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# A Comparative Study of Different Strains of *Trichoderma* under Different Conditions of Temperature and pH for the Control of *Rhizoctonia solani*

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## Abstract

Severe damage caused by *R. solani* in the potato crop and the current limitations for its control justify the assessment of potential biocontrol agents and their relationship with abiotic factors to be successfully included in sustainable production systems. The aim of this study was to test the effect of temperature (10˚C, 15˚C, 25˚C, 35˚C) and pH (5.5, 7, 8.5) on the growth and antagonist mechanisms of 26 native strains of 11 species of *Trichoderma* for the control of *R. solani*. The response obtained was dependent on the isolation, rather than on the *Trichoderma* specie that was tested. Most of them showed greater growth at 25˚C and pH 5.5, with overgrowth values between 75% and 100% and greater percentage of radial growth inhibition (PRGI) at 50%. A greater ability to compete for the substrate is observed, enhanced by its higher growth rate. Hyphal interaction mechanisms were varied and, at least, 92% of the isolations showed a minimum of two different types. Knowledge of the behavior of the different strains in front of varied abiotic factors will enable an understanding of the population dynamic of *Trichoderma* and the identification of the most efficient strains for the control of *R. solani*.

## Keywords

Biological Control, Abiotic Factors, Phytopathogen

## 1. Introduction

*Rhizoctonia solani* is a phytopathogen of worldwide distribution that causes se-

vere losses in the potato crop. Its underground presence and the lack of fungicides of high selectivity and efficiency make it difficult to chemically control this phytopathogen. Other alternative strategies are limited since it has a wide range of host plants and there are no varieties of potatoes available that can resist it. In this context, an alternative would be the implementation of a biological control.

In relation to this, the fungal genus *Trichoderma* has awakened great interest due to its high activity as a Biological Control Agent (BCA). Many of the studied strains have behaved as invader opportunists, of rapid growth and with high production of spores [1] [2]. This has let them be trade marketed to be included in the crop protection scheme [3].

Control efficacy of the different strains of *Trichoderma* may be affected by biotic (species and vegetable variety, edaphic microbial interactions) and abiotic factors (type of soil, water potential, temperature, pH) [4]. These factors not only have an effect on the survival of the BCA, but also on its disease control capacity [5].

Although studies have been conducted on the differential behavior among the *Trichoderma* species [2] [6] and particularly among strains [7] [8], there is no information available about the possible effect of the temperature and pH as environmental factors that influence its growth and mechanism of action for the control of *R. solani*.

In this respect, studies carried out [4] recorded that temperature and pH are two key factors in the growth, saprophytic capacity and antagonist mechanisms of *Trichoderma*. This biopesticide resists a wide range of temperatures and its growth rate depends on the species and on the isolation itself [9]. According to these authors, in general, optimal temperatures for the greatest growth would be between 25˚C and 30˚C and would not coincide with the greatest antagonistic activity. Karaoğlu *et al*. (2018) [10] communicated that the growth capacity depended on the studied strains with the highest values achieved between 37˚C and 42˚C, and without evidence of growth between 2˚C and 4˚C.

Studies have reported that the different strains of *Trichoderma* are active antagonists of phytopathogens in a wide range of pH [11] [12] or that its behavior is independent of its value [13]. Thereon, pH values between 4.6 and 5 favor the survival of *Trichoderma* and the control of *Fusarium* spp. in the cultivation of onion in a greenhouse [1]. In addition, the pH of the medium is a determinant of the enzymatic activity, more than the mycelial growth of *Trichoderma* as a control mechanism [11] [14]. Also, the production of the secondary metabolite 6 pentyl-alpha pyrone with antifungal function depends on the strain of *Trichoderma* studied, the culture medium and the pH value [15].

The aim of this study was to comparatively evaluate different native strains of *Trichoderma* under different conditions of temperature and pH for the control of *Rhizoctonia solani*.

## 2. Material and Methods

### 2.1. Biological Material

This study was carried out with 26 strains, corresponding to 11 species of *Tri-*

*choderma*, isolated from native forests and cultivated fields in the province of Córdoba (Argentina), with no history of application of *Trichoderma spp*. were used. Isolation was carried out according to the plate-dilution method [16]. From monosporic cultures, it was established the collection of pure strains that were identified by its morphologic features and its identity, tested by PCR, through the sequencing of the Internal Transcribed Spacer (ITS) region in Laboratory of the Institute of Biological Resources of INTA Castelar (Argentina). The sequences of the ITS were compared to those entered in the TRICHOBLAST database to confirm the observed pooling.

The *Rhizoctonia solani* Kühn AG-3 (Anastomosis Group 3) strain was isolated from affected potato tubers from Villa Dolores (Córdoba, Argentina) and then, it was molecularly characterized (Laboratory of the Institute of Biological Resources of INTA Castelar Argentina).

Fungal recovery at the time of assessment was done through sowing (mycelium and conidia of *Trichoderma* sp., mycelium and conidia of *Rhizoctonia* sp.) in Petri dishes with potato glucose agar (PGA), Britania, in growth chamber at 25˚C ± 2˚C for 5 days.

## 2.2. Assessments

Assessments in dual culture trials were done according to what was proposed by Martínez and Solano (1995) [17], in Petri dishes of 9 cm of diameter with 15 ml of PDA (potato dextrosa agar) medium (Britania). The pH value of the medium was adjusted by adding NaOH (0.1 N) and HCI (0.1 N) before autoclave processing [6] and with further control after cooling (20˚C).

Treatments assessed were the combination of three pH values (5.5, 7 and 8.5) and four temperatures (10˚C, 15˚C, 25˚C and 35˚C).

At each end, it was sowed an agar disk of 5 mm of diameter with *R. solani* mycelium and, on the opposite end, the mycelium corresponding to the *Trichoderma* spp. strain to be assessed, at equal distance of the periphery and incubated at each condition of combined temperature and pH for 12 hours.

Five repetitions by treatment were done, which included control samples of *R. solani* and *Trichoderma* spp.

## 2.3. Variables Measured

- Radial growth: Disks of 5 mm of diameter with *R. solani* Kühn AG-3 and with each strain of *Trichoderma* were sowed in Petri plates containing PDA medium (Britania). The incubation conditions were the combination of different temperatures (10˚C, 15˚C, 25˚C, 35˚C) and pH (5.5, 7, 8.5). Measurements were taken up to 5 days after sowing. Results were expressed in $mm \cdot day^{-1}$ using the growth rate (GR) formula = (final growth - initial growth) × $(\text{incubation time})^{-1}$ [18].
- Percentage of radial growth inhibition (PRGI): for confirming the antibiotic effect of the isolations of *Trichoderma* spp. over *R. solani*. The PRGI was as-

sessed in dual culture from the following 24 hours to the time physical contact was established between the antagonist and the pathogen [19]. The PRGI formula was used, PRGI = $[(R1 - R2) \times R1^{-1}] \times 100$; where R1 is the radial growth of the control and R2 the radial growth of the pathogen in the treatments [20].

- Competence for substrate: assessments in dual cultures were done after 96 and 120 hours. Sorting as antagonist of each tested strain was carried out taking into account the five-grade class scale [21].
- Mycoparasitism: It was determined as the overgrowth of *Trichoderma* spp. in relation to the pathogen, at 5 days from the sowing and the occupied surface was established [22]. Results were expressed as a percentage of the area occupied by *Trichoderma* in relation to the area occupied by the pathogen.

To establish the type of hyphal interaction (adhesion, winding, penetration, vacuolation, cytoplasm granulation, lysis), sample observations were carried out from the area of interaction of both fungi in binocular optic microscope at 400× magnification.

### 2.4. Design and Statistical Analysis

The experiment was carried out under a completely randomized. Percentage values were transformed by calculating 2arco$\sqrt{p}$ before the variance analysis. Grade 2 polynomial regression analysis was performed, where the significance of the quadratic and linear components was tested. The significance of the quadratic component would indicate the presence of an optimal and a minimum value, in relation to the percentage of inhibition and pH. Meanwhile, the significance of the lineal component (and the absence of a quadrant component) would indicate constant increments and decrements in the percentage of inhibition through the range of the pH examined. Analysis and visualizations of results were executed using the software R, version 3.2.2 [23].

## 3. Results

Based on the results (**Figure 1**), mycelial growth was observed in the *Trichoderma* strains tested at 15˚C and 25˚C and at all pH values (5.5, 7, 8.5). At 10˚C and 35˚C no growth was reported, regardless of pH. In general, growth was greater at 25˚C decreasing as pH was higher, except for *T. longibrachiatum* CBL1 and *T. konigiopsis* CBK2. While the strains *T. ghanense* CBGe1, *T. harzianum* CBH1, *T. konigiopsis* CBK6, *T. ovalisporium* CBO1, CBO2 and *T. sulpureum* CBS1, showed higher growth at 15˚C and at pH 5.5. It should be noted that the differential response in growth was dependent on the strain examined, more than on the *Trichoderma* species.

Regarding *R. solani* (**Figure 2**), no growth was observed at 10˚C and 35˚C. Greater values were achieved at 25˚C, rather than at 15˚C. This mycelial growth was not affected when varying pH conditions.

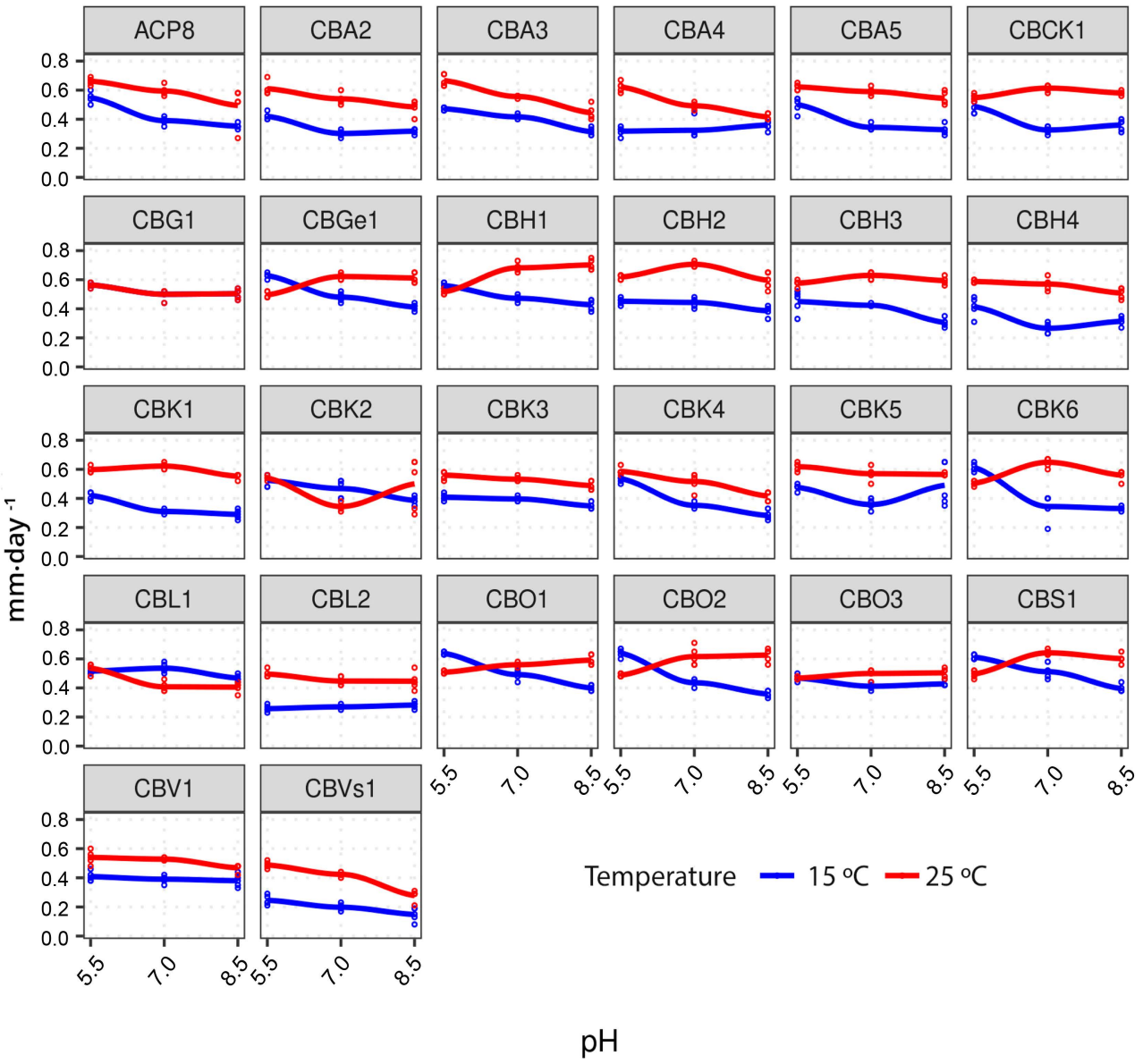


**Figure 1.** Mycelial growth of different strains of *Trichoderma* under different conditions of temperature and pH.

In dual growth trials (Table 1), by comparing the different strains of *Trichoderma* and *R. solani*, PRGI was, in general terms, lower at 15˚C and independent of pH. However, *T. atroviride* CBA3 and *T. sulpureum* CBS1 showed inhibition values significantly greater at pH 5.5 (7.6% of the tested strains). While 19.2% of the strains showed a trend for increasing the PRGI at lower pH (*T. harzianum* CBH3 and CBH4, *T. konigiopsis* CBK4 and CBK6, *T. ovalisporium* CBO2). At 25˚C, the observed behavior was similar, but more strains (15% of the tested strains) showed a greater PRGI at pH 5.5 (*T. atroviride* ACP8 and CBA3; *T. harzianum* CBH2 and CBH4; *T. konigiopsis* CBK3 and CBK4) and 27% of the strains evidenced greater PRGI with lower pH (*T. clado-koningii* CBCK1; *T. harzianum* CBH1, CBH3 and CBH4; *T. longibrachiatum* CBL1; *T. ovalisporium*

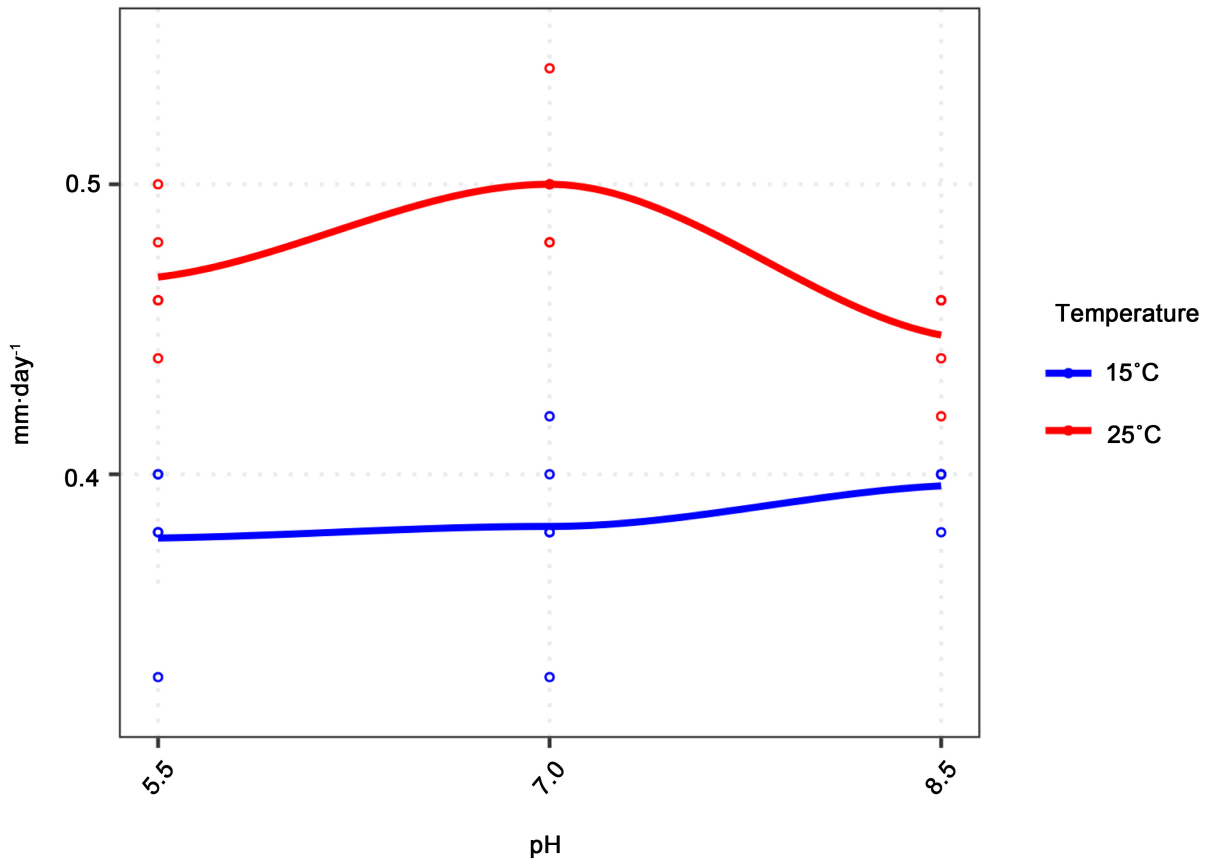


**Figure 2.** Mycelial growth of *R. solani* under different conditions of temperature and pH.

CBO3; *T. sulpureum* CBS1). It should be noted that *T. atroviride* CBA3 at 15 and 25˚C and pH 5.5 showed values of PRGI greater than 50%. Moreover, this same species, but CBA4 strain and *T. konigiopsis* CBK5, showed average values of inhibition of 32.6% and 31.9%, regardless of temperature and pH. Although *T. virens* CBVS1 evidenced a similar stable behavior, the inhibition percentage of *R. solani* was lower (4.6%). The differences between strains of *Trichoderma* in relation to the control mechanisms on *R. solani*, at the same temperature (25˚C) and different pH can be observed in Figure 3.

The capacity of the different strains to compete for the substrate at 25˚C and pH equal to 5.5 is shown in Table 2. According to the scale [21] at 96 hours after sowing, 88.5% of the tested strains grew over the pathogen, covering from 75% to 100% of the box surface (Class 1 and 2, respectively). The growth detention of both fungi (Class 3) was only observed in *T. longibrachiatum* CBL2 and *T. gamsii* CBG1. On the other hand, *T. virens* CBVs1 did not show any control effect over *R. solani* (Class 4). At 120 hours after sowing, 73% of the tested strains kept its competence for substrate capacity. It was kept even when time of the trial was extended.

The results of hyphal interaction with *R. solani* shown in Table 2 demonstrate that 96.2% of the strains under study revealed at least two types of mechanisms.

**Table 1.** Percentage of radial growth inhibition of *R. solani* vs. different strains of *Trichoderma* at different conditions of temperature and pH.

| Species | Strain | PRGI (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Temperature 15˚C | | | Temperature 25˚C | | |
| | | pH: 5.5 | pH: 7 | pH: 8.5 | pH: 5.5 | pH: 7 | pH: 8.5 |
| *T. atroviride* | ACP8 | 35.2 bc | 29.8 ab | 28.2 a | 41.8 c | 32.5 ab | 32.7 ab |
| | CBA2 | 19.5 b | 20.5 b | 12.3 a | 28.0 c | 23.2 bc | 20.7 b |
| | CBA3 | 59.0 c | 44.4 ab | 45.5 ab | 56.5 c | 49.6 b | 40.7 a |
| | CBA4 | 30.1 a | 30.9 a | 30.3 a | 35.4 a | 33.7 a | 35.2 a |
| | CBA5 | 25.62 a | 28.9 ab | 22.1 a | 38.6 c | 38.7 c | 33.3 bc |
| *T. clado-koningii* | CBCK1 | 19.9 ab | 17.1 a | 14.54 a | 29.3 c | 24.9 bc | 20.4 ab |
| *T. gamsii* | CBG1 | 13.3 ab | 16.2 b | 7.3 a | 16.0 b | 15.4 b | 9.6 ab |
| *T. ghanense* | CBGe1 | 31.6 ab | 27.6 ab | 25.5 a | 32.5 b | 29.5 ab | 28.4 ab |
| *T. harzianum* | CBH1 | 22.3 ab | 17.1 a | 16.4 a | 28.7 b | 26.7 b | 18.9 a |
| | CBH2 | 23.7 a | 19.8 a | 19.2 a | 33.9 b | 25.5 a | 20.1 a |
| | CBH3 | 15.3 bc | 12.2 ab | 6.17 a | 21.3 c | 15.1 bc | 12.2 ab |
| | CBH4 | 22.3 bc | 19.9 b | 12.9 a | 30.6 d | 29.0 cd | 21.4 b |
| *T. konigiopsis* | CBK1 | 19.0 abc | 17.9 ab | 13.9 a | 25.17 c | 23.1 bc | 22.0 bc |
| | CBK2 | 27.5 a | 27.5 a | 29.6 a | 38.6 b | 33.1 ab | 34.0 ab |
| | CBK3 | 30.1 cd | 28.2 bcd | 23.5 abc | 30.5 d | 22.8 ab | 20.4 a |
| | CBK4 | 31.6 cd | 26.8 bc | 17.4 a | 36.6 d | 26.8 bc | 22.5 ab |
| | CBK5 | 31.7 a | 33.9 a | 30.4 a | 34.0 a | 31.2 a | 30.0 a |
| | CBK6 | 30.2 bc | 24.9 ab | 21.3 a | 34.6 c | 34.9 c | 31.3 bc |
| *T. longibrachiatum* | CBL1 | 26.4 abc | 24.8 abc | 22.1 a | 31.3 c | 29.4 bc | 24.0 ab |
| | CBL2 | 3.6 a | 3.3 a | 2.7 a | 14.5 c | 6.9 ab | 11.9 bc |
| *T. ovalisporium* | CBO1 | 29.3 ab | 22.6 a | 24.1 ab | 30.6 b | 26.8 ab | 23.9 ab |
| | CBO2 | 38.2 b | 32.3 ab | 27.6 a | 38.4 b | 36.2 b | 32.0 ab |
| | CBO3 | 20.4 b | 19.9 b | 18.6 b | 21.2 b | 15.4 ab | 11.7 a |
| *T. sulpureum* | CBS1 | 30.9 bc | 22.7 a | 21.3 a | 33.2 c | 30.2 bc | 26.3 ab |
| *T. viride* | CBV1 | 25.7 ab | 22.0 ab | 24.8 ab | 28.8 b | 20.3 a | 24.0 ab |
| *T. virens* | CBVS1 | 5.6 a | 4.1 a | 3.4 a | 6.7 a | 5.8 a | 2.4 a |

Only *T. atroviride* ACP8 and *T. harzianum* CBH2 evidenced the five types of hyphal interaction detected: winding, penetration, vacuolization, cytoplasm granulation and lysis. *T. atroviride* CBA3 and CBA5, *T. ghanense* CBG1, *T. konigiopsis* CBK2 and CBK4, *T. ovalisporium* CBO1 and CBO2, *T. sulpureum* CBS1 showed four types of hyphal interaction. Only *T. virens* CBvs1 did not show any type of interaction with the pathogen, in accordance with absence of growth over *R. solani*.

**Table 2.** Competence for substrate and hyphal interaction of different strains of *Trichoderma* at 25˚C and pH 5.5.

| Species | Strain | Categories of Bell's Scale | | Overgrowth % | Hyphal Interaction |
|---|---|---|---|---|---|
| | | 96 hours | 120 hours | | |
| *T. atroviride* | ACP8 | 1 | 1 | 100 | E-P-V-G-L |
| | CBA2 | 2 | 1 | 100 | E-L-V |
| | CBA3 | 2 | 1 | 85 | E-V-G-L |
| | CBA4 | 1 | 1 | 100 | E-L-V |
| | CBA5 | 1 | 1 | 100 | E-P-L-V |
| *T. clado-koningii* | CBCK1 | 1 | 1 | 70 | L-V |
| *T. gamsii* | CBG1 | 3 | 3 | 20 | L-V |
| *T. ghanense* | CBGe1 | 1 | 1 | 100 | E-V-G-L |
| *T. harzianum* | CBH1 | 2 | 1 | 100 | L-V |
| | CBH2 | 1 | 1 | 100 | E-P-V-G-L |
| | CBH3 | 2 | 1 | 100 | L-V |
| | CBH4 | 1 | 1 | 100 | E-V |
| *T. konigiopsis* | CBK1 | 2 | 1 | 100 | E-L-V |
| | CBK2 | 2 | 1 | 100 | E-P-L-V |
| | CBK3 | 1 | 1 | 100 | E-L-V |
| | CBK4 | 2 | 1 | 100 | E-P-L-V |
| | CBK5 | 1 | 1 | 100 | E-L-V |
| | CBK6 | 1 | 1 | 100 | E-L-V |
| *T. longibrachiatum* | CBL1 | 2 | 2 | 75 | L-V |
| | CBL2 | 3 | 3 | 30 | L-V |
| *T. ovalisporium* | CBO1 | 1 | 1 | 100 | E-V-G-L |
| | CBO2 | 1 | 1 | 100 | E-V-G-L |
| | CBO3 | 1 | 1 | 100 | V-G |
| *T. sulpureum* | CBS1 | 1 | 1 | 100 | E-V-G-L |
| *T. viride* | CBv1 | 2 | 2 | 100 | L-V |
| *T. virens* | CBvs1 | 4 | 4 | 0 | Without interaction |

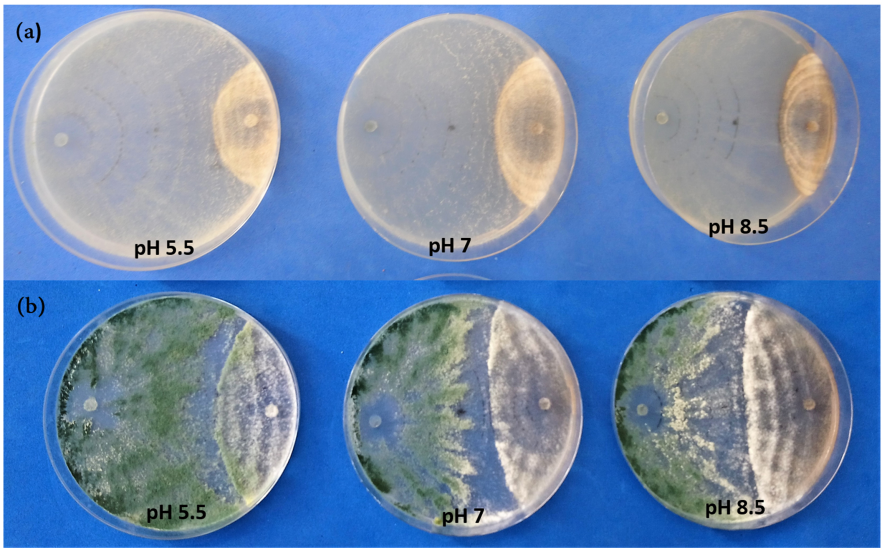


**Figure 3.** Differences in the action mechanisms for the control of *Rhizoctonia solani* of *T. atrovitidre* CBA3 (a) and *T. atroviride* ACP8 (b) at 25˚C and different Ph.

## 4. Discussion

The severe damage caused by *R. solani* in the potato production systems justifies the search for alternative control methods in the framework of a sustainability scheme. In this respect, *Trichoderma* has proven to be a cosmopolitan fungus, with high biological control activity over different phytopathogens [3]. Previous research proposes the inclusion of different species and strains of *Trichoderma* with variable results in the control of diseases, disregarding the effect of temperature and pH on the biopesticide-pathogen interaction mechanisms.

In this respect, studies carried out on pathogens other than *R. solani* have reported that the medium pH affects the growth and survival of *Trichoderma* [1] [6] [24]. In this regard, it has been reported that the mechanisms of biological control are maximized based on temperature, thus, improving the *Trichoderma*/phytopathogen interaction [25]. It is worth noting that both, temperature and pH are abiotic factors that determine the antagonist activity of *Trichoderma* [4].

According to our results, it was found that in 69% of the strains tested growth was greater at 25˚C, regardless of the pH value of the medium. This response is linked to the mesophilic nature of *Trichoderma* in accordance with that proposed by previous studies [4] [11]. The temperature range between 25˚C and 30˚C has been suggested for the greatest growth of *Trichoderma* [6] [9] [26] [27]. However, there are strains, such as *T. viridae* of Indian origin [28] and *T. harzianum* coming from Turkey [10] that show their greatest growth at temperature even higher than 40˚C. The isolations tested in our research did not evidence growth at the extreme temperatures tested (10˚C and 35˚C). According to the results obtained in the local isolations, the growth capacity was dependent on the strain, in consistency with what Kuc̈uk and Kivać (2004) [8] and Karaoglu *et*

*al*. (2018) [10] reported for strains of other origins.

Although *Trichoderma* grows in a wide range of pH [4] [13] [29], this was not observed when pH was increased in the tested strains. In 50% of the cases, a decrease of growth was reported as pH was increased, at 25˚C. It should be noted that in 26% of the strains, growth increased under conditions of 15˚C and pH 5.5.

According to our results, it was generally observed a greater growth of *Trichoderma* at 25˚C and pH 5.5, depending on the degree of response more related to the tested strain than to the species.

With respect to the pH values, similar results were found by Abeyratne and Deshappriya (2018) [1] when testing *Trichoderma* over *Fusarium* spp. and Bhai *et al*. (2010) [24] over *Phytophthora capsici* under warehouse conditions.

Likewise, the antagonist capacity of the different *Trichoderma* strains when faced to *R. solani* showed a higher PRGI value at 25˚C and pH 5.5. Favorable conditions in the growth medium would benefit *Trichoderma* and they would increase the production of metabolites and enzymes, since they promote the regulation of the genes involved in the biosynthesis [30] [31].

It should be highlighted that the strain behavior is a determinant factor since, as per our results, *T. atroviride* CBA4 (PRGI = 32.6%), *T. konigiopsis* CBK5 (PRGI = 31.9%) and *T. virens* CBvs1 (PRGI = 4.7%) kept their antagonistic capacity, regardless of the medium temperature and pH values.

In most of the tested strains, a pH value of 5.5 favored the control mechanisms over *R. solani*. This is due to the increase in the enzymatic activity that takes place on the cell wall [1] and to the increase in the production of volatile composites that suppress the growth of the pathogen [32].

It must be highlighted that a temperature of 25˚C and a pH value of 5.5 were not factors that, by themselves, decreased the growth of *R. solani*, since according to data reported by Raza *et al*. [33] and Ritchie *et al*. (2009) [34], such conditions would favor the mycelial growth and its survival.

## 5. Conclusions

In conclusion, this study demonstrated a greater growth of *Trichoderma* at 25˚C and pH 5.5, depending on the degree of response more related to the tested strain than to the species. Under these conditions, the control mechanisms over *R. solani* were favored.

The response of each strain in relation to the variation of abiotic factors (temperature and pH) in the growth environment would enable us to understand the population dynamics and the degree of antagonism of each strain faced by *R. solani*, thus, allowing the identification of the most efficient strains in their growing medium. In relation to the above, it is also clear that certain strategies of edaphic fertilization induce changes in certain *Trichoderma* strains as a biopesticide of *R. solani*, associated with a modification in the pH value of the soil.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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