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Evaluation of *Trichoderma harzianum* in controlling damping-off (*Pythium* spp) on tomato (*Solanum lycopersicum*) seedling varieties

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ABSTRACT

Losses in tomato (Solanum lycopersicum) production in Zimbabwe have been attributed to Pythium spp induced damping–off. Current research trends in seedling production suggest that Trichoderma harzianum has a positive effect on nursery diseases. T. harzianum uses a variety of mechanisms to colonise various ecological niches. Different plant varieties have different affinity levels for T. harzianum. In this research an evaluation was done on the effect of four different rates of T. harzianum (0cfu/ml, 10^5cfu/ml, 10^6cfu/ml and 10^7cfu/ml) and four tomato varieties namely Floradade, Money-maker, Rodade and Star 9064 in controlling Pythium spp damping-off. A 4x4 factorial experiment was laid in a completely randomized design with three replicates. Four different tomato varieties were primed with T. harzianum suspension for 24 hours and then sown in float trays according to the experimental design. Data was collected on emergence percentage, growth (number of true leaves and plant height); damping-off incidence and severity and dry weight. There was a significant interaction (P=0.001) between T. harzianum and variety on all parameters. T. harzianum 106cfu/ml and T. harzianum 10^7cfu/ml had no disease incidence and severity recorded on all tomato varieties. Emergence percentage, number of true leaves and dry weight increased on Money-maker and Star 9064 with an increase in T. harzianum rate. T. harzianum did not induce an increase on Rodade plant height. This study was carried out in the view of the use of T. harzianum as a bio-control strategy for Pythium-induced damping-off on tomato seedlings.

Key words: *Trichoderma harzianum*, damping-off, *Pythium*, tomato

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INTRODUCTION

Tomato (*Solanun lycopersicum*) is among the world's most popular vegetable crops because of its nutritive value [1]. Tomato is one of the most important nursery based vegetable crop, which is cultivated for its fleshy fruits. Success in tomato production is based on seedling quality [2]. Although tomato is commercially grown across the globe, there is hardly a place where the plant is free of disease [3]. Tomato seedlings are prone to attack by several soil borne fungal pathogens that cause serious diseases such as damping-off, wilt and root rot [4]. One of the major causes of seedling loss is damping-off, which is mostly a *Pythium*-induced root rot disease. When the fungi kills newly emerged or emerging seedlings it is known as damping-off, and is a very common problem in fields and greenhouses [5]. Damping-off is an important disease of tomato, which causes significant losses in nurseries on young susceptible transplants [6]. Damping-off is caused by a number of fungi including, *Pythium* species (spp), *Rhizoctonia* spp, *Fusarium* spp and *Phytophthora* spp [7]. Species of the soil organism *Pythium* are more often responsible for damping – off [8].

Pythium spp. tends to be very generalistic and unspecific in their host range. They infect a large range of hosts. For this reason, *Pythium* spp. are more devastating in the root rot they cause as *Pythium* spp. are also good saprotrophs, and will survive for a long time on decaying plant matter), [6]. Conditions for the development of this disease are

high temperature, high humidity, high soil moisture, poor aeration, high levels of nitrogen fertilizer, and closely sown seed [9] damping-off diseases caused by *Pythium* spp usually begins as a root rot, [10] .The use of chemicals has been the most widely used control strategy but due to their negative effects on the environment and human health, there is need to come up with alternative approaches that are environmentally friendly [11]. Resistance of the target pathogens to chemical active ingredient also affects the efficacy and usefullness of fungicides, [12]

Biological control of soil borne plant pathogens by the addition of antagonistic microorganisms to the soil is a potential non-chemical means for plant disease control [13]. The species of *Trichoderma* capable of hyper-parasitising pathogenic fungi are highly efficient antagonists [14]. Several fungi have the potential to become biological control agents under normal horticultural conditions. The major fungi used as biological control agents against soil borne diseases include *Trichoderm*a and *Gliocladium*, [15]. They are used in potting mixtures and used as seed treatments and they are effective against diseases caused by species of *Pythium*, *Phytophthora*, *Botrytis*, *Sclerotium*, *Sporidesmium*, and *Coniothyrium* [16]. Therefore, the use of *Trichoderma harzianum* as a biological control of damping-off becomes an interesting alternative control measure.

Biological control offers an environmentally friendly approach to the management of plant disease [17] Biological control can be incorporated into cultural methods thereby limiting chemical usage for an effective integrated disease management system [18]. Nursery inoculation with fungal inoculants such as *Trichoderma harzianum* is aimed to control the incidence and severity of soil borne pathogens such as *Pythium* spp and *Rhizoctonia* spp as well as to improve seedling growth and alleviate transplanting shock. In this study an attempt was made to test the feasibility of biological control ability of *Trichoderma harzianum* against tomato seedling *Pythium*-induced damping-off.

MATERIALS AND METHODS

Study site

The research was carried out in a nursery at Midlands State University, Gweru, Zimbabwe. The area falls under Natural Region III of Zimbabwe's Agro-ecological zones, located 19.48º S and 19.48º W with an altitude of about 1425m above sea level. The average annual rainfall ranges from 600 mm to 750mm and the mean temperature is around 20-25 ºC.

Experimental design

The experiment was a 4 x 4 factorial structure, replicated three times, and laid out in a Complete Randomized Design.

Culturing *Pythium* species

The fungus *Pythium* spp was isolated from already infected tomato seedlings and was cultured on Potato Dextrose Agar (PDA) at an incubation temperature of 25°C for a week to multiply. The mycelia was then flooded with distilled water. The spore suspension was adjusted to 107 cfu/ml, using a haemocytometer. The pathogen was inoculated in the growth media; this was done by thoroughly mixing the *Pythium* spp with the growth media before sowing of seeds (which were in Petri dishes before they were sown).

Culturing *Trichoderma harzianum*

Trichoderma harzianum was cultured on PDA for seven days until dark green conidia appeared. Conidial suspension was prepared by flooding the culture surface with sterile water and the conidia of *Trichoderma* spp was scrapped from medium surface by a sterile spatula. The conidia concentration was determined with haemocytometer and adjustment was done with sterile water. The final concentration of 105cfu/ml, 106cfu/ml and 107cfu/ml was used for the experiment on damping-off control of tomato seedlings.

Seed Priming

Different *Trichoderma* rates and distilled water (control) were used when priming seeds before sowing. The seeds were primed for 24 hours before sowing. A soluble formulation of Kutsaga float-fert (N 4.5%: P_2O_5 2.1%: K_2O 4.7%). From emergence nutrifol 4 (N 14%; K 5%; S 0.9%) foliar fertilizer was applied three times a week at a rate of 4ml nutrifol/1L water. The seedlings were watered twice or thrice a day depending with the weather to cater for the moisture regimes optimum for *Pythium* spp.

Damping-off incidence and severity

Damping-off incidence and severity was first recorded 10 days after sowing and repeated after every four days. Disease incidence refers to number of diseased plants or proportion of plants diseased out of the total number of plants observed, this was recorded as a percentage. Disease severity was assessed based on the 0 to 5 scale.

0 Healthy seedlings
1 One or two light brown lesions (1mm) on the crown root
2 Light brown-dark brown lesion 2-10mm on root
3 Dark brown lesions 10-25mm
4 Dark lesion 26mm water soaked hypocotyls
5 Collapsed hypocotyls with leaves or dead seedling

Plant height
The data on plant height was recorded two weeks after sowing, the seedlings were measured using a 30cm ruler and this was repeated after every four days for four weeks.

Data analysis
Analysis of Variance (ANOVA) was done using Gen stat Discovery 14th Edition (Gen Stat, 2005) and means were separated using the least significant difference (LSD) at 5% level of significance.

RESULTS AND DISCUSSION

Effect of *T. harzianum* and variety on damping-off incidence and severity 6 weeks after seed sowing
There was significant interaction (P=0.001) between *T. harzianum* and variety on disease incidence 6 weeks after seed sowing. Fig 1 shows disease incidence decreased with an increase *T. harzianum* rate on all varieties. Rodade primed with *T. harzianum* (0cfu/ml) had the highest disease incidence of 91.3% and *T. harzianum* (10^6cfu/ml) and *T. harzianum* (10^7cfu/ml) when inoculated on all seedling varieties; there was no disease incidence noted.

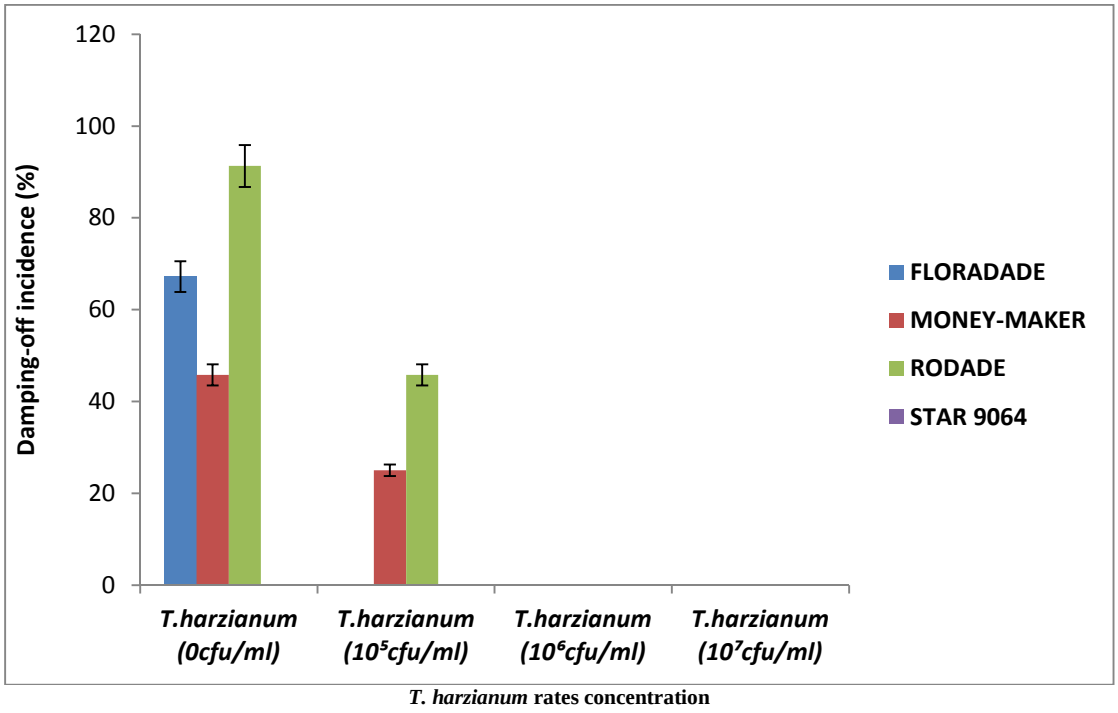


Fig 1: Effect of *T. harzianum* and variety on damping-off incidence

There was a significant interaction (P=0.001) between *T. harzianum* and variety on disease severity. As the *T. harzianum* rate increased there was also a decrease in disease severity on all seedling varieties(Fig 2). Floradade treated with *T. harzianum* (0cfu/ml) and Rodade treated with *T. harzianum* (0cfu/ml) scored 5 which meant collapsed hypocotyls with leaves or dead seedlings. All varieties treated with *T. harzianum* (10^6cfu/ml) and *T. harzianum* (10^7cfu/ml) scored 0 and they all had healthy seedlings. This is in line with results from Tucci *et al*., (2010), [18] when he evaluated the effect of *Trichoderma* strains on *B. cinerea*. The interaction at the rhizosphere level between *S. lycopersicum* lines and *Trichoderma* spp increased resistance against *B .cinerea* leaf infection. When tomato plants are infected with a pathogen (*Pythium* spp) soon after they have been primed with *Trichoderma harzianum*, they are poised to react more strongly, increasing defence gene expression and the activity of protective enzymes sooner and to higher levels than in untreated plants [19].

Genotypic differences in any of the plant components of the complex cross-talk with *T. harzianum* can be evoked to explain this effect, including the genotype ability to attract and sustain root colonization by the fungus [18]. The disease control and the inhibitory effect by *T. harzianum* were probably due to hyper-parasitism/ myco-parasitism, competition for space and nutritional source and antagonistic chemicals produced and released into the environment, [20]. *Trichoderma* spp. have been reported to produce antibiotic compounds (Trichodermin) extracellular enzymes (chitinase, cellulose) unsaturated monobasic acids (Dermadine) and peptides that either damage plant pathogen or enhance their population in biota [21 and 22].

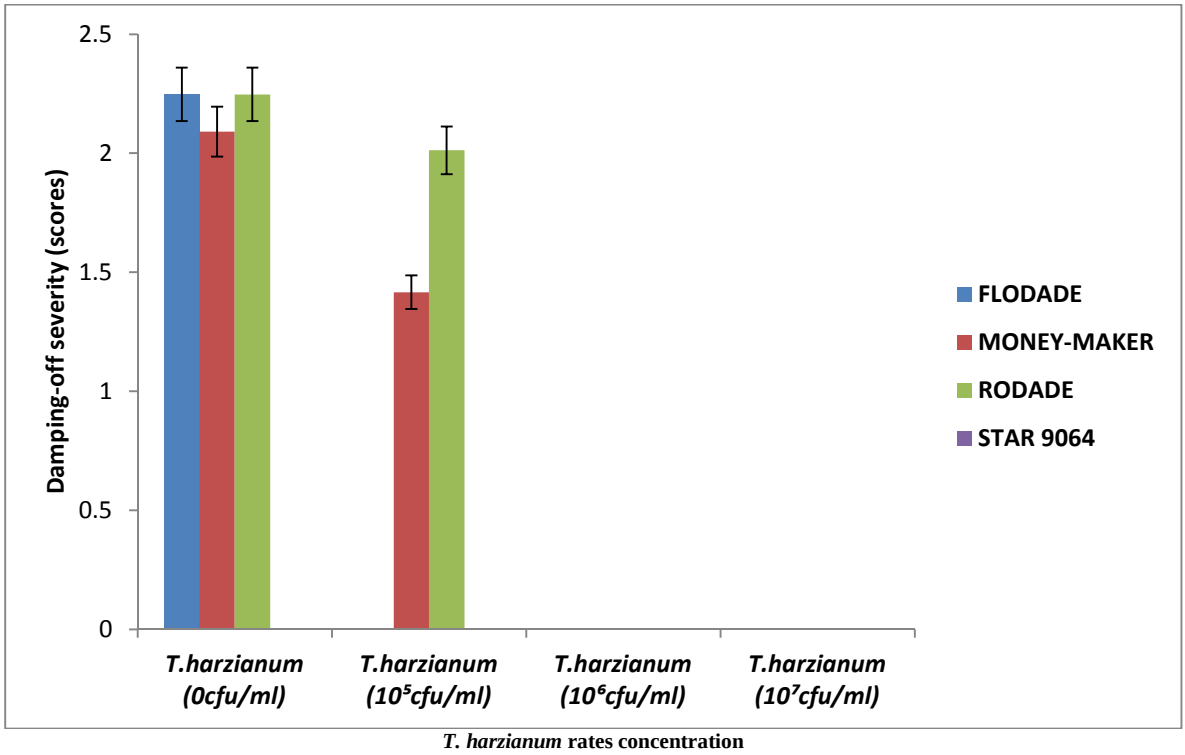
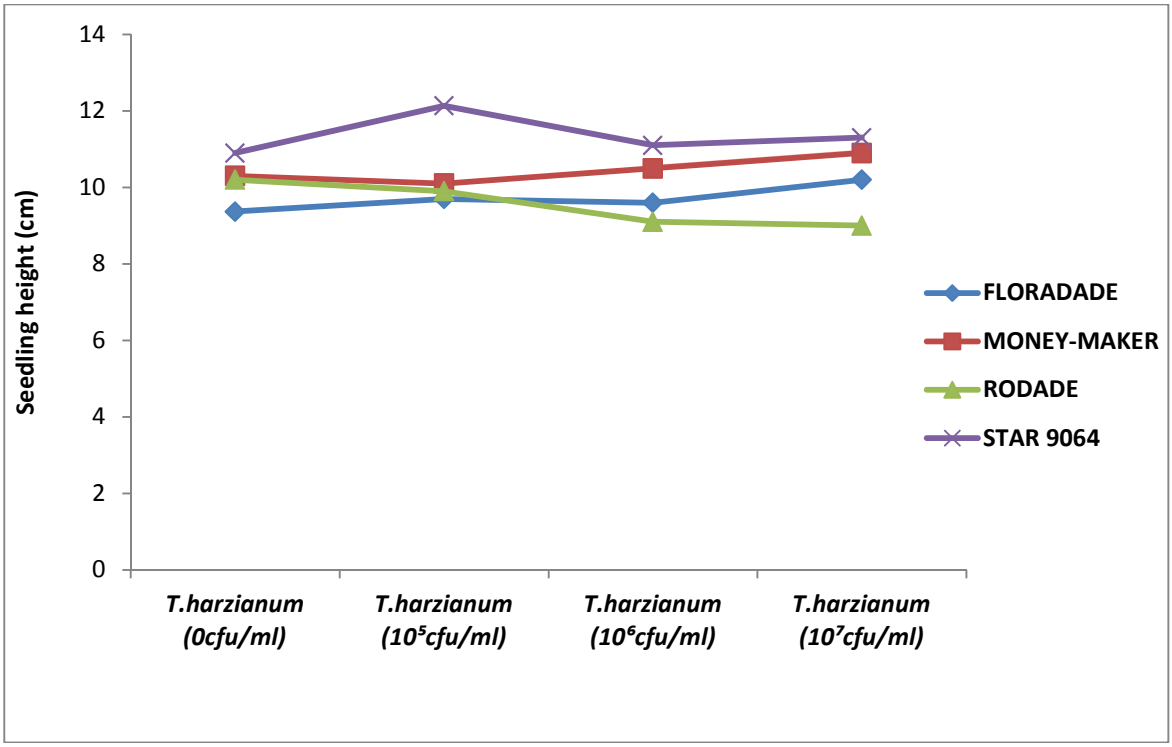


Fig 2: Effect of *T. harzianum* and variety on damping-off severity 6 weeks after seed sowing

Effect of *T. harzianum* and variety on plant height 4 weeks after seed sowing

There was significant interaction (P=0.001) between *T. harzianum* and variety on plant height 4 weeks after seed sowing. Star 9064 inoculated with *T. harzianum* (10^5cfu/ml) had the highest plant height (12.1cm). All varieties primed with T. harzianum (0cfu/ml) the seedling height ranged from 9cm to 10cm. Rodade as the *T. harzianum* rate increased there was no significant increase in plant height, (Fig 4). This can be explained by findings by Tucci *et al*., (2010) [18] which demonstrated that the extent of growth stimulation is largely dependent on the tomato genotype, suggesting that the response to *Trichoderma* spp. is under genetic control. The importance of the plant genetic background has already been reported for the interaction between maize and *T. harzianum*. Genetic analysis has demonstrated that the maize response is largely conditioned by dominant genes [23]. On plant height, an increase in *T. harzianum* on Rodade caused a decrease in plant height. With Money-maker, Floradade and Star 9064 plant height increased with an increase in *T. harzianum* rate.



***T. harzianum* rates concentration**

Fig 4 Effect of *T. harzianum* and variety on plant height 4 weeks after seed sowing

CONCLUSION

All seedling varieties when inoculated with *T. harzianum* (10^6cfu/ml) and *T. harzianum* (10^7cfu/ml) no disease incidences were recorded and the untreated plants showed the highest disease incidences and severity. However, the interaction between variety and *T. harzianum* had caused differences in seedling growth. An increase in *T. harzianum* rate also increased emergence percentage, number of true leaves and dry weight of Money-maker and Star 9064.

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