Effects of Temperature on *in vitro* Response of *Trichoderma* Strains Against Strawberry Pathogen *Rhizoctonia solani* Kühn.

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Abstract

Effect of temperature on growth and antagonistic ability of *Trichoderma* spp. isolated from local strawberry culture and commercial product, against *Rhizoctonia solani*, strawberry pathogen, was studied *in vitro*. Trials were carried out twice, at 10, 25 and 30°C. Inhibitor effect was evaluated by radial growth measures of established duals on PDA's dishes, using Royse and Ries formula, to evaluate the percentage inhibition of radial growth. Design of dishes was a randomized complete block, considering 10 replicates. Data were analyzed statistically by two-way analysis of variance. The objective has been to determine the most competitive *Trichoderma* strain and the best temperature that produce the inhibiting effect on the pathogen growth. Local strain has the best behavior at 10 and 25°C.

Keywords: *Rhizoctonia solani*; *Trichoderma* spp.; biocontrol; strawberry; temperature

INTRODUCTION

Trichoderma antagonistic activity is influenced by abiotic environmental factors. Temperature supposes an outstanding factor in the natural distribution of *Trichoderma* spp. in soil and their antagonistic activity. Hyphal interactions between *T. harzianum* Rifai and *Rhizoctonia solani* Kühn had been described like a mycoparasitic process (ELAD *et al.* 1981; BENHAMOU & CHET 1993).

The objective of this work had been to determine the most competitive *Trichoderma* strain and the best temperature rank that produces an inhibiting effect on the studied pathogen growth.

MATERIALS AND METHODS

Inhibition on the growth of *R. solani* by the six *Trichoderma* strains (Table 1) was evaluated by dual culture. Disks (6 mm diameter) were removed from the edge of expanding colonies (seven days old cultures grown on potato-dextrose agar – PDA at 25°C) to Petri dishes (85 mm diameter) with PDA. Dual cultures were placed on opposite side of the dishes containing

Table 1. Organisms used in this study, their isolation origin and their abbreviations

Organism	Isolation origin	Abbreviation
Rhizoctonia solani Kühn	strawberry fruit in Moguer (Huelva)	R
Trichoderma harzianum Rifai	commercial product Hors-Solsain	TA
Trichoderma atroviride P. Karsten	commercial product Tusal	TB
Trichoderma atroviride	commercial product Tusal	TC
Trichoderma inhamatum Veerkamp & W. Gams	commercial product Tusal	TF
Trichoderma viride Pers.	soil from local strawberry field	TJ
Trichoderma harzianum	commercial product Hors-Solsain	TM

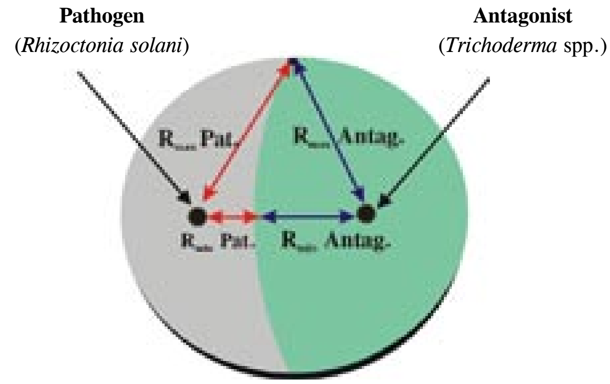


Figure 1. Graphic diagram of the measured parameters of the dual cultures on PDA plates

PDA and separated 5 cm in between the disks. Dishes were incubated in dark at 10, 25 and 30°C, according to the registered soil temperatures in the field at Huelva (SW of Spain). Growth parameters, minimum and maximum radial growth (Figure 1), were read for each isolate and dual culture after 24, 48, and 72 h, 4, 7 and 10 days. The percentage inhibition of radial growth (ROYSE & RIES 1978) was used to measure the inhibition of *R. solani* in dual cultures.

$$\% \text{ inhibition} = \frac{R_{max} - R_{min}}{R_{max}} \times 100$$

The statistic design of the dishes was a randomized complete block, considering 10 replicates per dual culture. The data were assessment and two-way analysis of variance were performed. Mean separation of data was determinated by Duncan's multiple rank test (PETERSON 1985; BANKOLE & ADEBANJO 1996). Values in percentages were analyzed statistically after carrying out angular transformation.

RESULTS

Effect of temperature on the isolated growth. *R. solani* shown its best growth conditions in PDA at 25 and 30°C, covering the 100% dishes surfaces 7 days after the sowing, and had a slow growing at 10°C, reaching the 41% growing area in 10 days. It is according with PARMETER (1970) who reported that optimum growth rate usually goes from 20 to 30°C. Minima temperature may range from at least 0–10°C and maxima from at least 29–38°C. Seven days after the sowing, all considered *Trichoderma* strains reached 90–100% of growing surface at 25°C, and 97–100%, at 30°C, except TB with a slow growing (60%) at this last temperature. At 10°C, the observed growing was smaller, fluctuating between 6 and 54% in 10 days. These data agree with the optimum growth reported by other authors (WIDDEN & SCATTOLIN 1988; KLEIN & EVELEIGH 1998). *Trichoderma* TJ, isolated in strawberry fields located in Moguer (Huelva), reached the greatest growing percentage for all the temperatures, with an optimum at 25°C in which the 99% of the growing surface was reached after 3 days of the sowing.

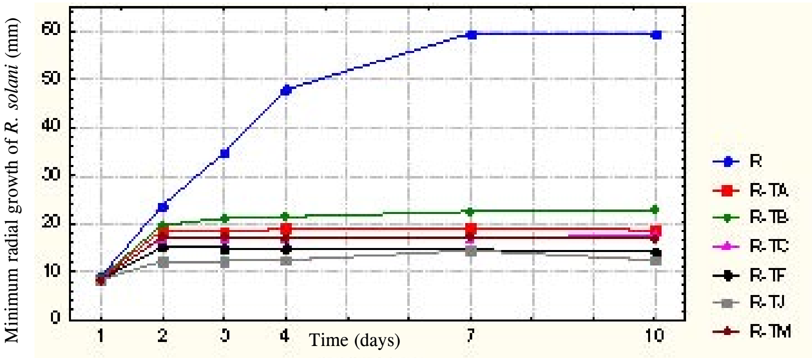
Dual culture of* Trichoderma *strains and* R. *solani (Table 2). At 10°C the greatest inhibition of *R. solani* was showing by TJ, with 67% inhibition of radial growth, which gave significant differences with the all rest of *Trichoderma* strains. The greatest inhibition radial growth at 25°C was showing by TF, TM and TA, with more than 60% of the inhibition area. There were significant differences with TB and TC. The best data at 30°C were about 60% inhibition, belong to TB and TA, and there were significant differences with TC. Figures 2 and 3 show the evolution

Table 2. Percentages inhibition of radial growth of *R. solani* after 10 days on dual culture against *Trichoderma* strains (measured by Roise and Ries formula)

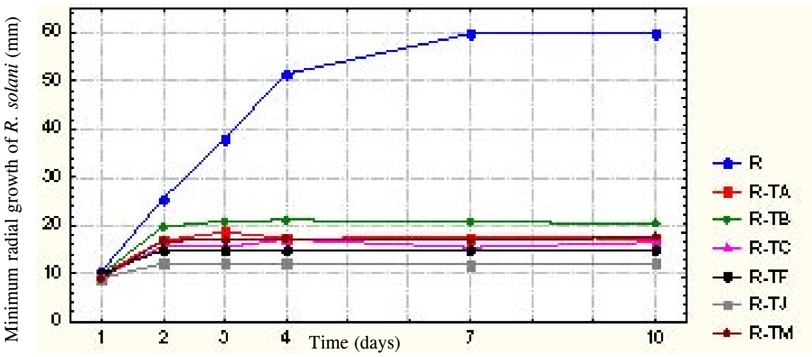
Rhizoctonia–Trichoderma	10°C[b]	25°C[a]	30°C[a]	S.D. between 10–25°C	S.D. between 10–30°C
R–TA	46.51bc	60.08[a]	59.00a	P = 0.000068**	P = 0.000154**
R–TB	26.93e	56.15b	60.27a	P = 0.000144**	P = 0.000063**
R–TC	50.22b	52.72c	51.33b	P = 0.411964	P = 0.702187
R–TF	43.16c	60.25[a]	56.11ab	P = 0.000067**	P = 0.000271**
R–TJ	67.06a	56.77ab	55.36ab	P = 0.000809**	P = 0.000306**
R–TM	37.09d	60.12a	57.53ab	P = 0.000630**	P= 0.000144**

Data followed with different letters indicate significant differences ($P < 0.05$)

**Following indicate high significantly differences ($P < 0.01$)



Figures 2. Evolution on time of the minimum radial growth of *R. solani* "per se" and against different *Trichoderma* strains, at 25°C



Figures 3. Evolution on time of the minimum radial growth of *R. solani* "per se" and against different *Trichoderma* strains, at 30°C

on time of the minimum radial growth of *R. solani* per se and against *Trichoderma* strains, at 25 and 30°C, respectively. There were significant differences between the inhibition effects of each *Trichoderma* isolate tested at 10 and 25°C, and between 10 and 30°C over the pathogen radial growth, except by TC. Between 25 and 30°C there were not statistically differences.

DISCUSSION

Trichoderma presence inhibits the growth of *R. solani* at all considered temperatures.

When each dual is observed at the three temperatures, in all of them the best inhibition was achieved at 25°C, except for TJ and TB strains. TJ produces the greatest inhibition at 10°C, and TB, at 30°C. Although, both strains are the most inhibiters at each temperature in comparison with the other strains.

Local strain (TJ) could be considered as the best *Trichoderma* strain to control *R. solani*, it had been in the greatest inhibition group at all tested temperatures. It could be as one of the best biocontrol agents. Forward, it must be considered the initial inoculum of the local strains and the possibility of the artificial inoculation of the fields with the autochthon *Trichoderma* strains.

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