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THE INTERACTIVE EFFECTS OF THREE *TRICHODERMA* SPECIES AND DAMPING-OFF CAUSATIVE PATHOGEN *PYTHIUM APHANIDERMATUM* ON EMERGENCE INDICES, INFECTION INCIDENCE AND GROWTH PERFORMANCE OF SWEET PEPPER

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ABSTRACT

This study is designed to investigate the effectiveness of these three *Trichoderma* species in controlling damping off disease caused by *Pythium aphanidermatum* in sweet pepper seedlings and to evaluate the contribution of the three *Trichoderma* species on overall growth performance and emergence indices in sweet pepper seedlings. Sweet pepper seeds treated with 2.5 x 10^{6} conidia/ ml of *Trichoderma harzianum*, *Trichoderma atroviride* and *Trichoderma koningii* were sown to determine the emergence percentage at 7 Days after planting (DAP), 9 DAP and 14 DAP. Emergence Index and Emergence Rate Index were computed for each *Trichoderma* treatment. Data on plant growth parameters such as stem height, number of roots, root length, number of leaves, fresh weight, dry weight, seedling vigour assessment, and disease incidence percentage were measured on 30 day old seedlings inoculated with 5 X 10^{5} spores/ ml *Pythium aphanidermatum* at 15-day old sweet pepper seedlings. Seedlings of sweet pepper treated with both *Trichoderma atroviride* and *Trichoderma harzianum* had higher emergence percentage than the un-treated seedlings, which was not significantly lower in emergence percentage from *Trichoderma koningii* treated pepper seedlings. The presence of these *Trichoderma* species in *P. aphanidermatum* infected sweet pepper seedlings, reduced disease incidence significantly than when there was none. Higher seedling vigour assessments and improved growth rates were recorded on 30 day old seedlings when treated with *Trichoderma* species either with or without *P. aphanidermatum* were observed, especially in *T. atroviride* plus pathogen. *Trichoderma* species, when presence enhance the growth of the developing plant root system *viz-a viz* the plant growth and yield through better nutrient uptake, production of growth promoting compounds and solubilization of phosphates, micronutrients and mineral cations like iron, manganese and magnesium necessary for plant metabolism.

INTRODUCTION

Pepper (*Capsicum annum* L.) belongs to the family *Solanaceae* and genus *Capsicum*, and it is believed to be native of America and tropical Africa. The family consists of about 90 genera and about 3000 species (Vidyarth and Tripatha, 2002). About 20 *capsicum* species of pepper were found out to be distributed worldwide, out of which *Capsicum annum*, *Capsicum frutescens*, *Capsicum chinense*, *Capsicum pendulum* and *Capsicum pubescens* are the five main species cultivated (Saikat and Amit, 2003). *Capsicum* species is among the world's most popular vegetable crop and it is being used as spices and condiments. It is an important crop that is produced and consumed either fresh or processed in salads (Sonago, 2003). It is also consumed in fresh form or as paste, mixed or grounded with other vegetables in preparation of stew and soup. Sweet pepper is rich in mineral elements such as sodium, calcium, iron, phosphorus, potassium, zinc, copper, cobalt, manganese, sulphur and magnesium (Mehnet *et al.*, 2006) with good quantities of vitamins B and C, carotene and folic acid (USDA, 2008). Pepper is an important vegetable consumed in Nigeria and is mainly grown around the savannah zones as a mono or mixed crop. This is grown during the rainy season while in dry season it is usually grown under irrigation. According to Gruben and Tahir (2004), Food and Agriculture Organization (FAO, 2001) statistics world production of pepper was estimated to be 21.3 million tons from 1.6 million ha harvested area (i.e. an average yield of 13.4 t/ha) in 2001, with Nigeria being one of the major producers of pepper in the world accounting for about 50% of the African production and

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rated 5th in the world (Business day, 2007). The production of pepper in Sub- Sahara Africa is hampered by many external factors which are biotic and a-biotic in nature. Among the factors are salinity, low fertility, water stress, weeds, pest and diseases.

Disease infection is one of the major constraints to pepper production in sub-Saharan Africa (Nsabiyera *et al*., 2012). The major organisms causing diseases of most peppers in Sub-Sahara Africa are phyto-pathogenic fungi, bacteria, and viruses (Melanie *et al*., 2004). Attacks by fungal, bacterial or viral diseases, nematodes, mites and many insect pests cause significant losses in pepper production (Ochoa-Alejo and Ramirez-Malagon, 2001). There are several fungal diseases of pepper crops, which are caused by *Sclerotinia spp, Cercospora* spp, *Colletotrichum* spp, *Fusarium* spp, *Phytophthora* spp, *Pythium* spp e.t.c. Among these diseases affecting pepper are damping-off disease caused by *Pythium* species and *Rhizoctonia solani*, which are very common in nurseries and greenhouses. Damping-off disease by *Pythium* species causes more than 60 percent mortality of seedlings both in nursery and main field (Manoranjitham *et al*., 2000). *Pythium aphanidermatum* (Edson) Fitzp. is a soil borne plant pathogen in this genus, belonging to the order Peronosporale in the class Oomycetes. It is also known as water moulds (Sutton *et al*., 2006). It is responsible for pre- and post-emergence damping-off. Pre-emergence damping-off occurs when the seed is infected prior to germination, resulting in poor or no germination (Whipps and Lumsden, 1991). According to Salami (2002), this pathogen causes diseases such as root rot and damping-off of seedlings. This fungus may cause root rot of seedlings or kill the seedlings before they emerge (Roberts, 2003). It also attack the soft stems of young seedlings after emergence causing water soaking and shriveling of the stem at the ground level. Plant seedlings attacked will soon fall over and die. This is particularly common in cool, over-watered and moist soil and the organisms are essentially soil-borne. *Pythium* species tends to be very generalistic and unspecific in their host range (Owen-Going, 2002). The brown coloration of root is the main symptom of *Pythium* root rot, which is a destructive disease of peppers, accounting for about 42% plant mortality in bell pepper (Chellemi *et al*., 2000). Symptoms of root rot disease include stunted growth, chlorotic leaves formation, leaf dropping, and wilting. This is associated with sudden decrease in the growth rate of the roots and foliage of affected pepper plants, indicating the initiation of the necrotrophic phase of the disease cycle. Sporangia of *Pythium* species rapidly germinates after 1.5–2.5 hours of exposure to exudates from seeds or roots (Osburn *et al*.,1989), followed by immediate infection; it has been observed that the pathogen is very aggressive and highly pathogenic, thus, making management of the pathogen very difficult (Ramamoorthy *et al*., 2002).

Although fungicides have shown promising results in controlling the damping-off disease, phytotoxicity as a result of fungicide residues causes environmental pollution leading to health hazards of both human and animals (Ramamoorthy *et al*., 2002). Additionally, that *Pythium spp* develops resistance to fungicide, and this further discourages the use of chemicals for the control of plant diseases (Punja and Yip, 2003). Also, global interest has been shifted towards the use of eco-friendly methods for protecting crops against pests and diseases (Paranthaman *et al*., 2009). This simply encourages the application of biological control method which involves the use of antagonists or bio-control agents to improve plant health. Suppression of diseases by bio-control agents is as a result of sustainable balance in interactions among the plant, bio-control agent and the microbial community on and around the plant (Handelsman and Stabb, 1996).The bio-control methods of soil borne pathogens causing plant diseases, have been deemed to be more natural and environmentally acceptable as an alternative to the existing chemical control methods (Eziashi *et al*., 2007). Examples of bio-control organisms are *Pseudomonas spp* used against *Rhizoctonia* diseases, *Streptomyces spp, Bacillus spp, Trichoderma spp* against *Colletotrichum capsici, Aspergillus spp, Glomus mosseae* against *P. aphanidermatum* e.t.c. (Jeyaseelan *et al*., 2012 and Salami *et al*., 2011).

Among the species of fungal isolates that possess the ability to control pathogens is *Trichoderma* species. It has an easy growth pattern and wide host range (Whipps and Lumsden, 2001). *Trichoderma* is a free living, filamentous fungi reproduced asexually. It is an excellent example of bio-control agent that possesses necessary attributes, such as; easy isolation and culturing, rapid multiplication on many substrates, myco-parasitism, and strong opportunistic invaders, avirulent plant symbionts, better competitor for food and site. It is also a prolific producer of spores and powerful antibiotics, antifungal compounds, secondary metabolites and enzymes (Kubicek *et al*., 2002), hereby making it ecologically successful. *Trichoderma* is a versatile microbe, characteristically distinguished as green mould fungus and common in soil.

The proposed mechanisms used by these rhizosphere biocontrol agents to suppress *Pythium* spp are; competition for nutrients, hyperparasitism or mycoparatism, antibiosis and production of cell-wall degrading enzymes, siderophore-mediated competition for iron and induction of systemic resistance in the host plant (Nakkeeran *et al*., 2006). For example, *T. hamatum*, *T. harzianum*, *T. koningii* and *T. viride* are known to control damping-off caused by *Rhizoctonia* and *Pythium* species in the laboratory, glasshouse and in the field (Matroudi *et al*., 2009). *Trichoderma hamatum* and *T. virens* effectively controlled *Sclerotinia* lettuce drop, reducing disease by 30-50% in New Zealand (Rabeendran *et al*., 2006), while *T. harzianum* controlled *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Pythium* species (Devaki *et al*., 2012). Jebessa and Ranamukhaarachchi (2006) showed that *T. harzianum*, *T. koningii* and *T. psedokoningii* could control *Colletotrichum gloeosporioides* causing pepper anthracnose in Ethiopia. *Trichoderma* isolates are able to induce resistance against *Botrytis cinerea* in tomato, tobacco, lettuce, pepper and bean plants, with reduction in disease symptom ranging from 25 to 100% (Tucci *et al*., 2011). The reduction in disease incidence and severity of sesame plant against *Alternaria* leaf spot treated with *T. harzianum* due to the antimicrobial activities towards the pathogen was also reported. *Trichoderma harzianum* protected bean seedlings against pre-emergence damping-off infection, reducing the disease severity and increased the plant growth in the presence of *R. solani* pathogen (Paula *et al*., 2001). Soil application of bio-control agents in *Fusarium* wilt-nematode interaction reduced the wilt incidence significantly and those of root lesion and knot index, in addition to the fact that, the nematode population of *Pratylenchus coffeae* and *Meloidogyne incognita* reduced by 50 to 82% as a result of application of bio-control agents. Gonzalez *et al*. (2005) reported that the field application of *T. viride* with/without *T. harzianum* as soil inoculants gave the same effectiveness (99%) against *R. solani* pathogen as with the seed immersion method. Soil amendments with *T. harzianum* significantly increased plants' height and weight and showed significant reduction in the *R. solani* infection. Application of *T. harzianum* as seed treatment significantly reduced the incidence of damping-off diseases in some leguminous crops, i.e., faba bean, lentil, and chickpea, when planted in a soil naturally infested with *Fusarium spp*. and *R. solani*. *Trichoderma harzianum*, *T. koningii* and *T. viride*, as seed dressing, improved the seedling emergence and health of runner bean (Pieta *et al*., 2003). It has been reported that *Trichoderma* species participated actively in the nutrient cycle, especially in decomposition of organic matter and availability of normally inaccessible elements for plant uptake. Yedidia *et al*. (2001) reported that the presence of *Trichoderma* species increased the concentration of essential nutrients such as copper, phosphorus, iron, manganese and sodium around the root area of plants grown in hydroponic culture and consequently their uptake by plant. This indicated an improvement in plant active-uptake mechanisms.

More so, *Trichoderma* species increases uptake of nitrates and other necessary ions by plant roots and may increase the rate of degradation of various toxic metals and metalloids, that may be harmful to plants (Harman, 2000). Most *Trichoderma* strains are implicated in acidifying their surrounding environment by secreting organic acids, such as citric, gluconic or fumaric acids (Gomez-Alarcon and de la Torre, 1994). These organic acids, produced from the metabolism of other carbon sources, mainly glucose, are in turn, able to digest phosphates, micronutrients and mineral cations such as iron, manganese and magnesium (Harman *et al*., 2004). The root colonization by *Trichoderma* isolates often increase root growth and development, resulting into higher crop productivity, increase resistance to abiotic stresses and the better uptake and use of nutrients (Arora *et al*., 1992). It was reported that crop productivity in the fields increased up to about 30 % after the addition of *Trichoderma hamatum* or *Trichoderma koningii* (Benitez *et al*., 2004). *Trichoderma* strains produced cytokinin-like molecules, e.g. zeatin and gibberellin GA3 or GA3-related molecules. The controlled production of these compounds could be utilized biological means of fertility to crops (Osiewacz, 2002). For example, Chacon *et al*. (2007) showed that *Trichoderma harzianum* is able to promote tomato plant growth by colonizing the roots, increasing the foliar area and secondary roots, as well as changing the root system architecture under sterile condition. *Trichoderma* species employs several mechanisms in influencing seed germination and seedling vigor of plants (Zheng and Shetty, 2000; Clear and Valic, 2005). Seed germination rate, rapid elongation of plant root and development during seed germination, plant height, root fresh and dry weight, and shoot fresh and dry weight of seedling are the most important indicators of seedling vigor. According to El-Kafrawy, (2002), the treatment of crops with bio-control agents improved the plant heights, fresh and dry weight and increased dry seeds yield, when compared with the control.

Therefore the objectives of the study were:

1. To determine the effectiveness of these three *Trichoderma* species in controlling damping off disease caused by *Pythium aphanidermatum* in sweet pepper seedlings.; and
2. To evaluate the contribution of the three *Trichoderma* species to overall growth performance and emergence indices in sweet pepper seedlings.

MATERIAL AND METHODS

Collection and identification of experimental materials

Sweet pepper (California wonder) seed was obtained from a subsidiary of Premier Seed Company in Ibadan. The mycelium of *P. aphanidermatum* was taken from infected tomato seedlings showing the symptom of damping-off disease at the Teaching and Research Farm, Obafemi Awolowo University (O.A.U.). The mycelia of the three *Trichoderma* species used for this study were collected from rotten root residue and soil compost around the screen-house of Faculty of Agriculture, O.A.U.

The identification of these organisms was done by observing the morphological features under compound microscope using standard protocol by Watanabe, (2002).

Treatment of pepper seeds with Trichoderma species for in vivo studies

The spores concentration of each *Trichoderma* species were standardized through serial dilution to 2.5 x 10^6 conidia/ml using haemocytometer, 25 g of pepper seeds were soaked in 100 ml of the suspension for 2 hours, after which they were air-dried overnight. Seeds for the control were soaked in sterile water and were sown immediately in an experimental pot filled with sterilized soil.

Effect of the three *Trichoderma spp* on germination percentage, emergence index and emergence rate index of sweet pepper evaluated in the screen house

The effect of the three *Trichoderma species* on seed germination were evaluated under screen house conditions for 7th Day after planting (DAP), 9th DAP and 14th DAP. Fifty (50) seeds were sown per pot in 4 replications. Emergence index (EI), a measure of the rate of emergence, was calculated according to Adetimirin (2008) as:

$$EI = \frac{\sum(\text{newly emerged seedlings on a day})\ (\text{Days after sowing})}{\text{Seedlings emerged 14 DAP}}$$

Seedling vigour assessments and disease incidence percentage

More so, seedling vigour was assessed on these seedlings by scoring on a scale of 1 to 9, where 1= strong seedlings with large green leaves indicating very high vigour and 9 = weak seedlings with small yellow leaves indicating very poor vigour (Adetmirin *et al*., 2006; Adetimirin, 2007; 2008).

Expression of post emergence infection of pepper was also conducted under screen house conditions, by estimating the percentage disease incidence:

Percentage disease incidence = (No. of diseased plants X 100)/Total no. of plants (Hassanein, 2012).

Experimental design for growth parameters

The experimental layout used for this screen house experiment was Randomised Complete Block Design (RCBD). The following treatments were included in the experiment; Seedlings raised from untreated seeds (CON); seedlings raised from untreated seeds and inoculated with *P. aphanidermatum* (PO), seedlings raised from each of the *Trichoderma species* treated seeds (T1, T2 and T3) seedlings raised from each *Trichoderma species* treated seeds and inoculated with *P. aphanidermatum* (T1+P, T2+P and T3+P). Fifteen day old seedlings of the treated and untreated seeds were inoculated with 5 ml of suspension containing 5x 10^5 spores/ml of *P. aphanidermatum*.

Therefore, the treatments are:

CON- Seedlings without *Trichoderma* treatment and *P. aphanidermatum*
T1 - Seedlings raised from *T. harzianum* treated seeds
T2 - Seedlings raised from *T. koningii* treated seeds
T3 - Seedlings raised from *T. atroviride* treated seeds
T1 + P - Seedlings raised from *T. harzianum* treated seeds inoculated with *P. aphanidermatum*
T2 + P- Seedlings raised from *T. koningii* treated seeds inoculated with *P. aphanidermatum*
T3 + P- Seedlings raised from *T. atroviride* treated seeds inoculated with *P. aphanidermatum*
PO- Seedlings inoculated with *P. aphanidermatum.*

Plant growth data such as stem height, number of roots, root length, number of leaves, fresh weight and dry weight were measured for all the eight treatments on 30 day old seedlings with 3 representative seedlings in 3 replicates.

Statistical analysis

Data collected were analysed using SAS 9.0. All data from screen-house experiments were analyzed separately and subjected to analysis of variance (ANOVA). Fischer's LSD at 5% was used to test means comparison for significant effects of the treatments.

RESULTS

Germination percentage, emergence index and emergence rate index as influenced by the treatment of three Trichoderma species

Significant effect of *Trichoderma* was recorded on emergence percentage of sweet pepper seedlings at day 9 after planting (9DAP), at p=0.05. Although, sweet pepper seedlings emerged at 7DAP for all the treatments, application of different *Trichoderma* species was not significant on emergence percentage of the seeds on 7DAP and 14DAP. Also, both Emergence Index (EI) and Emergence Rate Index (ERI) of the pepper seedlings were not affected by the treatment of seeds with three *Trichoderma* species (Table 1).

The significant effects of *Trichoderma* treatments (Table 1) on 9DAP revealed that, the seedlings of sweet pepper treated with *Trichoderma atroviride* had highest emergence percentage (80.5), which was not different from the emergence percentage of *Trichoderma harzianum* treated pepper seedlings (73.0), but definitely different from emergence percentage of *Trichoderma koningii* treated seedlings (67.5). Seedlings of sweet pepper treated with both *Trichoderma atroviride* and *Trichoderma harzianum* had higher emergence percentage than the control (un-treated) seedlings (65), which was not lower in emergence percentage when compared with *Trichoderma koningii* treated pepper seedlings. The treatment of seeds with these three *Trichoderma* species recorded no significant difference on the speed of emergence (EI), which indicated that the speed of emergence was the same for all the treatments. Moreover, all the treated seedlings seemed to emerge within the first 9- 11 days of planting.

Table 1 Comparison of means for germination percentages at 7, 9 and 14 DAP; emergence index and emergence rate index of *Trichoderma* treated seeds of sweet pepper.

Trt	7 DAP	9 DAP	14 DAP	EI	ERI
T1	56.0	73.0	85.5	8.4	9.9
T2	58.0	67.5	84.0	8.6	10.3
T3	62.0	80.5	88.0	8.0	9.1
CON	52.0	65.0	80.0	8.6	10.9
L S $D_{0.05}$	7.9	10.8	10.9	0.6	1.5

T1- Seedlings from *T. harzianum* Treated seeds; T2- Seedlings from *T. koningii* Treated seeds; T3- Seedlings from *T. atroviride* Treated seeds
CON-Seedlings from Untreated seeds; 7 DAP- Emergence percentage at 7 days after planting; 9 DAP- Emergence percentage at 9 days after planting; 14 DAP- Emergence percentage at 14 days after planting; EI- Emergence Index; ERI- Emergence Rate Index
LSD- Least Significant Difference at α=0.05

Disease incidence and seedling vigour assessments on Trichoderma treated sweet pepper seedlings

Highly significant differences (p=0.01) were recorded for the effects of three *Trichoderma* species on Disease Incidence Percentage (DI) and Seedling Vigour Assessment (SV), indicating that the application of these *Trichoderma* species has the ability to adversely affect the development of damping-off disease in sweet pepper and in the process contributed significantly to the seedling vigour (Table 2).

Table 2 Mean comparison of the three *Trichoderma* species with or without pathogen on the disease incidence and the seedling vigour assessments.

Treatment	Disease Incidence (%)	Seed Vigour Assessments
PO	56.7	6.3
T1+ P	26.7	4.3
T2+ P	23.3	4.3
T3+P	13.3	2.0
T1	0.0	3.0
T2	0.0	3.3
T3	0.0	3.0
CON	0.0	3.3
L S $D_{0.01}$	7.3	1.4

PO- Pepper seedlings inoculated with *Pythium aphanidermatum*; T1- Pepper seedlings with *Trichoderma harzianum*
T2- Pepper seedlings with *Trichoderma koningii;* T3- Pepper seedlings with *Trichoderma atroviride;* T1+P- Pepper seedlings with *Trichoderma harzianum* inoculated with *Pythium aphanidermatum;* T2+P- Pepper seedlings with *Trichoderma koningii* inoculated with *Pythium aphanidermatum;* T3+P- Pepper seedlings with *Trichoderma atroviride* inoculated with *Pythium aphanidermatum;* CON- Pepper seedlings treated with sterile water.; LSD- Least Significant Difference at α=0.01.

It was indicated that Pathogen alone (PO) recorded the highest percentage disease incidence (56.7), which was significantly higher in infection rate than all the dual inoculations (13.3-26.7%), although the disease incidence percentages of *T. harzianum* plus pathogen (26.7) and *T. koningii* plus pathogen (23.3) were not different from each other, but both recorded higher infection percentage than *T. atroviride* plus pathogen (13.3). All the singular inoculations of *Trichoderma* and Control recorded no infection based on the values of percentage disease incidence.

Plate 1 Picture showing the symptom of damping-off by *Pythium aphanidermatum* in sweet pepper.

The seedling vigour assessment also indicated that PO had the highest value of rating (6.3), therefore producing seedlings with poorest vigour strength as compared with all other singular inoculations, which recorded almost the same rating of (3-3.3). However, equal seedling vigor assessments were obtained for dual inoculations of *T. harzianum* (4.3) and *T. koningii* (4.3), as the singular inoculations. These two dual inoculations were lower in vigor strength when compared with dual inoculation of *T. atroviride* which was rated 2, but not different from all the singular inoculations. The seedling vigour assessment, however, recorded no significant different among the singular inoculations of *Trichoderma* and control. *Pythium aphanidermatum* infected seedlings performed extremely poor, when compared with each of the remaining treatments. However, *T. atroviride* plus pathogen performed better than dual inoculation of the other two species. For this variety of sweet pepper to produce strong and high vigour seedlings in the presence of *P. aphanidermatum*, there must be application of *Trichoderma* species.

From this table; both singular inoculation of *T. atroviride* and when challenged with *P. aphanidermatum* recorded the highest length in stem height of 9.77 cm and 9.73 cm respectively, and these values were significantly higher than other treatment combinations. Additionally, control recorded 8.46 cm and *T. harzianum* (8.35 cm), these seedlings were of the same height with other treatments, except for *P. aphahidermatum* infected pepper seedlings (6.64 cm) which was the shortest. For root length, *T. atroviride* challenged with *P. aphanidermatum* produced the highest length of 4.00 cm, which was significantly higher than Control plant seedlings (3.00 cm), seedlings of *T. koningii* with the pathogen (2.98 cm) and *T. harzianum* with the pathogen (2.90 cm), but were of the same root length with *T. atroviride* (3.76 cm), *T. harzianum* (3.37 cm) and *T. koningii* (3.15 cm). Seedlings with pathogen alone recorded the least and lowest value of 2.05 cm. For the counting of leaf number, all combinations of treatment performed equally (6.33- 7.33) except for pathogen alone (4.67). Also on the number of roots, *T. atroviride* recorded the highest value of 20.67, which was higher than other treatment combinations, however, *T. koningii* with pathogen (17.33) recorded equal effect in number of roots with other treatments apart from the control which was 13.7 and pathogen alone (9.3). The measurements taken on the stem fresh weight indicated that singular and dual inoculations of *T. atroviride* (1.39 g and 1.19 g), *T. harzianum* (1.42 g and 1.12 g) and *T. koningii* (1.20 g and 1.09 g) were of equal weight range. *T. harzianum* alone (1.42 g) weighed better than *T. koningii* plus pathogen (1.09) and control (1.05) although these two were not different from each other, however, control stem outweighed pathogen alone (0.44). Moreover, for stem dry weight, pathogen alone (0.08) recorded the least weight, although it was not different from *T. koningii* with pathogen (0.13) and control (0.13) in weight, but significantly lower than others. For the root fresh weight, *T. atroviride* alone (0.18) outweighed all other treatments except for *T. koningii* plus pathogen (0.16) which was of equal root weight. However, *T. koningii* plus pathogen was only higher than pathogen alone (0.07).

Plate 2 Picture showing the root system of treated and control seedlings of sweet pepper.

Plant growth parameters

Significant differences were recorded for the effects of singular and dual inoculations of the three *Trichoderma* species and *P. aphanidermatum* on the stem height, root length, number of leaves, number of root, stem fresh weight, fresh root weight and stem dry weight (Table 3).

Table 3 Mean comparisons of the three *Trichoderma* species and *Pythium aphanidermatum* on plant growth parameters of 30 day old pepper seedlings.

Olftnemtaert	S.H (cm)	R.L (cm)	N.L	N.R	Fresh Wt (g)	Stem Wt(g)	Root Wt(g)	Stem Dry Wt (g)
PO	6.64	2.05	4.67	9.33	0.48	0.44	0.07	0.08
T1	8.35	3.37	7.33	14.00	1.53	1.42	0.11	0.20
T2	7.84	3.15	7.33	14.33	1.33	1.20	0.11	0.16
T3	9.77	3.76	7.00	20.67	1.57	1.39	0.17	0.18
T1+P	7.84	2.90	7.00	15.00	1.32	1.12	0.10	0.16
T2+ P	7.91	2.98	7.33	17.33	1.30	1.09	0.16	0.13
T3+P	9.73	4.00	7.00	15.67	1.34	1.19	0.12	0.15
CON	8.46	3.00	6.33	13.67	1.16	1.05	0.10	0.13
LSD $_{0.05}$	1.20	0.81	0.97	3.10	0.27	0.29	0.05	0.06

S.H- Stem Height ; R.L- Root Length; N.L- Number of Leaves; N.R- Number of Root; PO- Pathogen Only; T1- *T. harzianum* treated pepper seedlings; T2- *T. koningii* treated pepper seedlings; T3- *T. atroviride* treated pepper seedlings; T1+P- *T. harzianum* treated pepper seedlings + Pathogen; T2+P- *T. koningii* treated pepper seedlings + Pathogen; T3+P- *T. atroviride* treated pepper seedlings + Pathogen CON- Sterile water treated Seedlings.

DISCUSSION

It was obtained from this study, that 30 day old seedlings of pepper infected with *P. aphanidermatum* recorded 56.67% infection rate, showing symptoms of stunted growth, chlorosis and wilting. Despite the fact that all other singular inoculations recorded zero percent infection, the dual inoculation of *T. atroviride* and the pathogen (13.33%) produced healthier seedlings than the other two species of *Trichoderma*. Chang *et al*. (2002) reported that both seed and soil treatment with *T. virens* was found to be more effective in enhancing the growth and suppressing the wilt disease incidence. It was observed that *T. harzianum* and *T. viride* were able to reduce disease incidence of *Fusarium* crown root rot in spite of the physical barrier between the antagonists and the pathogen. *Trichoderma* species have been shown to reduce disease incidence of several pathogens by stimulating vegetative growth and enhancing the root development of the treated plants (Daami-Remadi *et al*., 2006). However, the infection rate reduced by 50-75% in the presence of *Trichoderma* species, which got the ability to reduce pathogenic infections (Harman and Kubicek, 1998). This result was supported by Narasimha-Murthy *et al*. (2013) who reported that bacterial wilt disease incidence in *Trichoderma asperellum* treated tomato seedling inoculated with *Ralstonia solanacearum*, produced healthier plant than when *Trichoderma* was absent and thus produced seedlings with about 50% reduction in infection rate under greenhouse conditions. The reduction of disease infection rate and proportion, caused by soil borne pathogens after treatment with *T. harzianum* and subsequent enhancement in the yield of different crops have been reported by several workers (Harman *et al*., 2004; Singh and Singh, 2004). This was explained in study done by Abd-El-Khair *et al*. (2010), who reported that application of *Trichoderma* species significantly reduce the disease incidence of damping-off disease caused by *Rhizoctonia solani* in bean.

The efficacies of *Trichoderma* species in controlling *P. aphanidermatum* post emergence infection of pepper plants in screen house were evaluated and found to significantly increase the plant growth and development compared to the control. Analysis of stem height, root length, number of roots and dry weight of 30 day old seedlings, showed increased growth rate in the three *Trichoderma* treated seedlings over control plants. The stem heights were higher in *T. atroviride* seedlings with or without pathogen than all other treatment combinations, although the presence of *T. harzianum* and *T. koningii* did not increase the stem height than when they are absent. Root length was longer in *T. atroviride* plus pathogen than in control and pathogen alone, but all other *Trichoderma* species with/without pathogen were not different from control except for the pathogen alone. In numbering of leaves produced by pepper plants, *T. koningii* with/without pathogen, *T. harzianum* alone produced better than the control, which was not different from others except the pathogen alone with the least amount of leaves. *T. atroviride* produced more roots than all others. In total fresh weight, however, *T. atroviride* and *T. harzianum* outweighed control, this was also the same in the seedlings stem and root dry weights, which showed that they contribute significantly to the pepper plant weight.

The improved plant growth may be due to higher production of growth stimulators that been reported in plant-*Trichoderma* interactions, like Gibberellic acids and Indo-Acetic Acid (Gravel *et al*., 2006) or in the effort to reduce pathogens deleterious activities. In general, *Trichoderma* produced more vigorous and healthy seedlings (Kaveh *et al*., 2011). The observed increase in plant growth after inoculation of the antagonist may be through the enhancement of the plant root system (Benitez *et al*., 2004; Vinale *et al*., 2007). *Trichoderma* species, when added to soil or applied as seed treatment grow readily along with the developing plant root system; have many positive effects on plant growth, yield, nutrient uptake, fertilizer utilization efficiency and systemic resistance to plant diseases (Harman, 2006; Howell *et al*., 2000). Formulations of different products of *Trichoderma* in organic substrates were recorded to significantly promote plant growth, vigour, biomass and yields. A study carried by Yedidia *et al*. (1999) reported that *T. harzianum* inoculation improved the uptake of nutrients by plants at the early stage of growth. Vinale *et al*. (2004) investigated in greenhouse studies, the abilities of *T. harzianum* and *T. atroviride* to improve the growth of lettuce, tomato and pepper plants. Comparatively, few authors had reported the ability of antagonistic fungal strains to produce compounds acting as growth promoting factors. Cutler *et al*. (1986, 1989) reported that the isolated and identified secondary metabolites produced by *T. koningii* (koninginin A) and *T. harzianum* (6-pentyl-a-pyrone), acted as plant growth regulators.

Trichoderma species also produce organic acids, such as gluconic, citric or fumaric acids, which decrease soil pH and permit the solubilization of phosphates, micronutrients and mineral cations like iron, manganese and magnesium, and are necessary for plant metabolism (Benitez *et al*., 2004; Harman *et al*., 2004). The nutrients utilization by these antagonist deprived soil pathogens of access to nutrients and thus controlling the growth of pathogens. For example, biological strains of *Trichoderma* are able to produce highly efficient siderophores that chelate iron from other filamentous fungi. Such fungi like *Pythium* that need iron for survival will be killed (Benitez *et al*., 2004).

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