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GROWTH AND YIELD RESPONSES OF TOMATO (LYCOPERSICON ESCULENTUM) CULTIVATED IN TRICHODERMA AMENDED MEDIUM

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GROWTH AND YIELD RESPONSES OF TOMATO (*Lycopersicon esculentum*) CULTIVATED IN *Trichoderma* AMENDED MEDIUM

BY

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To my late grandmother, Hajah Fatimah Othman,
Who made my world magical

To my handsome father, Haji Abd Hafiz Haji Yunos,
Who shares my passion of gardening

To my elegant mother, Hajah Azlini Haji Zakariah,
Whose invariable support lasted till the end

To my cute sister, Nur Azrin Hazny,
Whose carefree attitude inspires me

To my little brothers, Mohamad Azraei Hazny & Mohamad Azmirul Hazny,
Whose enormous care always lifted my spirit

ABSTRACT

Growth and yield responses of tomato to *Trichoderma* inoculant in soilless growing medium (coco peat) were studied. *Trichoderma viride* was used in the study. Coco peat has low microbial populations comprising of mainly the genera *Aspergillus* and *Penicillium*. *T. viride* inoculants at the rate of 94 x 10^6 cfu/g dry weight of coco peat have shown an increment on seed germination and seedling emergence of tomato. Seedling emergence was 90% for *T. viride* treatment compared to untreated control (80%). Plants grown in *T. viride* amended coco peat showed high value of chlorophyll content (56.43). Overall, there was no significant difference in vegetative growth and root development between treatment and control, might due to the influence of line spacing on light interception and leaching effect of *Trichoderma* inoculants in coco peat with time. Tomato plants cultivated in *T. viride* amended coco peat resulted in higher fruit production (16.35) and total fresh weight of fruits (283.6 g) as compared to control. *T. viride* as amendment to soilless growing medium shown reduction in population on week 7 after application. *Trichoderma* population was high on the roots throughout the experimental period, suggesting that *T. viride* can colonize the germinating root and live on the root exudates. Application of *T. viride* as amendments to the growing medium gave positive effects on tomato's growth and yield.

ABSTRAK

Respon pertumbuhan dan hasil tomato terhadap kulat *Trichoderma* dalam medium tidak bertanah (sabut kelapa) dikaji. *Trichoderma viride* digunakan dalan kajian ini. Sabut kelapa mempunyai populasi mikroorganisma yang rendah di mana kebanyakannya terdiri daripada genera *Aspergillus* dan *Penicillium*. *T. viride* pada kadar 94 x 10^6 cfu/g sabut kelapa menunjukkan peningkatan terhadap percambahan dan pertumbuhan biji benih tomato. Pertumbuhan anak pokok yang dirawat dengan *T. viride* adalah 90% berbanding anak pokok kawalan (80%). Anak pokok yang ditanam dalam sabut kelapa yang dirawat dengan *T. viride* menghasilkan kandungan klorofil yang tinggi (56.43). Secara keseluruhannya, tiada perbezaan signifikan dalam pertumbuhan vegetatif dan perkembangan akar antara rawatan dan kawalan, disebabkan oleh pengaruh susunan pokok terhadap penerimaan cahaya dan kesan *Trichoderma* dalam sabut kelapa seiring dengan masa. Pokok tomato yang ditanam dalam sabut kelapa dicampur dengan *T. viride* menghasilkan jumlah bilangan (16.35) dan berat basah buah yang lebih tinggi (283.6 g) jika dibandingkan dengan pokok kawalan. *T. viride* sebagai rawatan kepada medium tidak bertanah menunjukkan pengurangan dari segi populasi pada minggu ketujuh selepas pengaplikasian. Populasi *Trichoderma* adalah tinggi pada akar sepanjang tempoh eksperimen, menunjukkan *T. viride* mampu untuk mengkoloni akar muda dan hidup pada akar dan persekitarannya. Aplikasi *T. viride* sebagai bahan tambahan dalam medium tidak bertanah menunjukkan kesan positif terhadap pertumbuhan dan hasil tomato.

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I certify that this project report entitled "Growth and Yield Responses of Tomato (*Lycopersicon esculentum*) Cultivated in *Trichoderma* Amended Medium" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus.

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INTRODUCTION

1.1 Background

Since the beginning of the "Green Revolution" in the early 1970's, which focused on food crop productivity, through high-yielding varieties, agrochemicals and irrigation system, chemical fertilizers were extensively used throughout most of agricultural Asia. In fact, Asia is the world's largest user of chemical fertilizers, consuming around 40% of the global total each year. The emphasis on chemical fertilizers, which sometimes led to injudicious application, has meant that the soil be regarded as an inert substrate for plant roots, instead of a living biosphere, the rhizosphere, containing a myriad of organisms. It is now realized that in agricultural lands under intensive monoculture system, including paddy rice, which receive heavy applications of chemical fertilizers alone, productivity is slowly declining, and environmental quality is deteriorating too. In the light of these problems, the use of organic fertilizers, biofertilizers and other microbial products is crucial in the current attempt to make the agriculture industry a viable component of a healthy and pleasant ecosystem (Khairuddin, 2002).

Trichoderma is a kind of anaerobic, facultative fungus, which can be found naturally in a large number of agricultural soils and other media. It belongs to the subdivision Deuteromycetes, which is characterized by not having, or not showing a determinate sexual state. There are more than 30 species of this microorganism, all with beneficial effects for agriculture and other fields (Chet *et el.*, 1988).

This fungus is widespread throughout the world, and appears in different zones and habitats, especially in those that contain organic matter or decomposing plant matter, and also in crop waste, especially when attacked by other fungi (Benitez, 2004). Development is encouraged by the presence of high density roots, which are rapidly colonized by these microorganisms. Their capacity to adapt to different environmental conditions and substrata means that *Trichoderma* can be used in different soils, crops, climates and technological processes (Harman, 2000).

It has been proved that *Trichoderma* produces substances that stimulate plant growth and development (Benitez *et al.*, 2002). These substances act as catalysts or accelerators in the primary meristem tissues in the young parts of plants, accelerating cell reproduction, so that the plants achieve faster growth than those which have not been treated with this microorganism (Harman, 2004).

Some species of *Trichoderma* have been reported to stimulate growth in species such as carnations, chrysanthemums, marigolds, petunias, cucumbers, aubergines, peas, peppers, radishes, tobacco, tomatoes, lettuce, carrots, potatoes, cotton, kidney beans and ornamental grass (Chet, 1988; Harman *et al.*, 2001; Benitez *et al.*, 2002; Harman *et al.*, 2004).

Cucumber seeds germinate two days earlier than those that have not been inoculated with the fungus (Chet, 1988). The numbers of flower buds per Madagascar periwinkle plant increased (Harman *et al.*, 2001). The chrysanthemum also has more

buds, and the plant height and weight is greater than of untreated plants (Benitez *et al.*, 2002). These results have been constant with concentrations of 108 colony-forming units per gram of soil, and these population densities are easily applied to soil as formulations, which equally favour growth in the *Trichoderma* population in this medium.

Some preliminary research has been carried out with *Trichoderma*, to stimulate growth in kidney bean plants, where the selected isolates stimulated germination and showed an increase in plant height of between 70% and 80%, and a weight gain of approximately 60%, which implies an increase in yield (Yedidia *et al.*, 2000). A similar test carried out on Estrella grass showed that the gain in dry weight with certain test specimens was close to 23%, with increases of 30% in root length and stolons (Altomare, 1999).

1.2 Problem Statement

Tomato or *Lycopersicon esculentum* is a common vegetable consumed by people around the world and it is normally cultivated in countries with tropical climate (Siemonsna and Kasem, 1994). Tomato is categorized as a fruit vegetable and through various scientific researches proven to have numerous nutritional elements beneficial to human kind. In 2000, Malaysia's imported tomato value reaches nearly RM600, 000 with a decrease of 15% from the imported value of the previous year (Department of Agriculture, 2000). Although tomato can be cultivated through conventional and fertigation method, nowadays more than 70% of the tomato cultivation method is done in greenhouses controlled by computer to oversee the environment and temperature that is favourable for the growth and cultivation of tomato (Smale, 2004).

Several factors affect tomato production and ultimately the cost of tomato cultivation. For one, tomato producers obtain seed either from their own fields, from neighbors and friends, or from women's groups that maintain and distribute varieties that are in high demand in the marketplace. This recycling drives down seed price, but has a negative effect on seed quality (Horna, 2004). Also, most tomato varieties used in commercial production are introduced varieties, which are not well adapted to local conditions in Malaysia. This, along with the seasonality of tomato production, creates periods of abundance and scarcity, which dramatically affect market prices. Chemical fertilizer is often used as fertilizer by producers across the region. On average, chemical fertilizer usage represents more than 70 percent of total production

costs for commercialize tomato producer in Malaysia (Department of Agriculture, 2000). Services, equipment and labor (land preparation, transplanting and harvesting) costs together account for only 30 percent of the total expenses incurred by a tomato producer. During the rainy season, fungal diseases and pests are common. Access to irrigation facilities also conditions production (Horna, 2004).

In Malaysia, 3.8 million tonnes of N, P, K fertilizers were imported in 2004, valued at US$529 million. Urea, ammonium sulphate and ammonium nitrate are the main nitrogen fertilizers. Malaysia produces high quality (prilled) urea compared to the quality of imports. The prilled urea is mostly exported instead and Australia and Thailand are the two largest importers. Urea remains the cheapest N-source and is largely used in paddy and vegetable cultivation. The main types of phosphorus fertilizers are rock phosphate, ammonium and superphosphate. High usage of fertilizers has led to non-sustainability in crop growth and productivity. Coupled with this, concern for the environment and spiraling petroleum prices have led farmers to revert to cheaper and safer alternatives for crop production (Zakaria, 2006).

1.3 Objectives

i. To evaluate growth and yield responses of tomato plants to *Trichoderma viride* inoculants.

ii. To assess the proliferation and survival of *Trichoderma viride* inoculants on roots and in medium.

CHAPTER 2
LITERATURE REVIEW

2.1 *Lycopersicon esculentum*

Tomato (*Solanum lycopersicum*) is a plant in the Solanaceae or nightshade family, as are its close cousins tobacco, chili peppers, potato, and eggplant. The leaves are 10–25 cm long, pinnate, with 5–9 leaflets, each leaflet up to 8 cm long, with a serrated margin; both the stem and leaves are densely glandular-hairy. The flowers are 1–2 cm across, yellow, with five pointed lobes on the corolla; they are borne in a cyme of 3–12 together. Tomato is a variable annual herb, up to 2 meter tall or taller (Siemonsna and Kasem, 1994).

Tomato originated from the Andean region of South America, in the area now covered by parts of Bolivia, Chile, Ecuador, Colombia and Peru. Archaeological and circumstantial evidence all suggest that tomato was domesticated in Mexico, outside its centre of origin, and the most likely ancestor is the primitive cherry tomato (*L. esculentum* var. *cerasiforme*). Tomato was introduced into Europe in an already fairly advanced stage of domestification soon after the discovery of the new world. From there, it was taken to other parts of the world at various times; in the 17th Century to China, South and South-East Asia; and in the 18th Century to Japan and the United States (Siemonsna and Kasem, 1994).

Total area of tomato planted annually worldwide is about 2.7 million ha, 80-85% in market gardens, producing an estimated 68 million tones. Tomatoes are

produced in the open field, under plastic shelter or in greenhouse, dependent on the climate and season. Most of the world trade in tomatoes comes from the Mediterranean region, the United States and the South and Central America. The area used for tomato production in the Philippines is about 18000 ha, in Thailand 8300 ha and in Malaysia 700 ha (Siemonsna and Kasem, 1994).

The tomato dry seed (5.5% moisture content) will maintain a high viability (90-95% germination) after several years of storage at ambient temperatures (18-24°C), provided the seeds have been extracted from fully matured fruits. Seed germinate within six days of sowing at optimum soil temperature of 20-25°C and the first true leaf formed one week later. The first flowering starts under optimum conditions about 5-7 weeks after sowing (Siemonsna and Kasem, 1994).

Ideally, tomato requires a relatively cool, dry climate for high yield and premium quality. However, it is adapted to a wide range of climatic condition. Tomatoes can be grown in many soil types ranging from sandy loam to clay-loam soils that are rich in organic matter. The ideal soil pH range is 6.0-6.5; higher or lower pHs can cause mineral deficiencies or toxicities. Long periods of flooding are detrimental to tomato growth and development (Siemonsna and Kasem, 1994).

Tomatoes are attacked by many diseases and insect pests. Of about 60 pathogens that attack tomatoes, 15 are considered to be major diseases in the hot and humid tropics. Bacterial wilt (*Pseudomonas solanacearum*) has often been reported

as the most serious handicap for tomato in the tropics. The most important fungal diseases in tomatoes in the tropics are early blight (*Alternaria solani*), black leaf mould (*Pseudocercospora fuligena*), late blight (*Phytophthora infestans*), leaf mould (*Cladosporium fulvum*), powdery mildew (*Leveillula taurica*), southern blight (*Sclerotium rolfsii*) and target spot (*Corynespora casiicola*). Important virus diseases are tomato mosaic, cucumber mosaic, tomato yellow leaf curl, tomato yellow dwarf and tomato spotted wilt virus (Siemonsna and Kasem, 1994).

Among the insects, the polyphagous tomato fruit worm (*Heliothis armigera*) is one of the most destructive, causing as high as 70% yield loss due to fruit boring. Cotton aphid (*Aphis gossypii*) is the major pest during dry season. It injures the plant by sucking the sap and by acting as a vector for the cucumber mosaic virus. White fly (*Bemisia tabaci*) is a serious pest, not only because of its foraging on the tomato plants but also because it acts as a vector of the tomato yellow leaf curl. Root knot nematodes (*Meloidogyne incognita*) invade the tomato roots and cause galling (Siemonsna and Kasem, 1994).

2.2 Coco Peat

Coco peat is an established organic substrate and widely used in the production of fruit vegetables in Malaysia. It is a natural and used to be a renewable resource produced from coconut husks by coconut industries in Sri Lanka, Malaysia and Indonesia (Mahamud, 2002). Coco peat is the 'coir fiber pith' or 'coir dust' produced as a bi-product when coconut husks are processed for the extraction of the long fibers from the husk. Coco peat is the binding material that comes from the fiber fraction of the coconut husk (Manisah, 2002).

The coir dust is washed, heat treated, screened and graded before being processed into various coco peat products for horticultural and agricultural applications. Coco peat was first introduced into English horticulture as a growing medium more than 135 years ago (Murphy, 2004). In horticulture, coco peat is recommended as substitute for peat because it is free of bacteria and fungal spores, and is sustainably produced without the environmental damage caused by peat mining (Mahamud, 2002).

Coco peat is a multi-purpose soil conditioner and growing medium. It is consistent and uniform in texture. It is a completely homogenous material composed of millions of capillary micro-sponges, which absorb and hold up to eight times its own weight in water. Coco peat has an unusually high Cation Exchange Capacity (CEC) and 27% of Easily Available Water assures that coir will hold and release nutrients in solution over extended periods without rewatering (Yau, 2004).

Coco peat by itself does not have any nutrients for plant growth. Necessary nutrients will have to be added according to the plant that is to be grown exclusively in coco peat (Mahamud, 2002). Coco peat may also be mixed with sand, compost and fertilizer to make good quality potting soil. Coco peat generally has acidity in the range of pH 5.5 to 6.5. It is a little on the acidic side for some plants, but many popular plants can tolerate this pH range (Yau, 2004).

Coco peat has high lignin and cellulose content which makes it ideal for growing mushrooms which thrive on the cellulose. Mahamud *et al.*, (2003) reported that 3-month biodegraded coco peat has lower C/N ratio, higher CEC and humic acid than the raw coco peat. The lowering of C/N ratio was due to addition of N and the reduction of carbon, mainly the hemicellulose, cellulose and to a lesser extent the lignin components. In the greenhouse trial, tomato plants grew well in the 3-month 'composted' coco peat. Being a good absorbent, dry coco peat can be used as an oil absorbent on slippery floors. Coco peat is also used as bedding in animal farms and pet houses to absorb animal waste so the farm is kept clean and dry (Manisah, 2002).

2.3 Biopesticide and Biofertilizer

Modern agriculture is highly dependent on the use of chemical pesticides to control plant pathogens. Fungicides and fumigants commonly have drastic effects on the soil biota, as they are intentionally applied at much higher rates than herbicides and insecticides (Fraser, 1994). These methods are time-consuming and uneconomical, pollute the atmosphere, and are environmentally harmful, as the chemicals build up in the soil (Nannipieri, 1994). Furthermore, the repeated use of such chemicals has encouraged the development of resistance among the target organisms (Goldman *et al.*, 1994). This has resulted in the use of ever-increasing amounts of pesticides and has prompted the search for new strategies of pest control to reduce or eliminate the use of pesticides (Lorito *et al.*, 1994).

Biological disease control is a promising strategy for managing soilborne or foliar diseases in a wide range of crops (Nelson and Craft, 1992). To be effective, biological disease control depends not only on suitable biocontrol organisms but also on methods and strategies for introducing and maintaining population levels and activities of these organisms in associations with crops and plants (Kenerley and Pettit, 1988). Regardless of the qualities of the biocontrol agents, the methods used to produce, formulate, and deliver these organisms may profoundly influence their efficacy (Hayes and Harman, 1991).

Bio-effectors based on plant-growth-promoting soil microorganisms are increasingly distributed on the world market. Mobilization of sparingly available

plant mineral nutrients, stimulation of root growth, enhanced resistance to environmental stress factors and direct or indirect suppression of plant pathogens and induced resistance are discussed as possible mechanisms for the effectiveness of these products (Akter *et al.*, 2007). Successful biocontrol requires an understanding of many factors; the cropping system; the epidemiology of the diseases to be controlled; the biology, ecology, and population dynamics of the biocontrol organisms; and the interactions among these variables (Sutton and Peng, 1993).

Alok and Deepak (2002) defines biofertilizer as a product containing carrier based (solid or liquid) living micro-organisms which are agriculturally useful in terms of nitrogen fixation, phosphorus solubilization or nutrient mobilization, so as to increase the productivity of the soil and/or the crop. These broadly include the nitrogen fixers (symbiotic and nonsymbiotic bacteria), phosphate solubilizing fungi and bacteria and the mycorrhizal fungi that are capable of mobilizing nonlabile nutrients from soil and transporting them to and across plant roots.

A recent addition is the plant growth promoting rhizobacteria (PGPR), specifically the fluorescent *Pseudomonads*, which stimulate plant growth and repress root diseases by a variety of mechanisms. So far emphasis has been given only to certain types of biofertilizers such as *Rhizobium*, *Azotobacter*, *Azospirillum* and Phosphate Solubilizing Bacteria (PSB). Usually carrier material such as peat, lignite, peat soil, humus, wood charcoal or similar material favoring the growth of microorganisms is used. However, in practice a large variety of microbial inoculants

are available and being used as biofertilizers. These include Vesicular Arbuscular Mycorrhiza (VAM), *Azolla, Frankia* and *Trichoderma* (Chet and Inbar, 1994).

Increasing public awareness of health issues has increased the demand for certified organic fresh produce, since pests and diseases of vegetable crops can be controlled by biological control agents (Chet and Inbar, 1994). A number of plant-associated microbes are free-living and strongly beneficial to plants. Fungi in the genus *Trichoderma* (Harman *et al.*, 2004) and rhizobacteria in the genera *Pseudomonas*, *Bacillus*, *Streptomyces*, *Enterobacter*, and others (Baker, 1987) have evolved multiple mechanisms that result in improvements in plant resistance to disease and plant growth and productivity.

2.4 *Trichoderma* spp.

Trichoderma is a filamentous fungus that is widely distributed in the soil, plant material, decaying vegetation, and wood. *Hypocrea* spp. is the teleomorph of some *Trichoderma* species. The genus *Trichoderma* has five species; *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma pseudokoningii*, and *Trichoderma viride* (Harman and Bjo¨rkman, 1998).

Colonies of *Trichoderma* grow rapidly and mature in 5 days. At 25°C and on potato dextrose agar, the colonies are wooly and become compact in time. From the front, the color is white. As the conidia are formed, scattered blue-green or yellow-green patches become visible. These patches may sometimes form concentric rings. They are more readily visible on potato dextrose agar compared to Sabouraud dextrose agar. Reverse is pale, tan, or yellowish (Harman and Bjo¨rkman, 1998).

Septate hyaline hyphae, conidiophores, phialides, and conidia are observed. *Trichoderma longibrachiatum* and *Trichoderma viride* may also produce chlamydospores. Conidiophores are hyaline, branched, and may occasionally display a pyramidal arrangement. Phialides are hyaline, flask-shaped, and inflated at the base. They are attached to the conidiophores at right angles. The phialides may be solitary or arranged in clusters. Conidia (3 µm in diameter, average) are one-celled and round or ellipsoidal in shape. They are smooth- or rough-walled and grouped in sticky heads at the tips of the phialides. These clusters frequently get disrupted during routine slide

preparation procedure for microscopic examination. The color of the conidia is mostly green (Harman and Bjo¨rkman, 1998).

The fungal genus *Trichoderma* is cosmopolitan in soils, and the ecological adaptability of *Trichoderma* species is evidenced by their widespread distribution, including under different environmental conditions and on various substrates. This physiological flexibility together with the antagonistic action of *Trichoderma* spp. against phytopathogenic fungi and the ability of these fungi to promote plant growth have made them attractive biological control agents (Harman and Bjo¨rkman, 1998). *Trichoderma* spp. is beginning to be used in reasonably large quantities in plant agriculture, both for disease control and yield increases (Harman and Bjo¨rkman, 1998).

2.4.1 *Trichoderma* spp. as Biocontrol Agent

Besnard and Davet (1993) found a number of *Trichoderma* strains that were simultaneously plant growth promoters (tomato and cucumber) and biocontrol agents (*Pythium*). Chet and Inbar (1994) reported that integration of biocontrol agents with reduced doses of chemical agents has the potential to control plant pathogens with minimal impact on the environment. *Trichoderma harzianum* is an efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. Among the target fungi that the *Trichoderma* spp. is effective against are *Phytium* spp., *Rhizoctonia solani*, *Fusarium* spp., *Botrytis cineria*, *Sclerotinia homeocarpa* and *Perenospora destructor* (Poldma *et al.*, 2001). Different

mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism, production of inhibitory compounds (Harman *et al.*, 1996), antibiosis (Chet, 1987) and induction of defense responses in host plants (Yedidia *et al.*, 1999).

It has been known for many years that *Trichoderma* can sense the presence of target fungi and appeared to grow tropically toward them (Chet *et al.*, 1981). More recently, the gene encoding green fluorescent protein was inserted downstream of the regulatory regions of genes encoding an endo- and an exochitinase that have biocontrol abilities. When paired with a target fungus, the endochitinase gene is activated before the fungi come into contact, while the activation of the exochitinase occurs only after contact is made (Zeilinger *et al.*, 1999). Different strains may follow different patterns of induction but the fungi apparently always produce low levels of an extracellular exochitinase. Diffusion of this enzyme catalyzes release of cell wall fragments from target fungi and this, in turn, induces expression of fungitoxic cell wall degrading enzymes (Bjorkman *et al.*, 1994) that also diffuse and begin the attack on the target fungi before contact is actually made (Viterbo *et al.*, 2002).

A recent review lists 11 separate reports demonstrating control by *Trichoderma* spp. of a wide range of plant pathogens, including fungi, oomycetes, bacteria, and one virus, by elicitation of induced systemic or localized resistance

(Harman *et al.*, 2004). The fungi, especially rhizosphere competent ones, colonize root surfaces and penetrate the epidermis and into the cortex (Yedidia *et al.*, 1999).

Along the way, the fungi may coil about root hairs in a manner reminiscent of mycoparasitism (Yedidia *et al.*, 1999). Once *Trichoderma* hyphae penetrate the roots, a series of bioactive metabolites from the fungus is produced that induces walling off and biochemical mechanisms that limit growth of the *Trichoderma* to a small area. This may be similar to responses of other root colonizing biocontrol fungi including binucleate *Rhizoctonia* species (Hwang and Benson, 2003) and nonpathogenic Fusaria (Benhamou *et al.*, 2002).

Lumsden *et al.*, (1996) discovered that *Trichoderma virens* is an effective biological control agent for plant diseases caused by soilborne fungi. The disease-suppressive ability of *T. virens* is presumably related to its antagonistic behavior towards other fungi. Multiple traits may contribute to this antagonistic activity, particularly since the modes of interaction appear to differ for different target fungi (Howell, 1987). Production of gliotoxin, an epidithiodiketopiperazine antibiotic, is associated with *T. virens* suppression of damping-off caused by *Pythium ultimum* (Lumsden and Straney, 1994). Production of additional antibiotics, competition for resources in the soil or rhizosphere, and hyperparasitism of target fungi are traits that may account for other antagonistic interactions (Cook and Baker, 1983).

Furthermore, plant-cell-wall-degrading enzymes produced by *Trichoderma*, such as xylanases and cellulases, are able to directly induce ethylene biosynthesis in plants, a well-known response to the presence of pathogens (Kubicek *et al.*, 1998). There is extensive evidence for the induction of plant defense systems by *Trichoderma* species in a number of plants (Hanson and Howell, 2004). Taken together, these data indicate that *Trichoderma* is able to colonize plant roots, inducing changes in plant defense systems and plant physiology without destroying plant tissues (Yedidia *et al.*, 2000).

Porras (2003) reported that *Trichoderma* spp. applications reduced soil populations of *P. cactorum* and reduced leather rot incidence 76.6% in year 1 and 33.8% in year 2 compared with the untreated control. The combination of solarization and *Trichoderma* spp. reduced *P. cactorum* soil population the most each year, 88.9% in January 2001, 97.6% in 2002, and 99.0% in 2003. Harman *et al.*, (2004) conducted an experiment where *T. harzianum* strain T22 (Stasz *et al.*, 1988) was added at transplanting to tomato roots. After 90 to 120 days, symptoms of late blight appeared on the leaves, but in trials over 2 years, there was up to 80% reduction in disease in the presence compared with the absence of T22 even though T22 was present only on roots.

2.4.2 *Trichoderma* spp. as Plant Growth Promoters

For about 70 years, *Trichoderma* spp. has been known to be able to attack other fungi, to produce antibiotics that affect other microbes, and to act as biocontrol microbes

(Weindling *et al.*, 1936). Some landmarks along the way include the discoveries that these fungi frequently increase plant growth and productivity (Lindsey and Baker, 1967) either in the presence (Chang *et al.*, 1986) or absence (Lindsey and Baker, 1967) of other microorganisms and that they can induce disease suppression in soils (Chet and Baker, 1981).

The ability of *Trichoderma* to recognize and parasitize phytopathogenic fungi in the rhizosphere (Benítez *et al.*, 2004) has been ascribed to several complex mechanisms, such as nutrient competition, antibiosis, mycoparasitism, induction of systemic resistance, and increased plant-nutrient availability (Naseby *et al.*, 2000). Species of the *Trichoderma* genus are characteristically saprophytes (Kubicek *et al.*, 1998); however, root colonization by some *Trichoderma* strains is a common phenomenon in the field (Harman *et al.*, 2004). The relationship between *Trichoderma* and plants has long been studied (Lindsey and Baker, 1967) and the promotion of plant growth, under axenic conditions and in natural soils (Harman *et al.*, 1999), is among the many reported effects of the plant-fungus interaction (Naseby *et al.*, 2000). *Trichoderma* are thought to promote plant growth by at least two different mechanisms: (i) by controlling the population of pathogenic microorganisms in the rhizosphere (Benítez *et al.*, 2004), and (ii) by influencing plant physiology through mineral solubilization (Harman *et al.*, 2004) or hormone secretion (Blanchard and Björkman, 1996).

According to Kloepper *et al.* (1988), numerous microorganisms, especially those associated with roots, have the ability to increase plant growth and productivity. The increased growth response of plants caused by *Trichoderma* depends on the ability of the fungus to survive and develop in the rhizosphere (Kleifield and Chet, 1992). A possible mechanism for increased plant growth is an increase in nutrient transfer from soil to root, which is supported by the fact that *Trichoderma* can colonize the interior of roots (Kleifield and Chet, 1992). Many microbial treatments of plants have in the past been shown to have positive or negative influence on plant growth (Lynch *et al.*, 1991). However, Ousley *et al.*, (1993) found that autoclaving *Trichoderma* inocula did not remove growth-promoting properties and suggested that seedling growth promotion by *Trichoderma* could be a balance between growth inhibition and growth promotion properties, with the balance altered in some strains by autoclaving.

In a few cases, this effect has been suggested to involve solubilization of otherwise unavailable mineral nutrients. In soil, both macro- and micronutrients undergo a complex dynamic equilibrium of solubilization and insolubilization that is greatly influenced by the soil pH and microflora and that ultimately affects their accessibility to plant roots for absorption (Goldstein, 1995).

According to Cuevas (2002), *Trichoderma* has been demonstrated in the field to solubilize unavailable phosphorus and zinc in the soil. The organism also decomposes soil organic matter making nutrients such as calcium, potassium and

nitrogen available for plant use. The crops receive balanced fertilization. Nitrogen is needed by crops to synthesize chlorophyll, green pigments in leaves and other biomolecules important to plants' growth and development. Potassium is essential for osmotic balance of the cell and in grain formation. Calcium makes the crops sturdy and strong since this element is directly incorporated in plant cell wall. The crops are able to withstand stresses brought about by natural weather phenomena such as heavy rains and winds. Phosphorus, on the other hand, is needed in fast vegetative growth of the crops and in energy relations of the plants. Zinc is important in pollination and fruit formation and in proper functioning of the enzyme system (Cuevas, 2002). This summation indicates that *Trichoderma* possesses a range of different mechanisms to solubilize and, in some cases, chelate various plant nutrient compounds (Altomare, 2002).

Poldma *et al.*, (2000) reported that the application of *Trichoderma* has not only an antagonistic effect on plant pathogens but also positive effect on plant growth and yield in some vegetable crops. The increase growth response is mainly due to increased solubilisation of uptake of minor and other minerals as well as improvement in the root morphology enabling the roots to exploit a larger volume of soil (Yedidia *et al.*, 2001). It is possible that an increase growth response may subsequently result in enhanced yield, especially in those plants grown for vegetative parts.

Tomatoes (*Lycopersicon esculentum*) are an important vegetable crop that requires chemical fertilizers and pesticides under protected cultivation to maintain high production (Anon, 2003). The production of *Trichoderma* into those production practices may offer protection of plants as a healthier alternative. However, reports on the effect of *Trichoderma* on the yield of vegetable crops are variable; for example, onion production was not increased by its use (Poldma *et al.*, 2001), whereas in 4-year study on greenhouse cucumber production, *Trichoderma* significantly increased yield only in the last year (Poldma, 2002). Published data are not available on the effects of *Trichoderma* on tomato production and yield, especially when the fungus is used against plant pathogenic fungi under conventional and relatively poor growing conditions.

However, some studies have also shown that *Trichoderma* can stimulate the growth of a number of vegetable and bedding plant crops (Lynch *et al.*, 1991). Lynch *et al.*, (1991) investigated the effect of *Trichoderma* on the growth of lettuce, and its ability to control damping off diseases caused by *Rhizoctonia solani* and *Pythium ultimum*. They investigated whether a number of *Trichoderma* strains had a direct effect on lettuce establishment and growth in the absence of pathogens. It was found that the fungal treatments reduced the emergence time of seedlings compared to the controls. From their results, and those of Ousley *et al.*, (1994), they concluded that specific *Trichoderma* strains have the potential to consistently increase plant growth (Lynch *et al.*, 1991).

Naseby *et al.*, (2000) investigated the effect upon pea growth and their antagonistic activity against large *Pythium ultimum* inocula of five strains of *Trichoderma* with known biocontrol. *Pythium* inoculation significantly reduced the root length and the number of lateral roots and nodules, and significantly increased the root and rhizosphere soil fungal populations. *Pythium* inoculation also significantly reduced the plant wet and dry shoot weights and significantly increased the wet and the dry shoot/root ratio. All the *Trichoderma* strains reduced the number of lesions caused by *Pythium* and increased the number of lateral roots.

The effect of the *Pythium* on emergence and shoot growth was significantly reduced by all the *Trichoderma* strains. They also found that *Pythium* increased the activity of C, N and P cycle enzymes, whilst four *Trichoderma* strains reduced this effect, indicating reduced plant damage and C leakage. Overall, two of the *Trichoderma* strains had the greatest beneficial characteristics, as both these strains improved plant growth in the absence of *Pythium* and reduced plant damage in the presence of *Pythium* (Naseby *et al.*, 2000).

Chacón *et al.*, (2007) reported that *T. harzianum* CECT 2413 promoted the growth of tomato and tobacco plants, increasing foliar area and secondary roots, among other parameters. The significant promotion of plant growth observed in this study was likely due either to the secretion of phytohormones by *T. harzianum* (Blanchard and Bjōrkman, 1996) or to the development of a plant-fungus

relationship similar to that described for mycorrhizal fungi, in which plant growth is promoted by nutrient exchange (Osiewacz, 2002).

The results showed that *T. harzianum* CECT 2413 is able to promote plant growth and colonize roots. Furthermore, the fungus undergoes morphological changes that are specific for plant-fungus interactions, such as the formation of papilla-like hyphal tips and yeast-like cells. Transcriptome analysis showed that the gene-expression patterns accompanying these interactions are similar to those of phytopathogenic and mycorrhizal fungal-plant associations. *Trichoderma* is a biotrophic symbiont that is more closely related to mycorrhyzal than to plant pathogenic fungi, since it does not damage host plants, but instead promotes plant growth (Chacón *et al.*, 2007).

CHAPTER 3
MATERIALS AND METHOD

3.1 Preparation of Soilless Growing Medium

Agriculture by-product, coco peat, was used as the soilless growing medium and was filled into in the polybags measuring 12 inches in height and 8 inches in diameter each.

3.2 Preparation of *Trichoderma viride* Inoculants

Indigenous isolates of *Trichoderma viride* was isolated from black pepper roots and maintained on Potato Dextrose Agar (PDA) at 20°C-23°C were used to prepare the microbial inoculants. The *T. viride* inoculants consisted of hyphae, conidia and chlamydospores were raised on sago pith. Three hundred grams of sago pith was put into an autoclavable polyethylene bag with 300 ml-distilled water and autoclaved for 1 hour at 103 Kpa and allowed to cool. Three mycelial plugs of 0.5 mm from 7 day-old cultures of *T. viride* were transferred into the bags after cooling and incubated at room temperature (28±2°C) for three weeks.

The infested sago pith was then air-dried and ground through 1 mm mesh sieve. Colony forming units (cfu) per gram of air-dried preparation were determined before used by serial dilution plating technique on *Trichoderma* selective media (TME) (Benitez, 2004). Twenty grams of the powder were mixed with 200 ml sterilized distilled water, incubated on mechanical shaker for 30 minutes and serial dilutions prepared. One ml aliquot was pipetted and spread on TME. The plates were

then incubated for 7 days under room temperature (28±2°C). Cfu of *T. viride* was determined and expressed as cfu/g as dried preparation.

3.3 Seed Germination Test

Tomato (*Lycopersicum esculentum*) was used as the test plant. Tomato seeds were randomly sampled from the seed lot (ISTA, 1987), surfaced disinfected for 10 minutes with 5% sodium hypochloride solution (Clorox®), and then air-dried at room temperature. Germination of seeds was assessed by placing the seeds on moist blotter paper and incubated at room temperature (28±2°C) for 7 days. Seeds were considered germinated when the radicle was equal to half the length of the seed. Seed viability was expressed as the percentage of germination 7 days after incubation.

3.4 In Vitro Screening for *Trichoderma viride*

A five-mm-diameter agar disc taken from the edge of 4-day-old PDA culture of *Trichoderma viride* was placed 3 cm from the periphery of the 9 cm PDA cultures plate. The characteristics of *T. viride* that were observed are its colour and growth pattern for seven days.

3.5 Determination of Initial *Trichoderma* Population in Growing Medium

Trichoderma spp. population of growing medium (coco peat) was determined by serial dilution platings on *Trichoderma* selective media (TME). 10 g of soilless media were suspended in 100 ml of sterilized distilled water and incubated in a mechanical shaker for 30 minutes. Serial dilution was prepared and 1 ml aliquot was pipetted into

TME plates with swirling. Fungal population was expressed as cfu/g dry weight growing medium (coco peat).

3.6 Growth and Yield Responses of Tomato to *Trichoderma viride* Inoculants

This study was undertaken to evaluate the growth and yield responses of tomato to the *Trichoderma viride* inoculants. This study was carried out under a rain proof structure located besides UPMKB's Department of Crop Science building with coco peat as the growing medium. The growing medium was filled into polybag measuring 12 inches in height and 8 inches in diameter. The average weight of growing medium for each bag was about 1kg. Treatments were replicated four times. There are a few holes punctured around the polybag for drainage. One month old air-dried preparation of *T. viride* was mixed with the growing medium giving cfu of 94 x 10^6 /g dry weight of growing medium and placed into individual germination pot measuring 5 cm in diameter and height. Individual tomato seed was sown into each germination pot. Seeds sown in non-amended growing medium served as control. 2 weeks after germination, tomato seedlings (5-6 leaves stage) was transferred to the respective cultivation polybags. Planting distance was 30 cm between polybags.

3.7 Plant Maintenance

Tomato plants were trained vertically, as single stemmed plants. Plants were staked or trained by using raffia string tied to an overhead support. At weekly intervals, shoots that developed from the auxiliary buds were removed. The shoot was bent sharply to one side until it snapped and was pulled off in the opposite direction. When the plants

produce a total of three trusses, the main growing stem was terminated at the point of two leaves after the final truss. No hormone was used to stimulate the flowering of tomato so as not to interfere with the assessment of the performance of *Trichoderma viride* as a plant growth enhancer.

Sampling was carried out weekly for 10 weeks with 4 replications for every sampling time. For each sampling time, the following parameters were assessed:

3.8 Physiology Response

3.8.1 Chlorophyll Content

Total chlorophyll was determined by using Minolta Chlorophyll Meter Spad-502. Reading was taken as an average of four readings for each leaf.

3.9 Growth Responses

3.9.1 Plant Height

Plant height was measured by using measuring tape, and represented as the total length from the top edge of the polybag to the tallest leaf.

3.9.2 Number of Leaves

Total number of leaves/plant was counted excluding the leaf less than 1 inch in breadth and length.

3.9.3 Fresh and Dry Weight of Leaves

The plants were separated into the leaf and root sections. Fresh weight of leaves was recorded and then placed in the oven at 65°C for 48 hours for determination of dry weight.

3.9.4 Root Length and Root Mass

The plants were carefully separated from the growing media. Root length was measured between root crown and rot tip using mechanical ruler. Fresh weight was determined and later placed in the oven at 65°C for 48 hours for determination of the dry weight.

3.9.5 Yield

Total yield (average number of fruits, total fresh weight) of fruit was determined at the end of the experiment (11th week). Yield was based on the production of 3 fruit trusses per plant.

3.10 Proliferation and Survival of *Trichoderma*

3.10.1 Cfu in Soilless Medium (Coco Peat)

Determination of the *Trichoderma* population in soilless growing medium (coco peat) around the tomato plants (within 5 cm radius and 3 cm depth) was carried out for each sampling time. The soilless growing medium was oven dried at room temperature for 24 hours. Twenty grams of the air-dried soilless media was randomly taken, and were transferred into a 250 ml Erlenmeyer flask containing 200 ml of

sterilized distilled water and serial dilutions were prepared from the suspension. 1 ml of the aliquot of each dilution from 10^3 to 10^6 was spread onto TME and incubated at room temperature (28±2°C) for 7 days. The population of *Trichoderma* in soilless growing medium (coco peat) was expressed as cfu/g weight soilless media.

3.10.2 Cfu on Roots

Roots of tomato were also sampled to determine the colonizing activity of *Trichoderma* inoculants. Roots were carefully excised to remove the adhering substrate from the root segments. 6 roots per plant with similar length were randomly taken out and cut into segments, starting 2 cm from the root tip and 2 cm in the middle (Parke, 1991) and air-dried. The scalpel used to cut the roots was flamed between cuts to avoid transfer of inoculums from one segment to another. Roots from each segment were weighted and transferred to a 20 ml vial containing 1 ml sterilized distilled water and the content of the vial was stirred vigorously to dislodge the propagules of the antagonists from the roots. Determination of the cfu (g/dry wt) of the antagonists was carried out by plating 1 ml aliquot of the dilution onto TME and the plates were incubated for 7 days.

3.11 Statistical Analysis

There were 2 treatments (*T. viride* and control) with 4 replications. All data were analysed using Statistical Analysis System (SAS) Version 9.1. The treatment means were statistically compared by Independent T-test at a specified probability level of 5% or 0.05.

CHAPTER 4

RESULTS AND DISCUSSION

The ability of *Trichoderma* to recognize and parasitize phytopathogenic fungi in the rhizosphere (Benítez *et al.*, 2004) has been ascribed to several complex mechanisms, such as nutrient competition, antibiosis, mycoparasitism, induction of systemic resistance, and increased plant-nutrient availability (Naseby *et al.*, 2000). Poldma *et al.*, (2000) reported that the application of *Trichoderma* has not only an antagonistic effect on plant pathogens but also positive effect on plant growth and yield in some vegetable crops.

Bio-effectors based on plant-growth-promoting soil microorganisms are increasingly distributed on the world market. Mobilization of sparingly available plant mineral nutrients, stimulation of root growth, enhanced resistance to environmental stress factors and direct or indirect suppression of plant pathogens and induced resistance are discussed as possible mechanisms for the effectiveness of these products (Akter *et al.*, 2007).

4.1 In-vitro screening of *Trichoderma viride*

Trichoderma is a filamentous fungus that is widely distributed in the soil, plant material, decaying vegetation, and wood. The genus *Trichoderma* has five species; *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma pseudokoningii*, and *Trichoderma viride*. Morphological features of the conidia and phialides help in differentiation of these species from each other.

Trichoderma viride usually recognized by fast-growing colonies (Table 1) producing white, green, or yellow cushions of sporulating filaments (Table 2). The fertile filaments or conidiophores produce side branches bearing whorls of short phialides. Figure 1 showed the mycelial growth stages of *Trichoderma viride* on potato dextrose agar (PDA).

Colonies of *Trichoderma* grow rapidly and mature in 5 days. At 25°C and on potato dextrose agar (PDA), the colonies are woolly and become compact in time. From the front, the colour is white. As the conidia are formed, scattered blue-green or yellow-green patches become visible. These patches may sometimes form concentric rings. Reverse is pale, tan, or yellowish. The colour of the conidia is mostly green (de Hoog *et al.*, 2000).

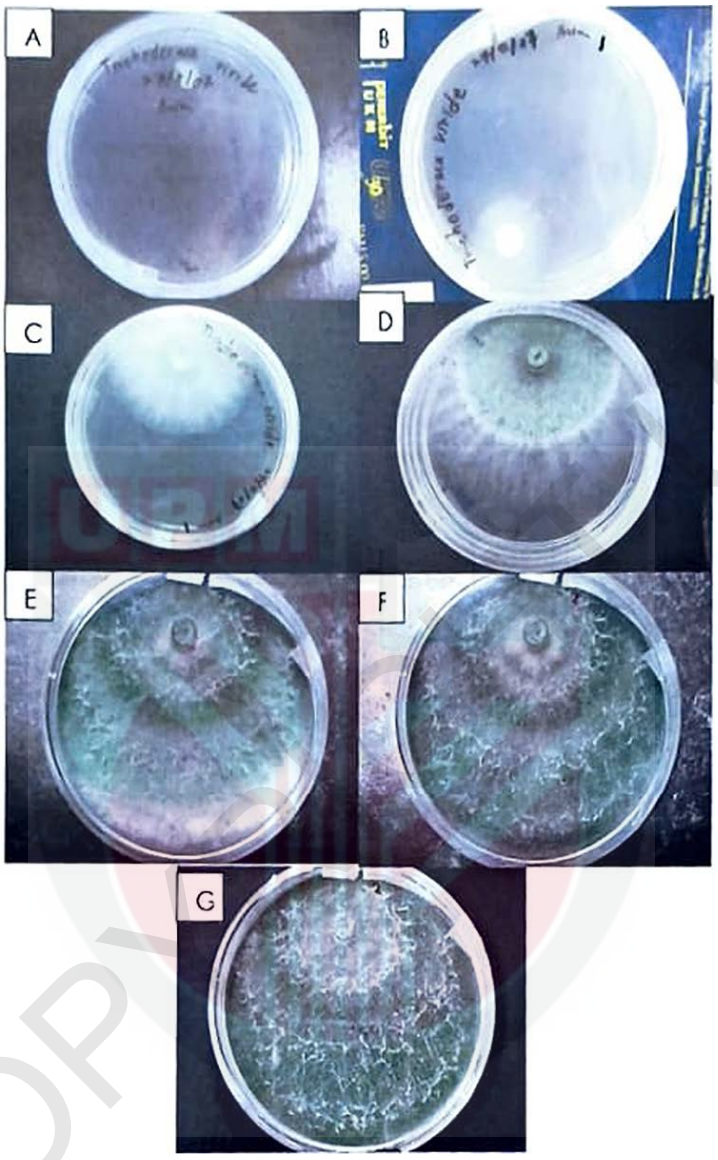

Figure 1: Mycelial growth stages of *Trichoderma viride* at day one (1) until day seven (7). Day 1 (A), Day 2 (B), Day 3 (C), Day 4 (D), Day 5 (E), Day 6 (F) and Day 7 (G). The white areas do not contain spores, while the green areas are covered with dense masses of spores (conidia).

Table 1: In vitro screening of *Trichoderma viride* mycelial growth for seven (7) days

Plate	Mycelium length (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	0.40	0.90	2.40	4.90	Petri dish fully covered	Petri dish fully covered	Petri dish fully covered
2	0.40	0.85	2.35	4.70	Petri dish fully covered	Petri dish fully covered	Petri dish fully covered
3	0.50	0.70	2.20	4.70	Petri dish fully covered	Petri dish fully covered	Petri dish fully covered
4	0.45	0.85	2.30	4.60	Petri dish fully covered	Petri dish fully covered	Petri dish fully covered
5	0.45	0.70	2.20	4.75	Petri dish fully covered	Petri dish fully covered	Petri dish fully covered
Average	0.40	0.80	2.30	4.70	-	-	-

Note:
Results were not statistically analysed.

Table 2: In-vitro screening of *Trichoderma viride* for colour of forming colony

Plate	Colour(s)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	-White	-White	-Green -White	-Green -Greenish white -White	-Green -Greenish white	-Greenish white -Green	-Greenish white -Green
2	-White	-White	-Green -White	-Green -Greenish white -White	-Green -Greenish white	-Greenish white -Green	-Greenish white -Green
3	-White	-White	-Green -White	-Green -Greenish white -White	-Green -Greenish white	-Greenish white -Green	-Greenish white -Green
4	-White	-White	-Green -White	-Green -Greenish white -White	-Green -Greenish white	-Greenish white -Green	-Greenish white -Green
5	-White	-Whitish green -White	-Green -White	-Green -Greenish white -White	-Green -Greenish white	-Greenish white -Green	-Greenish white -Green

4.2 Initial *Trichoderma* Population in Growing Medium

Growing medium for tomato (coco peat) recorded 4.5 x 10^6 cfu/g initial CFU count for *Trichoderma*. Inert materials generally contain low counts of microbial populations. *Trichoderma* spp. populations detected by plating of serial dilutions of aqueous suspensions of the substrate on TME confirmed that the medium contained low *Trichoderma* spp. populations.

Microbial population detected by plating were mainly saprophytic fungi such as *Aspergillus* and *Penicillium*. Thus, opportunity exist to introduce beneficial microorganisms such as *Trichoderma* into coco peat, which could limit the spread of diseases that might be introduced from outside.

4.3 Seed Germination Test

Incorporation of air dried *Trichoderma viride* inoculants into coco peat have shown to increased seed germination and seedling emergence of tomato (Table 3).

Percentage of emergence was higher in *T. viride*-treated medium compared to non-treated medium. After 7 days, more than 90% of the seed emerged in *T. viride*-treated medium compared to control, which gave only 80%.

Table 3: Effect of *T. viride* inoculation on percentage of seedling emergence, 7 days after sowing

Treatments	Control	*T.viride*
Percentage of Seedling Emergence	80	90

The results obtained above concurred to a report by Chet (1988) on the effect of *Tricoderma* inoculation on cucumber seeds. The seeds germinated two days earlier than those that have not been inoculated with the fungus. Lynch *et al.*, (1991) investigated the effect of *Trichoderma* on the growth of lettuce, and its ability to control damping off diseases caused by *Rhizoctonia solani* and *Pythium ultimum*. They investigated whether a number of *Trichoderma* strains had a direct effect on lettuce establishment and growth in the absence of pathogens. It was found that the fungal treatments reduced the emergence time of seedlings compared to the controls. From their results, and those of Ousley *et al.*, (1994), they concluded that specific *Trichoderma* strains have the potential to consistently increase plant growth (Lynch *et al.*, 1991).

4.4 Physiology Response

4.4.1 Chlorophyll Content

Chlorophylls help to convert radiant energy from the sun into chemical free energy that can be stored in various ways. SPAD reading were taken for 10 weeks experimental period after transplanting (Figure 2, Appendix 1). The chlorophyll content did show a significant difference between treatment and control except for week 1, 5, and 6. Treatment with *Trichoderma viride* at week 8 gave the highest level of chlorophyll content with the value of 56.43.

Value in week 1 was not significant between treatment and control probably due to the fact that *T. viride* inoculants were still in its early stages of developing in the plant root. At the beginning of week 5, the plants produced a total of three trusses; which resulted in the main growing stem being terminated at the point of two leaves after the final truss. This resulted in non significant difference between week 5 and 6 values which might due to the possibility that nutrient uptake and consumption in plants in both treatments were almost the same.

Results from this study showed that the presence of *T. viride* increased the chlorophyll content of tomato. *T. viride* inoculants could improve the physiological responses of tomato, therefore might have the potential role as plant growth promoting fungi besides its bioprotectant effect against soil-borne diseases.

This is according to a report by Cuevas (2002) which stated that *Trichoderma* has been demonstrated in the field to solubilize unavailable phosphorus and zinc in the soil. The organism also decomposes soil organic matter making nutrients such as

calcium, potassium and nitrogen available for plant use thus allowing plants to received balanced fertilization.

Nitrogen is needed by crops to synthesize chlorophyll, green pigments in leaves and other biomolecules important to plants' growth and development. Potassium is essential for osmotic balance of the cell and in grain formation. Calcium makes the crops sturdy and strong since this element is directly incorporated in plant cell wall. The crops are able to withstand stresses brought about by natural weather phenomena such as heavy rains and winds. Phosphorus, on the other hand, is needed in fast vegetative growth of the crops and in energy relations of the plants. Zinc is important in pollination and fruit formation and in proper functioning of the enzyme system (Cuevas, 2002).

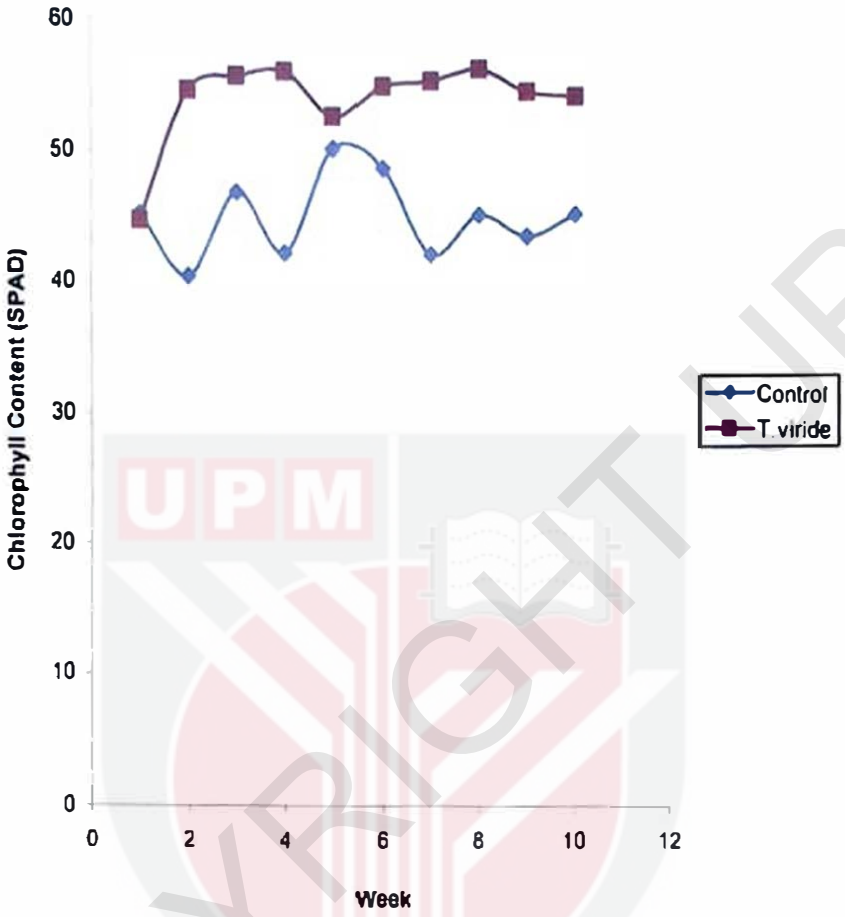

Figure 2: Effect of *T. viride* inoculants on chlorophyll content of tomato leaf

4.5 Growth Responses

4.5.1 Plant Height, Number of Leaves, Fresh and Dry Weight of Leaves

Trichoderma viride incorporated into growing medium generally did not have a significant effect on plant height, number of leaves, fresh and dry weight of leaves. Results from the experiments showed that although the plant height, number of leaves and fresh and dry weight of leaves were higher in plants treated with *T. viride* but most values were not significantly different as compared to control (Figure 3A-3D, Appendices 2A-2D).

Plants of *T. viride* amended medium developed better than control plants, as evidenced by the difference in plant height with treatment in week 10 gave the highest value of 140.25 compared to control which recorded the value of 131.50. *Trichoderma* strains that produce cytokinin-like molecules, e.g. zeatyn and gibberellin GA3 or GA3-related have been recently detected. The controlled production of these compounds could improve biofertilization (Osiewacz, 2002).

These statements were supported by Harman *et al.*, (2004) in their report which stated that together with the synthesis or stimulation of phytohormone production, most *Trichoderma* strains acidify their surrounding environment by secreting organic acids, such as gluconic, citric or fumaric acid. They also concluded that these organic acids result from the metabolism of other carbon sources, mainly glucose, and, in turn, are able to solubilize phosphates, micronutrients and mineral cations including iron, manganese and magnesium.

These reports were also concurred with the conclusion made by Altomare *et al.*, (1999) which suggested that insoluble calcium phosphate can be dissolved and made available to plants by soil and rhizosphere microorganisms via a mechanism that is thought to involve the release of organic acids.

Meanwhile, treatment with *T. viride* at week 10 recorded the highest amount of leaves produced with the value of 179.50 compared to control which gave the value of 159.25. However, leaf production of plants in both treatments from week 6 onwards were observed as having almost non significant increase, due to the fact that pinching of the main stem was executed in week 5.

It has been proved that *Trichoderma* produces substance that stimulates plant growth and development. These substances act as a catalysts or accelerators in the primary meristem tissues in young part of plants, accelerating cell reproduction, so that the plant achieve faster growth than those which have not been treated with this microorganism (Paez, 2006). Benitez *et al.*, (2007) reported that *Trichoderma* promoted tobacco plant growth by increasing the foliar area, the number of leaves and secondary roots, and plant fresh weight. They also did the experiment with tomato plants which yielded effects on growth promotion similar to those detected in tobacco plants. Furthermore, compared to tobacco plants, tomato plant sizes were more homogenous and the root system grew more profusely.

Values for leaf fresh weight at week 10 for *T. viride* and control were 292.88 and 262.48 respectively. Meanwhile for leaf dry weight at week 10 for *T. viride* and control was 51.87 and 49.83 respectively.

The results obtained from the experiment we conducted were consistent with the findings of Benitez *et al.*, (2007) that tested the effect of *Trichoderma* application on tobacco and tomato plants. They reported that *Trichoderma* promoted the growth of tobacco and tomato plants, increasing the foliar area and secondary roots, among other parameters. The increased of foliar area suggested that it could be consistent with the increase of fresh and dry weight of leaves and subsequently resulted in the promotion of plant growth itself. The result obtained from this experiment also concurred with a report by Harman *et al.*, (2002) that produced some conclusions from their experiment that crop productivity in fields can increase up to 300% after the addition of *Trichoderma*. Furthermore, they reported that experiments carried out in greenhouses yielded considerable increase in fresh weight of leaves when the plants were inoculated with *Trichoderma* strains.

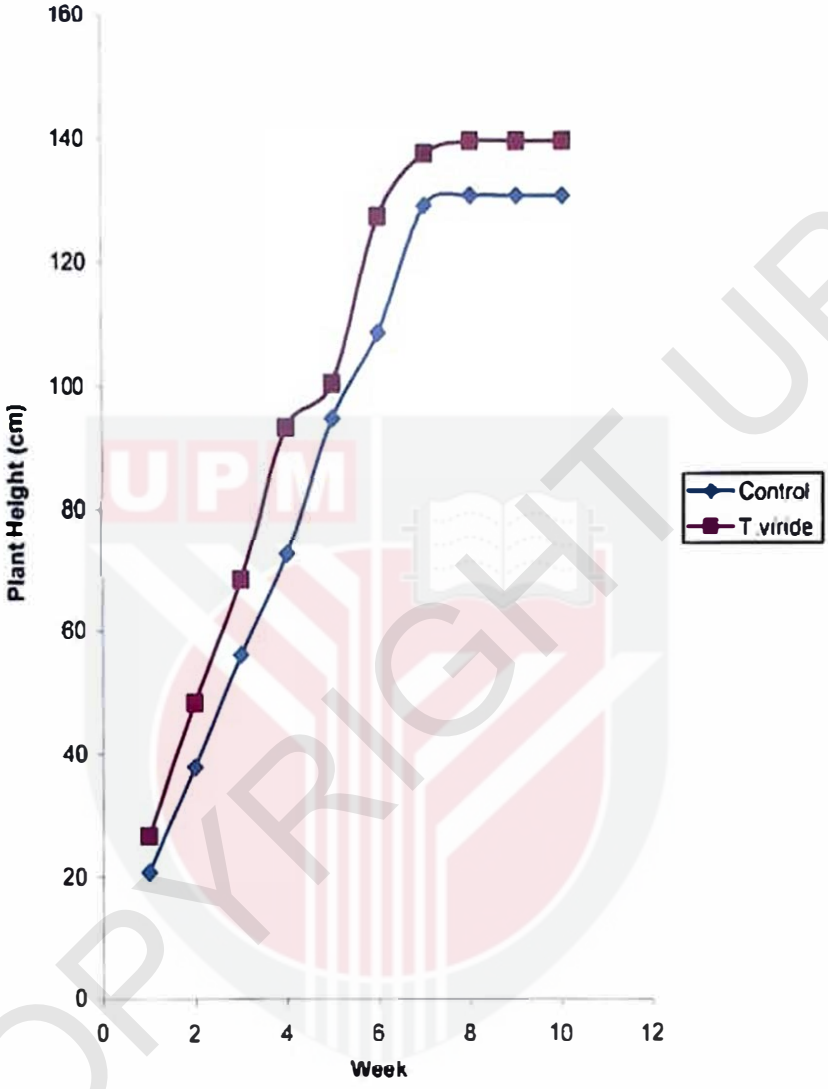

Figure 3A: Effect of *T. viride* inoculants on plant height of tomato

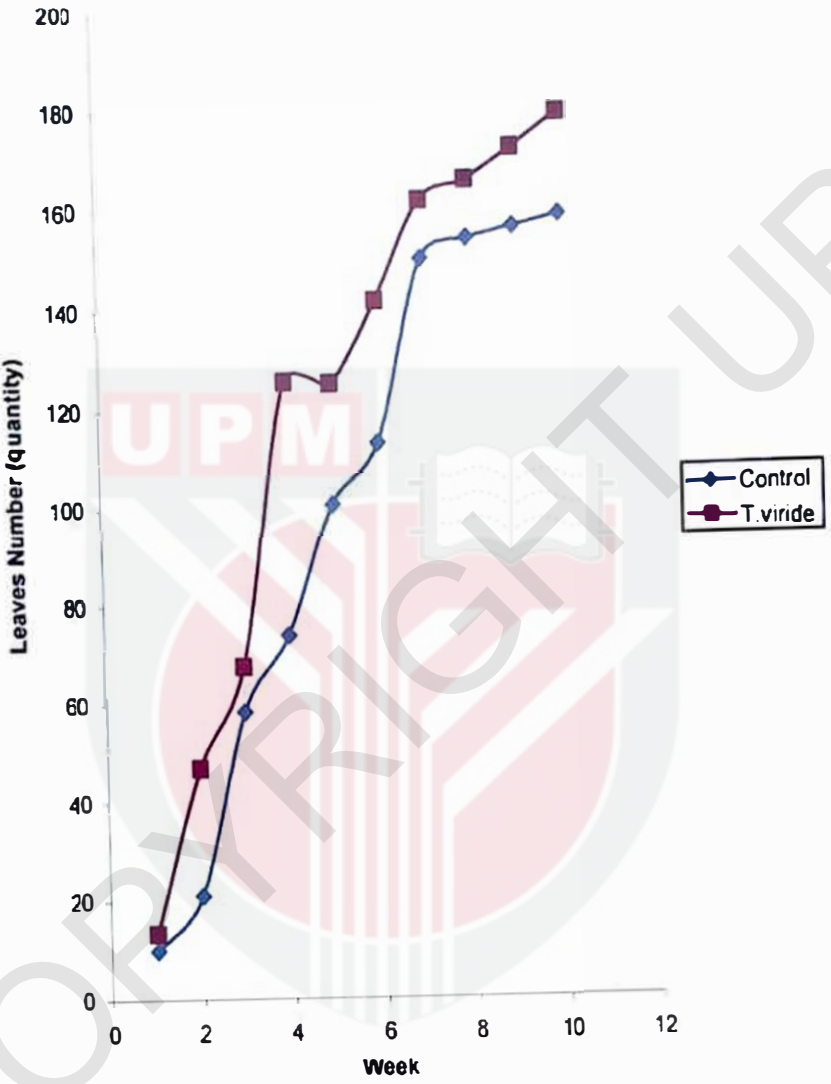

Figure 3B: Effect of *T. viride* inoculants on number of tomato leaves

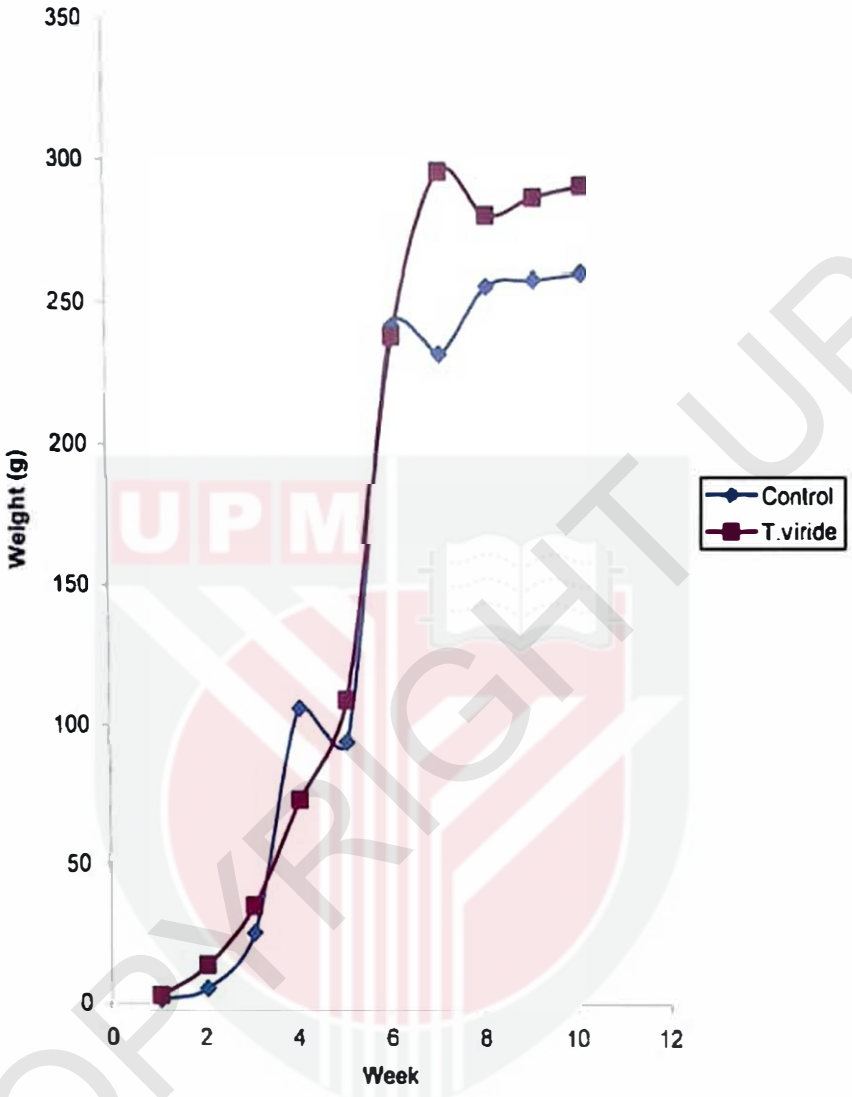

Figure 3C: Effect of *T. viride* inoculants on tomato leaf fresh weight

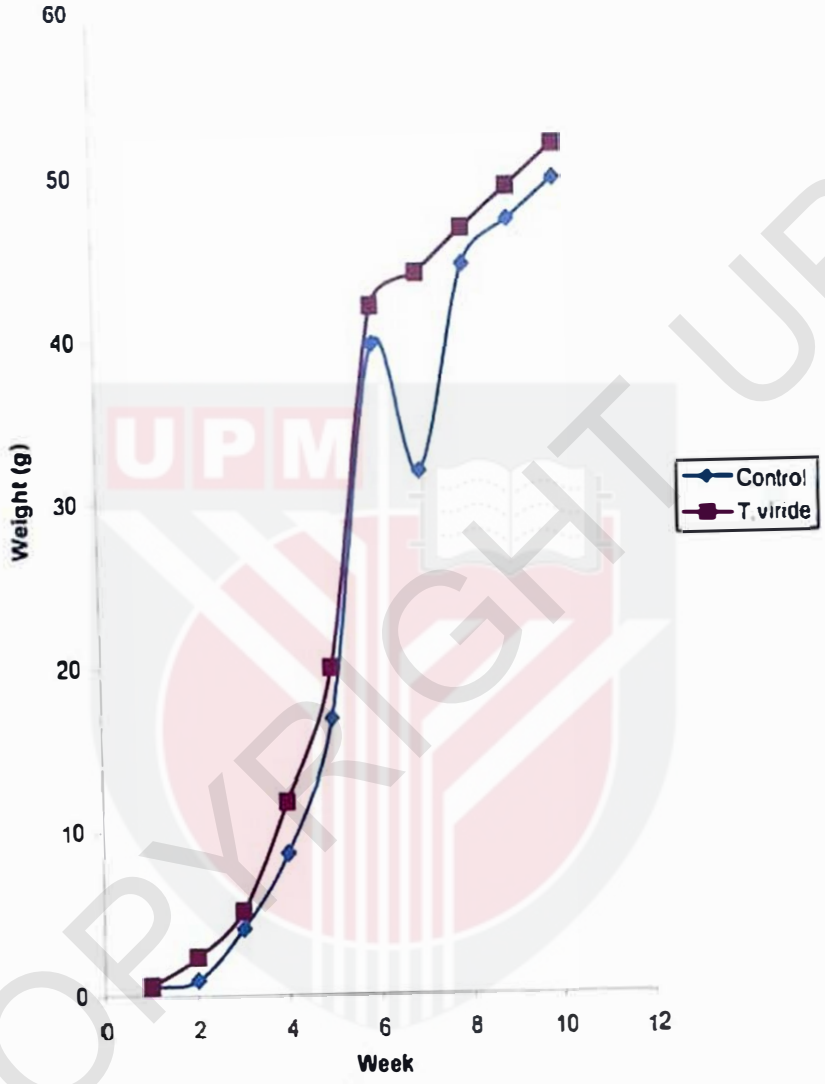

Figure 3D: Effect of *T. viride* inoculants on tomato leaf dry weight

4.5.4 Root Length, Fresh and Dry Weight of Root

Root length was not much affected by *Trichoderma viride* inoculants, probably due to limited size of the cultivation polybags (Figure 5A, Appendix 3A). However, plants grown in *T. viride*-treated medium generally gave a higher fresh and dry root mass (Figure 5B-5C, Appendices 3B-3C). Plants grown in *T. viride* amended coco peat showed a better performance during the early establishment of seedlings before being transferred into cultivation pot.

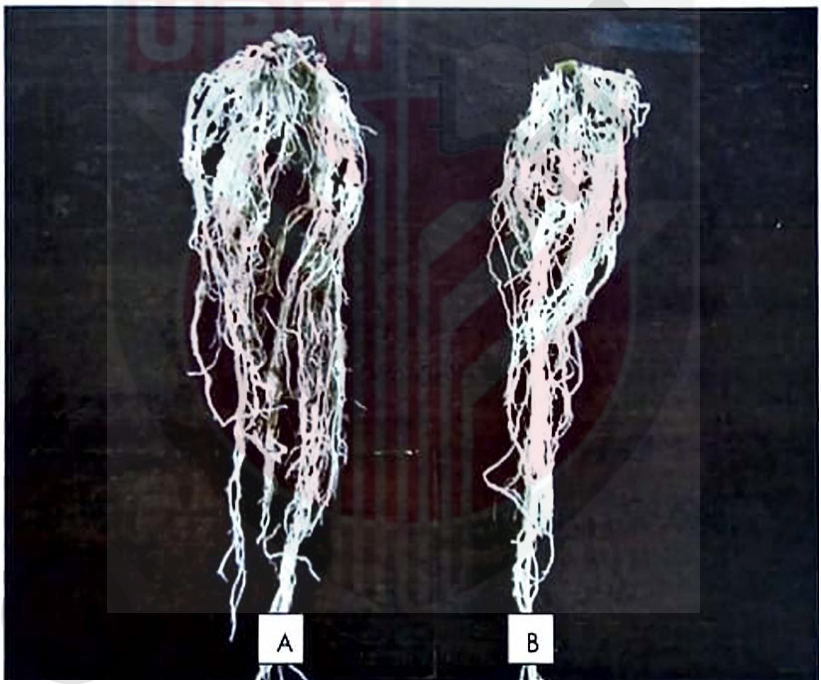

Figure 4: Effects of *T. viride* inoculants on tomato root development. *T. viride* amended medium (A) and control (B)

T. viride gave the highest value in root length at week 10 and there was significant difference in root length compared to control. However, during early growth there was no significant difference in root length between *T. viride* and control. Root of tomato plants grown in *T. viride* amended coco peat was generally better than non-amended coco peat, although the difference was not significant.

This shows that a major feature of *Trichoderma* is its capability to grow along roots during their elongation, thus colonizing the whole root system and benefiting the crop for its entire life. Colonization implies the ability to adhere and recognize plant roots, penetrate the plant, and withstand toxic metabolites produced by the plants in response to invasion by a foreign organism, whether pathogen or not (Franken, 2001).

Kleifield and Chet (1992) reported that the increased growth response of plants caused by *Trichoderma* depends on the ability of the fungus to survive and develop in the rhizosphere. They also suggested that the possible mechanism for increased plant growth is an increase in nutrient transfer from soil to root, which is supported by the fact that *Trichoderma* can colonize the interior of roots. In an experiment conducted by Kershaw and Talbot (1998) revealed, genes in *Trichoderma* strains that encode hydrophobins and other cell-wall structural proteins are specifically expressed, or their expression is up-regulated when applied in soil.

Hydrophobins and repellents are small, functionally similar hydrophobic proteins, that play fundamental roles in fungal morphogenesis, including infection structures, hyphal aggregation, cell to cell communication, and attachment of hyphae

to hydrophobic surfaces and adhesion. They also concluded that *Trichoderma* stimulates growth of at least tobacco, tomato and cotton plants, and also protects them against several fungal plant pathogens. Results that were obtained from the experiments that we conducted also concurred with a report made by Harman *et al.*, (1998) which indicated that some strains of *Trichoderma* are known to increase the numbers of even deep roots (at as much as a meter below the soil surface) in plants such as corn and ornamental plants.

Values for root fresh weight at week 10 for *T. viride* and control were 113.98 and 79.13 respectively. Meanwhile for leaf dry weight at week 10 for *T. viride* and control was 27.75 and 19.21 respectively. Enhancement of root growth improve nutrient uptake and the increase in physiological responses could be attributed to increase in water uptake as a consequence of increased root growth.

A recent review lists 11 separate reports demonstrating control by *Trichoderma* spp. of a wide range of plant pathogens, including fungi, oomycetes, bacteria, and one virus, by elicitation of induced systemic or localized resistance (Harman, 2006). The fungi, especially rhizosphere competent ones, colonize root surfaces and penetrate the epidermis and into the cortex (Chet *et al.*, 2007). Along the way, the fungi may coil about root hairs in a manner reminiscent of mycoparasitism (Chet *et al.*, 2007). Once *Trichoderma* hyphae penetrate the roots, a series of bioactive metabolites from the fungus is produced that induces walling off and biochemical mechanisms that limit growth of the *Trichoderma* to a small area.

This also concurred with a previous report made by Altomare *et al.*, (1999) which stated that nutrient in soil could be solubilized and stored in *Trichoderma* biomass to be released in a readily available form in close proximity to the roots after lysis of the mycelium with age.

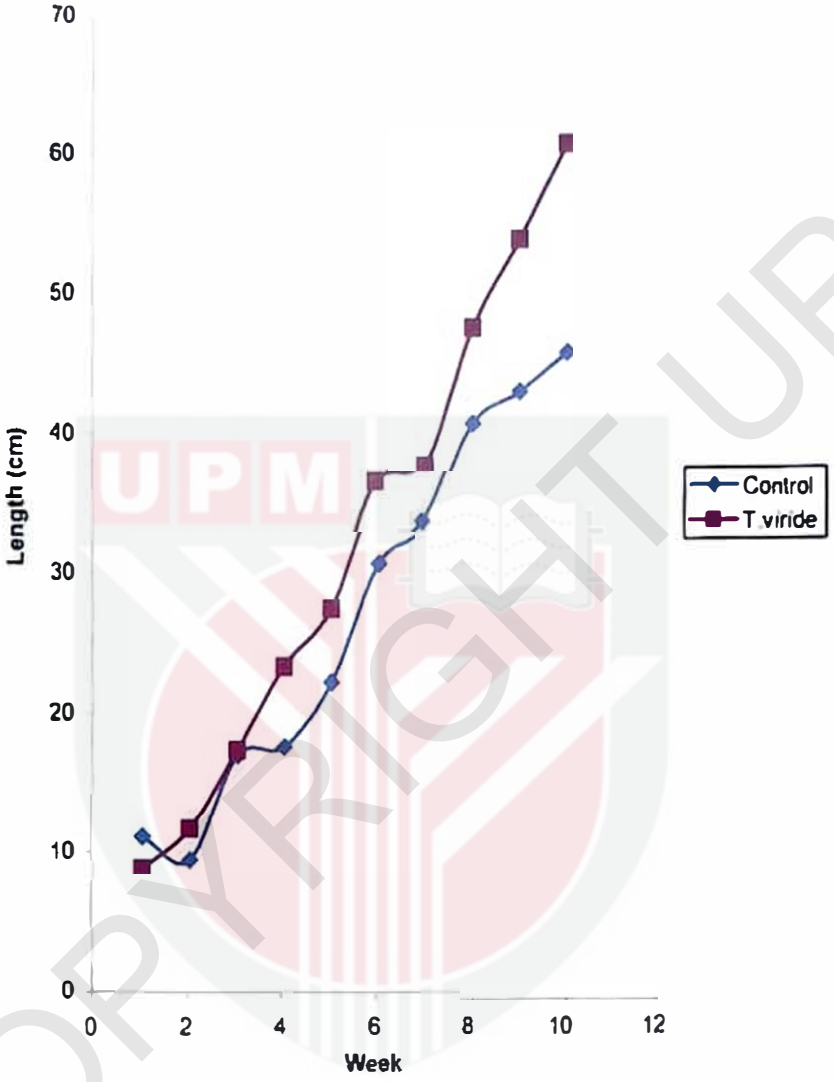

Figure 5A: Effect of *T. viride* inoculants on tomato root development

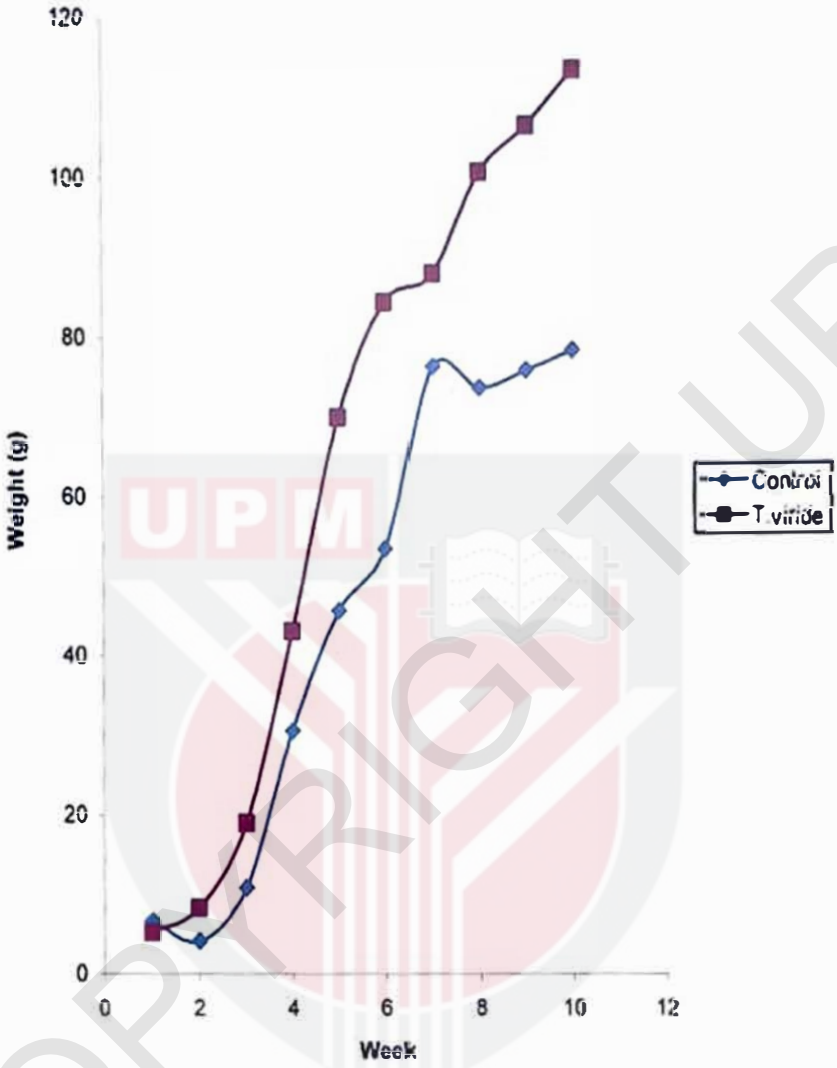

Figure 5B: Effect of *T. viride* inoculants on tomato root fresh weight

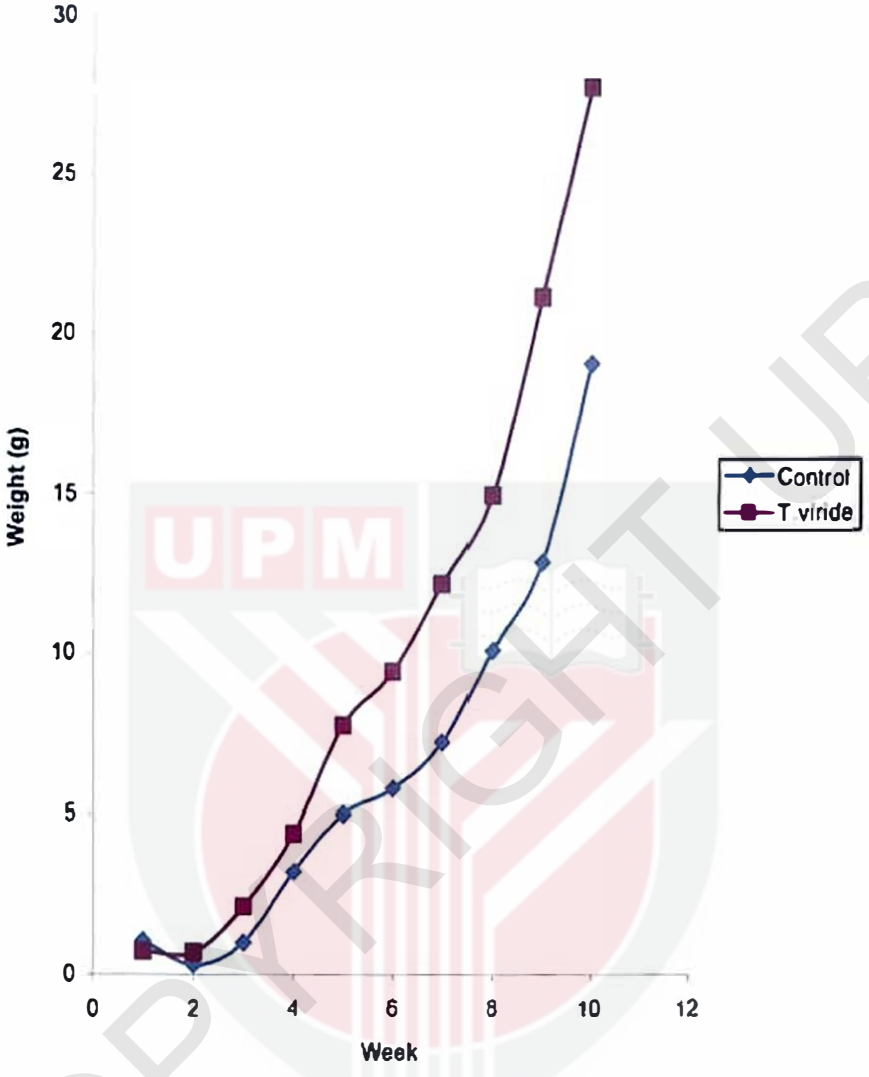

Figure 5C: Effect of *T. viride* inoculants on tomato root dry weight

4.5.5 Yield

The time of flowering, number of flowers and number of fruits were affected by the presence *Trichoderma viride*. Plants grown in coco peat amended with *T. viride* inoculants took 5 weeks for fruit setup meanwhile control treatment took 6 weeks. Tomato plants grown in *T. viride* amended coco peat showed difference in fruit production as compared to control (Table 4).

Table 4: Effect of *T. viride* inoculants on fruit setup, number of fruits and fruit average fresh weight

Treatment	Week of Fruiting (Fruit Setup)	Average Number of Fruits	Average Fresh Weight (g)
T. viride	5	16.35	283.6
Control	6	9.03	108.53

The average number of fruits produced at week 10 was 16.35 and 9.03 *T. viride* and control respectively. Tomato plants grown in *T. viride* amended coco peat also showed difference in total fresh weight of fruits produced as compared to control. Values for total fresh weight at week 10 for *T. viride* and control were 283.60 and 108.53 respectively.

Poldma *et al.*, (2000) reported that the application of *Trichoderma* has not only an antagonistic effect on plant pathogens but also positive effect on plant growth and yield in some vegetable crops. The increase growth response is mainly due to increased solubilisation of uptake of minor and other minerals as well as

improvement in the root morphology enabling the roots to exploit a larger volume of soil (Yedidia *et al.*, 2001). It is possible that an increase growth response may subsequently result in enhanced yield, especially in those plants grown for vegetative parts.

A possible mechanism for increased plant growth is an increase in nutrient transfer from soil to root, which is supported by the fact that *Trichoderma* can colonize the interior of roots (Kleifield and Chet, 1992). In a few cases, this effect has been suggested to involve solubilization of otherwise unavailable mineral nutrients. In soil, both macro- and micronutrients undergo a complex dynamic equilibrium of solubilization and insolubilization that is greatly influenced by the soil pH and microflora and that ultimately affects their accessibility to plant roots for absorption (Goldstein, 1995).

4.6 Proliferation and Survival of *Trichoderma*

4.6.1 CFU of Medium (Coco Peat) and Roots

Proliferation and survival of *Trichoderma* spp. in *Trichoderma viride* amended coco peat decreased significantly with time (Figure 6A, Appendix 4A). The *Trichoderma* population was at the lowest at week 10 with recovery of 44 x 10^6 and 3.5 x 10^6 cfu/g dry weight of coco peat for *T. viride* and control respectively.

Although *Trichoderma* population in coco peat fluctuates with time, population was maintained in the magnitude of 10^6 throughout the period of experiment. *Trichoderma* population in soil mixture at the magnitude of 10^6 was reported to have a beneficial effect on plant growth and disease suppression (Papavizas, 1985). This observation was supported by the fact that *Trichoderma* strains must colonize plant root prior to stimulation of plant growth and protection against infections (Benitez, 2004).

Although the population level of *T. viride* was maintained in the magnitude of 10^6, however they failed to exhibit a continuous significant effect on growth parameters of tomato. This may due to higher population of indigenous microbes in coco peat, which might be antagonistic to the effect of the introduced *T. viride* on plant growth.

The rhizosphere competency of *Trichoderma* was determined by plating serial dilutions of tomato roots. The *Trichoderma* populations of the roots showed a fluctuating pattern (Figure 6B, Appendix 4B). Total population of *Trichoderma* spp. recovered from the root at week 10 was 190.5 x 10^6 and 6.5 x 10^6 cfu/g dry weight of

root for *T. viride* and control respectively. *T. viride* population recovered on TME was significantly different throughout the experimental period. Generally, the population of *Trichoderma* spp. was lower during the initial stage of plant growth and gradually increased in time. At week 6 populations was at the highest giving cfu/g of dry weight roots; 300.75 x 10^6 and 9 x 10^6 cfu/g dry weight roots for *T. viride* and control respectively.

This result supported the earlier observation that *Trichoderma* spp. survived and proliferate in coco peat and colonized roots as the source of organic matter in coco peat was depleted. A similar observation was reported by Jensen (1997) when the organic materials in the cultivation slab was only able to support microbial activity for limited time, after which the organic substrate needed by antagonists would have originated mainly from the root exudates.

This experiment also suggests that *T. viride* could persist as a potential bioprotectant in coco peat. According to Harman (1996), a highly effective biocontrol agent should a) be able to compete and persist in the environment in which it must operate, and b) be able to colonize and proliferate on existing and newly formed plant parts at times well after application. Bjorkman (1999) reported that *Trichoderma* has shown little host specificity, colonizing most plants and has been tested on potatoes, radish, cucumber, potted flower and greenhouse tomatoes. Yedidia *et al.*, (1999) concluded that *Trichoderma* inoculated plants may be sensitized to respond faster and to greater extent to potential pathogen attacks.

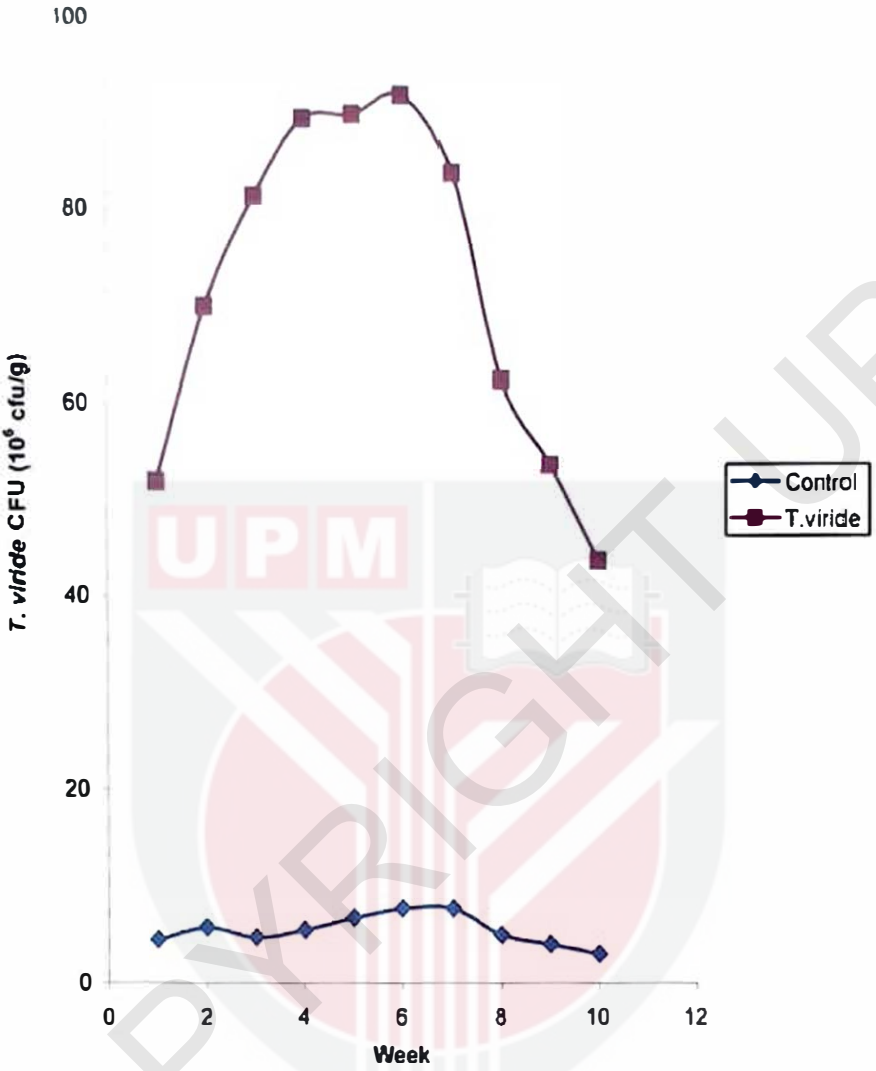

Figure 6A: Assessment on population of *T. viride* in inoculated medium (coco peat)

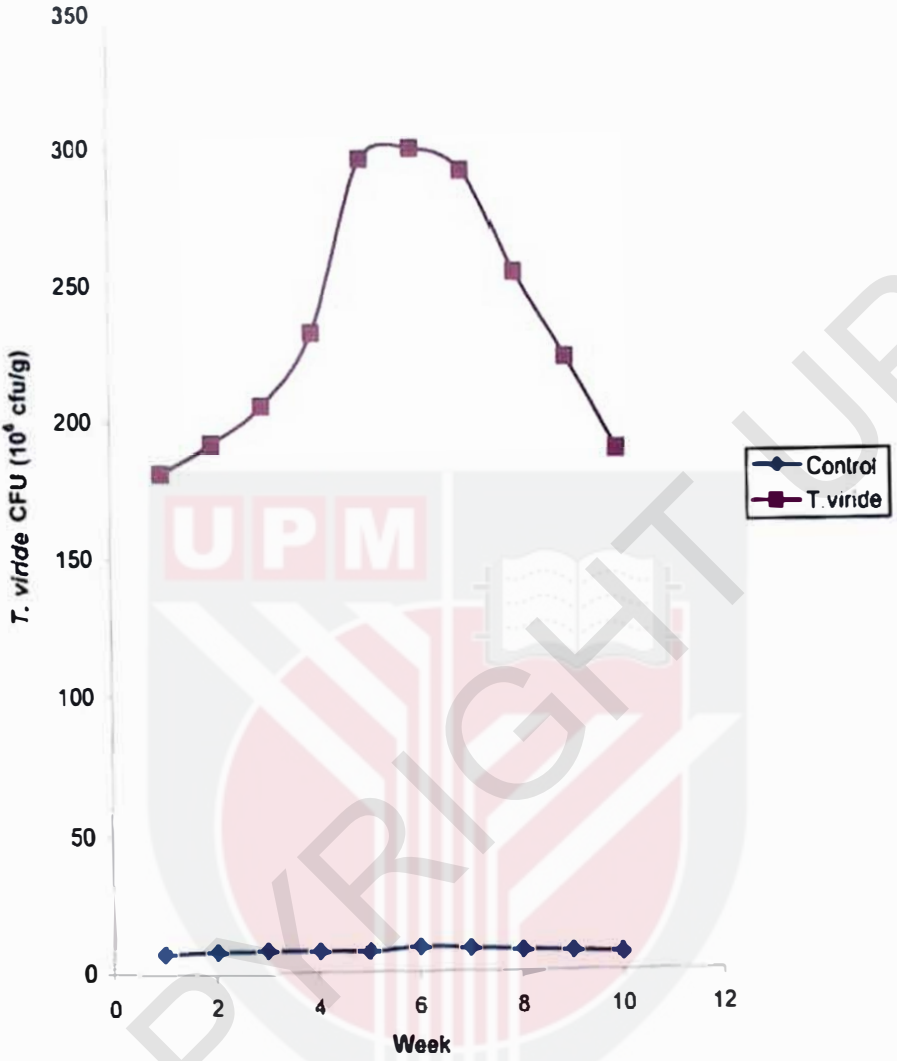

Figure 6B: Assessment on population of *T. viride* on root of tomato

CHAPTER 5

CONCLUSION

The problems of agriculture waste disposal such as burning in the open could be solved as it could be used as cultivation materials and furthermore it is environmentally-friendly. Utilization of substrates could be improved by incorporation of beneficial microbes such as *Trichoderma* for growth enhancement and disease suppression. Mass production of *Trichoderma* inoculums has become a focus research and industrial development in the search for alternatives to chemical treatments to control soil-borne pathogens and plant growth enhancer.

A laboratory experiment was carried out to evaluate the proliferation and survival of *T. viride* inoculants in coco peat. The cfu counts decreased with time in the substrate, but gradually increased on the growing roots, suggesting that *T. viride* was moving towards the actively growing roots.

T. viride inoculants were then incorporated into coco peat for the production of tomato under rain-proof structure. Air-dried preparations of *T. viride* inoculants were added into coco peat and transferred to germination pots. Individual tomato seed was sown in each pot. *T. viride* increased seed germination and seedling emergence.

Incorporation of *T. viride* also gave significant increased on chlorophyll content of tomato leaf. However, it did not have a significant effect on plant height, number of leaves, fresh and dry weight of leaves. The non significant result might be due to the influence of plant spacing on light interception of tomato plants. The

presence of indigenous *Trichoderma* spp. in the soilless medium, could also give a confounding effect on the beneficial effects of the introduced *T. viride* inoculants in coco peat especially at the later stage of assessment. However, *T. viride* significantly increased the root length and root mass of tomato suggesting that *T. viride* may serve as plant growth promoting organism.

Tomato plants grown in *T. viride* amended coco peat showed higher fruit production and total fresh weight of fruit as compared to control. This indicates the enhancements of plant growth by *T. viride*. *T. viride* improved mineral uptake, nutrient release from soil and organic matter through better root development and enhanced plant hormone production which stimulated the growth responses of plant. *T. viride* by preventing the root from certain physical stresses, root damage and soil-borne pathogen infection, an expanded root system ensure additional nutrients and moisture will be able to find their way up to the leaves, flowers and fruits resulting in an improved crop health.

The recovery rate of *T. viride* in substrate and on roots until week 10 indicates that it could proliferate and survive in coco peat and live in and around tomato roots. These also suggest that *T. viride* was able to compete and persists in the environment in which it operated and able to colonize and proliferate on the plant parts at times well after application.

As a recommendation, evaluation on other crop to see the effect of *Trichoderma* as biocontrol agent and plant growth enhancer should be further explored. Application methods should also be verified to ensure that the *Trichoderma* inoculants would grow well and able to achieve their purpose, such as improving the

quality of compost prepared from agricultural wastes such as sago waste. *Trichoderma* spp. is a lignocellulolytic fungus that can enhance biodegradation of agro-wastes providing suppressive effect against soil-borne plant pathogen.

Overall, *T. viride* in terms of plant growth enhancement resulted in an increase of crop and establishment, harvestability and crop yield. The recovered *T. viride* has a fluctuating pattern compared to initial population, resulting in failure to exhibit a continuous significant effect on growth parameters of tomato, suggesting that *T. viride* was not a strong root colonizer or due to increased in root mass. High spore viability is also an important aspect on the performance of the inoculants as a plant protectant and as a growth promoter.

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APPENDICES

Appendix 1: Effect of *T. viride* inoculants on chlorophyll content of tomato leaf

Treatment	Means (SPAD reading)									
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Control	45.28^{a}	40.58^{b}	47.05^{b}	42.45^{b}	50.45^{a}	48.93^{a}	42.38^{b}	45.40^{b}	43.78^{b}	45.40^{b}
T. viride	44.80^{a}	54.78^{a}	55.93^{a}	56.25^{a}	52.90^{a}	55.18^{a}	55.56^{a}	56.43^{a}	54.70^{a}	54.28^{a}

Means within column with the same letter are not significantly different by Independent T-test at $p \leq 0.05$

Appendix 2A: Effect of *T. viride* inoculants on plant height of tomato

Treatment	Means (cm)									
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Control	20.75^{b}	37.88^{b}	56.25^{b}	72.75^{b}	95.25^{a}	109.25^{a}	129.75^{a}	131.50^{a}	131.50^{a}	131.50^{a}
T. viride	26.63^{a}	48.38^{a}	68.50^{a}	93.75^{a}	101.00^{a}	128.00^{a}	138.25^{a}	140.25^{a}	140.25^{a}	140.25^{a}

Means within column with the same letter are not significantly different by Independent T-test at $p \leq 0.05$

Appendix 2B: Effect of *T. viride* inoculants on number of tomato leaves

Treatment	Means (quantity)									
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Control	10.00[a]	21.25[b]	58.75[a]	74.25[b]	101.25[a]	113.75[b]	150.75[a]	154.75[a]	157.00[a]	159.25[a]
T. viride	13.50[a]	47.25[a]	68.00[a]	126.25[a]	126.00[a]	142.50[a]	162.50[a]	166.50[a]	172.75[a]	179.50[a]

Means within column with the same letter are not significantly different by Independent T-test at $p \leq 0.05$

Appendix 2C: Effect of *T. viride* inoculants on tomato leaf fresh weight

Treatment	Means (g)									
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Control	4.28[a]	8.11[b]	28.22[a]	75.83[b]	96.43[a]	224.16[a]	234.20[b]	258.11[a]	260.37[a]	262.48[a]
T. viride	5.74[a]	16.67[a]	37.85[a]	108.43[a]	111.51[a]	240.43[a]	297.60[a]	282.55[a]	288.84[a]	292.88[a]

Means within column with the same letter are not significantly different by Independent T-test at $p \leq 0.05$

Appendix 2D: Effect of *T. viride* inoculants on tomato leaf dry weight

Treatment	Means (g)									
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Control	0.53[a]	0.90[b]	4.08[a]	8.72[b]	16.90[a]	39.85[a]	32.07[b]	44.68[a]	47.35[a]	49.83[a]
T. viride	0.60[a]	2.36[a]	5.18[a]	11.88[a]	20.03[a]	42.16[a]	44.37[a]	46.85[a]	49.34[a]	51.87[a]

Means within column with the same letter are not significantly different by Independent T-test at $p \leq 0.05$

Appendix 3A: Effect of *T. viride* inoculants on tomato root development

Treatment	Means (cm)									
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Control	11.58[a]	9.88[a]	17.50[a]	18.13[b]	22.75[b]	31.25[a]	34.00[a]	41.25[a]	43.50[b]	46.25[b]
T. viride	9.25[a]	12.13[a]	17.88[a]	23.88[a]	28.00[a]	36.88[a]	38.25[a]	48.00[a]	54.25[a]	61.00[a]

Means within column with the same letter are not significantly different by Independent T-test at $p \leq 0.05$

Appendix 3B: Effect of *T. viride* inoculants on tomato root fresh weight

Treatment	Means (g)									
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Control	6.71[a]	4.28[b]	11.03[b]	30.92[b]	46.15[a]	53.97[a]	77.03[a]	74.39[a]	76.63[a]	79.13[a]
T. viride	5.30[a]	8.53[a]	19.22[a]	43.50[a]	70.53[a]	85.15[a]	88.74[a]	101.35[a]	107.11[a]	113.98[a]

Means within column with the same letter are not significantly different by Independent T-test at $p \leq 0.05$

Appendix 3C: Effect of *T. viride* inoculants on tomato root dry weight

Treatment	Means (g)									
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Control	1.07[a]	0.31[b]	1.01[b]	3.25[a]	5.05[a]	5.83[a]	7.30[a]	10.20[a]	13.00[b]	19.21[b]
T. viride	0.74[a]	0.70[a]	2.15[a]	4.44[a]	7.82[a]	9.53[a]	12.30[a]	15.10[a]	21.26[a]	27.75[a]

Means within column with the same letter are not significantly different by Independent T-test at $p \leq 0.05$

Appendix 4A: Assessment on population of *T. viride* in inoculated medium (coco peat)

Treatment	Means (10^6 cfu/g)									
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Control	4.50^b	5.75^b	4.75^b	5.50^b	6.75^b	7.75^b	7.75^b	5.00^b	4.00^b	3.50^a
T. viride	52.00^a	70.00^a	81.50^a	89.50^a	90.00^a	92.00^a	84.00^a	62.75^a	54.00^a	44.00^a

Means within column with the same letter are not significantly different by Independent T-test at $p \leq 0.05$

Appendix 4B: Assessment on population of *T. viride* on root of tomato

Treatment	Means (10^6 cfu/g)									
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Control	7.00^b	7.75^b	8.00^b	8.00^b	7.50^b	9.00^b	8.50^b	7.75^b	7.00^b	6.50^b
T. viride	181.00^a	192.00^a	206.00^a	232.75^a	296.75^a	300.75^a	272.50^a	225.50^a	204.25^a	190.50^a

Means within column with the same letter are not significantly different by Independent T-test at $p \leq 0.05$

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Growth and Yield Responses of Tomato (*Lycopersicon esculentum)* cultivated in *Trichoderma* Amended Medium" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus and published in the form approved by the Faculty.

MOHAMAD AZIM HAZNY ABD HAFIZ

Date : 25/04/2008