



Cultivos Tropicales

ISSN: 1819-4087

Ediciones INCA

Mora-Quilismal, Segundo Ramiro; Cuaical-Galárraga, Emma Teresa;
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Mejía, Luis Miguel; Aguila-Alcantara, Edith; Ruiz-Sánchez, Michel
Biofertilización con bacterias solubilizadoras de fósforo y
hongos micorrízicos arbusculares en el cultivo de la papa
Cultivos Tropicales, vol. 42, no. 2, e02, 2021
Ediciones INCA

Available in: <https://www.redalyc.org/articulo.oa?id=193268052002>

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Original article

Biofertilization with phosphorus solubilizing bacteria and arbuscular mycorrhizal fungi in potato culture

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ABSTRACT

The present investigation evaluated biofertilization alternatives with the use of phosphorus-solubilizing bacteria and arbuscular mycorrhizal fungi, through the commercial products Fosfotic® and Safer Micorrizas®, respectively, in potato crop cv. Superchola, in Andisol soils from Carchi, Ecuador. The experiment was carried out under production conditions in an area of 360 m², subdivided into six plots of 60 m² each (5 m wide x 12 m long), where six treatments corresponding to a fertilized control and different phosphorus doses plus inoculation with Fosfotic® and Safer Micorrizas®. The planting distance was 1 m between rows and 0.50 m between plants. Variables were evaluated: stem length, number of sprouted stems, total number of tubers per plant and their classification by size, as well as the total yield and size, in addition to performing an

economic analysis of the treatments studied. The best results were obtained with 100 % of the fertilization based on NK+75 % P+Fosfotic®+Safer Micorrizas® and with 100 % of the fertilization based on NK+25 % P+Safer Micorrizas®; which also showed the highest economic benefits. These results demonstrate the feasibility of using these biofertilizers in the Carchi region, Ecuador.

Key words: biofertilizers, fertilization, *Solanum tuberosum*, yield

INTRODUCTION

Carchi province in the north of Ecuador is located in an inter-Andean valley, which has based its social and economic development on the soil resource exploitation, traditionally characterized by their high productivity due to their volcanic origin. However, the sustainability of agricultural development in Carchi is currently at risk, due to the degradation processes of its soils and water resources. It is an imminent need to innovate in agricultural production systems to stop environmental degradation and maintain economic growth and social well-being in the region ⁽¹⁾.

Potato (*Solanum tuberosum* L.) cultivation generally requires high amounts of phosphate fertilizer to achieve economically acceptable yields, particularly in volcanic ash soils. This is a consequence of the low roots density of their plants and the low diffusion rate of phosphorus (P) in these soils ⁽²⁾. The yield and production quality are the results of the action and interaction of genetic factors (variety or genotype), the environment (climate, soil and biota) and the empirical technological knowledge applied to the process ⁽³⁾.

In Ecuador, potatoes have been a high priority crop, in 2018 approximately 66,000 ha of this crop were planted. The conditions of intensive production and monoculture have contributed to facing many problems that endanger the economic well-being of producers and the country's food security. This crop occupies the seventh place of production at the national level and it is cultivated in 12 provinces, but Carchi, Pichincha, Tungurahua, Chimborazo and Cotopaxi, represent 89 % of the national production, with the highest productivity: 22.43 t ha⁻¹, 14.72 t ha⁻¹, 14.04 t ha⁻¹ and 13.80 t ha⁻¹, respectively ⁽⁴⁾. Carchi province ranked first in tuber production nationwide in the 2012-2016 period, with an approximate yield of 19.7 t ha⁻¹.

Phosphorus solubilizing microorganisms convert insoluble phosphates into soluble forms, generally through acidification, chelation and exchange reactions, so their use as biofertilizers can not only offset the higher cost of fertilizers, but also mobilize those added to the ground ⁽⁵⁾. A biofertilizer is a substance that contains living organisms that,

applied to the seed, the plant or the soil, colonize the rhizosphere or the interior of the plant and promote growth through a greater supply or availability of primary nutrients for the plant host ⁽⁶⁾. Mycorrhizal fungi, on the other hand, improve the absorption of phosphorus by the plant and photosynthesis through AMF-root symbiosis, mainly due to the increase in the transport of inorganic elements from the soil to the plants ^(7,8). There is evidence showing that arbuscular mycorrhizal fungi (AMF) can also transfer nitrogen to their host, and that the host plant with its supply of carbon stimulates this transport, in addition to the fact that the host's peri-arbuscular membrane is capable of facilitating active nitrogen uptake from the mycorrhizal interface ⁽⁹⁾. In general, AMF inoculation studies have shown that increased yields and larger tuber size are feasible compared to conventional chemical fertilization ⁽¹⁰⁾. Likewise, it has been shown that the combined application of biofertilizers based on AMF and BSF could partially replace chemical fertilizer in the potato cultivation system, thanks to the mobilization of nutrients in the soil, especially phosphorus, available to plants ⁽¹¹⁾.

Although these biological fertilization alternatives have demonstrated their effectiveness in different regions and crops, and particularly in potatoes. In the Andean region, these biofertilization practices have not been incorporated into the productive dynamics as new environmentally-friendly alternatives, to improve the yield and quality of production, partly due to ignorance and also because there are no scientific bases suitable technologies for the edaphoclimatic conditions of the region that can demonstrate the effectiveness of their use to the producers.

Taking into account the aforementioned, the objective of this research was to evaluate biofertilization alternatives with the use of phosphorus solubilizing bacteria and arbuscular mycorrhizal fungi, through the commercial products Fosfotic® and Safer Micorrizas®, respectively, in potato culture the cv. Superchola, in Andisols soils from Carchi, Ecuador.

MATERIALS AND METHODS

The research was carried out in areas of San Francisco Experimental Center, from Carchi State Polytechnic University, an area with high potato production and located at Latitude N: 86°13'10'', Longitude W: 100°68'43.7'', at a height of 2750 meters above sea level. The soils in the study area are classified as Andisols ⁽¹²⁾. The experiment under

production conditions was developed from November 2017 to July 2018, with six treatments (Table 1). The potato cultivar Superchola was used ⁽¹³⁾.

Table 1. Treatments used in potato biofertilization on Andisol soil in Carchi province, Ecuador.

Campaign 2017-2018				
Treatments		Nutrient quantity		
		N	P ₂ O ₅	K ₂ O
		Kg ha ⁻¹		
T1	100 % NPK (control)	135.00	335.00	225.00
T2	100 % NPK+Fosfotic®	135.00	335.00	225.00
T3	100 % NPK+Safer Micorrizas®	135.00	335.00	225.00
T4	100 % NK+75% P+Fosfotic®+Safer Micorrizas®	135.00	251.25	225.00
T5	100 % NK+50% P+Fosfotic®	135.00	167.50	225.00
T6	100 % NK+25% P+Safer Micorrizas	135.00	83.75	225.00

Nitrogen (N), Phosphorus (P) and Potassium (K)

An area of 360 m² was used for the investigation, subdivided into six plots of 60 m² each (5 m wide x 12 m long). Each plot was subdivided into four equal parts of 15 m² (5 m long x 3 m wide), constituting four replicas of each treatment, for a total of 24 plots. The planting distance was 1 m between rows and 0.50 m between plants, with a total of six rows per plot. The phytotechnical work was carried out according to the Technical Manual of the crop ⁽¹³⁾. No irrigation was made to the crop.

Chemical fertilization was carried out in two stages, according to the Crop Technical Manual ⁽¹³⁾. The carriers used were Urea (46-0-0), Diammonium Phosphate (18-46-0) and Potassium Muriate (0-0-60). The first application was in the broom 20 days after planting (dap) where 50 % of the fertilization foreseen for each treatment was incorporated and later, in weeding and hilling (60 dap) the remaining quantity was incorporated, concluding with the raising of the soil towards the plants (hilling) and covering the fertilizer. All the tasks were carried out manually and with a hoe.

The Fosfotic® product is composed of *Azotobacter vinelandii*, *Bacillus cereus*, *Bacillus licheniformis* and *Pseudomonas fluorescens*. It has the ability to solubilize phosphorus retained in the soil and convert it into available and assimilable phosphorus for the plant ⁽¹⁴⁾. The Safer-Micorrizas® product is a biofertilizer based on arbuscular mycorrhizal fungi (AMF), which contains colonized rootlets, free mycelium and AMF spores ⁽¹⁴⁾.

The inoculation with the biofertilizers was carried out according to the manufacturer's recommendations. In the case of Fosfotic® was applied in three moments: in the

plantation, at 20 and 90 dap; in a dose of 5 ml L⁻¹ of water ⁽¹⁴⁾. Safer-Micorrizas® was applied only once, at sowing, at a dose of 10 g for each seed tuber ⁽¹⁵⁾.

Variables evaluated

At nine weeks after sowing, 10 plants were selected and stem length was evaluated, with the use of a millimeter ruler from the soil surface to the apical bud projected in the stem direction and the number of sprouted stems. At harvest (24 weeks after sowing), six plants were taken from the central area of each plot and the total number of tubers was determined, later separating them according to their size for classification by size: where first size (greater than 10 cm), second caliber (5 to 10 cm) and third caliber (less than 5 cm). The total production and by size of each plot (kg) was also determined. This allowed the calculation of the total yield and by caliber following the formula:

$$\text{Yield (kg m}^{-2}\text{)} = \text{Total production (or of each size) (kg)/plot area (15 m}^2\text{)}$$

$$\text{Yield (t ha}^{-1}\text{)} = \text{Yield kg m}^{-2} \times 10$$

Statistical analysis

The data were processed by Simple Classification Analysis and the differences between the means were compared by the LSD test. In all cases the significant differences were established for $p \leq 0.05$. The statistical package STATGRAPHICS PLUS Version 5.1, in Windows environment (Statistical Graphics Corp., 2000) was used.

Economic analysis

An economic analysis was carried out from the results achieved in each treatment after harvest. The sale price established at the time of marketing was USD 400 t⁻¹ for the first size, USD 200 t⁻¹ for the second size and USD 100 t⁻¹ for the third size. For the calculation of the total gross profits (USD ha⁻¹) of each treatment, the calculation was first carried out independently for each caliber and later they were added. The net profits were then calculated from the difference between the total gross profits and the production costs (USD ha⁻¹), which were determined for each treatment from the sum of the expenses generated by the different phytotechnical tasks carried out to cultivation. From the division of the production cost by the net profit, the cost/benefit ratio was obtained.

RESULTS AND DISCUSSION

The application of solubilizing bacteria (Fosfotic®) and/or arbuscular mycorrhizal fungi (Safer Micorrizas®), in combination with different doses of phosphoric fertilizer in potato crop, caused variations in plant growth. The treatment where 100 % of the fertilization with N and K and 75 % of phosphorus was applied. In combination with the two biofertilizers (T4) showed superior results in stem length and the number of sprouted stems (Table 2) followed and without statistical differences from the one where 100 % N and K+25 % P+Safer Micorrizas® (T6) was used, although the latter did not differ from the control treatment (T1).

Table 2. Treatment effect on stem length and sprouted stem number in potato crop cv ‘Superchola’ on Andisol soil in Carchi province, Ecuador. Campaign 2017-2018

	Treatments	Stem length (cm)	Nu. of sprouted stems
T1	100 % NPK (control)	60.12 b	4.83 bc
T2	100 % NPK+Fosfotic®	59.38 b	4.67 c
T3	100 % NPK+Safer Micorrizas®	63.21 a	4.92 bc
T4	100 % NK+75 % P+Fosfotic®+Safer Micorrizas®	65.17 a	5.50 a
T5	100 % NK+50 % P+Fosfotic®	59.83 b	4.88 bc
T6	100 % NK+25 % P+Safer Micorrizas®	63.12 a	5.21 ab
	SEx	1.457	0.226

Means with different letters in the same column differ significantly according to the LSD test for $p \leq 0.05$, $n = 24$.

The positive results obtained in these treatments (T4 and T6), can then be attributed to the beneficial effect of arbuscular mycorrhizal fungi on plant growth promotion, as has been previously demonstrated ⁽¹⁶⁾. These fungi promote nutrient exchange, particularly the absorption of low-mobility nutrients such as phosphorus ⁽¹⁷⁾, which justifies the substitution of up to 75 % of phosphoric fertilizer under these conditions.

The use of Fosfotic® (T5) allowed substituting 50 % of the phosphorus, as it did not show significant differences with the control. The solubilizing bacteria contained in the product, in addition to making this nutrient available to plants, produce growth regulators (auxins and gibberellins), hormones that play an important role in bud growth and germination ⁽¹⁸⁾.

Although these beneficial effects have been recognized and identified, the inoculation effectiveness with these microorganisms individually or in combination can be affected by various factors, such as soil pH, available P, pesticides application and the chemical fertilization used. High levels of nitrogen and phosphoric fertilization, as well as the

residuality of the latter, inhibit the establishment and AMF development and phosphorus-solubilizing bacteria ^(19,20).

At the time of harvest, the number of tubers per plant (Table 3) reached the highest results in the control treatment (T1), followed by the treatment where 25 % of the P was reduced and the two biofertilizers were applied (T4), although this treatment did not differ from T3 or T6, treatments where mycorrhizae were applied. The lowest values corresponded to the treatment where it was fertilized with 100% NPK and biofertilized with Fosfotic (T2). However, when classifying the total tubers according to the size, it was observed that the best results were obtained in the treatments where the P was reduced to 75% and the two products were inoculated (T4), and where the P plus was reduced to 25 % mycorrhizae (T6), treatments that did not differ from the fertilized control in the quality of the first caliber, but exceeded it in the quality of the second.

Table 3. Fertilization effect and the use of biofertilizers on tuber number and their quality according to the size, in the potato crop cv ‘Superchola’ on Andisol soil in Carchi province, Ecuador. Campaign 2017-2018

Treatments		Nu. tubers	Size		
			First	Second	Third
T1	100 % NPK (control)	20.00 a	6.67 ab	5.75 b	7.58 a
T2	100 % NPK+Fosfotic®	17.58 d	6.25 bc	5.62 b	5.71 cd
T3	100 % NPK+Safer Micorrizas®	18.71 bc	6.25 bc	6.42 a	6.04 bc
T4	100 % NK+75 % P®+Fosfotic+Safer Micorrizas®	19.08 b	6.88 a	6.58 a	5.62 cd
T5	100 % NK+50 % P+Fosfotic®	17.92 cd	5.71 c	5.67 b	6.54 b
T6	100 % NK+25 % P+Safer Micorrizas®	18.42 bcd	6.88 a	6.42 a	5.12 d
SEx		0.441	0.287	0.291	0.312

Means with different letters in the same column differ significantly according to the LSD test for $p \leq 0.05$, $n = 24$

The production of third caliber tubers was higher in the control treatment compared to the rest of the treatments, and lower in the treatment where only 25 % of the P and mycorrhiza were applied.

In summary, the complete mineral fertilization (T1) allowed to obtain a greater number of tubers, but the use of both biofertilizers, with doses of 75 % of P (T4) and of the mycorrhizal biofertilizer with only 25 % of this element (T6) , allowed achieving similar values of first calibers and superior to the control in second calibers. The tuber size is an important indicator for Ecuadorian producers, since it influences the harvest commercialization, specifically the sale prices.

Phosphorus has been reported to have a marked effect on tuber quality ⁽²⁾, due to its influence on cell division and therefore on their size. In contrast, high dose application of phosphoric fertilizer can have the opposite effect, producing a decrease in tubers' size. The phosphoric fertilization benefit will be greater under conditions of low to medium availability of this element in the soil ⁽²¹⁾.

Another important aspect to take into account is that the highest demand for phosphorus in potatoes occurs at the crop tuberization beginning. At this time, the phosphorus applied in the first weeks has lost between 30 and 60 % of its assimilability, depending on the source used and the element's fixation phenomena ⁽²²⁾. Therefore, the use of mycorrhizal fungi, in combination with reduced levels of phosphorus application, can be a viable alternative that allows reducing the doses of chemical fertilizer, while maintaining the availability of the element in the soil, at the higher demand time for the crop ^(7,8,10,23) and, therefore, can contribute positively to agricultural yield.

In relation to the agricultural yield obtained, it could be seen that the best results were found in the treatments where the mycorrhiza-based biofertilizer (T3, T4 and T6) was included, which equaled or exceeded the control (T1) in yield total and first and second calibers (Table 4).

Table 4. Treatments effect on total agricultural yield and by potato size cv 'Superchola' on Andisol soil in Carchi province, Ecuador. Campaign 2017-2018

Treatments		Agricultural yield (t ha ⁻¹)			
		Size			
		Total	First	Second	Third
T1	100 % NPK (control)	25.28 a	13.98 a	6.41 bc	4.9 a
T2	100 % NPK+Fosfotíc®	23.51 b	13.25 ab	6.38 bc	3.88 c
T3	100 % NPK+Safer Micorrizas®	24.91 a	13.44 ab	7.37 a	4.1 bc
T4	100 % NK+75 % P+Fosfotíc®+Safer Micorrizas®	26.04 a	14.64 a	7.65 a	3.75 c
T5	100 % NK+50 % P+Fosfotíc®	22.88 b	12.09 b	6.26 c	4.52 ab
T6	100 % NK+25 % P+Safer Micorrizas®	25.32 a	14.49 a	7.12 ab	3.7 c
SE _x		0.613	0.668	0.354	0.249

Means with different letters in the same column differ significantly according to the LSD test for $p \leq 0.05$, $n = 36$

The results of the research show that it is possible to reduce the phosphoric fertilization of the crop up to 25 % of the total dose to be applied with the use of the biofertilizer Safer Micorrizas®, which shows a better mycorrhizal functioning when the applied phosphorus contents are low ^(19,23).

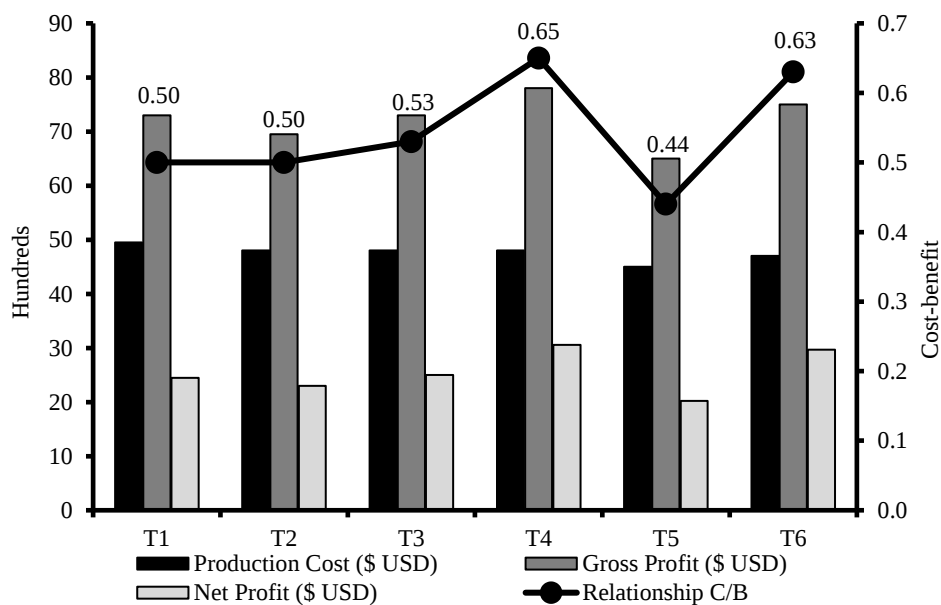
It should be noted that all the treatments in the study achieved yields higher than the 22.52 t ha⁻¹ reported for the potato crop in Ecuador ⁽²⁴⁾. Treatments 4 and 6 obtained satisfactory results in the conditions of Carchi region, where the yields for this cultivar oscillate between 21.0 and 25.0 t ha⁻¹, with an average of 23 t ha⁻¹ ^(8,25).

In general, the highest agricultural yields per size were reached for the first category, where treatments T4 and T6 once again stand out.

Other investigations have reported for potato crop that biological fertilization also influenced crop yields, increasing the availability of phosphates for plants and enhancing their development, not only with the increase in the number of tubers per plant, but rather with obtaining higher quality tubers (first and second calibers) ^(16,26,27).

Economic analysis

In all the treatments imposed in the experiment, economic benefits were obtained (Figure 1).



T1 (100% NPK), T2 (100 % NPK+Fosfotic®), T3 (100% NPK+Safer Micorrizas®), T4 (100% NK+75 % P+Fosfotic®+Safer Micorrizas®), T5 (100% NK+50 % P+Fosfotic®) y T6 (100% NK+25 % P+Safer Micorrizas®)

Figure 1. Economic analysis per hectare of each treatment used in the investigation in potato crop cv 'Superchola' on Andisol soil in Carchi province, Ecuador. Campaign 2017-2018

The cost benefit analysis of production shows that all treatments generated income, highlighting treatments T4 (100 % NK+75 % P+Fosfotic®+Safer Micorrizas®) and T6 (100 % NK+25 % P+Safer Micorrizas®) with the greatest economic benefit, surpassing the control treatment (T1), which is identified with the chemical fertilization that is

currently applied in the Carchi region. The increase of these treatments in the C/B ratio was 30 and 26 %, respectively, with respect to the control treatment. With such similar benefits, it is advisable to use only the mycorrhizal biofertilizer, which manages to replace up to 75 % of phosphoric fertilization.

CONCLUSIONS

The use of Safer Micorrizas® product constitutes a promising alternative for potato biofertilization in Andisol soils of Carchi, Ecuador. Its application allows reducing the use of phosphoric fertilizer by up to 75 %, without affecting yields, thus contributing to sustainable agriculture, while achieving greater profitability in the production of potato crops.

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