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Identification and Evaluation of *Trichoderma*spp Native, Present on eroded soils in Tetela de Ocampo, Puebla-Mexico

Romero Omar[1*], Tello Isaac[2], Damián M.Angel[1], Villareal Oscar[1], Aragón Agustin[1] and Parraguirre Conrado [3]
[1]Centro de Agroecologia del Instituto de Ciencias de la Benemérita Universidad Autónoma de Puebla, MÉXICO
[2]Instituto de Ciencias Biologiocas de la Universidad Autónoma de Morelos, MÉXICO
[3]Instituto de Ciencias de la Benemérita Universidad Autónoma de Puebla, MÉXICO

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Abstract

Is reported the isolation and identification of 9 native strains of Trichoderma *spp., on eroded soils in the municipality of Tetela de Ocampo Puebla-Mexico, pH ranges from 5.3 to 6.8, mainly related to the pine-oak vegetation. It evaluated the growth rate of the strains according to the nomenclature of the collection site: TS1P1, TS1P2, TS1P3, CS2P2, PS3P1, P3P3, SS4P2, RS5P2 and RS5P2 in PDA culture medium, where the strain had the highest TS1P1 growth rate of 3.1 mm / day and a growth rate of 4.0 mm / day, followed by strains TS1P2 with 2.9 mm / day and 3.7 mm / day, CS2P2 with 3.2 mm / day and 4.0 mm / day and underrepresented by strain PS3P1 with 2.4 mm / day and 3.5 mm / day test was applied Tukey's multiple comparison ($\alpha = 0.05$) for comparing treatment means and determine their significance in relation to the growth rate and rate development according to their degree of soil erosion.*

Keywords: isolation and identification, growth rate, rate of development, soil erosion**.**

Introduction

In Latin America and the Caribbean, there are few studies of diversity of *Trichoderma* spp[1], despite the great biotechnological potential that has this kind. *Trichoderma* is often found in decaying wood and in almost all soil types[2-4], in the rhizosphere being a great place to find strains of *Trichoderma*[5]. The Northern Sierra of Puebla presents significant problems in forest degradation, mainly caused by deforestation, expansion of the agricultural frontier on land and steep slopes from poor management soil[6,7]. In soil exist various microorganisms with antagonic capability towards phytopathogens microorganism, the most studied is *Trichoderma* spp., due to its easy and fast growth[8,9]. This is a natural inhabitant of the soil that is characterized by its behavior saprophyte, this property gives antagonic advantages, such as antibiosis, competition for nutrients, mycoparasitism to other fungi, etc., allowing selection and use for biocontrol in certain crops agricultural[10-12].

In the last 10 years there has been research in which has been isolating, evaluating and selecting native species *Trichoderma* spp., with potential for a biological control against various pathogens, which have proposed mechanisms for innovation the implementation of this fungus with satisfactory results, highlighting mainly the inhibition percentage of other fungi as well as plant growth stimulation, providing quality as the most important parameters used in certain production systems[13,14]. *Trichoderma* spp. are highly successful colonizers of their habitats, which is reflected both by their efficient utilization of the substrate at hand as well as their secretion capacity for antibiotic metabolites and enzymes[15,16].

The objective of this research is to identify and evaluate native strains of *Trichoderma* spp., present in eroded soils in the municipality of Tetela de Ocampo (Puebla-Mexico), so as to preserve the acquis in the culture collection of the Mycology Laboratory of the Center for Agroecology the BUAP.

Material and Methods

The study area is located in the municipality of Tetela de Ocampo-Puebla, among the geographical coordinates: 19 ° 43 '00 "to 19 ° 57' 06" and 97 ° 38 '42 "to 97 ° 54' 06" (figure 1), the sampling area was selected based on the degree of soil erosion present in the town and in different locations, such as: La Cañada, El Puerto, Tonalapa, Rancho Alegre and 3rd. section of San Nicolas. After identification the dominant vegetation in this case Pine-Oak, were established three plots per each site. The soil at the site is origin from forest, with a humid temperate climate, the average temperature is between 12 and 18ºC, precipitation of the driest month is less than 40 millimeters and winter precipitation over the year is less 5%. This type of climate covers a large swath of south-central study area[7].

Sampling sites: The samplings being carried in spring 2010, were used plots with nomenclature plot 1 (P1), plot 2 (P2) and plot 3 (P3), for each selected site, five samples were obtained at depths of 20 cm, taking 3 samples of soil from each site, giving a total of 15 samples for the 5 sites (figure 2), according to the classification of the degree of soil erosion (table 1) and adapted this classification to the municipality of Tetela de OcampoPuebla, was derived the following terminology: i. mild-moderate, ii. moderate-strong, iii. strong, iv. strong-very strong, v. very strong[17]. Was determined the pH reading with help from

a potentiometer Thermo Cientific® brand, this to observe the relationship between the degree of acidity and the presence of a microorganism, was made according to the Official Mexican Norm[18], which establishes the specifications of fertility, salinity and the soil classification. The soil samples were kept in jars with capacity of 250 g., at temperature of 8°C, to preserve present microorganisms.

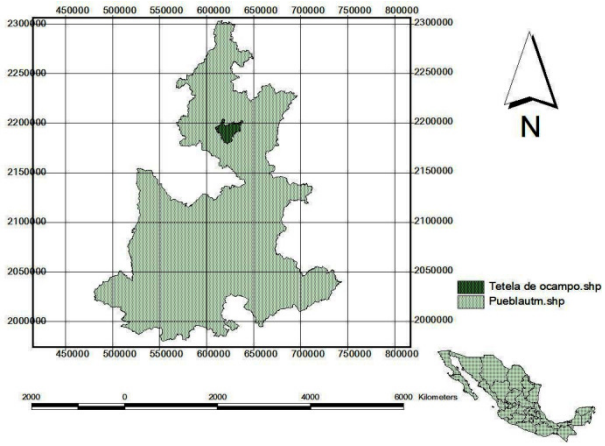

Figure-1
Location of Tetela municipality of Ocampo, Puebla-Mexico

Table-1
Description of the degrees of erosion in relation to profile loss on the horizon, Taking five categories to define the degree of erosion, taken in the manual description of soil profiles and assessing the environment[1]

Grade	Description
Null	When there is no difference with the pattern profile, on the assumption that it is not eroded.
Mild	If when compared to the standard profile, the land in question has lost at least 25% of its original thickness on the horizon A.
Moderate	When soil loss in A is 75% or more.
Strong	When compared soil has lost all the horizon A and at least 25% of B.
Very strong	When the loss of horizon A is total and the B has lost up to 75% or more.

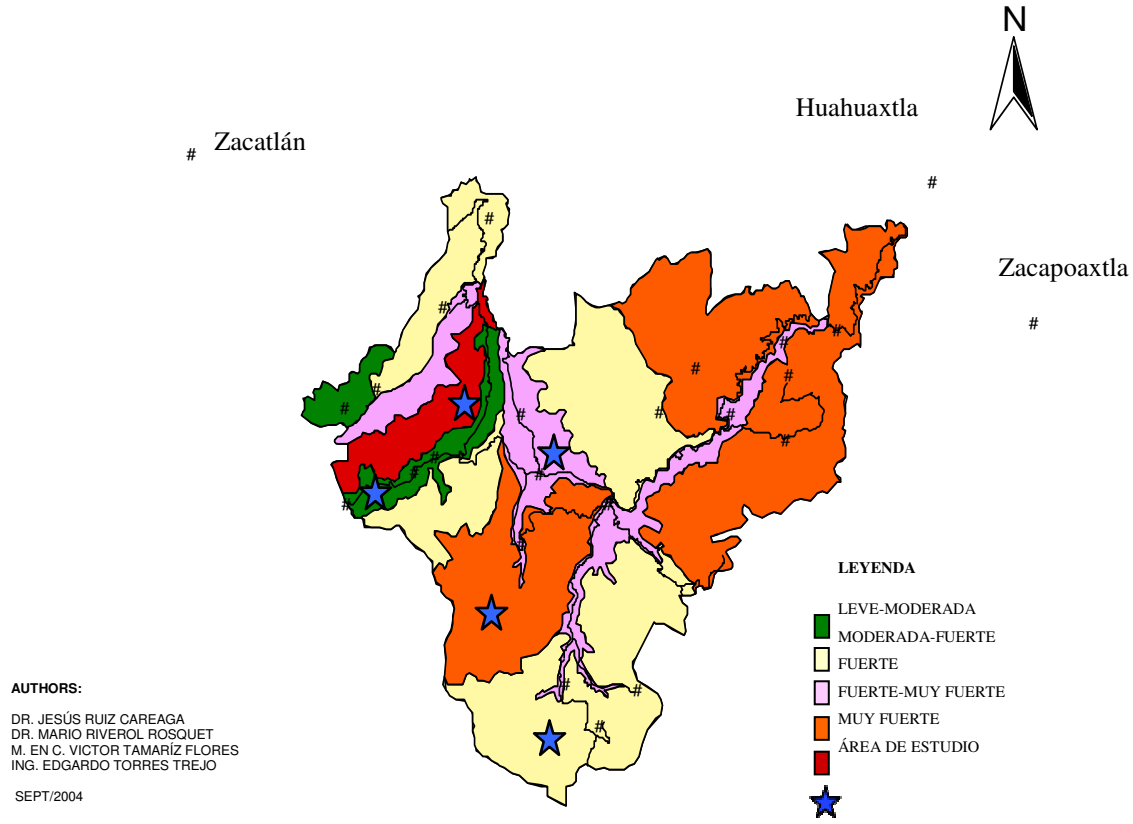

Figure-2
Map of current erosion of soils in the municipality of Ocampo Tetela of Puebla. Scale 1: 50 000

Classification of study sites in Municipality of Tetela de Ocampo Puebla-Mexico: TS1 (Site Tonalapa): According to FAO / UNESCO (1975)[19] this soil is classified as Leptosol, in which the bedrock and the underlying are shales. It is a young soil from formation in situ with a type of laminar erosion of mild-moderate. Its environment is hilly with slopes ranging from about 60% depending on the area, with a very strong human influence due to agricultural activities that caused the loss of horizon A in most of the region and many of these soils are in rejuvenation process since these soils have remained at rest and in other areas with incipient reforestation.

The land use is subsistence agriculture, especially corn, beans and chili, establishing crops without any soil conservation measure, so the soil loss is meaningful.

CS2 (Site Cañada): The plot located in La Cañada, lies on a soil type that is classified by FAO / UNESCO (1975)[19] as Leptosol, the forest sits on a bedrock of limestone and the underlaying rock is also shale. This is a rejuvenated soil from formation in situ with a grade strong-very strong of laminar erosion. Its terrain is hilly and its slope is 66%. This forest has been conditioned by a strong human influence on the environment, which has favored large-scale erosion in many areas due to indiscriminate felling of forests to make way for the continued cultivation of plots on slopes. Are soils with good internal drainage, and very drained externally, present rocky outcrops, 70% of stones and 70% in surface rocks. Land use in subsistence farming and pine forests.

PS3 (Site El Puerto): El Puerto is classified according to FAO / UNESCO (1975)[19] as Cambisol. The bedrock and the underlying rock is limestones. It is a young soil from formation in situ with a strong degree of erosion and laminar type, which, causes you to lose lots of ground superficial runoff due to soil unprotected by indiscriminate logging leaving it exposed risk to erosion caused by rain and wind. Its relief is hilly with slopes of up to 70%, is an internally drained soil, and very drained externally and no rock outcrops. Land use is shared among temporary agriculture and forestry, as water resources has sprinkler and water channel.

SS4 (Site 3rd. section of San Nicolas): According to FAO / UNESCO (1975)[19] is classified as Cambisol soil in which, the bedrock and the underlying, are limestones. It is a young soil, formation in situ with laminar erosion of moderate-strong. Its outline is mountainous with some slopes ranging from 40-60% depending on the area. It is a very drained soil both internally and externally, and no presence of rocky outcrops. Land uses are divided into agroforestry uses emphasis on subsistence agriculture. It goes directly from the litter layer to the B horizon since deforestation allowed the soil bare and unprotected, which made it to miss the horizon A.

RS5 (Site Rancho Alegre): This plot is located on a soil classified by FAO / UNESCO (1975)[19] as a Cambisol. These soils are characterized by soils that originate and evolve in the same place, medium-depth, well-drained, low fertility and very susceptible to erosion due to lack of vegetation cover. Both bedrock as the underlying rock on which is sustained all this forest are acidic. This soil is characterized as a young soil of formation in situ. Due to strong human influence and relief features, with slopes elongated and often above 70%, the rate of erosion is very strong and laminar type and gully. The high rainfall regime and the intense deforestation have caused the loss of an excessive amount of soil through the surface particle entrainment by these steep slopes. The land uses stand out forest use and subsistence agriculture. The lack of vegetation makes it very vulnerable to soil erosion, because soils are shallow and underdeveloped. This has caused the loss of horizon A and AB.

Isolation, Purification and Identification: Samples were processed in the department of mycology (DICA-BUAP), by the method of isolating granules from soil and planted in potato dextrose agar (PDA)[20]. To isolate the fungus was placed 1 mL of culture medium in a Petri box (90 mm diameter)[21,22]. This method consists in using two watch glasses, one with the soil sample and one with sterile distilled water, was taken a sample portion with dissecting needle wetted then the granules of ground were placed in the surface of the Petri dishes with culture medium (PDA) and incubated for 7 days at 26°C[23,24].

After isolation of the fungus and to obtain a pure culture was proceeded to identify was visually observed coloration of the colony in the culture medium (PDA) which turned green, which is one of the characteristics of the genus *Trichoderma*; subsequently with the staining method: preparations in fresh[20], which involves placing a drop of dye (blue lactophenol) in the center of a slide. It took a small portion of the culture with a sterile loop and placed on the drop of dye preparation extending gently with a pair of dissecting needles, the cover plate was covered and it was observed under a microscope (x1600, x1300 y x3300), microscopic structures were identified as characteristics of *Trichoderma* spp., such as spores, conidiophores and chlamydospores[11].

Evaluation of biological activity of *Trichoderma*: The data considered in this research include the macroscopic characteristics of strains, 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). We obtained the average of three independent readings.Data was processed using the program SPSS Statistics version 17 (Statistical Package for the Social Sciences) 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.

Conservation of strains in solid medium culture: Were prepared solid culture media in agar nutrient broth following the instructions of the supplier, and was added 1g of Czapeck Dox,

subsequently were poured 20 mL of culture medium in test tubes with thread capacity of 45 mL, then sterilized at 120 °C in an autoclave, ALL AMERICAN mark, with 20 L for 15 minutes. Once the sterilization time elapsed, the test tubes were positioned in the neck of flute with a hose and allowed to cool at ambient temperature for 12 hrs. Later with the aid of a needle bacteriological is reseeding the previously identified fungus as *Trichoderma* spp., into the nutrient medium and incubated at 26°C for 8 days[25].

Results and Discussion

Were obtained 9 native strains of *Trichoderma* spp., in the municipality of Tetela de Ocampo, Puebla-Mexico of 20 samples taken of each erosion grade from mild-moderate, very strong, strong, strong moderate. Whose classifications are: TS1P1, TS1P2, TS1P3, CS2P2, PS3P1, P3P3, SS4P2, and RS5P2 RS5P2 (table 2), whose macroscopic characteristics in culture medium (PDA) at 26 °C incubation, included cottony colonies from rapid concentric growth within five days with a greenish yellow color and seven days after, full completion of colonization (figure 3).

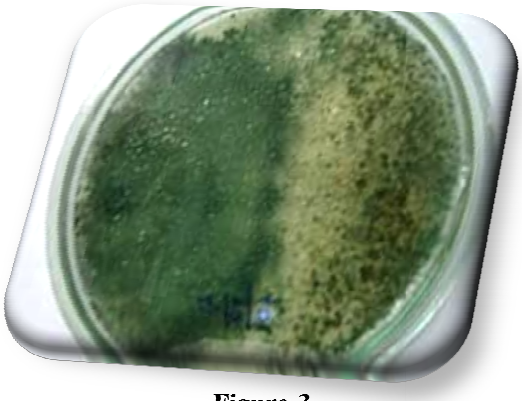

Figure-3
Growth of *Trichoderma*spp native in agar PDA

The pure strains with relevant macroscopic characteristics of the genus *Trichoderma*, taxonomically identified with the aid of a dyeing test, performed by a lactophenol blue staining, and where presented mycelium branched and hyphae; bottle-shaped phialides, and conidia ovoid 3µm, chlamydospores of 11µm in diameter (figure 4). These microscopic characteristics allow an easy identification and *Trichoderma* genus relative, subsequently identified as strains *Trichoderma* spp., were preserved in solid culture medium.

The above data allow to represent the percentages of each degree of soil erosion, 34% obtained mild-moderate erosion, 22% strong and very strong, and 11% strong, moderate-strong and Very Strong.

Evaluation of biological activity of *Trichoderma*spp: The growth rate of strain TS1P1 was 3.1 mm / day, TS1P2 of 2.9 mm / day, TS1P3 with 2.4 mm / day, the strain CS2P2 of 3.2 mm / day, PS3P1 with 2.8 mm / day, PS3P3 with 2.4 mm/ day, the strain SS4P2 presented 2.2 mm / day, RS5P1 with 2.5 mm / day and RS5P2 of 2.2 mm / day (table 3). It had a higher rate of development in strain TS1P1, CS2P2 with 4.1 mm / day, PS3P1 with 3.9 mm / day, TS1P2 of 3.8 mm / day, TS1P3 with 3.6 mm / day, PS3P3 with 3.4 mm / day, RS5P2 and RS5P1 with 3.5 mm / day, finally the lowest growth rate was strain SS4P2 with 3.6 mm / day. The macroscopic characterization of strains *Trichoderma* spp., concerning the texture of the colonies of strains showed differences between the same; observing variations from cottony to woolly, the density and the aerial mycelium of most of the strains was abundant, two strains with regular feature and; two low. The dark green color of the mycelium was observed in most strains, turning to olive green in CS2P2 strain, radial shape of the colonies was characteristic for all treatments (figure 6).

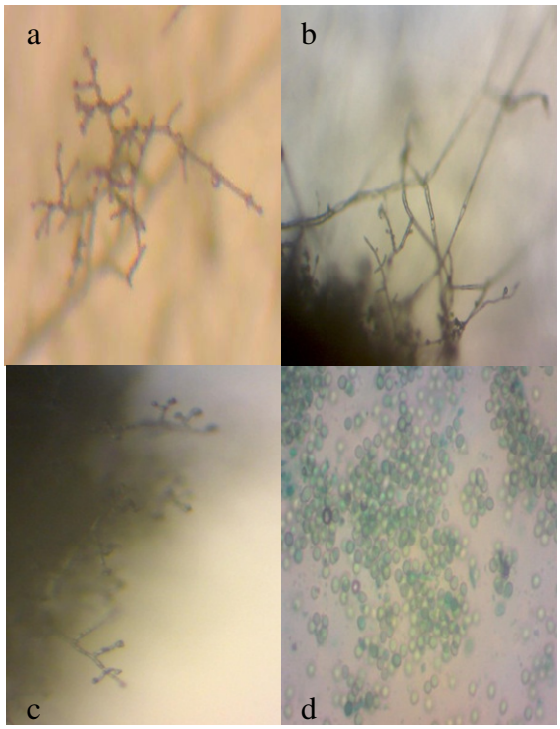

Figure-4
Description: T. harzianum (Rifai), b. conidiophores of pyramidal shape, c, d. phialides, and conidia. Resolution. a, d. x1600 b. x1300 c. X3300

The soil acidity is factor which affects the presence, density and longevity of *Trichoderma* spp., including some species that prefer a determined pH, for example *T. viride* and *T. polysporum* prevail in acid soils and forest soils contaminated with alkali powders, pH 6.6 at low populations recover *T. viride* and *T. harzianum* predominant neutral soils[18]. In a study conducted by Otalora*et al*., [26], obtained a growth rate of 4.35 and 1.66 mm / day in PDA culture medium, in the M45 treatment with a speed of 3.72 mm / day and vel. Long. 1.05

(cm / day) and radial growth 1.52 cm / day. Romero *et al*.[27] in the *Trichodermaviride* (CP-T4) showed growth rates of 86.33 in PDA culture medium and in CYM 88.33 respectively, with abundant sporulation. Guigon*et al*.[10], obtained the radial growth (mm) of *Trichoderma* spp., in strains TC74; 90.0, TvVA; 82.2, TbVA; 72.0, TvB 90.0 and TS01 9.0. Growth rate (mm / h) of 4.77, 4.34, 3.86, 4.64 and 4.71, a witness with 6.84 and 3.67 Wakelin *et al*.[28], obtained the GK7 strain with a growth rate of 1.70 mm / day, the TCR (1.62 mm / day) and a growth rate of 9.33 mm / day. In *Trichodermaharzianum* and *Trichodermakoningii* growth rates were 3.83, 4.37 and 3.43 mm / day, similar in TS1P2 and TS1P1 found in soils with a mild moderate erosion at pH 5.9.

Table-2
Degree of erosion in relation to the presence of *Trichoderma* spp., with the type of vegetation and pH

Code Site	Degree of erosion	pH	Geographic coordinates		Height	Vegetation type	Presence of strains
			N	W			
S1P1	Mild-Moderate	5.9	624070	2186952	1810	Pine	TS1P1
S1P2	Mild-moderate	6.6	627734	2190216	1882	Pine	TS1P2
S1P3	Mild-Moderate	6.8	627690	2190234	1920	Pine	TS1P3
S2P1	Strong- Very Strong	6.7	624049	2187020	1881	Pine-Oak	S/T
S2P2	Strong-Very Strong	6.4	625188	2193020	2155	Pine-Oak	CS2P2
S2P3	Strong-Very Strong	6.7	624035	2186992	2239	Pine	S/T
S3P1	Strong	6.5	625086	2191052	2155	Pine	PS3P1
S3P2	Strong	6.2	627731	2190262	2193	Pine	S/T
S3P3	Strong	5.3	625062	2191061	2239	Pine-Oak	PS3P3
S4P1	Moderate-Strong	6.2	625581	2192965	1711	Pine	S/T
S4P2	Moderate-Strong	6.8	618151	2189996	1751	Pine	SS4P2
S4P3	Moderate-Strong	6.9	625091	2193142	1914	Pine	S/T
S5P1	Very Strong	6.4	617578	2189820	1974	Pine-Oak	RS5P1
S5P2	Very Strong	5.9	618100	2190091	1988	Pine	RS5P2
S5P3	Very Strong	5.8	625048	2191045	1998	Pino	S/T

S1= Tonalapa Site, S2 = La Cañada Site, S3 = El Puerto Site, S4 = 3rd section of San Nicolás and S5 = Rancho Alegre. P = Plots. 1, 2,3 plot number, S/ T = absence of *Trichoderma* spp.

Table-3
Characterization macroscopic colonies of native strains of *Trichoderma* spp. in culture medium and potato dextrose agar (PDA)

Identification key	Macroscopic Characteristics of *Trichoderma* spp.						Development rate (mm/day) *	
	Texture	Density	Aerial mycelium	Color	Shape	Growth rate (mm/day)		
TS1P1	Cottony	abundant	abundant	Green /dark	Radial	3.1	4.1	a
TS1P2	Cottony	abundant	abundant	Green/claro	Radial	2.9	3.8	ab
TS1P3	Velvety	Regular	scarce	Green	Radial	2.4	3.6	bc
CS2P2	Woolly	abundant	abundant	Green/ dark	Radial	3.2	4.1	abd
PS3P1	Cottony	abundant	abundant	Green/ dark	Radial	2.8	3.9	abcde
PS3P3	Cottony	scarce	abundant	Green/ light	Radial	2.4	3.4	bcef
SS4P2	Woolly	Regular	Regular	Green	Radial	2.2	3.6	cfg
RS5P1	Woolly	scarce	scarce	Green	Radial	2.5	3.5	bcfgh
RS5P2	Cottony	scarce	Regular	Green	Radial	2.2	3.5	bcfgh

*Different letters in columns mean statistical difference between the native strains of *Trichoderma* spp., eroded soils Municipality of Tetela de Ocampo, Puebla (Tukey 0.05)

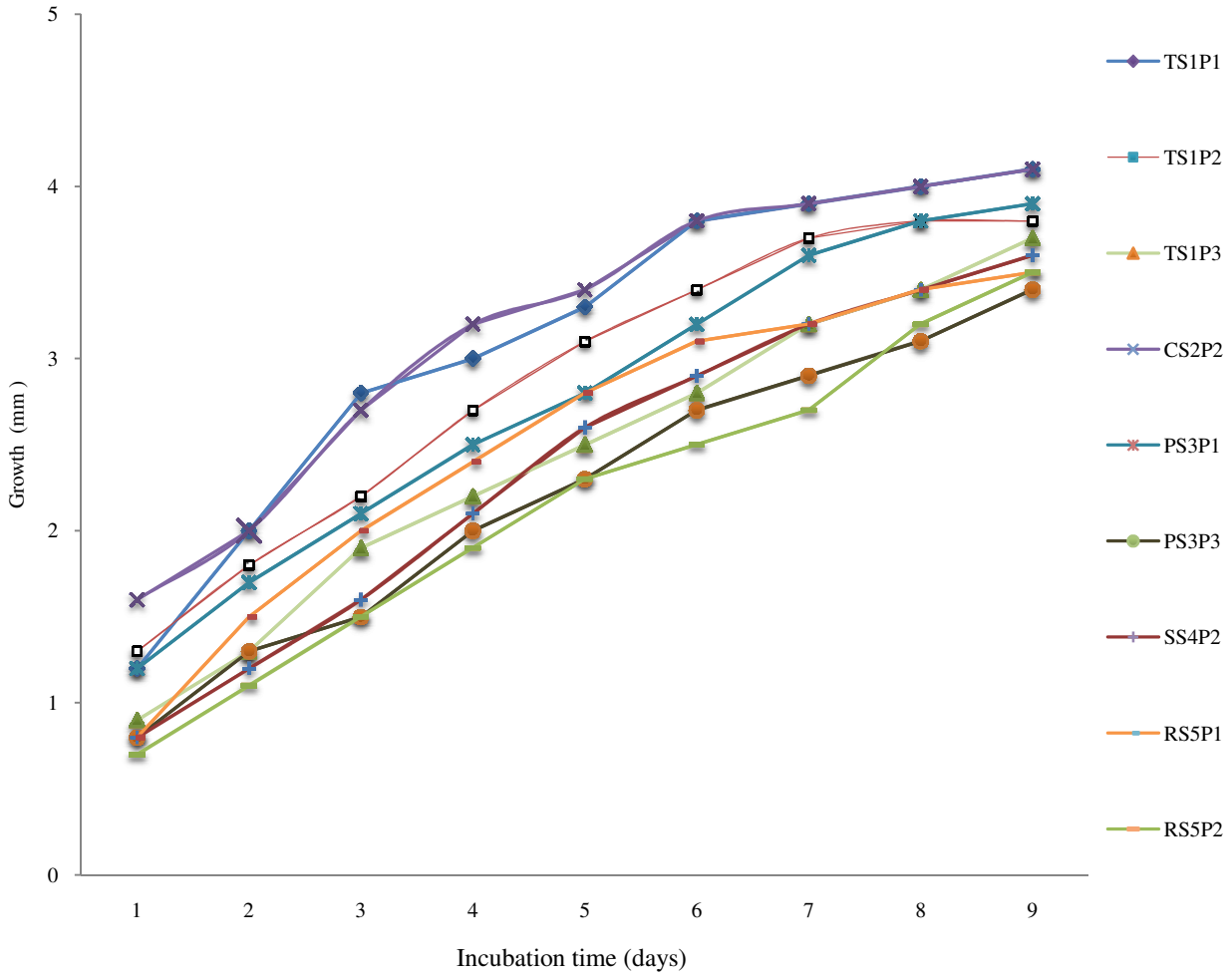

Figure-5
Growth (mm/days) of the strains of *Trichoderma spp.*, in potato dextrose agar (PDA)

Conclusion

From 20 samples tested in the areas of highest degree of erosion, were obtained 9 strains of *Trichoderma* spp., mild-moderate (strains TS1P1, TS1P2 and TS1P3), strong-very strong (CS2P2), strong (PS3P1 and PS3P3), moderate -strong (SS4P2) and the degree of erosion very strong, was found in RS5P1 and RS5P2. It was determined that *Trichoderma* spp., inhabits soil with pH range from 5.3 to 6.8.

The strains identified and evaluated, presenting a large capacity sporulation by its high speed growth, demostrated being highly aggressive organisms regarding the competition for space in culture media PDA and substrates where mainly include TS1P1, with a growth rate of 3.1 mm / day and a growth rate 4.0 mm / day, TS1P2 with 2.9 mm / day and 3.7 mm / day, CS2P2 with 3.2 mm / day and 4.0 mm / day and PS3P1 with 2.4 mm / day and 3.5 mm / day, compared to the strain, proved to be strain SS4P2 the most vulnerable, which, was obtained a growth rate of 2.2 mm / day and a development rate of 3.3 mm / day.

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