# Efficacy of *Trichoderma harzianum* as A Bio-control Agent Compared with Traditional Chemical Fungicide for Controlling the Soil Borne Pathogen (*Fusarium oxysporum* f. sp*. Lycopersici*) Infesting Tomato Plants

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**ABSTRACT :** Laboratory and greenhouse experiments were conducted to investigate the efficacy of a biological control agent (*Trichoderma harzianum*) against the soil-borne plant pathogen (*Fusarium oxysporum* f. sp*. lycopersici*) as compared with a traditional fungicide (Vitavax®-200). Results indicated that *T. harzianum* was more effective than Vitavax®-200 for radial growth inhibition. Through seedling dip and soil application, Vitavax®-200 was more effective than *T. harzianum* where it recorded the least wilt incidence comparing to untreated control, but it gave a shorter height of tomato plant than *T. Harzianum* with significant differences between them. Also, application of *T. harzianum* as antagonistic agent significantly increased the plant height and increased fruit yield/plant without significant differences with Vitavax® when compared with untreated control.

**Keywords:** *Trichoderma harzianum*, Disease severity, soil-borne plant pathogen, *Fusarium oxysporum*.

## INTRODUCTION

Tomato *(Lycopersicon esculenturn* Mill) is one of the most important Solanaceous economic vegetable crops in Egypt for local consumption and exportation. Its popularity is due to its high nutritive value, diversified use, and nutritional significance as a source of vitamins A and C. It is affected by several diseases, reflecting negatively on plant growth and the produced yield (Anonymous, 2007). Fungal pathogens are considered as damaging agents causing a considerable reduction of its production.

The wilt disease caused by the soil-borne fungi [*Fusariurn oxysporum* f. sp. *Lycopersici* (Sace.)] has serious effects on tomato plants either in nurseries or in the fields (Besri, 1982). It remains to be a challenging task in terms of management, because of nature, application of fungicides to control this disease is not practical. Besides, chemicals pose serious health hazards to the applicator as well as to the consumer.(Agrios, 2005;Srinon*et al*., 2006 and Cal *et al.*, 2004).In addition to target organism, pesticides also kill various beneficial organisms. Their toxic forms persist in soil and contaminate the whole environment (Hayes and Laws, 1991).

*Trichoderma* species that are common inhabitants of the rhizosphere are useful as they can be used as biological control organisms against a wide range of soil borne pathogens and also have been known to provide plant growth promotion. Successful reductions of *Fusarium* wilt in many crops with application of different species of *Trichoderma* have been found (Bell *et al*., 1982; Elad and Kapat, 1999 and Ramezani 2009). However, it is also reported that all the isolates of *Trichoderma* spp. are not equally effective in control of pathogen *in vitro* (Biswas and Das, 1999 and Ramezani, 2008) and *in vivo*

conditions to control diseases. Therefore, *T. harzianum* gave a successful control of a particular pathogen. *T. harzianum* Rifai have been known to show antagonism to various root pathogens such as *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. (Baker, 1989 and Chet *et al*., 1987) and has been described as a most promising biocontrol agent, (Morsy *et al*., 2009 and Sabalpara *et al*., 2009). Some strains of *T. harzianum* establish robust and long lasting colonization of root surfaces penetrating into the epidermis (Harman, 2000). This colonization by *T. harzianum* frequently enhances root growth development, crop productivity and resistance to abiotic stresses through enhancement of mineral absorption.

Therefore, the objectives of the present study were to assess the ability of *T. harzianum* as a bio-control agent in suppressing the populations of *Fusariurn oxysporum* f. sp. *Lycopersici* (Sace.) in tomato under *in vitro* and *in vivo* conditions, comparing with standard chemical traditional fungicide [Vitavax®-200 75% WP] which has been recommended by the Egyptian Ministry of Agriculture.

## MATERIALS AND METHODS

Tested fungi were : *Fusariurn oxysporum* f. sp. *Lycopersici* (Sace.) and *T. harzianum* obtained from the Plant Pathology Institute laboratory, Agricultural Research Center, Egypt.

Tested chemical : [Vitavax®-200(37.5% Carboxin + 37.5% thiram)] was also obtained from Central Agricultural Pesticide Laboratory, Agricultural Research Center, Egypt.

*In vitro* comparing effect of *T. harzianum* antagonist and a traditional fungicide (Vitavax®-200) against *F. oxysporum* f. sp. *lycopersici* pathogen

The tested fungi were placed and cultured on potato dextrose agar (PDA) medium separately, and incubated at the laboratory conditions at 25°Cfor 4 days for *T. harzianum* and 7 days for *F. oxysporum* f. sp. *lycopersici*. Nine millimeters discs of fifteen days old *F. oxysporum* fungal culture were placed on PDA medium one cm away from the edge of the plate, separately and anther (9 mm) disc of *T. harzianum* or traditional fungicide (Vitavax®-200) were placed at the opposite side of the petri plate. Four replicated plates for each treatment was maintained and incubated at 25°C. Inhibition percentage over control was calculated (Vincent, 1927) as the following formulae :

$$IP = \frac{C-T}{C} X\ 100$$

Where :IP = Inhibition percentage over control, C = Growth of tested pathogen with absence of antagonist (mm) and T = Growth of the tested pathogen with antagonist (mm)

Greenhouse experiment: A field experiment was conducted to test the efficacy of *T. harzianum* and Vitavax®-200against *F. oxysporum* f. sp. *lycopersici*. Soil Mixture (clay: sand1:1 w/w) was prepared and autoclaved for 1hr for two consecutive days and was put in plastic pots of 5 kg capacity. Tomato (supper strain B) seeds were sown in autoclaved soil mixture in the plastic pots. After 25 days, the seedlings were pulled out from the pots and washed in tap water then

immersed in the *T. harzianum* conidial suspension ($10^7$/ml) or dipped in the Vitavax®-200 solution (0.15%), ensuring that the roots alone were immersed in the solutions and then transplanted in pots at the rate of four seedlings per pot (5 kg capacity which were infested with the wilt pathogen *F. oxysporum* f. sp. *lycopersici* at a concentration of $10^7$ conidia/ml allowed to establish in the soil for a period of 6 days.). Soil drenched with treatments after 15and 30 days of transplantation.

Disease severity (DS) assessment was done 21 days after transplanting when symptoms of infection were observed. Such symptoms included clearing of the veins and drooping of petioles followed by yellowing of lower leaves (Agrios, 1988). Four plants were selected at random, marked with pieces of string and were used to evaluate disease severity after every 3 weeks. Disease severity was determined using a modification of a scale proposed and designed by Waudo *et al*., (1995). This was based on the wilt severity rated as follows; (% of shoot wilted, using a scale of 0–5 where, 0=No symptoms, 1=One leaf wilted (1%-25%), 2= 2 or 3 leaves wilted (26%-49%), 3=half plant wilted (50%-74%), 4= all leaves wilted (75%-100%) and 5=Plant dead). Four seedlings from treatments had their roots were stained by the method of Kormaik and McGraw (1982).

The observation on the disease incidence percentage was recorded at the harvest time. Each treatment was replicated six times in Completely Randomized Block Design (CRBD).Table 1 shows the treatments details.

**Table (1) : The treatments of the biological control agent and the fungicide Vitavax®-200**

| Treatments | Treatment details |
|---|---|
| *Trichoderma harzianum* | Seedling dip at 0.2 % + Soil application at 15 and 30 DAT*at 0.2 % |
| Vitavax®-200 (0.15%) | Seedling dip at 0.15 % + Soil drenching at 15 and 30 DAT at 0.15 % |
| Inoculated control | (with pathogen) |
| Healthy control | (without pathogen) |

*DAT : day after treatment

Severity of infection and treatment efficacy percentages were recorded as loss of weight according to the equation suggested by Spalding and Reeder (1974) :

DI (Disease Index %) = (No. of leaves wilted / Total No. of leaves) x 100

Statistical analysis : The data were statistically analyzed and the treatment means were compared by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

## RESULTS AND DISCUSSION

The efficiency of the tested treatments (*In vitro*) on the radial mycelial growth of *F. oxysporum* f. sp. *lycopersici*

*T. harzianum* and Vitavax®-200weretested for their *in vitro* effect against *F. oxysporum* f. sp. *Lycopersici* by traditional dual cultural technique.

The obtained data in Fig. (1) indicated that *T. harzianum* inhibited the mycelial growth of *F. oxysporum* f. sp. *lycopersici* to an extent of 79.0% over control followed by traditional fungicide Vitavax®-200 by 64.0% radial growth inhibition. There for, the bio-control agent (*T. harzianum*) was more effective than the traditional fungicide in inhibiting the radial growth of *F. oxysporum*.

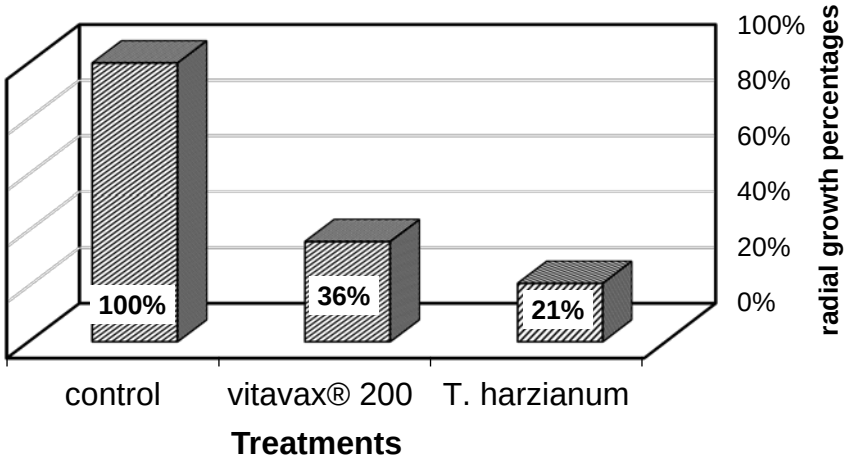


**Fig. (1) : Soil-borne fungus *F. oxysporum* f. sp. *Lycopersici* radial growth percentages affected by tested bio-control agent and traditional fungicides Vitavax®-200.**

Effectiveness of *T. harzianum* antagonist on wilt incidence and yield parameters under glasshouse conditions

Obtained data in Table (2) showed that application of *T. harzianum* antagonist through seedling dip and soil application was effective in suppressing wilt incidence giving a disease incidence percentage of16.9%.It was clear that Vitavax®-200 (0.15%) was found to be more effective than *T. harzianum,* where it recorded the least wilt incidence of 11.3% compared to untreated control, but it gave a shorter height of tomato plant than *T. Harzianum* (62.4 cm) with significant differences between them. Also, the results of this experiment revealed that the application of *T. harzianum* antagonistic agent significantly increased the plant height (by 15.1 cm) and increased fruit yield/plant without significant differences with Vitavax®-200when compared to untreated control. *T. harzianum* recorded the highest fruit yield (235 g/plant) followed by Vitavax®-200 (228.5 g/plant) with no significant differences. The use of the bio-control agent would be useful and recommended to reduce environmental pollution that resulted from chemical fungicides.

**Table. (2): Efficacy of tested treatments in the management of *Fusarium* wilts of tomato under greenhouse conditions.**

| Treatments | Plant height (cm) | Disease incidence Percentage | Fruit yield g/plant |
|---|---|---|---|
| *T. harzianum* | 69.6[a] | 16.9[b] | 235.0[a] |
| Vitavax®-200 (0.15%) | 62.4[b] | 11.3[a] | 228.5[a] |
| Inoculated control (with pathogen) | 48.3[d] | 59.6[d] | 123.5[c] |
| Healthy control (without pathogen) | 54.5[c] | 21.4[c] | 183.5[b] |

- Mean of six replications
- In a column, means followed by the same letters are not significantly different at the 5% level by DMRT.

The potential of *Trichoderma* species as bio-control agents against various plant diseases has been reported by several workers (Wells *et al*., 1972 and Sharon *et al*., 2001). In the present investigation, fungal antagonist *T. harzianum* caused highly significant reduction in tomato wilt incidence under *in vitro* and *in vivo* conditions. The inhibitory effect of these bio-agents against tested pathogen was probably due to competition and/or antibiosis.

*In vitro* effectiveness of *T. harzianum* against species of *Fusarium* have been reported (Padmadaya and Reddy 1996). The antagonist *Trichoderma harzianum*, *T. coningi* and *T. viride* were reported to be equally antagonistic to *F. udum in vitro* (Bahatnagar, 1986).Sivan and Chet, (1987) reported that *Trichoderma* spp. successfully controlled *Fusarium* spp. on cotton, wheat and muskmelon. Sesame seeds treated with three isolates of *T*. *viride* reduced the pre- and post-emergence damping off caused by *R*. *solani* and *F. oxysporum* f. sp. *sesame* under pot culture and field conditions.

In the present investigation, the plant height and fruit yield were also increased in *T. harzianum* treated plants. Similar results on increased plant growth due to application of *Trichoderma gamsii* in cereals and legume crops were reported (Rinu *et al*., 2013). The increase in plant growth might be associated with secretion of auxins, gibberellins and cytokinins.

The increase in biomatter production may be due to the production of plant growth promoters or through indirect stimulation of nutrient uptake and by producing siderophore or antibiotics to protect plants from deleterious rhizosphere organisms. Therefore, the antagonist *T. harzianum* is chosen to be the most promising bio-control agent for *F. oxysporum* f. sp. *lycopersici*. On the base of present study the bio-agents of fungi, might be exploited for sustainable disease management programs to save environmental risk (Margaret *et al*., 2011 and Sundaramoorthy and Balabaskar, 2013).

Finally, the present evaluation thus gave clear indication that *T. harzianum* is effective and virulent antagonist, which can be effectively used in the management of tomato wilt. Combination of seedling dip and soil application appears to be most effective.

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## الملخص العربى

# فعالية التراكوديرما هارزيانم كعنصر مكافحة حيوية مقارنة مع مبيد فطرى كيميائى تقليدى لمكافحة ممرض التربة فيوزاريم اكسيسبوريم الذى يصيب نباتات الطماطم

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تم تنفيذ التجارب المعملية وفى الصوب للتحقق ومقارنة كفاءة عنصر مكافحة حيوية لممرضات التربة (التراكوديرما هارزيانم) مع مبيد فطرى تقليدى فيتافاكس–200. وقد أوضحت النتائج أن التراكوديرما كانت أكثر كفاءة من مبيد الفيتافاكس فى تثبيط النمو الفطرى بالتجربة المعملية. وخلال تجربة نقع الجذور ومعاملة التربة فى الحقل تحت ظروف الصوبة تبين أن المبيد الفطرى فيتافاكس–200 كان أكثر كفاءة من التراكوديرما المختبرة حيث سجل أقل نسبة إصابة بالذبول لنباتات الطماطم مقارنة بالكنترول الغير معامل، وبالقياسات النباتية كانت نباتات الطماطم الناتجة من المعاملات أطول معنوياً من الناتجة من الكنترول الغير معامل حيث كانت النباتات الناتجة من معاملة الفيتافاكس أقصر طولاً من النباتات الناتجة من معاملة التراكوديرما بفارق معنوى، بينما لم تظهر أى فروق معنوية فى إنتاجية محصول الطماطم الناتج من المعاملتان (العامل الحيوى والمبيد الفطرى) رغم تضاعف انتاجهما تقريباً عن الكنترول (غير المعامل).