Scholars Journal of Agriculture and Veterinary Sciences

OPEN ACCESS

Abbreviated Key Title: Sch J Agric Vet Sci
ISSN 2348–8883 (Print) | ISSN 2348–1854 (Online)
Journal homepage: https://saspublishers.com

Isolation and Characterization of *Trichoderma* spp., for Their Potential Use in Peanut (*Arachis hypogaea*) Crops

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DOI: 10.36347/sjavs.2023.v10i06.002 | **Received:** 14.05.2023 | **Accepted:** 22.06.2023 | **Published:** 28.06.2023

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Abstract **Original Research Article**

A study was conducted isolation and characterization of *Trichoderma* spp. for its potential use in a study of isolation and characterization of mycoparasite fungi from soil was carried out by means of rice traps installed in commercial peanut plantations in the west of Nicaragua. From a total of 100 samples, 37 native isolates of *Trichoderma* spp. were obtained and subjected to different studies. Macroscopic physiological and microscopic morphometric characteristics were evaluated. It was observed that the conidiophores emerge from hyaline hyphae with tubular septate mycelium, these presented characteristics of conical form and verticillate an angle of 450 towards the apical zone of hyaline aspect, with straight ramifications, normally in the form of alternate branches or in pair and sometimes in the form of candelabra, the conidiophores presented a size at the moment of the measurements that oscillated between 25.42 µm - 57.50 µm of length and 1-3 µm of width. The phialides presented a thin citric-form shape at the base, then swollen and curved in the middle part, then becoming thin and pointed in the apical part where the conidia are found in clusters or individually, the sizes at the time of measurement ranged from 9.59 µm - 15µm long. The conidia of all native isolates on the three means of crop propagation, Malt Extract, Papa Dextrose Agar, and Sabouraud Dextrose Agar were 2.92 µm - 5.10 µm long with a green globose to sub-globose coloration. The culture medium did not determine the growth rate, the radial growth rate of the isolates under study depended on time, obtaining the highest growth peak 48 hours after the experiment was established, the length of the conidia did not depend on the isolate or the culture medium, while the size of the phialides did depend on the isolate, in the same way as the conidiophore.

Keywords: Entomopathogenic fungi, mitosporic fungi, biological control, pests.

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INTRODUCTION

Mycoparasitic or antagonistic fungi are widely distributed in nature in diverse ecosystems, where they play an important role in the regulation of epiphytic populations (Infante *et al.,* 2009). *Trichoderma* is considered a biological controller and natural antagonist of phytopathogens and has a wide host range, including important phytopathogenic fungi su.ch as: *Fusarium oxysporum, Botrytis* spp, *Rhizoctonia solani*, *Sclerotium* spp, *Sclerotinia* spp, *Pythium* spp, *Phytophthora* spp, *Alternaria* spp, among others (Sandoval and Lopez, 2000). For most biological controllers, the infection process begins with mycoparasitism of the pathogen (Gürlek *et al.,* 2018), some species belonging to the genus *Trichoderma* cause antagonism; they are facultative pathogens that attack a wide range of hosts (Hu *et al.,* 2014). Agricultural field soils have been reported to harbor high densities of *Trichoderma* spp. compared to different ecosystems within the same region, such as forests and pastures, so they are frequently isolated from soil samples (Steinwender *et al.,* 2014).

The success of a biological pest control program based on biocontrol fungi depends on the production of inoculum for field applications, the selection of strains or isolates with high virulence, good growth, sporulation, and resistance to adverse environmental conditions (García *et al.,* 2015), (Castillo-Arévalo, 2023). In the search for biological control agents, one of the basic strategies is the initial exploration of native natural antagonists, before introducing exotic agents, because native strains are adapted to the environmental conditions of the area; unlike strains of transient microorganisms, which due to

their lack of adaptability may be ineffective in different agroecosystems (Castillo-Arévalo, 2021).

Nicaraguan peanut producers face many phytosanitary problems, according to De Araujo *et al.,* (2015) farmers use less advanced technologies both in the cultivation and post-harvest of peanuts, this has increased phytosanitary problems, among which fungal contamination and aflatoxins stand out, this contamination can occur during seed formation, harvest, transport and storage that added to the interaction with environmental factors, are able to accelerate losses due to product deterioration.

Rojas Jaimes, *et al.,* (2021), indicate that "fungi such as *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., *Alternaria* spp. and *Claviceps* spp. that colonize grains and fruits produce metabolites that are carcinogenic, immunosuppressive, neurotoxic and allergenic in humans. These metabolites are known as aflatoxins. Like many mycotoxins, aflatoxins are secondary metabolites of fungi that contaminate agricultural raw materials and can cause disease in humans and animals. The consequences of aflatoxin poisoning are wide ranging, ranging from high exposure effects to acute effects, including rapid death and chronic effects such as hepatocellular carcinoma (Him *et al.,* 2021).

To reduce aflatoxin contamination produced by fungi, the use of biological products has emerged as an alternative, in the framework of promoting sustainable crop management to improve productivity through nutrient enhancement and protection to phytopathogens (Sherathia, *et al.,* 2016). These effects have motivated the search for more environmentally compatible strategies to regulate these Pathogens. Native isolates of *Trichoderma* have been the most investigated recently (Rezende *et al.,* 2015). *Trichoderma* is the antagonistic fungus that is widely produced and used for the control of aflatoxin precursor fungi in peanut plantations, with annual applications in approximately two million hectares (Iwanicki *et al.,* 2019; Tiago *et al.,* 2012), considering both the germination percentage, as well as the ability to form a colony.

Trichoderma has a rapid and efficient growth capacity to compete with and displace crop pathogens, as well as inhibit the growth of other microorganisms by releasing growth inhibitory compounds. It acts as a bio fungicide against soil pathogens and as a bio stimulant favoring the growth of the plant root system (Meyer *et al.,* 2022). In the Pacific region of Nicaragua, this fungus can be a promising alternative for the control and management of aflatoxin precursor microorganisms in tropical conditions. In this zone, high relative humidity prevails throughout the year, factors that favor the growth of antagonistic fungi (Castillo-Arévalo, 2023).

To explore the agroecosystems of peanut in the western zone of Nicaragua can be viable to isolate native species of *Trichoderma* spp. with potential for the biological control against fungi producers of aflatoxins, they can present variability as for the range virulence of pathogens. For this, the isolation and characterization of antagonistic fungal species is necessary to select promising isolates as biological control agents (Torres de la C. *et al.,* 2013). These studies constitute the basis for the production and scaling of bioproducts that contribute to sustainable agricultural processes in fragile agroecosystems, such as those of western Nicaragua. For the characterization of the strains, it was necessary to consider the macroscopic and microscopic characteristics of the colony (conidiophore, conidium, conidiogenous cell).

MATERIAL AND METHODS

Collection of samples in the field of native isolates of *Trichoderma* spp.

The isolation and purification of *Trichoderma* spp. was carried out in the biopesticide laboratory of the Department of Agricultural and Forest Protection of the Universidad Nacional Agraria, located at Km 12.5 North Highway, Managua, Nicaragua. Five peanut farms located in the department of León were selected for sample collection. Figure 1. The traps were set up in plastic containers, the size of each container was 11 cm in diameter x 12 cm high. Each trap contained 300 grams of sterile parboiled rice. The upper part of the trap was covered with a fine mesh and secured with a 3 mm elastic band to prevent the introduction of excess soil. The depth at which the traps were left was 20 cm and 10 cm from the root system of the peanut plant. These traps were left in the field for eight days. When the traps were removed, they were labeled, then placed in individual plastic bags and stored in thermos flasks to avoid damage due to environmental conditions such as temperature, after which they were transferred to the laboratory for incubation and subsequent processing.

Isolation and purification of native isolates of *Trichoderma* spp.

The isolation method of *Trichoderma* spp was a dry procedure, for this purpose we used a sterile disposable bacteriological loop, a portion of the colonies was taken from the traps brought from the field with the desired characteristics. Before sowing in the Petri dishes each sample of *Trichoderma* spp. was observed under the microscope to confirm that it was the desired colony. The culture medium used for the isolation was Papa Dextrose Agar, to this medium 800 milligrams of amoxicillin were added per liter to avoid bacterial growth, and it was replicated five times for each sample. These Petri dishes were incubated at 25 ± 1 ºC with eight hours of light daily and left for eight days under these conditions. Quality control was

performed every two days, then passages were made in PDA medium again to purify the culture of each isolate.

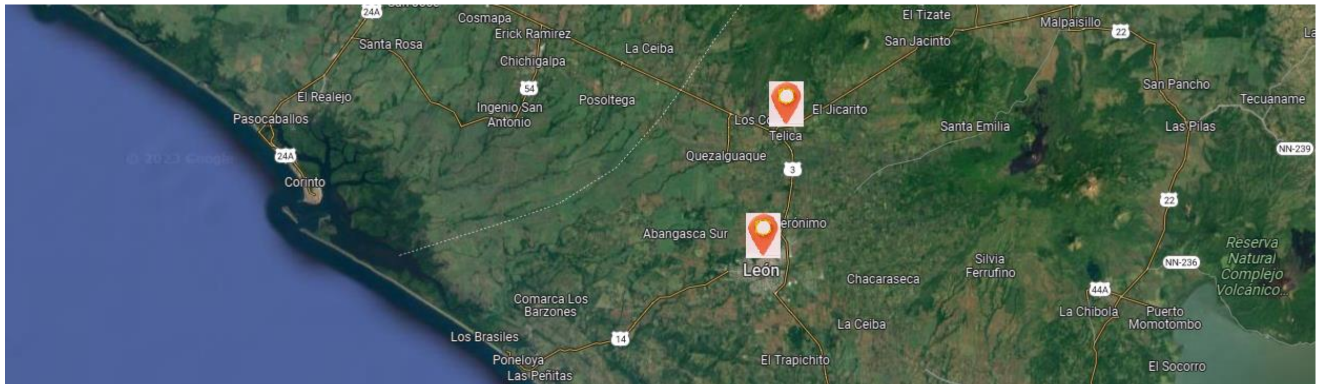

Figure 1: Geographical location of the farms under study

Morphometric and microscopic characterization of native isolates of *Trichoderma* spp.

Microscopic measurements and characterization of structures of each isolate were performed in 90 mm diameter Petri dishes containing acidified Sabouraud Dextrose Agar, Papa Dextrose Agar, and Malt Extract Agar culture media. To avoid contamination with bacteria, an antibiotic based on 2% chloramphenicol was used. The methodology consisted of inoculating a 20 µl aliquot in the center of the plate at a concentration of $1x10^8$ conidia, this study was carried out at a temperature of 26 ± 1°C. The variables considered for the measurements were the reproductive asexual structures such as: phialides, conidiophores and conidia, a previously calibrated Westover-Scientific light microscope and an ocular micrometric lens were used to take the samples, the 40x lens was used for the measurement.

The data were taken 48 hours after the test was established in the culture medium. Thirty structures were measured for each variable for each culture medium (French and Hebert, 1982). Transparent adhesive tape was used in portions of two cm wide and four cm long, which was placed on the colony growth of the isolate, so that the reproductive structures were fixed, 20 µl of lactophenol blue was applied to ensure better visualization of the structures, then the portion of adhesive tape was placed on the slide to perform the reading in the Westover-Scientific light microscope.

Spore production of different native isolates of *Trichoderma* spp. in Sabouraud Dextrose Agar, Papa Dextrose Agar, and Malt Extract Agar media.

Spore production of *Trichoderma* spp isolates was determined on the three-culture media: SDA, PDA and EMA without acidification. An aliquot with a spore concentration of $1x10^4$ was inoculated in the center of the Petri dishes, two dishes per treatment were used for this test in the three means of crop propagation for the 37 isolates for a total of 222 experimental units, incubated at a temperature of 24 ± 1°C. The evaluation of spore production was performed 20 days after the test was established. The methodology for harvesting the spores in the Petri dishes consisted of collecting with the help of a spatula all the contents of the spores from the surface of the Petri dish of 90 mm in diameter, the collected spores were deposited in an Erlenmeyer with 27 ml of water, then the surface of the dish was washed with 23 ml to collect almost all the spores. Once deposited all the spores were shaken for 20 seconds in a vortex at 3000 rpm to homogenize the suspension. From this 1x10-5 suspension, spore counts were performed in a Neubauer chamber and a 40x lens was used for counting. To fill the counting chamber, an aliquot of 20 µl was deposited and the spores were left to rest for five minutes so that the conidia could settle, and the reading could be taken more accurately. For the count, the secondary squares were used, and it was calculated with the following formula described by: (French and Hebert, 1982)

Concentration of conidia=Total conidia x chamber factor x dilution factor Equation 1.

Radial growth of different native isolates of *Trichoderma* spp in culture media Sabouraud Dextrose Agar, Papa Dextrose Agar, and Malta Extract Agar

Once the cultures of each isolate of *Trichoderma* spp were purified, we proceeded to the measurement of the radial growth, this test was done based on the methodology described by French and Hebert (1982), which allows to determine the growth rate in Petri dishes. The technique consisted of taking a portion with a five mm diameter punch from the edge of the stem culture of the colony coming from the culture medium to the corresponding dishes of the same medium and the inoculum was placed in the center of the dish containing the culture medium. Each plate was marked with a cross on the back that delimits four radii identified with letters A, B, C and D on which the readings were taken with a ruler graduated in millimeters. For data collection, a growth margin of 12 hours was left and after this margin, readings were taken every 12 hours; this test was carried out at a

temperature of 26 ± 1^{O}C. For the 37 isolates, five replicates per three culture media were used for a total of 555 experimental units. The resulting data were sorted in a Microsoft Excel database and then analyzed in an analysis of variance.

$$\text{Growth rate} = \frac{(\text{Final growth} - \text{Initial growth})}{(\text{Incubation time})} \quad \text{Equation 2}$$

Macroscopic physiological characterization of native isolates of *Trichoderma* spp. in Sabouraud Dextrose Agar, Papa Dextrose Agar and Malt Extract Agar culture media.

The characterization of the isolates was performed on PDA, SDA and EMA culture media. For this test, a total of 111 Petri dishes of 90 mm in diameter were used, where visual characteristics such as: colony color and growth type were determined. For each isolate 37 Petri dishes were prepared for each culture medium. The Petri dishes were inoculated in the center with the help of a five mm diameter punch, which were incubated with eight hours of light at a temperature of 26^{0}C ±. Data were taken 20 days after inoculation.

Statistical analysis

The data were processed using the statistical package InfoStat (2020). The normality of the data and homogeneity of variances were analyzed. Comparison of means was performed by analysis of variance (ANOVA) and Tukey's test ($p < 0.05$).

RESULTS

Morphometric and microscopic characteristics of native isolates of *Trichoderma* spp. in culture media Sabouraud Dextrose Agar, Papa Dextrose Agar, and Malta Extract Agar

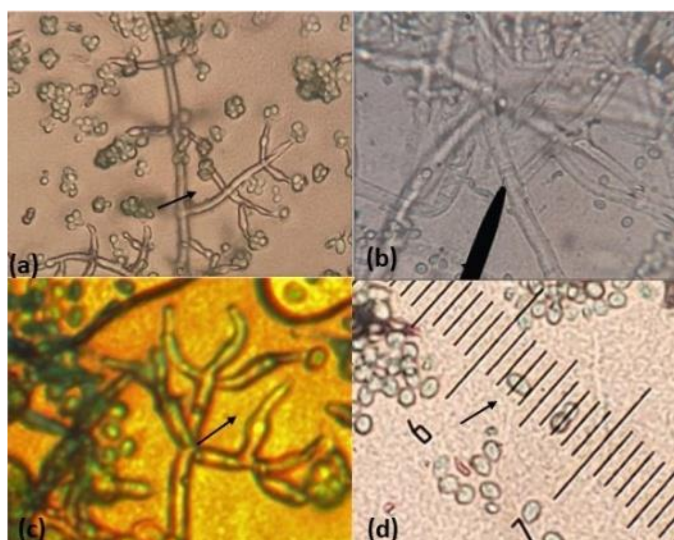

Figure 2: Reproductive structures of native isolates of *Trichoderma* spp in different culture media. Conidiophores (a). Septate mycelium (b). Phialides (c) Conidia (d)

It was observed that the conidiophores emerge from hyaline hyphae with tubular septate mycelium, these presented characteristics of conical shape and verticillate an angle of 450 towards the apical zone of hyaline aspect, with straight ramifications, normally in the form of alternate branches or in pair and sometimes in the form of candelabra, the conidiophores presented a size at the moment of the measurements that oscillated between 25.42 µm - 57.50 µm in length and 1-3 µm in width. The phialides presented a thin citric form shape at the base, then swollen and curved in the middle part, then becoming thin and pointed in the apical part where the conidia are found in clusters or individually, the sizes at the time of measurement ranged from 9.59 µm - 15µm long. Conidia of all native isolates on the three culture media Malt Extract, Papa Dextrose Agar and Sabouraud Dextrose Agar ranged in size from 2.92 µm - 5.10 µm long with a globose to sub-globose green coloration (Figure 1).

Radial growth of different native isolates of *Trichoderma* spp in culture media Sabouraud Dextrose Agar, Papa Dextrose Agar, and Malt Extract Agar at 48 hours of growth at 27 ± 1°C.

The analysis of variance carried out for the variable radial growth of the native isolates of *Trichoderma* spp on culture media under study indicates that the radial growth was different among the isolates, likewise there were statistical differences among the sampling hours ($p<0.0001$), which indicates that the growth of the isolates presents different growth rates, in the same way the hours influence the growth rate of the isolates. The best growth rates were presented by the isolates TSASA4, TFSA5 and TER2 with 11.0 mm every 12 hours (Table 1).

Table 1: Radial growth of native isolates of *Trichoderma* spp. on culture media

Isolated	Growth Radial	Categories Tukey(0.05)	Isolated	Growth Radial	Categories Tukey(0.05)	Isolated	Growth Radial	Categories Tukey(0.05)
TSASA4	11.0	a	TSAA5	10.5	de	TER8	9.8	klm
TFSA5	11.0	a	TEB6	10.5	de	TSAA9	9.8	klm
TER2	11.0	a	TEB3	10.5	de	TFSA1	9.8	klmn
TEB4	11.0	ab	TSAA6	10.4	def	TEB1	9.8	klmn
TSAA8	10.8	bc	TER10	10.3	efg	TFSA2	9.7	lmn
TSASA1	10.8	bc	TSAA2	10.3	egh	TFA1	9.7	lmno
TSASA2	10.6	cd	TFA2	10.1	ghi	TFSA6	9.7	lmno
TER7	10.5	de	TFS3	10.1	ghi	TSASA7	9.6	mno
TFA8	10.5	de	TER6	10.1	hij	TER4	9.6	no
TEBR2	10.5	de	TER1	10.0	hijk	TER5	9.5	o
TFSA4	10.5	de	TEB5	10.0	ijk	TSAA1	7.7	p
TG8	10.5	de	TEB7	9.9	jkl			
TSAA3	10.5	de	TFA4	9.9	jkl			

Means with a common letter are not significantly different ($p > 0.05$)

Effect of the hours on the radial growth of native isolates of *Trichoderma* spp.

The results obtained in the radial growth test of different isolates of *Trichoderma* spp, show that the growth rate of the colonies is determined by time ($p < 0.0001$) which indicates that as the hours pass the growth rate increases (Figure 2). At 48 hours the growth rate was 12.4 mm compared to 12 hours after the test was established with a growth rate of 8.1 mm.

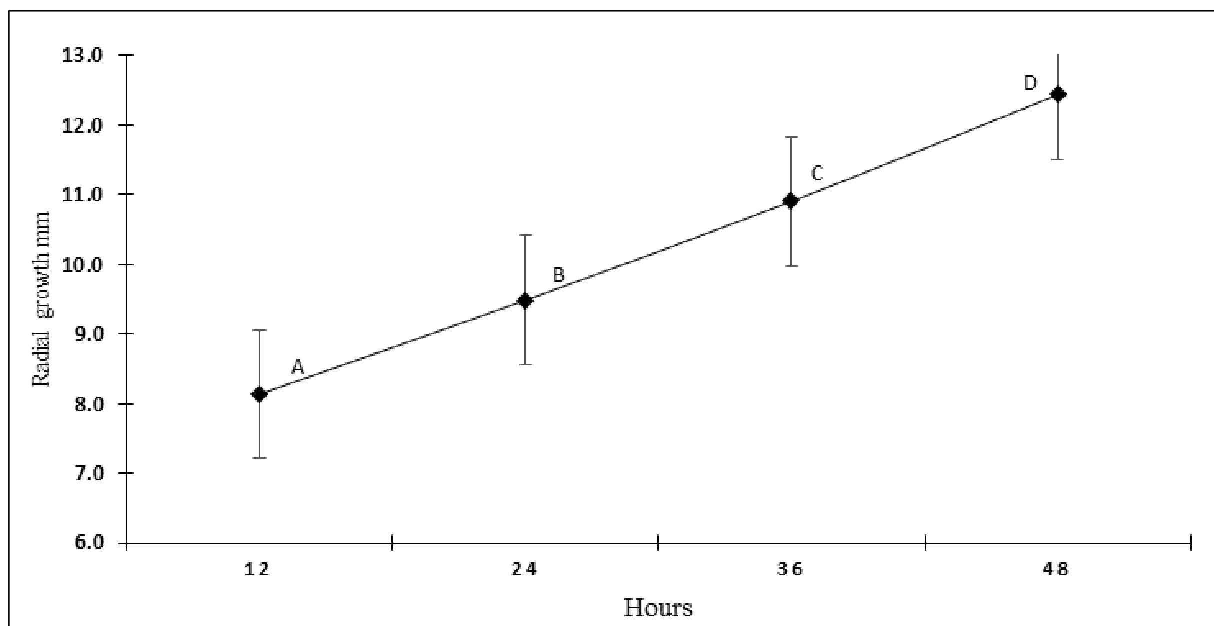

Figure 3: Radial growth behavior of native *Trichoderma* isolates in Sabouraud Dextrose Agar, Papa Dextrose Agar, and Malt Extract Agar media at 48 hours

Macroscopic physiological characteristics of native isolates of *Trichoderma* spp. in Sabouraud Dextrose Agar, Papa Dextrose Agar, and Malt Extract Agar culture media

The colonies of all isolates were white at the beginning with abundant mycelium, at 48 hours they presented green colorations due to the sporulation of the asexual structure's conidia, at 12 days different tonalities and forms of the colonies were observed in the three culture media. For most of the isolates on Papa Dextrose Agar the colonies were intense green without concentric rings, with a white coloration on the back and no exudates. Most of the colonies on Sabouraud Dextrose Agar medium were green with concentric rings and the colony was amber on the back side. The colonies of the isolates on Malta Extract Agar were olive green, most of them without concentric rings and the back of the colony was white.

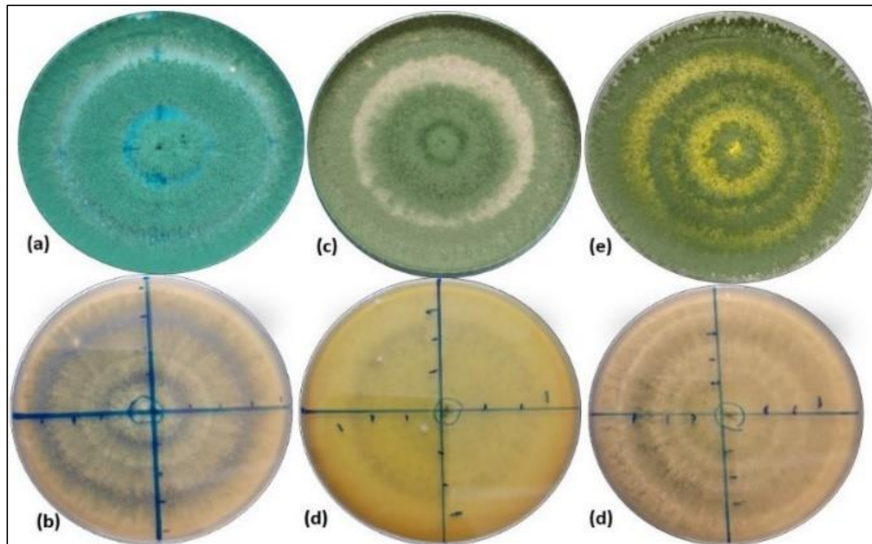

Figure 4: Macroscopic physiological characteristics of native isolates of *Trichoderma* spp. in Sabouraud Dextrose Agar, Papa Dextrose Agar, and Malt Extract Agar culture media

At 12 days of growth at 27 ± 1°C. The colonies of the isolates in PDA were of intense green color with concentric rings, in SDA culture medium the colonies were green with concentric rings and in the culture medium the colonies presented olive green coloration with yellow tones and presence of concentric rings.

Radial growth of *Trichoderma* spp. isolates on artificial media

Table 2: Effect of culture media on the radial growth of native isolates of *Trichoderma* spp.

Culture media	Growth Radial	Categories Tukey (0.05)
SDA	10.240	A
PDA	10.220	A
EMA	10.210	A

Means with a common letter are not significantly different (p > 0.05).

The results of the separation of means by Tukey (0.005) indicate that the effect of the culture media was not significantly different in the radial growth of the isolates, which shows that they can grow indistinctly in any of the three media evaluated.

Spore production of different native isolates of Trichoderma spp. in Sabouraud Dextrose Agar, Papa Dextrose Agar, and Malt Extract Agar culture media.

Table 3: Spore yield of native isolates of *Trichoderma* spp.

Isolated	Spore yield	Categories Tukey (0.05)	Isolated	Spore yield	Categories Tukey (0.05)	Isolated	Spore yield	Categories Tukey (0.05)
TSAA8	3.4E+12	a	TEB3	1.5E+12	bcd	TER7	1.2E+12	bcd
TSASA7	3.4E+12	a	TEB5	1.4E+12	bcd	TEB7	1.2E+12	bcd
TEB1	2.8E+12	a	TFA1	1.4E+12	bcd	TFSA6	1.2E+12	bcd
TFA2	1.8E+12	b	TEB4	1.4E+12	bcd	TER10	1.1E+12	bcd
TFSA2	1.8E+12	b c	TSAA9	1.4E+12	bcd	TFSA4	1.1E+12	bcd
TFA4	1.7E+12	b c	TEB6	1.4E+12	bcd	TER4	1.1E+12	bcd
TER6	1.7E+12	b c	TSAA1	1.3E+12	bcd	TSASA4	1.0E+12	bcd
TFA8	1.7E+12	b c	TFSA5	1.3E+12	bcd	TFSA1	9.9E+11	bcd
TSASA2	1.7E+12	b c	TSAA3	1.3E+12	bcd	TSAA6	8.8E+11	cd
TER2	1.6E+12	b c	TER1	1.3E+12	bcd	TG8	8.6E+11	cd
TFA3	1.6E+12	b c	TEBR2	1.2E+12	bcd	TSASA1	6.2E+11	d
TER5	1.5E+12	bcd	TSAA2	1.2E+12	bcd			
TER8	1.5E+12	bcd	TSAA5	1.2E+12	bcd			

The resulting analysis of variance for the spore production variable showed statistical differences between the different native isolates ($p < 0.0001$) at 26 ± °C, likewise the culture media presented statistical differences in yield ($p < 0.0001$), the culture medium that favored the highest spore production was SDA with 2.26E+12 (Figure 3). Likewise, the isolates that showed the highest spore production were TSAA8 with 3.41E+12, TSASA7 with 3.37E+12 and TEB1 with 2.82E+12 spores per milliliter (Figure 5 and Table 3).

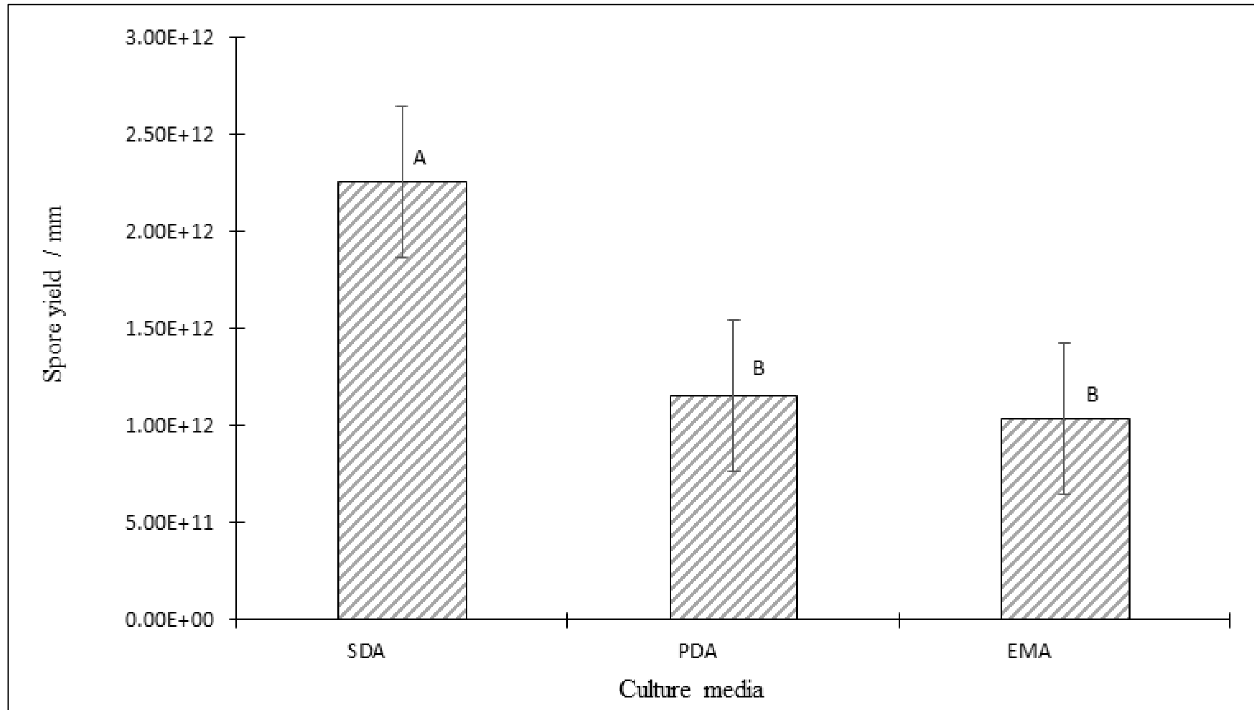

Figure 5: Spore yield of different native isolates of *Trichoderma* spp. in Sabouraud Dextrose Agar, Papa Dextrose Agar, and Malt Extract Agar culture media

DISCUSSION

Mycelial growth is an important characteristic for the selection and multiplication of mycoparasitic fungi (Ekesi *et al.,* 1999). In the results presented by Ummidi *et al.* (2013) reported that the strains studied presented similar growth to that of this study. The growth rate values obtained in the present study are higher than those reported by García-Gutiérrez *et al.* (2020), who when evaluating the radial growth of *Trichoderma* recorded a growth rate of 2.62 ± 8.0 mm at 48 hours and the size of the conidia of the isolates evaluated, was 5.08 to 7.19 µm long and 2.02 to 3.24 µm wide, which are in the range of the values reported by Driver *et al.* (2000), Fernandes *et al.* (2010) and Humber (2012), relating to the results found in this study.

The growth rate allows the selection of an efficient strain and its subsequent application in biological control (Faria *et al.,* 2015). Vélez *et al.* (1997) pointed out that one of the quality parameters of biopesticides is a 90% germination within 24 hours of incubation which makes the isolates studied in this research stand out. Torres de la C. *et al.* (2013) pointed out that the higher the speed, the greater the opportunity for the fungus to exert effective antagonism on the aflatoxin-producing pathogen, especially when environmental conditions are adverse. According to Schapovaloff *et al.* (2015), the soil environment constitutes an important reservoir for a diversity of entomopathogenic fungi, which can contribute significantly to the regulation of microorganisms affecting crops.

Iwanicki *et al.* (2019), when monitoring the presence of *Trichoderma* in crop soils, obtained 70 % of isolates from soil, 23 % from insects and 7 % from roots. Discrepant of this research. These morphological characteristics found in this study agree with that described by Humber (2012) and Bischoff *et al.* (2009), who described that colonies of the *Trichoderma* species complex are initially white, yellow during early conidial growth (typically 4-7 days) and green with conidial maturation (10-14 days). Driver *et al.* (2000) noted that isolates generally have green cylindrical conidia, 5 ± 7 µm long, which form in columns of chains, like what was presented in each of the isolates in this study, which allowed classifying all isolates within the *Trichoderma* spp. species complex. Hernández-Domínguez *et al.* (2016) found the mycosporic

pathogen in several of the plantations sampled throughout Mexico.

Valle-Ramírez *et al.,* 2022 show us in their research that the isolates obtained presented differences in colony diameter, growth rate, dimensions of reproductive structures and germination percentage of conidia, also the colonies presented a moderately fast growth where it reached an average diameter of 67, The difference in the length and width of the conidia between the different fungal isolates was highly significant (length and width), relating to the data obtained in this study. These results demonstrate the importance of isolate selection based on pathogenicity due to their wide variation among strains. The growth of targeted antagonistic fungi in phytopathogenic aflatoxin precursor fungi requires selecting the most effective isolates, which can play an important role in regulating these (Barrios *et al.,* 2016).

On the other hand, the use of native isolates in biological control programs is advisable, as they can have a reduced risk of significant impact on pathogenic organisms, compared to exotic isolates; moreover, they can adapt to the habitat and soil types where they are found, which increases the chances of success in the control of pest insects (Gürlek *et al.,* 2018). In this context, the isolates obtained in this study are potential candidates for *Trichoderma* control in peanut plantations in León and Chinandega and can be validated in other agroclimatic conditions.

CONCLUSION

The main microscopic morphometric and macroscopic physiological characteristics were characterized with taxonomic keys, in addition they were submitted to different tests where the variables radial growth, production of conidia in different artificial culture media were evaluated. The 37 monosporic isolates of *Trichoderma* spp. obtained from soils through parboiled rice in peanut farms in the department of León, Nicaragua, showed variability in terms of colony diameter, growth rate, length and width of conidia and conidia production. In general, the culture medium did not determine the growth rate; the radial growth rate of the isolates under study was time-dependent, obtaining the highest growth peak 48 hours after the experiment was established.

The length of the conidia did not depend on the isolate or the culture medium, while the size of the phialides did depend on the isolate, in the same way as the conidiophore. The five best isolates evaluated under laboratory conditions that showed high radial growth rate and conidia production will be selected as agents that could be used in future biological control programs of aflatoxin precursor pathogens under field conditions in peanut producing areas in western Nicaragua. The coloration of fungal colonies depends on the type of culture medium in relation to the carbon-nitrogen ratio.

ACKNOWLEDGMENTS

The authors of this research would like to thank the Universidad Nacional Agraria for its financial support for this study and CUKRA INDUSTRIAL, S.A., for allowing us to take samples from their farms in production and for the logistics provided.

REFERENCES

- Barrios, C., Bustillo, A., Ocampo, K., Reina, M., & Alvarado, H. (2016). Eficacia de hongos entomopatógenos en el control de *Leptopharsa gibbicarina* (Hemiptera: Tingidae) en palma de aceite. *Revista Colombiana de Entomología*, *42*(1), 22-27.
- Bischoff, J., Rehner, S., & Humber, R. (2009). A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia*, *100*(4), 512-530.
- Castillo Arévalo, T. (2021). Incidencia y severidad de Mal de Panamá (Fusarium Sp) en Guineo (Musa balbisiana ABB). *Ciencia E Interculturalidad*, *29*(02), 152–165.
- Castillo-Arévalo, T. (2023). Alternatives for the Biocontrol of *Fusarium oxysporum* f. sp. Cubense, Causal Agent of *Fusarium* wilt or Panama Disease in Guineo (*Musa balbisiana* ABB) Under Field Conditions. *Sch J Agric Vet Sci, 2*, 12-18.
- Castillo, T. (2023). Effect of Shade on the Severity of Moko (Ralston Solanacearum) on Guineo (Musa Balbisiana ABB). *European Journal of Agriculture and Food Sciences*, *5*(1), 1-5.
- Bischoff, J., Rehner, S., & Humber, R. (2009). A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia*, *100*(4), 512-530.
- De Araujo, D., Cardoso, F., Palmeira, J., Figueiredo, A. & Cesar, N. (2015). Incidencia de hongos y producción de aflatoxina en semillas de maní crioconservadas. *Revista Iberoamericana de Tecnología Postcosecha, 16(*1), 136-147.
- Driver, F., Milner, R., & Trueman, J. (2000). A taxonomic revision of *Metarhizium* based on a phylogenetic analysis of rDNA sequence data. *Mycological Research*, *104*(2), 134-150.
- Ekesi, S., Maniania, N., & Ampo, N. K. (1999). Effect of temperature on germination, radial growth, and virulence of *Metarhizium anisopliae* and *Beauveria bassiana* on *Megalurothrips sjostedti*. *Biocontrol Science and Technology*, *9*(2), 177-185.
- Erper, I., Saruhan, I., Akca, I., Aksoy, H. M., & Tuncer, C. (2016). Evaluation of some entomopathogenic fungi for controlling the green shield bug, Palomena prasina L. (Heteroptera: Pentatomidae). *Egyptian Journal of Biological Pest Control, 26*(3), 573.
- Fernandes, E. K., Keyser, C. A., Chong, J. P.,

Rangel, D. E., Miller, M. P., & Roberts, D. W. (2010). Characterization of *Metarhizium* species and varieties based on molecular analysis, heat tolerance and cold activity. Characterization of *Metarhizium* species and varieties based on molecular analysis, heat tolerance and cold activity. *Journal of Applied Microbiology*, *108*, 115- 128.

- Faria, M., Lopes, R., Souza, D., & Wraight, S. (2015). Conidial vigor vs. viability as predictors of virulence of entomopathogenic fungi. *Journal of Invertebrate Pathology*, *125*, 68-72.
- French, E., & Hebert, T. (1982). Phytopathological Research Methods. *IICA. San Jose, Costa Rica*, *275*, 209.
- García, N., Tlecuitl, S., Favela, E., & Loera, O. (2015). Production and quality of conidia by *Metarhizium anisopliae* var. *lepidiotum*: critical oxygen level and period of mycelium competence. *Applied Microbiology and Biotechnology*, *99*, 2783-2791.
- García-Gutiérrez, C., García-Guajardo, I., Vejar-Cota, G., Meza-García, L., Chávez-Medina J. (2020). Macromorfología y crecimiento radial de cepas de hongos entomopatógenos suplementado con polvo de lepidópteros. *Revista Colombiana de Entomología*, *46*(1), e10164.
- Gürlek, S., Sevim, A., Sezgin, F., & Sevim, E. (2018). Isolation and characterization of *Beauveria* and *Metarhizium* spp. from walnut fields and their pathogenicity against the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). *Egyptian Journal of Biological Pest Control*, *28*, 50.
- Hernández-Domínguez, C., & Guzmán-Franco, A. (2017). Species diversity and population dynamics of entomopathogenic fungal species in the genus *Metarhizium* a spatiotemporal study. *Microbial Ecology*, *74*, 194-206.
- Him, F., Tuñón, J.J., & Rodríguez, D.Y. (2021). Detección de aflatoxinas en maní y maíz para palomitas en tres marcas locales en panamá. *Revista Colegiada de Ciencia Universidad de Panamá*, Panamá ISSN-e: 2710-7434 Periodicidad: Semestral vol. 3, núm. 1.
- Hu, X., Xiao, G., Zheng, P., Shang Y., Su, Y., Zhang, X., Liu, X., Zhan, S., St Leger, R. J., & Wang, C. (2014). Trajectory and genomic determinants of fungal-pathogen speciation and host adaptation. *Proceedings of the National Academy of Sciences*, *111*(47), 16796-16801.
- Humber, R. (2012). Identification of entomopathogenic fungi. En L.A. Lacey (Ed.), *Manual of Techniques in Invertebrate Pathology* (2nd ed., pp. 151-187).
- Infante, D., Martínez, B., González, N., & Reyes, Y. (2009). Mecanismos de acción de *Trichoderma* frente a hongos fitopatógenos. *Revista de protección vegetal*, *24*(1), 14-21.
- Iwanicki, N., Pereira, A., Botelho, A., Rezende, J., Moral, A., Zucchi, M., & Júnior, I. (2019). Monitoring of the field application of *Metarhizium anisopliae* in Brazil revealed high molecular diversity of *Metarhizium* spp in insects, soil, and sugarcane roots. *Scientific Reports*, *9*, 4443.
- Meyer, M.., Mazaro, S., da Silva, J. (2022). *Trichoderma*: su uso en la agricultura. https://scholar.google.com/scholar_url?url=https://www.alice.cnptia.embrapa.br/bitstream/doc/1146280/1/LV-*Trichoderma*-espanhol-2022.pdf&hl=es&sa=T&oi=gsb-ggp&ct=res&cd=0&d=4131356743302085652&ei=smt7ZJLQNouGmgHon7D4DA&scisig=AGlGAw-oVgg3fvjrxkMcD1ILHeW3
- Rezende, J., Zanardo, A., Lopes, S., Delalibera, I., & Rehner, S. (2015). Phylogenetic diversity of Brazilian *Metarhizium* associated with sugarcane agriculture. *BioControl*, *60*, 495-505.
- Rojas Jaimes, J., Chacón Cruzado, M., Castañeda Peláez, L., Díaz, & Tello, A. (2021). Cuantificación de aflatoxinas carcinogénicas en alimentos no procesados y su implicación para el consumo en Lima, Perú. *Nutrición Hospitalaria*, 38(1), 146–151.
- Sandoval, I., & López, M. O. (2000). Antagonismo de *Trichoderma* harzianum A34 hacia Macrophomina phaseolina y otros patógenos fúngicos del frijol. *Fitosanidad*, *4*(3-4), 69-72.
- Schapovaloff, M., Alves, A. Luis, F., Urrutia, M., & López, C. (2015). Ocurrencia natural de hongos entomopatógenos en suelos cultivados con yerba mate (*Ilex paraguariensis* St. Hil.) en Misiones, Argentina. *Revista Argentina de Microbiología*, *47*(2), 138-142.
- Sherathia, D., Dey R., Thomas, M., Dalsania, T., Savsani, K. & Pal, K.K. (2016). Biochemical and molecular characterization of DAPG-producing plant growth- promoting rhizobacteria (PGPR) of groundnut (Arachis hypogaea L.). *Legume Research* 39 (4), 614-622.
- Steinwender, B., Enkerli, J., Widmer, F., Eilenberg, J., Kristensen, K., & Meyling, N. (2014). Molecular diversity of the entomopathogenic fungal *Metarhizium* community within an agroecosystem. *Journal of Invertebrate Pathology*, *123*, 6-12.
- Tiago, P., Leao, M., Malosso, E., Oliveira, N., & Lima, E. (2012). Persistence and effect of Metarhizium anisopliae in the fungal community of sugarcane soil. *BioControl,* 57, 653-661.
- Torres de la C., Cortez, M., Ortiz, G., Cappello, G., & De La Cruz, P. (2013). Caracterización de aislamientos nativos de *Metarhizium anisopliae* y su patogenicidad hacia Aeneolamia postica, en Tabasco, México. *Revista Colombiana de Entomología, 39*(1), 40-46.
- Ummidi, V., Josyula, U., & Vadlamani, P. (2013). Germination rates of *Beauveria bassiana* and *Metarhizium anisopliae* its possible correlation

with virulence against *Spodoptera litura* larvae. *International Journal of Advanced Research*, *2*(1), 625-630.

- Valle-Ramírez, S., Torres-Gutiérrez, R., Caicedo-Quinche, W., Abril-Saltos, R., & Sucoshañay-Villalba, D. (2022). Aislamiento y caracterización de *Metarhizium* spp. de cultivos de caña de azúcar y su patogenicidad contra Mahanarva andigena (Hemiptera: Cercopidae). *Ciencia y Tecnología Agropecuaria*, *23*(1).
- Vélez, A. P. E., Posada, F. F. J., Marín, P., González, G. M. T., Osorio, V. E., & Bustillo, P. A. E. (1997). *Técnicas para el control de calidad de formulaciones de hongos entomopatógenos* [Boletín Técnico Cenicafé N.º 17]. Cenicafé. https://biblioteca.cenicafe.org/handle/10778/709