

## *Pseudomonas* of the rhizosphere of avocado (*Persea americana* Mill.) with biocontrol activity of *Phytophthora cinnamomi* Rands isolated in the central coast of Peru

### *Pseudomonas* de la rizosfera del Palto (*Persea americana* Mill.) con actividad de biocontrol de *Phytophthora cinnamomi* Rands aisladas en la costa central del Perú

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#### Abstract

In the rhizosphere, as the zone of biological activity, diversity of microorganisms can be found like bacteria of the genus *Pseudomonas*. They are characterized for controlling pathogens like *Phytophthora cinnamomi*, also to be promoters of growth. For this reason, in the present study, bacteria of the genus *Pseudomonas* were isolated from the avocado rhizosphere of the provinces of Casma, Huaral, and Lima. A total of six strains (R2, R5, R7, R10, S10 and S6) were selected for evaluating their biocontrol capacity against *P. cinnamomi* under in vitro and greenhouse conditions. In the in vitro test, strains S6 and S10 controlled 30.3 and 44 %, respectively. Under greenhouse conditions, *Pseudomonas* strains were inoculated on 4-month-old avocado cv. "Zutano" plants. Five months later, we evaluated the following variables: severity in roots, height increase, fresh root and leaf weight, and percentage of root and leaf dry matter. In greenhouse, the best strains in the control of *P. cinnamomi* were S6, R2, R7 and R10, controlling 55.2, 39.5, 33.7 and 31.0 %, respectively. In the increase of height, the strains S6, R2, R7 and R10 reached 11.4, 9.3, 7.6 and 5.1 cm, respectively. The percentage of dry matter of roots, strains S10, R10 and R7 obtained 29.6, 27.5 and 27.9 %, respectively. In this study, it was observed that although the application of *Pseudomonas* controls *P. cinnamomi*, it also induces the root and apical growth of avocado.

**Keywords:** avocado, promoter of growth, *Pseudomonas*, rhizosphere.

#### Resumen

En la rizósfera como zona de actividad biológica, se pueden encontrar diversidad de microorganismos como bacterias del género *Pseudomonas* que se caracterizan por controlar patógenos como *Phytophthora cinnamomi*, también por ser promotores de crecimiento (PGPR). Por esta razón en la presente investigación se aislaron bacterias del género *Pseudomonas* de la rizósfera de palto de las provincias de Lima, Huaral y Casma. Se seleccionaron 6 cepas (R2, R5, R7, R10, S10 y S6) con las que se realizaron pruebas para evaluar su capacidad biocontroladora frente a *P. cinnamomi* in vitro e invernadero. En la prueba *in vitro* las cepas S6 y S10 controlaron un 30.3 y 44 %; respectivamente. En condiciones de invernadero, se inocularon cepas de *Pseudomonas* en plántones de palto cv. Zutano de 4 meses de edad; transcurridos cinco meses, se evaluó variables como severidad en raíces, incremento de altura, peso fresco radicular y foliar y porcentaje de materia seca radicular y foliar. En invernadero, las mejores cepas en el control de *P. cinnamomi* fueron S6, R2, R7 y R10 que controlaron un 55.2, 39.5, 33.7 y 31.0 %; respectivamente. En el incremento de altura, las cepas S6, R2, R7 y R10 alcanzaron 11.4, 9.3, 7.6 y 5.1cm; respectivamente. El porcentaje de materia seca de raíces, las cepas S10, R10 y R7 obtuvieron 29.6, 27.5 y 27.9 %; respectivamente. En este estudio se observó que si bien la aplicación de *Pseudomonas* ejerce un control sobre *P. cinnamomi* también induce el crecimiento radicular y apical de la planta.

**Palabras Claves:** palto, PGPR, *Pseudomonas*, rizósfera.

#### Introduction

Peru has an avocado producing area of around 30,320 hectares with a total production of 349,317 tons. The coastal departments of Ancash, Ica, La Libertad, and Lima have the biggest cultivated area. (MINAGRI, 2014).

Peru is the second exporter of avocado worldwide, with 175.6 million kilos exported (Arteaga, 2016). Therefore, avocado production in Peru will continue to be an attractive business due to the opening of new markets and new consumers who value their nutritional properties.

The production of the avocado depends on climatic, edaphic, nutritional and sanitary factors. Within the sanitary aspect, diseases are one of the factors that augment the production costs of the fruit. Among the most important, is "root rot" caused by *Phytophthora cinnamomi* (Chromista, Heterokontophyta). This pathogen limits tree development, reduces fruit production and quality, directly affecting profitability.

Currently, this oomycete is controlled by chemical products, such as fosetil-Al and metalaxyl (Mora, 2007),

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but the constant use of chemical products diminishes its effectiveness over time due to the resistance that this phytopathogen develops towards the fungicide. For these reasons, biological control is considered to take advantage of soil microorganisms with antifungal activity, including bacteria of the genus *Pseudomonas*, responsible for the suppression of some soil pathogens (Raaijmakers *et al.*, 2002).

Bacteria of the genus *Pseudomonas* have been widely studied as biological controllers for their ability to colonize the root, compete aggressively with other microorganisms, adapt to different situations of environmental stress, synthesize antibiotics and enzymes, and activate systemic resistance in plants (Weller *et al.*, 2006).

For this reason, in the present work, we evaluated under in vitro conditions the antagonistic effect of strains of *Pseudomonas* spp. isolated from the rhizosphere of avocado on *P. cinnamomi* by scoring the progress of the pathogen in centimeters. In addition, in the greenhouse; the severity of the infection of *P. cinnamomi* in avocado seedlings previously inoculated with the strains of *Pseudomonas* spp. was measured by the percentage of lesions in roots, and the effect of these strains on the growth of the avocado seedlings was measured by the increase of height of the stem in centimeters.

## Materials and methods

**Field work.** The sampling was carried out in commercial avocado fields that reported root rot problems in Casma, Huaral and Lima. In each field, 10 avocado trees were randomly selected. Roots were collected from each tree with a volume of rhizospheric soil from four cardinal points, with a total equivalent of 250g of soil. These samples were transported and kept in cold conditions by using gel packs to maintain their temperature and humidity.

**Laboratory work.** The laboratory work (in vitro isolation) was carried out in the facilities of the Laboratory of the Department of Phytopathology of the National Agrarian University of La Molina (UNALM for its acronym in Spanish), located in the district of La Molina, Province and Department of Lima, at 12° 05' 06" S of latitude, 76° 57' 07" WG of longitude and 243.7 m.a.s.l.

**Sample Treatment.** The microflora of both the rhizosphere and the soil was analyzed separately. Under aseptic conditions, 3 g of root of each sample was placed in test tubes with 9 mL of peptonated water in continuous agitation, performing 7 serial dilutions ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$  and  $10^{-7}$ ), adapted from Andres (1991). For the soil sample near the root, 10 g were placed in 90 mL of peptone water (ratio 1: 9), following the same previous procedure.

Count of *Pseudomonas* spp. In regard to the quantification of *Pseudomonas* spp., 1 mL of the dilution ( $10^{-2}$  to  $10^{-5}$ ) was seeded in triplicate in tubes containing 9 mL of asparagine broth. These tubes were incubated for 36

four days at 37°C (Burgess, 1960). This procedure was performed for both root and soil.

**Isolation and identification of *Pseudomonas* spp.** The tubes that were positive (fluorescent) to the asparagine broth culture were striated in the King-B culture medium and incubated for 48 hours, and fluorescence was evaluated in ultraviolet light at 366 nm (Schaad, 2001) together with the morphology of the colonies (Palleroni, 2005). The colonies that were positive for fluorescence were then selected.

From each isolate, a colony was selected, which was striated in the Tryptone Soy Agar (TSA) culture medium to ensure the purity of each isolate and to be stored in TSA agar wedges at 4°C for the next tests (Martínez, 2010).

## Pathogen isolation (*Phytophthora cinnamomi* Rands).

Rootlets of 1-3 millimeters in diameter that presented the typical symptomatology corresponding to a black and firm rot that originated from the zone of elongation were selected. They were washed with distilled water and segments of about 1 cm long that presented the area of pathogen progress were cut. Then, they were immersed for a few seconds in a 70% alcohol solution in order to prevent possible contamination (Zentmyer, 1980).

These pieces of 1 to 2 mm<sup>2</sup> of diseased and asymptomatic tissue were seeded in selective culture medium CMA (Corn Meal Agar) with PARB (Pimaricin-Ampicillin-Rifampicin-Benomyl) (Erwin & Ribeiro, 1996). They were incubated at 22°C for seven days, and the growth rate of the colony and its characteristics were observed (Alvarado-Rosales *et al.*, 2007).

Each pure colony in CMA was transferred in V8 juice agar medium for four days at 21°C. From slices of grown mycelium in V8 juice agar that was introduced in Petri dishes containing 1% soil solution, structures of the pathogen were visualized as sporangia.

The identification of *P. cinnamomi* was conducted by following the Dichotomous Key to Taiwan Species of *Phytophthora* (Ho, 1992).

## Antagonism test against *Phytophthora cinnamomi*.

From each previous isolate, the strains that controlled *Phytophthora cinnamomi* were identified. These strains were used in the challenge test in Nutrient Agar culture medium. It was necessary to prepare culture medium in which the corresponding reagents were included because the results of this test were not separately obtained. In each plate containing medium PDA + King-B + Nutrient Agar a controller was included (*Pseudomonas* spp. strain) and a pathogen disk.

This methodology was carried out with the objective of identifying strains of *Pseudomonas* spp. with greater control over *P. cinnamomi*, demonstrating a higher percentage of inhibition of the radial mycelial growth of the pathogen (% I).

**Greenhouse phase.** The greenhouse phase was carried out in the Greenhouse Research Station of the Department of Phytopathology of UNALM.

**Vegetal material.** We used for four-month old plants of avocado var. 'Zutano' which were treated before to the germination (immersed in Homai fungicide solution in 5 g/l dose of product), according to the disinfection protocol of the nursery of the Fruit Trees Research Program of National Agrarian University - La Molina. The transplant to bags was made in January 2016 to be taken to the greenhouse.

**Preparation of the Substrate.** The substrate mixture consisted of 50% soil, 25% river sand and 25% organic matter (Herrera & Narrea, 2011). Then, it was sterilized at 121°C for 15 minutes. The substrate was used in black polyethylene bags measuring 8x16x2.2 cm<sup>3</sup> (5.5 l), where the sterile soil was placed. Then, we proceeded to transplant the avocado.

**Preparation, density and inoculation of the controller.** Selected strains of *Pseudomonas* were seeded in peptone water at 27°C and 150 rpm (Revolutions per Minute) until reaching a population of 6x10<sup>8</sup> (Colony Forming Units) CFU/mL (Martínez, 2010).

**Pathogen inoculation.** According to Drenth & Sendall (2001), it is recommended to use mycelium developed in wheat previously sterilized at 121°C and a pressure of 1.1 bar (15 lb/in<sup>2</sup> or 15 psi) for 30 minutes for two consecutive times.

The inoculation was done in five-month-old avocado plants. The application was made around the root system of the avocado at a depth of 5 cm (the dose of inoculum was 2.5 g of wheat with mycelial development/kg of soil) (Zentmyer & Richards, 1952). The humidity of the substrate was maintained with periodic irrigation to favor the development of the pathogen.

The mycelium of *Phytophthora cinnamomi* was inoculated ten days after the inoculation of the controller, according to Martínez (2010).

#### **Evaluated parameters.**

**Disease severity and root length.** The severity and root length were measured by processing digital photographs of avocado roots using ASSESS software (Lamari, 2002).

**Plant Height.** We used a millimeter ruler. Measurement (length of the main stem) was taken from the neck of the plant to the terminal bud at the time of inoculation of the biocontroller and at the end of the experiment. The results were expressed in centimeters.

**Fresh weight, and dry root and foliar matter.** To obtain the percentage of dry matter, firstable the roots and leaves of the plant were placed in paper bags to be weighed; then they were taken to the stove at a temperature of 70°C for 48 hours to obtain the dry weight. The weights were expressed in grams using electronic balance.

**Biochemical characterization of *Pseudomonas* spp.** The following biochemical tests were carried out, according to Palleroni (2005): (i) production of fluorescent pigments, (ii) resistance to high and low temperatures, (iii) and gelatin liquefaction, in order to characterize strains of non-pathogenic fluorescent *Pseudomonas* from the rest of species

**Fluorescence in King-B culture medium.** The culture medium of King-B is used for the detection of fluorescein, a green or blue fluorescent soluble in water. After growth of 24-48 hours at 27°C, the colonies, previously striated in King-B, were observed with an ultraviolet lamp (366 nm).

**Growth at 4 and 41°C.** Strains of *Pseudomonas* spp. were planted in tubes containing TSA culture medium, which were incubated at 4 and 41°C.

**Jellied liquefaction.** This test is used to determine the capacity of a microorganism to produce enzymes of the proteolytic type (gelatinases) that liquefy the gelatin. It is positive for *Pseudomonas aeruginosa* (pathogenic) (Mac Faddin, 1980).

The tubes containing nutrient gelatin were prepared considering three replications per treatment. The strains of *Pseudomonas* spp. were then inoculated by puncture and incubated at 37°C for 48 hours. Finally, they were placed in the refrigerator at 4°C for 2 hours. The test was positive if the inoculated medium became liquid, and the test was negative if the inoculated medium maintained its characteristics (Mac Faddin, 1980).

**Experimental design.** The statistical design used in the antagonism test against *Phytophthora cinnamomi* was a completely randomized statistical design (CRD), with ten treatments of strains isolated from *Pseudomonas* spp., four dishes per treatment and control treatment of *P. cinnamomi* pathogen (Table 1). The parameter that was evaluated in this test was the percentage of inhibition of grown radial mycelial, and the differences between the average of cultures analyzed out through the Tukey test (P = 0.05).

In greenhouse, the statistical analysis of variance (ANOVA) and Tukey test with a level of significance at 5% were conducted to identify whether or not there are significant differences between the results obtained with each evaluated strain. All statistical analyses tools were applied with minimum confidence of 95% and SAS 9.2 software (SAS, 2009).

**Table 1.** Treatments used in the present study.

Treatment	Description
T1	<i>P. cinnamomi</i> + strain of <i>Pseudomonas</i> spp. (R2)
T2	<i>P. cinnamomi</i> + strain of <i>Pseudomonas</i> spp. (R5)
T3	<i>P. cinnamomi</i> + strain of <i>Pseudomonas</i> spp. (R7)
T4	<i>P. cinnamomi</i> + strain of <i>Pseudomonas</i> spp. (R10)
T5	<i>P. cinnamomi</i> + strain of <i>Pseudomonas</i> spp. (S10)
T6	<i>P. cinnamomi</i> + strain of <i>Pseudomonas</i> spp. (S6)
T7	Control treatment: <i>P. cinnamomi</i> (Phy)
T8	Absolute control treatment (T)

**Results**

**Isolation, purification and identification of *Pseudomonas* spp.** A bacterial density of *Pseudomonas* spp. was obtained from the sampled areas which varied between 10<sup>5</sup> and 10<sup>8</sup> NMP/g of roots and dry soil. In Table 2, it can be observed that the Casma area presented the highest levels of bacterial density because the sampled farm promotes the microbial flora with applications of organic matter. We worked with Casma strains because they had a higher inoculum potential compared to the other areas sampled.

**Table 2.** Count of *Pseudomonas* spp. (NMP/g) isolated from the central coast of Peru.

Location		NMP/g
Lima	Soil	2.3x10 <sup>6</sup> organisms /g of soil
	Root	2.3x10 <sup>6</sup> organisms/g of root
Huaral	Soil	9.3x10 <sup>5</sup> organisms /g of soil
	Root	4.3x10 <sup>5</sup> organisms/g of root
Casma	Soil	>1.1x10 <sup>8</sup> organisms /g of soil
	Root	>1.1x10 <sup>8</sup> organisms/g of root

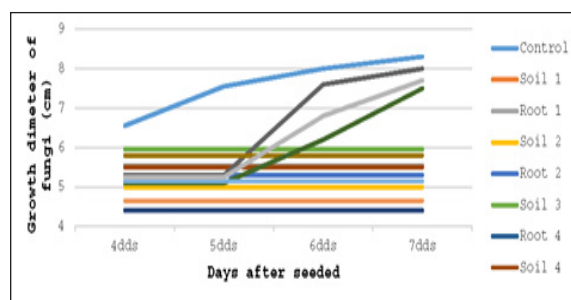
The isolates that grew and emitted fluorescence in asparagine broth were striated in King-B culture medium (also called *Pseudomonas* agar F) for the isolation of all *Pseudomonas* spp. (Schaad, 2001), thus differentiating them from other gram-negative microorganisms that do not emit fluorescence. A total of 14 positive isolates were obtained for this test.

**Antagonic effect in vitro test.** Figure 1 shows that the isolates from soil S7, S10, S2, S9, S4, S1, S6 and S3 inhibit the advance of *P. cinnamomi*; being constant in time, while in isolations from roots (R7, R10, R4 and R5) there is a strong inhibition at 4 DAS (days after sowing), but it is lost quickly over time.

All the evaluated strains reported some control varying the percentages of inhibition between 28.3 to 47% (Figure 2).

The isolates R2, R5, R7, R10, S6 and S10 showed growths above the mycelium of *P. cinnamomi*, being reduced to a yellow gelatinous mass. From there, samples were taken to be observed in a microscope. In isolates from the confrontation *P. cinnamomi* and *Pseudomonas*

spp. fluorescence observed in a microscope (40X), the disintegration of the cytoplasmic content of the mycelium of *P. cinnamomi* was observed.



**Fig. 1.** Advance of *P. cinnamomi* (in centimeters) against soil and root isolates of *Pseudomonas* spp. for 7 days after seed in vitro test.

In the biochemical characterization tests, according to the Bergey Manual (Palleroni, 2005), carried out on strains of *Pseudomonas* spp. selected (R2, R5, R7, R10, S6 and S10) the results were obtained (Table 3) in which it is determined that the strains evaluated do not correspond to *Pseudomonas aeruginosa* (pathogenic to man).

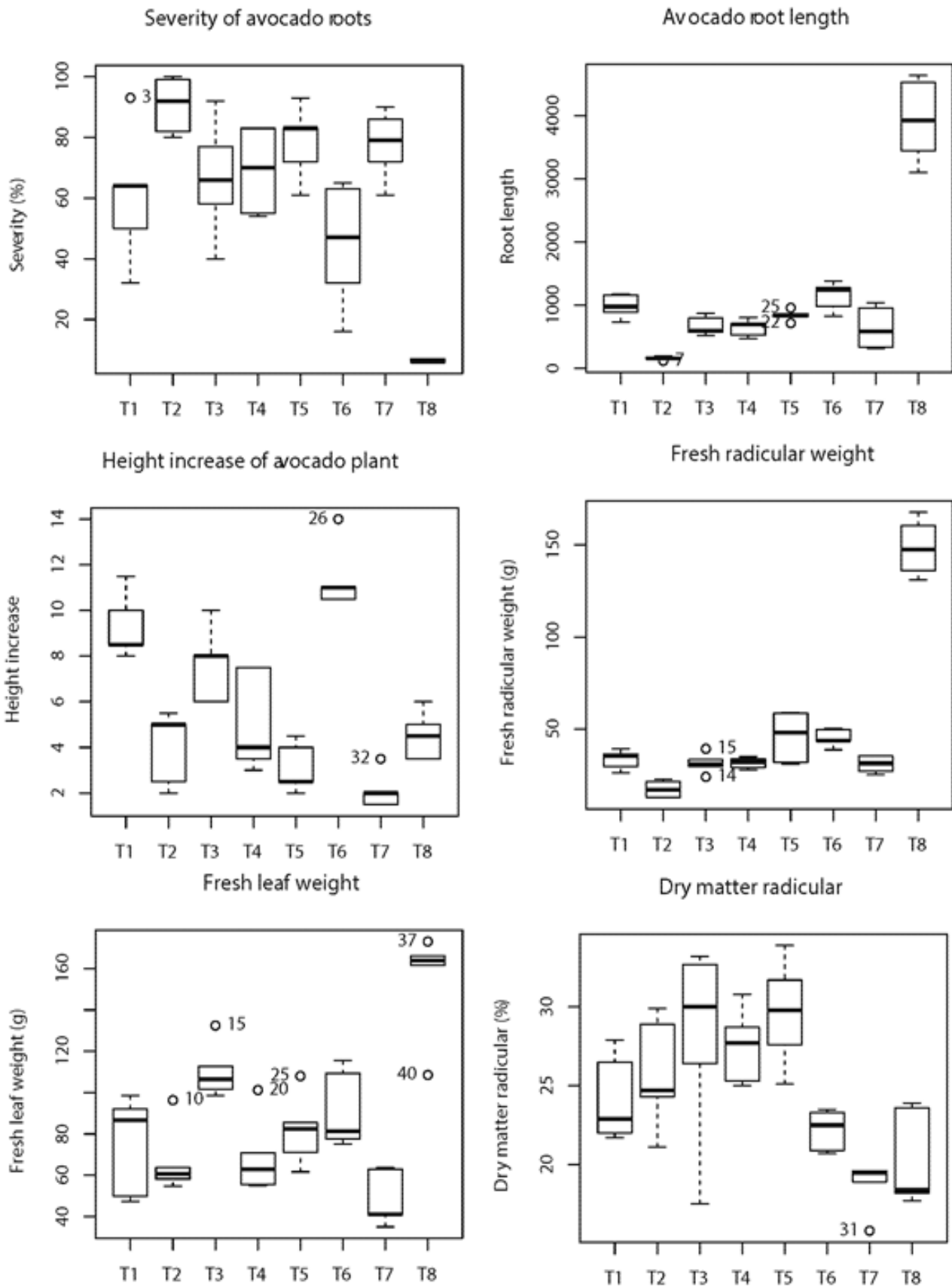
**Table 3.** Reaction of different strains of *Pseudomonas* spp. to the biochemical tests.

	Fluorescent pigments	Grows at 4°C	Grows at 41°C	Gelatin liquefaction
<i>P. fluorescens</i>	+	+	-	+/-
<i>P. putida</i>	+	+	-	-
<i>P. aeruginosa</i>	+	-	+	+
Strain R2	+	+	-	-
Strain R5	+	+	-	-
Strain R7	+	+	-	-
Strain R10	+	+	-	-
Strain S10	+	+	-	-
Strain S6	+	+	-	-

**Greenhouse evaluation.**

**Evaluation of the severity of avocado roots.** Treatments R5, S10, R10, R7 and R2 (60.4, 66.2, 69.0, 77.5, 78.4 and 90.7%, respectively) show percentage of severity that is not significantly different from the treatment inoculated with the pathogen. On the other hand, the S6 treatment has a significant difference with the control treatment and the treatment inoculated with *P. cinnamomi* with only 44.8% of severity in avocado roots (Figs. 2 and 3, Table 4).

Figure 2, strains R5 and S10 showed severity values (90.7 and 78.4%, respectively) above the control inoculated with *P. cinnamomi* (77.4%), this loss of antagonistic capacity could be due to population decline of these strains (Van et al., 1997).



**Fig. 2.** Measurement of severity of avocado roots (%), avocado root length, height increase of avocado plant (cm), fresh radicular weight (g), fresh leaf weight (g) and dry matter radicular (%) of treatments inoculated with strains *Pseudomonas* spp. against *P. cinnamomi*, under greenhouse conditions.

It is also observed that the best treatment in the control of *P. cinnamomi* in greenhouse was T6 (inoculated with the S6 strain of *Pseudomonas* spp.) which showed 59.0% of control.

**Table 4.** Measurement of avocado variables with the comparison test of means (Tukey) of treatments inoculated with strains *Pseudomonas* spp. against *P. cinnamomi*, under greenhouse conditions.

Treatments	Strains	Severitya (%)	Increase in heightb (cm)	Foliar biomassc (g)	Root lengthd (cm)	Biomass of rootse (g)	Root dry matterf (%)
T1	R2	60.462 AB	9.3 AB	74.86 BCD	989.1 B	33.320 BC	24.200 ABCD
T2	R5	90.720 A	4.0 D	66.70 CD	160.6 C	17.340 C	25.780 ABC
T3	R7	66.274 AB	7.6 BC	110.38 B	672.2 BC	31.500 BC	27.960 AB
T4	R10	69.034 AB	5.1 CD	69.04 CD	640.5 BC	31.500 BC	27.500 AB
T5	S10	78.456 A	3.1 D	81.68 BCD	841.1 B	45.680 B	29.620 A
T6	S6	44.848 B	11.4 A	91.76 BC	1142.2 B	45.220 B	22.180 BCD
T7	Phy	77.466 A	2.1 D	48.68 D	648.1 BC	30.880 BC	18.680 D
T8	T	6.578 C	4.5 D	154.68 A	3924.6 A	148.640 A	20.360 CD

Coefficient of variation (alpha 0.05): 24.86<sup>a</sup>, 25.17<sup>b</sup>, 22.07<sup>c</sup>, 26.17<sup>d</sup>, 17.55<sup>e</sup>, 13.98<sup>f</sup>.

**Avocado root length evaluation.** The effect of *Pseudomonas* sp. on the length of avocado roots showed a significant difference between treatments. In all treatments, shorter root length was observed compared to the control treatment (Figs. 2 and 3, Table 4). Therefore, inoculated *Pseudomonas* strains influenced root growth but did not achieve significant difference with respect to the control.

Treatments S6, R2 and S10 presented longer root lengths (1142.2, 841.1 and 989.1, respectively) than the control inoculated with the pathogen (648.1).

**Evaluation of the height increase of avocado plant.**

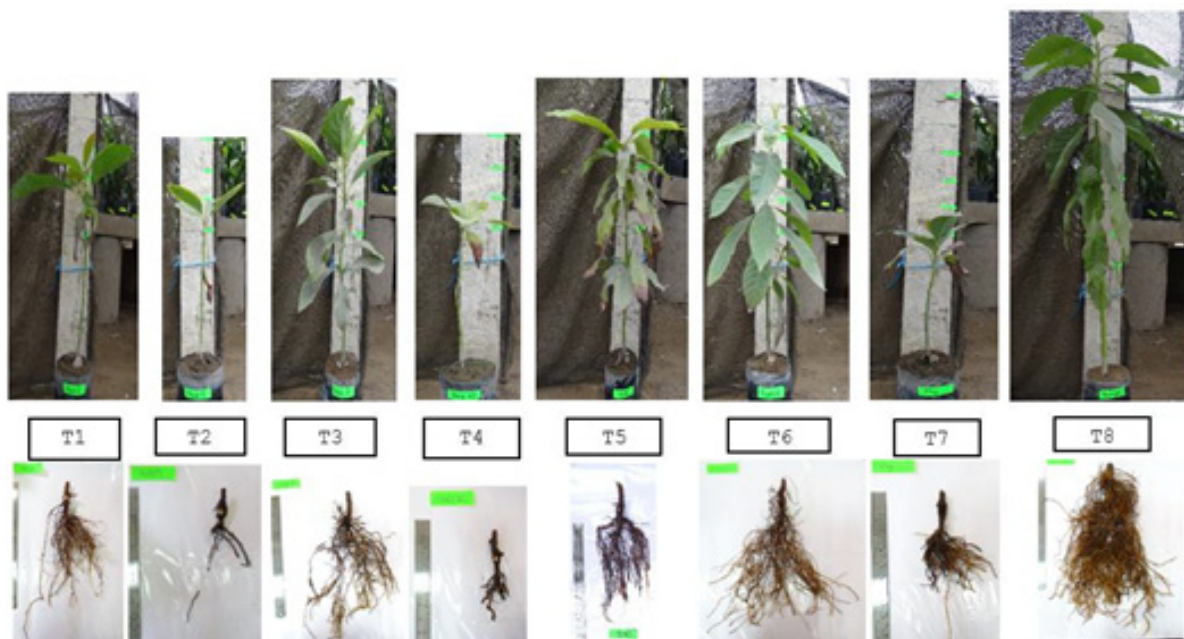
Inoculation of strains S6 and R2 showed a greater increase in height, 11.4 and 9.3 cm respectively, compared to the control treatment (Fig. 2). There is no significant difference between treatments S6 and R2, according to the Tukey test at 95% (Table 4). Figures 2 and 3 show all treatments with inoculation of *Pseudomonas* spp. have values that are above the control inoculated with *P. cinnamomi* (2.1 cm). The treatments inoculated with strains of *Pseudomonas* spp. present greater amplitude of the interval of variance of their repetitions.

**Evaluation of fresh radicular weight.**

Regarding fresh radicular weight, treatments S10 and S6 (45.6 and 45.2 g) show a significant difference compared to the treatment inoculated with *P. cinnamomi* (30.8 g), but lower than the absolute control treatment (148.6 g), as observed in Figs. 2 and 3. For this variable, the repetitions of each treatment were close values, thus a shorter interval of variance.

**Evaluation of the dry matter radicular.**

On the percentage of dry matter of roots, there is a significant difference between the treatments. Treatment S10 with 29.0% of dry matter of roots is the best compared to the control inoculated with *P. cinnamomi* (18.6%).



**Fig. 3.** Photograph of foliar and root area of avocado cv. ‘Zutano’, six months after inoculated with soil and root isolates of *Pseudomonas* sp. and *P. cinnamomi*, under greenhouse conditions.

**Evaluation of fresh leaf weight.** Treatments R7 and S6 (110.4 and 91.8 g) show a significant difference compared to the treatment inoculated with *P. cinnamomi* (48.7 g), but lower than the absolute control treatment (156.6 g), as observed in Figs. 2 and 3.

**Dry leaf matter.** Figure 2 shows there is no significant difference among treatments, and that the repetitions of treatments T2 (R5), T3 (R7) and T5 (S10) present higher values than the absolute control (T).

## Discussion

This study shows the inhibition of *Phytophthora cinnamomi* against *Pseudomonas* in vitro and greenhouse. Villa *et al.* (2005) found that strains of *Pseudomonas* sp. inhibited the growth of *Sclerotium rolfsii* between 60 and 90%. Other studies also report that *Pseudomonas cepacia* and *P. fluorescens* were significant for suppressing *P. cinnamomi* that grew in vitro and in vivo (Yang *et al.*, 2001).

The coagulation of the cytoplasm was observed; this same partial degradation or coagulation of the cytoplasmic content was observed in cultures of *Phytophthora capsici* with *Pseudomonas fluorescens* in which Diby *et al.* (2005) attributes it to hydrolytic enzymes. Broadbent & Baker (1974) also demonstrated that *P. putida* and *P. fluorescens* isolated from avocado soils caused massive mycelia lysis of *P. cinnamomi* in vitro.

The strains of isolated *Pseudomonas* were not pathogenic for humans according to Rodríguez *et al.* (2005) who demonstrated that to isolate *Pseudomonas aeruginosa* (opportunistic pathogen in humans) the bacteria should be cultured in a gelatin culture medium, thus demonstrating the presence of gelatinases. The promising strains of *Pseudomonas* gave a negative result to this test. Therefore, they are not pathogenic to man. *Pseudomonas putida* and *Pseudomonas fluorescens* are unable to grow at 42°C and do not produce pyocyanin unlike *P. aeruginosa*, corroborated by the UK Standards for Microbiology Investigations (2015).

Several possible mechanisms have been described by which soil *Pseudomonas* suppresses conditions related to pathogenicity. De la Fuente *et al.* (2000) identified three native strains of fluorescent *Pseudomonas* (UP61, UP143 and UP148) producing HCN, fluorescent siderophores such as pyoverdine proteases and antibiotics [2, 4-diacetylchloroglucinol (DAPG), PLT (piolterine), PRN (pyrrolnitrine) and phenazine derivatives] with antifungal activity (Julisch *et al.*, 2001). Thomashow and Weller (1996) also described the production of antibiotics by fluorescent *Pseudomonas*.

Therefore, the control effect of treatment T6 (S6) could be explained by mechanisms such as competition for iron, competition for colonization sites and nutrients exuded from the root, as well as the induction of plant defense mechanisms (Van Weels *et al.* 1997). Another mechanism of control is the production of extracellular enzymes, such

as chitinases, laminases and glucanases that can degrade the walls of fungal cells. Van Weels *et al.* (1997) isolated a strain of *Pseudomonas stutzeri* that produced extracellular chitinase and laminase, and found that these enzymes digest and lyse the mycelium of *Fusarium solani*.

Marques *et al.* (2010) who mentioned that the production of IAA by PGPR generally causes the elongation and accumulation of P and N in the tissues of the plant would explain the increase in root length. In the root system, it has been seen that high levels of IAA increase the formation of lateral and adventitious roots, but inhibits the growth of the primary root.

This improvement in growth was also observed in the 1970s when some fluorescent *Pseudomonas* strains improved the growth of potatoes and sugarcane when applied to the seeds (Schroth & Hancock, 1982).

The increase in height is explained because *Pseudomonas* can act as promoters of plant growth in two ways: directly by suppression of pathogens or indirectly through the secretion of phytohormones and vitamins, or by increasing the absorption of minerals per plant.

Glick (1995) proposes that *Pseudomonas* can manifest its growth by promoting effects indirectly, stimulating the beneficial actions of other microorganisms associated with the roots, such as mycorrhizae. When the stimulation of plant growth occurs in the absence of other microorganisms, it has been attributed to the increase in the availability of mineral nutrients, such as phosphate or nitrogen, due to the production of phytohormones stimulating plant growth or to the degradation of ethylene precursors in the root by these bacteria.

Faggioli *et al.* (2007) also observed that the inoculation of corn plants with PGPR does not significantly influence the height but the percentage of dry matter (Fig. 2).

## Conclusions

The soil sampled from Casma area had a higher quantity of *Pseudomonas* spp. organisms. The *Pseudomonas* spp. isolated from soil showed greater controlling effect on *Phytophthora cinnamomi* compared to isolates from roots, evaluated in vitro. The strain of *Pseudomonas* spp. S6 (T6) showed greater control of *P. cinnamomi* in the greenhouse and the strain of *Pseudomonas* spp. S10 (T5) showed greater root system development in the greenhouse. It was ruled out that all *Pseudomonas* spp. inoculated were pathogenic to humans (*Pseudomonas aeruginosa*).

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