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Full Length Research Paper

Effect of pH on growth of the mycelium of *Trichoderma viride* and *Pleurotus ostreatus* in solid cultivation mediums

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In the cultivation of oyster mushroom (*Pleurotus* spp), various contaminants moulds such as *Trichoderma* spp that are often found in production plants, leading to losses exceeding 77% in a short periods of production were identified. This research studied the effect of pH on two solid cultivation mediums, potato dextrose agar (PDA) and yeast complete medium (YCM), evaluating mycelial growth and the rate of development of strains of *Trichoderma viride* and *Pleurotus ostreatus*. The pH levels studied were 7, 9 and 11 approximately which were obtained with sodium hydroxide (NaOH-1N), $Ca(OH)_2$ and $CaS0_4$ (lime and gypsum commercial); the control group has a pH of 5.7 according to the manufacturer. The strain of *P. ostreatus* (CP-50) obtains a development rate of 6.10 mm / day at pH 11.2 presenting tolerance at alkaline pH; however, the strain of *T. viride* (CP-T4) has a rate of development of 0.41 mm / day at pH 11.2 showing negative effects on growth and development in alkaline pH.

Key words: *Trichoderma* spp., *Pleurotus* spp., pH, development rate, mycelial growth.

INTRODUCTION

The production of *Pleurotus ostreatus* has been reduced to small and medium scale, due to the fact that technology is not known to a vast majority of rural producers. The technology used so far by the small and medium producers is based on the inappropriate use of information for cultivation, besides the problems of contamination by green mold causing economic losses to producers (Martinez-Carrera et al., 2000; Ortega-Garrido, 2002; Aguilar et al., 2002).

Proper management in the cultivation of oyster mushroom can produce large amount of edible mushrooms in a little space and it is widely accepted in both urban and rural areas, because of its high nutritional value. Given that the oyster mushroom is a food with 350 calories, it is richer than red meat that contains 150 or the fish that contains 101 calories, and has immunological and antitumor properties (Del Toro et al., 2006; Sarangi et al., 2006).

The concern to avoid the occurrence of *Trichoderma* spp in the cultivation of oyster mushroom is also reflected in the methods used in preparing the substrate for growing (Fermor, 1987; Flegg et al., 1987). Most of them consist in the selection of strains of high yield and good biological selection or fostering the development of thermophilic bacteria such as *Bacillus subtilis*, *Bacillus macerans* and *Bacillus pumilus* during fermentation of the substrate to reduce pollution (Bonilla, 2006).

The fungicides have also been used to protect the substrate from competitor's moulds, such as selectivity against *Trichoderma* spp by adjusting the pH and the substrate to values higher than 7.5 through limestone fermentation, but none of these methods fully solve the problem (Angelika and Riesen, 1998; Harman, 2000; Romero, 2007; Rojo et al., 2007).

This study assessed the effect of pH on the growth and development of strains of *Trichoderma viride* (CP-T4) as compared to strains of *P. ostreatus* (CP-50) in two solid cultivation mediums, with the addition of sodium hydroxide

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Table 1. Treatments studied in this investigation, its code and the amount of substance or product added to the cultivation medium, YCM and PDA to adjust the pH.

Treatment	Code	Expected initial pH	Medium quantity (ml)	Substance and product added to the culture medium	
				Substances	Quantity
PDA + SQ	Control	5.6	100	-	-
	NaOH -7	7.0	100	NaOH 1 N	210 µl
	NaOH -9	9.0	100	NaOH 1 N	990 µl
	NaOH -11	11.0	100	NaOH 1 N	2,500 µl
PDA + PQIND	Control	5.6	100	-	-
	PQIND -7	7.0	100	$Ca(OH)_2$: $CaSO_4$	0.09 g: 0.4 g
	PQIND -9	9.0	100	$Ca(OH)_2$: $CaSO_4$	0.9 g: 3.5 g
	PQIND -11	11.0	100	$Ca(OH)_2$: $CaSO_4$	1.32 g: 5.3 g
YCM + SQ	Control	5.3	100	-	-
	NaOH -7	7.0	100	NaOH 1 N	650 µl
	NaOH -9	9.0	100	NaOH 1 N	1,030 µl
	NaOH -11	11.0	100	NaOH 1 N	1,620 µl
YCM + PQIND	Control	5.3	100	-	-
	PQIND -7	7.0	100	$Ca(OH)_2$: $CaSO_4$	0.05 g: 0.18 g
	PQIND-9	9.0	100	$Ca(OH)_2$: $CaSO_4$	0.5 g: 1.8 g
	PQIND-11	11.0	100	$Ca(OH)_2$: $CaSO_4$	0.7 g: 2.5 g

PDA= Potato dextrose agar, YCM= yeast complete medium, SQ= chemical substance, NaOH= sodium hydroxide, PQIND= industrial chemical products, $Ca(OH)_2$= lime, $CaSO_4$= gypsum.

1N, lime and commercial plaster.

MATERIALS AND METHODS

In the present study we used the strains called CP-T4 of *T. viride* Pers and CP-50 of *P. ostreatus* (Jacq, ex Fr)Kumm. The strains were kept on potato dextrose agar (PDA) and were deposited at the School Laboratory of Agroforestry Engineering, Regional Unit Tetela-BUAP (Meritorious Autonomous University of Puebla). The chemical (SQ) used to adjust the desired pH in the cultivation mediums was sodium hydroxide at 1 Normal (NaOH-1N, Biochemika, Switzerland). In the case of industrial chemicals (PQIND), these were purchased in the market town of Puebla as commercial lime or calcium hydroxide, with chemical formula $Ca(OH)_2$ and a level of 82% purity, and the commercial gypsum or calcium sulfate, with chemical formula $CaSO_4$ and 88% purity level. Both the SQ and the PQIND were added directly to the culture medium to adjust the pH to 7, 9 and 11 approximately. Strains CP-50 and CP-T4 were studied in the following cultivation mediums: a) Potato Dextrose Agar (PDA) and b) Yeast Complete Medium (YCM), which were prepared in line with the manufacturer's instructions (Sobal et al., 1989; Romero, 2007).

The cultivation medium was poured into 500 ml bottles (Duran, Germany) and sterilized at 121^{o}C for 20 min. Later, under aseptic conditions in a laminar flow chamber (Vecco, Mexico) were added specific amounts of chemical (SQ) and industrial chemicals product (PQIND) using a micropipette (Pipetman, USA) to adjust the required pH (Table 1). The bottles with culture medium were gently shaken with the help of a magnet and a stir plate (Corning, USA) for homogeneity. The cultivation medium was poured into sterilized plastic Petri dishes of 90 mm in diameter, depositing a volume of 20 ml of PDA and yeast complete medium YCM, within a laminar flow hood (Vecco, México).

The data considered in this research include the macroscopic characteristics of strains CP-T4 and CP-50 as texture, density, aerial mycelium, color of the mycelium, as well as the growth speed (VC= mm / Number of day), rate of development (TD= VC_{Final} - $CV_{Initial}$ / Number of days), the initial pH was determined homogenizing a sample (ca. 20 ml) of cultivation medium in the time of inoculation. We obtained the average of three independent readings. The final pH was determined using a sterile agar plate without inoculation and with an average time of the final day of the experiment, was homogenized with the aid of a sterile spatula. We obtained the average of three independent readings (ca. 20 ml). The experimental design used is completely random with ten replicates per treatment, as in previous studies conducted by Martinez-Carrera et al. (1986) and Sobal et al. (1989). Data was processed using the program GraphpadInstat tm. V 3.0 for Windows. Thereafter we performed an analysis of variance (ANOVA) subsequently and we applied test Tukey-Kramer of multiple comparisons ($\alpha = 0.05$) to determine the statistical differences between treatments.

RESULTS

T. viride

The macroscopic characterization of the colonies of strain of *T. viride* Pers. (CP-T4) in the different treatments studied, showed effect of pH on the texture density of the

Table 2. Macroscopic characteristics of strain of *Trichoderma viride* Pers.(CP-T4) and the rate of development after inoculation in potato dextrose agar (PDA) and yeast complete medium (YCM) adjusted with SQ and PQIND.

Treatment	Code	Initial pH	Macroscopic characteristics of the strain CP-T4				Development rate (mm/day)*	Final pH
			Texture	Density	Aerial Mycelium	Color		
PDA	CONTROL	5.7	Al	Ab	Ab	Green	11.10 a	5.4
+	NaOH-7	7.3	Al	Ab	A	Green	10.10 b	6.9
SQ	NaOH-9	9.2	A	Re	E	Light Green	4.80 c	9.0
	NaOH-11	11.2	La	E	E	Yellow Green	0.70 d	11.1
YCM	CONTROL	5.4	Al	Ab	Ab	Dark Green	10.80 a	5.1
+	NaOH-7	7.3	Al	Ab	Ab	Green	11.20 b	7.0
SQ	NaOH-9	8.9	La	Re	Re	Light Green	6.20 c	8.7
	NaOH-11	10.8	La	E	E	Yellow Green	0.50 d	10.7
PDA	CONTROL	5.6	Al	Ab	Ab	Green	11.30 a	4.9
+	PQIND-7	7.2	Al	Ab	Ab	Green	10.10 b	6.1
PQIND	PQIND-9	9.3	Al	Ab	Re	Light Green	8.80 c	8.6
	PQIND-11	11.2	A	Re	E	Light Green	0.41 d	8.8
YCM	CONTROL	5.4	Al	Ab	Ab	Green	11.20 a	5.0
+	PQIND-7	7.4	A	Re	E	Light Green	9.70 b	6.3
PQIND	PQIND-9	9.2	A	Re	Re	Light Green	6.60 c	8.4
	PQIND-11	11.3	A	Re	E	Light Green	0.80 d	9.9

SQ = Sodium Hydroxide 1N, PQIND = Chemical Industrial Products, A = Velvety, Al = Cottony, Ab = Abundant, La= Woolly, Re = Regular, E = low. Different letters in the same column indicate significant differences with the multiple ranges Tukey Kramer test (α 0.05).

colony, aerial mycelium and spores color, varying with respect to the alkalinity (Table 2). The mycelial growth speed of strain CP-T4 of *T. viride* in cultivation medium PDA + SQ, was affected by pH in the treatments studied, showing significant differences between control and alkaline treatments. It registered a growth of 86.33 mm in the control group, followed by NaOH-7, NaOH-9 and NaOH-11 with 77.33 mm, 37.33 mm and 05.50 mm at 9 days (Figure 1).

The final values of pH, decreased in all cases with respect to the initial pH, registering variations of 0.4 - 0.1, in the control group from 5.7 to 5.4, NaOH-7 of 7.3 to 6.9, NaOH-9 from 9.2 to 9.0 and NaOH-11 of 11.2 to 11.1 respectively.

In the cultivation medium YCM + SQ, the rate of mycelial development was affected by pH in the studied treatments, showing significant differences between control and alkaline treatments. It registered a growth of 88.33 mm in the control group, followed by NaOH-7 with 71.33 mm, NaOH-9 with 39.00 mm, and NaOH-11 with 04.03 mm at 8 days (Figure 2). The final values of pH decreased in all cases with respect to the initial pH, registering variations of 0.3 - 0.17, in the control group from 5.4 to 5.1, NaOH-7 from 7.3 to 7.0, NaOH-9 from 8.9 to 8.7 and NaOH-11 from 10.8 to 10.7.

In the cultivation medium PDA + PQIND, the rate of development was affected by the pH of the studied treatments; there was a growth of 88.33 mm in control group, followed by PQIDN-7 with 79.00 mm, PQIDN-9 with 67.00 mm and PQIDN-11 to 03.04 mm at 9 days of development. The final values of pH decreased in all cases with respect to the initial pH, registering variations of 0.4 to 1.3 in the control group from 5.6 to 4.9, in PQIND-7 from 7.2 to 6.1, in PQIND-9 from 9.3 to 8.6 and PQIND-11 from 11.2 to 8.8 at 9 days (Figure 3).

In the cultivation medium YCM + PQIDN, the rate of development was affected by pH in the treatments studied, showing significant differences between the control and alkaline treatments. In the control group grew by 82.33 mm, followed by PQIDN-7 with 68.00 mm, in the treatment PQIDN-9 was recorded 42.33 mm and in the treatment PQIDN-11 was 04.93 mm on the 8th day of development. The final values of pH decreased in all cases with respect to the initial pH, registering variations of 0.4 - 1.4 in the control group from 5.4 to 5.0, PQIND-7 from 7.4 to 6.3, PQIND-9 from 9.2 to 8.4 and PQIND-11

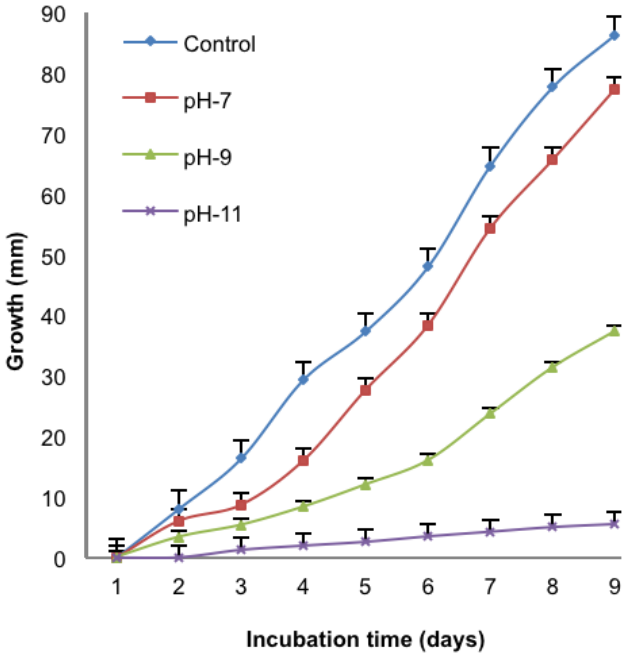

Figure 1. Growth (mm/days) of the strain of *T. viride* (CP-T4) in potato dextrose agar (PDA) with NaOH-1N.

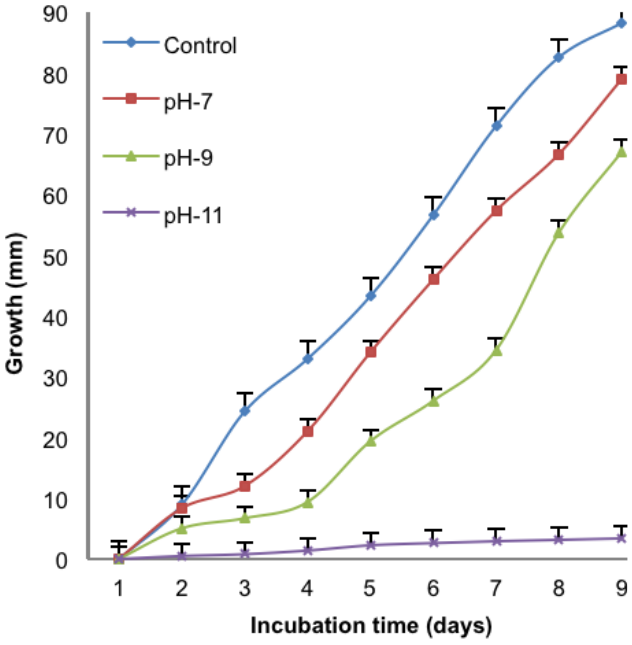

Figure 3. Growth (mm/days) of the strain of *T. viride* (CP-T4) in potato dextrose agar (PDA) with PQIND.

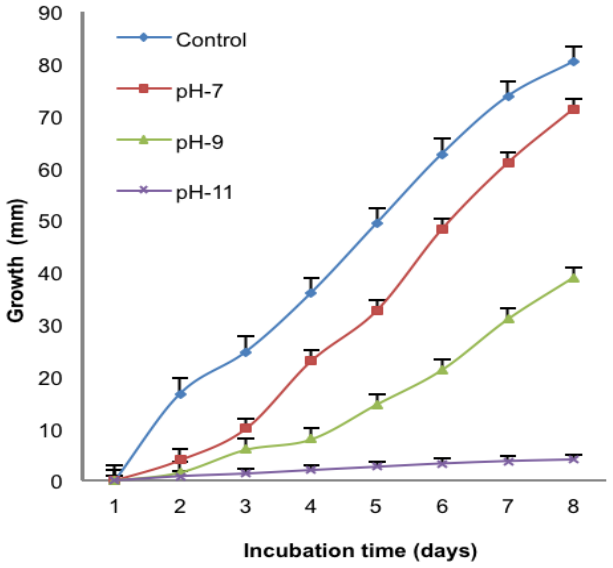

Figure 2. Growth (mm/days) of the strain of *T. viride* (CP-T4) in yeast complete medium (YCM) with NaOH-1N.

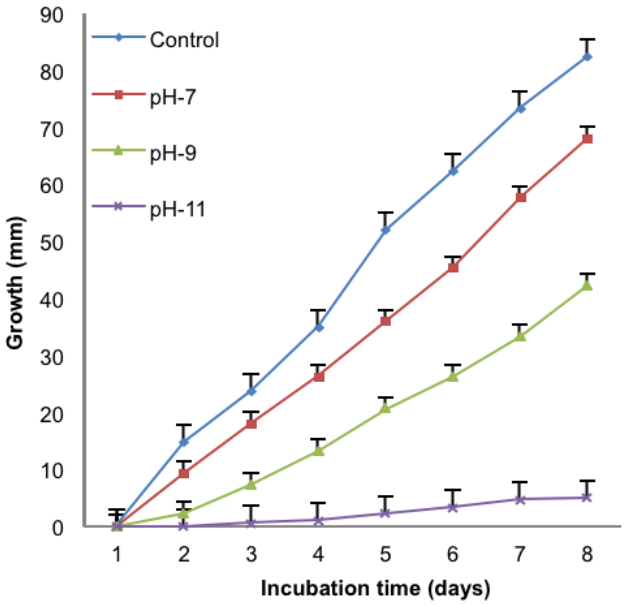

Figure 4. Growth (mm/days) of the strain of *T. viride* (CP-T4) in yeast complete medium (YCM) with PQIND.

from 11.3 to 9.9 on the 8th day (Figure 4).

P. ostreatus

The macroscopic characterization of the colonies of strain of *P. ostreatus* (Jacq. ex Fr) Kumm (CP-50) in the different treatments studied, showed effect of pH on the texture density of the colony, aerial mycelium and spores color, varying with respect to the alkalinity (Table 3). The mycelial growth speed of strain CP-50 of *P. ostreatus* in cultivation medium PDA + SQ was affected by pH in the treatments studied, showing significant differences

Table 3. Macroscopic characteristics of strain of *Pleurotus ostreatus* (Jacq. ex Fr) Kumm (CP-50)and rate of development after inoculation in potato dextrose agar (PDA) and yeast complete medium (YCM) adjusted with SQ and PQIND.

Treatment	Code	Initial pH	Macroscopic characteristics of the strain CP-50				Development rate (mm/day) *	Final pH
			Texture	Density	Air mycelium	Color		
PDA	CONTROL	5.7	Al	Ab	Re	B	10.30a	5.3
+	NaOH-7	7.3	Al	Ab	Re	B	9.40ab	7.1
SQ	NaOH-9	9.5	La	Re	Re	B	8.60b	9.4
	NaOH-11	11.2	La	Re	Re	B	7.00c	10.9
YCM	CONTROL	5.4	Al	Ab	Ab	B	13.20a	4.9
+	NaOH-7	7.3	Al	Ab	Ab	B	12.20b	7.0
SQ	NaOH-9	8.9	Al	Ab	Ab	B	10.60c	8.6
	NaOH-11	10.8	La	Re	Ab	B	8.10 d	10.6
PDA	CONTROL	5.6	Al	Ab	Ab	B	9.80a	4.8
+	PQIND-7	7,2	Al	Ab	Ab	B	7.70b	6.5
PQIND	PQIND-9	9,3	Al	Ab	Ab	B	6.70c	7.8
	PQIND-11	11.2	La	Ab	Ab	B	6.10c	9.6
YCM	CONTROL	5.4	Al	Ab	Ab	B	14.20a	4.8
+	PQIND-7	7.4	A	Ab	Ab	B	13.40b	6.6
PQIND	PQIND-9	9.2	A	Ab	Ab	B	12.00c	8.5
	PQIND-11	11.3	A	Ab	Ab	B	11.10d	9.4

NaOH= Sodium Hydroxide 1N, PQIND= Industrial Chemical Products, A= Velvety, Al= Cottony, Ab=Abundant, B = White, A= Woolly, E=low.
*Different letters in the same column indicate significant differences with the multiple range test of Tukey Kramer (α 0.05).

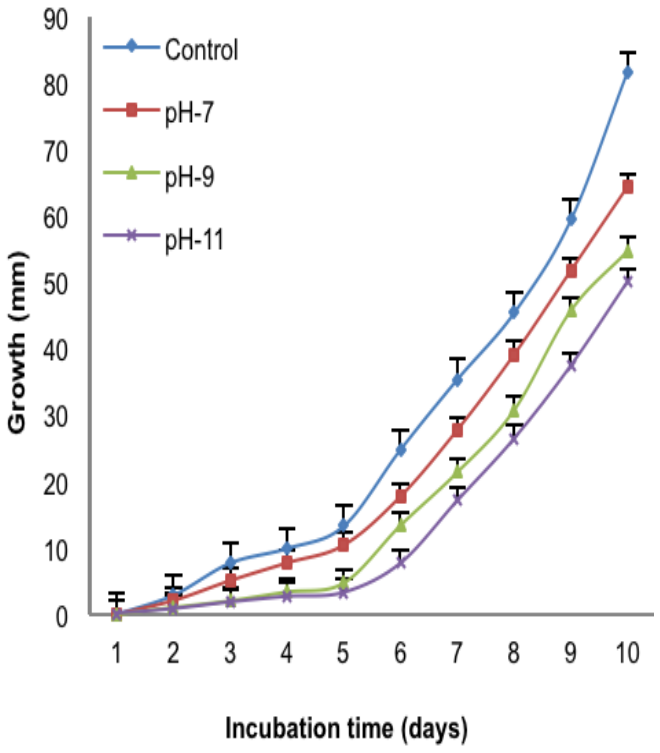

Figure 5. Growth (mm/days) of the strain of *P. ostreatus* (CP-50) in potato dextrose agar (PDA) with NaOH.

between control and alkaline treatments. It registered a growth of 85.33 mm in the control group, followed by NaOH-7 with 77.67 mm, NaOH-9 to 70.30 mm and NaOH-11 to 57.33 mm at 10 days. The final pH values remained almost stable in all cases with respect to the initial pH, registering slight variations in the control group from 5.7 to 5.3, NaOH-7 from 7.3 to 7.1, NaOH-9 from 9.2 to 9.4 and NaOH-11 from 11.2 to 10.9 (Figure 5).

In the cultivation medium YCM + SQ, the rate of development was affected by pH in the treatments studied, showing significant differences between control and alkaline treatments. It registered a growth of 85.67 mm in the control group, followed by NaOH-7 with 76.33 mm, to NaOH-9 66.00 mm and NaOH-11 to 50.00 mm at 10 days. The final pH values remained almost stable in all cases with respect to the initial pH, registering variations of 0.2-0.5 in the control group from 5.4 to 4.9, NaOH-7 from 7.3 to 7.0, NaOH-9 from 8.9 to 8.6 and NaOH-11 from 10.8 to 10.6 (Figure 6).

In PDA + PQIND cultivation medium, the rate of development was affected by the pH of the treatments studied, showing significant differences between the control and alkaline treatments. It registered a growth of 81.67 mm in the control group, followed by PQIND-7 with 64.33 mm, PQIND-9 with 54.67 mm and PQIND-11 with 50.00 mm on the 8th day. The final values of pH decreased in all cases with respect to the initial pH,

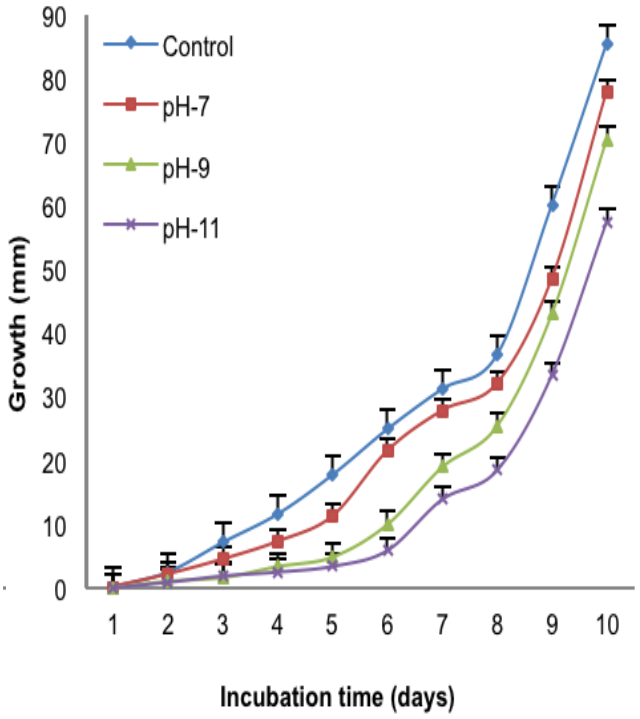

Figure 6. Growth (mm/days) of the strain of *P .ostreatus* (CP-50) in yeast complete medium (YCM) with NaOH.

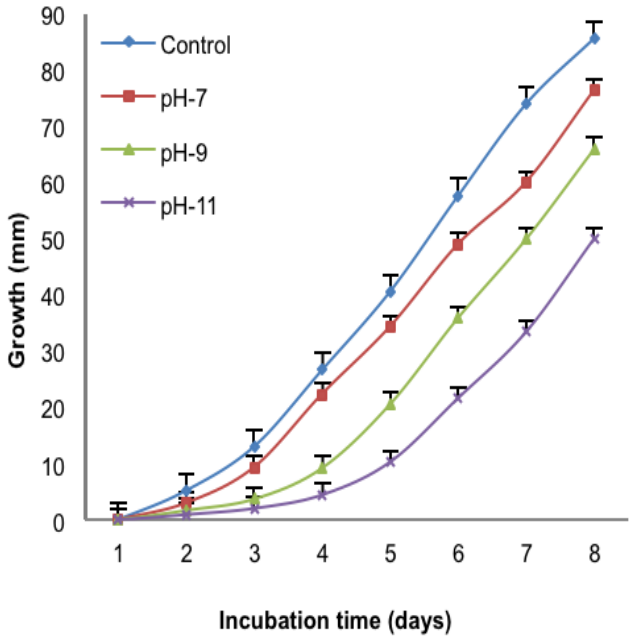

Figure 7. Growth (mm/days) of the strain of *P. ostreatus* (CP-50) in potato dextrose agar (PDA) with PQIND.

registering variations of 0.4-1.3 in the control group from 5.6 to 4.8, PQIND-7 from 7.2 to 6.5, PQIND-9 from 9.3 to 7.8 and PQIND-11 from 11.2 to 9.6 (Figure 7).

In the YCM + PQIND cultivation medium, the rate ofdevelopment was affected by pH in the treatments studied, showing significant differences between the control and alkaline treatments. The control group grew by 89.11 mm, followed by PQIND-7 with 82.67 mm, PQIND-9 for 73.33 mm and PQIND-11 with 67.77 mm on 8th day. The final values of pH decreased in all cases with respect to the initial pH. In the control group low from 5.4 to 4.8 in PQIND-7 from 7.4 to 6.6, in PQIND -9 from 9.2 to 8.5 and PQIND-11 from 11.3 to 9.4 (Figure 8).

DISCUSSION

According to these results, this research showed that there is an effect of pH in its alkaline form in the substances and products studied, inhibiting the growth of the strain of *T. viride* (CP-T4), on the other hand, strain of *P. ostreatus* (CP-50) presented varying degree of tolerance to alkaline pH, in the cultivation medium tested. This means an important advance in the prevention, management and control of the biotypes of *Trichoderma* spp. The studies conducted by Przybylowicz and Donoghue (1988) state that the optimum pH for the development of *Trichoderma* spp is 4.5 to 5.0 in a moist environment. In this research, physiological studies of *T. viride* (CP-T4) in cultivation medium of potato dextrose agar (PDA) and yeast complete medium (YCM) at different pH values adjusted with chemical (SQ) and industrial products (PQIND) indicated a higher rate of development in acidic to neutral values, although the highest rate of development was obtained in the control (11.30 mm / day) on PDA + PQIND medium with an initial pH of 5.6, and lowest (0.41 mm / day) was obtained in the same medium at an initial pH of 11.2.

Bonilla (2006) in *Trichoderma harzianum*, had the highest rate of development in the control group (18.94 mm / day) on PDA medium at pH 5.24, and the lower rate of development was obtained in treatment PDA + SQ (2.0 mm / day) with an initial pH of 11.42.

Romero (2007) in *T. harzianum* had the highest development rate (11.5 mm / day) on PDA medium with an initial pH of 5.6. The lower rate of development was obtained in the NaOH-11 treatment (0.5 mm / day) with a pH of 10.8 in the medium YCM. The results show variation because the strains are different and also the pH levels. The macroscopic characterization of colonies of *T. harzianum* in potato dextrose agar PDA + NaOH; the texture of the colonies was influenced by pH ranging from cottony in the control group to woolly in pH-11. The density varied from abundant in the control group to regular in pH-9 and low in pH-11. The aerial mycelium was affected by pH in the different treatments, the color of the spores varied from light green to yellow green with pH-11. The results show similarity to those found with Romero, 2007 and shows the effect of pH on the development of the strain of *T. viride* on cultivation mediums alkaline.

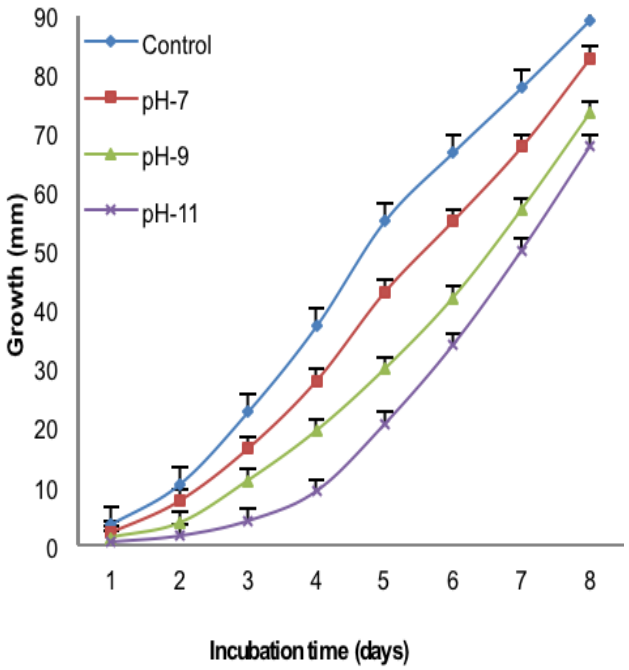

Figure 8. Growth (mm/days) of the strain of *P. ostreatus* (CP-50) in yeast complete medium (YCM) with PQIND.

In the case of *P. ostreatus*, Sobal et al., (1989) was reported that the optimum pH for mycelial development was 5 to 7. However in this investigation, the strain of *P. ostreatus* (CP-50) tolerated cultivation medium with alkaline pH (9-11) in PDA and YCM media adjusted with a chemical (SQ) and industrial chemical products (PQIND).The highest development rates were observed in acidic to neutral values, although the highest rate of development was obtained in the YCM control medium with (14.2 mm / day) at pH 5.1 and the lowest in the middle PDA + PQIND with (6.1 mm / day) to a pH of 10.9. Bonilla (2006) had the highest growth rate in treatment B-7 (9.08 mm / day) on PDA medium at pH 6.9 and the lower rate of development was obtained in treatment B-11 (5.0 mm / day) with an initial pH of 11.1. Romero (2007) had the highest growth rate in the control (14.2 mm / day) in YCM medium, with an initial pH of 5.4, the lower rate of development was obtained in the treatment PQIND -11 (6.1 mm / day) with a pH of 11.2 in PDA medium.

There is little research on this aspect; however, the general tendency seems to indicate that alkaline pH levels (above 8) inhibit the growth of species of the genus *Trichoderma*, as shown in the results obtained in this investigation.

Conclusions

Alkaline pH levels adjusted with sodium hydroxide (NaOH-1N) and industrial chemicals (PQIND) in the cultivation medium inhibited the development of strains of *T. viride* (CP-T4) to 98%; however, the strain of *P. ostreatus* (CP-50) showed levels of resistance, which did not significantly affect their development.

According to studies conducted in this investigation, the strain of *T. viride* (CP-T4) in potato dextrose agar (PDA) and yeast complete medium (YCM) showed slower growth and inhibitor at alkaline pH.

Studies with strains of *P. ostreatus* (CP-50) in potato dextrose agar (PDA) and yeast complete medium (YCM) showed different growth rates and did not affect significantly the development and morphology of their colonies in alkaline pH.

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