

## ORIGINAL RESEARCH ARTICLE

# Combination of phosphorus mobilizing and solubilizing fungi with phosphoric rocks and volcanic materials for growth promotion of lettuce (*Lactuca sativa* L.) plants

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## ABSTRACT

Arbuscular mycorrhizal fungi (AMF) increase the uptake of soluble phosphates, while phosphorus solubilizing fungi (S) promote the solubilization of insoluble phosphate complexes, together benefiting plant nutrition. The use of these organisms in combination with minerals or rocks that provide nutrients is another alternative to maintain crop productivity. The objective of this work was to combine AMF and S with pyroclastic materials (ashes and pumicites) from the Puyehue volcano and phosphoric rocks (PR) from the Río Chico Group (Chubut) and to evaluate the performance of these mixtures as substrates for potted production of *Lactuca sativa*. To formulate the substrates, a mixture of Ter-rafertil® with ashes was used as a base. *Penicillium thomii* was the S and spores of the fungus *Rhizophagus intraradices* (AEGIS® Irriga) served as the source of AMF. Various combinations of microorganisms and the addition or not of RP were evaluated. The treatments were: (1) substrate; (2) substrate + AMF; (3) substrate + S; (4) substrate + AMF + S; (5) substrate: PR; (6) substrate: PR + AMF; (7) substrate: PR + S, and (8) substrate: PR + AMF + S. There were 3 replicates per treatment. The parameters evaluated were total and assimilable P content in the substrate, P in plant tissue and dry biomass. All of them were significantly higher in the plants grown in the substrate added with PR and inoculated with S and AMF. This work confirms that the S/AMF combination with volcanic ashes from Puyehue and PR from Grupo Río Chico formulated with a commercial substrate promote the growth of *L. sativa*. Thus, it is possible to increase the added value of geomaterials of national origin.

**Keywords:** Solubilizing Fungus DEP; P-mobilizing Fungi; Phosphate Rock; Volcanic Ashes

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## 1. Introduction

Arbuscular mycorrhizal fungi (AMF) and phosphorus (P)-solubilizing microorganisms present in the rhizosphere play an important role in the growth and development of their host plants<sup>[1]</sup>. AMF (phylum Glomeromycota) increase the uptake of soluble phosphates in the plant surroundings<sup>[2,3]</sup>, while P-solubilizing fungi (S) promote the solubilization of insoluble phosphate complexes<sup>[1,4-7]</sup>. This process is achieved through acidification and chelation, as well as by ion exchange reactions and production of low molecular weight organic acids, such as gluconic, citric and oxalic acids<sup>[8,9]</sup>.

Another alternative use to maintain plant productivity is the combination of different mineral materials in the growth substrate, which

can provide nutrients, among other desirable properties. P and nitrogen (N) are the most important nutrients in the soil that limit plant growth. It is estimated that 5.7 billion hectares in the world do not contain a sufficient amount of P available to sustain the productivity of their crops<sup>[10]</sup>. Our country does not have phosphoric deposits with high P contents to produce phosphate fertilizers; however, in different regions there are large deposits of phosphoric rocks (PR) with low to medium contents of this nutrient, which do not allow the production of soluble fertilizers (e.g., superphosphates) with current technologies.

Soilless cropping systems in Argentina are relatively recent. They have gained importance and grown steadily, especially in the last decade. These systems require the use of substrates<sup>[11]</sup>. These are porous materials that, placed in containers, provide anchorage and sufficient levels of water and oxygen for plant growth. Among the advantages of soilless cultivation are the elimination of pathogens, tight control of nutrient availability and uniformity in production. As a result, this system has spread rapidly in the production of horticultural, forestry, fruit, floricultural, tobacco and ornamental plant seedlings. Soilless cultivation requires inputs such as peat, perlite or expanded clays, among others, mostly of high cost. The incorporation of pyroclastic materials (ashes, pumicites and other volcanic materials) in substrates is common in other countries that have them. Given the ample resources of volcanic materials existing in different regions of our country, it is of interest to expand the possibilities of their agricultural use, especially in intensive crops, and to replace the use of imported and high-cost products.

The eruption of the Puyehue-Cordón Caulle volcanic complex (June 2011) expelled into the atmosphere large quantities of pyroclastic materials that covered large areas of Argentina. In the provinces of Río Negro and Neuquén, the most affected, the accumulation of these materials covered 5,658 hm<sup>2</sup>; in 45% of this surface the ash thickness reached 0.2 to 1.5 cm and in the remaining 55% the accumulation was greater than 1.5 cm<sup>[12]</sup>.

Alternatives have been tested for the use of these materials as plant growth substrates, as well as vehicles or carriers of beneficial microorganisms.

These pyroclastic materials have been tested by Barbaro *et al.*<sup>[13]</sup>, who obtained encouraging results by combining them with Sphagnum peat as a growth substrate for *Salvia splendens*. On the other hand, Schalamuk *et al.*<sup>[14,15]</sup> evaluated the potential of these materials as carriers of the entomopathogenic fungus *Beauveria bassiana* for use as a mycoinsecticide.

Direct application of low-grade PR in agriculture may be technologically feasible when combined with beneficial microorganisms, given their ability to promote solubilization and mobilization of P to the plant in some soils<sup>[16]</sup>.

The PR from the Río Chico Group (Chubut), of sedimentary origin, is a resource of interest as a source of phosphorus for agriculture<sup>[17,18]</sup>. Although these PRs have a low P content, an economic and ecological alternative could be the direct application of PRs together with microorganisms capable of solubilizing P through the production of organic acids<sup>[19]</sup>. In this sense, these phosphorites have been tested through the inoculation of substrates with microorganisms<sup>[7,20,21]</sup>. Biological studies have shown that native strains of geofungi such as *Penicillium thomii* and *Aspergillus niger* solubilize the aforementioned PRs, which has been corroborated in in vitro studies in culture media and in substrates for plant growth. On the other hand, it has been found that the application of these PRs together with the aforementioned S and AMF generated positive responses in the growth of horticultural species<sup>[9]</sup>.

Taking into account this background, the objective of this work was to combine AMF and S with Argentine geological materials—ashes and pumicites from the Puyehue volcano and PR of the Río Chico Group—in the elaboration of a substrate for the potted production of *Lactuca sativa* plants.

## 2. Materials and methods

The trial was carried out under controlled conditions in the greenhouse belonging to the Spigazzini Institute, Faculty of Natural Sciences and Museum, National University of La Plata, Argentina.

### 2.1 Substrate preparation and characteristics

A mixture of the commercial product

Terrafertil® (Terrafertil Sociedad Anónima Moreno, Province of Buenos Aires, Argentina) with ashes (1:1 v/v) was used to formulate the substrate. The ashes came from Neuquén, Villa La Angostura (40°45'48" S; 71°38'46" W); their physical and chemical properties are shown in **Table 1**. The ashes were sieved with a 2 mm mesh before mixing with the commercial substrate. The substrate was toasted before being placed in 377 cm<sup>3</sup> pots for the greenhouse trial. The paste pH of the Terrafertil®-ash mixture, determined at 23.7 °C, was 5.7.

To half of the substrate thus obtained, PR from the Río Chico Group was added as a source of phosphorus. The amount of PR added to the substrate was calculated to provide 22.7 mg of PR per plant as a phosphorus nutritional supplement.

**Table 1.** Physical and chemical properties of the ashes used to mix with the commercial product and use as a substrate for growing *Lactuca sativa* plants

Properties	Ashes
pH	5.18
Electrical conductivity (dS/m)	0.01
Calcium (mg/L)	5
Magnesium (mg/L)	3
Potassium (mg/L)	9
Sodium (mg/L)	90
Bulk density (kg/cm <sup>3</sup> )	474
Pores with air (%)	44
Water holding capacity (%)	39
Total pore space (%)	83
Particles > 3.35 mm (%)	0
Particles 3.35–1 mm (%)	38
Particles < 1 mm (%)	62

## 2.2 Chemical and mineralogical studies of phosphoric rocks

RPs were analyzed by X-ray diffraction (Philips 3020 Goniometer with PW 3710 controller, Cu-K $\alpha$  radiation and Ni filter). Quantitative analyses were performed by ICP-AES (ALS Chemex Lab, Canada) and by infrared photometry (FT-IR). The CO<sub>3</sub>/PO<sub>4</sub><sup>[22]</sup> molar ratio was calculated. Solubility of PR was also determined using different extractants<sup>[23]</sup>.

## 2.3 Inoculation with phosphorus solubilizing fungi (S) and arbuscular mycorrhizal fungi (AMF)

A strain of *P. thomii* deposited at the Spegazzini Institute, La Plata (LPS culture n.º 743), isolated from a soil of Tierra del Fuego, Argentina<sup>[24]</sup> was used as S. For isolation, identification, determination

of P solubilizing capacity and colony forming unit (CFU) count, the methodology described by Cabello *et al.*<sup>[20]</sup> was followed. 5 g of *P. thomii* inoculum was added at a concentration of 10<sup>[4]</sup> cells/mL.

For inoculation with AMF, the biological fertilizer AEGIS® Irriga (lot No. ASIR311HL, Grupo Giten, Tarragona, Spain), which contained AMF spores, was used. The product was analyzed at the Spegazzini Institute using the sucrose gradient sieving and centrifugation technique<sup>[21]</sup>. It was determined that it contained 192 spores/g of inoculum and the morphotype of those spores corresponded to Rhizophagus intraradices (Schenck & Smith) Walker & Schuessler. This inoculum was mixed with the growth substrate, following the dosification indicated on the label, so that each plant received 3 g of inoculant. When the assay was finalized, the root systems were carefully washed, clarified and stained according to the Phillips and Hayman<sup>[25]</sup> technique. Microscopic counting of mycorrhizal root segments was carried out according to McGonigle *et al.*<sup>[26]</sup>

## 2.4 Test with plants

The trial consisted of 8 treatments (3 pots per treatment): (1) substrate (control without PR); (2) substrate + AMF; (3) substrate + S; (4) substrate + AMF + S; (5) substrate: PR (control with PR); (6) substrate: PR + AMF; (7) substrate: PR + S, and (8) substrate: PR + AMF + S.

*L. sativa* seeds were superficially disinfected with a solution of sodium hypochlorite (10% v/v) for 10 min, and then rinsed with sterile distilled water. Seeds were sown in a sterile substrate composed of perlite: vermiculite (1:1 v/v), placed in 20 × 5 × 5 cm plastic trays. After germination, after 15 days, the seedlings were selected for size uniformity and transplanted as follows.

The experimental unit was a pot (10 × 12 cm, 377 cm<sup>3</sup>). Each pot was filled with 315 g of the corresponding substrate and a pregerminated *L. sativa* seedling was transplanted, as mentioned above. The pots were arranged in a temperature-controlled greenhouse (24 ± 1 °C day/20 ± 1 °C night) and a 16-h photoperiod provided by cool-white and incandescent lamps. The trial finalized at 60 days. At that time, the dry and fresh mass of the aerial and root part was

measured.

## 2.5 Analysis of P content

The total and assimilable P content in the substrate and the P content in plant tissue were determined according to the norms standardized by the Standardization and Accreditation Commission–Chilean Society of Soil Science, methods of analysis of plant tissue (revision 2004). The analyses were performed in an OPDS-approved laboratory, Reg. No. 017 Res. 640/02.

## 2.6 Experimental design and statistical analysis

The data obtained for total and assimilable P in the substrate, P in the plant tissue, aerial and root dry weights, radical colonization by AMF and CFU of *P. thomii* were evaluated by analysis of variance (ANOVA) using a completely randomized design. The percentage of colonization was transformed using arcsine and those of CFU applying  $\log(x + 1)$ , to fit the data to a normal distribution and normalize the residuals of variance. Means were compared using

Tukey's test.  $p < 0.05$  was considered as a value of statistical signification. The software used was the InfoStat program version 2009<sup>[27]</sup>.

## 3. Results

### 3.1 Chemical and mineralogical studies of the phosphate rock

PR analyses by X-ray diffraction recorded major phosphate reflections belonging to carbonatohydroxylapatite (2.78; 2.68; 1.96 Å) and carbonato fluorapatite (2.69; 2.79; 2.24; 3.05 Å); calcite, quartz and kaolinite were also identified. Quantitative analyses by ICP-AES revealed the major elements expressed as oxides (**Table 2**). The FT-IR spectra of the phosphorites showed well defined absorption bands of the C-O bond at 1,427 and 1,456/cm. In addition, the bands at 603 and 578/cm, corresponding to the P-O bond, which are characteristic of carbonate-apatites, stood out. The CO<sub>3</sub>/PO<sub>4</sub> molar ratio allowed establishing that the phosphoric component has a high isomorfic replacement of PO<sub>4</sub><sup>-3</sup> by CO<sub>3</sub><sup>-2</sup>, with values of 18%.

**Table 2.** Quantitative analysis (ICP-AES) of the Rio Chico phosphate rocks in terms of percentage of oxides and BPL (Bone Phosphate of Lime) values

	P <sub>2</sub> O <sub>5</sub>	Al <sub>2</sub> O <sub>5</sub>	SiO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	CaO	MgO	Na <sub>2</sub> O	K <sub>2</sub> O	F	Cl	CO <sub>2</sub>	SO <sub>3</sub>	TiO <sub>2</sub>	Corg	BPL
<i>Samples</i>															
1	19.2	5.75	32.4	2.15	30.1	0.45	1.7	1.5	2.1	0.5	2.8	0.8	0.4	0.1	42.4
2	16.4	6.1	34.5	1.85	29.7	0.65	1.82	2.55	2.15	0.42	2.93	0.45	0.32	0.05	36.12

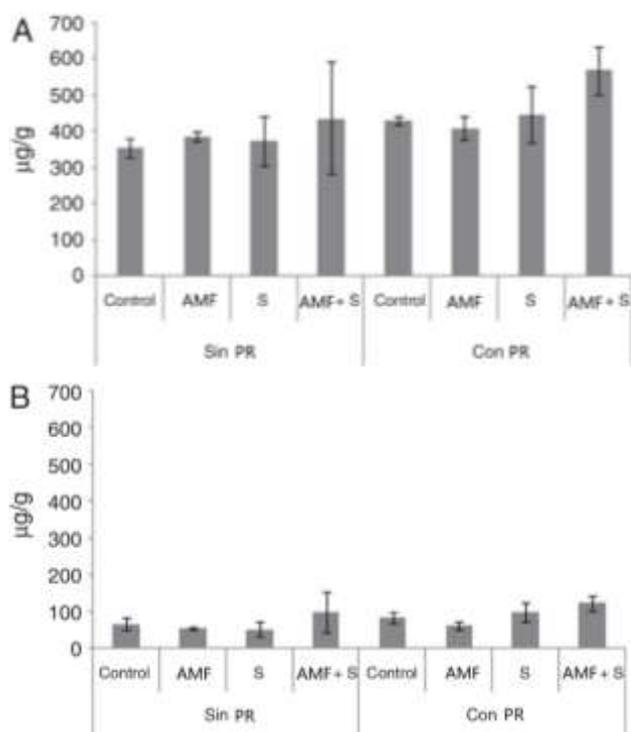
### 3.2 Total and assimilable phosphorus in the substrate

**Figure 1** shows the contents of total P and assimilable P in the growth substrate of *L. sativa* plants.

Total P contents in the substrate (**Figure 1A**) did not show significant differences in treatments inoculated with AMF, with S or with AMF + S ( $F = 3.15$ ;  $gl = 3$ ;  $p = 0.0542$ ). When the effect of PR addition was analyzed, significant differences were observed ( $F = 6.75$ ;  $gl = 1$ ;  $p < 0.05$ ), with higher values in the treatments with PR with respect to those in which PR was not added (464.15 µg/g and 388.03 µg/g of total P, respectively). The PR-microorganisms interaction presented significant differences ( $F = 7$ ;  $gl = 0.59$ ;  $p < 0.05$ ). The treatment with PR + S + AMF showed the highest total P content in the substrate (569.5 µg/g), while the control without PR showed the

lowest content (355 µg/g).

**Figure 1B** shows the contents of assimilable P in the substrate. The treatments inoculated with microorganisms and those in which PR was added presented significant differences ( $F = 4.63$ ;  $gl = 3$ ;  $p < 0.05$  and  $F = 5.27$ ;  $gl = 1$ ;  $p < 0.05$ , respectively). The highest values of assimilable P (110.86 µg/g) were obtained with AMF + S inoculation, while the lowest values (57.46 µg/g) were recorded in the AMF treatment. However, no significant differences ( $F = 0.7$ ;  $gl = 7$ ;  $p = 0.0675$ ) were observed in the microorganism × PR interaction.



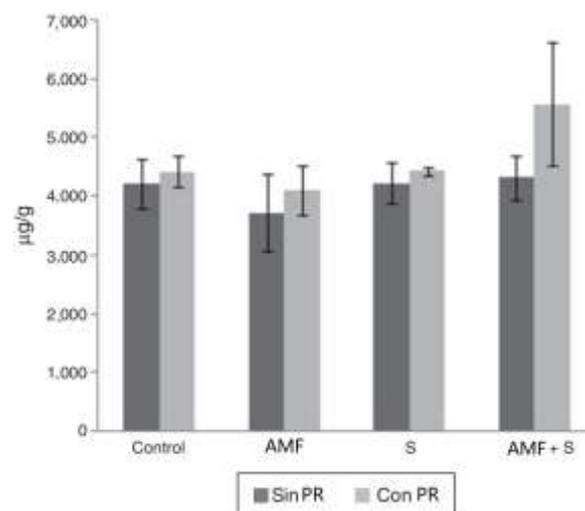
**Figure 1** Phosphorus concentration in 2 substrates for *Lactuca sativa* plant growth, consisting of a mixture of Terrafertil® with ashes from the Puyehue volcano, without phosphate rock (without PR) or added with phosphate rock (with PR). **A)** Total phosphorus. **B)** Assimilable phosphorus. Data are average of 3 pots. The lines above the bars indicate standard deviation from the average. AMF: arbuscular mycorrhizal fungus; S: phosphorus solubilizing fungus.

### 3.3 Phosphorus in plant tissue

The presence of microorganisms led to significant differences in this nutrient ( $F = 3.98$ ;  $gl = 3$ ;  $p < 0.05$ ), the highest content was recorded in the treatment with AMF + S ( $4,952.83 \mu\text{g/g}$ ), while the lowest in the treatment with AMF ( $3,913.83 \mu\text{g/g}$ ). The addition of PR in the substrate led to significant differences ( $F = 5.71$ ;  $gl = 1$ ;  $p < 0.05$ ): the highest content was verified in the treatment with PR ( $4,639.58 \mu\text{g/g}$ ) and the lowest in the one without addition of this source ( $4,126.67 \mu\text{g/g}$ ). The PR-microorganisms interaction was significantly different ( $F = 1.33$ ;  $gl = 7$ ;  $p < 0.05$ ), with the highest P contents observed in the AMF + S PR treatment and the lowest in the treatment with AMF alone ( $5,575.33 \mu\text{g/g}$  and  $3,720.62 \mu\text{g/g}$ , respectively).

The P content in plant tissue was significantly higher in plants grown on substrate with PR compared to those grown without PR addition. The P content in plants grown on substrate combined with PR was significantly higher in the treatment in which

AMF and S were incorporated ( $5,575 \mu\text{g/g}$ ) and a significantly lower value was recorded in the treatment associated with AMF ( $4,107 \mu\text{g/g}$ ). The same trend was observed in the plants grown on the substrate, to which PR was not added: the highest P content was observed in those in which AMF and S were consociated ( $4,225 \mu\text{g/g}$ ) and significantly lower values ( $3,720 \mu\text{g/g}$ ) were recorded in those inoculated only with AMF.



**Figure 2.** Phosphorus in the tissue of *Lactuca sativa* plants grown for 60 days in 2 growth substrates, consisting of a mixture of Terrafertil® with ashes from the Puyehue volcano, without phosphate rock (without PR, dark gray series) or added with phosphate rock (with PR, light gray series). Lines above the bars indicate standard deviation of the averages of 3 replicates. AMF: arbuscular mycorrhizal fungus; S: phosphorus solubilizing fungus.

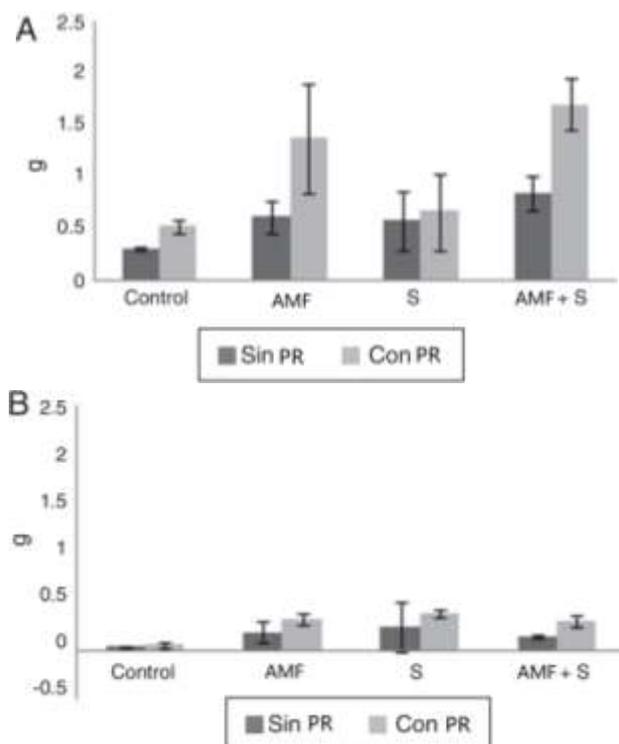
### 3.4 Evaluation of growth parameters of *L. sativa* plants

**Figure 3** shows the results obtained for aerial and root dry biomass of *L. sativa* plants.

Aerial dry biomass (**Figure 3A**) showed significant differences when analyzing the simple effect of the presence of microorganisms ( $F = 11.61$ ;  $gl = 3$ ;  $p < 0.05$ ). The highest value was recorded in the AMF + S treatment ( $1.27 \text{ g}$ ), while the lowest was in the control treatment ( $0.41 \text{ g}$ ). When analyzing the effect of PR, significantly higher values ( $F = 18.1$ ;  $gl = 1$ ;  $p < 0.05$ ) were recorded in the treatments with PR and lower in those in which PR was not added ( $1.06 \text{ g}$  and  $0.58 \text{ g}$ , respectively). For the PR-microorganism interaction, the differences were significant ( $F = 2.86$ ;  $gl = 7$ ;  $p < 0.05$ ); the highest aerial biomass was recorded in the AMF + S PR treatment and the

lowest in the control without PR (1.69 g and 0.3 g, respectively).

Root dry biomass (**Figure 3B**) only showed significant differences ( $F = 3.92$ ;  $gl = 3$ ;  $p < 0.05$ ) when analyzing the simple effect of microorganism treatments; the highest value was observed when inoculated with AMF and the lowest in the control treatment (0.25 g and 0.05 g, respectively). The presence of PR ( $F = 0.58$ ;  $gl = 1$ ;  $p = 0.45$ ) and the PR-microorganism interaction ( $F = 2.74$ ;  $gl = 7$  and  $p = 0.07$ ) showed no significant differences.



**Figure 3.** Aerial (**A**) and root (**B**) dry weight of *Lactuca sativa* plants grown for 60 days in 2 growth substrates, consisting of a mixture of Terrafertil® with ashes from the Puyehue volcano, without phosphate rock (without PR, dark gray series) or added with phosphate rock (with PR, light gray series). The lines above the bars indicate standard deviation of the average of 3 replicates. (AMF) Arbuscular mycorrhizal fungus; (S) Phosphorus solubilizing fungus.

### 3.5 Root colonization by arbuscular mycorrhizae-forming fungi

The structures observed were external mycelium, internal mycelium, entry points and arbuscules (**Table 3**). The presence of AMF structures was found only in treatments inoculated with arbuscular mycorrhizae. The number of arbuscules was the only mycorrhizal parameter that presented significant differences between the treatment inoculated only with AMF and those in which AMF + S were present. The

highest percentage of colonization with arbuscules (26%) was observed in the treatment with PR and both microorganisms (AMF + S), while a significantly lower value was observed in the treatment without PR and mycorrhizae (4%).

The percentages of colonization with the presence of both external and internal mycelium showed the same trend: the highest percentages were found in the treatment without PR and with the presence of both microorganisms (50% with external mycelium and 52% with internal mycelium), and the lowest values were found in the treatment without PR and with AMF (21% and 20% with external and internal mycelium, respectively). With respect to the percentage of entry points, the highest value was obtained in those treatments in which PR + AMF was added (31%) and the lowest value was reported in the treatment without PR and with the 2 types of microorganisms consociated (7.3%).

### 3.6 Colony-forming units of P-solubilizing fungi

Significant differences were found for *P. thomii* CFU counts when analyzing the simple effect of PR addition and the presence of microorganisms. The PR-microorganism interaction showed no significant differences (**Table 3**). The highest count of these microorganisms was obtained in the AMF + S treatment (1.43 CFU/g substrate) and the lowest in the one where only solubilizers were present (1.31 CFU/g substrate). Considering the addition of PR, a higher count was obtained in the treatment without PR than in the one that received PR (1.51 CFU/g substrate and 1.23 CFU/g substrate, respectively).

## 4. Discussion

Volcanic ashes from the Puyehue volcano showed favorable physicochemical properties for use as a substrate for potted crops. However, there is a need to combine them with other materials to optimize their properties. Barbaro *et al.*<sup>[13]</sup> pointed out that these pyroclastic materials in combination with substrates such as Sphagnum peat increase water retention capacity by improving the ratio of pores to air and water.

The contents of total and assimilable P in the

substrate increased in those treatments in which PR was added and combined with *P. thomii* and *R. intraradices*. This shows the potential of the PRs of the Río Chico Group to be used in direct application in containers. Although the PRs used in this trial have low P contents, they constitute an economic and ecological alternative in combination with microorganisms capable of solubilizing phosphate, such as *P. thomii*, and with phosphate mobilizers, as in the case

of the arbuscular fungus *R. intraradices*. The interactions that occur between these microorganisms are essential for understanding the dynamics of the processes that take place in the rhizosphere<sup>[28]</sup>. Thus, a large number of rhizosphere microorganisms develop activities that alter nutrient availability and act synergistically with AMF to increase plant growth<sup>[1,4,5,29,30]</sup>.

**Table 3.** Results of 2-way ANOVA (F and P values) for the effect of the addition of phosphate rock, the presence of microorganisms and the interaction between both factors on the variables of colonization by arbuscular mycorrhizal fungi and colony forming units of phosphorus solubilizing fungi.

	External mycelium		Internal mycelium		Entry point		Arbuscles		Colony forming units	
	F	P	F	P	F	P	F	P	F	P
Phosphate rock	0.04	0.84	0.11	0.75	4.21	0.07	0.19	0.67	74.09	<0.01
Presence of microorganisms	2.92	0.12	2.62	0.14	0.77	0.41	5.90	<0.05	13.15	<0.01
Phosphate rock × microorganisms	0.41	0.54	1.02	0.34	0.15	0.71	0.11	0.75	1.69	0.22

Significance level  $p < 0.05$ .

Likewise, the highest P content in plant tissue was recorded in those plants in which Puyehue volcano ash, PR and the S/AMF consortium were combined. Goenadi *et al.*<sup>[31]</sup> reported a better absorption of both native P and P contained in PR when plants were co-inoculated with both microorganisms. The solubilizing capacity of *P. thomii* and the positive synergism of this fungus with *Funneliformis mosseae*, an AMF, in the growth of *Mentha piperita* plants were verified by Cabello *et al.*<sup>[20]</sup>

Plant growth parameters, measured as dry aerial biomass, were higher in those plants with the addition of PR plus the inoculation of *P. thomii* and *R. intraradices*. These results are consistent with the increases in P detected in the plant tissue of these plants. The increase in aerial biomass can be attributed to both improved phosphorus nutrition and the effect of co-inoculation with microorganisms. The positive effect with the co-inoculated treatment also included the treatment without PR and with *P. thomii*/*R. intraradices*, which presented the highest aerial dry weight. This result evidences that the synergism between these microorganisms was beneficial for the biomass production of *L. sativa*, even under this condition. Velázquez *et al.*<sup>[9]</sup> also reported an increase in the biomass of tomato plants when they were co-inoculated with *A. niger*/*F. mosseae*, both in substrates that received contributions from a

phosphorus source and those substrates that did not.

Regarding the production of root dry biomass, no significant differences were found between treatments with or without the addition of PR. However, plants associated with AMF presented the highest value of root biomass in both conditions (with PR and without PR), while the lowest dry weight was found in the control treatments. The increase in root biomass due to the effect of inoculation with *R. intraradices* has been previously reported by Salgado-Barreiro *et al.*<sup>[32]</sup> The increase in the production of root biomass, as well as aerial biomass, is part of the positive response that the host plant obtains from the mycorrhizal symbiosis. In our work, this increase in root biomass is a positive effect of colonization by arbuscular fungi and was independent of the addition of a phosphorus source.

All plants that received AMF-based inoculum showed characteristic structures of this association in their roots: external and internal mycelium, entry points and arbuscles. However, the number of arbuscles showed significant differences between treatments: the highest number of arbuscles was observed in the treatments with PR and with the associated microorganisms. In our work, it is observed that the amount of P, both total and assimilable in the substrate, is positively related to an increase in arbuscular colonization. These observations contrast with

the findings of other authors, who indicate a negative correlation between P availability and mycorrhizal colonization<sup>[18]</sup>. Velázquez *et al.*<sup>[9]</sup> found that the percentages of root colonization decreased in co-inoculated treatments and with the addition of PR.

The results provided in this assay confirm the solubilizing capacity of *P. thomii* against an insoluble P source, previously reported by Cabello *et al.*<sup>[20]</sup> Kucey *et al.*<sup>[10]</sup> and Kapoor<sup>[33]</sup> described that the excretion of organic acids and chelating substances are the pathways by which solubilizing microorganisms dissolve these unavailable forms of P. The CFU values of *P. thomii* obtained in the counts were influenced by the addition of PR and by the microorganisms. The treatments in which PR was added evidenced the lowest counts, relative to the treatments without PR. The release of organic acids produced during the solubilization process decreases the pH of the substrate<sup>[34]</sup>. Possibly, this increase in acidity affects the germination of *P. thomii* spores. Thus, the treatment with PR and the *P. thomii* and *R. intraradices* consortium presented the highest soluble P values. In relation to the presence of microorganisms, the co-inoculated treatments, with or without PR, presented the highest counts, which highlights the positive synergism between both microorganisms. These synergistic effects have been reported in previous studies<sup>[2,20]</sup>.

This work confirms that volcanic ashes from the Puyehue volcano and phosphoric rocks from the Río Chico group, formulated with a commercial substrate (Terrafertil®) and with the combination of P solubilizing/mobilizing microorganisms, represent a favorable alternative for the partial replacement of conventional materials (perlite, peat, expanded clays, among others) used in the cultivation of potted *L. sativa* plants. In this way, the added value of geological materials of national origin is also increased.

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## Conflict of interest

The authors declare that they have no conflict of

interest.

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