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INFLUENCE OF ABIOTIC FACTORS ON *IN VITRO* GROWTH OF *TRICHODERMA* STRAINS

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The effect of temperature and pH on the radial growth rate and biomass yield of two strain of *Trichoderma* (Td85 and Td50) with hight biocontrol potential was studied. Four incubation temperature (15°C, 25°C,30°C,35°C) and four values of pH (4.5; 5.5;7.5;8.5) were tested. A significant difference in the biomass production was recorded between strains and among the pH levels tested.The most favorable pH range was between 4.5 and 5.5 in which biomass yield varies between 1.5 g and 2.46 g. The fungus studied tolerates a different range of pH levels but growth was reduced on alkaine media between 7.5 and 8.5. Also, our results showed that both strains studied was grown better at 25–30°C and very slow at 15 °C.

Keywords: temperature, pH, mycelial growth, fungi.

INTRODUCTION

Trichoderma spp. is a fungus that exists in almost all soils type and a wide range of habitats. *Trichoderma* spp. strains are of great importance as biocontrol agents, should have better stress tolerance levels than the plant pathogens against which they are going to be used for biological control under field conditions[7]. Microorganisms such as filamentous fungi *Trichoderma* regulate their metabolism in response to changing environmental conditions.

Temperature, pH, water activity and soil type are key factors in the growth and development of the fungal mycelium. Understanding the influence of this abiotic factors is relevant for biotechnological and agronomic application of *Trichoderma* fungi.

The abiotic factors can deteriorate the antagonistic properties of *Trichoderma* spp. against the phytopathogenic fungi. As in all micro-organisms, in *Trichoderma* spp. the external factors modify its morphological characteristics as well as physiological functions[13, 17]. Among these factors, pH is probabil the most important environmental parameters affecting the mycoparasitic activities of *Trichoderma* spp.[8, 14]. The studies on the variation of pH by different researchers revealed that *Trichoderma* spp. showed optimum growth and biomass production at different pH values ranging from 4 to 7[2, 4, 7, 8, 11, 12, 17]. However some authors[9, 10] showed that different strains of *Trichoderma* grow under high values of pH between 9–11 range.

Different studies are avilable on the effects of temperature on the spore germination and mycelial growth. The optimum temperature for growth differs among *Trichodema* species[7, 18]. *Trichoderma* strains with biocontrol potential are applied in agricultural soils with different pH value and in areas with variation of temperature. Therefore , it is also of great importance to collect information about ecophysiological of this genus.

The present study investigated effects of abiotic factors (pH and temperature) on the radial growth and biomass yield of some *Trichoderma* strains (Td85 and Td50).

MATERIAL AND METHODS

The experimental material consisted of two strains of *Trichoderma* spp. obtained from RDIPP collection. Strains were identified on the basis of morphological characteristics as Td50 (*Trichoderma viride*) and Td85 (*Trichoderma pseudokoningii*). Test fungi were mantained on PDA slants and stored at 4 °C in refrigerator.

A five days old colony culture of both strain studied grown on potato dextrose agar (PDA) was used to obtain spore suspension. Ten milliliters of sterile distilled water containing 0.05 % Tween 80 was added to Petri plate and conidia were carefully scraped from the surface of the colonies before filtration through sterile cloth. Spore suspension were ajustated to 10^6 spores/mL used heamo-cytometer Neubauer and 20 µL aliquotes of suspensions were inoculated at the centre of Petri plate and then incubated for 4 days at 15 °C, 25 °C, 30 °C, 35 °C.

The radial growth of each mycelial colony was measured in two perpendicular directions until the plate were completely colonized. Three replicates were used for each experimental temperature conditions.

The two strains of *Trichoderma* were assessed for biomass yield at different pH on potato dextrose medium (PDB). PDB medium with different pH values 4.5; 5.5; 7.5; 8.5 were prepared and ajusted with 0.1N NaOH and HCl 3% before autoclaved at 121 °C for 20 minute. PDB medium was inoculate with spore suspension with concentration of 10^6 spore/mL previously obtained. All the treatments were carried out in triplicate. The mycelium was collected after 7 days of incubation at 25°C and 150 rpm by filtration through sterile cloth and fresh weight of biomass was measured in grams.

RESULTS AND DISCUSSION

Both strains studied were developed at all pH values examined but registered variation both between strains and between pH values. Observations made at 7 days after incubation at 25°C revealed that the amount of biomass of Td85 strain was maximum 1.60 g at pH=5.5 followed by 1.52 g at pH=4.5. In other variants (pH=7.5–8.5) of pH, amount of biomass (1.35–1.36 g) produced by Td85 did not differ significantly (Figure 1).

From the data presented in Figure 1 we observed that for Td50 strain a maximum amount of biomass (2.46 g) was recorded at pH=4.5 this quantity decrease linearly to other values of pH attain 1.7–1.9 g. We observed a decreased production of biomass at basic values of pH. This results are in accordance with those of[3, 5, 6, 15]. However, (10) found that maximum weight of mycelium of *Trichoderma harzianum* strains was recorded at pH=7–7.5 and observed a decrease grow at pH=6 and a minimal grow at pH=5.

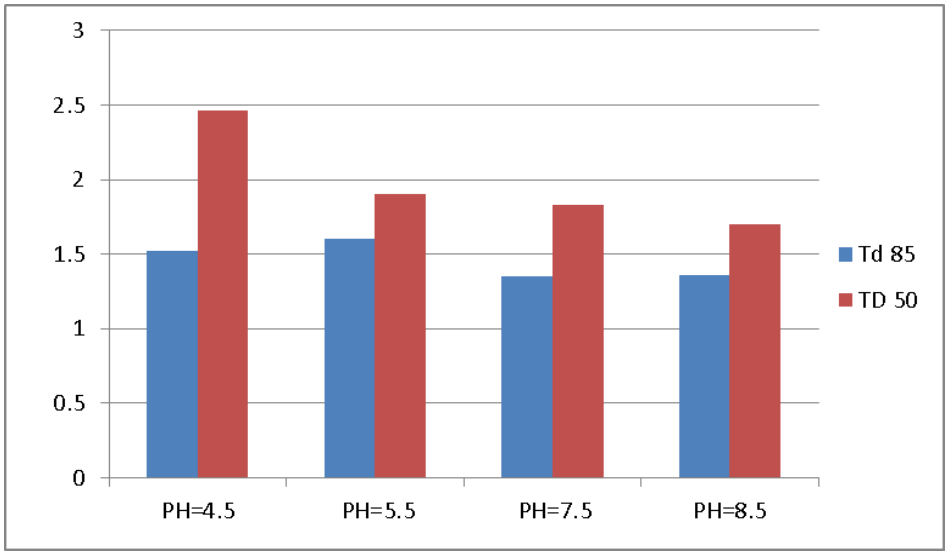

Figure 1. Effect of pH on biomass amount produced by *Trichoderma* strains after 7 days of incubation at 25°C.

Our results reflected that strains analyzed (Td85, Td50) had an optimum amount of biomass at pH values between 4.5 and 5.5. Lower values of the amount of biomass produced by the two strains of *Trichoderma* were recorded between 7.5 and 8.5 range of pH.

Kredics, 2003, 2004[7, 8] have shown that *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma aureoviride* isolates are able to develop in a large range of pH between 2 and 6. They establish that some strains have a maximum growth at pH 4 under acidic conditions. Then

studies of [4, 11, 12, 15] supported that acidic soil favored *Trichoderma* strains growth rather than alkaline and change soil rhizosphere pH by acidification. In studies conducted by Singh *et al.*[16] has beeen demonstrated the ability of some *Trichoderma harzianum* strains to grow in a wide range of pH[3–11] and so was claimed that this feature makes them suitable to be applied in different types of soil with variable pH.

Study of the influence of temperature on growth of two strains of *Trichoderma* studied showed that after two days of incubation at 15 °C growth radius was around 0.5 cm. At 25 °C, the TD 85 strain had a growth radius of 2.7 cm, significantly higher than the growth radius of TD 50 strain which was 1.3 cm (Figure 2).

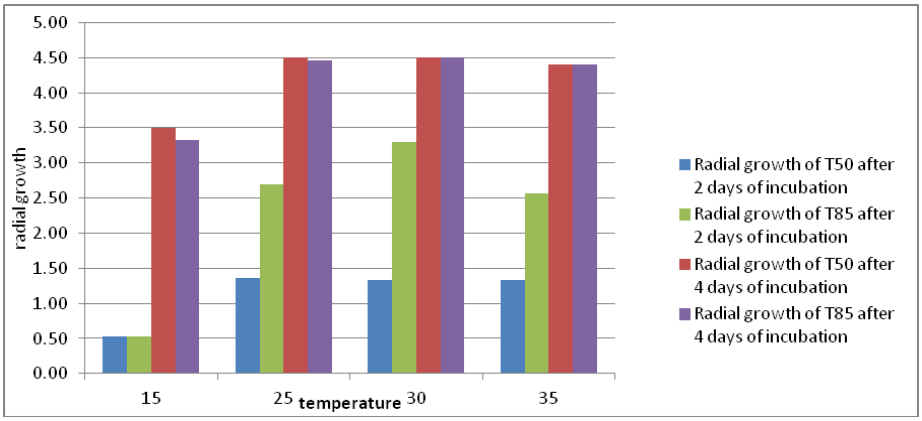

Figure 2. The influence of temperature on the radial growth of fungal microorganisms analyzed.

At a temperature of 30 °C was recorded also growing more pronunced of Td85 strain (3.3 cm) compared with TD 50 (1.3 cm). At a temperature of 35 °C growth of Td85 strain was slower, growth radius after 2 days of incubation was less than 3 cm while the Td50 strain had a similar growth with that of the 30 °C.

The observation performed at 4 days of incubation at 15°C revealed that growth radius of two strains studied ranged between 3.3 and 3.5 cm. In the variants with temperature 25 °C, 30 °C, 35 °C after 4 days, there was an increase of 100% for both strains of *Trichoderma* spp. studied (Figure 3).

Td85 strain had in most variants of temperature, faster growth compared to Td50 strain. The optimum temperature for growth for both strains was between 25 °C and 30 °C. These results are in accordance with those of[17] who reported that the most favorable temperature for growth and sporulation of *Trichoderma atroviride* strain was between 25–30 °C.

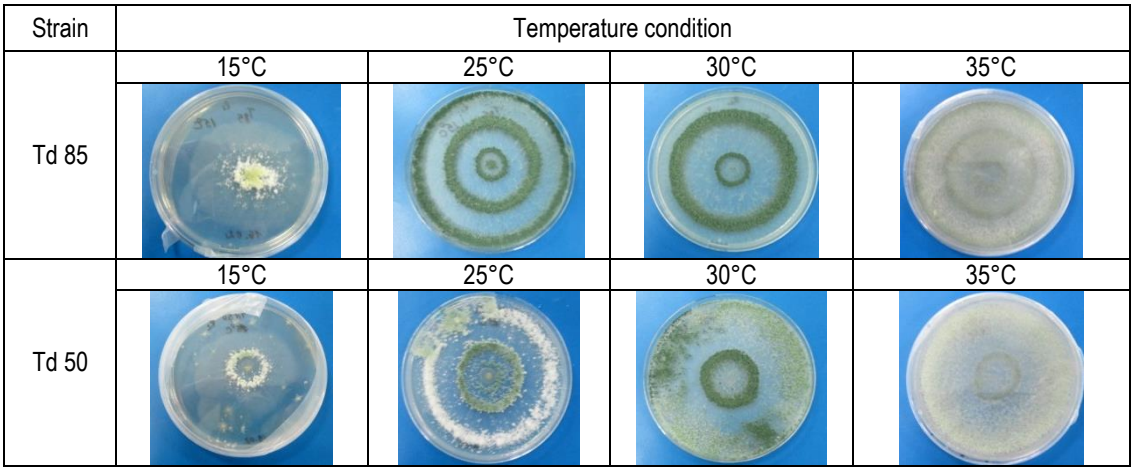

Figure 3. Growth and sporulation of *Trichoderma sp*. Td50 şi Td85 after 4 days of incubation on PDA medium at different temperatures.

Also, our studies are similar with the results obtained by[3] with different *Trichoderma asperellum* strains. Studies of[10, 18] establish that a *Trichodrerma harzianum* strain has a maximum growth at temperature between 25–30 °C and a minimum increase at 35 °C and have not observed any growth at 45 °C. Similar results were found on the other species of *Trichoderma* by[8]. However[1], testing 360 *Trichoderma* strains for cold tolerance establish that strains of *Trichoderma aureoviride*, *Trichoderma viride* grew well at 5°C.

CONCLUSIONS

The optimum temperatures for growth of the strains *Trichoderma viride* (Td50) and *Trichoderma pseudokoningii* (Td85) was between 25 °C and 30°C.

Both strains studied had a very slow growth at 35°C.

The strain of *Trichoderma pseudokoningii* (Td85) had higher rate of growth compared to the strain *Trichoderma viride* (Td50).

The Td85 si Td50 strains showed optimum mycelial growth between pH 4.5 to 5.5.

We observed that the amount of biomass produced by the two strains decreases between pH 7.5 to 8.5.

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