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## **Effect of the application of nematophagous fungus *Purpureocillium lilacinum* over nutrients availability on agricultural soil and yield of *Avena sativa***

### **Efecto de la aplicación del hongo nematófago *Purpureocillium lilacinum* sobre la disponibilidad de nutrientes en un suelo agrícola y el rendimiento de *Avena sativa***

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#### **ABSTRACT**

In the present work the application effect of nematophagous fungus *Purpureocillium lilacinum* over the availability of soil nutrients and the yield of oats plants, using an agricultural soil was evaluated. Two experiments were conducted under greenhouse conditions, one with soil in natural conditions and other with autoclaved soil. In each experiment were handled two treatments with 20 replicates in a completely randomized design; one with the application of *P. lilacinum* and the other without the fungus application as a control. The soil chemical characteristics: pH, C, N, P, K, Ca, Fe, Cu and Zn were analyzed at the beginning and at the end of the experiment. The variables evaluated on oat plants were: height, fresh and dry weight, nutrients, number of spikelets per plant and the spikelets weight. There were no significant differences between treatments in plants height, the nutrient content of the soil and plants in both treatments, neither in fresh and dry weight in the experiment with natural conditions soil. The positive effect of fungus application was reflected on the greater fresh and dry weight in the experiment with autoclaved soil and the greater number and weight of spikelets of oat plants in both experiments.

#### **Keywords**

soil fertility • phosphorous • filamentous fungi

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## RESUMEN

En el presente trabajo se evaluó el efecto de la aplicación del hongo nematófago *Purpureocillium lilacinum* sobre la disponibilidad de nutrimentos del suelo y el rendimiento de plantas de avena, utilizando un suelo agrícola. Se realizó dos experimentos bajo condiciones de invernadero, uno con suelo en condiciones naturales y otro con suelo esterilizado en autoclave. En cada experimento se manejó dos tratamientos con 20 repeticiones en un diseño completamente aleatorizado; uno con la aplicación de *P. lilacinum* y el otro sin aplicación del hongo como control. Las características químicas del suelo: pH, C, N, P, K, Ca, Mg, Na, Fe, Cu y Zn fueron analizadas al inicio y final del experimento. Las variables evaluadas en las plantas de avena fueron: altura, peso fresco y seco, contenido de nutrimentos, número de espiguillas por planta y el peso de espiguillas. No hubo diferencias significativas entre tratamientos en la altura, contenido de nutrimentos del suelo y de las plantas en ambos tratamientos, ni en peso fresco y seco en el experimento con suelo en condiciones naturales. El efecto positivo de la aplicación del hongo se reflejó en el mayor peso fresco y seco en el experimento con suelo esterilizado y en el mayor número y peso de espiguillas por planta en ambos tratamientos.

### Palabras clave

fertilidad del suelo • fósforo • hongos filamentosos

## INTRODUCTION

In the conventional agriculture, one of the main issues is to provide the crops with the available phosphorus, which has been compensated with the continuous application of fertilizers (42). The phosphorous (P) after the nitrogen, it is the most important nutrient for the plants and they only can absorb it in orthophosphates form. In nature, most of the soils have a vast reserve of organic and inorganic phosphorus forms (40). Nevertheless, most part of P is not available for the plants, because it forms insoluble compounds with iron and aluminum in acid soils and with calcium in alkaline soils (5, 20). In soils with high fixation capacity, when P contained within the fertilizers passes to the soil solution, it is fixed in the mineral fraction, without being available for the plants. Besides, when the fixation capacity of the soil has been saturated, the

excess of phosphorus is leached towards underground waters, turning into a contamination source (16, 40).

Although there are many saprobes fungi in the soil able to phosphate solubilizing (19), it is not enough to supply the demand for nutrients of the crop. Some species of saprophytic fungi like *Penicillium* spp. and *Aspergillus* spp. have been studied with agricultural interest for their capacity of solubilizing phosphate compounds, improving the P availability in the soil and consequently enhancing crops yield (29, 35). These fungi affect directly the minerals solubilization and the release of cations like  $\text{Fe}^{2+}$ ,  $^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Sr}^{2+}$ , by the secretion of organic acids or proton liberation  $\text{H}^+$  (22, 23). Another species of fungi, used in the agricultural systems as phytoparasitic nematodes control

agents, are facultative saprophytes and take part in organic matter degradation and there its importance in nutrients cycling, like nitrogen and carbon (8). Some works concerning laboratory and greenhouse with *Arthrobotrys oligospora* Fres. have demonstrated the effect of this nematophagous fungus on the availability of the P using phosphoric rock (13).

*Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (= *Paecilomyces lilacinus*) is known since 1970's as a biological control agent of several phytoparasitic nematodes (27, 30). Also its solubilizing activity of inorganic forms of P is known and was evaluated *in vitro*, like calcium and iron phosphates (21).

However, the application of this fungus in the field had generated questions about its function in the agrosystem, because the previous observations (unpublished data) of a better development in oats plants grown (height, fresh and dry weight) in soil treated with *P. lilacinum*, applied to mitigate the levels of the phytoparasitic nematode populations. The aim of this work was to evaluate in greenhouse the effect of an application of *P. lilacinum*, on the availability of phosphorus, calcium and iron in an agricultural soil and its influence on the yield of oats plants (*Avena sativa*).

## MATERIALS AND METHODS

### Soil sampling

The soil used in this study was collected in the rural village of Los Pescados, located in the municipality of Perote, Veracruz, Mexico (19°34'41" N, 97°9'30" W, altitude 2.946 m), where the dominant soils are humic, mollic and

haplic phaeozem andosols (24). Four soil samples (10 Kg each) were taken from potato crop rows at a depth of 10-15 cm in a plot of 2 ha. In the laboratory the soil was dried and sieved with a 5 mm mesh, in order to remove large organic and mineral particles. Subsequently, it was mixed with sterile perlite at a ratio of 1:1 in order to avoid soil compaction. Soil texture was a sandy clay loam (sand = 63%, silt = 15% and clay = 22%) according to Bouyoucos method.

### Microorganism

In this study one strain of *P. lilacinum* (IE-430) was employed, which it is native of the study area and was isolated from juveniles J2 of the potato cyst nematode *Globodera rostochiensis* (Woll. 1923) Skarbilovich, 1959. This fungus was cultivated on oatmeal-agar plate during three weeks before its use.

### Experimental design

Two experiments were carried out, the first one with soil under natural conditions (NS) and the second one with autoclaved soil (AS).

Two treatments were applied in each of both experiments: 1) with *P. lilacinum* inoculated to soil (F), and 2) without fungus (C), as a control. All experiments were conducted simultaneously and distributed in a completely randomized design, under greenhouse conditions (natural light and 30±3°C). For ASF and ASC treatments, soil was sterilized in autoclave, into plastic bags for one hour at 121°C, then cooled at room temperature. This process was repeated.

Each treatment had 20 replicates, which consisted of polyethylene pots (1 kg capacity) with 1000 cm<sup>3</sup> of soil-perlite substrate.

For NSF and ASF pots, 200 mL of *P. lilacinum* spore suspension ( $1 \times 10^6$  spores  $\text{ml}^{-1}$ ) prepared with sterile distilled water were added so the applied dose was  $2 \times 10^8$  spores  $\text{pot}^{-1}$ .

For the controls (NSC and ASC), the same amount of sterile distilled water was added. Three oat seeds (*cv.* Chihuahua) previously disinfected with a 1% sodium hypochlorite solution were planted in each pot. Once the plants emerged, two were selected for each pot removing the other one. The plants were watered (85 mL per pot) every 72 h.

### Soil chemical characterization

Before applying the fungus and 14 weeks later, rizosphera soil samples were taken from five pots (200 g) of each treatment.

The following chemical analyses were made for each soil sample: pH was measured in water (1:2 w/v) using a potentiometer (Jenco Electronics Ltd., Model 1671, USA). Inorganic nitrogen (nitrate  $\text{NO}_3^-$  and ammonium  $\text{NH}_4^+$ ) was extracted with potassium chloride (KCl 2N) and quantified with the micro-Kjeldahl technique. Phosphorus was measured according to Bray and Kurtz 1 methodology with a spectrophotometer at 882 nm (Spectronic 21D, Milton Roy, USA). Exchangeable bases (K, Ca, Mg and Na) were extracted with ammonium acetate ( $\text{CH}_3\text{COONH}_4$  1N pH7), and their concentration of potassium and sodium was determined by flame photometry (Flame Photometer 410, Corning, UK) and concentration of calcium and magnesium by atomic absorption (AA-6501 Shimadzu, Japan).

The extraction of microelements (Fe and Cu) was achieved with DTPA and its quantification was carried out by atomic absorption. The percentage of organic carbon and organic nitrogen was determined using the C/N TruSpec analyzer (LECO, USA). Aluminum was determined

by the technique of exchangeable acidity, accomplishing extraction with potassium chloride (KCl 1M) and titration with sodium hydroxide (NaOH 0.1 M) and hydrochloric acid (HCl 0.1 M) (37). Reference values from SEMARNAT (2002) were consulted for interpreting soil tests according to Fassbender and Bornemisza (1987) for assessing organic carbon content. In the autoclaved soil treatments, tests were performed after the sterilization process.

### Yield and nutrient content evaluation in oat plants

At the end of the experiment (after 14 weeks) height, fresh and dry weight (root and stem) and number of spikelets  $\text{plant}^{-1}$  of all oat plants and weight of 200 spikelets per treatment were evaluated. After that, the oat plants corresponding to the pots where soil samples were taken to determine nutrient content were selected. These plants were dried in an oven at 70°C for 24 h and were ground up in order to quantify their nutrients content. The carbon and nitrogen were evaluated using a C/N TruSpec analyzer.

For phosphorus, potassium, calcium, magnesium, sodium, iron and copper a dissolving technique involving digestion with perchloric acid ( $\text{HClO}_4$ ) and nitric acid ( $\text{HNO}_3$ ) was used. Each element was analyzed from the acidic matrix. For phosphorus, we used a colorimetric method using phosphovanadomolybdic complexes and reading was performed with a spectrophotometer at 470 nm.

The contents of potassium and sodium were measured using a flame photometer. In the case of calcium, magnesium, iron and copper, atomic absorption spectrophotometry and flame emission were applied.

### Statistical analysis

Differences between final and initial amounts of most nutrients in all treatments were calculated and statistically tested. Inorganic nitrogen and magnesium were depleted at the end of the experiment, so their data was only analyzed in the first sampling.

For magnesium, potassium and inorganic nitrogen, the statistical analysis was performed only on samples with detectable nutrients.

The data expressed as a percentage were transformed by applying the function arccosine. NSF and NSC treatments were compared between them, and likewise ASF and ASC treatments. In order to determine differences between treatments in terms of the nutrient content of soil, plant and crop yield, the Student's t test was used, if the samples had the norms for normality and homogeneity of variances. Otherwise, we applied the non-parametric Mann-Whitney U test ( $p < 0.05$ ). All analyses were performed using the MINITAB 14 and Statistica 8.0 program for Windows.

## RESULTS

### Chemical characteristics of Soil

At the beginning of the experiments, the pH of NS (NSF, NSC) and of AS (ASF, ASC) treatments was strongly and moderately acidic respectively. At the end of the study a tendency of slight increase was found in all treatments, without presenting any significant differences. In the NS experiment, NSF treatment had a medium level (20-40 mg kg<sup>-1</sup>) of initial inorganic nitrogen (NO<sub>3</sub>-NH<sub>4</sub>), whereas NSC nitrogen level was high (40-60 mg kg<sup>-1</sup>), according to reference values from SEMARNAT (2002). Therefore, differences were found in inorganic nitrogen concentration ( $t = -4.36$ ,  $p = 0.002$ ) between NSF and

NSC. In the experiment AS, control (ASC) and fungus treatments (ASF) had medium and high level of initial inorganic nitrogen, respectively. Thus, ASF and ASC showed significant differences in ammonium concentration ( $t = 2.69$ ,  $p = 0.027$ ) at the beginning of the experiment. Potassium level was low (0.2-0.3 cmol<sub>(+)</sub> kg<sup>-1</sup> in soil) in both experiments at first sampling and was depleted in all treatments at the end of the experiments.

In both experiments (NS and AS), the amount of phosphorus was high (>30 ppm) and its concentration increased in all treatments, but no significant differences were registered between them (table 1, page 6 and table 2, page 7).

The calcium content of NS and AS treatments was very low (<2 cmol<sub>(+)</sub> kg<sup>-1</sup> in soil) in the initial and final sampling.

In AS, the calcium content decreased significantly in ASF (-0.36 cmol<sub>(+)</sub> kg<sup>-1</sup> in soil) compared with ASC (-0.14 cmol<sub>(+)</sub> kg<sup>-1</sup> in soil). In both experiments magnesium was not detected at the beginning, but it increased significantly ( $t = -2.59$ ,  $p = 0.031$ ) in NSC compared with NSF at the end of the experiment. Also was higher ( $t = 3.03$ ,  $p = 0.016$ ) in ASF (0.05 cmol<sub>(+)</sub> kg<sup>-1</sup>) than in ASC (0.01 cmol<sub>(+)</sub> kg<sup>-1</sup>). Copper and iron contents were adequate (Cu:>0.2 mg kg<sup>-1</sup>; Fe:>4.5 mg kg<sup>-1</sup>), in both samplings (initial and end) and in all treatments.

The concentration of copper at the end of experiment was lower ( $t = -2.42$ ,  $p = 0.04$ ) in ASF (0.56 mg kg<sup>-1</sup>) than in ASC (0.68 mg kg<sup>-1</sup>), while there were not differences between NS treatments. The concentration of active aluminum was less than 0.5 cmol kg<sup>-1</sup> in all treatments.

For both AS, and NS the percentage of carbon increased in all treatments, except for ASF.

**Table 1.** Soil chemical characteristics of the NSF and NSC treatments at the beginning and at the end of the experiment.**Tabla 1.** Características químicas del suelo de los tratamientos NSF y NSC al principio y al final del experimento.

Variable	Treatments						Statistic	p
	NS F			NS C				
	Start	End	End-Initial †	Start	End	End-Start †		
pH	4.71	4.82	+0.11±0.16	4.69	5.03	+0.34±0.16	t= -2.26	0.05
N (mg kg <sup>-1</sup> )	39.05	1.49	-37.6±9.01b	47.6	0.00	-47.6 ±3.02a	t= 2.36	0.04
P (mg kg <sup>-1</sup> )	32.86	36.07	+3.21±8.63	33.35	34.49	+1.14±9.51	t=0.36	0.73
K (cmol kg <sup>-1</sup> )	0.30	0.0	-0.30±0.01	0.29	0.0	-0.29 ±0.0	U= 10	0.60
Ca (cmol kg <sup>-1</sup> )	0.67	0.67	0.0±0.04	0.66	0.68	+0.02±0.03	t= -1.03	0.33
Mg (cmol kg <sup>-1</sup> )	0.0	0.03	+0.03±0.02b	0.0	0.08	+0.08±0.03a	t= -2.60	0.03
Na (cmol kg <sup>-1</sup> )	0.14	0.0	-0.14 ±0.02	0.13	0.0	-0.13 ±0.03	U= 11.5	0.83
Cu (mg kg <sup>-1</sup> )	0.29	0.74	+0.45±0.07	0.27	0.73	+0.46±0.06	t= -0.42	0.68
Fe (mg kg <sup>-1</sup> )	13.15	17.28	+4.13±2.31	13.06	16.40	+3.34±1.58	t=0.63	0.55
Al (cmol kg <sup>-1</sup> )	0.47	0.45	-0.02±0.04	0.42	0.43	+0.01±0.05	t= -0.86	0.41
% organic C	2.11	2.18	+0.07±0.26	1.97	2.14	+0.17±0.09	U=12	0.60
% organic N	0.18	0.18	0.0±0.0	0.14	0.15	+0.01±0.02	U= 12.5	1.00
C/N	11.91	12.28	+0.37±1.44	13.61	13.93	+0.32±1.36		

The data of beginning and end are averages of five replicates.

NSF = Natural Soil + *P. lilacinum*, NSC = Natural Soil Control.

Different letters in different columns indicate statistical difference ( $p \leq 0.05$ ) among treatments according to Student's t or Mann Whitney U tests. †(+/-) Indicates the increase or decrease ± standard deviation of element between the first and the second assessment.

Los datos de inicio y final son promedio de cinco repeticiones.

NSF = Suelo Natural + *P. lilacinum*, NSC = Suelo Natural Control.

Letras diferentes en diferentes columnas indican diferencias estadísticas ( $p \leq 0,05$ ) entre los tratamientos de acuerdo con la prueba t de Student o U de Mann Whitney. † (+/-) indica el aumento disminución ± desviación estándar del elemento entre la primera y la segunda evaluación.

Treatments where *P. lilacinum* was applied (NSF and ASF) presented the same content of organic nitrogen at the beginning as at the end of the experiment. Control treatments (NSC and ASC) increased organic nitrogen percentage at the end of the study, but in neither case were significant differences between treatments observed (table 1; table 2, page 7).

The C/N relationship showed no variation between treatments and assessments.

### Plants nutrient content and yield

The presence of *P. lilacinum* in soil did not significantly influence the content of nutrients in plants from the NS and AS treatments (table 3, page 7).

The elements N, P, K, Mg and Zn were at low-deficient levels in oat plants from both experiments (N <1.5%, P <0.15%, K <1.26-1.5%, Mg <0.15% and Zn <15 ppm), according to the reference values provided by Westfall *et al.* (1990) and Jones *et al.* (1991).

**Table 2.** Soil chemical characteristics of the ASF and ASC treatments at the beginning and at the end of the experiment.

**Tabla 2.** Características químicas del suelo de los tratamientos ASF y ASC al principio y al final del experimento.

Variable	Treatments						Statistic	p
	ASF			ASC				
	Start	End	End-Start †	Start	End	End-Start †		
pH	5.03	5.24	+0.21±0.11	5.12	5.40	+0.27±0.05	t= -1.23	0.25
N (mg kg <sup>-1</sup> )	52.96	0.25	-52.71±6.62	36.74	1.65	-34.08 ± 17.5	U= 2.0	0.03
P (mg kg <sup>-1</sup> )	40.33	45.18	+4.84 ±7.62	35.25	45.5	+11.25±9.34	t= -1.19	0.27
K (cmol kg <sup>-1</sup> )	0.26	0.0	-0.26	0.26	0.0	-0.26	U=11.5	0.83
Ca (cmol kg <sup>-1</sup> )	1.08	0.72	-0.36±0.05a	0.94	0.80	-0.14±0.11b	t= -3.85	0.00
Mg (cmolkg <sup>-1</sup> )	0.0	0.05	+0.05±0.03a	0.0	0.01	+0.01±0.01b	t= 3.03	0.02
Na (cmol kg <sup>-1</sup> )	0.45	0.02	-0.43±0.03	0.43	0.04	-0.39±0.02	U=5	0.12
Cu (mg kg <sup>-1</sup> )	0.24	0.56	+0.3±0.09b	0.23	0.68	+0.45±0.08a	t= -2.43	0.04
Fe (mg kg <sup>-1</sup> )	10.27	14.92	+4.65±2.93	10.14	13.92	+3.78±1.11	U=0.10	0.60
Al (cmol kg <sup>-1</sup> )	0.43	0.38	-0.05±0.10	0.42	0.36	-0.06±0.06	t=0.23	0.83
% organic C	1.94	1.9	-0.04±0.12	1.90	1.98	+0.08±0.11	U=8	0.35
% organic N	0.16	0.16	0.0±0.02	0.14	0.15	+0.01±0.02	U=8	0.35
C/N	12.19	11.58	-0.61±0.97	13.98	13.43	-0.54±1.11		

The data of beginning and end are averages of five replicates. ASF= Autoclaved Soil + *P. lilacinum*, ASC = Autoclaved Soil Control. Different letters in different columns indicate statistical difference (p<0.05) among treatments according to Student's t or Mann Whitney U tests. † (+/-) Indicates the increase or decrease ± standard deviation of element between the first and the second assessment.

Los datos de inicio y final son promedio de cinco repeticiones. ASF = Suelo Autoclavado + *P. lilacinum*, ASC = Suelo Autoclavado Control. Letras diferentes en diferentes columnas indican diferencias estadísticas (p<0,05) entre los tratamientos de acuerdo con la prueba t de Student o U de Mann Whitney. † (+/-) indica el aumento o disminución ± desviación estándar del elemento entre la primera y la segunda evaluación.

**Table 3.** Nutrient content in oat plants under different treatments.

**Tabla 3.** Contenido de nutrientes en las plantas de avena bajo diferentes tratamientos.

Nutrients	Treatments			
	NSF	NSC	ASF	ASC
% C	45.76±0.19	47.64±3.39	45.88±0.31	47.7±3.52
% N	1.20±0.03	1.25±0.13	1.29±0.03 b	1.44±0.12 a
% P	0.15±0.02	0.14±0.01	0.09±0.01	0.09±0.01
% K	1.49±0.2	1.57±0.09	1.35±0.09	1.44±0.06
% Ca	0.32±0.03	0.36±0.11	0.36±0.04	0.34±0.04
% Mg	0.08±0.0	0.10±0.0	0.09±0.0	0.10±0.0
% Na	0.86±0.05	0.94±0.06	1.06±0.09	1.01±0.04
Fe mg kg <sup>-1</sup>	79.98±20.1	72.73±15.14	80.07±21.27	92.31±28.28
Cu mg kg <sup>-1</sup>	5.84±2.07	5.51±2.09	4.61±1.07	4.19±0.53
Zn mg kg <sup>-1</sup>	13.21±1.84	12.98±3.75	10.75±2.33	10.59±1.51

The results are averages from five replicates ± standard deviation. NSF = Non-sterilized soil + *P. lilacinum*, NSC= Non-sterilized soil (control). ASF = Autoclaved soil + *P. lilacinum*, ASC = Autoclaved soil control. Different letters in the columns indicate the statistical difference (p = 0.05) between treatments (NSF-NSC) and (ASF-ASC) according to the Student's t or Mann Whitney U test.

Los resultados son el promedio de cinco repeticiones ± desviación estándar. NSF = Suelo Natural + *P. lilacinum*, NSC = Suelo Natural Control, ASF = Suelo Autoclavado + *P. lilacinum*, ASC = Suelo Autoclavado Control. Letras diferentes en las columnas indican la diferencia estadística (p = 0,05) entre los tratamientos (NSF-ASC) y (ASF-ASC) de acuerdo con la prueba t de Student o la prueba U de Mann Whitney.

The Ca and Fe content of the plants was enough (Ca = 0.2-0.5% and Fe = 40-150 ppm) in all treatments.

The level of Cu was sufficient in treatments NSF and NSC (5-25 ppm), whereas in ASF and ASC it was low (<5 ppm).

In NS experiment, NSF spikelets were heavier (>20%) than NSC ones ( $U = 36267.5, p < 0.05$ ). Also, NSF treatment had more spikelets plant<sup>-1</sup> than NSC.

In contrast, NSF oat plants had a lower fresh weight ( $U = 862, p = 0.011$ ) and dry weight ( $U = 874.5, p = 0.014$ ) than in NSC plants.

There were no significant differences in plant height and number of spikelets between these treatments. In AS experiment, ASF treatment had greater fresh weight ( $U = 938, p = 0.009$ ) and dry weight ( $U = 1017.5, p = 0.024$ ) as well as number of spikelets per plant ( $U = 112.5, p = 0.017$ ) and spikelet weight (9%) ( $U = 42467, p = 0.04$ ), than in ASC. There was no difference in plant height between treatments (table 4).

## DISCUSSION

Although the pH of the soil used in this study remains acid, the slight changes registered in a short time are interesting data to consider in the soil fertility, because a pH close to seven allows a greater availability in most of the essential nutrients for the plants (1, 31).

Our data concur with the published values (3.9 y 6.1) for the Perote municipality soils (6, 7, 17).

The low content of K, Ca, Mg and C in our experiment (15, 37) is related to the intensive farming of potato in the soil used in our experiments for more than 70 years.

In the soils of this region, chemicals characteristics such as the cation exchange capacity, the original carbon content, total nitrogen and exchangeable bases have progressively diminished due to soil use change, from forest to agricultural fields (7, 17).

**Table 4.** Yield of oat plants under different treatments.

**Tabla 4.** Rendimiento de las plantas de avena bajo diferentes tratamientos.

Variables	Treatments			
	NSF	NSC	ASF	ASC
Height (cm)	83.23±11.58	87.19±13.65	95.70±14.04	94.30±12.64
Fresh weight (g)	6.28±2.40 <b>b</b>	7.57±2.54 <b>a</b>	10.55±3.53 <b>a</b>	8.77±1.93 <b>b</b>
Dry weight (g)	1.75±0.65 <b>b</b>	2.14±0.84 <b>a</b>	2.65±1.03 <b>a</b>	2.24±0.60 <b>b</b>
Spikelets plant <sup>-1</sup>	8.62±3.49	7.20±4.75	10.54±6.24 <b>a</b>	8.05±4.07 <b>b</b>
Spikelet weight † (mg)	36.35±20.23 <b>a</b>	29.11±14.72 <b>b</b>	35.38±18.07 <b>a</b>	32.06±16.69 <b>b</b>

The results are averages from 20 replicates ± standard deviation. † average of 200 spikelets weighth. NSF = Non-sterilized soil + *P. lilacinum*, NSC= Non-sterilized soil control. ASF= Autoclaved soil + *P. lilacinum*, ASC = Autoclaved soil control. Different letters in the columns indicate the statistical difference ( $p = 0.05$ ) between treatments (NSF-NSC) and (ASF-ASC) according to the Mann Whitney U test.

Los resultados son el promedio de 20 réplicas ± desviación estandar. † promedio del peso de 200 espiguillas. NSF = Suelo Natural + *P. lilacinum*, NSC = Suelo Natural Control, ASF = Suelo Autoclavado + *P. lilacinum*, ASC = Suelo Autoclavado Control. Letras diferentes en las columnas indican la diferencia estadística ( $p = 0,05$ ) entre los tratamientos (NSF-ASC) y (ASF-ASC) de acuerdo con la prueba U de Mann Whitney.



The high concentrations of P registered at the beginning and end of the experiment (32-45 mg kg<sup>-1</sup>) in comparison to the found (3 y 12 mg kg<sup>-1</sup>) in the soils of the same study area (6), could be related with the application of granulated fertilizers in the farming fields as is the case of soil used in this experiment.

The lowest amount of P in ASF treatment compared with the control treatment may be due to better P assimilation by plant, and this was reflected in more spikelets production (26).

The depletion of inorganic nitrogen and potassium observed in the soil of all treatments at the end of experiment, is attributed to the consumption realized by the oat, since cereals absorb more nitrogen and potassium than any other element (10, 14, 34). Crops as oat require chemical fertilization to obtain acceptable yields, mainly when they are seeded in depleted soils. This crop absorbs 34, 5 and 20 kg of nitrogen, phosphorous and potassium respectively, for each tonne of dry matter produced (9). In addition, the consumption of nutrients by the microorganisms and the leaching of minerals, also contribute to the elements loss of the soil reserve (5).

The increment of magnesium in the soil at the end of this experiment, although in low quantities, is important, because of this element is essential for the chlorophyll production (4). This raise of magnesium could be due to the activity of the microorganisms of the soil in the NS treatments and to the activity of *P. lilacinum* in the ASF treatment (11).

The degrading action of the soil biota contribute to the availability of cations, like magnesium when it mineralizes organic matter and dissolves the inorganic sources like the dolomite, hornblende and serpentine (5), however it is a subject of study to know if it is related to the activity of *P. lilacinum*.

Moreover, the concentration of active aluminum is presented in tolerable levels of plants (<2 cmol kg<sup>-1</sup>), despite of the acidness of the soil of all treatments (pH = <5.5) (28).

The less quantity of organic C and N of the soil, presented in the treatments with *P. lilacinum* regarding the controls, could point that the fungus used this elements for its own growth.

The organic matter in the soil represents the principal energetic resource for the fungus (3, 12) and it is important for the production of organic acids by the phosphates solubilizing organisms (20).

Even though the relation of C/N of the soil was the right one (between 8 and 15) to allow the release of nitrogen through the degradation of the organic matter, the content of organic carbon was low, this could be a limiting factor on microorganisms development (5, 31).

Low nutrients content of plants obtained is a consequence of the soil nutrients deficiencies.

The high of the oat plants, fresh and dry weigh, and the content of P on vegetal tissue, in this experiments, are variables that do not indicate the activity *P. lilacinum* like solubilizer of phosphates, coinciding with Bashan *et al.* (2013).

However, the highest number and weight of spikelets per plant were the variables that indicate in a indirect way, the positive effect of *P. lilacinum* application in the soil, because the plants may have absorbed a greater quantity of P, nutrient related to the production of seed (18, 33). This increment in the production of seeds has being obtained when applied *Aspergillus brasiliensis* Varga, Frisvad & Samson, *Penicillium citrinum* Thom and *Cladosporium herbarum* (Pers.) Link in different plants (32, 38, 43).

Phosphorus uptake by plants is influenced and maximized by the soil microbiota. Promoting microbial activity by the addition of organic amendments to agricultural soils, significantly influences the availability and absorption of this nutrient (36, 39). The low content of organic matter in the soil used in this experiment (<4%), could negatively influence the establishment of *P. lilacinum* and subsequent solubilization activity.

We hypothesized that availability of P, Ca y Fe measured by nutrients amount of the soil and the oats plants, would indicate solubilizing capacity of *P. lilacinum*, as well as higher data in its vegetative variables. Contrary to our predictions only the number of spikelets plant<sup>-1</sup> and spikelets weight were indicators that the plants

had a greater availability of phosphorus. An important point in the field is the establishment of organisms applied to the crop soil to obtain the benefits expected in the yield. However, there are many factors that influence for conditioning the success of biological agents.

## CONCLUSION

The application of *P. lilacinum* has not produced a clear effect on the soil and plants nutrient content. However, according to our results, the fungus could have favored the better absorption of P, which was reflected on yield by stimulating the seeds formation.

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