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Improving the nutritional values of plant products through the use of biological agents such as *Trichoderma viride* in tomato plantations

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1 ABSTRACT

The purpose of this study was to determine whether the use of *Trichoderma* has a beneficial protective effect against pathogens existing on the above ground parts of tomato plants. Samples obtained from isolated and multiplied fungal cultures of *Trichoderma viride* were used for treatment of the above ground parts of tomatoes. In one embodiment, the plants were treated with pathogenic strain of *Fusarium* sp that commonly damages crops. Influence of the fungus *Trichoderma* was determined by quantitative presence of necrosis on leaves in various groups of cultivated tomatoes compared to control samples. The development of seedlings was checked by the length and weight of fresh and dry plants. The experiments were conducted in pot cultures and controlled conditions. The results showed the protective role of *Trichoderma viride* in relation to the *Fusarium* and statistical significant positive effect on the growth and development of tomato seedlings. The group of seedlings treated only with parasitic fungi is characterized to have greater presence of necrosis compared to the group treated with saprophytic fungi and control. *Trichoderma viride* effectively fights plant pathogenic fungus of the genus *Fusarium*. The use of this biological agent in plant protection may restrict the use of chemicals and thus contribute to improve consumers' health.

2 INTRODUCTION

Excessive chemicals used in agriculture and market gardening are a problem that affects the health of consumers. Intensification of production, however, requires the use of pesticides. Long-term exposure to pesticides has a negative impact on human health, leading to instability of various organs and systems, especially, the nervous system, endocrine, immune system, reproductive system (Martenies and Perry 2013), urinary, cardiovascular and respiratory (Mostafalou and Abdollahi 2013). The relationship between exposure to pesticides and increased incidence of cancer (breast, prostate, lung, brain, colon, testis, pancreas, oesophagus, stomach, skin, non-Hodgkin's lymphomas, leukaemia's, multiple myeloma (Alavanja *et al.* 2012; Alavanja *et al.* 2013), Parkinson's disease (Moretto and Colosio 2013), Alzheimer's disease

(Zaganas *et al.* 2013), multiple sclerosis, diabetes (Zeliger 2013) was determined. An alternative to chemical agents, particularly valuable in so-called ecological farming, can be application of biological agents to remove pathogens. Beginning in the 1930s (Wiendling 1932), *Trichoderma* fungi have been noted to be factors that inhibit or reduce the occurrence of phytopathogens. This naturally growing fungus belongs to the kingdom of Fungi, type *Ascomycota*, class *Sordariomycetes*, the order of *Hypocreales* and the family of *Hypocreaceae.* They are potent antagonists of parasitic microorganisms of the genus: *Rhizoctonia, Phytophthora, Pythium, Fusarium, Alternaria, Sclerotinia, Gaeumannomyces, Thielaviopsis, Verticillium, Botrytis*, and numerous bacteria and viruses (Benìtez *et al.* 2004; Howell 2003). In recent years, there has been increased interest in those micro-organisms which may be components in the formulations of plant protection products (Alfano *et al.* 2007; Ebtsam *et al.* 2009; Pietr 1997; Świerczyńska *et al.* 2011). It is also important that mentioned fungus never attacks plants and even stimulate their growth and resistance (Kowalska *et al.* 2012; Wojtkowiak-Gębarowska 2006). The increased interest in this fungus is also because it is extremely common in nature, and has ability to adapt in different environments. Moreover, it is easy to isolate and culture in artificial conditions that encourage rapid growth and abundant production of conidiospores and often spores of type chlamydospores. Most of the published works that have appeared on *Trichoderma* indicate antagonism to pathogenic fungi colonizing the root systems (Ebtsam 2009; Pietr 1997; Pięta 2002). Few reports, however, show that the fungus may be an effective means to protect above ground parts of plants, including fruits, even during storage (Benìtez *et al.* 2004; Howell 2003).

3 MATERIALS AND METHODS

The experiment was conducted in pot cultures between the months of April and May 2013 on strains of fungi previously isolated from natural environments. From the garden soil, eight strains of *Trichoderma* were isolated. The pathogenic strain of *Fusarium* was obtained from the dried stems of tomato plants. Isolation, selection and propagation of fungi was carried out on mineral medium (created in our laboratory) of the following composition: agar 25 g; glucose 15 g; maltose 2 g; ammonium tartrate 0.5 g; potassium dihydrogen phosphate 0.1 g; zinc sulfate (1:500 solution) 0.5 ml; 30µg of thiamine; distilled H_2O up to 1000 ml. Selection was based on the antagonism of the genus *Trichoderma* against the *Fusarium* fungus, and was performed in petri dishes. Tested fungi were implanted in pairs - two-week culture inocula were placed at a distance of 3 cm apart on the prepared and solidified medium. Strain designated as T08, characterized by high virulence and strong antagonistic properties against *Fusarium*, was selected for further study. Fungi were grown on a petri dish surface at a temperature of 22°C leading to sporulation. Spores and aerial mycelium were washed off the surface by saline solution and then centrifuged in a physiological salt gradient. Thickened precipitate was diluted in sterile water to the application level of spores concentration of 10^8 cfu /ml. Pot culture was carried out under controlled conditions: temperature 25°C, humidity 70%, photoperiod of 12 hr/day and 12 hr/night. For all variants of experiments, non-sterile commercial garden soil (Natura) was used. Tomato seeds Slonka F1 (*Lycopersicon lycopersicum*) were germinated in a growth chamber (Biosell) on MS medium (Sigma-Aldrich) with addition of 0.8% agar and 1% sucrose (Murashige and Skoog 1962). Two-week old seedlings were pricked out in 250 cm^3 containers of soil. After a week of adaptation entire above ground part of plants were carefully sprayed in the following combinations:

1) Control seedlings – no fungi spraying

2) Seedlings were sprayed with a solution containing *Fusarium* sp spores
3) Seedlings were sprayed with solutions containing *Fusarium* sp and *Trichoderma* sp spores. In the last condition, spraying with *Trichoderma* was conducted 3 days after spraying with *Fusarium* solution. After 6 weeks of growth, seedling growth parameters were measured including: dry and fresh weight of seedlings, number of necrosis on leaves, the length of stems and roots. The results were analyzed statistically using STATISTICA 10.0. Normality of distribution was checked by the W Shapiro-Wilk test, and the significance of differences was determined by the t-Student test for independent samples. Statistically significant data was present when $p < 0.05$.

4 RESULTS

Macroscopic analysis of the second phase of mycelial growth led to the identification of T08 strain as: *Trichoderma viride* PERS (1829), which has shown a strong antagonism against *Fusarium* sp (Fig 1). The results obtained after 6 weeks of aerial spraying of tomatoes in the conditions described in the section of materials and methods were analyzed for the presence of the resulting necrosis on plant leaves. The group treated only with a parasitic fungus is characterized as having the presence of greater necrosis as compared to the group treated with saprophytic fungus and control (Fig 2). Another parameter analyzed was the dry weight of stems (Fig 3). As the chart shows, it was the largest in the case of tomato plants treated with saprophytic fungus. However, stem length did not differ between tomatoes treated with saprophytic and parasitic fungus (Fig 4). Analysis of the dry weights of the roots was conducted, and results were similar to stem analysis (Fig 5). There were no significant differences in root length between groups (Fig 6).

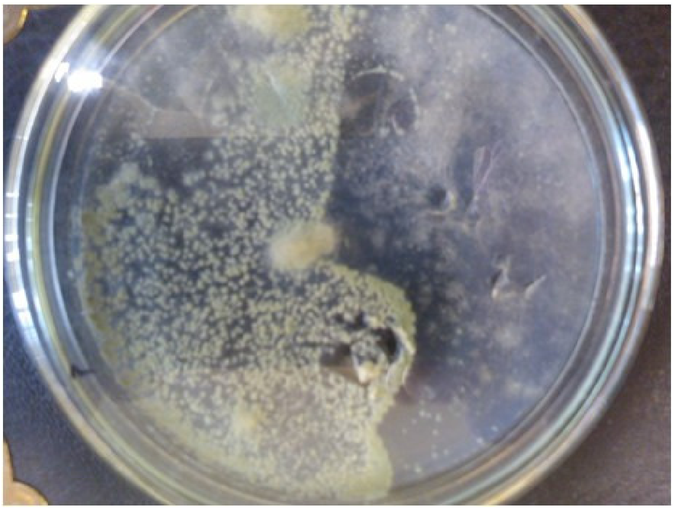

Figure 1: Antibiosis of *Trichoderma viride* (left) against *Fusarium* sp. Visible zone of reduced growth so-called zone of brightening.

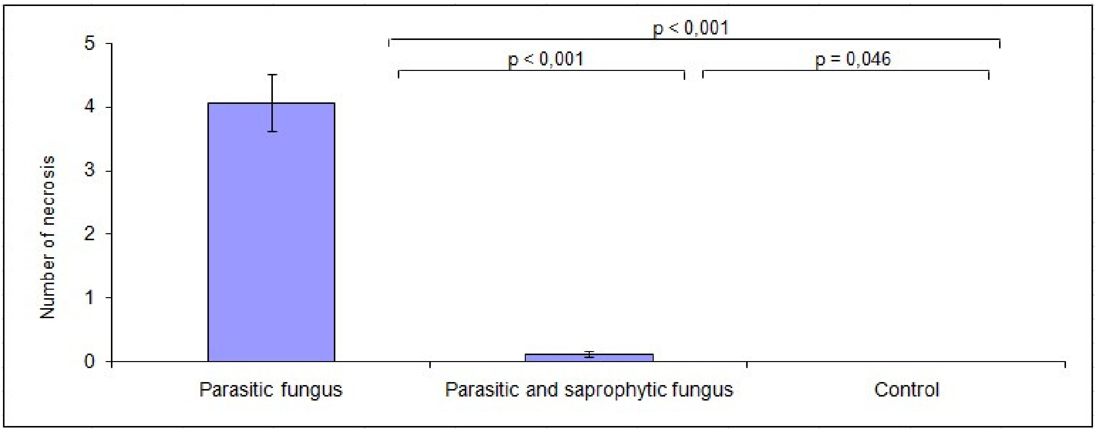

Figure 2: An average number of necrosis presence on the tomato leaves sprayed with various combination of fungus cultures compared to control group. Bars: Mean ±SD

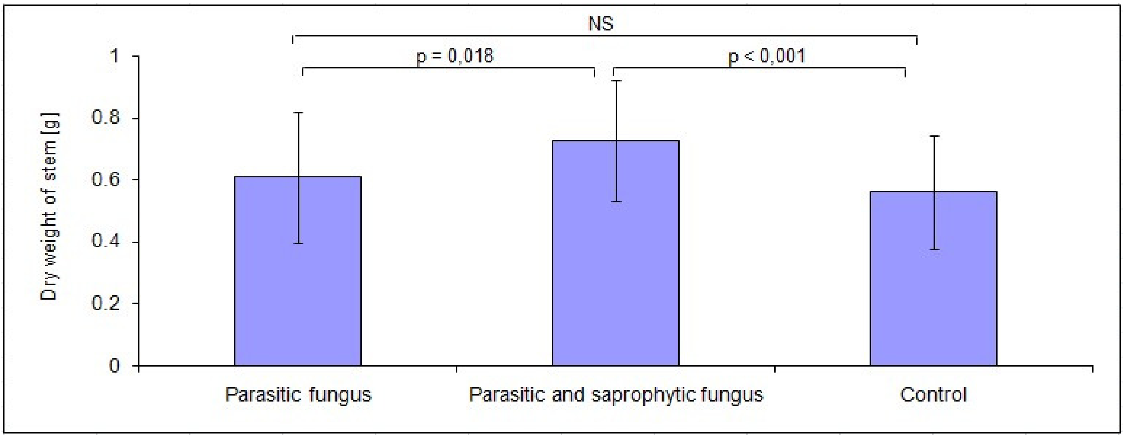

Figure 3: An average dry weight of tomatoes stem after 6 weeks of spraying with various combination of fungus culture compared to control group. Bars: Mean ±SD

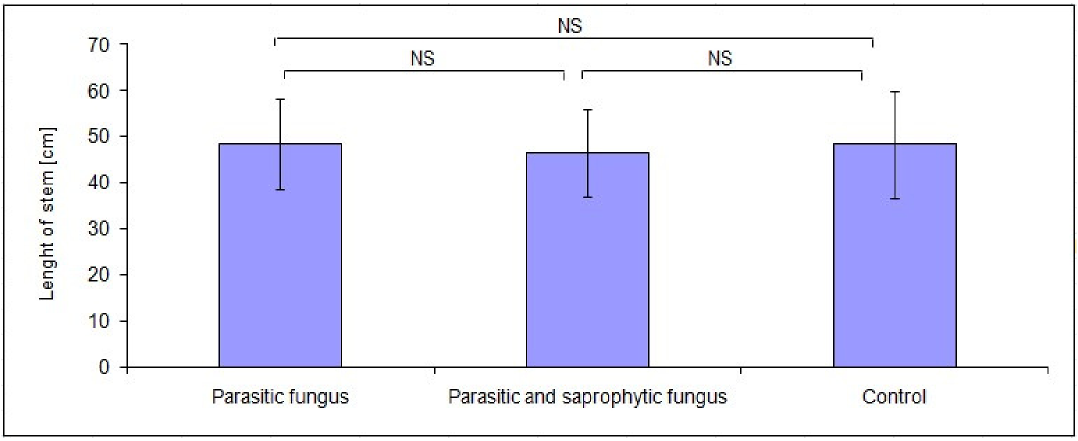

Figure 4: An average length of tomatoes stem after 6 weeks of spraying with various combination of fungus culture compared to control group. Bars: Mean ±SD

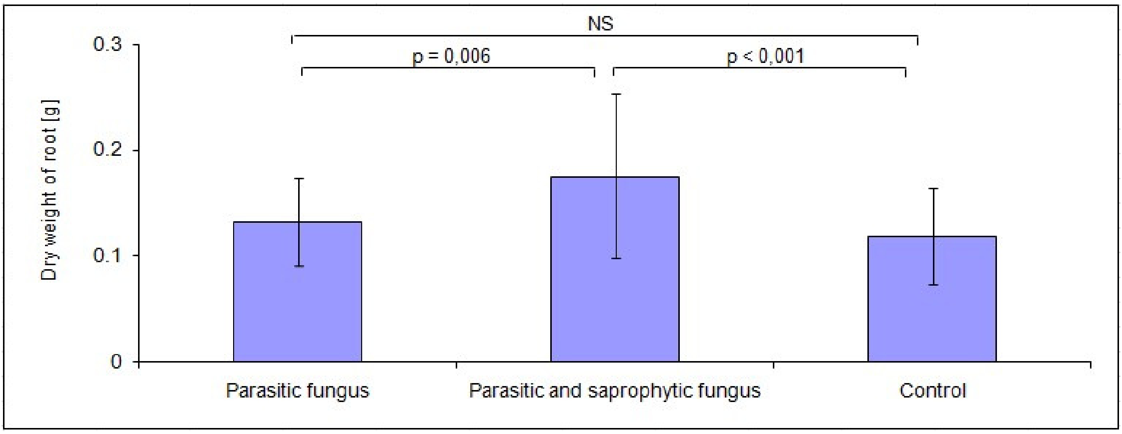

Figure 5: An average dry weight of tomatoes root after 6 weeks of spraying with various combination of fungus culture compared to control group. Bars: Mean ±SD

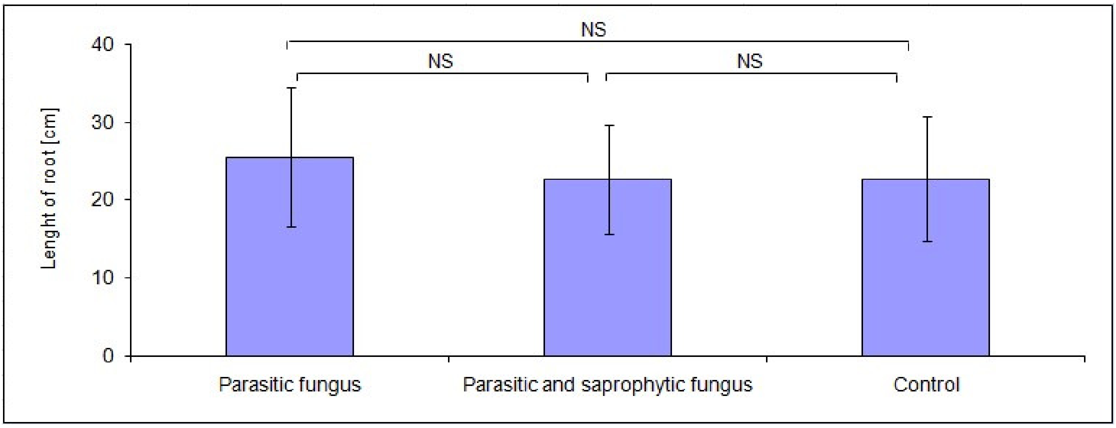

Figure 6: An average length of tomatoes root 6 weeks of spraying with various combination of fungus culture compared to control group. Bars: Mean ±SD

5 DISCUSSION

The results concerning the health and mass of tomato seedling growth indicate showed that antagonistic microorganisms such as *Trichoderma viride* might influence the reduction of diseases caused by plant pathogens. This is consistent with other literature (Hag and Khan 2002; Kowalska *et al.* 2012; Mesta and Amaresh 2000; Świerczyńska *et al.* 2011). In the fight against pathogens, *Trichoderma* fungi uses different mechanisms - the best documented is the production of various antibiotics and volatile compounds such as harzianic acid, alamethicins, tricholin, peptaibols, 6-pentyl-α-pyrone, massoilactone, viridin, gliovirin, gliotoxin, glisoprenins and heptelidic acid toxic to microorganisms (Benìtez *et al.* 2004; Howell 2006; Reino *et al.* 2008; Vey *et al.* 2001). These factors may limit competition by giving an advantage in the struggle for ecological niche. In addition, a synergistic effect of antibiotics and hydrolytic enzymes as particularly effective in inhibiting the germination of competitive conidia has been observed (Howell 2003; Rey *et al.* 2001). Chet and Inbar (1994) had shown that certain strains are capable of producing siderophores by scavenging iron ions from the environment, which inhibits the growth of other fungi. Although in the experiments in this study, substance in the form of spray was applied to the above ground parts of plants, some formulation may have fallen on the ground, therefore affecting the root systems. Spores from the surface of the soil were deposited near the roots by the water, which is possible because of the shallow root system of tomato plants. Root colonization by *Trichoderma* was examined by analyzing the results of dry mass of tomatoes plants (Fig 5). These types of positive aspects are confirmed by numerous literature reports showing growth stimulation and increased nutrient absorption (Benìtez *et al.* 2004; Chet *et al.* 1997). Yedida *et al.* (2003) found that treatment of a cucumber by *T. harzianum* T-203 increases the weight of the roots and the above ground parts of plants due to better availability of microelements. Harman *et al.* (2004) point out that this is one of the mechanisms leading to increased bioavailability of the chemical elements. They also indicate that these fungi can produce weak organic acids such as gluconic acid, citric and coumaric acids, which release phosphates and microelements that can be used by plants. Multiple research findings related to the positive effects of plants treated by *Trichoderma* mixtures, including the results of this study, allow us to conclude that these fungi are excellent antagonists in relation to multiple pathogens. They stimulate the growth and resistance of plants. Use of *Trichoderma viridae* as a biological agent in tomato plant cultivation can significantly reduce the use of chemical pesticides without the risk of contamination of the environment and compromising the health of consumers. It is worth noting that it can significantly improve the nutritional value of crop production and efficiency.

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