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PHOSPHATE SOURCES ASSOCIATED WITH FILTER CAKE AND BIOFERTILIZER ON PHOSPHORUS SOLUBILIZING MICROORGANISMS AND ITS CONTENT IN THE SOIL

Fuentes de fosfato asociadas a la cachaza y el biofertilizante sobre los microorganismos solubilizadores de fósforo y su contenido en el suelo

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ABSTRACT. Soils from tropical regions have high adsorption of phosphorus, which is hardly available to plants, so limiting crop production. This study was aimed to evaluate the effect of acidulated phosphate and ground rock phosphate sources associated with filter cake, enriched or not with biofertilizer, on the population of phosphate solubilizing microorganisms and P concentration in the soil. The experiment was carried out under laboratory conditions at "Carlos Rafael Rodríguez" University of Cienfuegos, Cuba. Experimental units were pots of 0,5 dm^3 containing Luvisol. A completely randomized design with 3x2+1 factorial arrangement and three repetitions was used; also, three phosphorus sources: triple superphosphate, mono-ammonium phosphate and natural phosphate of Cuba; filter cake enriched or not with Azotofos biofertilizer and a control treatment (just soil). The amount of P solubilizing microorganisms, soil P levels, organic matter and soil pH value were assessed at 30 and 60 days after applying treatments. The biofertilizer added to filter cake did not have any effect on P levels and soil organic matter. Phosphorus applied as natural phosphate increased the population of microorganisms and soil P level in presence of filter cake, enriched or not with biofertilizer, in relation to acidulated phosphate sources.

RESUMEN. Los suelos de regiones tropicales presentan una elevada adsorción del fósforo, el cual se encuentra poco disponible para las plantas y limita la producción de los cultivos. Este estudio tuvo como objetivo evaluar el efecto de las fuentes de fosfato acidulado y fosfato natural asociadas a la cachaza, no enriquecida y enriquecida con biofertilizante, sobre la población de microorganismos solubilizadores de fosfatos y los tenores de P en el suelo. El experimento se realizó en condiciones controladas a nivel de laboratorio en la Universidad "Carlos Rafael Rodríguez" de Cienfuegos, Cuba. Las unidades experimentales fueron macetas de 0,5 dm^3 conteniendo suelo Ferralítico Calcítico. El diseño experimental utilizado fue completamente aleatorizado con arreglo factorial 3x2+1 y tres repeticiones; tres fuentes de fósforo: superfosfato triple, fosfato monoamónico y fosfato natural de Cuba; cachaza no enriquecida y enriquecida con el biofertilizante Azotofos y un tratamiento control (solo suelo). A los 30 y 60 días después de la aplicación de los tratamientos, se evaluaron la cantidad de microorganismos solubilizadores de P, los niveles de P, la materia orgánica y el pH del suelo. La adición de biofertilizante a la cachaza no tuvo ningún efecto en los niveles de P y la materia orgánica del suelo. La aplicación de fósforo en forma de fosfato natural en presencia de cachaza no enriquecida y enriquecida con biofertilizante proporcionó una mayor población de microorganismos e incrementó el nivel de P en el suelo, en relación con las fuentes de fosfato acidulado.

Key words: organic fertilization, phosphorus, soil microbiology, solubilization

Palabras clave: fertilización orgánica, fósforo, microbiología del suelo, solubilización

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INTRODUCTION

The soils of tropical regions, with intense weathering, have high adsorption of phosphorus (P) in the oxides and hydroxides of iron and aluminum, being little available to plants, which implies that P is one of the most limiting nutrients the production and longevity of crops under such conditions.

Therefore, an alternative to increasing levels of P in soils would add organic acids such as sluggishness associated with phosphate fertilizer waste. The filter cake contains high amounts of P, which can reduce the use of mineral fertilizers (1, 2).

Another benefit of the use of filter cake is associated with soil microbiology, once it causes an increase of microorganism colonies producing enzymes responsible for solubilization of P adsorbed on soil organic acids (3, 4, 5).

Currently, it is spreading the application of biofertilizers associated with fertilization. Some authors, using products of microorganisms, found increased levels of P in soil, contributing to more pronounced phosphate fertilization efficiency (6, 7, 8, 9). However, others reported that this association is not reflected in the increased concentration of P in the soil (10).

Consequently, it is necessary to conduct more studies on solubilization of P associated with microorganisms, so that new possibilities for application in agriculture are established, which may be reflected in an improvement of P fertilization (4). There are factors that can influence the efficiency of phosphorus fertilization associated with bio-fertilizers, such as the source of P used (acidulated or natural phosphate) and the population of microorganisms involved in the process.

An important aspect of the efficient use of organo-mineral, phosphorus fertilization would verify the hypothesis that conventional sources of phosphorus, such as monoammonium phosphate (MAP) and triple superphosphate, compared with the natural phosphate, associated with an organic compound can change the population of microorganisms in the soil, reflecting on the P available. Also, check for increased activity of microbiota soil and the availability of P when the filter cake is used, compared with the filter cake enriched with bio-fertilizers containing microorganisms.

Therefore, the aim of the study was to evaluate the effect of the acidulated and natural phosphate sources associated with filter cake with and without enrichment biofertilizer on the population of phosphate solubilizing microorganisms and the tenors of P in the soil.

MATERIALS AND METHODS

The experiment was conducted under controlled conditions in the laboratory of chemistry at the "Carlos Rafael Rodriguez" University from Cienfuegos, Cuba, in the period from September to November 2013.

Samples, collected at the depth of 0 to 0,40 m, from a Ferralitic calcitic (11), corresponding to a Luvisol one (12), cultivated with sugar cane in the sugar mill "Carlos Baliño", Villa Clara province, Cuba were used. Immediately after harvest, the soil was dried in air; the lumps were pulverized and sieved (mesh 4 mm). Then, the chemical analysis was performed to evaluate fertility and the following results were obtained: pH (KCl)=5,5; organic matter=5,4%; P content in soil (Bray-2) = 21 mg 100 g^{-1} soil (13).

The experimental design was completely randomized, in factorial arrangement with three replicates, corresponding to the following treatments: three sources of phosphorus (60 mg dm^{-3} of soluble P in citric acid 2 %): triple superphosphate (P_2O_5 soluble=41 %), MAP (monoammonium phosphate, P_2O_5 soluble =48 %) and Cuban natural phosphate (P_2O_5 soluble=6,5 %; P_2O_5 Total=24,1 %); filter cake enriched and non-enriched biofertilizer and control (no treatment, only with ground). Cuba natural phosphate is apatite from magmatic (granulometry powder) of the site of Trinidad de Guedes, Matanzas, Cuba.

Evaluations were performed at two points: at 30 and 60 days after application of treatments. Each experimental unit consisted of plastic containers with 0,5 dm^3 of soil and the respective treatments.

Filter cake decomposed was used and for its enrichment, Azotofos biofertilizer (480 mL kg^{-1} of filter cake) containing the microorganism *Pseudomonas fluorescens* and *Azotobacter fluorescens* (108 cfu mL-1), according to the methodology proposed (14) was added. The biofertilizer was obtained in the laboratory of the Research Institute of Soil and Fertilizer from Barajagua in Cienfuegos.

Chemical analysis of filter cake was performed (15) and the following results were obtained, expressed in g kg^{-1}: N=18; P=12,1; K=4,3; Ca=96,4; Mg=10,2; S=3,4, while the microbiologic one[A] showed the following results: 2,5x102 cfu g^{-1} of phosphorus solubilizing microorganisms. Filter cake dose was 25 t ha^{-1} dry basis (12,5 g dm^{-3} of soil).

[A] Martínez, R. V.; López, M.; Brossard, F. M.; Tejeda, G. G.; Pereira, A. H.; Parra, Z. C.; Rodríguez, S. J. y Alba, A. *Procedimientos para el estudio y fabricación de Biofertilizantes bacterianos*. Instituto Nacional de Investigaciones Agrícolas, Maracay, Venezuela, 2006, p. 88.

In all treatments was applied uniformly, nitrogen (200 mg dm^{-3}) in the form of urea (44 % N) and potassium (150 mg dm^{-3}) in the form of potassium chloride (60 % K_2O). Throughout the experiment they were conducted frequent watering, keeping moisture near field capacity soil.

At 30 and 60 days after the start of the experiment, soil samples from each container (0,5 dm^3), for the determination of organic matter, the pH and levels of soil P (Bray-2) were collected (13); moreover, phosphate solubilizing microorganisms were quantified. The collected samples were placed in nylon bags, protected from light and kept in coolers until arrival at the laboratory; then, (mesh of 2 mm) were sieved, adjusting the moisture to 60-70 % of field capacity, packed in plastic bags with opening for ventilation and then kept in a cold chamber (4 °C).

For quantification of phosphate solubilizing microorganisms, the culture medium of Pykoskaia[A] was used. The plates were incubated at 30 ºC for 96 hours and counting microorganisms was performed using a colony counter.

Data phosphate solubilizing microorganisms were transformed into logarithm (Log. X). To compare treatment means, the Tukey test (P<0,05) was used.

RESULTS AND DISCUSSION

Interaction was found between P sources and the filter cake for phosphorus solubilizing microorganisms (MSF) at 30 and 60 days (Table I). Filter cake application, regardless of enrichment or not with biofertilizer, generated MSF largest colonies compared to control treatment.

In the presence of filter cake enriched biofertilizer 30 days, monoammonium phosphate increased the number of MSF, in relation to other sources of P and 60 days no differences between sources of monoammonium phosphate and rock phosphate were verified; however, there was an increase both sources of SPS regarding the use of triple superphosphate (Table II). Meanwhile, when only filter cake was applied (without enrichment with biofertilizer), the largest number of SPS was obtained with the addition of Cuban rock phosphate at 30 and 60 days.

The use of bio-fertilizer increased the population of MSF on the soil with the application of the monoammonium phosphate source. These results may be related to the chemical characteristics of the fertilizer, since it has nitrogen in its composition, in addition to its solubility and interaction with microorganisms in the biofertilizer, as these are factors that influence the efficiency of phosphorus fertilization associated to the addition of microorganisms (16).

For other sources of P, no effect on the amount of SPS between treatments with or without enrichment biofertilizer was verified. A similar result has been obtained by other authors (10).

Table I. Phosphorus solubilizing microorganisms (MSF) in a soil treated with different sources of P and the filter cake in presence or not of biofertilizer, at 30 and 60 days after the application the treatments. Cienfuegos, Cuba, 2013

Sources of P (P)	Phosphorus solubilizing microorganisms (MSF)	
	30 days	60 days
	UFC g^{-1} soil	
Triple Superphosphate	6,89 c	6,89 b
Monoammonium phosphate	7,11 a	6,95 a
Natural phosphate	7,01 b	6,99 a
DMS	0,06	0,04
Filter cake (FC)		
Filter cake	6,92 b	6,93 b
Filter cake +biofertilizer	7,09 a	6,96 a
DMS	0,04	0,02
	Values of F	
Filter cake of P	55,56**	18,18**
Filter cake	95,72**	4,61*
P x FC	69,20**	7,67**
Factorial x control	94,63**	66,14**
Mean of the control	6,78 b	6,79 b
Mean of the factorial	7,00 a	6,95 a
DMS	0,09	0,07
CV (%)	0,53	0,43

Average followed of different letters in column differ due to Tukey test (P<0,05)
* and **: significant to level1 % and 5 % of probability

The fact that the addition of biofertilizer in filter cake has not significantly increased the number of MSF in most sources of P, can be attributed to the high amount of microorganisms in the composition of filter cake (2,5x10^2 ufc g^{-1}).

For the tenor of P, organic matter and pH value in soil no interaction effects were verified among the sources of P and filter cake, at 30 and 60 days after application of treatments (Table III).

Although the use of the monoammonium phosphate source has shown higher MSF in relation to other sources of P (Table II) amounts, was the application of phosphate rock which provided a higher level of P in soil, corresponding to 98 % and 94 % at 30 days and 99 % and 91 % at 60 days, in relation to the application of monoammonium phosphate and triple superphosphate, respectively (Table III).

The results can be attributed to the combined effect of two main elements; first, the presence of insoluble P in phosphate rock, which with the addition of the microorganisms contained in the filter cake and the biofertilizer, favors solubilization, increasing P levels in soil. The mechanisms involved in phosphate solubilization are the production of organic acids and increased enzymatic action, which is attributed to the activity of microorganisms (3, 4, 5).

Moreover, with the extractor (Bray-2) of P used at work, since by this method (13) the complex combined with phosphorus cations is removed, so it can be extracted the P from source phosphate rock that has that element connected with Al and Ca (17, 18); therefore, it is assumed that the extractor determined the P available and unavailable portion of phosphate rock.

Table II. Phosphorus solubilizing microorganisms (MSF) at 30 and 60 days, after the application the treatments. Cienfuegos, Cuba, 2013

Sources of P (P)	Filter cake+biofertilizer	Filter cake
	30 days	
	UFC g^{-1} soil	
Triple Superphosphate	6,90 cA	6,88 bA
Monoammonium phosphate	7,34 aA	6,88 bB
Natural phosphate	7,03 bA	6,99 aA
DMS (P)	0,08	
DMS (FC)	0,06	
	60 dias	
Triple Superphosphate	6,89 bA	6,89 bA
Monoammonium phosphate	7,01 aA	6,90 bB
Natural phosphate	6,98 aA	6,99 aA
DMS (P)	0,06	
DMS (FC)	0,05	

The same common letters in column do not differ for Tukey test at 5 % level of probability
The same capital letters in the lines do not differ among them for Tukey test at 5 % level of probability

Table III. Phosphorus tenor (P), organic matter (OM) y pH in the soil associated to P Sources and Filter cake in presence and in absence of biofertilizer, at 30 and 60 days after the application of the treatments Cienfuegos, Cuba, 2013

Sources of P (P)	P		MO		pH	
	30 days	60 days	30 days	60 days	30 days	60 days
	mg 100g soil^{-1}		%		u	
Triple Superphosphate	30,56 b	30,34 c	5,76	5,62	5,48 b	5,48 b
Monoammonium phosphate	31,58 b	33,12 b	5,74	5,63	5,47 b	5,50 b
Natural phosphate	59,61 a	60,29 a	5,75	5,63	5,68 a	5,70 a
DMS	2,44	1,68	0,17	0,13	0,13	0,08
Filter cake (FC)						
Filter cake	40,23	41,60	5,74	5,61	5,59 a	5,61 a
Filter cake+biofertilizer	40,94	40,90	5,76	5,64	5,50 b	5,51 b
DMS	1,63	1,13	0,11	0,09	0,09	0,05
Values of F						
Sources of P	627,14**	1323,20**	0,04 ^{ns}	0,01*	12,21**	30,53 **
Filter cake (FC)	0,86^{ns}	1,80^{ns}	0,22 ^{ns}	0,31^{ns}	4,98*	15,75 **
P x FC	2,64^{ns}	3,73^{ns}	0,42 ^{ns}	0,15^{ns}	0,08 ^{ns}	1,75 ^{ns}
Factorial x control	378,87**	800,45**	17,62 **	44,74**	16,04**	46,69 **
Mean of control	21,01 b	21,59 b	5,46 b	5,27 b	5,33 b	5,33 b
Mean of factorial	40,58 a	41,25 a	5,75 a	5,63 a	5,54 a	5,56 a
DMS	3,83	2,65	0,26	0,20	0,20	0,13
CV (%)	4,27	2,90	1,92	1,53	1,53	0,97

Averages with different letters in column differ to Tukey test (P<0,05)
**; * and ns: significant (P <0,01); (O <0,05) and not significant respectively

The results concide with other studies that verify a greater recovery of P applied on the ground with the use of the extractor (17).

The phosphate rock has a slow solubilization in the soil. However, when this is applied associated with the addition of organic matter and biofertilizers, it can be an alternative source of soluble phosphate fertilizers; several studies have proven the effectiveness of this source in the supply of P to the plant (6, 19).

For the level of soil organic matter, there was no significant effect among sources of P, at 30 and 60 days (Table III). However, the soil pH value increased with the application of phosphate rock compared to other sources.

With the addition of biofertilizer to filter cake no effect on levels of P and soil organic matter, in both periods evaluated (30 and 60 days) were verified. These results are similar to those found in other studies (10).

The enrichment of filter cake with biofertilizers provided decrease in pH values in the soil at 30 and 60 days; similar results have been found by other authors, who attributed this to the production of organic acids by microorganisms (6).

It is observed that the pH variation agronomically (up to 0,2) because of soil treatments was relatively small; therefore, it is not important to explain the results.

In both periods evaluated, adding filter cake (enriched or not with biofertilizer) promoted the increase in the colonies of MSF, levels of P, organic matter and soil pH value, relative to control. The results show the benefits of filter cake for improving chemical and biological soil quality.

CONCLUSION

The application of phosphorus in form of natural phosphate in the presence of filter cake, with or without enrichment with biofertilizer, provided a population of microorganisms and increased the level of soil P in relation to the sources of acidulated phosphate.

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