

Biocontrol of *Fusarium oxysporum* by *Trichoderma* spp. in *Aloe vera* under greenhouse and field conditions

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Abstract

During the last decade, an increase in the incidence of the fungus *Fusarium oxysporum* in aloe [*Aloe vera* (L.) Burm.f.] plants has been observed, reducing the national production by 14%. A study was conducted to evaluate five native strains of *Trichoderma* against *F. oxysporum* in *A. vera* plants, under greenhouse and field conditions. The experiment in the greenhouse consisted of evaluating 1-year-old *A. vera* plants, which were inoculated in the root with a suspension of 10 mL of 1×10^8 conidia of *Trichoderma* spp. This experiment consisted of six treatments with ten repetitions; five strains of *Trichoderma* spp. and the control (without treatment). The evaluation in field was conducted for one year (2016-17) to test five strains of *Trichoderma* spp. and the control, under a randomized complete block design with five replications. The evaluation under greenhouse conditions showed that when *A. vera* plants were treated with *Trichoderma asperellum*, the lowest incidence (5%) of *F. oxysporum* was observed and plants had the highest number of leaves than the rest of the treatments with 0.9% of total solids. Under greenhouse conditions, plants inoculated with *Trichoderma asperellum* and *T. harzianum* reduced the incidence of *F. oxysporum* by 30% and 40%, respectively. In the field, when evaluating *T. asperellum*, a lower incidence (9%) of *F. oxysporum* was observed in *A. vera* plants, and the leaves showed 0.9% of total solids. The plants inoculated with *T. asperellum* and *T. harzianum* decreased the incidence of the phytopathogen by 40% and 50%, respectively. In addition, an increase was observed in plant height, number of leaves, total solids, weight of root and leaves of *A. vera* with different *Trichoderma* species both under greenhouse and field conditions.

Keywords: *Aloe vera*, Biological control, *Fusarium oxysporum*, *Trichoderma asperellum*, *Trichoderma harzianum*.

Introduction

Aloe vera is a crop of agricultural and industrial importance and Mexico is one of its main producing countries around the world (Zakia *et al.*, 2013). The national production is dedicated for export mainly to the United States of America, Latin America and Europe, where it is used mainly for the pharmaceutical and cosmetology industries (SIAP, 2014). However, one of the main limitations during aloe production is the damage caused by different phytopathogens namely *F. oxysporum* (Gajera *et al.*, 2013; Dinolfo *et al.*, 2017), *Fusarium solani*, *Phythium ultimum*, *Aspergillus verocosa*, *Plectosphaerella cucumerina*, *Mammeria ehinobotryoides*, *Torula herbariu*, and *Pectobacterium chrysanthemi* (Mandal and Maiti,

2005; Ji *et al.*, 2007; Ayodele and Ilondu, 2008; Kawuri *et al.*, 2012). During the last decade, an increase in the incidence of the fungus *F. oxysporum* in this crop has been observed that causes root necrosis, yellowing and wilting of plants, which limits the quantity and quality of *A. vera* leaves (Kawuri *et al.*, 2012), reducing the national production by 14% (SIAP, 2014). Currently, the control of phytopathogens in the field is based on the intensive use of synthetic agrochemicals, which has led to the appearance of resistance in plant pathogens to the agrochemical's active ingredient, in addition to being an expensive control method and generating risks to environment and public health (Yadav *et al.*, 2015).

One of the alternatives for the control of

phytopathogens is the use of native antagonistic microorganisms, which offer high percentages of pathogen inhibition, due to their ability to produce molecules such as enzymes, acids and polyphenols that inhibit the growth of phytopathogens, and also present adaptation to environmental factors. They are harmless to the environment, economical and do not represent a risk to health, furthermore (Osorio-Hernández *et al.*, 2014). These characteristics confer to the biological control an advantage over synthetic agrochemicals, because they do not show residuality in the crop. It is worth mentioning that *A. vera* gel is intended for the pharmaceutical, and cosmetic industry, therefore, it must be certified as 100% organic product in order to be exported (IASC, 2018).

Many greenhouse and field studies have shown the antagonistic effect of different microorganisms against phytopathogens. Some important antagonist microorganisms include *Bacillus* and *Pseudomonas* species (Vega, 2001; Sharf *et al.*, 2021), actinomycetes (Ezziyyani *et al.*, 2004), *Aspergillus* spp. (Khan and Javaid, 2021), *Penicillium oxalicum* (Javaid *et al.*, 2020) and *Trichoderma* species (Akhtar and Javaid, 2018), causing biological control of different phytopathogens especially those causing root diseases such as *F. oxysporum*, *Sclerotium rolfsii*, *Macrophomina phaseolina* and *Phytophthora parasitica* (Yasmeen and Siddiqui, 2017; Javaid *et al.*, 2018; Ali *et al.*, 2020). Moreover, importance of *Trichoderma* lies in its rapid growth, and production of metabolites with antimicrobial properties (Osorio-Hernández *et al.*, 2014; Khan *et al.*, 2021), in addition to induction of endogenous plant defense mechanisms (Gajera *et al.*, 2013; Osorio-Hernández *et al.*, 2016). *Trichoderma* is one of the most used antagonists due to its different mechanisms of action (Khan and Javaid, 2020; Khan *et al.*, 2021). Infante *et al.* (2009) consigned the effects of *Trichoderma* on plants, including induction of systemic or localized resistance, due to the production of a rich mixture of antifungal enzymes such as chitinases and β -1, 3 glucanases. These fungi colonize the epidermis of the root, and outer cortical layers, releasing volatile compounds such as ethylene, alcohols, aldehydes, ketones, and non-volatile compounds such as peptides, highlighting the molecule called λ -amino acid oxidase, which have been shown to inhibit the mycelium growth of *Rhizoctonia solani* (Yang *et al.*, 2011). Another mechanism of action of species of *Trichoderma* genus is the production of growth regulators (auxins and gibberellins) that promote development of plant root system, which increases its tolerance to water stress (Mukherjee *et al.*, 2013). In addition, it has been proven that *Trichoderma* species produce compounds related to the activation of systemic resistance, the elimination of toxins excreted by pathogens, deactivation of these enzymes during the

infection process, and the solubilization of soil nutrients, which improve plant growth (Liu *et al.*, 2016). The objective of this research was to evaluate the biological efficiency of native strains of *Trichoderma* on the incidence of *F. oxysporum* under greenhouse and field conditions, and to assess the effects of inoculation on the physiological state of *A. vera* plants.

Materials and Methods

Plant material and experimental location

The greenhouse trial was established at the Institute of Applied Ecology, the Autonomous University of Tamaulipas, located in Cd. Victoria, Tamaulipas, Mexico. The field work was carried out in the Juan Capitan Town Victoria, Tamaulipas. The phytopathogenic strains were isolated from *A. vera* plants with symptoms of root necrosis and leaves yellowing, while the *Trichoderma* strains were isolated from agricultural soils of Victoria, Xicoténcatl, Padilla, Jaumave, and Llera de Canales, counties, Tamaulipas State, Mexico.

Greenhouse trial

Five strains of *F. oxysporum*, four strains of *T. asperellum* (TV, TLL, TX, TP) and one strain of *T. harzianum* (TJ) were isolated from *A. vera* plots. The native strains of *F. oxysporum* and *Trichoderma* spp. were purified by monospore culture in Petri dishes with PDA medium (potato-dextrose-agar, Difco) and kept at 25 °C for eight days. Once the inoculum was increased, each strain was transferred to 250 mL flasks, in which five slices with mycelium of 1 cm in diameter were placed in 50 mL of liquid medium added with potato broth, sucrose and yeast extract (5%) and kept at 27±2 °C and 150 rpm in a shaker (Labco®) for seven days. Subsequently, 20 cm- *A. vera* seedlings were planted in plastic bags with a capacity of 2 kg in which peat moss or vermicompost were used as substrate, roots of each plant were inoculated with a suspension of 10 mL of conidia at a concentration of 1×10⁸ mL⁻¹ of *Trichoderma* spp., the next day, a suspension of 10 mL of *F. oxysporum* conidia at a concentration of 1×10⁸ mL⁻¹ was inoculated in the plant roots. *Aloe vera* plants without inoculum were evaluated as a control. The variables to be measured were disease incidence, colony-forming units (CFU) of *F. oxysporum*, height, number of leaves, total foliar gel solids, weight of root and *A. vera* leaves. This evaluation was carried out from June 2016 to June 2017. The experiment was established under a completely randomized design with six treatments (five strains of *Trichoderma* spp. and one control) and ten replications. The substrates used in the greenhouse experiment were peat moss and vermicompost. Subsequently, the data were analyzed using an analysis of variance (ANOVA), when necessary, the comparison of means was performed

with the Tukey method ($P = 0.05$), by using statistical program SAS (Statistical Analysis System, version 9).

Field trial

The field experiment was carried out in a plot with a history of the presence of *F. oxysporum*, which reported a concentration of 1×10^8 mL. Sowing of one-year-old *A. vera* plants was carried out in June 2016, using five native strains of *Trichoderma* (Four strains of *T. asperellum* (TV, TLL, TX, and TJ) and a strain of *T. harzianum* (TP), and a treatment without inoculum (as a control). First, *Trichoderma* strains were inoculated in *A. vera* roots by immersing the plant in a bath containing a 3,000 mL solution with conidia of the antagonist at a concentration of 1×10^8 mL⁻¹, the plants were kept in contact with the solution for 10 min, and then planted. Two months later, a second inoculation was performed with *Trichoderma* spp. in the basal part of the plant with 20 mL at a concentration of 1×10^8 mL⁻¹ conidia in each treatment. The variables evaluated were disease incidence, colony-forming units (CFU) of *F. oxysporum*, height, number of leaves, total foliar gel solids, weight root and leaves.

This experiment was established under a randomized complete block design, with six treatments and five replications. The experimental unit consisted of plots with 5 rows, 5 m long, 50 cm row to row and 30 cm plant to plant distance. An analysis of variance (ANOVA) was performed with all the obtained data and the treatment means were compared with Tukey's test ($P = 0.05$), using the statistical program SAS (Statistical Analysis System) version 9.0.

Soil physical and chemical characteristics

Soil physical and chemical characteristics (texture, pH, electrical conductivity, sodium adsorption ratio, organic matter, total nitrogen, extractable phosphorus, exchangeable potassium, extractable iron, extractable zinc and total carbonates) were determined at the Agricultural Research and Diagnosis Laboratory of the School of Engineering and Sciences-the Autonomous University of Tamaulipas. In addition, relative humidity, precipitation, and temperature were recorded for one year using a portable weather station (Davis Instruments 6351 Vantage Vue).

Results and Discussion

Greenhouse trial

In the evaluation of the native strains of *Trichoderma* under greenhouse conditions. There were significant differences ($P = 0.05$) among the substrate used in the experiment, observing higher values in the variables: height, number of leaves, root and leaf weight in plants grown on vermicompost. However, in the two substrates

inoculated with *Trichoderma* strains, optimal percentages were observed in the total solids' variable, but not in the control plants. In all the treatments where the native strains of *Trichoderma* spp were used, a decrease in the percentages of incidence with respect to the control was observed (Table 1 and 2).

It should be noted that the treatments in which the *T. asperellum* (TV) strain was applied, were those that promoted the highest plant growth in comparison to the other treatments, however, in the treatments with *T. asperellum* from Llera de Canales and Xicoténcatl localities (TLL and TX), the plants showed the highest number of leaves in the two substrates. The root weight varied in the substrates of peat moss and vermicompost from 428 to 1,623.1 g, respectively, being the plants inoculated with *Trichoderma harzianum* (TP) those that showed the highest root weight. Regarding the leaf weight variable, the values ranged from 2,397.3 g in peat moss and 4,687.5 g in vermicompost (Table 1 and 2). For this reason, the substrate effect on the crop was evidenced. Plants grown on vermicompost had higher values of height, number of leaves, root and leaf weight, and a decrease in the incidence of the disease caused by *F. oxysporum*, than those grown on peat moss.

On the other hand, it was observed that all treatments with *Trichoderma* strains reduced the CFU of the phytopathogen regardless of the substrate, this was achieved by inoculating concentrations of 1×10^8 of the antagonist to the *A. vera* plants. Similar results to this research were reported by Checa-Coral *et al.* (2017), who evaluated the antagonistic effectiveness of native strains of *Trichoderma* on *F. oxysporum* f. sp. pisi, in *Pisum sativum*, under greenhouse conditions. The *Trichoderma* strains were in doses of 20 mL at concentrations of 1×10^8 , and 10^6 conidia mL⁻¹, which promoted plant height, and fresh weight of the roots. However, it was reported that C12 and C21 strains of *T. harzianum*, were the ones that showed an antagonistic effect in the treatments, these data differ from what is reported in the present investigation, where, it was observed that the treatment TL1 (*T. asperellum*) was that with low percentages of *F. oxysporum* incidence (5%).

The antagonistic capacity of *T. asperellum* against phytopathogens had already been proven in studies carried out by Hoyos-Carvajal *et al.* (2008) who reported that *T. asperellum* (T-21 and T-7) decreased the incidence of *Sclerotium rolfsii* in bean seedlings by 90%. On the other hand, with respect to total solids, the values ranged from 0.8 to 0.9% between the *Trichoderma* strains. However, they were higher compared to the control 0.6%. It is important to point out that the acceptable values of total solids in the Aloe gel for exportation are from 0.8% to 0.9%, for which the results found in this investigation show that the native strains of

Trichoderma, constitute a viable option for biological control of *F. oxysporum* during *A. vera* production (Koyyappurath *et al.*, 2015).

Field trial

The Aloe plants that were inoculated by *F. oxysporum* presented necrosis in the root and stem. A change in color from dark green to orange was observed in the leaves and ended with plant death. These symptoms are associated with the presence of *F. oxysporum* in this crop (Leslie and Summerell, 2006). On the other hand, it was observed that *F. oxysporum* incidence is related to temperature, since average temperatures at the experiment site ranged between 19.8 and 36.9 °C. Another relevant factor was relative humidity, which fluctuated from 65 to 88.6% with 950 mm of annual precipitation (Fig. 1). These conditions were favorable for the development of this phytopathogen, since it proliferates in environments with relative humidity levels from 74 to 80% and temperatures ranging between 23 and 28 °C (Benaouali *et al.*, 2014; Ramos-Quintana *et al.*, 2017). On the other hand, *F. oxysporum* increased over time, this is related to changes in temperatures and relative humidity, since, in the months of higher and lower rainfall, a trend of increase disease over time was observed. In addition to the unstable precipitation that led to the development of the phytopathogen. The results observed in this research coincide with those reported by Batlle-Viera and Pérez-Vicente (2009), who consigned that there is a correlation between temperature, and optimal growth of the phytopathogen when evaluating populations of *F. oxysporum* f. sp. cubense in bananas from Cuba, where it was observed that the organism presents a growth plateau in the temperature range between 22.5 to 27.5 and 35 °C, with the optimum temperature being 27 °C; These data coincide with what was reported in the present investigation, since temperatures that ranged between 19.8 to 36.9 °C were observed, which is why the incidence of the disease grew over time.

Regarding the pluvial precipitation, it was observed that unstable precipitation influenced the incidence of the disease due to the correlation of this factor with relative humidity (Fig. 1), a necessary condition for the phytopathogen to spread in the soil and crop. In the present study, rains were recorded during the months of September to November 2016 and occurred again in March to April 2017, where an incidence that fluctuated between 12 and 27% was reported in the treatments with *Trichoderma* spp., however, an incidence of 62% was reported in the control treatment.

On the other hand, it was observed that temperature and relative humidity stand out for their importance during development of *Trichoderma* spp., since in optimal conditions of humidity (>75%) and temperature (28-30 °C) the *Chlamydozopore* production of *Trichoderma* spp. has been reported

(Infante *et al.*, 2009) (Fig. 1). Similar results to those mentioned by Jackson *et al.* (1991) who observed that *T. viride* and *T. pseudokoningii* isolates showed mycelial growth at temperatures ranging from 10 to 35 °C, with a maximum growth at 25 °C, this factor provides an advantage in the growth of the antagonist in a wide range of temperature, and with it, competition for space and nutrients against *F. oxysporum* is guaranteed; in this study, native strains of *Trichoderma* were reported to grow at temperatures ranging from 19.8 to 36.9 °C. On the other hand, Partridge *et al.* (2006) indicated that mycoparasitism of *S. minor* sclerotia by *C. minitans* occurred at temperatures ranging from 14 to 22 °C, and that only a small percentage of sclerotia were parasitized by the antagonist at temperatures above 28 °C. Therefore, it has been observed that commercial products formulated with *Trichoderma* spp. are applied during the warmer months of the year (May-August), generally before planting, when conditions are more favorable for sclerotia mycoparasitism (Domínguez *et al.*, 2016), and this system could be adapted for the management of Aloe. On the other hand, in relation to *Trichoderma* conidia concentration. Czech-Coral *et al.* (2017) found similar results to this work, when evaluating the antagonistic effectiveness of native strains of *Trichoderma* on *F. oxysporum* f. sp. pisi under greenhouse and field conditions in *Pisum sativum* L., the field evaluation showed that plants inoculated with strain C12 (*T. harzianum*), at a concentration of 1×10^8 had a lower incidence of *F. oxysporum* in comparison to control plants, but, without effect on biomass yields. A higher incidence of *F. oxysporum* was observed in the control treatment in the months of April to July 2017, with an incidence of 62%, due to the fact that there were percentages of relative humidity in the environment that varied between 68.9, and 87.6%, a determinant factor for proliferation of phytopathogens in the field (Fig. 2).

Another factor associated with the incidence of *F. oxysporum* in the field is clay soil which has drainage problems, causing an increase in soil moisture in percentages greater than 45%. It has been documented that this type of soil texture favors the presence of fusariosis, causing a 15% decrease in crop harvest (Duarte-Leal, 2016). This phenomenon has also been observed in the Mediterranean basin and California, where *F. oxysporum* destroyed crops that presented favorable conditions for their development, highlighting the pH above 6 and the texture of the clay soil, in addition, they highlighted that those soils with deficiencies of micro and macro nutrients (Na, K, Ca, F), are related to the proliferation of *F. oxysporum* strains in the crop (De la Fe and Hernández, 2011). In the present study (Table 3), the organic functional analysis showed that the experimental site has a type of soil that corresponds to clay, and that has an optimal pH for the proliferation of the phytopathogen, and the

antagonist, however, a deficit in macro and micronutrients was observed, which are determining factors for the infection process by the phytopathogen (Table 3). A plant deficient in micronutrients, generally has depressed defense capabilities against soil-borne diseases. However, in some cases, nutrients can have direct effects on soilborne pathogens. For example, manganese (Mn) applied to the soil can inhibit the growth of certain fungi. Furthermore, nitrites are toxic to some species of *Fusarium* and *Phytophthora*. Nitrites are formed from ammonium nitrogen, during the nitrogen cycle as it is converted to nitrates by beneficial soil bacteria (Buyer *et al.*, 2002).

The evaluation of plant variables under field conditions (Table 4), showed significant differences ($P = 0.05$), being the plants inoculated with the *T. asperellum* strain, those that showed greater height and number of leaves in comparison to the other treatments. However, in the root and leaf weight variables, differences were observed among treatments ($P = 0.05$), with the plants treated with the *T. asperellum* strain with the highest root weight, and the plants treated with *T. asperellum* had higher leaves weight. However, the treatments with *T. asperellum* strain showed the lowest percentage of phytopathogen incidence with 9%, so this variable oscillated between treatments of *Trichoderma* spp. from 9 to 27%, this difference would be due to differences in the activation of the mechanisms of action of each native strain. On the other hand, it was observed that the treatment where the antagonist was not inoculated, showed an incidence of the phytopathogen of 62%. These results are similar to those reported by Porras *et al.* (2007) in studies carried out on strawberry cultivation, where they found that the application of *Trichoderma* spp. reduces the incidence of *P. cactorum* by 76.6%. These results are based on the metabolites produced by *Trichoderma* spp. that have antifungal activity and constitute a group of volatile, and non-volatile compounds, very diverse in structure and function. That inhibit other microorganisms, with which physical contact is not established, and these inhibitory substances were considered antibiotics (Naglot *et al.*, 2015; Ng *et al.*, 2015).

The differences between the evaluated variables of the *Trichoderma* species are because biological agents are significantly affected by the environment, and seasonal or annual variation in their effectiveness can be observed. This has been corroborated in studies conducted by Poldma *et al.* (2002), who evaluated during four years, different *Trichoderma* strains on cucumber, they mentioned that the fourth year, it was observed increased production in the plants treated with *Trichoderma*. Studies conducted by Buyer *et al.* (2002), showed that the fungal populations present in the rhizosphere are strongly affected by type of soil and plant, plant-soil interaction, and activity of fungal and bacterial populations. In this sense, it has been reported that soils with a sandy clay loam texture have the optimal characteristics for the proliferation of the microbiota, because they have an optimal pH, and a source of organic matter.

Conclusion

In conclusion, under greenhouse conditions, the inoculated plants with *T. asperellum* and *T. harzianum* strains decreased *F. oxysporum* incidence by 30 and 40%. In addition, it was observed that the plants treated with the vermicompost substrate showed higher values in the variables: plant height, number of leaves, and root and leaf weight. Under field conditions, plants inoculated with *T. asperellum* showed a lower incidence of *F. oxysporum* in 9%, where the leaves showed 0.9% in total solids, this means an increase in this variable. The inoculated plants with *T. asperellum* and *T. harzianum*, decreased the incidence by 40 and 50%, respectively. In addition, it was observed that under greenhouse and field conditions, *Trichoderma* treatments increased height, number of leaves, total solids, roots and leaves weight of *Aloe* plants.

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Table 1: Means of variables evaluated in *Aloe vera* plantstreated with strains of *Trichoderma*, as protection against *Fusarium oxysporum* under greenhouse conditions.

Treatments Substratum Peat moss	Height (cm)	Number of leaves	Root weight (g)	Leaves weight (g)	Incidence (%)	Total solids (%)	UFC <i>F. oxysporum</i>
TP (<i>T. harzianum</i>)	32.5 c*	15 a	712.6 a	4256.8 a	12.5 d	0.8 a	10 ³ b
TLL (<i>T. asperellum</i>)	42.7 b	14 a	578.2 b	4034.8 a	12.5 d	0.9 a	10 ² b
TV(<i>T. asperellum</i>)	52.5 a	17 a	542.1 b	3472.2 b	12.5 d	0.8 a	10 ³ b
TX (<i>T. asperellum</i>)	38.7 b	14 a	428.8 c	2697.3 b	37.5 b	0.8 a	10 ³ b
TJ (<i>T. asperellum</i>)	28.2 c	14 a	598.5 b	2892.6 b	25.0 c	0.8 a	10 ⁴ b
Control (T0)	20.9 d	9 b	109.4 d	1263.2 c	62.5 a	0.6 b	10 ⁸ a

T: *Trichoderma* spp., P (Padilla), LL (Llera de Canales), V (Victoria), X (Xicoténcatl), J (Jaumave). Treatments

with the same letter are statistically the same according to Tukey's test ($P=0.05$). CFU = Colony forming units.

Table 2: Means of variables evaluated in aloe plants (*Aloe barbadensis* Miller) treated with strains of *Trichoderma* spp. as protection against *F. oxysporum* under greenhouse conditions with vermicompost substrate.

Treatments Substratum Vermicompost	Height (cm)	Number of leaves	Root weight (g)	Leaves weight (g)	Incidence (%)	Total solids (%)	CUF <i>Fusarium oxysporum</i>
TP (<i>T. harzianum</i>)	44.6 c	18 a*	1623.1 a	4687.5 a	16 c	0.9 a	10 ³ b
TLL (<i>T. asperellum</i>)	54.9 b	16 a	942.3 b	4267.2 a	4 e	0.9 a	10 ² b
TV (<i>T. asperellum</i>)	62.2 a	17 a	582.4 c	4578.3 a	12 d	0.8 b	10 ² b
TX (<i>T. asperellum</i>)	43.5 c	16 a	532.7 c	2457.3 c	24 b	0.9 a	10 ³ b
TJ (<i>T. asperellum</i>)	45.7 c	15 a	918.2 b	3897.2 b	16 c	0.8 b	10 ⁴ b
Control (T0)	23.1 d	10 b	214.7 d	1538.2 d	56 a	0.6 c	10 ⁸ a

T corresponds to *Trichoderma* spp. and the second letter corresponds to the collection site P (Padilla), LL (Llera de Canales), V (Victoria), X (Xicoténcatl), J (Jaumave). Treatments with the same letter are statistically same according to Tukey's test ($P = 0.05$). CFU = Colony Forming Units.

Table 3: Organic functional analysis of agricultural soil in the lot of Victoria, Tamaulipas.

Variable	Values
Texture	Clay
pH in water 1:2	8.4
Electrical conductivity (mS cm ⁻¹)	0.31
Sodium	0.17
Organic material (%)	2.32
Total nitrogen (%)	0.14
Extractable phosphorus (mg kg ⁻¹)	1.14
Potassium (meq 100 g ⁻¹)	0.34
Iron (mg kg ⁻¹)	2.82
Zinc (mg kg ⁻¹)	0.45
Total carbonate (%)	29.0

Table 4: Comparison of the variables evaluated in *Aloe vera* plants inoculated with *Trichoderma* spp. as an antagonist against *Fusarium oxysporum* under field conditions.

Treatments Substratum Lombricompost	Hight (cm)	Number of leaves	Root weight (g)	Leaves weight (g)	Incidence (%)	Total solids (%)
TP(<i>T. harzianum</i>)	54.1 b*	18 c	852.0 c	5978.5 a	12 e	0.9 a
TLL(<i>T. asperellum</i>)	51.5 b	18 c	962.0 b	5234.9 c	9 f	0.9 a
TV (<i>T. asperellum</i>)	67.8 a	22 a	745.9 d	5467.1 b	27 b	0.8 a
TX (<i>T. asperellum</i>)	51.3 b	19 b	1056.2 a	4587.4 d	23 c	0.9 a
TJ (<i>T. asperellum</i>)	47.5 c	16 d	492.7 e	4879.1 e	16 d	0.8 a
T0	29.5 d	15 e	246.2 f	1874.1 f	62 a	0.7 a

T corresponds to *Trichoderma* spp. and the second letter corresponds to the collection site TP (Padilla), TLL (Llera de Canales), TV (Victoria), TX (Xicoténcatl), TJ (Jaumave). Treatments with the same letter are statistically nonsignificant according to Tukey's test ($P = 0.05$).

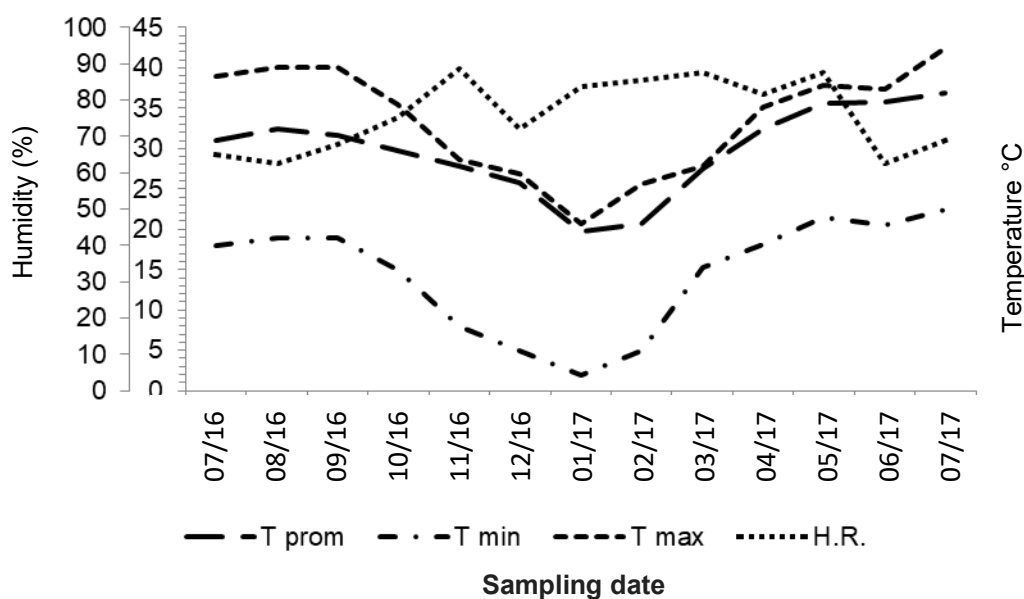


Fig. 1: Average of maximum, minimum, average and relative humidity data for each month (2016-2017), in Juan Capitan, Victoria, Tamaulipas. T max = maximum temperature; T min = minimum temperature; T avg = average temperature; R.H. = relative humidity.

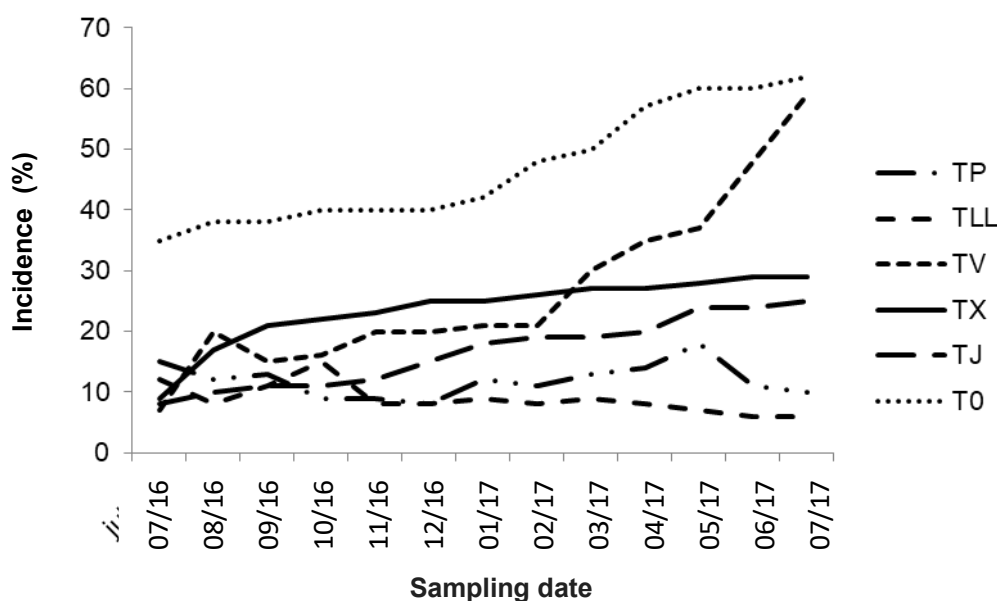


Fig. 2: Percentage of *F. oxysporum* incidence on *Aloe* under field conditions (2016-2017), in TP (*T. asperellum*), TLL (*T. asperellum*), TV (*T. asperellum*), TX (*T. harzianum*), TJ (*T. asperellum*) and T0 (control).

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