DOI: 10.13140/RG.2.2.17308.08324
http://dx.doi.org/10.13140/RG.2.2.17308.08324
Cultivos Tropicales, 2016, vol. 37, no. 4, pp. 145-151
October-December

ISSN impreso: 0258-5936
ISSN digital: 1819-4087

Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas
http://ediciones.inca.edu.cu

EFFECT OF THE FILTER CAKE, PHOSPHORIC ROCK AND BIOFERTILIZER ADDITION TO THE SOIL ON THE PHOSPHOROUS CONTENT AND SUGAR CANE SEEDLINGS

Efecto de la adición de cachaza, roca fosfórica y biofertilizantes en el suelo sobre el contenido de fósforo y el desarrollo de plántulas de caña de azúcar

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ABSTRACT: The sugar cane crop has global importance scale and it can be affected productively by the phosphorous deficit. This work aims to evaluate the effect of filter cake, phosphoric rock, and the Azotofo® and Ecomic® biofertilizer addition to the soil on the phosphorous content and the development of sugar cane seedlings CP 5243 variety. The investigation was carried out in pots with a Luvisol soil where two vitroplants of 60 days were planted. The treatments were prepared in a completely randomized design with three repetitions. Nine treatments were evaluated by the addition to the soil of filter cake, phosphoric rock and the biofertilizers in different combinations. The morphometric, biological and agrochemical evaluations were carried out 60 days after planting. The obtained data were submitted to variance analysis. The media were compared by Tukey´s test using the statistical program ASISTAT. The simbiosis of mycorrhizae with sugarcane seedlings was verified both at treatments with *Glomus cubense* and in which was not applied. The combination of filter cake and phosphoric rock with the biofertilizers Ecomic® and Azotofos® increased the number of buds in the sugar cane seedlings. The application of filter cake+Ecomic®, filter cake+Azotofos®, phosphoric rock+Ecomic®, phosphoric rock+Azotofos® and rock+filter cake+Ecomic® increases the soluble phosphorous in the studied soil.

Key words: organic fertilizer, nutrition, *Saccharum*

RESUMEN. El cultivo de la caña de azúcar tiene importancia a escala mundial y puede afectarse productivamente por el déficit de fósforo. El objetivo del trabajo fue evaluar el efecto de la adición al suelo de cachaza, roca fosfórica y los biofertilizantes Azotofos® y Ecomic® sobre su contenido de fósforo asimilable y el desarrollo de plántulas de caña de azúcar variedad CP 5243. La investigación se condujo en macetas con un suelo Luvisol, donde se plantaron dos vitroplantas de 60 días. Los tratamientos se dispusieron en un diseño completamente aleatorizado con tres repeticiones. Se evaluaron nueve tratamientos compuestos por la adición al suelo de cachaza, roca fosfórica y los biofertilizantes en diferentes combinaciones. Las evaluaciones morfométricas, biológicas y agroquímicas se realizaron a los 60 días después de la plantación. Los datos obtenidos fueron sometidos a un análisis de varianza. Las medias fueron comparadas por el test de Tukey utilizando el programa estadístico ASISTAT. Se verifica la simbiosis de las micorrizas con las plántulas de caña de azúcar, tanto en los tratamientos con *Glomus cubense* como en los que no se aplicó. La combinación de cachaza y roca fosfórica con los biofertilizantes Ecomic® y Azotofo® incrementa el número de brotes en las plántulas de caña de azúcar. La aplicación de cachaza+Ecomic®, cachaza+Azotofos®, roca fosfórica+Ecomic®, roca fosfórica+Azotofos® y roca+cachaza+Ecomic® incrementan el fósforo soluble en el suelo estudiado.

Palabras clave: abono orgánico, nutrición, *Saccharum*

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INTRODUCTION

Sugar cane is a crop that arouses interest worldwide for its importance, not only for the production of sugar and its derivatives, but the relationship with one of the most accessible biotechnologies, for the generation of clean energy to replace oil (1).

Phosphorus (P) is an essential element for all living organisms (2). As an essential plant nutrient is characterized by the high degree of interaction with the soil and it is one of the most deficient for crops in tropical soils, being critical in some countries like Brazil (3) and in some sugarcane areas in Cuba (4).

This element has a decisive effect on the growth and development of sugar cane, through its influence on aspects such as sprouting, root development, stem elongation, tillering and millable stalk population (2).

There are indications that the use of a source of phosphate fertilizer mixed with organic compounds results in increases in nutrient availability and microbial activity in soil and increased plant growth (5-8). In this sense, the use of filter cake (organic waste from the sugar industry) can reduce the doses of mineral fertilizer in sugarcane, increasing the availability of P in soil (9-11). The benefits obtained from the use of this compound can be attributed to increased activity of microorganisms and enzymes that facilitate the process of solubilization of P in the soil (11).

The filter cake can be used then to partially substitute the mineral phosphate fertilization (1, 12), but information on the use of natural phosphate effect, associated with filter cake and microorganisms, the availability of soil P and response the plant is still insufficient.

The phosphorus solubilizing microorganisms have great importance in soil microbiota, by having the ability to break the link among phosphate groups and compounds of organic or mineral origin. Many of the phosphorus solubilizing bacteria are within the group of promoting rhizobacteria plant growth. Particularly *Pseudomonas fluorescens* has been widely reported as solubilizing phosphor (13).

Although the results are contradictory as to the use of solubilizing microorganisms phosphorus, some authors report that their addition provides an increase in the content of this element on the ground (14), absorption by plants and productivity (15).

The direct application of ground natural phosphate rocks as a source of phosphorus for crops is an alternative to P fertilization in many tropical countries (2). The addition of phosphorus solubilizing bacteria phosphate rock is recommended as an alternative to increase the solubilization of this element (16).

It is recognized that many species of plants acquire nutrients through interactions established with microorganisms living in the rhizosphere, especially those who have been called symbionts, such as Arbuscular mycorrhizal fungi (AMF) (17). These microorganisms involved in the translocation of nutrients in the soil, mainly phosphorus. There is a great diversity of species of AMF in the soil and their behavior is conditioned on the characteristics of this or der substrates where they are and the culture that is used (18). This group is among the most studied biofertilizer microorganisms in Cuba and has some results in sugar cane (19).

From this background the hypothesis that the filter cake enriched with AMF and Azotofos® as well as the addition of a natural phosphate source, can increase the available soil P arises and therefore the absorption of this element in cane sugar. Therefore, the objective of this study was to evaluate the effect of adding to the soil of filter cake, phosphate rock, and AMF Azotofos® on the content of phosphorus in the soil and seedling development of sugar cane.

MATERIALS AND METHODS

The research was conducted in the near areas to the Faculty of Agricultural Sciences at the University of Cienfuegos, in the period from September to December 2013.

3 kg pots were used with Alitic soil with clayey low activity (20), corresponding to a Luvisol soil (21). The soil was collected in a field previously cultivated with sugar cane, the Sugar Company “Carlos Baliño” Villa Clara province, Cuba, at a depth of 0 to 0,40 m.

Immediately after harvest, soil air dried, the lumps were pulverized and sieved (mesh 4 mm). Subsequently chemical analysis was performed to assess fertility, presenting the following characteristics: pH (KCl) = 5.5; organic matter = 5,4 %; P content in soil = 21 mg per 100 g of soil (Bray-2 method) (22).

Nine treatments were evaluated:

1. Control
2. Soil + filter cake
3. Soil + phosphate rock
4. Soil + filter cake + Azotofos®
5. Soil +filter cake + EcoMic®
6. Soil + phosphate rock + Azotofos®
7. Soil + phosphate rock + Ecomic®
8. Soil + phosphate rock + filter cake + Azotofos®
9. Soil + phosphate rock + filter cake + EcoMic®

The treatments were conducted in a completely randomized design with three replications. The experimental units (repeats) were constituted by the pots. In all treatments was applied uniformly, nitrogen (200mg dm3); as urea (46% N) and potassium (150 mg dm^3); in the form of potassium chloride (60 % K_2O) (23).

Two vitroplants variety CP 5243, 60 days in each pot, obtained from laboratory in vitro reproduction of the Territorial Research Station of Sugarcane (ETHIC) in Villa Clara were planted. The seedlings were watered daily to 80 % of field capacity.

Phosphate rock was obtained from magmatic apatite (powder grain size) of the site of Trinidad de Guedes, Matanzas, Cuba; with a composition of 24 % P_2O_5 = total soluble P_2O_5 and citric acid 2 % = 6,5 %. It was applied at a rate of 0,175 g kg^{-1} soil, equivalent to 60 mg kg^{-1} of P_2O_5.

The filter cake decomposed 60 days old, obtained from the Company Azucarera "Elpidio Gómez", whose chemical analysis (24) showed the following characteristics, expressed in g kg^{-1} was used: N = 18; P = 12,1; K = 4,3; Ca = 96,4; Mg = 10,2; S = 3,4. Microbiological analysis showed 2,5 x 102 colony forming units (ufc) of phosphorus solubilizing microorganisms per gram (12). The dose used was 25 t ha^{-1} dry basis (12,5 g soil dm^3).

The mycorrhizal bioproduct used (EcoMic®) was obtained from the National Institute of Agricultural Sciences (INCA), based *Glomus Cubense*, with a concentration of 29 spores per gram of soil, apply 10 g thereof per pot (5 g per vitroplant), just below the roots of each seedling.

The Azotofos® biofertilizer was obtained in the laboratory Soil and Fertilizer in Barajagua and contained 10^8 ufc of *Azotobacter chroococcum* and *Pseudomonas fluorescens* per gram of substrate. The byproduct was applied at 1 kg ha^{-1}.

At 60 days the height, thickness and number of children of each seedling was evaluated. In addition, the concentration of P in soil, organic matter and pH in each experimental unit was evaluated.

Fungal variables, frequency and intensity mycorrhizal were determined at the end of the experiment by trypan blue staining (24). At the roots of each seedling was determined, the percentage of colonization, visual density and weight of the endophyte in INCA laboratory.

The data of the height, thickness, number of children of the plants, the percentage of colonization in roots, visual density, the weight of the endophyte, pH, organic matter and phosphorus concentration in soil, underwent to an analysis of variance. The means of the variables were compared by Tukey's test ($P < 0.05$) (25) using the ASISTAT (26) statistical program.

RESULTS AND DISCUSSION

The roots of cane seedlings showed mycorrhizal infection in all treatments, highlighting the percentage of colonization in those receiving filter cake + EcoMic®, with statistical difference in relation to the single fertilized with rock phosphate + Azotofos®, but did not differ of control (Table I).

Table I. Variables of fungal infection (colonization, visual density and endophyte weight)

Treatments	Colonization in roots (%)	Visual density	Weight of the endophyte (mg)
1. Control	19,33ab	0,68ab	1,37 ab
2. Filter cake	14,00b	0,61ab	1,23 ab
3. Rock	15,66b	0,51ab	1,02 ab
4. Filter cake+Azotofos®	14,67b	0,29ab	0,59 ab
5. Filter cake+Ecomic®	26,33a	1,78a	3,73 a
6. Rock+Azotofos®	3,33c	0,04b	0,08 b
7. Rock+ Ecomic®	14,33b	0,68ab	1,36 ab
8. Rock+Filter cake+Azotofos®	14,33b	0,61ab	1,23 ab
9. Roca+Filter cake+ Ecomic®	19,00b	0,57 ab	1,15 ab
Standar error*	1,26	0,10	0,20
Coefficient of variation (%)	19,2	18,7	19,5

Letters in uneven rows for each column differ for $P \leq 0.05$

[A] Crespo, A. B. *Efecto de micorrizas benéficas (Mycoral®) y cachaza, en el peso de la caña y rendimiento neto de azúcar, en la Compañía Azucarera Tres Valles, Honduras*. Tesis de Grado, Escuela el Zamorano, 2006, Honduras, 43 p.

The visual density and weight of endophyte seedlings not increased no treatment, compared to the control and statistical difference expressed only between seedlings fertilized with filter cake + EcoMic® and that received only phosphate rock + Azotofos®. This confirms the presence of native strains of AMF on the floor of Sugar Company “Carlos Baliño”.

It is shown that the filter cake can be a key enabler for the beneficial action of AMF substrate, although the natives did not need this to infect, even in the presence of phosphate rock, but was observed to some extent, decreased symbiosis in the presence of Azotofos®. Keep in mind that this biofertilizer consists of bacteria of the species *Azotobacter chroococcum* and *Pseudomonas*, on which is much debated whether may have synergistic or antagonistic effects with other microorganisms. In this regard, other authors point as positive, *Azotobacter chroococcum* adding *Bacillus megatherium* and AMF in guava (*Psidium guajava* L.) (27).

The presence of AMF in sugarcane soils was also studied by others, settling differences in colonization in roots, among treatments where filter cake and AMF were applied only[A]

For variables height and thickness of the plants there was no difference among treatments tested (Table II); however, the number of shoots of seedlings sugar cane increased in relation to the control, for the three treatments receiving EcoMic®, but also for receiving phosphate rock + filter cake + Azotofos®.

Table II. Height, thickness and shoots of sugarcane vitroplants according to the treatments. University of Cienfuegos/Cuba, 2013

Treatments	Height (cm)	thickness (mm)	Sprouts (unidad)
1. Control	16,50	8,09	4,33 b
2. Filter cake	18,33	9,01	8,66 ab
3. Rock	19,25	9,42	7,83 ab
4. Filter cake +Azotofos®	19,33	8,29	8,33 ab
5. Filter cake +Ecomic®	19,17	8,99	10,00a
6. Rock+Azotofos®	19,75	8,06	9,33 ab
7. Rock+ Ecomic®	18,00	9,30	10,50 a
8. Rock+Filter cake+Azotofos®	19,50	8,91	9,66 a
9. Roca+Filter cake+Ecomic®	19,25	9,68	10,16 a
Standard Error *	0,40 ns	0,32 ns	0,21
Coefficient of variation (%)	9,35	10,88	19,61

* Letters in uneven rows for outbreaks differ for P≤0.05
ns. No statistical significance

These results show, somehow, the efficiency of the application of mycorrhizae and the Azotofos®, but the latter biofertilizer only surpassed the witness when applied in the presence of filter cake[A].

Perhaps it requires longer follow up to experiments or assessments under field conditions, to verify the beneficial action of filter cake with EcoMic® or Azotofos®; as compared to the benefit of the addition of the filter cake to AMF, the criteria are shared. Some researchers report that the filter cake substrate + litonite produced a positive effect *of Glomus manihotis* on plantlets of sugarcane in the adaptation phase (28), while others, from tests in plant cane, do not recommend mixing filter cake with AMF because the latter do not show all their potential[A].

The phosphorus content in the soil was influenced by some treatments made to the sugar cane seedlings, not the pH and organic matter (Table III).

Soluble P increases over control was presented in treatments fertilized with filter cake + EcoMic®, filter cake with Azotofos®,.EcoMic® + rock phosphate, rock phosphate +Azotofos® and rock + filter cake + EcoMic®.

Treatments with presence of filter cake, enriched with mycorrhizae and Azotofos® also rock + EcoMic®, with or without addition of phosphate rock, showing that there are reserves of P in these soils, which can be solubilized and depending on the plant stand cane in different ways.

The soluble phosphorus in the soil increased in all treatments in relation to their concentration, before planting the plantlets, which manifested 21 mg per 100 grams of soil and minimum reached by the witness was 53,72 mg per 100 g of soil . This is explained in the treatments that received phosphate rock, because it provided 60 mg of P_2O_5 per kg of soil and receiving filter cake, because it had in its composition 12.1 g kg-1 of P_2O_5. To this, it must add the filter cake was associated with a population of 2,5 x 102 ufc of phosphorus solubilizing microorganisms per gram.

Control only be explained this increase by sufficient existence of fixed phosphorus, as referred to above and native microflora (AMF that were observed colonizing the roots and other bacteria, actinomycetes and fungi) associated rhizosphere plants, under specific conditions, produce organic acids in their metabolism that release the P fixed.

[A] Crespo, A. B. *Efecto de micorrizas benéficas (Mycoral®) y cachaza, en el peso de la caña y rendimiento neto de azúcar, en la Compañía Azucarera Tres Valles, Honduras*. Tesis de Grado, Escuela el Zamorano, 2006, Honduras, 43 p.

Table III. Soluble content P (P2O5) in the soil, pH and organic matter content

Treatments	Soluble P (mg per 100 g of soil)	pH (in water)	MO (G kg^{-1})
1. Control	53,72 c	7,6	0,58
2. Filter cake	66,29 abc	7,2	0,65
3. Rock	60,42 bc	7,3	0,58
4. Filter cake +Azotofos®	93,31 a	7,3	0,64
5. Filter cake +Ecomic®	91,66 a	7,2	0,63
6. Rock+Azotofos®	87,16 ab	7,2	0,63
7. Rock+ Ecomic®	93,29 a	7,3	0,65
8. Rock+Filter cake +Azotofos®	67,29 abc	7,5	0,59
9. Rock+Filter cake + Ecomic®	85,88 ab	7,4 ns	0,59 ns
Standard error*	5,11	0,03 ns	0,07 ns
Coefficient of variation (%)	12,60	2,22	4,62

*Uneven letters differ in columns for p≤0,05
ns. No statistical significance

In other trials in maize (*Zea mays* L.), filter cake increased the soluble P in soil, which was attributed to the amount of this nutrient in the same (146 mg kg^{-1} soil) and microbial mass associated (2,97 x 105 and 2,2 x 103 ufc of bacteria and fungi, respectively, per gram of filter cake) (4).

These results are the first of their kind on a soil of Sugar Company “Carlos Baliño”. show that there may be alternatives to phosphate fertilization with some variants addition of AMF or Azotofos® to the soil, although they must be verified in field conditions as well as to determine if there is or synergistic effect between the phosphorus-solubilizing bacterium and the AMF as other researchers have recommended (29).

Regarding phosphate rock, although soluble P levels are low, it is not necessary to apply this soil, as there are sufficient amounts of insoluble P in it, but would need to follow tested in other areas of the company.

With regard to the need to apply EcoMic®, it must continue to deepen the study, as some researchers suggest that native AMF certain environments, may have a level of adaptability such that they can be more effective than introduced species (30).

Other researchers point out that the species of mycorrhizal fungi introduced under field conditions may be more effective than native and encourage the development of plants (31); however, the ground studied, applied more solubilized native P.

It requires further identification and level of AMF inoculum in key cane areas of the Sugar Company “Carlos Baliño” where waste from the sugar industry are used, such as filter cake that might favor the inoculum of arbuscular mycorrhizal fungi. It would also be interesting to evaluate other AMF strains, to determine their effectiveness in different soils that have the Company.

CONCLUSIONS

- The presence of native mycorrhizae and the association of these are shown and seedlings inoculated with sugarcane.
- The combination of filter cake and phosphate rock with Azotofos® and EcoMic® biofertilizers increases the number of outbreaks in sugarcane seedlings.
- Applying filter cake + EcoMic®, filter cake + Azotofos®, rock phosphate + EcoMic®, rock phosphate + Azotofos® and rock + filter cake+ EcoMic® increase the soluble P in soil studied.

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Received: July 4th, 2015
Accepted: March 31rst, 2016

Revista Cultivos Tropicales
Instituto Nacional de Ciencias Agrícolas