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Trichoderma-induced Suppressive Soil for the Control of Fusarium Wilt of Tomato

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ABSTRACT

A study was carried out to evaluate the efficacy of Trichoderma-*induced suppressive soil on growth of tomato cv* Baccarat *322 and on wilt disease caused by* Fusarium oxysporum f. sp. lycopersici *(Fol). Dry preparation of* T. virens *(UPM 23),* T. harzianum *(UPM 40), singly and as mixtures (UPM 2340) with organic compost as carrier were amended into soil mixture as treatments to induce disease suppressiveness. The wilt fungus at the inoculum level of 100 mL plant^{-1} (9 x 10^6 spore mL^{-1}) caused significant suppression in growth and yield. However, the* Trichoderma-*induced suppressive soil checked the suppressive effect of the fungus leading to significant increase in root dry weight and yield compared with the inoculated control. Disease incidence expressed as the area under the disease progress curve (AUDPC) was highest for the control plants and lowest for plants treated with UPM 2340 followed by UPM 40 and UPM 23. The disease progress rate was significantly lower in plants treated with UPM 2340 (r_m = 0.01) compared to non-treated plants (r_m = 0.75). Rhizosphere population of the introduced* Trichoderma *(colony forming units g^{-1} soil) gradually decreased with an increase in frequency recovered from the tomato roots, suggesting an ability of the* Trichoderma *to colonize the roots.*

INTRODUCTION

The production of tomato (*Lycopersicum esculentum*, Mill.) in Malaysia, confined mainly to the highlands because of the mild temperature, is threatened by the wide spread of vascular wilt, a disease commonly associated with *Fusarium oxysporum* f. sp. *lycopersici* (Fol). Control measures such as the use of resistant varieties and chemicals have been found to be erratic or not long lasting. This could be due to the unavailability of the acceptable resistant cultivars and, moreover, Methyl Bromide Chloropicrin (MBC), a fumigant commonly used for the control of Fusarium wilt has been deregistered due to the implications for soil and water pollution.

The success of biological control through manipulation of antagonistic microorganisms such as of *Trichoderma* species, has been extensively studied in field and glasshouse crops. Introduction of *T. harzianum* (UPM 40) and *T. virens* (UPM 23) applied as granules or as dry powder has significantly promoted plant growth in several crops, with significant increase in the development of the root system (Ismail, 2001; Franklin, 2002). They have also been shown to suppress certain root diseases of vegetable crops (Jinantana and Sariah, 1998; Ibrahim, 2005). These antagonistic fungi can proliferate in the rhizosphere colonizing the roots, creating an environment unfavourable for the growth and sporulation of the pathogen. Henis *et al.* (1979) observed the development of suppressiveness after four to five successive plantings of radish, but observed no correlation between onset of suppressiveness and the *in vitro* antagonism of *Rhizoctonia solani* by the resident soil microflora. However, suppressive soils possessed higher populations of *Trichoderma* spp. than the corresponding conducive soil. This study was carried out with the aim of evaluating the effect of *Trichoderma*-induced suppressive soil for the control of Fusarium wilt of tomato.

MATERIALS AND METHODS

Preparation of Trichoderma *Inoculants*

Stock cultures of *Trichoderma virens* (UPM 23) and *T. harzianum* (UPM 40) were obtained from the Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. UPM 23 and UPM 40 were cultured on potato dextrose agar (PDA) and incubated at room temperature (28±2°C) for one week.

Molasses and rice flour were used as culture substrates for the mass production of the *Trichoderma* inoculants as it has been shown to produce abundant conidia and mycelial biomass (Ibrahim, 2005). Compost prepared from oil palm trunk and chicken dung (OPTCD) was used in this study as a carrier for the *Trichoderma* inoculants and/or to provide a ready food source for the initial establishment of the inoculants. The physical and microbiological properties of the compost were as determined (Ibrahim, 2005).The freeze-dried biomass of *Trichoderma* (UPM 23 and UPM 40) and the mixture of UPM 2340 (UPM 23: UPM 40; 1:1 w/w) were incorporated into the carrier (OPTCD) in the ratio 1:10 w/w fungal biomass: OPTCD. The initial colony forming units (cfu) of the formulated mixtures were determined and expressed as g^{-1} dry substrate.

Preparation of Trichoderma*-Induced Suppressive Soil*

Potting medium was prepared by mixing non sterilised soil mixture (3:2:1 v/v, mixture of top soil, sand and peat) with the respective *Trichoderma* preparations at 0.5% (w/w), respectively to induce 'suppressive soil'. Three kilograms of each mixture were placed in pots and allowed to incubate for four days at field capacity to allow for the proliferation and establishment of the *Trichoderma* inoculants in the soil. The populations of *Trichoderma* inoculants in the soil for each treatment were evaluated at the end of the experiment.

Effects of Trichoderma*-induced Suppressive Soil on Plant Growth and Disease Suppression*

Individual tomato seedlings cv Baccarat (4-6 leaves stage) were transferred into individual pots containing the *Trichoderma*-induced suppressive soil and allowed to establish for two weeks at field capacity. The seedlings were then inoculated with 100 mL Fol inoculant with a spore count of 9 x 10^6 conidia/mL. Treatments carried out include plants treated with compost alone (T1); UPM 23 (T2); UPM 40 (T3); mixture of UPM23 and UPM 40 (T4) and control (T4, sterilised distilled water) both for the infested and non-infested soil. The factorial experiment was conducted in a glasshouse with pots arranged in completely randomized design (CRD) replicated four times with three seedlings per replicate. Data were recorded as mean for each replicate. All seedlings were watered daily and fertilized fortnightly with NPK Blue (12:12:17) at a rate of 3 g seedling^{-1}.

Beneficial effects of *Trichoderma*-induced suppressive soil on plant vigor were assessed based on root mass and yield. Total yield of marketable fruits were harvested 12 weeks after transplanting and weighed. At the end of the experiment, three plants from each replicate were uprooted and shaken vigorously to remove all adhering potting medium. The roots were separated and placed in the oven at 65°C for 48 hours for determinations of dry weight.

Disease incidence (DI) was calculated based on the foliar-associated symptoms, according to the formula (modified from Campbell and Madden, 1990). Plants were considered infected when they expressed symptoms of epinasty, yellowing of lower leaves, wilting or marginal necrosis of the remaining leaves. The Area Under Disease Progress Curve (AUDPC) was then assessed using the same data plotted as disease progress curve based on the formula: AUDPC = $\sum_i^{n-1} (y_{(i+1)} + y_i /2) (t_{(1+i)} - t_i)$, where n = the number of assessment time; y = disease incidence and t = time (weeks). The disease progress rate expressed as the slopes of the disease progress curve was obtained by the multiple regression analysis using the Sigma Plot Software Program (SPS Version 9).

Proliferation and Establishment of Trichoderma *in the Rhizosphere and on Roots of Tomato*

Trichoderma population in the rhizosphere and roots of tomato was determined following the soil dilution plate technique on *Trichoderma* selective medium (TME) (Papavizas, 1981), and expressed as colony forming units g^{-1} soil (cfu g^{-1}). Roots of tomato were sampled according to Parke (1991) to determine the colonizing ability of the introduced *Trichoderma* inoculants and were expressed as cfu g^{-1} roots.

All data were arc-sine transformed and subjected to ANOVA using Statistical Analysis System (SAS) computer program. Results

showing significant differences were then subjected to mean comparison using Tukey's Studentized Range Test ($HSD_{0.05}$).

RESULTS

Effects of Trichoderma-*induced Suppressive Soil on Plant Growth and Disease Suppression*

Trichoderma-fortified compost incorporated into potting medium to induce soil suppression to Fusarium wilt had a significant effect on the vegetative growth of tomato plants based on root dry weight and yield. In the absence of *F. oxysporum f. sp. lycopersici*, plants grown in the *Trichoderma*-induced suppressive soil gave significantly higher values of root dry weight compared to the control (*Fig. 1A*). Fusarium infection had a detrimental effect on root dry weight of tomato plants (*Fig. 1B*). However, in the presence of *Trichoderma*-induced suppressive soil, the tolerance of tomato to Fusarium infection increased and this promoted vigor through an increase in root dry weight of the infected plants. Results showed that UPM 2340 and UPM 40 gave the highest effect with mean values of 14.41 g and 14.08 g, respectively. The control plants produced 2.83 g of root dry weight which was significantly lower than UPM 23 (9.85 g) and compost alone (5.09 g).

Tomato plants grown in the *Trichoderma*-induced suppressive soil showed a significant increase in total fresh weight of fruits. The total fresh weight of fruits was 512.08 g, 511.62 g, 454.97 g and 382.76 g for UPM 2340, UPM 40, UPM 23 and compost alone (Table 1), respectively for the non infested soil. The control plants produced only only 306.03 g. The wilt

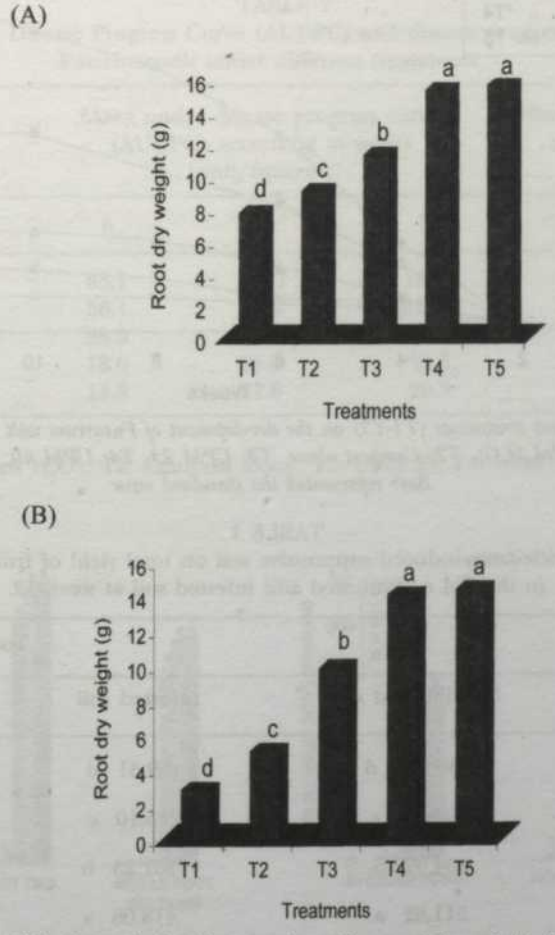

Fig. 1: Effect of Trichoderma-*induced suppressive soil on root dry weight of tomato plants in the Fol non-infested (A) and infested soil (B) at 12 weeks after treatment. Bars with different letters are significantly different using $HSD_{0.05}$. T1: Control (Sterilised distilled H_2O), T2: Compost alone, T3: UPM 23, T4: UPM 40, T5: UPM 2340*

fungus at inoculum level of 100 mL plant^{-1} (9 x 10^6 spore mL^{-1}) caused significant suppression in yield. However, the *Trichoderma*-induced suppressive soil checked the suppressive effect of the fungus leading to significant increase in root dry weight and yield compared to the inoculated control. The reduction in total fresh weight of fruits was highest with value of 64.28% (T1), followed by 25.51% (T2) as compared to 18.08%, 18.28% and 20.60% for T5, T4, and T3, respectively (Table 1).

Disease incidence was highest in control plants (T1) and lowest in plants treated with UPM 2340 (T5) followed by UPM 40 (T4), UPM 23 (T3) and compost alone (T2) (*Fig. 2*). Application of treatments T3, T4 and T5 significantly reduced the development of Fusarium wilt from week four until week 12. Disease incidence was highest for control (100%). Infected plants produced symptoms of yellowing of the older leaves. The yellowing gradually spread to affect most of the foliage and was normally accompanied by wilting of the plant during the hottest time of the day. The wilting became more extensive with time (days) until the plant collapsed and died. In cross-sections of the stem at the base of infected plants showed an evident brown ring in the vascular bundles area. Treatment T2 also suppressed the disease incidence caused by Fol but it was not as effective

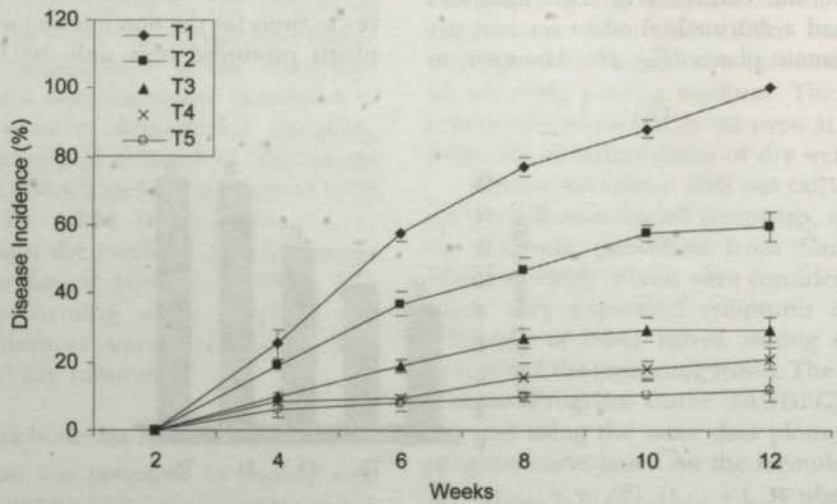

Fig. 2: Effect of different treatments (T1-T5) on the development of Fusarium wilt on tomato. T1: Control (Sterilised distilled H_2O), T2: Compost alone, T3: UPM 23, T4: UPM 40, T5: UPM 2340. Bars represented the standard error

TABLE 1
Effect of *Trichoderma*-induced suppressive soil on total yield of fruits per plant in the Fol non-infested and infested soil at week 12.

Treatment	Total fresh weight of fruits (g)		
	Non-infested soil	Infested soil	Reduction (%)
T1	306.03 d	109.31 d	64.28a
T2	382.76 c	285.10 c	25.51b
T3	454.97 b	361.23 b	20.60c
T4	511.62 a	418.08 a	18.28 c
T5	512.08 a	419.52 a	18.08c

Means with different letters within a column are significantly different using $HSD_{0.05}$. T1: Control (Sterilised distilled H_2O), T2: Compost alone, T3: UPM 23, T4: UPM 40, T5: UPM 2340.

as treatments T3, T4, and T5. This could possibly be due to the population of indigenous *Trichoderma* present in the compost and the presence of high saprophytic fungi that can cause strong competition for nutrients and space, as the soil mixture used in this study was not sterilized. In addition to disease incidence, the progress in disease development was also evaluated based on the area under the disease progress curve (AUDPC). The symptoms development was delayed in the *Trichoderma* treated plants as seen by the Area under Disease Progress Curve (AUDPC) values at week four until week 12 compared to control (Table 2), with the disease progress rate (slope) highest for T1, followed by T2, T3, T4 and finally T5 (r_m = 0.75, r_m = 0.09, r_m = 0.04, r_m = 0.02 and r_m = 0.01, respectively).

Proliferation and Establishment of Trichoderma *in the Rhizosphere and on Roots of Tomato*

Proliferation and survival of *Trichoderma* spp. was assessed at week 12 from both the rhizosphere of Fol non-infested and infested soil mixture and also the roots of tomato. The populations were higher on roots than rhizosphere for both main plots of Fol non-infested and infested soil (*Fig. 3*). The results showed that populations of *Trichoderma* in the rhizosphere were significantly lower than on roots due to the movement of *Trichoderma* populations from potting medium towards the roots.

TABLE 2
Area Under Disease Progress Curve (AUDPC) and disease progress rate of Fusarium wilt under different treatments

Treatment	[2]Area under disease progress curve (AUDPC) according to weeks (unit/square^2)				Disease progress rate (slope) at week 12 (unit/week)
	4[1]	6	8	10	12	
T1	25.3	83.1	134.7	164.9	188.0	0.75
T2	19.1	56.1	84.0	104.8	117.3	0.09
T3	10.0	28.9	45.8	56.4	59.0	0.04
T4	8.5	18.0	25.0	33.3	38.8	0.02
T5	6.0	13.8	17.6	20.3	22.5	0.01

[1] weeks [2] AUDPC values

T1: Control (Sterilised distilled H_2O), T2: Compost alone, T3: UPM 23, T4: UPM 40, T5: UPM 2340

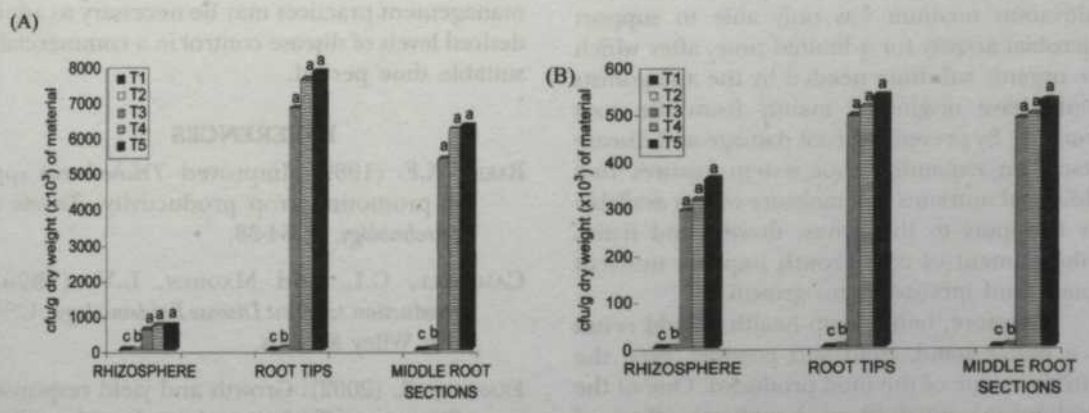

Fig. 3: Frequency of isolation of Trichoderma *from the rhizosphere, root tips and middle root sections of tomato grown in Fol non-infested (A) and infested soil (B) at 12 weeks after inoculation. Means with different letters within the same tested zone are significantly different using $HSD_{0.05}$. T1: Control (Sterilised distilled H_2O), T2: Compost alone, T3: UPM 23, T4: UPM 40, T5: UPM 2340*

DISCUSSION

This study showed that there was a positive relationship on the effects of *Trichoderma* (UPM 40 and UPM 23) on plant vigor and induced disease suppressiveness. Although there was a reduction in root mass in the presence of the pathogen, *Trichoderma*-induced suppressive soil maintain plant vigor and increased its tolerance to Fusarium wilt.

In recent studies, substrate amendment with *T. harzianum* resulted in enhanced plant growth throughout the growing season (Baker, 1989; Harman and Bjorkman, 1998; Ismail, 2001; Franklin, 2002; Ibrahim, 2005). It also protects the root from certain physical stresses thus allowing the roots to grow faster. Successful colonization of the tomato roots by *Trichoderma* involved the utilization of energy and nitrogen sources present in the root exudates. Movement of *Trichoderma* populations from substrates towards young germinated roots was due to root exudates, which serves as nutrient to *Trichoderma*. Studies by Sariah and Cheng (1999) showed that populations of UPM 23 incorporated into a mixture of coconut dust and peat (1:1 v/v) increased for the first four weeks in the growing medium, then decreased marginally until week 12 because of increased populations of *Trichoderma* detected on roots. Franklin (2002) also observed that *Trichoderma* spp. survived and proliferated in the growing medium consisting mixture of coconut dust and peat and colonized roots of tomato plants as the source of organic matter in the medium was depleted. In addition, Jensen (1997) reported that organic materials in cultivation medium was only able to support microbial activity for a limited time, after which the organic substrate needed by the antagonists would have originated mainly from the root exudates. By preventing root damage and disease attack, an expanded root system ensures that additional nutrients and moisture will be available for transport to the leaves, flowers and fruits. Enhancement of root growth improve nutrient uptake and increase plant growth.

Therefore, better crop health should result in a better stand, yield and possibly, even the nutritive value of the food produced. One of the mechanisms involved in beneficial effect of antagonistic microorganisms was increased uptake of nutrients (N, P and K), which have an important role on plant growth and subsequent yield. Mao *et al.* (1998) reported that tomato seeds treated with *Burkholderia cepacia* and *Gliocladium virens* individually and in combination significantly increased fruit yield of tomato and pepper in the field compared to the non-treated plants. Maman (2004) also reported that a mixture of *B. cepacia*, *Pseudomonas aeruginosa* 1 and *P. aeruginosa* 2 increased marketable fruit yield of tomato. In this study, application of mixture inoculants (UPM 2340) resulted in a greater yield increase, suggesting that the use of combinations or multiple antagonists may enhance yield and improve disease control over the use of single microorganisms. Such combinations may overcome inconsistencies in the performance of individual isolates. However, there was no significant difference between treatments of UPM 40 and the mixture of UPM 23 and UPM 40 (UPM 2340), suggesting that UPM 40 might have contributed significantly to the biological activity in the mixture.

The phenomena of disease suppressive soils have been documented for numerous plant-pathogen systems. Harnessing the potential of these soils as a practical means to manage diseases in conventional and organic growing conditions has long been a goal of plant pathologists. The findings reported here, demonstrate that the manipulation of microbial communities to induce a disease suppressive soil environment does possess potential as a tool in the management of soilborne plant diseases. The use of specific effective antagonists such as *Trichoderma* spp. can elicit the desired shifts in microbial community structure, but integration with additional management practices may be necessary to attain desired levels of disease control in a commercially suitable time period.

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