



DOI: [10.29298/rmcf.v15i83.1446](https://doi.org/10.29298/rmcf.v15i83.1446)

Research article

Control biológico de *Fusarium circinatum* Nirenberg & O' Donnell 1998 con *Trichoderma harzianum* Rifai 1969 en *Pinus devoniana* Lindl.

Biological control of *Fusarium circinatum* Nirenberg & O' Donnell 1998 with *Trichoderma harzianum* Rifai 1969 in *Pinus devoniana* Lindl.

María Alejandra Ortega Cerón¹, Elizabeth Hernández Acosta², Silvia Edith García Díaz^{1*}, Antonio Villanueva Morales¹

Fecha de recepción/Reception date: 10 de octubre de 2023.
Fecha de aceptación/Acceptance date: 16 de febrero de 2024.

¹División de Ciencias Forestales, Universidad Autónoma Chapingo. México.

²División de Suelos, Universidad Autónoma Chapingo. México.

*Autor para correspondencia; correo-e: edith65@gmail.com

*Corresponding author; e-mail: edith65@gmail.com

Abstract

The drying or seedbed evil caused by *Fusarium circinatum* is a problem of importance in plant production in forest nurseries. The aim of this study was to assess *Trichoderma harzianum* as an alternative to prevent *F. circinatum* with the method of application and the use of two mixtures of substrates: (1) Peat moss, agrolite and vermiculite, and (2) Sawdust, pine bark and peat moss. Three forms of application of *T. harzianum* were made: to the substrate, to the seed and without the fungus, which gave a total of 12 treatments with completely at random factorial arrangement (2×3×2). The effect of the substrate was evaluated, the form of application of *T. harzianum* and its interaction on the pathogenicity and incidence of *F. circinatum* and the morphological properties of the seedlings. The results indicate that the morphological characteristics in the growth of the strain coincide with those described for *F. circinatum* and turned out to be pathogenic when causing disease. The pathogenicity of *F. circinatum* decreased in those treatments where *T. harzianum* was applied to the substrate, showing a positive response in the sawdust-based mixture and ensuring a better plant quality. The application of *T. harzianum* to the substrate and the use of sawdust-based mixture can serve as an option for the *F. circinatum* fungus, providing better plant quality indexes.

Key words: Biocontrol, plant quality, phytopathogen, *Fusarium circinatum* Nirenberg & O' Donnell 1998, antagonistic fungus, incidence.

Resumen

La secadera o mal de almacigo causada por *Fusarium circinatum* es un problema importante en la producción de planta en viveros forestales. El objetivo de esta investigación fue evaluar a *Trichoderma harzianum* como

alternativa para controlar de manera preventiva a *F. circinatum* con el método de aplicación y la utilización de dos mezclas de sustratos: (1) turba de musgo, agrolita y vermiculita, y (2) aserrín, corteza de pino y turba de musgo. Se utilizaron tres formas de aplicación de *T. harzianum*: al sustrato, a la semilla y sin el hongo; lo que dio un total de 12 tratamientos con arreglo factorial completamente al azar (2×3×2). Se evaluó el efecto del sustrato, la forma de aplicación de *T. harzianum* y su interacción sobre la patogenicidad e incidencia de *F. circinatum* y las propiedades morfológicas de las plántulas. Los resultados indicaron que las características morfológicas en el crecimiento de la cepa coinciden con los descritos para *F. circinatum* y resultó ser patogénica, ya que causó enfermedad. La patogenicidad de *F. circinatum* disminuyó en aquellos tratamientos en los que se adicionó *T. harzianum* al sustrato, y presentó una respuesta positiva en la mezcla a base de aserrín con lo cual se aseguró una mejor calidad de planta. La aplicación de *T. harzianum* al sustrato y el uso de la mezcla a base de aserrín pueden servir como una alternativa para el control de *F. circinatum*, además de proveer mejores índices de calidad de planta.

Palabras clave: Biocontrol, calidad de planta, fitopatógeno, *Fusarium circinatum* Nirenberg & O'Donnell 1998, hongo antagonista, incidencia.

Introduction

Mexico loses around 47 770 hectares of forest coverage per year due to anthropogenic activities; thus, the development of reforestation programs that allow their recovery (Gómez, 2021) has been sought. Therefore, quality plant from a forest nursery that guarantees sanity and morpho-physiological characteristics necessary for its field establishment (Buamscha *et al.*, 2012; Sáenz *et al.*, 2014) is used. The quality of the plant is determined by physicochemical conditions that are suitable for its production.

One of the preferred species in reforestation programs is *Pinus devoniana* Lindl., from its adaptability and economic benefits. This species is generally produced in substrate based on Peat Moss, vermiculite and agrolite (55:35:10) with slow-release fertilizers (30-15-10); however, it has been observed that these mixtures favor the development of pests and diseases (Conafor, 2001; Sáenz-Romero, 2014).

Phytopathogenic fungi are the most harmful agents due to their fast reproduction, which makes it is necessary to perform constant samples to avoid diseases (Murace and Aprea, 2011). *Fusarium circinatum* Nirenberg & O'Donnell 1998 affects pine

seedlings from the seed stage, damage to the embryo and hypocotyl, stem bending, root rot, discoloration of needles and death. It can favor plant losses of 40 to 100 % (González *et al.*, 2008; García-Díaz *et al.*, 2017).

There are alternative substrates made from by-products of the plant production process in forest nurseries, which can reduce the unit cost of the plant (Aguilera *et al.*, 2016). For pathogen control there are options such as the use of the antagonist fungus of the *Trichoderma* Pers. 1794 genus (Okorski *et al.*, 2014).

Studies focused on analyzing the effect of substrate mixtures on the pathogenicity of *F. circinatum* such as García-Díaz *et al.* (2017), who evaluated the pathogenicity of the fungus in three mixtures of substrates and during the germination stage of *Pinus greggii* Engelm. ex Parl. On the other hand, Robles *et al.* (2016) assessed the *Fusarium oxysporum* Schltdl. 1824 attack at the nursery stage and *Fusarium solani* (Mart.) Sacc. 1881 in *Pinus* spp. seedlings. Also, Moraga-Suazo *et al.* (2011) compared the effectiveness of the control exercised on *F. circinatum* with *Trichoderma* spp. and *Clonostachys* spp. in *Pinus radiata* D. Don.

Based on the above, the aim of this study was to assess the effect of *Trichoderma harzianum* Rifai 1969 as an alternative to prevent *Fusarium circinatum*, with the method of application and the use of two mixtures of substrate.

Materials and Methods

Study area

The experiment was conducted from May 9th, 2021 to January 26th, 2022 in the greenhouse of the Forest Sciences Division (*Dicifo*) of the *Universidad Autónoma*

Chapingo (UACH) (*Chapingo* Autonomous University), in *Texcoco*, State of Mexico, located between 19°29'34" N and 98°53'38" W, at 2 240 masl. Inside the greenhouse, temperature remained at 24 °C during the study period.

Experimental design and statistical analysis

A completely at random factorial experiment (2×3×2) was established with four replicates; the assessed factors were: (A) Mixture of substrate, with two levels (S1 and S2, described below), (B) *T. harzianum* application method, with three levels (application to the substrate, application to the seed and without applying), and (C) Presence of *F. circinatum*, with two levels (presence or absence of the fungus). The combined effect of the factors was evaluated, so there were 12 treatments (Table 1). The experimental unit consisted of 12 *P. devoniana* plants with 220 mL pipes. In total 48 trays were used. The response variables were incidence, severity and plant quality.

Table 1. Established treatments for the evaluation of substrate mixtures, application method of *Trichoderma harzianum* Rifai 1969, absence and presence of *Fusarium circinatum* Nirenberg & O'Donnell 1998 in *Pinus devoniana* Lindl. plants.

Treatments	Description
T1	S1+ <i>T. harzianum</i> Rifai 1969 to the substrate
T2	S1+ <i>T. harzianum</i> Rifai 1969 to the substrate+ <i>F. circinatum</i> Nirenberg & O'Donnell 1998
T3	S2+ <i>T. harzianum</i> Rifai 1969 to the substrate
T4	S2+ <i>T. harzianum</i> Rifai 1969 to the substrate+ <i>F. circinatum</i> Nirenberg & O'Donnell 1998

T5	S1+ <i>T. harzianum</i> Rifai 1969 to the seed
T6	S1+ <i>T. harzianum</i> Rifai 1969 to the seed+ <i>F. circinatum</i> Nirenberg & O'Donnell 1998
T7	S2+ <i>T. harzianum</i> Rifai 1969 to the seed
T8	S2+ <i>T. harzianum</i> Rifai 1969 to the seed+ <i>F. circinatum</i> Nirenberg & O'Donnell 1998
T9	S1
T10	S1+ <i>F. circinatum</i> Nirenberg & O'Donnell 1998
T11	S2
T12	S2+ <i>F. circinatum</i> Nirenberg & O'Donnell 1998

S1 = Substrate 1 (peat moss+agrolite+vermiculite); S2 = Substrate 2 (pine sawdust+pine bark+peat moss).

An Anova was performed through the Glimmix of SAS procedure, version 13.1 (SAS Institute Inc., 2013) and multiple comparison of means through the Tukey test at a significance level of 0.05.

The following model was used:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + \varepsilon_{ijkl} \quad (1)$$

Where:

Y_{ijkl} = Variable to assess (incidence, severity and plant quality)

μ = General mean

A_i = Effect of the i^{th} level of the A factor (Substrate mixture)

B_j = Effect of the j^{th} level of the B factor (*T. harzianum* application method)

C_k = Effect of the k^{th} level of the C factor (presence of *Fusarium*)

$(AB)_{ij}$ = Interaction of i^{th} level of the *A* factor with j^{th} level of the *B* factor

$(AC)_{ik}$ = Interaction of i^{th} level of the *A* factor with k^{th} level of the *C* factor

$(BC)_{jk}$ = Interaction of j^{th} level of the *B* factor with k^{th} level of the *C* factor

$(ABC)_{ijk}$ = Interaction of i^{th} level of the *A* factor with j^{th} level of the *B* factor with k^{th} level of the *C* factor

ε_{ijkl} = Standard error

Seed and substrates

Two substrate mixtures were used, S1: peat moss, agrolite and vermiculite, and S2: pine sawdust from 15 days of sawing, composted pine bark and peat moss, 60:20:20 and Multicote® 18-6-12 fertilizer was added, at a dose of 7 g L⁻¹ of substrate (Table 1). Two *P. devoniana* seeds were placed per tube, previously disinfected with 10 % sodium hypochlorite (NaClO) for 10 minutes, followed by 24 hours of soaking with sterile water and a second disinfection with 5 % NaClO for 3 min. Surface irrigation was carried out for a month and then every third day.

Inoculation with *Trichoderma harzianum*

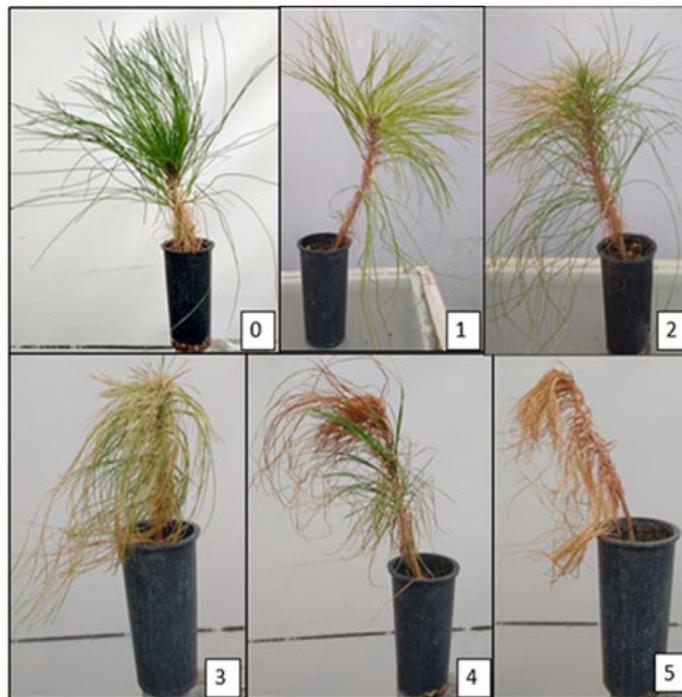
A 14 L of a *T. harzianum* (commercial strain PHC[®] T22) solution was prepared at 1×10^{-7} CFU/g dry weight. The application dose was 3.4 g L⁻¹ of water. The treatments with *T. harzianum* to the substrate were: T1, T2 (S1) and T3, T4 (S2). Those that received *T. harzianum* with seed soaking for 60 min. were: T5, T6 (S1) and T7, T8 (S2); while those without *T. harzianum* were the treatments: T9, T10 (S1) and T11, T12 (S2) (Table 1).

Inoculation with *Fusarium circinatum*

Strain SF11 of *F. circinatum* from the forest nursery of *Amealco*, state of *Querétaro*, provided by the *Dicifo* Forest Pathology Laboratory was used with seven days of growth at room temperature in Potato Dextrose Agar BD Bioxon[™] (PDA) culture medium; a solution of 1.6×10^6 spores mL⁻¹ was prepared. In seedlings of two months and 20 days (July 2021), corresponding to treatments T2, T4, T6, T8, T10 and T12 (Table 1), 20 mL were applied directly to the substrate of each tube. 24 h after inoculation, the counting of plants with the presence of symptoms began.

Determination of incidence and severity

The pathogenicity of *F. circinatum* was evaluated by its ability to cause disease with typical symptoms of *secadera*. The incidence was determined by the mortality of plants affected with symptoms for eight months. Severity was estimated using the Townsend and Heuberger (1943) diagrammatic scale (Figure 1) and the Formula 2:



Class 0 = Healthy or asymptomatic plant; Class 1 = Falling or distal yellowing needles; Class 2 = Partial plant wilting; Class 3 = Medium plant wilting; Class 4 = Advanced plant wilting; Class 5 = Dead plant.

Figure 1. Based on Townsend and Heuberger (1943), scale of symptoms caused by *Fusarium circinatum* Nirenberg & O'Donnell 1998 for severity estimation.

$$DS (\%) = \left[\frac{\sum nv}{NV} \right] X 100 \quad (2)$$

Where:

DS = Severity

n = Degree of infection according to the scale

v = Number of present plants by category

N = Maximun degree of infection

V = Total number of plants

The degrees of infection according to the scale allowed us to estimate the severity and a Pearson correlation analysis was carried out between incidence and severity.

Morphological identification of *Fusarium circinatum*

One plant with symptoms for each treatment inoculated with *F. circinatum* (a total of 24 plants) was selected for isolation and cultivation in PDA and stored at 28 °C for 24 h of light (model INCL-11 Prendo® incubator) to obtain re-isolations of the pathogen and demonstrate Koch's postulates.

The morphological identification of *F. circinatum* was carried out by obtaining the isolation, sporulation and development of macroconidia, microconidia, phialides and coloration of the mycelium, in three culture media: PDA medium, to examine the morphology and pigmentation of the colony, Water Agar medium with carnation leaf

for the formation of macroconidia and phialides on sporodochia, and for the development of circins the Synthetic Nutritive Agar medium (García-Díaz *et al.*, 2017). 10 permanent mounts were made on slides with 100 % glycerin and methylene blue to observe and measure the structures of the fungus in a DM 2 500 Leica® microscope. The keys of Barnett and Hunter (1998) and Leslie and Sumerell (2006) were used.

Morphological standards to evaluate plant quality

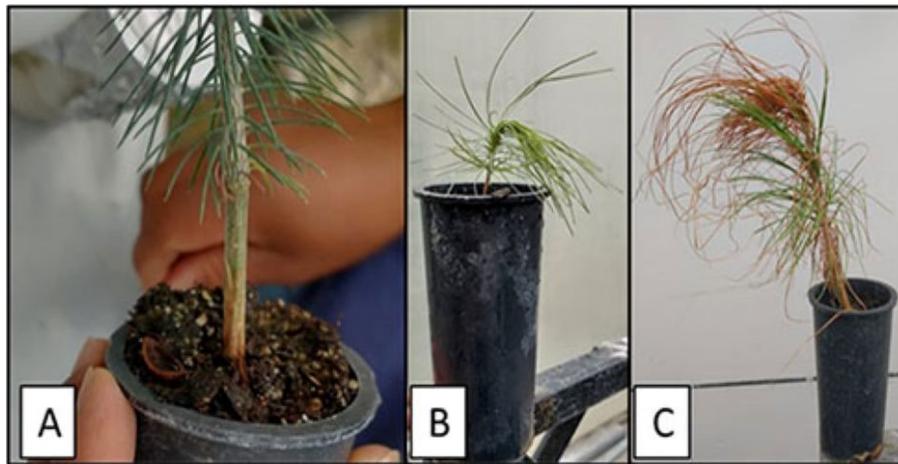
Eight months after sowing, five central plants were selected from each tray (20 plants per treatment, resulting in a total of 240); they were removed from the root ball and immersed in water to wash and not hurt the roots. Subsequently, measurements of Stem Diameter (D) (section in which it differs from the main root) (with TXD-a33 Lenfech® digital caliper), and Height of the aerial part (A) (section in which it differs from the main root to the apex of the terminal bud) (with H-6 560 Cline® steel rule) were made.

Each plant was sectioned at the neck level, and both sections, foliage and root, were placed in a paper bag labeled with the treatment data for drying in a Fe-143 Felisa® oven at 70 °C for 72 h. The dry weight of the root (PSR) and the dry weight of the aerial part (PSA) were evaluated. The dry weight ratio of the aerial part to the dry weight of the root (PSA/PSR), Slenderness index (IE), plant height (cm) between diameter (mm) and Dickson quality index (ICD) were determined, with the equation $PST/(A/D)+(PSA/PSR)$ (Dickson *et al.*, 1960). The seedlings were weighed on a model Galaxy 2 000 OHAUS® analytical balance.

Results

Determination of incidence and severity

F. circinatum symptoms in plants began in week 12 after inoculation; chlorotic needles, reddish brown color and drying of foliage, apical bending, root rot and death were present (Figure 2). These symptoms coincide with those reported by Flores-Pacheco (2017) and García-Díaz *et al.* (2017).



A = Rot at neck level; B = Chlorotic and twisted needles, tip bending; C = Descending death of the plant.

Figure 2. Symptoms observed in *Pinus devoniana* Lind. plants inoculated with *Fusarium circinatum* Nirenberg & O' Donnell 1998.

The analysis of variance revealed that there are no differences between the substrates (S1 and S2) and their interaction with the application of *T. harzianum* on the incidence. On the contrary, the application method of *T. harzianum*, the presence of *F. circinatum* and their interactions showed differences on the response variables at $\alpha=0.05$ (Table 2 shows the $Pr>F$ corresponding to the Anova of each variable).

Table 2. Determination of the effect of the mixture, application of *Trichoderma harzianum* Rifai 1969 and presence of *Fusarium circinatum* Nirenberg & O' Donnell 1998 on the incidence in *Pinus devoniana* Lindl. plants.

Effect	F-value	Pr>F
Application of <i>T. harzianum</i> Rifai 1969	11.32	0.0002
<i>Fusarium circinatum</i> Nirenberg & O' Donnell 1998	805.31	<0.0001
Mixture× <i>Fusarium circinatum</i> Nirenberg & O' Donnell 1998	1.21	0.2793
Application of <i>T. harzianum</i> Rifai 1969× <i>Fusarium circinatum</i> Nirenberg & O' Donnell 1998	12.23	<0.0001

Because there were differences between the form of application with *T. harzianum* and the presence of *F. circinatum* on the incidence of the disease, it was necessary to perform a Tukey test (Table 3).

Table 3. Evaluation of incidence and severity of the disease caused by *Fusarium circinatum* Nirenberg & O' Donnell 1998 in *Pinus devoniana* Lindl. seedlings.

Number of treatment	Description	Incidence %	Severity %
T1	S1+ <i>T. harzianum</i> Rifai 1969 to the substrate	5c	4.16d
T2	S1+ <i>T. harzianum</i> Rifai 1969 to the substrate+ <i>F. circinatum</i> Nirenberg & O' Donnell 1998	46b	26.16c
T3	S2+ <i>T. harzianum</i> Rifai 1969 to the substrate	3c	2d

T4	S2+ <i>T. harzianum</i> Rifai 1969 to the substrate+ <i>F. circinatum</i> Nirenberg & O'Donnell 1998	42b	25.16c
T5	S1+ <i>T. harzianum</i> Rifai 1969 to the seed	4c	2.83d
T6	S1+ <i>T. harzianum</i> Rifai 1969 to the seed+ <i>F. circinatum</i> Nirenberg & O'Donnell 1998	59a	33b
T7	S2+ <i>T. harzianum</i> Rifai 1969 to the seed	4c	2.73d
T8	S2+ <i>T. harzianum</i> Rifai 1969 to the seed+ <i>F. circinatum</i> Nirenberg & O'Donnell 1998	58a	24.83c
T9	S1	4c	2.33d
T10	S1+ <i>F. circinatum</i> Nirenberg & O'Donnell 1998	66a	33.99ab
T11	S2	3c	2.33d
T12	S2+ <i>F. circinatum</i> Nirenberg & O'Donnell 1998	63a	37.5a

S1 = Substrate 1 (peat moss, agrolite and vermiculite); S2 = Substrate 2 (pine sawdust, pine bark and peat moss). Equal letters per column indicate that there are no significant differences ($p \geq 0.05$) and different letters indicate significant differences between treatments according to the Tukey test ($p \leq 0.05$). Values expressed as the mean of each response variable.

The analysis of disease incidence indicated that the low values (3 to 5 %) were in the treatments in the absence of *F. circinatum*, and the treatments with the highest incidence were those in which *F. circinatum* was inoculated, that is, 46 % (T2), 42 % (T4), 59 % (T6), 58 % (T8), 66 % (T10) and 63 % (T12). This demonstrates the importance of the pathogen on the loss of plant production. Plants with application of *T. harzianum* directly to the substrate presented 13 to 16 % less incidence, compared to application to the seed. Where *T. harzianum* was not applied, it was 20 % lower, a behavior similar to that of S1 and S2. The application method of *T. harzianum* was the one that had the greatest effect, due to a lower incidence of the disease when it was applied to the substrate (Table 4).

Table 4. Determination of the effect of the application method of *Trichoderma harzianum* Rifai 1969 on the incidence of the disease in *Pinus devoniana* Lindl. plants.

Application method	Incidence %
Without <i>Trichoderma harzianum</i> Rifai 1969	8.5a
<i>Trichoderma harzianum</i> Rifai 1969 to the seed	7.8125a
<i>Trichoderma harzianum</i> Rifai 1969 to the substrate	6b

Equal letters per column indicate that there are no significant differences ($p \geq 0.05$) and different letters indicate significant differences between treatments according to the Tukey test ($p \leq 0.05$).

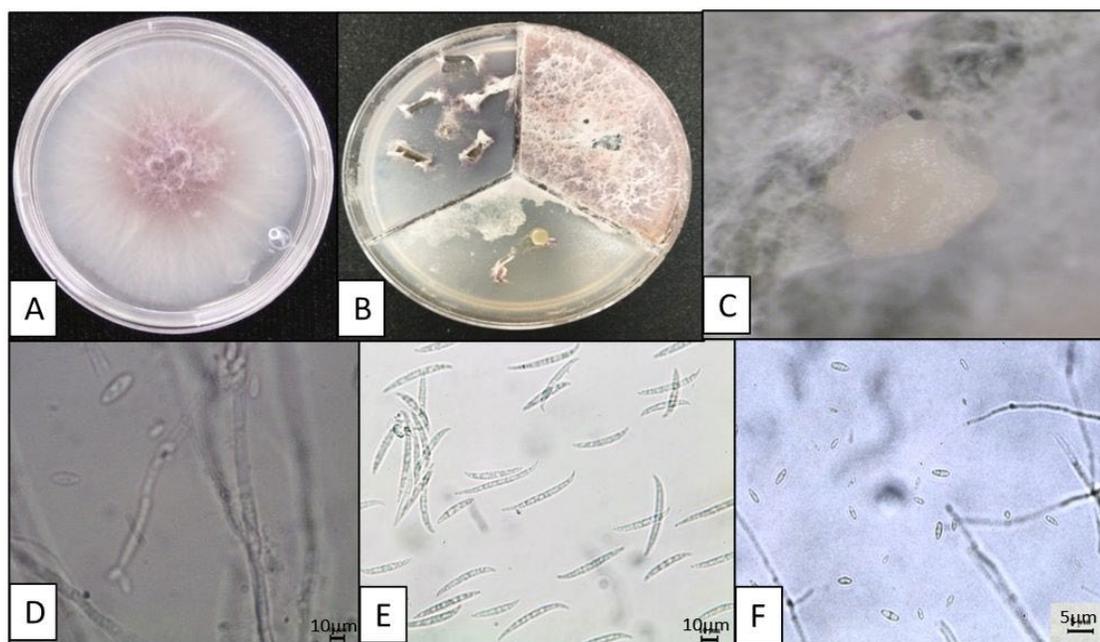
The estimation of the degree of infection allowed to carried out the Pearson correlation test in order to measure the relationship between incidence and severity, with a value of 0.98617 was obtained, which indicates that, the higher the incidence, the greater the severity (Table 5).

Table 5. Pearson correlation between incidence and severity of *Fusarium circinatum* Nirenberg & O 'Donnell 1998 with *P*-value of < 0.0001 .

	Incidence	Severity
Incidence	1	0.986 <0.0001
Severity	0.986 <0.0001	1

Morphological identification of *Fusarium circinatum*

White colonies with fuchsia hues were obtained, turning strong violet, with violet pigmentation (Figure 3A and 3B), little formation of pale orange sporodochia (Figure 3C), macroconidia with 2-4 septa, typically three-septate, thin and curved (Figure 3E), 28-44×3.3-3.9 nm⁻², 39×3.8 nm⁻² on average and with a curved apical and poorly developed basal cell; finally, ovoid to allantoic shaped, unicellular microconidia, which develop on mono- and polyphialids (Figure 3D and 3F), with spiral-shaped sterile hyphae known as circins. These characteristics and measurements coincide with those recorded for *F. circinatum* by Flores-Pacheco (2017), García-Díaz *et al.* (2019) and O'Donnell *et al.* (2022).



A = Purple cottony mycelium; B = Development in three media; C = Sporodochia;
D = Monophialides of the 40X microconidia; E = Allantoic macroconidia with poorly
developed basal cell; F = Microconidia.

Figure 3. Characteristics of *Fusarium circinatum* Nirenberg & O' Donnell 1998.

Morphological standards to evaluate plant quality

The assessment of plant quality with the individual variables did not show significant differences between the treatments, but, on the other hand, in the *ICD* analysis, there are differences in the S2 treatments with values >1 compared to S1, which are smaller. Saenz *et al.* (2014) consider values ≥ 0.5 as high quality for cespitose species; the numbers found in this study were higher, so they are classified as high quality. In the *ICD*, no significant differences were observed in the application method of *T. harzianum* (Table 6).

Table 6. Plant quality indexes of *Pinus devoniana* Lindl. in two substrates with *Trichoderma harzianum* Rifai 1969 and *Fusarium circinatum* Nirenberg & O' Donnell 1998.

No.	Treatment	Alt (cm)	Diam (mm)	PSA (gr)	PSR (gr)	IR	PSA/PSR	ICD
T1	S1+ <i>T. harzianum</i> Rifai 1969 to the substrate	16.105ab	7.3abc	3.875abcd	1.09f	2.245ab	3.554a	0.859e
T2	S1+ <i>T. harzianum</i> Rifai 1969 to the substrate+ <i>F. circinatum</i> Nirenberg & O' Donnell 1998	13.47ab	6.775c	3.08d	0.995f	2.125ab	3.054abcd	0.843e
T3	S2+ <i>T. harzianum</i> Rifai 1969 to the substrate	15.555ab	7.375abc	4.43ab	2.12a	2.145ab	2.096fg	1.538ab
T4	S2+ <i>T. harzianum</i> Rifai 1969 to the substrate+ <i>F. circinatum</i> Nirenberg & O' Donnell 1998	14.215ab	7.79abc	4.425ab	1.765abcd	2.073ab	2.522cdefg	1.448abc
T5	S1+ <i>T. harzianum</i> Rifai 1969 to the seed	13.745ab	7.92ab	4.1abc	1.28ef	1.765ab	3.20ab	1.095cde
T6	S1+ <i>T. harzianum</i> Rifai 1969 to the seed+ <i>F. circinatum</i> Nirenberg &	15.645ab	7.24bc	3.415cd	1.11f	2.245ab	3.137abc	0.864e

O'Donnell 1998								
T7	S2+ <i>T. harzianum</i> Rifai 1969 to the seed	16.585ab	8.145ab	4.54a	1.9abc	2.14ab	2.44defg	1.439abc
T8	S2+ <i>T. harzianum</i> Rifai 1969 to the seed+F. <i>circinatum</i> Nirenberg & O'Donnell 1998	12.56b	8.225ab	4.07abc	1.85abc	1.615b	2.231efg	1.628a
T9	S1	16.805ab	8.485a	4.255abc	1.54cde	2.043ab	2.849bdce	1.223bcde
T10	S1+F. <i>circinatum</i> Nirenberg & O'Donnell 1998	17.015a	7.2bc	3.525bcd	1.38ef	2.413a	2.598bcdef	1.005de
T11	S2	16.475ab	7.445abc	3.855abcd	2.04ab	2.315a	1.897g	1.478abc
T12	S2+F. <i>circinatum</i> Nirenberg & O'Donnell 1998	12.505b	7.205bc	3.345cd	1.635bcde	1.898ab	2.074fg	1.349abcd

S1 = Substrate 1 (peat moss+agrolite+vermiculite); S2 = Substrate 2 (pine sawdust+pine bark+peat moss); *Alt* = Height; *Diam* = Diameter; *PSA* = Air dry weight; *PSR* = Radical dry weight; *IR* = Robustness index; *ICD* = Dickson quality index. Equal letters per column indicate that there are no significant differences ($p \geq 0.05$) and different letters indicate significant differences between treatments according to the Tukey test ($p \leq 0.05$).

The analysis of variance showed that there are differences between S1 and S2. Substrate 2, based on sawdust, was significant for the *ICD*, with $\alpha=0.05$. Table 7 shows the $Pr > F$ corresponding to the Anova of each variable.

Table 7. Effect of the mixture of two substrates, with application of *Trichoderma harzianum* Rifai 1969 and presence of *Fusarium circinatum* Nirenberg & O'Donnell 1998 on the plant quality indexes of *Pinus devoniana* Lindl.

Effect	F-value	Pr>F
Mixture	40.58	<0.0001

Based on Tukey's multiple comparison of means, sawdust-based substrate 2 (S2), showed significant differences for the *ICD* (Table 8).

Table 8. Multiple comparison test of means per substrate mixture for the Dickson quality index in *Pinus devoniana* Lindl. plants.

Substrate Mixture	ICD
(S1) Peat moss, agrolite and vermiculite	0.9814b
(S2) Pine sawdust, pine bark and pear moss	1.4799a

According to Tukey's test. Equal letters per row indicate that there are no significant differences ($p \geq 0.05$).

Discussion

The results of this study indicate that *Fusarium circinatum* caused symptoms of apical bending, stem rot at the neck level and yellowish needles turning reddish, subsequently death, which coincides with the results of Flores-Pacheco (2017) and García-Díaz *et al.* (2017).

Gordon *et al.* (2015) point out that the use of peat moss is associated with greater susceptibility to *F. circinatum*, since they obtained a 97 % incidence when inoculating a pine isolate in corn plants, and in this research, such substrate (S1) inoculated in *P. devoniana* plants showed 66 %. Aguilera *et al.* (2016) mention that substrates with sawdust and pine bark are used with great success in nurseries in central Mexico, since sawdust is an abundant, economical by-product that allows the development of plants with good morphological characteristics. It can be added to this fact that the emergence of the disease is slightly lower; in this study, an incidence of 63 % was obtained with S2, compared to 66 % with S1, although

statistically there were no differences. However, García-Díaz *et al.* (2017) report that the presence of the disease is lower in sawdust-based substrates.

The analysis of variance showed that the significant factor is the application of *T. harzianum* to the substrate, presenting low incidence and severity; these results coincide with those of García-Díaz *et al.* (2017), in which the damage caused by *F. circinatum* was lower in those treatments in which *T. harzianum* was applied to *Pinus greggii* plants. Likewise, what is stipulated agrees with what is stated by Moraga-Suazo *et al.* (2011), who mention that when applying *Trichoderma* to *Pinus radiata* plants the incidence is reduced by more than 20 %. Okorski *et al.* (2014) concludes that *Trichoderma* is used as a biocontrol agent for forest diseases.

The *ICD* analysis indicates that there are differences in the treatments of S2 by obtaining values greater than 1 with respect to S1, which are lower. This is considered within the "high quality" classification according to the parameters established for cespitose species, as mentioned by Rueda *et al.* (2012) and Sáenz (2014). The results agree with those of Vicente-Arbona *et al.* (2019), who when evaluating the *ICD* in *P. greggii* plants in different substrate mixtures obtained better results in that mixture with a formulation similar to S2 (pine sawdust, pine bark and peat moss) (Aguilera *et al.*, 2016).

Conclusions

Fusarium circinatum was shown to be pathogenic in *Pinus devoniana* seedlings, it was observed that the application method with *Trichoderma harzianum* to the substrate can reduce the incidence and severity of the disease to be used preventively. It was

observed that the plant quality based on the Dickson quality index for cespitose species had higher values in those individuals that developed in the sawdust-based substrate and the application of *Trichoderma harzianum* to the substrate.

Acknowledgements

The authors thank the *Universidad Autónoma Chapingo* (Chapingo Autonomous University), the *Conahcyt* sectoral fund and project A-S-67865 of the *Conafor-Conahcyt* Sectoral Fund, entitled "Monitoring, damage assessment, preventive management and control of drying and root rot caused by *Fusarium* spp., and the fungus flies *Bradysia* and *Lycoriella*" for their support to accomplish this project.

Conflict of interest

The authors of this work declare that they do not present any type of conflict of interest.

Contribution by author

María Alejandra Ortega Cerón: field work, laboratory and writing of the manuscript; Elizabeth Hernández Acosta: research direction and review of the manuscript; Silvia Edith García Díaz: advice on laboratory and greenhouse research work and review of the manuscript; Antonio Villanueva Morales: statistical analysis, experimental design and drafting review of the manuscript.

References

- Aguilera R., M., A. Aldrete, T. Martínez T. y V. M. Ordaz C. 2016. Producción de *Pinus pseudostrobus* Lindl. con sustratos de aserrín y fertilizantes de liberación controlada. *Revista Mexicana de Ciencias Forestales* 7(34):7-19. Doi: 10.29298/rmcf.v7i34.79.
- Barnett, H. L. and B. B. Hunter. 1998. Illustrated genera of imperfect fungi. American Phytopathological Society. St. Paul, MN, United States of America. 218 p.
- Buamscha, M. G., L. T. Contardi, R. K. Dumroese, J. A. Enricci, ... y K. M Wilkinson. 2012. Producción de plantas en viveros forestales. Consejo Federal de Inversiones (CFI), Centro de Investigación y Extensión Forestal Andino Patagónico (CIEFAP) y Universidad Nacional de la Patagonia San Juan Bosco. Buenos Aires, C, Argentina. 220 p.
- Comisión Nacional Forestal (Conafor). 2001. *Pinus devoniana* Lindley. SIRE-Paquetes Tecnológicos. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (Conabio) y Programa Nacional de Reforestación (Pronare). <http://www.conafor.gob.mx:8080/documentos/docs/13/957Pinus%20devoniana.pdf>. (21 de mayo de 2021).
- Dickson, A., A. L. Leaf and J. F. Hosner. 1960. Quality appraisal of White spruce and White pine seedling stock in nurseries. *The Forestry Chronicle* 36(1):10-13. Doi: 10.5558/tfc36010-1.
- Flores-Pacheco, J. A. 2017. Chancro resinoso del pino (*Fusarium circinatum*): Historia, evolución, dispersión y estrategias de manejo. *Nexo Revista Científica* 30(1):19-42. Doi: 10.5377/nexo.v30i01.5170.
- García-Díaz, S. E., A. Aldrete, D. Alvarado-Rosales, D. Cibrián-Tovar, ... y A. Equihua-Martínez. 2017. Efecto de *Fusarium circinatum* en la germinación y crecimiento de plántulas de *Pinus greggii* en tres sustratos. *Agrociencia* 51(8):895-908. <https://www.redalyc.org/pdf/302/30253817006.pdf>. (13 de octubre de 2023).
- García-Díaz, S. E., A. Aldrete, D. Alvarado-Rosales, D. Cibrián-Tovar and J. T. Méndez-Montiel. 2019. *Trichoderma harzianum* Rifai as a biocontrol of *Fusarium*

circinatum Nirenberg & O'Donnell in seedlings of *Pinus greggii* Engelm. ex Parl. in three substrates. *Revista Chapingo Serie Ciencias Forestales y del Ambiente* 25(3):353-367. Doi: 10.5154/r.rchscfa.2018.12.088.

Gómez D., T. 2021. *Los desafíos ambientales de México en el 2021*. Mongabay. <https://es.mongabay.com/2021/01/desafios-ambientales-mexico-2021-acuerdo-de-escazu-nuevas-leyes-bosques-clima/>. (4 de septiembre de 2022).

González P., B., C. Pintos V., J. P. Mansilla V., O. Aguin C. y R. Pérez O. 2008. Presencia de especies de *Fusarium* sobre semillas de *Pinus* spp. en Galicia. *Cuadernos de la Sociedad Española de Ciencias Forestales* (26):149-154. Doi: 10.31167/csef.v0i26.9740.

Gordon, T. R., C. L. Swett and M. J. Wingfield. 2015. Management of *Fusarium* diseases affecting conifers. *Crop Protection* 73:28-39. Doi: 10.1016/j.cropro.2015.02.018.

Leslie, J. F. and B. A. Summerell. 2006. *The Fusarium laboratory manual*. Blackwell Publishing. Ames, IA, United States of America. 388 p.

Moraga-Suazo, P., A. Opazo, S. Zaldúa, G. González and E. Sanfuentes. 2011. Evaluation of *Trichoderma* spp. and *Clonostachys* spp. strains to control *Fusarium circinatum* in *Pinus radiata* Seedlings. *Chilean Journal of Agricultural Research* 71(3):412-417. Doi: 10.4067/S0718-58392011000300011.

Murace, M. y A. Aprea. 2011. *Enfermedades forestales. Generalidades*. Facultad de Ciencias Agrarias y Forestales de la Universidad Nacional de la Plata (UNLP). https://aulavirtual.agro.unlp.edu.ar/pluginfile.php/20946/mod_resource/content/1/2011Enfermedades%20forestales.%20Generalidades..pdf. (15 de noviembre de 2023).

O'Donnell, K., B. K. Whitaker, I. Laraba, R. H. Proctor, ... and D. M. Geiser. 2022. DNA sequence-based identification of *Fusarium*: A work in progress. *Plant Disease* 106(6):1597-1609. Doi: 10.1094/PDIS-09-21-2035-SR.

Okorski, A., T. Oszako, J. A. Nowakowska and A. Pszczółkowska. 2014. The possibilities of biologically protecting plants against diseases in nurseries, with

special consideration of Oomycetes and *Fusarium* fungi. Leśne Prace Badawcze (Forest Research Papers) 75(3):301-321. Doi: 10.2478/frp-2014-0029.

Robles Y., L., S. G. Leyva M., A. Cruz G., M. Camacho T., D. Nieto Á. y J. M. Tovar P. 2016. *Fusarium oxysporum* Schltdl. y *Fusarium solani* (Mart). Sacc. causantes de la marchitez de plántulas de *Pinus* spp. en vivero. Revista Mexicana de Ciencias Forestales 7(36):25-36. Doi: 10.29298/rmcf.v7i36.57.

Rueda S., A., J. de D. Benavides S., J. Á. Prieto-Ruiz, J. T. Sáenz R., G. Orozco-Gutiérrez y A. Molina C. 2012. Calidad de planta producida en los viveros forestales de Jalisco. Revista Mexicana de Ciencias Forestales 3(14):69-82. Doi: 10.29298/rmcf.v3i14.475.

Sáenz R., J. T., H. J. Muñoz F., C. M. Á. Pérez D., A. Rueda S. y J. Hernández R. 2014. Calidad de planta de tres especies de pino en el vivero "Morelia", estado de Michoacán. Revista Mexicana de Ciencias Forestales 5(26):98-111. Doi: 10.29298/rmcf.v5i26.293.

Sáenz-Romero, C. 2014. Guía técnica para la planeación de la reforestación adaptada al Cambio Climático. Comisión Nacional Forestal (Conafor). Zapopan, Jal., México. 72 p.

https://www.gob.mx/cms/uploads/attachment/file/80238/Guia_Tecnica_para_la_Planeacion_de_la_Reforestacion.pdf. (21 de mayo de 2021).

SAS Institute Inc. 2013. SAS/STAT® 13.1 User's Guide. The GLIMMIX Procedure. SAS Institute Inc. Cary, NC, United States of America. 375 p. <https://support.sas.com/documentation/onlinedoc/stat/131/glimmix.pdf>. (13 de octubre de 2023).

Towsend, G. R. and J. W. Heuberger. 1943. Methods for estimating losses caused by diseases in fungicides experiments. The Plant Disease Reporter 27(17):340-343. <https://eurekamag.com/research/025/008/025008582.php>. (15 de noviembre de 2023).

Vicente-Arbona, J. C., V. Carrasco-Hernández, D. A. Rodríguez-Trejo y A. Villanueva-Morales. 2019. Calidad de planta de *Pinus greggii* producida en sustratos

a base de aserrín. *Madera y Bosques* 25(2):e2521784. Doi: 10.21829/myb.2019.2521784.



Todos los textos publicados por la **Revista Mexicana de Ciencias Forestales** –sin excepción– se distribuyen amparados bajo la licencia *Creative Commons 4.0 Atribución-No Comercial (CC BY-NC 4.0 Internacional)*, que permite a terceros utilizar lo publicado siempre que mencionen la autoría del trabajo y a la primera publicación en esta revista.