

The Beneficial Effect of *Trichoderma* spp. in Seed Treatment of Four Maize (*Zea mays* L.) Genotypes

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Abstract

Maize is the main crop for Mexicans; however, it is affected by species of fungi causing ear rot. This research aimed to evaluate the effect of *T. asperellum* T11, *T. harzianum* T1 4 y *T. longibrachiatum* T1 40 on some agronomic variables of four maize genotypes. The seeds of the genotypes H-515, Zapata 7, and H-507 were treated with a suspension of *Trichoderma* spp. to 1×10^8 spores mL⁻¹, using a control (untreated seed), and Benomyl as chemical control. The planting was in Morelos, in a completely random block design with a factorial arrangement. The ear rot was natural. Data were obtained at the end of the crop cycle and processed in SAS 9.4[®]. H-515 genotype had the greatest effect on the treatment of maize seeds with *Trichoderma* spp. (5.562 kg); *T. asperellum* T11 was the strain that stood out with a mean yield of 50 ears in an area of 16 m² of 4.904 kg, and control of 4.448 kg. Our results are an economic option for farmers to contemplate the use of *Trichoderma* and obtain its benefits.

Keywords

Seeds, Treatment, *Trichoderma asperellum*, Ear Rot, Yield

1. Introduction

The relationship between Mexicans and *Zea mays* L. (Poaceae) maize is millenary and is intimately associated with the evolution of Mesoamerican civilizations, it

has been the basis of our diet, and its use diversifies from grains and fodder, which constitutes the basis for the elaboration of a vast number of foods, to the pharmaceutical and manufacturing industries [1]. The main maize producers worldwide are the United States (383,943,000 Mt), China (272,552,000 Mt), Brazil (88,461,943 Mt), and Mexico ranks seventh (27,503,477.88 Mt) [2].

Varón and Sarria [3] mentioned that the main diseases that attack the maize crops in Mexico are of fungal origin, spread throughout the country and their appearance is subject to the environmental conditions that favor the infection and multiplication of the pathogen, as well as the source of the inoculum and the susceptibility of the genotypes. In Mexico, the main genus of fungi involved in ear rot are: *Diplodia* Fries (Botryosphaeriaceae), *Gibberella* Link, *Fusarium* Link (Nectriaceae) y *Aspergillus* P. Micheli ex Haller (Trichocomaceae) [4] y *Penicillium* [5].

Rios-Velasco *et al.* [6] alluded that Biological Control (BC) represents an alternative to chemical control, based on the use of native antagonists, an aspect that confers it to be environmentally friendly and sustainable. *Trichoderma* genus contains species of fungi belonging to the order Hypocreales of the division Ascomycota [7], associated with soil ecosystems with a worldwide distribution [8], species of this genus are often used as biocontrol agents against phytopathogenic fungi [9] [10], note that the biological control of fungal phytopathogens is often more variable in efficacy compared to the suppression of diseases achieved through the use of conventional pesticides [10]. That being said, the use of this species for biocontrol is an alternative for sustaining high production with low ecological impact on different agricultural production systems [11], so BC is considered one of the important practices for pest and disease management [12].

Inoculation of seeds with *Trichoderma* spores in suspension or powder is probably the most economical and extensive method for the biocontrol of maize ear rot [13]. Argumedo *et al.* [14] reported on the species of fungi that belong to the genus *Trichoderma*, these have been fully characterized by having an application in the agricultural field, mainly for the biological control of other pathogenic organisms that attack crops, however, studies on their behavior and effect in contaminated terrestrial and aquatic environments have been scarcely studied. Furthermore, *Trichoderma* spp. promotes plant growth, improves fruit quality, as well as potentiating crop yields through the production of phytohormones while also promoting the availability of phosphates and other minerals necessary for plant metabolism [15], due to these reasons, several species of this genus are used as biofertilizers and phytostimulators [16] [17], therefore, this research aimed to evaluate the effect of *T. asperellum* T11, *T. harzianum* T1 4 and *T. longibrachiatum* T1 40 on some agronomic variables of four genotypes.

2. Materials and Methods

Location

The field research was carried out in the municipality of Tepalcingo, Morelos, Mexico (18°37'39.9"N and 98°52'08.0"W), while the laboratory stage was carried

out in the Phytopathology laboratory at Universidad Autónoma Agraria Antonio Narro (UAAAN) (25°21'16.0"N 101°01'51.7"W).

***Trichoderma* strains under study**

The strains used in this study were *T. asperellum* T11, *T. harzianum* T1 4, *T. longibrachiatum* T1 40.

Maize genotypes

In the present research, the commercial genotypes (hybrids) H-515, Zapata 7, H-507 and a native at Tepalcingo, Morelos, which is typically used by the community for its production and consumption, were used.

Treatment of *Trichoderma* spp. on maize seeds

The genotypes were hydrated with distilled water for 12 h, and 500 seeds were used per replicate, giving a total of four replicates per genotype and strain. To achieve spore adhesion to the seed, they were immersed in an aqueous solution of molasses at 5% for 5 min, the excess was removed and 1 L of suspension of *Trichoderma* spp. spores were added at 1×10^8 mL⁻¹ spores per replicate for 10 min. Benomyl was used as a chemical control and 2 g per 1 kg of seed L⁻¹ and water for 10 min was applied (Figure 1), coupled with control (untreated seeds).

Planting the genotypes

The planting of the 500 seeds per replicate of each treatment was carried out in a random block design and was on August 20, 2017, with a temperature of 17.76°C - 28.83°C and a relative humidity of 81.60% (humidity in the environment), in an irrigation system. Planting was done in furrows 17 m long by 80 cm wide, with a distance between furrows of 80 cm and between plants of 40 cm, sowing two seeds per stroke, at a depth of 3 cm.

Harvest

The harvest in Tepalcingo was done on December 28, 2017, selecting 50 corn



Figure 1. Treatment process in the different seeds maize genotypes. A = Genotypes with molasses, B = Genotype with *Trichoderma* spp., and C = Chemical control.

plants at random per replicate and harvesting only the main ear per plant, which were put in the sun for drying as the population normally does and the moisture content was verified every two days using a digital grade moisture meter (Lds-1g), until the maize presented 14% humidity (**Figure 2**).

Evaluated variables

At the end of the production cycle, the incidence and severity of maize rot was evaluated, as well as the height and diameter of the stem of 50 maize plants of each genotype by means of a flexometer and the yield of 50 ears per replicate, (only the main ear per plant and shell), done by means of an analytical scale.

Statistical analysis

Data were evaluated in a random block analysis with a factorial arrangement, with factor A as genotypes and factor B as treatments, employing four levels in factor A and five levels in factor B, with four replicates per treatment, analyzing data in the STATISTICAL program SAS version 9.4[®] [18], the separation of means done by the Tukey test ($p = 0.1$). Moreover, a correlation test of variables under study was done in the R software using the Pearson test [19].

3. Results and Discussion

Table 1 shows that there is no statistical difference between the treatments, however, the strains of *Trichoderma* applied to the seed showed an effect similar to the rest of the treatments (Tukey $p < 0.01$). The genotype H-515 (**Table 1**, **Figure 3**) presented the greatest effect in the treatment with the *Trichoderma* species (5.562 kg), and *T. asperellum* T11 was the strain that stood out with an average yield of 50 ears in an area of 16 m² of 4.904 kg, in other words, the inoculation of this strain presented 0.456 g (10.25%) more than the control, it



Figure 2. Maize ear drying process by replicate up to 14% moisture. A = Hybrid maize, B = Native maize at Tepalcin-go, Morelos.

probably seems significant, however, if we interpolate it to 1 ha, we would have a yield of 285 kg more with the application of this species of *Trichoderma*, while the native presented the lowest yield (2.750 kg).

If we extrapolate our results obtained to one ha with the same management and number of plants as normally sowed by producers in the region (60,000 plants/ha), we would have the H-515 genotype inoculated to seed with the strain *T. asperellum* T11, we would reach 5884.80 t/ha (Table 2), if we compare it with the control, which would present 5337.60 t/ha, we would have a difference of

Table 1. Comparison of means of Factors A and B in grain yield.

Genotype ¹	Mean (kg/50 ears)	Treatments ²	Mean (kg/50 ears)	Yield ³ (t/ha)
H-515	5.562 A	Benomyl	4.693 A	5631.60
Zapata 7	5.433 A	T1 4	4.821 A	5785.20
H-507	5.081 A	T1 40	4.662 A	5594.40
Native	2.750 A	T11	4.904 A	5884.80
		Control	4.448 A	5337.60

Same letters are not statistically different according to Tukey's test at 0.01 probability, ¹ = Factor A (genotypes), ² = Factor B (treatments), ³Yield extrapolated per ha.

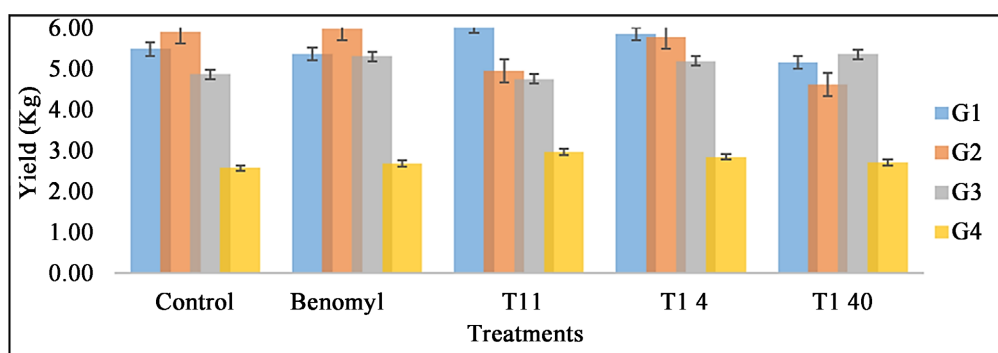


Figure 3. Effect of the inoculation of the different treatments on the seed of the maize genotypes under study (yield of 50 ears). G1 = H-515, G2 = Zapata 7, G3 = H-507, G4 = Native.

Table 2. Means of the yield obtained from each treatment by genotype under study.

T	G1		G2		G3		G4	
	R	±SD	R	±SD	R	±SD	R	±SD
1	5.466	0.52222	5.88	0.73549	4.85	0.51517	2.56	0.21524
2	5.347	0.50301	5.96	0.80076	5.29	0.25817	2.67	0.10489
3	6.007	0.18181	4.94	0.77952	4.74	0.57328	2.96	0.13711
4	5.834	0.26174	5.76	0.88213	5.17	0.58744	2.84	0.15944
5	5.145	0.44703	4.60	0.85444	5.33	0.57148	2.70	0.14993

T = Treatments; 1 = Control, 2 = Benomyl, 3 = *T. asperellum*, 4 = *T. harzianum*, 5 = *T. longibrachiatum*; G1 = H-515, G2 = Zapata 7, G3 = H-507, G4 = Control; R = Yield; ±SD = Standard Deviation.

547.20 kg, regularly the price of corn per kg in the study area is \$MX 5.00 to 6.00, that is, if we take the average (\$MX 5.50), the farmer would have a profit of \$MX 3009.60.

In **Figure 4**, we can see the variables that correlated with each other according to Pearson, the values according to this test are from -1 to 1 , meaning the closer to 1 the correlation is stronger. In other words, if one variable increases or decreases the other tends to present the same effect, for example, the strongest correlations they obtained were between incidence and severity, and stem thickness and performance with r -values of 0.85 and 0.8 , respectively. However, other negative correlations were presented as positive (**Figure 4**).

Global food security is of great importance, so an increase in investment in agricultural research and development is necessary to increase the productivity of the world's farms, especially in developing countries [20]. Among the most important microorganisms are the bacteria of the genus *Pseudomonas* Migula (Pseudomonadaceae) and *Bacillus* Cohn (Bacillaceae) and fungi of the genus *Gliocladium* Corda and *Trichoderma* Persoon (Hypocreaceae), the latter is the most used for the control of an important group of soil pathogens [21]. This genus includes cosmopolitan, free-living or mycoparasite fungi that are very common in soil and root ecosystems [22]. By way of explanation, *Trichoderma* is probably the more beneficial, more versatile fungus that abounds in soils and there are more than

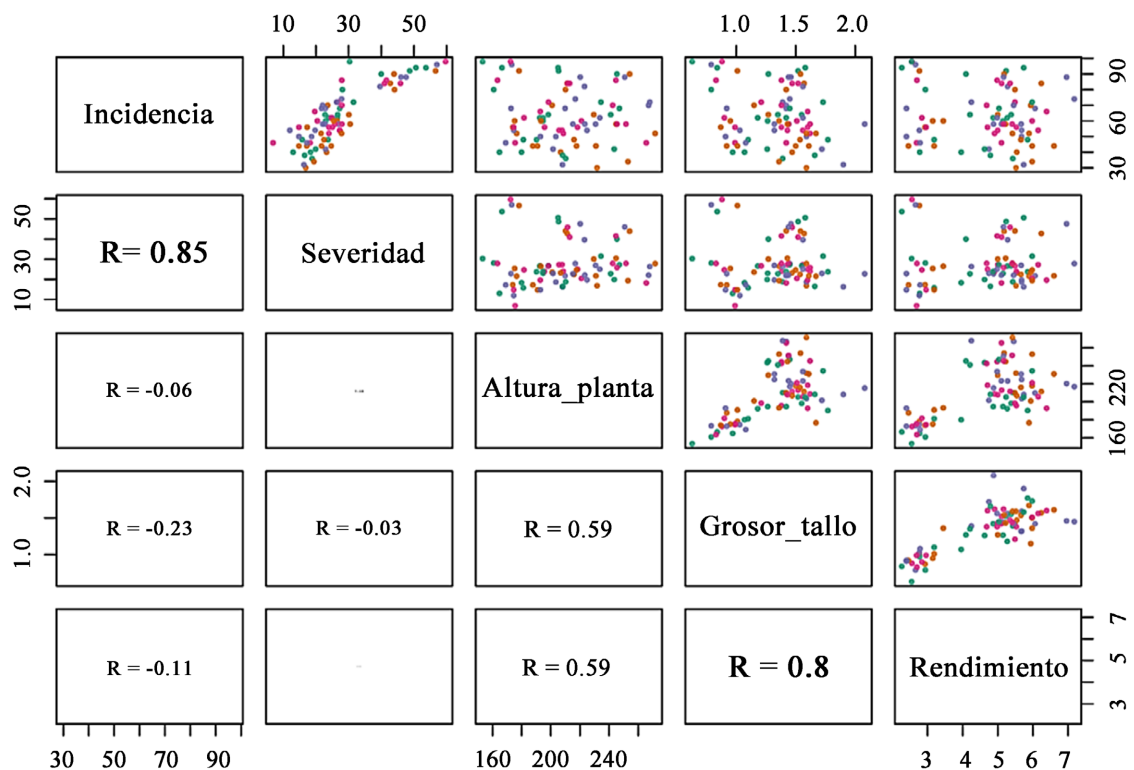


Figure 4. Correlation matrix of the variables under study. Pearson correlation coefficient is shown in the upper triangular matrix. Significant levels are represented by larger and more visible numbers, whereas values that are smaller or not shown in the tables are low and/or negative correlations. The scatter plots are shown in the upper diagonal and triangular matrix, respectively.

100 species [23].

Note that in some maize stalks (in the interior), the strain of *Trichoderma* with which the seed was treated was developed (especially those treated with *T. asperellum* T11) (Figure 5). In addition, some species of this genus have direct effects on plants, increasing their potential for growth and nutrient absorption, fertilizer efficiency, a higher rate and percentage of seed germination, and stimulating plant defense against biotic and abiotic damage [24].

Buysens *et al.* [25] mentioned that *Trichoderma* spp. has also been used in agriculture as a biofertilizer to increase both plant growth and crop yields. Several species of *Trichoderma* are associated with the rhizosphere of plants, meaning they can promote the growth and development of plants, by the production of auxins and gibberellins. Moreover, they can also produce organic acids such as gluconic, fumaric, and citric acid, which can lower the pH of the soil and promote the solubilization of phosphates, magnesium, iron and manganese, which are vital for the metabolism of the plant [23] [26].

Camargo *et al.* [27] reported that the application of commercial *Trichoderma* sp. in the cultivation of pea *Pisum sativum* L. (Fabaceae) significantly improved its growth and development, influencing physiological variables such as germination, leaf area, dry weight of the root, fresh weight of the root, dry weight of the aerial part, fresh weight of the aerial part and length of root, favoring the productive yield of the crop, when applying the same treatment to the seed. However, the seeds of this experiment were exposed to spore suspensions for 24 h and in this study, the seeds were kept only for 10 min, presenting no significant difference in the variables of height, diameter and yield. Kamilova *et al.* [28] reported that in previous studies carried out in the greenhouse, treating the seeds of a plant with a suspension of spores of *Trichoderma* spp. increased performance. Nevertheless, Tlapal *et al.* [29] informed that when *T. harzianum* and other bacteria-based treatments were inoculated, no significant differences were found in plant height, in our research, plant height was correlated with stem thickness, as well as corn yield ($r = 0.59$). On the other hand, Xue *et al.* [30] reported that with the inoculation of *Trichoderma* spp. in wheat crop, the yield



Figure 5. Presence of *T. asperellum* mycelium in a cross section of the stem of the corn plant, which in seed was treated with *T. asperellum*.

increased by 11.0% compared to the control, López *et al.* [31] also reported that some of the treatments where *Trichoderma* was applied led to a greater growth of the maize plant.

In addition, it should be noted that producers can obtain these products made from the species of this genus at affordable prices (approximately from \$MX 300.00). Some species have the ability to increase nutrient availability and crop yields [32], However, interactions between microorganisms and the plant have a better response when these are affected by abiotic factors [33]. On the other hand, Strange and Scott [34] commented that despite the continuous release of resistant cultivars and chemical fungicides, it is estimated that 10% of crop yields are lost due to fungal diseases and in this research due to the use of fungicides 0.245 g (5.50%) where more than the control was obtained.

Chemical fertilizers have played an important role in improving crop yields in intensified agriculture [35], also modern agriculture has become dependent on the use of chemical pesticides to control phytopathogenic organisms, which has led to resistance to pests. Likewise, improper and indiscriminate applications of pesticides have been subject to numerous restrictions in several countries where there is strong pressure from consumers who demand the limitation of the use of these products [36]. Correspondingly, the genus *Trichoderma* forms sclerotia as resistance structures, which survive in the soil and are difficult to destroy [37]. The use of *Trichoderma* spp., it is not only an act of respect for the environment, but also one of the most effective actions against other genera of phytopathogenic soil fungi such as *Fusarium*, *Pythium* Pringsheim (Pythiaceae), *Rhizoctonia solani* J.G. Kühn (Ceratobasidiaceae), *Sclerotinia sclerotiorum* (Lib.) de Bary (Sclerotiniaceae) and *Sclerotium rolfsii* Sacc. (Atheliaceae) and aerial phytopathogens like *Botrytis* P. Micheli ex Haller (Sclerotiniaceae). It is even extremely effective against species of phytoparasitic nematodes such as *Meloidogyne javanica* (Treb.) Chitwood and *M. incognita* (Kofold & White) Chitwood (Meloidogynidae) [38], correspondingly it is advisable to apply *Trichoderma* in crops, not only for the control of phytopathogens, but also for the benefits in the development and yield of crops. In actuality, it is easier to carry out this process, since several products are on the market based on mushrooms of the genus *Trichoderma* [39] [40], however, in the application of *Trichoderma* in the field, several important aspects that allow its adequate expression must be taken into account, which is related to the host plant interaction, susceptible phytopathogen, favorable environment (soil temperature, humidity, presence of oxygen, pH), soil conditions (structure, content of organic matter and nutrients) and time [41]. Another advantage is that some species of *Trichoderma* are capable of degrading pesticides due to their enzymatic activity and biochemical capacity, this allows us to glimpse the potential of application of this genus in the bioremediation of contaminated sites, thus having an ecological relevance [42] [43].

On the other hand, fumonisins, mainly produced by *Fusarium verticillioides* (formerly *F. moniliforme*) (Saccardo) Nirenberg (Nectriaceae) causative agent of ear rot by *Fusarium* [44] [45] [46], from which the name of the toxin is derived,

frequently contaminated grains [47] produce fumonisins B1, B2, B3 and B4 [48]. Causing in animals, decreased feed conversion, decreased appetite, diarrhea, weakness and increased mortality (poultry), decreased intake, and milk production (ruminants), as well as equine leukoencephalomalacia (horses), leukoencephalomalacia, cerebral hemorrhages and nephrotoxic alterations (rabbits), esophageal cancer, liver tumors, defects in neural tube development while also causing esophageal cancer in humans [49]. B1 fumonisin is a possible carcinogen for humans (IARC Group 2B) [50] and still holds its place as the least resistant maize in the formation of fumonisins although, the production of fumonisins is influenced by the seed variety used for cultivation by the different fertilizer formulations, as well as variations in storage conditions and environmental characteristics such as temperature, humidity and precipitation [51] [52]. Some *Trichoderma* species reduce fumonisin levels, for example: Chandra [53] reported that *T. harzianum* spores as a powder and liquid formulation, under field conditions, seed treatment and foliar spraying, alone and in combination reduced *F. verticillioides* infection in three maize genotypes and fumonisin levels. These species present different modes or mechanisms of action as competition for the substrate, mycoparasitism, antibiosis, deactivation of pathogen enzymes, and induced resistance, among others [54]. The application of *Trichoderma* has a positive effect on maize yield and *T. asperellum* was the species with the greatest effect on maize genotypes. The H-515 genotype presented the greatest effect of the *Trichoderma* species with the seed treatment.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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