

Etiology and control of *Gypsophila paniculata* L. stem base rot in the Caraz Valley, Ancash

Etiología y control de la pudrición del cuello en el cultivo de *Gypsophila paniculata* L. en el valle de Caraz, Ancash

Aguilar-Ancocota, R.^{(1)*}; Mattos-Calderon, L.⁽¹⁾

*Corresponding author: raancocota@hotmail.com
<https://orcid.org/0000-0002-3965-6096>

Abstract

Gypsophila is an ornamental plant whose flowers are economically important, which is cultivated in the Callejón de Huaylas valley-Ancash. Recently, cultivated *Gypsophila* fields have shown diseased plants characterized by stem base rot, which has been followed by a reduction in vigor and the collapse and death of plants. Therefore, the objectives of this research were to describe the symptomatology of the disease, identify the causative agent of the disease, and prove how effective fungicides and biological control agents (BCA) are in controlling the disease using *in vitro* and field experiments. To isolate the pathogen, symptomatic plant tissue samples were washed, cut into small pieces, disinfected in 1% sodium hypochlorite solution for 1 min, rinsed twice with sterilized water, and air-dried on paper towels. The samples were seeded on Petri dishes containing potato dextrose agar media and incubated at 25 °C. A pathogenicity test was conducted in healthy *Gypsophila* seedlings, which were grown in a sterilized substrate, using mechanical inoculation on the stem base and agar disks colonized by the pathogen-mycelium. Then the pathogen was reisolated from symptomatic inoculated *Gypsophila* seedlings. The “poisoned medium” technique was used to conduct the *in vitro* fungicide test, while the “dual method” was used to conduct the bio controller’s test. The results of the pathogenicity test and *in vitro* and field experiments showed that *Rhizoctonia solani* is the causative agent of the stem base *Gypsophila* disease, and at both assayed doses, the fungicides Rovral, Benopoint, Parachupadera, Vitavax, and Homai completely inhibited the mycelial growth of *R. solani*. Moreover, the BCAs *Trichoderma harzianum* and *T. viride* showed higher *in vitro* growth rates than *R. solani* and completely colonized the pathogen-mycelium. Under field conditions, the incidence of the disease in field plots treated with *T. harzianum* was 12.5% lower than in the control treatment, which showed 51.28% incidence of the disease. In addition, *Gypsophila* plants harvested from plots treated with *T. harzianum* exhibited higher numbers of flower stalks per plant and a higher fresh weight compared to the control treatment.

Keywords: *Gypsophila*, stem base rot, *Rhizoctonia solani*, *Trichoderma* spp.

Resumen

Gypsophila paniculata L. es una planta ornamental cuyas flores son de importancia económica en el valle del callejón de Huaylas-Ancash, en los últimos años, se ha presentado una enfermedad caracterizada por la presencia de pudrición en el cuello de planta, seguida de pérdida de vigor, colapso y muerte. Por lo tanto, los objetivos de esta investigación fueron: describir la sintomatología, identificar al agente causal de la enfermedad y probar la efectividad de control de fungicidas y antagonistas biológicos *in vitro* y en campo. Para aislar el patógeno, muestras sintomáticas fueron lavadas, y cortadas en porciones pequeñas de la zona infectada, luego se sumergieron en hipoclorito de sodio al 1% por un minuto, se enjuagaron por dos veces en agua esterilizada y se orearon sobre papel toalla; luego los tejidos fueron sembrados en placas de Petri con PDA e incubadas a 25°C. Para las pruebas de patogenicidad se inocularon a plántulas sanas de *Gypsophila* crecidas en sustrato estéril, utilizando discos de agar con micelio del hongo dirigidas al cuello de la cada plántula. De las plántulas que mostraron síntomas de decaimiento y marchitez, se realizó el reaislamiento del patógeno. Para el ensayo de fungicidas *in vitro* se empleó el “medio envenenado”, y para la prueba de biocontroladores el “método dual”. El ensayo de patogenicidad nos permitió determinar que el agente causal es *Rhizoctonia solani*, mientras que los ensayos *in vitro* de fungicidas y biocontroladores demostró que los fungicidas Rovral, Benopoint, Vitavax, Parachupadera y Homai a dosis baja y alta *in vitro* inhibieron al 100% el crecimiento micelial de *R. solani*, y los biocontroladores *Trichoderma harzianum* y *T. viride* alcanzaron mayor velocidad de crecimiento frente a *R. solani*, observándose invasión total de la colonia y esporulación, alcanzando grado 4 de antagonismo. En campo, la menor incidencia de la enfermedad se obtuvo con el tratamiento *T. harzianum* registrándose 12.5% inferior al testigo que alcanzó 51.28%. Asimismo, las plantas tratadas con *T. harzianum* alcanzaron mayor peso fresco y mayor número de tallos florales por planta.

Palabras clave: *Gypsophila*, pudrición del cuello, *Rhizoctonia solani* y *Trichoderma* spp.

¹Universidad Nacional Agraria La Molina. Lima, Perú.

Introduction

Gypsophila paniculata L. is an ornamental plant whose flowers are in high demand in the markets, generates economic income for Peru. Therefore, it is a crop of substantial importance to the producers in the Callejón de Huaylas valley in the Department of Ancash. Nationally, 80% of all flowers are grown in the Province of Caraz to supply flowers for Mother's Day, day of the dead, and Valentine's Day, which are the dates of highest demand. The Roots Perú Company S.A. Enterprise has been growing 100 ha of flowers, including *Gypsophila*, *Liatris*, and *Helianthus*. Each year, the company exports more than two million bouquets to the United States, Europe, Canada, Panama, and Costa Rica, with a single flower stem costing \$ 2–3. In the Callejón de Huaylas valley, the death of *Gypsophila* plants has been occurring due to the presence of rot in the neck of the plant, with dry, brown lesions. In addition, in the aerial part of the plant, the basal leaves sag, and there is a loss of vigor, generalized wilt, and collapse. As a consequence, the plants die, and there are economic losses. Given the importance of neck rot, this research was conducted to identify the causative agent of the disease and test the efficacy of fungicides and biocontrollers in *in vitro* and field conditions. The results of the study will contribute to solving the phytopathological problems of the floriculturists in the valley.

Materials and Methods

The research was conducted in the Phytopathology Laboratory at the National Agrarian University of La Molina (UNALM) and in the fields of the Roots Perú Company, located in the Callejón de Huaylas valley, Province of Caraz, Department of Ancash, at an altitude of 2400 m a.s.l.

Isolation and identification of pathogens

Whole *Gypsophila* plants, with root, stem, and leaves and symptoms of decay and generalized wilt, were collected from fields in production of the Roots Perú Company. Nine samples were placed in brown Kraft paper bags and taken to the Phytopathology Laboratory for processing. The samples were washed with distilled water and cut into small pieces using a sterile scalpel, taking portions of infected and healthy tissue of 0.5 × 0.5 cm in size. These samples were subsequently disinfected with 1% NaClO for 1 min and then rinsed twice with distilled water and finally placed on a sterile paper towel for drying. The pieces of these samples were seeded in Petri dishes with potato dextrose agar (PDA) medium and 50 mg of streptomycin. The seeded dishes were incubated at 25 °C for 48 h. The isolates obtained were then purified on PDA and cornmeal agar media. The pure isolates were seeded in test tubes with nutrient medium and then incubated at 5 °C for preservation (French & Hebert, 1980). Then the phytopathogens were identified using the keys proposed by

Sneh *et al.* (1991), Barnett and Hunter (1998) and Erwin and Ribeiro (1996).

Pathogenicity test

Seedlings of *G. paniculata* were grown in a sterile substrate of agricultural soil and humus in a 1: 2 ratio. The inoculation of the isolated fungi was performed seven days after transplantation. Agar disks with mycelium were placed on the neck of the plant at 2–3 cm below the surface, and irrigation was conducted every two days in each of the pots containing the inoculated *G. paniculata* seedlings. Four seedlings were inoculated by isolation. The pathogen was then reisolated from the seedlings that showed symptoms of decay, wilt, and the presence of rot in the neck.

Test and evaluation of fungicides in *in vitro* conditions

The *in vitro* efficacy of each fungicide was determined using the “poisoned medium” technique, for which PDA was prepared in an Erlenmeyer's flask. Fungicide was added to each Erlenmeyer's flask containing the medium, and then it was homogenized before pouring 20 ml of the mixture into sterile Petri dishes and allowing it to solidify (French & Hebert, 1980). The fungus was isolated from the growth margin of a 10-day incubation culture. Using a sterile punch, 0.8 cm disks containing the fungus were cut and placed in the center of each Petri dish, placing the mycelium in contact with the culture medium. In the case of the control, the Petri dishes only contained culture medium without fungicide. The seeded Petri dishes were incubated at 25 °C. The trial had 15 treatments, including the control with four repetitions in a complete randomized design (Table 1).

For the evaluations, the base of each Petri dish was divided into four parts by drawing two perpendicular lines. The letters a, b, c, and d were written at each end of the lines, and daily measurements of the diameter (cm) of the mycelial growth of the fungus were made. The evaluations culminated when the control covered the entire surface of the medium. The efficacy of the fungicides was determined by calculating the percentage of inhibition in the mycelial growth of the colony using the **formula**

$$\text{Inhibition (\%)} = (C - T / C) \times 100$$

Where:

T: mycelial growth (cm) in each treatment

C: mycelial growth in the control

A completely randomized design (DCA) was applied with 17 treatments and four repetitions, and an analysis of variance (ANOVA) and a multiple means comparisons Tukey's test at P<0.05 were performed on the data.

Table 1. Fungicides used on the efficacy essays against *Rhizoctonia solani* *in vitro* conditions and in the field.

N° of treatments	Commercial Name and Formulation	Active Ingredient	Commercial dose kg / 200 L of water
1	Rovral 50% WP	Iprodione	0.3
2	Kaptan 83 WP	Captan	0.3
3	Manzate 200 WP	Mancozeb	0.3
4	Benopoint 50 WP	Benomil	0.3
5	Vitavax 300 PM	Carboxin + Captan	0.2
6	Parachupadera PM	Captan + Flutolanil	0.4
7	Homai WP	Tiofanate + Thiram	0.3
8	Rovral * 50% WP	Iprodione	0.5
9	Kaptan* 83 WP	Captan	0.5
10	Manzate* 200 WP	Mancozeb	0.5
11	Benopoint* 50 WP	Benomil	0.5
12	Vitavax* 300 PM	Carboxin + Captan	0.4
13	Parachupadera* PM	Captan + Flutolanil	0.6
14	Homai* WP	Tiofanate + Thiram	0.5
15	Control	---	Water

* = High dose of fungicides, WP = Wettable powder

Test and evaluation of the efficacy of *Trichoderma* spp. *in vitro*

The efficacy of the biocontrollers *Trichoderma harzianum* and *T. viride* was evaluated. These fungi were provided by the Mycothec fungal collection of the Diagnosis Clinic of the UNALM. To evaluate the effect of the antagonists, the “Dual Method” was used [Correa et al. \(2007\)](#), which consisted of sowing 0.8 cm disks of PDA medium at two opposite points in the same Petri dish with mycelial development of *Trichoderma* spp. and *R. solani*. The seeded Petri dishes were incubated at 25 °C, and the degree of antagonism was observed every 24 h, according to the scale proposed by [Ezziymani et al. \(2004\)](#) (Table 2).

Table 2. Evaluation scale of *in vitro* antagonism, taking into account the invasion, colonization and sporulation of *Trichoderma* spp. on the colony of the pathogenic fungus *Rhizoctonia solani*.

Grade	Antagonism (Characteristics)
0	No invasion of <i>Trichoderma</i> spp. on the surface of the colony of the pathogenic fungus.
1	Invasion of <i>Trichoderma</i> spp. ¼ of the colony surface of the pathogenic fungus.
2	Invasion of <i>Trichoderma</i> spp. ½ of the colony surface of the pathogenic fungus.
3	Total invasion of <i>Trichoderma</i> spp. on the surface of the colony of the pathogenic fungus.
4	Total invasion of <i>Trichoderma</i> spp. on the surface of the colony of the pathogenic fungus and sporulation on it.

Effect of fungicides and *Trichoderma* spp. on *Rhizoctonia solani* in the field

In the study site at the Roots Perú Company, fungicides that inhibited the growth of the pathogen were used for the field test. Seven fungicides were evaluated in low and high doses (Table 1), and applications were made every 15 days by spraying with a manual backpack with a 12 L capacity, which was directed to the neck of the plant.

As with the *in vitro* study, the fungi *Trichoderma harzianum* and *T. viride* were seeded in Petri dishes containing PDA medium. After 10 days of cultivation, disks of 0.8 cm in diameter were prepared, and then 15 disks were placed inside polypropylene bags containing 500 g of sterile peeled wheat. The bags were then incubated at 25 °C for 15 days for colonization and sporulation. Biocontroller applications were made every 15 days, totaling six applications. The concentration used was 1×10^8 CFU/g of substrate and the amount applied was reduced with each application: 5 g/plant on the 1st, 10 g/plant on the 2nd, 15 g/plant on the 3rd, 20 g/plant on the 4th, and 25g/plant on the 5th and 6th. Applications were first made manually and directed to the neck of the plant before applications were made to small holes at the height of the neck of the plants. In the field, two treatments *T. harzianum* and *T. viride* were installed, which were included in the fungicide treatments. Before each application, the incidence of the disease was evaluated. An area of 400 m² (5 m × 80 m) was used. A completely randomized block design was used, with 17 treatments of 14 fungicides, two biocontrollers, and one control, with four repetitions of 10 plants. An ANOVA and Tukey’s test were performed at P<0.05 using the Statistical Analysis System program.

Evaluation parameters

Incidence of disease

The incidence of the disease was evaluated before each application of fungicides and biocontrollers using the formula

$$\text{Incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total of evaluated plants}} \times 100$$

Calculation of the area under the disease progress curve

The area under the disease progress curve (AUDPC) was calculated based on the formula suggested by Shaner and Finney (1977).

$$\text{AUDPC} = \sum_i^{n-1} \left[\frac{y_i + y_{i+1}}{2} \right] (t_{i+1} + t_i)$$

Where:

Σ = summation, n = total number of evaluations

Y_i = incidence percentage at i days after evaluation

Y_{i+1} = incidence percentage at $i+1$ days after evaluation

$(T_{i+1} + T_i)$ = number of days from the first to the second evaluation

Evaluation of the fresh weight of the aerial part of the plant

At the end of the phenological phase, the green weight of the aerial part of the *Gypsophila* plants was evaluated and recorded for all treatments.

Performance evaluation (Number of flower stalks/plant)

At the time of harvest, the number of flower stems was counted in all treatments.

Results

Symptomatology

In the first stages of phenological development, plants showed sagging and loss of turgidity in the leaves of the

lower third of the plant, and two days later, advanced symptoms were observed that consisted of decay, generalized wilt, collapse, and death of the plant (Fig. 1). Initially, at the neck level, the plants presented slightly sunken lesions, and when longitudinal cuts were made at the height of the neck, light brown discoloration was observed. In plants with more advanced symptoms, there was rot in the dark brown neck that covered the entire diameter of the stem and caused the collapse and death of the plant.



Fig. 1. *Gypsophila paniculata* plants with symptoms of leaf sagging, collapse, generalized wilt, and death due to *Rhizoctonia solani* infection.

Fungi isolation and identification

Seven isolates of *Rhizoctonia solani* developed hyaline mycelium at the beginning of growth in the PDA medium. Then, they became cream-colored, and after 10 days, the mycelium was brown. Septated hyphae were observed, under the compound microscope, with right-angled branches (90°) and a constriction at the base of the branching. The diameter of the hyphae was 6–8 μ , and sclerotia formation was not observed. The characteristics that we observed correspond to the description of the fungus *R. solani* by Sneh *et al.* (1991). Five isolates of *Fusarium* spp. developed cottony mycelium in the Petri dishes with PDA medium. The conidiophores of the mycelium were straight, simple, and short, the filialides were simple and macroconidia were formed on them, which were elongated and curved with 3–4 septa, and the microconidia were ovoid with 1–2 septa. These characteristics correspond to those of the fungus *Fusarium* spp., according to the Barnett and Hunter (1998) key. Four isolates of oomyceta *Pythium* spp. developed cenocytic mycelium, with sporangia that were rounded, not papillated, and the liberation of zoospores was through the vesicle. These characteristics correspond to the oomyceta *Pythium* spp., according to the key of Erwin and Ribeiro (1996).

Pathogenicity test

At 10 days after the inoculation (DAI), the lower third of leaves in the *Gypsophila* seedlings that had been inoculated with *R. solani* were flaccid and yellowish, and there

was generalized decay and wilt. At 13 DAI, symptoms advanced and collapse and seedling death were observed (Fig. 2). While the seedlings inoculated with *Fusarium* spp. and *Pythium* spp. did not show any symptoms until 45 DAI. When conducting the re-isolations from the seedlings of *Gypsophila*, *R. solani* was reisolated. The pathogenicity test determined that *R. solani* is the causative agent of rotting neck disease in the culture of *G. paniculata*.



Fig. 2. Pathogenicity test in *Gypsophila paniculata* seedlings a) inoculated with *Rhizoctonia solani*, showing collapse and death, b) *Fusarium* spp., c) *Pythium* spp., and d) control without inoculation, showing healthy seedlings without symptoms of the disease.

Effect of fungicides in vitro against Rhizoctonia solani

Table 3 and Fig. 3 show the results of the fungicide effect against the fungus *R. solani*, where the Homai*, Parachupadera*, Vitavax*, Benopoint*, Rovral*, Homai, Parachupadera, Benopoint, and Rovral treatments inhibited 100% of mycelial fungus growth, followed by Kaptan, Kaptan*, Mancozeb that inhibited 80.87%, 79.57%, and 76.09% of mycelial growth, respectively. Thereby significantly exceeding the control and demonstrating the efficacy of the inhibitory activity of these fungicides.

Table 3. Comparison of the means of the effect of fungicides under *in vitro* conditions in the inhibition of mycelial growth of the fungus *Rhizoctonia solani*, a causative agent of neck rot in the culture of *Gypsophila paniculata*, using Tukey's test ($P < 0.05$).

Treatments	Fungicides	Media (Inhibition %)	Significance Tukey ($P \leq 0.05$)
T 14 16	Homai*	100	a
T 13	Parachupadera*	100	a
T 12	Vitavax*	100	a
T 11	Benopoint*	100	a
T 8	Rovral*	100	a
T 7	Homai	100	a
T 6	Parachupadera	100	a
T 5	Vitavax	100	a
T 4	Benopoint	100	a
T 1	Rovral	100	a
T 2	Kaptan	80.80	b
T 9	Kaptan*	79.57	b
T 10	Mancozeb*	76.09	c
T 3	Mancozeb	72.52	d
T 15	Control	0	e

* = High dose of fungicides

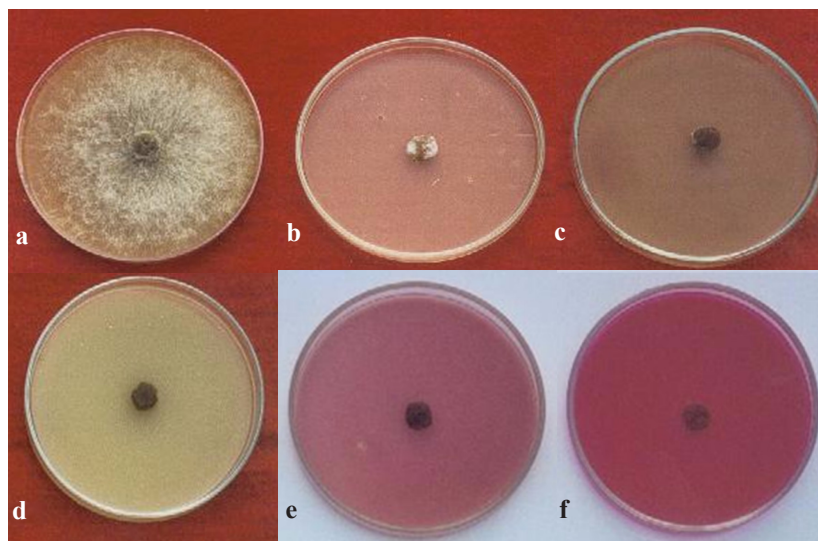


Fig. 3. Inhibition of the mycelial growth of *Rhizoctonia solani* by a) the control, b) Rovral, c) Benopoint, d) Parachupadera, e) Homai, and f) Vitavax at low and high doses.

Effect of biocontrollers in in vitro conditions

Three DAI competition antagonism of *T. harzianum* and *T. viride* was observed with mycelial growth of 6.17 and 5.78 cm in diameter, respectively. While *R. solani* grew 2.81 and 2.75 cm in diameter (Fig. 4a and Fig. 5a). Later, at four and five DAI, *T. harzianum* and *T. viride* colonized over the colony of the *R. solani* (Fig. 4b and Fig. 5b), and at six DAI, there was total invasion of the antagonists on the surface of the *R. solani* colony and dark green sporulation (Fig. 4c and Fig. 5c), so that grade four antagonism was recorded for the two biocontrollers.

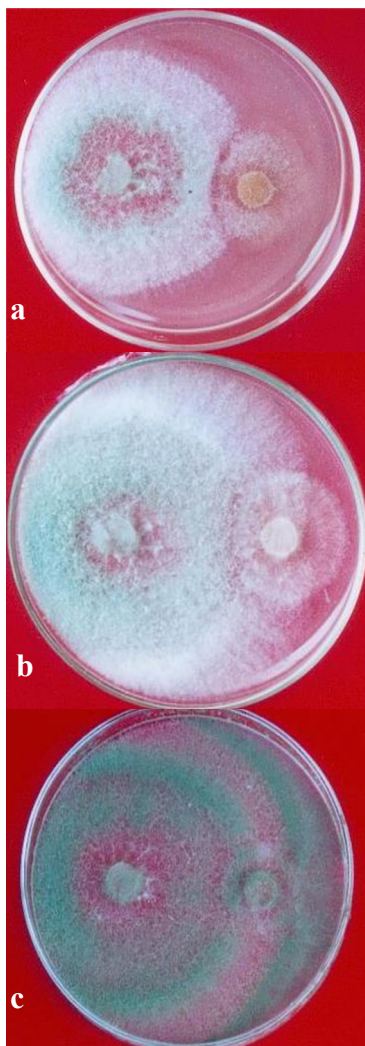


Fig. 4. Antagonism of *Trichoderma harzianum* against *Rhizoctonia solani* a) three days after incubation (DAI) showing competition antagonism, and b) at four DAI, and c) six DAI showing colonization and sporulation of *T. harzianum* on the colony of *R. solani*

Effect of fungicides and Trichoderma spp. in the field*The area under the disease progress curve of incidence*

The treatments T16 (*T. harzianum*), T10 (Kaptan*), T15 (Homai*) and T17 (*T. viride*) showed the lowest values for the AUDPC, with values of 3.3, 4.7, 5.4, and 7.4

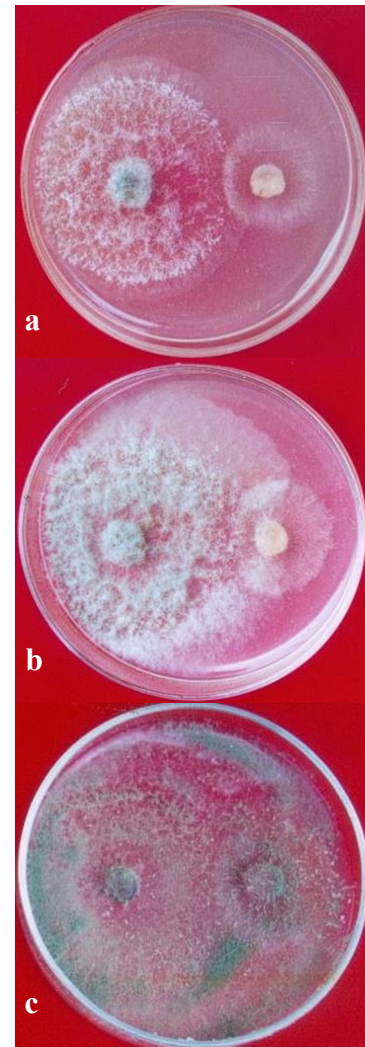


Fig. 5. Antagonism of *Trichoderma viride* against *Rhizoctonia solani* a) at three days after incubation (DAI) showing competition antagonism, and b) at four DAI, and c) six DAI showing colonization and sporulation of *T. viride* on the colony of *R. solani*

respectively, and these values were not statistically different, according to Tukey's test, although they were better than the control and lowered the incidence of disease (Table 4 and Fig. 6). In contrast, treatments T6 (Parachupadera), T8 (control), T5 (Vitavax), and T7 (Homai) showed the highest values for the AUDPC with values of 18.7, 16.5, and 14.9, respectively.

Fresh weight of the aerial part of Gypsophila

Treatment with *T. harzianum*, Homai*, *T. viride*, and Rovral, showed higher fresh weights of 13.4, 12.5, 12.3, and 12.1 kg, respectively, than the control treatment, which reached 6.1 kg (Table 5).

Efficiency (Number of floral stems harvested/plant)

The *T. harzianum* and *T. viride* treatments resulted in harvests of 6.25 and 5.5 flower stems/plant, respectively, unlike the control that only reached 2.25 floral stems/plant (Table 5).

Table 4. The area under the disease progress curve (AUDPC) of neck rot disease incidence and comparison of the effect of fungicides and *Trichoderma* spp. in the control of neck rot disease in *Gypsophila paniculata*, in the valley of the Callejon de Huaylas, Ancash, using Tukey's test (P <0.05).

N° de treats.	Fungicides	AUDPC	Comparison of Tukey test(P ≤ 0.05)
T1	Rovral	13.0	a
T2	Kaptan	7.0	bf
T3	Manzate	12.8	ac
T4	Benopoint	8.6	abcdf
T5	Vitavax	14.9	ace
T6	Parachupadera	18.7	e
T7	Homai	14.9	ace
T8	Control	16.5	ace
T9	Rovral*	8.6	abcdf
T10	Kaptan*	4.7	f
T11	Manzate*	9.6	af
T12	Benopoint*	10.2	a
T13	Vitavax*	8.4	af
T14	Parachupadera*	11.0	a
T15	Homai*	5.4	f
T16	<i>T. harzianum</i>	3.3	f
T17	<i>T. viride</i>	7.4	f

* = High dose of fungicides

Discussion

The results of the pathogenicity test and *in vitro* and field experiments showed that *Rhizoctonia solani* is the causative agent of the stem base *Gypsophila* disease, the symptoms observed in *G. paniculata* coincide with the typical symptomatology produced by *R. solani* in other crops it affects, such as beans, potatoes, cotton, and beetroot (Agrios, 1995). These symptoms include sunken lesions in the form of cankers because the pathogen produces enzymes such as Polygalacturonase (PG) and Pectin Methyl Esterase, which cause degradation of the pectic compounds of the cell wall and damage to plant tissues, causing cell death. In addition, the sunken lesions caused by *R. solani* are also a consequence of the production of other enzymes, such as endocellulases, which cause the breakdown of cellulose fibrils, which make up the cell wall, and death of plant tissue (Goodman *et al.*, 1986).

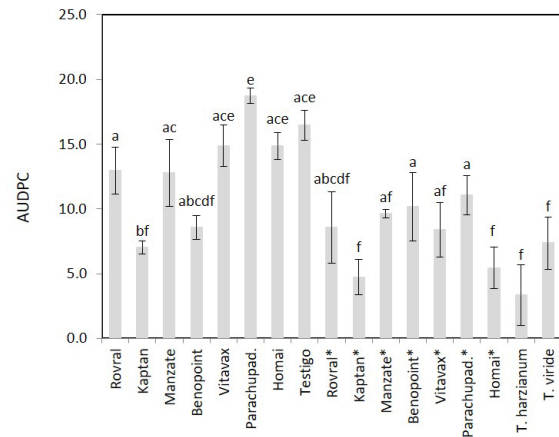


Fig. 6. The area under the disease progress curve (AUD-PC) of neck rot disease incidence with the effect of fungicides and *Trichoderma* spp. in the control of crown rot disease in *Gypsophila paniculata* cultivation, in the valley of the Callejon de Huaylas, Caraz Province in Ancash Department..

Table 5. Fresh weight of the aerial part of plants and Tukey's test (P <0.05) of the number of harvested flower stalks/*Gypsophila paniculata* plant to determine the effect of low and high doses of fungicides and *Trichoderma* spp., Province of Caraz, Department of Ancash.

Treatments	Fresh weight of the aerial part (K.g.)	N° of harvested flower stalks/plant	Significance Tukey (P ≤ 0.05)
T16 <i>T. harzianum</i>	13.4	6.25	a
T17 <i>T. viride</i>	12.3	5.5	ab
T01 Rovral	12.1	4.5	bc
T15 Homai*	12.5	4.5	bc
T05 Vitavax	10.5	4.25	bcd
T11 Manzate*	11.2	4.25	bcd
T14 Parachupadera*	9.6	4.0	bcd
T06 Parachupadera	11	3.75	cde
T10 Kaptan*	11.3	3.75	cde
T03 Manzate	6.9	3.5	cde
T04 Benopoint	9.7	3.5	cde
T02 Kaptan	11.9	3.0	cde
T09 Rovral*	10.5	3.0	cde
T12 Benopoint*	7.2	3.0	cde
T13 Vitavax*	10.9	3.0	cde
T07 Homai	9.2	2.75	de
T08 Control	6.1	2.25	e

* = High dose of fungicides

In vitro it was observed that the fungicides Iprodione, Benomil, Carboxin + Captan, Flutolanil + Captan and Thiofanate methyl + Thiram at low and high doses inhibited 100% the mycelial growth of *R. solani*; these results are probably a consequence of the fact that each fungicide is located in a different group of fungicides, with different active ingredients and modes of action (Mont, 2002). The fungicide Rovral, which is a contact fungicide, completely inhibited the development of the fungus *R. solani*. Rovral belongs to the Dicarboximides group, which interfere with the activity of DNA synthesis and alter cell permeability. In interaction with thiols, they block oxidative phosphorylation and alter the breathing process, according to Mont (2002). *R. solani* also belongs to this division, so the response of 100% inhibition was expected. Benopoint is a systemic fungicide that belongs to the Benzimidazoles group. It is postulated that in aqueous solution, benomyl is hydrolyzed into methyl benzimidazole carbamate, which is fungitoxic and fungistatic and acts to inhibit mycelial development. The most affected and inhibited process in fungal cells is cell metabolism and DNA synthesis, without which the pathogen is prevented from growing and developing. The fungicides Vitavax (carboxin + captan), Parachupadera (captan flutolanil) and Homai (thiofanate + thiram) are compound fungicides, and all of them inhibited 100% of the mycelial growth of *R. solani*, probably because these fungicides are composed of two types of active ingredients (curative-systemic), which affect various metabolic processes of the pathogen, thus having a more substantial range of toxic action on several species of fungi belonging to the Deuteromycota division (Mont, 2002).

The *in vitro* results of 2 biocontrollers against *R. solani*, reached degree 4 of antagonism, which coincide with several studies that indicate that different strains of *Trichoderma* spp. have the ability to grow more rapidly than phytopathogenic fungi, and this increases their antagonistic capacity (Chet, 1990). Cook and Baker (1983) also indicate that the growth rate presented by *Trichoderma* species is a reason for using these fungi as an antagonist for the control of *R. solani* and *Sclerotium rolfsii*. In addition, Guédeza *et al.* (2012) concluded that the main modes of action of *T. harzianum* are parasitism and competition for space and nutrients, surpassing the growth of *R. solani* and *S. rolfsii* and preventing normal development and inhibiting more 50% of their development. *T. harzianum* and *T. viride* cause destruction of the hyphae of the *R. solani*, due to the action of enzymes, such as glucanase, chitinase, and protease, through which they degrade the cell wall of the hypha, causing death (Chet, 1990).

The establishment of the antagonistic capacity or antagonistic degree of *Trichoderma* strains under *in vitro* conditions is a priority and constitutes a premise for the selection of strains, not only for subsequent tests in greenhouse and field conditions but also because information on a possible formulation can also be generated with an appropriate vehicle that gives them better stability and efficiency in field conditions.

In the Callejón de Huaylas valley during the months of January to May, a maximum temperature of 23.1 °C, average temperature of 16.8 °C and a minimum temperature of 10.3 °C were recorded, it is likely that these conditions favored the viability of the conidia of *T. harzianum*, and this protected the inoculation zone, of the perimeter of the neck/crown of the plant, from the attack of *R. solani*, which concurs with the report of Nampoothiri *et al.* (2004), who indicate that the optimum temperature for the germination of conidia, growth of the germination tube, mycelium growth, competitive abilities, and the production of volatile and non-volatile metabolites of *Trichoderma* spp. 15–30 °C. Therefore, it can produce a chitinase enzyme over a wide range of environmental conditions. In the present study, peeled wheat was used as a substrate for the application of *T. harzianum*. However, because the fungi were saprophytes, it is possible that the conidia were viable for several days or even weeks. According to Lewis and Papavizas (1987), conidia and clamidospores may form on natural substrates, such as cereal grains, inoculated in the soil. In our study, it is likely that these structures were formed and that the biocontroller may have been protecting the tissue of the plant from the attack of *R. solani*. Therefore, there were fewer dead plants and 12.5% incidence of the disease. These results also agree with the reports of Elad *et al.* (1980), who found that with the application of *T. harzianum* in the soil infested by *R. solani*, the incidence was reduced by 7.5% in a bean crop. Likewise, Hohmann *et al.* (2011) reduced the mortality of pine seedlings by 29%.

According to Harman (1996), *Trichoderma* spp. applied to the soil spreads and shows rapid colonization in the area of inoculation, thereby reducing the inoculum of the population of phytopathogens. We found that the application of fungicides was not successful in the control of the disease. This could be due to the precipitation that fell during the execution of the field experiment, which could have washed the treatment off the tissue surface. In addition, contact fungicides have low residuality, and although applications were made every 15 days, this could have influenced the results. However, with the Kaptan* treatment, an incidence of 18.42% was recorded, which is similar to the results obtained by Poddar *et al.* (2004), of 13.26% incidence in chickpea seedlings, and Silveira *et al.* (2003), of 14% incidence in eucalyptus seedlings, demonstrating that Kaptan has protective properties in plant tissues. With the fungicide Homai* (Thiofanate-methyl + Thiram), an incidence of 22.50% was obtained, which is consistent with results reported by Goulart (2002) for the application of Homai of 82.5% germination of cottonseed seeds (*Gossypium hirsutum*) for the control of *R. solani*, demonstrating a smaller number of plants infected by fungus sucking post-emergence. The reduction in incidence was due to the active ingredients (systemic-contact) that affect various metabolic processes of the pathogen. Mont (2002) indicates that Homai fungicide has systemic action and is translocated by the xylem, penetrating the tissues of

the plant, and affecting the fungus at the level of tubulin. In the future, these products could be incorporated into an integrated management plan for rotting neck disease in *Gypsophila* culture to reduce plant mortality.

In this study, the effect of the antagonist fungi *T. harzianum* and *T. viride* was evaluated, a higher fresh weight and a greater number of harvested flower stems were obtained, these results agree with studies conducted by Hohmann *et al.* (2011) that indicate that *Trichoderma* spp. promotes root growth, which implies a taller and larger aerial part of the plant. Other researchers, such as Galeano *et al.* (2009), indicate that the mechanisms of action of *T. harzianum* are based on its role as a plant growth promoter and that this antagonist is associated with the roots of the plant through hyphae and grows as the root system does, feeding on the waste products and exudates that the roots secrete. This leads to an increase in the uptake of water and nutrients, and extends to a greater volume of soil, increasing the solubilization of organic nutrients, such as phosphorus, thus providing higher vigor and plant growth. This increased vigor of the plant provides improved tolerance against different abiotic and biotic stresses. This is supported by Harman's (1996) research, which shows that *Trichoderma* spp. can colonize the area of the rhizosphere of plants, contributing to improved root and foliar development, and greater productivity in corn and cultivated pastures with lower requirements for nitrogen fertilizers. This could explain why the application of *T. harzianum* and *T. viride* to *Gypsophila* resulted in vigorous development and an increased number of flower stems. Likewise, this study coincides with the report by Galeano *et al.* (2009), which indicates that the *Trichoderma* spp. promotes the development and growth of the root system. Therefore, better development of the aerial part of the plant is evident in cucumber, bean, and pepper crops. These characteristics are attributed to the production of hormones and vitamins and the assimilation of nutrients, such as P, Zn, Mg, and K (Galeano *et al.*, 2009). Likewise, Mont (2002) indicates that *Trichoderma* spp. consistently stimulates plant development through the production of metabolites. This has been found in petunia and marigold, for which the incorporation of 1% of dry biomass containing 60–80% of clamidospores of the fungus increased the size and weight of flowers by 100%.

Treatments with Homai* and Rovral obtained increased fresh weight compared with the rest of the fungicides under study. Homai*, being a compound fungicide, has a broad spectrum of preventive and curative control acting on respiration and preventing normal oxygen uptake by pathogenic fungi (Rubio-Reque *et al.*, 2008). In addition, with fungicides Rovral and Homai* both resulted in harvests of 4.5 floral stems/plants, which was less than those obtained from the biocontrollers, possibly because of the rainfall in the months of January to April, which could have washed off these fungicides, as well as the low residuality, toughness, and frequency of application at every 15 days.

Conclusions

The causative agent of neck rot disease in *Gypsophila paniculata* was *Rhizoctonia solani* fungus. *In vitro*, low and high doses of Rovral, Benopoint, Vitavax, Rovral, and Homai inhibited 100% of the mycelial growth of *R. solani*. *Trichoderma harzianum* and *T. viride* showed a higher growth rate than *R. solani*, and a total invasion of the *R. solani* colony and sporulation was observed, reaching degree four of antagonism. In the field, *T. harzianum*, Kaptan*, Homai*, and *T. viride* obtained the lowest values for the AUDPC, with values of 3.3, 4.7, 5.4, and 7.4, respectively, which were statistically the same, according to Tukey's test ($P>0.05$), with these treatments showing better control and lower incidence of disease. However, the treatments of Parachupadera, the control, Vitavax, and Homai, had the highest values for the AUDPC with values of 18.7, 16.5, and 14.9, respectively. Higher fresh weight and a higher number of flower stalks/plant were obtained with the *T. harzianum* treatment recording 13.4 kg fresh weight and an average of 6.25 flower stalks/plant, whereas the control reached 6.1 kg of fresh weight and an average of 2.25 flower stalks/plant.

Acknowledgments

To Corporación Roots Perú, for the financing and Ing. José Terry for the logistical support in Caraz.

References

- Agrios, G. N. (1995). *Fitopatología*. Tercera edición traducida del inglés por Manuel Guzmán Ortiz. S.A. de C.V. México. Editorial Limusa. 837 p.
- Barnett, H. L., & Hunter, B. B. (1998). *Illustrated genera of imperfect fungi*. Editorial Burgess Publishing Company.
- Chet, I. (1990). Biological control of soil-borne plant pathogens with fungal antagonists in combinations with soil treatments. In D. Hornby (Ed.), *Biological Control of Soil-borne Plant Pathogens*. (pp. 15–25). Wallingford, UK: C.A.B. International.
- Cook, R. J., & Baker, K. F. (1983). The nature and practice of biological control of plant Pathogens. In *Phytophthora. Its biology, taxonomy, ecology and pathology* (pp. 190–195). St. Paul, MN: APS. Publishing.
- Correa, S., Mello, M., Ávila, Z., Minare, L., Pádua, R., & Gomes, D. (2007). Cepas de *Trichoderma* spp. para el control biológico de *Sclerotium rolfsii* SAAC. *Fitosanidad*, 11(1), 3–9.
- Elad, Y., Chet, I., & Katan, J. (1980). *Trichoderma harzianum*: A biocontrol agent effective against

- Sclerotium rolfsii* and *Rhizoctonia solani*. *Phytopathology*, 70(2), 119–121. doi:10.1094/Phyto-70-119
- Erwin, D. C., & Ribeiro, O. K. (1996). *Phytophthora diseases worldwide*. St. Paul, MN: American Phytopathological Society.
- Ezziyyani, M., Pérez, C., Requena, M., & Rubio, L. (2004). Biocontrol por *Streptomyces rochei-ziyani*, de la podredumbre del pimiento (*Capsicum annum* L.) causada por *Phytophthora capsici*. *Anales de Biología*, 26, 69–78.
- French, E. R., & Hebert, T. T. (1980). *Métodos de investigación fitopatológica*. San José, Costa Rica. Editorial IICA. 277 p.
- Galeano, M., Méndez, F., & Urbaneja, A. (2009). Efecto de *Trichoderma harzianum* Rifai (Cepa T-22) sobre cultivos hortícolas. Departamento I+D. Koppert Biological Systems.
- Goodman, R. N., Zoltan, K., & Wood, K. R. (1986). *The biochemistry and physiology of plant disease*. United States of America. University of Missouri Press.
- Goulart, A. C. P. (2002). Efeito do tratamento de sementes de algodão com fungicidas no controle do tombamento de plântulas causado por *Rhizoctonia solani*. *Fitopatología Brasileira*, 27(4), 399–402. <http://dx.doi.org/10.1590/S0100-41582002000400011>
- Guédeza, C., Cañizaleza, L., Castilloa, C., & Olivarb, R. (2012). Evaluación *in vitro* *Trichoderma harzianum* para el control de *Rhizoctonia solani*, en plantas de tomate. *Revista de la Sociedad Venezolana de Microbiología*, 32, 44–49.
- Harman, G. E. (1996). *Trichoderma for biocontrol of plant pathogens: From basic research to commercialized products*. 6p. NY: Cornell University NYSAES Departments of Horticultural Science and of Plant Pathology. <http://web.entomology.cornell.edu/shelton/cornell-biocontrol-conf/talks/harman.html>
- Hohmann, P. E., Jones, E. E., Hill, R. A., & Stewart, A. (2011). Understanding *Trichoderma* in the root system of *Pinus radiata*: Associations between rhizosphere colonization and growth promotion for commercially grown seedlings. *Fungal Biology*, 115(8), 759–767. <https://doi.org/10.1016/j.funbio.2011.05.010>
- Lewis, J. A., & Papavizas, G. C. (1987). Permeability changes in hyphae of *Rhizoctonia solani* induced by gremlin's preparations of *Trichoderma* and *Gliocladium*. *Phytopathology*, 77(5), 699–703. doi:10.1094/Phyto-77-699
- Mont, R. M. (2002). *Manejo Integrado de Enfermedades de las Plantas*. Lima-Perú. Ministerio de Agricultura. Servicio Nacional de Sanidad Agraria. 210 p.
- Nampoothiri, K. M., Baiju, T. V., Sandhya, C., Sabu, A., Szakacs, G., & Pandey, A. (2004). Process optimization for antifungal chitinase production by *Trichoderma harzianum*. *Process Biochemistry*, 39(11), 1583–1590. [https://doi.org/10.1016/S0032-9592\(03\)00282-6](https://doi.org/10.1016/S0032-9592(03)00282-6)
- Poddar, R. K., Singh, D. V., & Dubey, S. C. (2004). Management of chickpea wilt through combination of fungicides and bioagents. *Indian Phytopathology*, 57, 39–43.
- Rubio-Reque, G., Baltodano-Sánchez, F., Abanto-Campos, L., Wilson-Krugg, J., & Muñoz-Ríos, M. (2008). Resistencia *in vitro* de *Rhizoctonia solani* y *Fusarium oxysporum* a los fungicidas Benzomil 500. Rhizolex-T y Homai. *REBIOL*, 28(2).
- Shaner, G., & Finney, R. E. (1977). The effect of nitrogen fertilization on the expression of slow mildewing resistance in Knox wheat. *Phytopathology*, 77(8), 1051–1056. doi:10.1094/Phyto-67-1051
- Silveira, S. Fd, Alfenas, A. C., Maffia, L. A., & Suzuki, M. S. (2003). Controle químico da mela de estacas e da queima de folhas de eucalipto, causadas por *Rhizoctonia* spp. *Fitopatología Brasileira*, 28(6), 642–649. <https://doi.org/10.1590/S0100-41582003000600010>
- Sneh, B., Burpee, L., & Ogoshi, A. (1991). *Identification of Rhizoctonia species*. St. Paul, MN: American Phytopathological Society.
- Van Der Plaats-Niterink, A. (1981). Monograph of the genus *Pythium*. In *Studies in Mycology* N° 21. http://www.wi.knaw.nl/publications/1021/content_files/content.htm