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Influence of beneficial microorganisms on the agronomic behavior of potato crop cv. "Bicentenaria"

Influencia de microorganismos benéficos en el comportamiento agronómico del cultivo de la papa cv. "Bicentenaria"

Influência de microrganismos benéficos no comportamento agronômico da batata cv. "Bicentenária"

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Abstract

The excessive use of chemical fertilizers causes alterations in soil microbial activity, environmental pollution and high production costs in potato cultivation (*Solanum tuberosum* L.). One way to avoid this effect is with the use of beneficial microorganisms, due to their ability to capture atmospheric nitrogen, produce growth-promoting substances such as indole acetic acid and solubilize inorganic phosphorus from insoluble compounds, which in turn time improves crop yields. The objective of this research was to evaluate the effect of the beneficial microorganisms as biofertilizers on growth and yield of potato crop. In a population of 1,600 potato plants cv. "Bicentenaria" four treatments were evaluated: *Trichoderma harzianum* (0.5 $g.L^{-1}$), *Glomus* spp. (30 $g.plant^{-1}$), efficient microorganisms (EM) (50 $mL.L^{-1}$) and a control treatment (no application of microorganisms). A completely randomized experimental design with four repetitions per treatment was used. The effect of the treatments was evaluated using vegetative and reproductive variables. It was evident that the treatment with *T. harzianum* significantly favored the rest of the treatments in plant height at 90 days (43.60 cm), tuber weight (154 g) and yield (57.13 $t.ha^{-1}$). The use of *Glomus* spp. and EM, had only a partial effect on the growth of the plants. The treatment with *T. harzianum* could represent an ecological agricultural alternative for potato production.

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Resumen

El uso excesivo de fertilizantes químicos provoca alteraciones en la actividad microbiana del suelo, contaminación ambiental y alto costo de producción del cultivo de papa (*Solanum tuberosum* L.). Una forma de evitar este efecto es con la utilización de microorganismos benéficos, por la capacidad que tienen de captar el nitrógeno atmosférico, producir sustancias promotoras del crecimiento como el ácido indolacético y solubilizar el fósforo inorgánico a partir de compuestos insolubles, lo que a su vez puede mejorar el rendimiento de los cultivos. El objetivo de la presente investigación fue evaluar el efecto de microorganismos benéficos como biofertilizantes sobre el crecimiento y producción del cultivo de la papa. Para ello, se empleó un total de 1.600 plantas de papa cv. "Bicentenaria", donde se evaluaron cuatro tratamientos: *Trichoderma harzianum* (0,5 $g.L^{-1}$), *Glomus* spp. (30 $g.planta^{-1}$), microorganismos eficientes (EM) (50 $mL.L^{-1}$) y un testigo (sin aplicación). Se usó un diseño experimental completamente al azar con cuatro repeticiones por tratamiento. El efecto de los tratamientos se evaluó mediante variables vegetativas y reproductivas. Se evidenció que el tratamiento con *T. harzianum* favoreció significativamente al resto de los tratamientos en altura de la planta a los 90 días (43,60 cm), masa del tubérculo (154 g) y rendimiento (57,13 $t.ha^{-1}$). El empleo de *Glomus* spp. y los EM sólo ejercieron un efecto parcial sobre el crecimiento de la planta. El tratamiento con *T. harzianum* podría representar una alternativa agrícola ecológica para la producción de papa.

Palabras clave: *Solanum tuberosum*, micorrizas, *Trichoderma* spp., *Glomus* spp., microorganismos eficientes, bioestimulantes del crecimiento vegetal.

Resumo

O uso excessivo de fertilizantes químicos provoca alterações na atividade microbiana do solo, poluição ambiental e elevados custos de produção no cultivo da batata (*Solanum tuberosum* L.). Uma forma de evitar esse efeito é com o uso de microrganismos benéficos, devido à sua capacidade de capturar o nitrogênio atmosférico, produzir substâncias promotoras de crescimento, como o ácido indol acético, e solubilizar o fósforo inorgânico a partir de compostos insolúveis, o que por sua vez pode melhorar o rendimento das culturas. O objetivo desta pesquisa foi avaliar o efeito de microrganismos benéficos como biofertilizantes no crescimento e produção da cultura da batata. Para isso foram utilizadas 1.600 plantas de batata cv. "Bicentenaria", onde foram avaliados quatro tratamentos: *Trichoderma harzianum* (0,5 $g.L^{-1}$), *Glomus* spp. (30 $g.planta^{-1}$), microrganismos eficientes (EM) (50 $mL.L^{-1}$) e uma testemunha (sem aplicação). O delineamento experimental utilizado foi inteiramente casualizado, com quatro repetições por tratamento. O efeito dos tratamentos foi avaliado através de variáveis vegetativas e reprodutivas. Evidenciou-se que o tratamento com *T. harzianum* favoreceu significativamente os demais tratamentos na altura das plantas aos 90 dias (43,60 cm), masa do tubérculo (154 g) e produtividade (57,13 $t.ha^{-1}$). O uso de *Glomus* spp. e o EM teve apenas um efeito parcial no crescimento das plantas. O tratamento com *T. harzianum* pode representar uma alternativa agrícola ecológica para a produção de batata.

Palavras-chave: *Solanum tuberosum*, micorrizas, *Trichoderma* spp., *Glomus* spp., microrganismos eficientes, bioestimulantes de crescimento vegetal.

Introduction

Potato (*Solanum tuberosum* L.) cultivation constitutes an important item in the agricultural, economic and social sector in Peru, since according to the Instituto Nacional de Estadística e Informática (INEI) (INEI, 2023), potato cultivation has increased its production particularly in the departments of Cusco, Lima and Huánuco. Díaz-Canseco (2018) noted that about 87 % of farmers in the peruvian highlands depend on this crop for both production and consumption. There are a high number of commercial varieties, among them, the new variety "Bicentenaria" has high agronomic value due to its adaptability and production capacity, with an average yield of over 40,000 $kg.ha^{-1}$ of tubers (Contreras-Liza *et al*., 2023). This cultivar represents an excellent alternative for potato producers due to its agronomic properties (short vegetative period, high yield and phenotypic stability) and characteristics suitable for industrial use (tuber suitable for frying into sticks and flakes) with low content of reducing sugars [International Potato Center (CIP, 2017)]. However, given that there is little knowledge among producers about the role of nutrients in plants and alternative sustainable agricultural practices, conventional agriculture is used with excessive use of chemical fertilizers, which causes alterations in soil microbial activity, environmental pollution and high production costs (Dash and Jena, 2015).

Therefore, it is necessary to implement strategies to improve sustainability indexes, especially economically, in order to generate greater profitability and environmentally develop technological practices such as the use of biofertilizers that are not polluting or harmful to the health of farmers and consumers. In this sense, the use of beneficial microorganisms, among which are the so-called plant growth-promoting microorganisms, which can favor their development, generate tolerance to biotic and abiotic stresses in the plant and facilitate its nutrition due to their capacity to capture atmospheric nitrogen, has become increasingly important, produce growth-promoting substances such as indoleacetic acid and solubilize inorganic phosphorus from insoluble compounds, and at the same time can antagonize phytopathogens in host plants (Cruz-Cárdenas, 2021). Several studies have demonstrated the importance of these microorganisms in potato production and quality (Sawicka *et al*., 2021).

In another important group of beneficial microorganisms are, firstly, *Trichoderma harzianum*, which is a cosmopolitan facultative anaerobic fungus, known as the most effective pathogen controller (Srivastava *et al*., 2016). Secondly *Glomus* spp. is a mycorrhizal fungus that inhabits most agricultural soils and helps in the absorption of water and nutrients by the plant (Luna-Quecaño, 2020).

Therefore, the objective of this research was to evaluate the effect of beneficial microorganisms as biofertilizers on the growth and production of potato cv. "Bicentenaria".

Materials and methods

The research was carried out in the Grau annex, Viñac district, Yauyos province, Lima Region, Peru (12°48' S; 75°49' W, 3,420 m. a.s.l.), in soils whose physical and chemical analysis results are presented in table 1.

Table 1. Physical and chemical analysis of soil cultivated with potato cv. “Bicentenaria” subjected to treatments with beneficial microorganisms in Yauyos, Lima Region, Peru.

Element	Value	Method
Sand (%)	32.8	Bouyoucos
Clay (%)	42.4	Bouyoucos
pH	6.94	Potentiometry
Electrical conductivity (CE) ($dS.m^{-1}$)	0.67	Conductimetry
Organic matter (%)	2.28	Walkley-Black
Nitrogen (N) ($g.kg^{-1}$)	1.30	Kjeldahl
Phosforus (P) ($mg.kg^{-1}$)	12.80	Olsen
Potasium (K) ($mg.kg^{-1}$)	177.00	Flame photometry

Source: Agricultural Chemistry Laboratory. Valle Grande. San Vicente de Cañete, Lima, Peru. 2022.

The trial occupied an effective area of 600 m^2 with 20 experimental units of 30 m^2 each, for a total of 1,600 plants established at 0.9 m between rows, 0.3 m between plants and 0.1 m depth. Seed potatoes obtained from the Universidad Nacional José Faustino Sánchez Carrión, Huacho, Peru (Contreras-Liza *et al*., 2023) were used.

The climatic information recorded during the trial period was low temperature, with an average of 14.0 °C, average relative humidity of 82 % and total precipitation of 1,728 mm (figure 1).

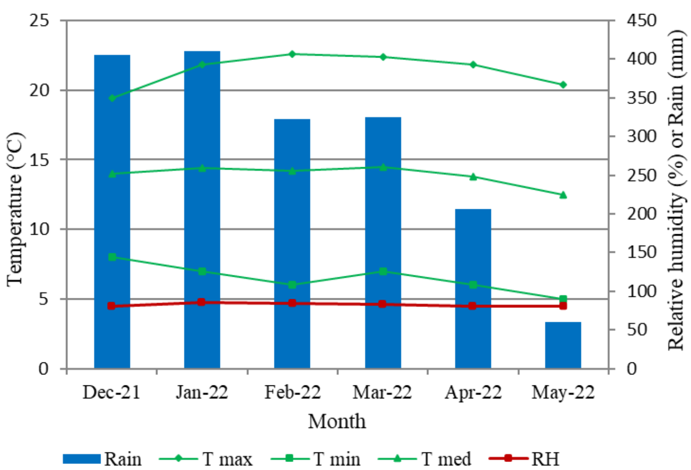

Figure 1. Climogram of temperature, humidity and precipitation (period December 2021-May 2022) during the evaluation with beneficial microorganisms on potato cv. “Bicentenaria” in Yauyos, Lima Region, Peru. Source: National Service of Meteorology and Hydrology (SENAMHI, 2022).

The plants received applications of three growth-promoting microorganisms and a control treatment without any application (Table 2). The trial consisted of four treatments and four replicates, using a completely randomized experimental design.

Preparation and application of treatments. *T. harzianum* was applied using the commercial product Tricho-Tab® ($1x10^7$ $spores.g^{-1}$) of which 10 g were weighed (Ohaus mod. Adventurer Pro., USA) and diluted in 20 L of water. The solution was applied to the potato tuber-seed before sowing and then at the stages of vegetative development (75 days), stolonization (45 days), tuberization (43 days) and pre-flowering (7 days), by foliar spraying using a 20 L capacity backpack sprayer (JACTA, Peru).

For the application of *Glomus* spp. 1 kg of the commercial product Mico-Tab® (300 $CFU.g^{-1}$) was used. This mycorrhiza was purchased in solid form (sterile peat), mixed with 12.5 kg of decomposed sheep manure and applied during planting, incorporating 30 g of the mixture around each tuber-seed.

For the application of effective microorganisms, 1 kg of the commercial product EM/19® containing photosynthetic bacteria (*Rhodopseudomonas* spp.), lactic acid bacteria (*Lactobacillus* spp.) and yeasts (*Sacharomyces* spp.) was used, which was activated in 10 L of water and 1 kg of molasses, for 4 days at 40 °C, until reaching a pH of 3.8 (Orion mod. Star A211, USA). Then a 1:20 dilution of this mixture was made and 5 $g.L^{-1}$ of EM-ceramica soil®, a mineral fertilizer that acts as a source of natural micronutrients and phytoprotectant (mineral rock composed of 88.15 % SLO_2 and 11.85 % inerts) was added. It was then applied at the time of planting using the “drench” technique to the soil, which consists of applying a solution near the stem of the plant, and also by foliar application during the stages of vegetative development, stolonation, tuberization and pre-flowering. Table 2 describes the treatments evaluated in this research.

Table 2. Description of treatments with beneficial microorganisms evaluated on potato cv. “Bicentenaria” in Yauyos, Lima Region, Peru.

Treatment	Microorganism	Commercial product	Dose
T1	*Trichoderma harzianum*	Tricho-Tab®	0.5 $g.L^{-1}$
T2	*Glomus* spp.	Mico-Tab®	30 $g.plant^{-1}$
T3	Effective microorganisms (EM)*	EM/19®	50 $mL.L^{-1}$
T4	Control	None	-

*EM: *Rhodopseudomonas* spp., *Lactobacillus* spp., *Sacharomyces* spp.

Soil preparation and cultivation

Soil preparation included plowing to a depth of 30 cm, two passes of harrowing and furrowing. Fertilization was carried out using diammonium phosphate (40 $g.m^{-2}$), potassium chloride (25 $g.m^{-2}$) and ammonium nitrate (32 $g.m^{-2}$), in a continuous stream and conveniently covered with soil. One week later, the seed tuber was sown. Irrigation was applied in furrows twice a week, enough to moisten the root zone. Manual weeding was carried out to control weed growth and hilling at 60 and 90 days to favor tuberization. Given the low incidence of pests during the study, no phytosanitary controls were required on the crop.

Harvesting was done manually when the leaves began to turn yellowish, at 170 days after sowing (das) (physiological maturity), and at the same time it was verified that the epicarp of the tubers did not detach with the friction of the fingers (Akello *et al*., 2022).

The evaluations were carried out on 10 plants taken at random from the two central furrows of each experimental unit. The variables evaluated were the following:

Plant height

It was measured from the base to the apical part of the plant at 30, 60 and 90 das, with measuring tape (Stanley, China).

Number of stems

At flowering, the number of stems sprouted from the soil surface was counted.

Tuber number, mass and size

At harvest, the number and mass of tubers per plant were quantified. Size was assessed as a function of tuber polar and equatorial diameter using a vernier caliper (Mitutoyo, Japan). Crop yield was determined from the mass of tubers per plant and the number of plants per hectare (37,037 $plants.ha^{-1}$).

Sampling of soil microorganisms

For this analysis, at the time of harvest, isolations were made from soil samples taken at a depth of 20 cm. From these, 10 g of soil were taken and placed in a flask with 90 mL of sterile distilled water, and homogenized using an orbital shaker (Biosan mod. ES-20/60, Latvia), at 110 rpm for 5 min. Serial dilutions were then performed up to the 10^{-3} dilution. From this last dilution, 1 mL was extracted for the isolation of bacteria and actinomycetes and placed in Petri dishes containing nutrient agar for bacteria and selective medium for actinomycetes (Oatmeal Agar - OAT). A 10^{-2} dilution on potato dextrose agar medium was used to isolate the fungi. The plates were incubated at 25 °C for 2 and 7 days to count CFU of bacteria and fungi, respectively (Herrera *et al*., 2022).

Statistical analysis of the data

Prior to data analysis, the assumptions of homogeneity of variances and normality were checked by means of the Shapiro-Wilk test. For the evaluation of the microorganism count, the data were transformed using log x. All the information was compared and interpreted, through analysis of variance (ANOVA) and means comparison by Tukey's test at 5 % using the statistical program InfoStat version 2018 (Di Renzo *et al*., 2018).

Results and discussion

Vegetative growth variables

Plant height, obtained at the different crop stages, showed that the treatment with *T. harzianum* significantly outperformed the control ($p \leq 0.05$) (table 3), as well as the rest of the treatments applied at 30 and 90 das. Similarly, Singh *et al*. (2017) found that foliar spraying of *T. viride* on the tuber-seed stimulated the growth of the vegetative part of the potato plant. Likewise, Abdirahman *et al*. (2022) found that *Trichoderma* application on tuber-seed stimulated bud sprouting with increased stem production per plant. Recent studies demonstrated significant effects of *Trichoderma* use on vegetative growth of potato plants (Rakibuzzaman *et al*., 2021).

Regarding the mechanism of action, Contreras-Cornejo *et al*. (2016) pointed out that different strains of *Trichoderma* induce branching of roots and shoots in the plant due to cell division, expansion and differentiation that occurs in the presence of auxin-like fungal compounds, which facilitates nutrient uptake by the plant. For his part, Cano (2011) indicated that fungal elicitors in the rhizosphere can solubilize organic compounds and nutrients, fulfilling a dual function by activating the defense system and promoting plant growth.

Regarding the *Glomus* spp. treatment, its effect was very limited, since it only promoted plant height increase in the first stage (30 das) of plant growth (table 3); however, Rafiq *et al*. (2015) found that the application of this mycorrhizal fungus achieved increases in leaf chlorophyll levels and potato tuber size.

On the other hand, the treatment that included efficient microorganisms (EM) did not manage to overcome the control in any of the evaluations (table 3); however, there are other experiences that show that with the application of EM, increases in plant height were achieved (Ranjani *et al*., 2018). The variable number of stems did not show differences between treatments ($p>0.05$), suggesting that this variable was not a good indicator of response to the application of beneficial microorganisms (table 3).

Table 3. Vegetative growth variables in potato cv. "Bicentenaria" subjected to treatments with beneficial microorganisms in Yauyos, Lima Region, Peru.

Treatment	Plant height (cm)			N° stems
	30 das	60 das	90 das	
T. harzianum	8.79a	27.75a	43.60a	3.75a
Glomus spp.	6.06b	23.25ab	31.88b	3.00a
*Effective microorganisms	4.93bc	23.60ab	31.63b	3.50a
Control	3.68c	21.15b	28.20b	2.50a

*Effective microorganisms: *Rhodopseudomonas* spp., *Lactobacillus* spp., *Sacharomyces* spp. [a,b, c]Means with different letters are statistically different according to Tukey's test. (p≤0,05).

Reproductive growth variables

he plant yield variables, together with tuber number, tuber mass and tuber size, reached the highest values when applying the treatments with *T. harzianum* with respect to the control treatment ($p \leq 0.05$) (table 4). Additionally, *T. harzianum* outperformed the rest of the treatments in tuber mass and yield. In this regard, Mamani-Rojas *et al*. (2016) argue that the effect of *Trichoderma* on plant yield is due to the fact that this fungus synthesizes phytohormones and phyto-regulators important for the growth of stolons, tubers, in addition to improving nutrient absorption in the plant.

The effect of *Glomus* spp. was manifested only on tuber mass (table 4). Likewise, Rahmani and Aboutalebian (2021) observed that under water stress conditions the application of the mycorrhizal fungus, *G. mossea*, was able to increase tuber yield.

In the case of EM bacteria, they showed their effect on the yield variable compared to control (table 4). Pathak *et al*. (2019) mentioned that EM have exerted significant effect on potato tuber yield when acting in combination with mycorrhizal fungi. In this regard, Saini *et al*. (2021) found that combining *Bacillus subtilis* and *Glomus* spp. promoted an increase in tuber mass, so the authors recommend replacing a percentage of the commonly used chemical fertilizer with this combination. Similarly, Wang *et al*. (2019) reported that EM *B. subtilis* in consortium with *Trichoderma* was very effective in promoting yield increase in potato plants.

Table 4. Variables response in tubers of potato cv. "Bicentenaria" subjected to treatments with beneficial microorganisms in Yauyos, Lima Region, Peru.

Treatment	Number of tubers	Average mass (g)	Equatorial diameter (mm)	Polar diameter (mm)	Yield ($t.ha^{-1}$)
T. harzianum	11.20a	154a	64.18a	70.01a	57.13a
Glomus spp.	9.28ab	116b	57.03ab	67.63ab	43.11b
*EM	8.80ab	107b	56.23b	66.53ab	39.54b
Control	5.94b	77c	51.54b	60.15b	28.65c

*EM: *Rhodopseudomonas* spp., *Lactobacillus* spp., *Sacharomyces* spp. [a,b, c]Means with different letters are statistically different according to Tukey's test (p≤0.05).

The application of beneficial microorganisms showed that the control treatment had, in all cases, the lowest values and was significantly exceeded ($p \leq 0.05$) (table 5) by the treatment with *T.*

harzianum in all variables, and by the treatments with *Glomus* spp. and EM for the case of ascomycetes and total bacteria, suggesting that the treatments used favor the development of these beneficial microorganisms.

According to Beltrán-Pineda (2014), the presence of actinomycetes in the rhizosphere of crops is beneficial, which allows suggesting their application in an integrated conservation management together with mycorrhizae and beneficial fungi.

Table 5. Number of beneficial microorganisms (per gram of soil) after the application of treatments to the substrate cultivated with potato cv. "Bicentenaria" in Yauyos, Lima Region, Peru.

Treatment	Actinomicetos	Aerobic bacteria	Total bacteria	Fungi
T. harzianum	$3.333x10^4$a	$4.507x10^6$a	$73.950x10^6$a	$5.4524x10^4$a
Glomus spp.	$1.916x10^4$a	$1.573x10^6$ab	$20.125x10^6$b	$5.100x10^4$ab
EM	$1.917x10^4$a	$2.472x10^6$ab	$20.555x10^6$b	$4.792x10^4$ab
Control	$0.583x10^4$b	$0.960x10^6$b	$6.850x10^6$c	$4,466x10^4$b

*EM: *Rhodopseudomonas* spp., *Lactobacillus* spp., *Sacharomyces* spp. [a,b,c]Means with different letters are statistically different according to Tukey's test (p≤0,05). Data were log x-transformed for analysis.

Conclusions

The treatment with *Trichoderma harzianum* produced the highest growth in potato plants cv. "Bicentenaria" compared to the control treatment. The use of *Glomus* spp. and EM only exerted a partial effect on plant growth. Therefore, the use of *T. harzianum* may represent an ecological agricultural alternative for potato production.

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