilizer, which

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can be applied directly to the soil without any treatment in solid form [6, 7] or in liquid form after of the digestion process of biogas, increasing the concentration of nutrients [7-9] and showing potential for biofertilizer production [10].

Chemical analysis on the soil, leaves, fruits and tubers after harvest have determined that *Jatropha* cake used as organic fertilizer in cabbage (*Brassica oleracea* L.), tomato (*Solanum lycopersicum* L.) and sweet potato (*Ipomoea batatas* L.) production, does not have phorbol ester residue, indicating that it is safe to use [8]. Therefore, this cake can contribute to bean plant development for its higher nutritional content in soil and plants, showing up as an alternative to expand the exploitation of less fertile areas.

The common bean (*Phaseolus vulgaris* L.) is one of the most widespread crops in Brazil, having besides an economic character, a high social significance, being a staple food for the population. Despite its importance, it still has relatively low productivity, considering the low soil fertility as one of the responsible factors [11].

Due to the small, shallow root system and short cycle, the common bean is considered a demanding nutrient crop. Among the most demanding nutrients for its development are nitrogen (N), phosphorus (P) and potassium (K) [12] – components present in significant amounts in *Jatropha* cake [5].

There are some positive results from the application of organic fertilizers in improving soil properties; however, few studies evaluate the effects of this application on other attributes influenced by the organic matter increase, for example clay dispersion. Organic matter in the soil is important as a nutrient source, retention of cations, improving microbial activity and the soil physical properties which, in turn, influence the availability of air and water to plant roots [13].

Phosphorus (P) is abundant in soils, in both organic and inorganic forms, and its availability is restricted as it occurs mostly in insoluble forms [14]. Low availability is common in most tropical soils [15], which makes this element a frequent object of study, which seeks to analyze the regulatory mechanisms of P supply to plants and the influence of soil properties on crop response to P application.

The hypothesis of this work is that *Jatropha* cake application, digested or non-digested as fertilizer for the common bean plants will increase organic matter, promoting a decline in clay dispersion and phosphorus availability. The aims were to evaluate the effect of *Jatropha* cake application, digested and non-digested, on common bean crop development, P uptake and clay soil dispersion.

Material and Methods

A greenhouse experiment was conducted at the Agronomic Institute of Paraná - IAPAR in Londrina, PR (latitude 23º23'S and longitude 51º11'W) during the period of October to November 2015. It used a sand-clay-loam soil (Oxisol perudic) classified as typic Rhodic Haploperox [16] and in Brazilian classification as Dystrophic Red Latosol, which were collected from 0-0.20 m depth, in the experimental station of IAPAR in Ponta Grossa, Parana. Soil samples were dried by air, sieved with 4 mm and placed in 3.5 kg plastic pots. The selected physico-chemical characteristics of soil subsamples after liming are shown in Table 1.

Characterization of Physic Nut Seed Cake

The physic nut seed cake was extracted on 9 January of 2014 in the National Institute for Agricultural Research (INIAP) from the Experimental Portoviejo Station, Manabi, Ecuador (lat 01°14'S, long 80°16'W, 44 m.a.s.l.), using the press system type "expeller", developed by the German company REINARTZ CompacTropha [17], and cold extraction.

In a low-density polyethylene bag with a thickness of 0.10 mm and 5 kg capacity, the cake produced was packed and transported by air to the Agronomic Institute of Paraná – IAPAR, located in Londrina in northern Parana State, Brazil (lat. 23°08'47 "S, long. 51°19'11"W, 640 m.a.s.l.). The bags containing the cake, which was named in this work as non-digested cake, were stored in an incubator (BOD brand FANEM 347.FG. model) at 25±2.0ºC.

For obtaining the cake digested, a system type "batch" with four digesters (1.5 L capacity each one) was constructed, using 200 g of the cake containing approximately 20% of total solids [9] and added 823.40 mL of water. The digester was sealed and maintained for 21 days at 30ºC.

Chemical analyses of macro and micronutrients of the cake, digested and non-digested (Table 2), were performed according to the methodology described by Miyazawa [18].

Table 1. Selected physical-chemical properties of soil samples from layer 0.0 to 0.20 m that were used in the greenhouse experiment.

pH[a]	H+Al	Ca^{2+}	Mg^{2+}	K$^+$	CEC	P	C$_{tot}$	BCS	Clay	Silt	Sand
	cmol$_c$ dm^{-3}					g kg^{-1}		%	g kg^{-1}		
5.7	4.96	5.45	3.41	0.3	14.12	5.1	32.21	64.87	545.10	316.40	138.50

[a]CaCl$_2$ 0.01 mol L^{-1}; H+Al in SMP; Ca, Mg and Al by KCl 1 mol L^{-1}. CEC = cation exchange capacity at pH 7.0;
P and K in Mehlich-1; Total Carbon (C$_{tot}$) by Walkley and Black; BCS = Base-cation saturation.

Table 2. Organic carbon (C_{org}), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), cupric (Cu), zinc (Zn), boron (B) and manganese (Mn) concentrations of physic nut seed cake (*Jatropha curcas* L.) non-digested and digested, and amounts of macro and micronutrients applied per pot.

Jatropha cake	C_{org}	N	P	K	Ca	Mg	Cu	Zn	B	Mn
	g kg^{-1}						mg kg^{-1}			
Non-digested	347.26	35.15	5.18	11.91	3.44	3.48	7.89	13.46	16.42	22.36
*Digested	369.02	50.55	13.33	25.46	10.09	8.10	23.41	52.27	35.3	48.94
Non-digested										
g pot-1	g pot^{-1}						mg pot^{-1}			
17.37	6.03	0.21	0.09	0.21	0.06	0.06	0.14	0.23	0.29	0.39
34.75	12.07	0.42	0.18	0.41	0.12	0.12	0.27	0.47	0.57	0.78
67.57	23.46	2.38	0.35	0.80	0.23	0.24	0.53	0.91	1.11	1.51
102.32	35.53	3.60	0.53	1.22	0.35	0.36	0.81	1.38	1.68	2.29
Digested										
6.75	2.49	0.34	0.09	0.17	0.07	0.05	0.16	0.35	0.24	0.33
13.5	4.98	0.68	0.18	0.34	0.14	0.11	0.32	0.71	0.48	0.66
26.26	9.69	1.33	0.35	0.67	0.26	0.21	0.61	1.37	0.93	1.29
39.76	14.67	2.01	0.53	1.01	0.40	0.32	0.93	2.08	1.40	1.95

*P = 0,064

Treatments and Experimental Design

The treatments evaluated consisted of the *Jatropha* cake applied to soil as organic P fertilizer in different amounts, which were calculated according to P content of each jatropha cake (non-digested and digested) in order to achieve the following P doses: 50; 100; 200 and 300 kg P ha^{-1}, equivalent to 17.37; 34.75; 67.57 and 102.32 g for non-digested cake and to 6.75; 13.5; 26.26 and 39.76 g for digested cake based on the volume of soil in each pot.

Two treatment controls were also included, with mineral fertilizers and without. The treatment with mineral fertilizer containing 4.72 g of triple superphosphate, 1.33 g of potassium sulphate and micronutrient solutions, in mg per pot with: 18.54 of H_3BO_3, 13.08 of $ZnSO_4.7H_2O$, 0.79 of $CuSO_4.5H_2O$, 9.23 of $MnSO_4.H_2O$, 0.18 de $(NH_4) 6Mo7O_{24}4H_2O$, 24.2 de $FeCl_2.6H_2O$ [19]. These 10 treatments were evaluated using a completely randomized design, with four replications.

The physic nut (digested and non-digested cake) was applied into the soil according to each treatment seven days before sowing the common bean seeds. In each pot containing 3.5 kg of soil were seeded, approximately to 1 cm deep, 10 common bean seeds of IPR-Colibri variety (carioca group, early cycle and erect).

Inoculation of seeds with *Rhizobium tropici*, strain (CIAT899, SEMIA-4077) was performed with an aliquot of 1 mL of suspension per pot, containing on average 10^9 cells mL^{-1}. As in the treatments with 200

and 300 kg P ha^{-1} of non-digested cake, there was no plant emergence, seeds were sowing again twice without success.

During the experiment the temperature ranged from 27±3ºC day to 22±3ºC night, and soil moisture was maintained between 60 to 70% holding water capacity by the addition of deionized water. The experiment was harvested at flowering when more than 50% of the plants were in the R5 stage, which corresponds to the emission of floral buds.

Plant Evaluation

To estimate the percentage of plant emergence, eight days after sowing, seedlings were counted and then thinning was performed by leaving three seedlings per pot. At flowering, in the end of the experiment we evaluated leaf area estimation in cm^2 [20]. Main branch length was evaluated using a graduated ruler in cm, from the distance of the lap to the apex of the plant and stem diameter was measured in mm between the lap and the first node using a pachymeter.

After harvesting the plants, roots and shoots were separated and washed. Nodules were removed from the roots, washed in a fine-mesh sieve (1 mm) and dried on paper tissue for counting.

Dry matter (DM) of roots and shoots were determined on samples after oven-drying for 72 h using a forced ventilation oven at 65±2ºC. Shoot samples were milled and P concentration was determined following procedures described by Miyazawa [18]. The

Table 3. Seedling emergence (%), plant height (cm), stem diameter (mm), estimates of foliar area (cm²) and number of nodules of common bean (IPR - Colibri) grown in soil with the application of *Jatropha* cake, not digested (ND) and digested (D); values are means of four replicates.

Treatments	Plant		Stem	Foliar	Nodules
	Emergence (%)	Height (cm)	Diameter (mm)	Area (cm²)	Nº
1- ND50*	82.50a	28.13ab	4.02a	27.24a	0.00c
2- ND100*	57.50b	16.92cd	3.55b	14.76bc	0.00c
3- ND200*	—	—	—	—	—
4- ND300*	—	—	—	—	—
5- D50*	77.50ab	22.67bc	3.18bc	13.53cd	56.00a
6- D100*	72.50ab	22.84bc	3.24bc	13.25cd	37.00b
7-D200*	80.00ab	29.44a	3.27bc	17.18bc	54.00a
8-D300*	87.50a	25.50ab	3.47b	17.54bc	58.00a
9- Without fertilizer	85.00a	15.59d	2.93c	9.62d	31.00b
10- With fertilizer	77.50ab	27.46ab	3.39b	18.55b	34.00b
CV (%)	**13.58**	**11.53**	**5.15**	**12.36**	**16.27**

Means followed by the same letter in the column do not differ by Tukey test ($\alpha = 0.05$). *Doses of phosphorus in kg of P ha⁻¹ calculated according to P content in *Jatropha* cake. Data from treatments ND200 and ND300 were not included in statistical analysis.

P uptake in the shoot was calculated by multiplying the concentration of shoot P measured by the amount of DM of plant to express in mg plant⁻¹.

Soil Chemical and Physical Analyses

After harvest of plants, soil samples were collected from each vase and we determined Δ pH (pH-KCl - pH-H$_2$O); Ca^{2+} (KCl 1 mol L⁻¹), Mg^{2+} (KCl 1 mol L⁻¹) and Al^{+3} (KCl 1 mol L⁻¹), expressed in cmol$_c$ dm⁻³; total carbon (Walkey-Black); and, water-dispersed clay (WDC), according to EMBRAPA [21]. These analyses were performed in the Laboratory of Soil of the State University of Londrina.

Statistical Analyses

The data of common bean plant growth, nodulation and of soil chemical characteristics were subjected to analysis of variance. The treatment means were compared by Tukey test at 5%. The linear correlation analysis was carried out between WDC and Al, Ca, C and Pearson coefficients were obtained. All statistical analyses were performed using the software SISVAR [22]. The software SIGMA PLOT 11.0 was used to create bar graphics.

Results and Discussion

Analysis of selected macro and micronutrient of the cake, digested and non-digested, are shown in Table 2. The concentrations of N, P, Ca, Mg, Cu, Zn, B and

Mn are higher in digested *Jatropha* cake than in non-digested ($P = 0,064$) by Mann-Whitney rank sum test.

Plant Evaluation

For the percentage of plant emergence, we observed that there are significant differences according to the treatments. Using non-digested cake with a dose of 100 kg P ha⁻¹ had only 57.40% of plant emergence, which was significantly lower than the control with 85.00%, the ND50 with 82.50% and D300 with 87.50% (Table 3).

Treatments in which non-digested cake was applied at doses of 200 kg ha⁻¹ P and 300 kg ha⁻¹ P did not show plant emergence. Treating seeds with higher plant extract concentrations has a negative effect on plant cell integrity, which consequently results in low emergence percentage and increased mean emergence time [23], a fact that probably happened in this study with higher doses of *Jatropha* non-digested cake.

At the height of plants at 30 days after emergence we found significant differences, and found that performing the application of digested cake, dose equivalent 200 kg P ha⁻¹, to the soil, obtained the greatest height, 29.44 cm and, with non-digested cake, dose equivalent 50 kg P ha⁻¹, 28.13 cm (Table 3).

We found significant differences for some growth common bean IPR-Colibri variables, with the treatment applying non-digested cake equivalent dose of 50 kg P ha⁻¹ having the highest value of stem diameter of 4.02 cm, and the largest leaf area estimated at 27.24 cm² (Table 3). Plant height, stem diameter and estimated leaf area values were possibly influenced

by the increased availability of nutrients from the physic nut cake, emphasizing the non-digested cake with 50 kg ha^{-1} P (Table 3).

The variable number of nodules in common bean roots in treatments using digested cake with 300, 50 and 200 kg ha^{-1} P obtained the highest values with 58, 56 and 54 nodules, respectively (Table 3). However, *Jatropha* non-digested cake inhibited nodules formation, even the seeds which were initially inoculated with the SEMIA-4077 strain, which may express that the fixing microorganisms presented some sensitivity to *Jatropha* non-digested cake.

We noted differences between the two *Jatropha* cakes regarding nutrient content. As the content of P in the non-digested cake is lower than digested, the amount per pot was higher in order to achieve the same dose of P (Table 2). The *Jatropha* non-digested cake had an N:P ratio of 6.8:1.0 and digested cake of 3.8:1.0, which may be the reason for inhibition of nodule formation in the common bean plants with the application of non-digested cake (Table 3), for the higher concentration of N.

Nitrogen fertilization is detrimental to nodulation of common bean as shown by a study of response to inoculation in greenhouse [19] and field experiments [24] with different cultivars with a lower number of nodules. Field evaluation at Popayan with soil having high organic matter and high N availability reduced the amount of nitrogen derived from the atmosphere by biological fixation [25].

Regarding shoot production, there were significant differences showing that applying the non-digested cake with 50 kg ha^{-1} P, it can be obtained 6.24 g of dry matter, higher than that obtained with chemical fertilizers (Fig. 1a).

The development of the common bean IAC-Carioca under use of swine biofertilizers and mineral fertilizer was analyzed by Galbiatti and Silva [26], who obtained more dry matter content in the shoot in treatments using biofertilizers, indicating the importance of using organic compounds for fertilization. The use of *Jatropha* press cake as organic fertilizer reduced lettuce production and the accumulation of nutrients in the shoots [27].
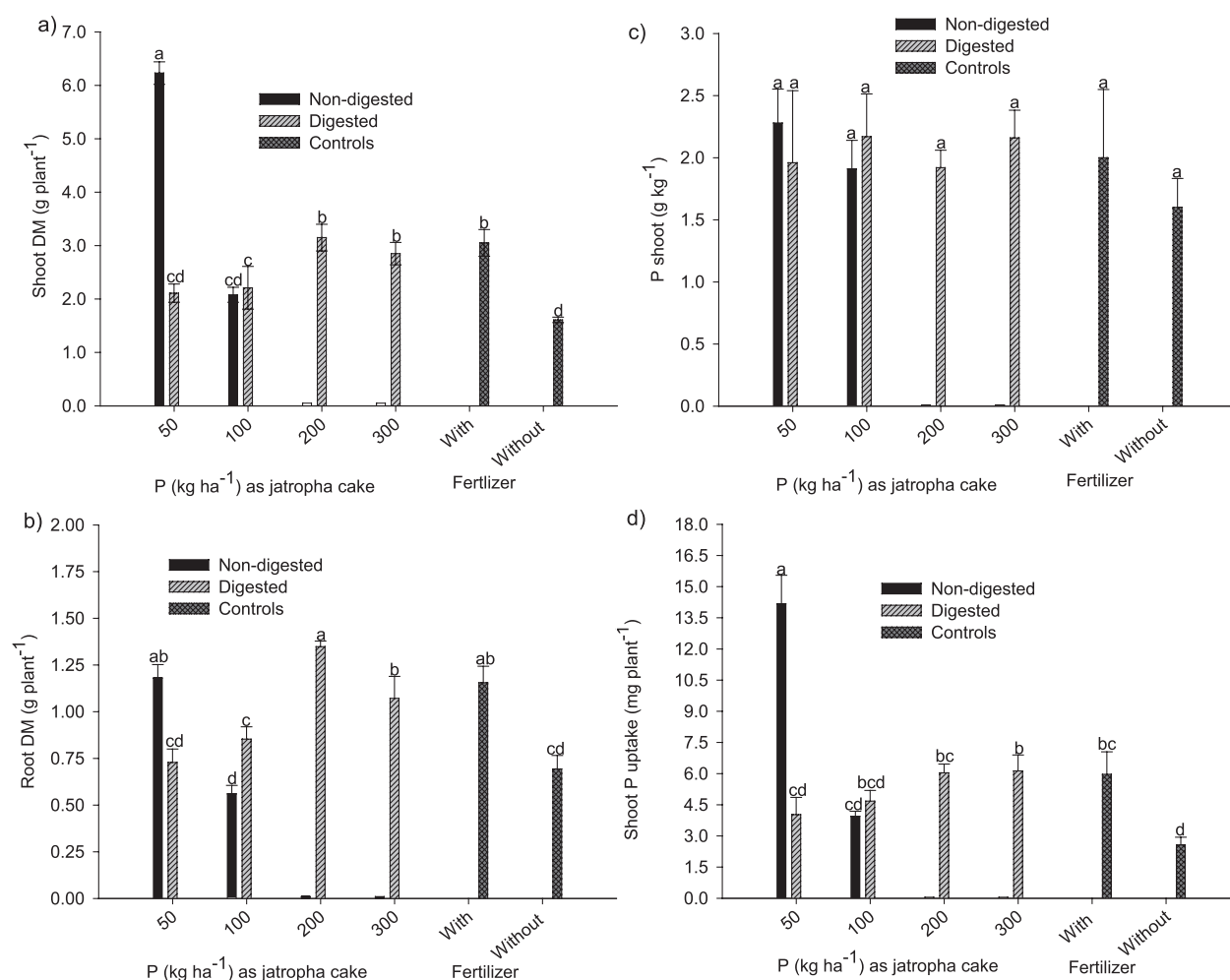

Fig. 1. Effects of *Jatropha* cake (non-digested and digested) application on common bean, (IPR-colibri): dry matter (DM) in g plant^{-1} a) shoot (CV = 9.45), b) root (CV = 10.51), c) concentration of P in g kg^{-1} of DM in shoot (CV = 20.61), and d) P uptake in mg plant^{-1} (CV = 16.43); values of bars are means (n = 4) ± standard deviation and when followed by the same letter do not differ significantly from Tukey's test (α = 0.05).
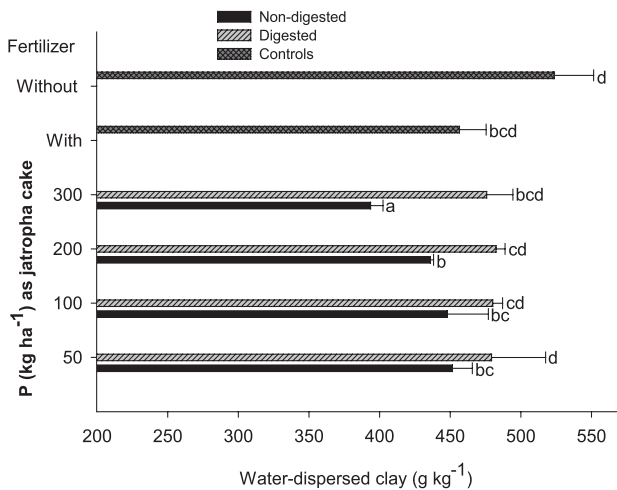
Fig. 2. Water-dispersed clay (WDC) content (g kg⁻¹) of soil due to doses and types of *Jatropha* cake (non-digested and digested) compared to treatments with and without mineral fertilizers after common bean (IPR-colibri) cultivation; values of bars are means (n = 4) ± standard deviation and when followed by the same letter do not differ significantly from Tukey's test (α = 0.05; CV = 5.15%).

It is observed in Fig. 1b) that greater root dry mass per plant is found in the treatment with digested cake at 200 kg ha⁻¹ P dose, value that can be associated with

a balanced macro and micronutrients supply in the root environment. The bio-fertilizer increases the absorption of water and nutrients by plants due to greater root area [26].

Phosphorus, in the dry matter g kg⁻¹, showed no significant differences to 5% in the Tukey test; even so, we can highlight the fact that the treatment using non-digested cake with 50 P kg ha⁻¹ presented higher P absorption with 2.28 g kg⁻¹ (Fig. 1c). Melém Júnior, Rodrigues Brito [28] in the study of mineral nutrition, using IPR-Colibri beans, found a better response in organo-mineral fertilizer with P content of 1.91 g kg⁻¹; such value is lower than that found in our study using *Jatropha* non-digested cake in lower doses.

In phosphorus analysis there were significant differences, the best response being that obtained with the *Jatropha* non-digested cake application at the 50 kg P ha⁻¹ dose, with 14.00 mg of P per plant, observing that the *Jatropha* cake application in low doses can be a phosphorus alternative supply to common bean fertilization (Fig. 1d).

Soil Characteristics

We observed a reduction of water-dispersed clay values with *Jatropha* non-digested cake applications

Table 4. Total carbon (C_{tot}), ΔpH, calcium (Ca), magnesium (Mg) and aluminum (Al) contents in the soil after 33 days in the greenhouse; values are means of four replicates; Pearson correlation coefficients of water-dispersed clay (WDC) with C, ΔpH, Ca, Mg e Al, n = 40.

Treatments	WDC	C_{tot}	ΔpH	Ca	Mg	Al
	g kg⁻¹	g kg⁻¹			cmol$_c$ dm⁻³	
1-ND 50 P*	451.65bc	40.40cde	-0.51a	2.09bcd	3.46d	0.03c
2-ND100 P	447.96bc	45.86b	-0.70ab	2.15bcd	4.53bcd	0.10c
3-ND 200 P	435.96b	44.11bc	-0.68ab	2.46abc	5.47ab	0.13bc
4-ND 300 P	393.64a	51.68a	-0.66ab	2.55ab	6.17a	0.38a
5-D 50 P	494.13d	38.47ef	-0.94bc	2.06cd	4.13cd	0.02c
6-D 100 P	473.82cd	38.90def	-0.93bc	2.15bcd	4.26bcd	0.05c
7-D 200 P	480.67cd	42.35bcde	-0.95bc	2.31abcd	4.42bcd	0.05c
8-D 300 P	467.32bcd	42.97bcd	-0.93bc	2.35abcd	5.01abc	0.22b
9- Without fertilizer	492.97d	35.01f	-1.10c	1.94d	2.03e	0.02c
10-With ferti-lizer	464.80bcd	35.71f	-0.78abc	2.76a	1.44e	0.03c
CV (%)	2.98	4.12	16.45	8.57	12.50	36.75
			r (Pearson)			
	WDC	C_{tot}	ΔpH	Ca	Mg	Al
WDC	1.00	-0.7123	-0.5468	-0.3932	-0.5503	-0.686
(*p*)	-	< 0.0001	0.0003	0.012	0.0002	< 0.0001

Means followed by the same letter in the column do not differ statistically by Tukey test (α = 0.05). *Doses of phosphorus in kg of P ha⁻¹ calculated according to P content in *Jatropha* cake; ND = non-digested *Jatropha* cake and D = digested *Jatropha* cake.

in relation to the control treatment – particularly in the treatment that was using cake at 300 kg ha^{-1} P (Fig. 2). This reduction is due to increased soil organic carbon content, promoted by this residue – a fact observed by the significant correlation (p - value <0.001) in the water-dispersed clay with organic carbon (Table 4).

Marchuk and Rengasamy [29] mentioned that when organic matter is high in soils, water interaction leading to clay dispersion is minimal because charge on clays is reduced by clay-organic bonds, which are mostly covalent, or soil aggregates are enveloped by organic materials formed by covalent bonding. Igwe and Udegbunam [30] and Nguetnkam and Dultz [31] observed that the organic matter is one of the soil properties that influences the dispersed clay content in the soil, causing particle flocculation.

For the digested cake, water-dispersed clay did not show the same behavior (Fig. 2), because there was no reduction of the clay dispersion with the addition of this residue compared to the control. In this case, the organic carbon increment was not enough to promote the water-dispersed clay reduction. The carbon organic matter content can lead to dispersion when the proportion of fulvic acids in relation to humic acids is increased, thus making more soluble organic matter [32]. de Cesare Barbosa and de Oliveira [33] observed that after applying two sources of organic matter, the clay dispersion behaved differently for the other materials.

The water-dispersed clay has a significant negative correlation with the ΔpH (Table 4), showing that the increase in flocculation of the clays is associated with this attribute of the soil. Besides that, this residue has the ability to modify the ΔpH, leaving it near zero and indicating a change in the clay particle surface electric potential, with a reduction of the clay dispersion [34]. Tavares Filho, Barbosa [35] in experiments in the greenhouse showed that the ΔpH was the variable that has the best correlation with clay content dispersed in the soil.

The cations calcium (Ca), magnesium (Mg) and aluminum (Al) had significant correlation with the water-dispersed clay (Table 4), because the increases in these cation levels in the soil promotes the reduction of the dispersion. It is suggested that the residue applied, although considered a phosphorus source, may not provide sufficient amounts of these elements to meet the nutritional needs of the plant, but probably contributed to the flocculation of clays. Calcium ion is considered flocculent; according to Marchuk and Marchuk [36], smaller dispersed clay values in water were because of increased calcium saturation. Igwe and Udegbunam [30] also observed that the level of Ca^{2+} is one of the factors that influence the dispersion of clay. In acid soils, according to Singh, Sarkar [37], aluminum is the main flocculating agent.

Conclusions

The *Jatropha* non-digested cake in doses of 200 and 300 kg P ha^{-1} inhibited the emergence of bean seeds. The application of non-digested cake with a dose of 50 kg P ha^{-1} had positive influences on the development of the common bean plant and in the P uptake in shoot. There was no presence of nodulation on common bean roots with non-digested cake; in contrast, when the digested cake was applied the common bean roots had nodules. It is concluded that for better development of inoculated common bean crop, *Jatropha* digested cake should be applied at the dose of 50 kg P ha^{-1}. There was a reduction in the values of water-dispersed clay and an increase in organic carbon with *Jatropha* non-digested cake applications in the soil.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

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