CONTROL OF *FUSARIUM VERTICILLIOIDES* (SACCARDO) NIRENBERG IN GENOTYPES OF CORN (*ZEA MAYS* L.) WITH *TRICHODERMA HARZIANUM* RIFAI, *BACILLUS SUBTILIS* (JANSEN) AND COPPER IN GREENHOUSE

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ABSTRACT

The objective this research was to determine the control of *Trichoderma harzianum* Rifai, *Bacillus subtilis* (Ehrenberg) Cohn and Copper against *Fusarium verticillioides* (Saccardo) Nirenberg in roots of three types of corn in greenhouse. *F. verticillioides* was isolated at Universidad Autonoma Agraria Antonio Narro, and was purified using monosporic culture. Inoculation was into the seeds was performed, using different treatments, was evaluated 85 days after seedtime and data was displayed as percentage. An analysis of variance with comparison between means was performed with a Tukey test (p=0.05).Was analyzed using a SAS 9.1. software. *T. harzianum* was the most efficient treatment; plants presented a better germination, height, stem diameter, with an effect of control of *F. verticillioides* of 75% and 31.97% in severity.

Keywords: Incidence, severity, control, crops.

INTRODUCTION

Corn in Mexico is necessary for rural and urban population nourishment, cattle raising and industry (PROMEAR, 2009). Production during the 2019 cycle was 593, 180 t and occupates the eighth place of production after United States, China, Brazil, Argentina, Ukraine, Indonesia and India (FAO, 2020). *Fusarium* in corn causes root of stem and cob rot, in sugar cane the rotting of the stem known as pokka-boeng (Romero, 1993) and in rice causes bakanae disease or gigantism by the gibberellin produced by this fungi (Rojas, 1984).

F. verticillioides is a pathogen that affects roots and corn cobs (McKeen 1953, Visconti and Doko 1994), it can disease on every stage of development of the plant and can affect different parts of the same, impacting on yield and quality of the seed (Schulthess et al., 2002). Gleen and colleagues (2002) showed that *F. verticillioides* is a fungus whit great economic impact, due to its negative effects on the plant and animal health, additionally, its direct or indirect effect on human population, since corn consume is high.

Trichoderma shows several advantages as biologic control agent (Argumedo-Delira et al., 2009) by rapid growth and development. INTAGRI (2016) mencionated that its biocontrol effects are due to is high reproductive capacity, efficiency in nutrient use, capacity to modify the rhizosphere, strong aggressivity against phytopathogenic fungus, efficiency of inducing plant growth and self-defense mechanism induction and its ability to grow under poor conditions. Due to the aforementioned, the objective of this research was to determine the control capacity of *T. harzianum*, *B. subtilis* and Copper against *F. verticillioides* in roots of three different genotypes of corn in greenhouse.

MATERIALS AND METHODS

Sampling

Sampling of corn roots was performed in June of 2017 at Universidad Autonoma Agraria Antonio Narro (25°21'30.7"N 101°02'20.8"W). Samples were placed in paper bags and taken to the phytopathology laboratory.

Isolation, identification and purification of *F. verticillioides*

Three samples of 1 cm were taken (ealthy and diseased tissu), later were washed with 3% sodium hypochlorite for 2 min and washed for 1 min with sterile distilled water (three times). Four root cuts samples were put equidistantly per Petri dishes with PDA and ketp at 25°C ± 2°C for 240 h. Purification was performed by monosporic culture (Fernandez, 1993) and identified morphologically following Nelson et al. (1983) and Leslie and Summerell (2006).

Genetic and biologic material

Corn seeds used were Creole of Arteaga, Creole of Guanajuato and Jaguan genotype; and the biologic material was *F. verticillioides*, *T. harzianum*, *B. subtilis* and copper as commercial control.

Inoculum preparation

F. verticillioides and *T. harzianum* were produced in sterile rice and *B. subtilis* in enriched broth, later suspended in water until obtaining a spore suspension of *T. harzianum and F. verticillioides* of 1×108 spores/mL^{-1} and *B. subtilis* 1×108 CFU/mL^{-1} suspension in the McFarland scale; for copper it was used the Cupravit (85% tribasic copper chloride).

Planting

Planting consisted four corn seeds to a depth of 2.5 cm in polyethylene bags whit 10 kg of soil, whit 4 replicates per variety, later they were inoculated with the different treatments (Table 1), after 15 days a second inoculation of *B. subtilis* was applied 1 mL/500 mL of water, *T. harzianum* 5 mL and 2g/1 L^{-1} of copper.

Table 1. Treatments in study

Treatments	Components
T1	*T. harzianum* a 1×10^{8} spores/mL^{-1} + *F. verticillioides* a 1×10^{8} spores/mL^{-1}
T2	*B. subtilis* a 1×10^{8} UFC/mL^{-1} + *F. verticillioides* a 1×10^{8} spores/mL^{-1}
T3	Cupravit a 2 g/L^{-1} + *F. verticillioides* a 1×10^{8} spores/mL^{-1}
T4	*F. verticillioides* a 1×10^{8} spores/mL^{-1}
T5	Control (Sterile water)

Evaluated variables

Eighty-five days after germination, height, stem diameter and incidence of *F. verticillioides* in roots ((number of roots with symptoms/total roots)*100) were evaluated by observing transversal cut of the root (reddish to brown color) and the severity by weight of the roots ((weight of affected root/total weight of roots)*100).

Statistical Analysis

The results were analyzed by the SAS version 9.1 statistical program, the mean separation whit the Tukey test to 0.05 of significance. (α=0.05).

RESULTS

White-violet mycelium with hyaline macroconidia, long and thin with approximately 5 septa and basal foot-shaped cell, besides, the hyaline macroconidia in abundance bludgeon and chain-shaped observed are characteristic for F. verticillioides results that agree whit Torre-Hernández et al., 2014 and Solano-Báez, 2011 reports.

Germination

Variance analysis did not show any significantly difference between treatments (p=0.8571). Inoculation with suspension 10^8 spores mL^{-1} of *T. harzianum* + 10^8 spores mL^{-1} of F. *verticillioides* (Treatment 1) and 2 g/L-1 Cupravit + suspension 10^8 spores mL^{-1} of F. *verticillioides* (Treatment 3) resulted higher of germination (72.92%) (Figure 1).

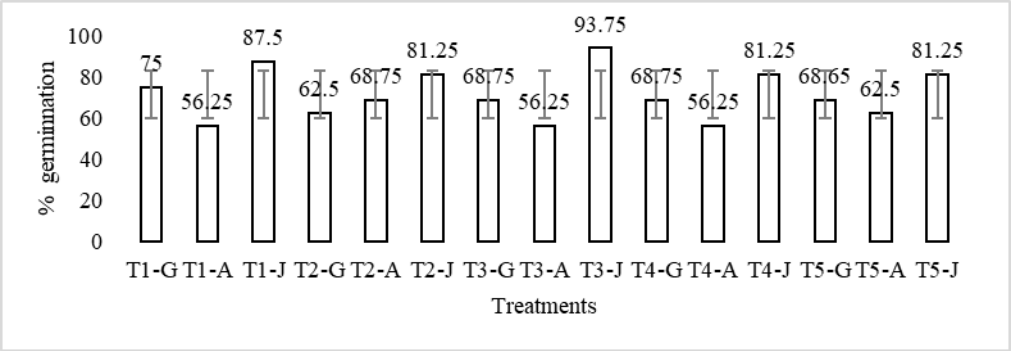

Figure 1. Effect of treatment in germination of three genotypes**.** T1-G=Treatment 1 in Creole Guanajuato, T1-A=Treatment 1 in Creole Arteaga, T1-J= Treatment 1 in Jaguan, T2-G=Treatment 2 in Creole Guanajuato, T2-A Treatment 2 in Creole Arteaga, T2-J= Treatment 2 in Jaguan, T3-G=Treatment 3 in Creole Guanajuato, T3-A Treatment 3 in Creole Arteaga, T3-J= Treatment 3 in Jaguan, T4-G=Treatment 4 in Creole Guanajuato, T4- Treatment 4 in Creole Arteaga, T4-J= Treatment 4 in Jaguan, T5-G=Treatment 5 in Creole Guanajuato, T5-Treatment 5 in Creole Arteaga, T5-J= Treatment 5 in Jaguan.

Height

Variance analysis did not show any significantly difference between treatments (p=0.8571). Inoculation with suspension 10^8 spores mL^{-1} of *T. harzianum* + 10^8 spores mL^{-1} of *F. verticillioides* (Treatment 1) and suspension 10^8 CFU mL^{-1} of *B. subtilis* + 10^8 spores mL^{-1} of *F. verticillioides* (Treatment 2) tend to show a greater height (Figure 2).

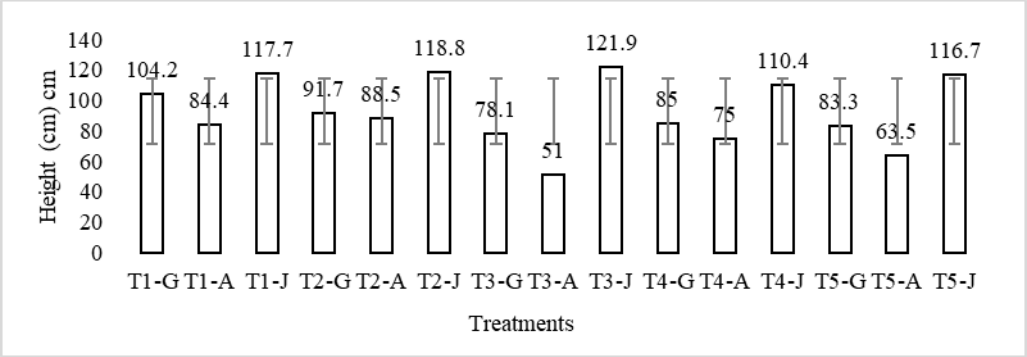

Figure 2. Effect of treatments in height of three genotypes. T1-G=Treatment 1 in Creole Guanajuato, T1-A=Treatment 1 in Creole Arteaga, T1-J= Treatment 1 in Jaguan, T2-G=Treatment 2 in Creole Guanajuato, T2-A Treatment 2 in Creole Arteaga, T2-J= Treatment 2 in Jaguan, T3-G=Treatment 3 in Creole Guanajuato, T3-A Treatment 3 in Creole Arteaga, T3-J= Treatment 3 in Jaguan, T4-G=Treatment 4 in Creole Guanajuato, T4- Treatment 4 in Creole Arteaga, T4-J= Treatment 4 in Jaguan, T5-G=Treatment 5 in Creole Guanajuato, T5-Treatment 5 in Creole Arteaga, T5-J= Treatment 5 in Jaguan.

Diameter

Variance analysis did not show any significantly difference between treatments (p=0.8571). Plants with suspension 10^8 spores mL^{-1} of *T. harzianum* + 10^8 spores mL^{-1} of *T. harzianum* (Treatment 1) and suspension 10^8 CFU mL^{-1} of *B. subtilis* + 10^8 spores mL^{-1} of *F. verticillioides* (Treatment 2) tend to induce a thicker stem compared to other treatments (Figure 3).

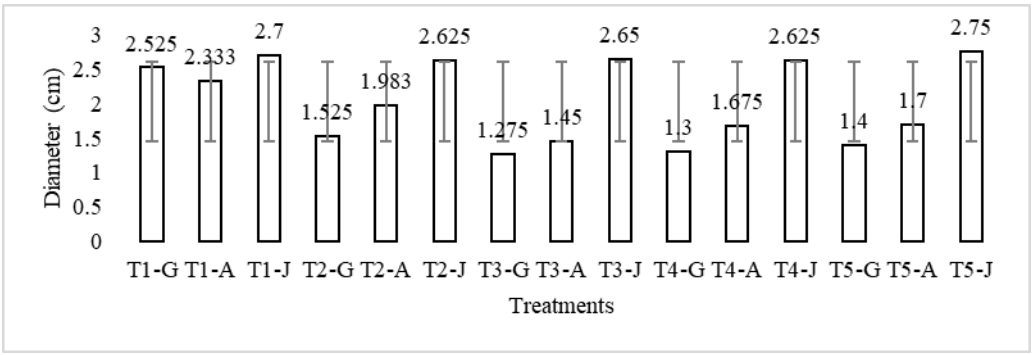

Figure 3. Effect of treatments in the diameter of three genotypes**.** T1-G=Treatment 1 in Creole Guanajuato, T1-A=Treatment 1 in Creole Arteaga, T1-J= Treatment 1 in Jaguan, T2-G=Treatment 2 in Creole Guanajuato, T2-A Treatment 2 in Creole Arteaga, T2-J= Treatment 2 in Jaguan, T3-G=Treatment 3 in Creole Guanajuato, T3-A Treatment 3 in Creole Arteaga, T3-J= Treatment 3 in Jaguan, T4-G=Treatment 4 in Creole Guanajuato, T4- Treatment 4 in Creole Arteaga, T4-J= Treatment 4 in Jaguan, T5-G=Treatment 5 in Creole Guanajuato, T5-Treatment 5 in Creole Arteaga, T5-J= Treatment 5 in Jaguan.

Incidence of *F. verticillioides* in roots

Variance analysis showed an important statistically significant difference between treatments (p=0.0030). Table 2 displays plants with suspension 10^8 spores mL^{-1} of *F. verticillioides* (Treatment 4) showed the higher incidence in roots, whereas plants treated with suspension 10^8 spores mL^{-1} of *T. harzianum* (Treatment 1) showed the least incidence of *F. verticillioides* in roots.

Table 2. Means of the incidence of *F. verticillioides*

Treatments	Means (%)	Ag		
4	100.00000	A		
5	91.670000	A	B	
2	66.670000	A	B	C
3	50.000000		B	C
1	25.000000			C

T1=*T. harzianum* a 1×10^8 spores/mL^{-1} + *F. verticillioides* a 1×10^8 spores/mL^{-1}, T2= *B. subtilis* a 1×10^8 UFC/mL^{-1} + *F. verticillioides* 1×10^8 spores/mL^{-1}, T3= Cupravid a 2 g/L^{-1} + *F. verticillioides* a 1×10^8 spores/mL^{-1}, T4= *F. verticillioides* a 1×10^8 spores/mL^{-1}, T5= Control (Sterile water), Ag= Statistical aggrupation, equal letters are not statistically different according to Tukey test to 0.05 probability.

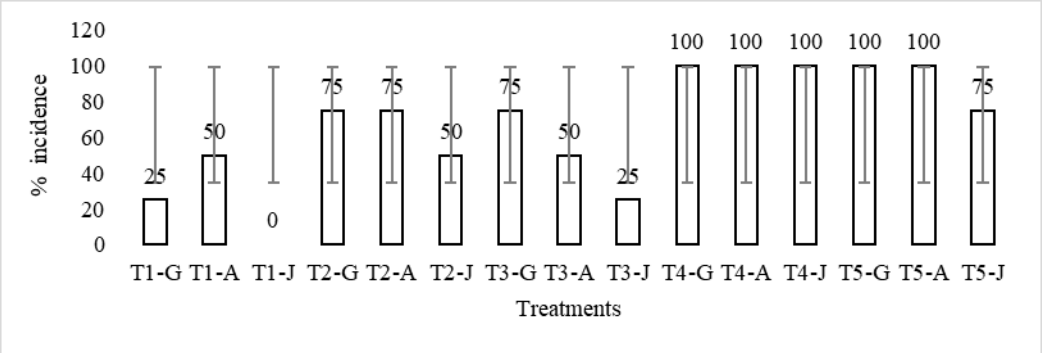

Figure 4. Effect of treatments in the incidence of three genotypes. T1-G=Treatment 1 in Creole Guanajuato, T1-A=Treatment 1 in Creole Arteaga, T1-J= Treatment 1 in Jaguan, T2-G=Treatment 2 in Creole Guanajuato, T2-A Treatment 2 in Creole Arteaga, T2-J= Treatment 2 in Jaguan, T3-G=Treatment 3 in Creole Guanajuato, T3-A Treatment 3 in Creole Arteaga, T3-J= Treatment 3 in Jaguan, T4-G=Treatment 4 in Creole Guanajuato, T4- Treatment 4 in Creole Arteaga, T4-J= Treatment 4 in Jaguan, T5-G=Treatment 5 in Creole Guanajuato, T5- Treatment 5 in Creole Arteaga, T5-J= Treatment 5 in Jaguan.

Severity of *F. verticillioides* in roots

Variance analysis did not show any important statistically significant difference between treatments (p=0.0406). Uninoculated plants (Treatment 5) displayed worst severity to *F. verticillioides* in roots, whereas plant with suspension 10^8 spores mL^{-1} of *T. harzianum* + 10^8 spores mL^{-1} of *F. verticillioides* (Treatment 1) showed less severity to this pathogen.

Table 3. Means of severity of *F. verticillioides*

Treatments	Means	Ag	
5	35.433	A	
4	34.700	A	
3	28.167	A	B
2	26.567	A	B
1	11.333		B

T1=*T. harzianum* a 1×10^8 spores/mL^{-1} + *F. verticillioides* a 1×10^8 spores/mL^{-1}, T2= *B. subtilis* a 1×10^8 UFC/mL^{-1} + *F. verticillioides* 1×10^8 spores/mL^{-1}, T3= Cupravid a 2 g/L^{-1} + *F. verticillioides* a 1×10^8 spores/mL^{-1}, T4= *F. verticillioides* a 1×10^8 spores/mL^{-1}, T5= Control (Sterile water), Ag= Statistical aggrupation, equal letters are not statistically different according to Tukey test to 0.05 probability.

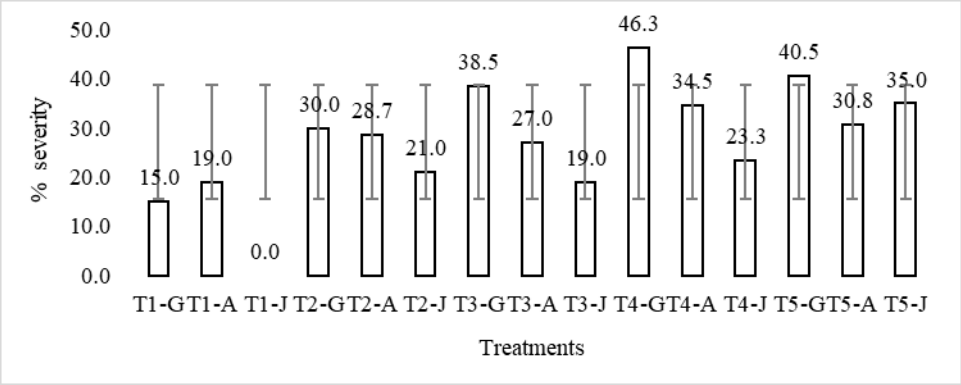

Figure 5. **Effect of treatments in the severity of three genotypes.** T1-G=Treatment 1 in Creole Guanajuato, T1-A=Treatment 1 in Creole Arteaga, T1-J= Treatment 1 in Jaguan, T2-G=Treatment 2 in Creole Guanajuato, T2-A Treatment 2 in Creole Arteaga, T2-J= Treatment 2 in Jaguan, T3-G=Treatment 3 in Creole Guanajuato, T3-A Treatment 3 in Creole Arteaga, T3-J= Treatment 3 in Jaguan, T4-G=Treatment 4 in Creole Guanajuato, T4- Treatment 4 in Creole Arteaga, T4-J= Treatment 4 in Jaguan, T5-G=Treatment 5 in Creole Guanajuato, T5- Treatment 5 in Creole Arteaga, T5-J= Treatment 5 in Jaguan.

DISCUSSION

Inoculation with suspension 10^8 spores mL^{-1} of *T. harzianum* + 10^8 spores mL^{-1} of *F. verticillioides* and suspension 10^8 CFU mL^{-1} of *B. subtilis* + 10^8 spores mL^{-1} of *F. verticillioides* showed a greater positive effect. Good germination was not achieved (72.92%) (Valdivia, 2020). Cubillos-Hinojosa et al. 2009 mencionated than benefic action of *T. harzianum* in early germination and growth of *Passiflora edulis* Sims (passion fruit) in greenhouse. Plants whit 10^8 spores mL^{-1} of *T. harzianum* + 10^8 spores mL^{-1} of *F. verticillioides* had a height of 1 84.4 to 117 cm and treatment 3, 2 g / L-1 Cupravit + suspension 10^8 spores mL $^{-1}$ of *F. verticillioides* presented the lowest height. High concentrations of copper might inhibit or delay germination and elongation of cotyledons and roots due of the osmotic effect or ionic toxicity that causes bad seeds (Castro-Díaz et al., 1998; Lopez et al., 2013; Belagziz and Romane, 2014). Administration of *T. harzianum* as biocontrol mechanism tend to stimulate growth (Bailey and Lumsden 1998, Kleifeld and Chet 1992, de Aguiar et al., 2014; Sandle 2014; Vargas-Hoyos and Gilchrist-Ramelli, 2015). It has been shown *Trichoderma* spp induce plant growth (Rojan et al., 2010; Fintenelle et al., 2011; Larsen et al., 2017). Rabeedran et al. 2000 demonstrated that not-mycorrhizal fungi stimulates plant growth. Candelero et al. 2015 showed that inoculation of *T. harzianum* to pepper seeds Capsicum Chinese Jacquin increased plant height. Trichoderma induces the production of organic acids such as gluconic, fumaric and citric acid; diminishes the soil pH and allows phosphate solubilization as well as micro and macronutrients such as iron, manganese and magnesium, which are essential for plant metabolism, improving its assimilation and promoting plant growth (KOPERT, 2020). *T. harzianum* promotes growth and plant development, producing metabolites that stimulate the developmental processes in the plants (WAS, 2018; Vera et al., 2002).

Incidence of *Fusarium* sp in the stems was from 25 to 100%, and the severity was from 11.33 to 35.43% and the best treatment in the control of desease was the suspension 10^8 spores mL^{-1} of *T. harzianum* + 10^8 spores mL^{-1} of *F. verticillioides*. Michael-Aceves et al. in 2008 showed the native strain Thzn-2 of *T. harzianum* has the potential to biocontrol *F. subglutinans* and *F. oxysporum* by inhibition and class 2 antagonism, previous selection of antagonist through dual culture is useful, but do not guarantee the biocontrol in greenhouse (Sid Ahmed et al. 2000, McLeod et al., 1995, Smith et al., 1990, Ezziyyani et al., 2004). *T. harzianum* protects roots to secondary infections by blocking the entry of the pathogen to the damaged roots (Bezano and Ancía, 2015). Trichoderma sp. antagonic effect against soil fungus such as *Rhizoctonia*, *Sclerotium* and *Fusarium* is already demonstrated (Fernández, 2001; Infante et al., 2009; Guédez et al., 2012, Chet et al., 1997, Sid-Ahmed et al., 2003).

CONCLUSIONS

T. harzianum was the most efficient treatment, plants presented a better germination, height and diameter stem, and a control effect of 75% in the incidence presented of *F. verticillioides* and a severity of 31.97%.

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