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COMBINED EFFECTS AND RELATIONSHIPS OF COMPOST TEA, FERTILISER, AND *GLOMUS INTRARADICES* INOCULATED-SUBSTRATE ON TOMATO SEEDLING QUALITY

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ABSTRACT: There is a paucity of information on the efficacy of nutrient amendments made from readily available local material on seedling quality. The objectives of this study were to evaluate the combinatory effects and relationships of compost tea, fertiliser, and *Glomus intraradices* inoculated-substrate on tomato seedling quality as defined by fresh and dry biomass, root to shoot ratio, length of root and stem, and selected root system architecture parameters. The factorial designed assays consisted of tomato sown in autoclaved or non-autoclaved peat-based substrates, which was either fortified (PM) or not fortified (PNM) with the endomycorrhizal fungi, *G. intraradices*, to which fertiliser only (FO), unfiltered compost tea + fertiliser (UCT + F), or filtered compost tea + fertiliser (FCT + F) were applied to these substrates. With the exception of the positive effect of FCT + F on leaf area, the application of compost teas + fertiliser to PM substrates (non-autoclaved or autoclaved) did not provide any additional benefits compared to FO treatment. However, FO applied to PM or autoclaved substrates resulted in lower or lowest seedling growth (root and shoot) compared to UCT + F applied to PM substrates. The application of FCT + F, which had similar nutrient supplying capacity as UCT + F but without microorganisms, to PM substrates, resulted in significantly lower seedling growth. Roots were not colonised with *G. intraradices* and results suggest that increased seedling growth was related to the biological properties of the UCT and non-autoclaved substrates. Network width to depth ratio was the most important factor affecting shoot growth. Quality of tomato seedlings resulting from non-autoclaved PM and PNM substrates applied with UCT + F was comparable.

Keywords: compost, *Solanum lycopersicum,* nutrient amendment, mycorrhiza, soil-less substrate, seedling performance.

Introduction

The use of compost tea as a nutrient amendment in commercial horticulture and factors affecting its efficacy, remains poorly investigated and farmers are still being primarily informed by popular literature. As such, the scientific evidence for the inclusion of compost tea as part of an integrated nutrient management system in commercial horticulture is not convincing.

To date, the limited peer-reviewed studies have focused on a few crop families or crops including Brassicas (Pant et al., 2012a; Pant et al., 2012b; Pant et al., 2009), Cucurbits (Akanbi et al., 2007), raspberries (*Rubus* spp.) (Hargreaves et al., 2008), strawberries (*Fragaria* spp.) (Hargreaves et al., 2009; Welke, 2005), wheat (*Triticum aestivum*) (Reeve et al., 2010), Canada yew (*Taxus canadensis*) (Smith et al., 2006),

and narrow leaf plantain (*Plantago arenaria*) (Hendawy, 2008). Scientific evidence on the efficacy of compost tea as a nutrient amendment in economically important solanaceous crops such as tomato and sweet pepper is limited.

Hargreaves et al. (2009) found that compost tea treatments provided similar amounts of most macro- and micronutrients compared to municipal solid waste compost, ruminant compost, and fertiliser treatments. Akanbi et al. (2007) showed that foliar spray of compost extracts from cassava (*Manihot esculenta*) peel and Mexican sunflower (*Tithonia rotundifolia*) help produce fluted pumpkin (*Telfairia occidentalis*) plants with comparable growth to those that received NPK fertiliser. Pant et al. (2009) demonstrated that vermicompost extracted with or without active aeration can increase yield and carotenoid content in pak choi (*Brassica rapa* cv Bonsai, Chinensis group) and the effect has been confirmed in multiple soil types. St. Martin et al. (2012) found that drench application of Miracle-Gro® or NCT made from BLC and brewed for 168 h resulted in a significant mean total dry matter increase of 122%, compared with the non-fertilised water control treatment. However, they reported that NCTs brewed for 56 h using BLC or LCC, and aerated compost tea produced from BLC brewed for 18 h, significantly reduced seed germination of sweet pepper. Concentration of copper in compost tea was identified as the most significant factor inhibiting seed germination (St. Martin et al., 2012).

Most researchers have attributed the plant growth promotion (PGP) effect of compost tea to its nutrient composition and/or microbial properties (Hargreaves et al., 2009; Pant et al., 2011). Hendawy (2008) reported that compost tea provides chelated micronutrients for easy plant absorption as well as nutrients in biological available form for both plant and microbial uptake. In addition, microbes in the compost tea produce plant growth hormones, mineralise plant available nutrients, and fix nitrogen (Hendawy, 2008). Humic substances present in compost tea may also cause hormone-like effects or stimulate root respiration leading to increased nutrient uptake both in controlled conditions and in the field (Arancon et al., 2003; Zhang et al., 2003). Pant et al. (2012b) found that the positive influence of compost on plant growth was largely associated with the mineral N and gibberellin (GA_4) present in the teas. Pant et al. (2012a) also reported that the application of compost tea resulted in increased soil respiration and dehydrogenase activity that implies more efficient organic decomposition and mineralisation in the rhizoshpere, which may have in turn contributed to better plant growth.

Essential to this understanding is determining the effect of these nutrient management strategies on root system architecture (RSA) including root system size, shape, and distribution traits along with the probable functional relationship between RSA, and seedling quality. In field and rhizotron studies, RSA has been shown to have important, yet distinct effects on seedling growth and nutrient uptake (Hodge, 2004; Robinson, 2001). These effects are particularly evident under plant stress conditions including drought, low soil fertility and microbial populations, and poor lighting (Hodge, 2004; Reich et al., 2002).

Arbuscular mycorrhizal (AM) fungi, such as *G. intraradices*, have been shown to improve plant growth and nutrition (Koide and Mosse, 2004). As such, many of the commercial horticultural potting mixes e.g. PRO-MIX 'BX'/Mycorise® PRO (Premier Horticulture Ltd., Dorval, Canada) (PM) are inoculated with *G. intraradices*, on the

premise that root colonisation with AM fungi will result in increased nutrient uptake, reduce water and other stresses associated with cultivation (Premier Horticulture, 2005). Though considered important primarily for phosphorus uptake, roots colonised with AM fungi have also been reported to result in increased uptake of NH_4^+ and NO_3 (Frey and Schüepp, 1993; Johansen et al., 2006), Zn, Cu, and K (Marschner and Dell, 1994). Improved nutrient uptake resulting from root colonisation with AM fungi, has been particularly evident under organically managed soil (Mäder et al., 2002) and in substrates with low-nutrient levels (Hetrick, 1991; Menge, 1983). Under such systems and or conditions, AM fungi effectively increase the absorptive surface of the plant root system thereby providing access to soil-derived nutrients from sources not necessarily otherwise accessible to roots (Menge, 1983). Research work on mycorrhizal root colonisation of plants grown in AM fortified commercial substrates, under wet-dry tropical climates is limited in comparison to the plethora of work done on the effect of fertilisers, and manure on crop growth (Belay et al., 2002; Ghosh et al., 2004).

It is hypothesised that compared to a fertiliser alone nutrient treatment, the drench application of compost tea and fertiliser to commercial peat-based substrates inoculated with *G. intraradices* will result in better seedling quality. The objectives of this study were therefore to: 1) investigate the combinatory effects of compost tea, fertiliser, and *G. intraradices* inoculated-substrate on tomato seedling emergence, RSA architecture (root system size, shape, and distribution traits), and seedling growth, 2) determine if the effects of compost tea and substrates on seedling growth were related to microbial populations and root colonisation with *G. intraradices* and 3) investigate the relationship between RSA parameters and shoot growth parameters.

Materials and Methods

Production of Compost Tea

Aerated compost tea produced from lawn clippings compost (LCC) and brewed for 36 hours (ACT-36 h) was made using methods previously described by St. Martin et al. (2012). As is commonly practiced by farmers and in accordance with recommendations by Scheuerell (2003), LCC was cured in rotary barrel composter for three months before sampling and use.

Physical, Physico-Chemical, Chemical, and Biological Properties of Substrates and Compost Teas

Physical, Physico-Chemical and Chemical Properties

BD, WHC, pH, EC, total Kjeldahl nitrogen (TKN), NO_3-N + NO_2^-N, P, K, total organic matter (TOM), total organic carbon (TOC), and C/N ratio of substrates were determined using protocols previously described by St. Martin et al. (2012). Dissolved oxygen, temperature, pH, and electrical conductivity of compost tea were recorded at the end of each batch by immersing probes into the bucket, prior to use in seedling growth assays. Protocols described by St. Martin et al. (2012) were used to determine ammonia, nitrate, nitrite, phosphorus, potassium, calcium, magnesium, copper, and zinc concentration in compost tea.

Microbiological Populations of Substrates and Compost Teas

Total culturable bacterial, fungal and yeast populations of substrates and compost tea were enumerated using methods described by Scheuerell and Mahaffee (2004). Total microbial population was determined by summing bacterial, fungal and yeast populations.

Seedling Emergence and Growth Assay

Seedling emergence and relative growth assays (Thompson et al., 2002) were used to evaluate the combined effect of compost tea, fertiliser, and *G. intraradices* on seedling emergence, growth, and RSA. Experiments were done in a conventional span-roof, naturally ventilated glasshouse (length -8.5m, width- 3.7, height - 2.7 m) located at the University of the West Indies, Department of Food Production, St. Augustine, Trinidad and Tobago. Mean day and night temperatures were 31 and 24°C, respectively, at a corresponding relative humidity of 61 and 85%.

An experimental unit consisted of a styrofoam container (top diameter -12.7cm, bottom diameter -10.2 cm and height -8.9 cm) with eight tomato (*Solanum lycopersicum* L. cv. Calypso) seeds sown 1 cm deep in non-autoclaved (N) or autoclaved (A) PM, or in non-autoclaved or autoclaved peat-based substrate not inoculated with *G. intraradices* (Sunshine Professional growing mix® Sun Gro Horticulture, British Columbia, Canada) (PNM). Nine days after sowing (DAS), seedling emergence was measured and seedlings were thinned-out to 4 seedling/container. Nutrient amendments, which included fertiliser only (Miracle-Gro®, Water Soluble All Purpose Plant Food, 24-8-16) (FO), unfiltered compost tea + fertiliser (UCT + F), or filtered compost tea + fertiliser (FCT + F) were applied to experimental units 21 DAS and every seven days thereafter, using a fine-spray watering can. In accordance with the Miracle Gro® label instructions, fertiliser treatments were applied to provide a total of 84 mg N/L (174 kg N/ha), 30 mg P/L (58 kg P/ha), and 54 mg K/L (116 kg K/ha) at the end of the six weekly applications.

Containers were arranged in a random order on seedling benches in the glasshouse with each treatment replicated six times. At 60 DAS, 12 plants from each treatment were harvested for seedling growth analysis. Fresh and dry root, stem, leaf, and total biomass were measured as well as, length and width of stem and root, number of leaves, and root/shoot ratio. Leaf area was determined using the non-destructive image scanning pixel method (Xiao et al., 2005).

Root Colonisation with *G. intraradices*

One plant per experimental unit was harvested for assessment of root colonisation. The total root system of individual plants was preserved in 50% alcohol prior to root staining. The root staining procedure described below was modified from the procedure outlined by Hebert et al. (1999). Root segments of 3-4 cm lengths and 2 mm in diameter were randomly selected from each root system and placed in separate test tubes. The roots were rinsed with distilled water before being covered with 10 ml 10% KOH and incubated in a water bath set at 80° C for 30 minutes to allow clearing of the roots. The tubes were removed from the water bath and 400 µl

of 9% H_2O_2 added and allowed to stand at room temperature for 10 min to remove root pigments. After 10 minutes, the solution was decanted and the root pieces rinsed three times with distilled water. The root segments were then transferred to fresh tubes containing 10% HCl and left at room temperature for 10 min. The HCl was removed and 10 ml of 0.05% (w/v) trypan blue in glycerol added; the tubes were then incubated at 80˚ C for 30 min in a water bath to allow staining. The trypan blue stain was removed and replaced with 10 ml of 50% lactic acid in glycerol to remove excess stain from the roots. The roots were left in the lactic acid solution until slide mounting.

A scalpel was used to cut root segments into 1cm lengths; three groups of four stained 1cm root segments were placed on a slide with a spacing of 5 mm between each root segment and 10-15 mm between each group. The roots were mounted in glycerol and covered with a cover slip; two slides were prepared for each treatment. The slide preparations were viewed using an Olympus BX5 microscope using bright field optics and images were photographed using a Pixera 5.8 megapixel 48-bit CCD camera hosted on a Dell 8300 graphics intensive computer. Roots were examined for the presence of hyphae, vesicles, and arbuscules. Root colonisation by the vesicular arbuscular mycorrhiza *G. intraradices* was confirmed if vesicles with associated hyphae and arbuscules were present in the cortex of the roots and colonisation levels determined (Brundrett, 2009). Absence of these structures is indicative no colonisation.

Root System Architecture

RSA measurements were done using 12 plants for each treatment, which were harvested 60 DAS. Root systems of plants, which were carefully harvested from substrates, were separated from the shoots. The majority of substrate particles attached to the roots, were removed with limited breakage of roots, by soaking roots in distilled water with a mild detergent (0.01% Tween®-20) for 10 minutes. Finer substrate particles still attached to the root were removed using a size-6-fan painting brush. Root systems (networks) free of substrate particles were imaged with a square of known size (2 x 2 cm) against a black velvet background using a Fujifilm, T410WM, 16-megapixels camera. Photographs were taken in a closed room, under the same lighting conditions (white fluorescence tube lamps, 60 WATTS). RSA parameters categorised into size, shape, and distribution traits (Table 1), were analysed using GiA Roots software® (Georgia Tech Research Corporation and Duke University, 2011) (Galkovskyi et al., 2012). The square of known size was used to set the scale parameter of pictures of root systems uploaded to the software. Root length density (RLD) was determined using the formula: RLD = total length of root system network / unit volume of substrate in which crop is planted (Ho et al., 2005). Root to shoot mass ratio was also determined.

Statistical Analysis

Univariate analysis of variance in SPSS (ver. 17.0, 2008, SPSS Inc., Armonk, NY) for Windows was used to determine treatment effects. Tukey's test was used to separate main effect means if interactions were not significant. Significant interactions were investigated by simple effects analyses using the EMMEANS subcommand (least square means analysis) (Green and Salkind, 2005). Zero-order

correlation analysis was used to determine linear interdependencies between RSA parameters and multiple regression analysis to formulate predictive models of shoot growth and root to shoot mass ratio using RSA variables. Before analyses were run, homogeneity of variance and normality of datasets were assessed using Levene's test and by inspection of histogram, distribution curve and skewness values. Data expressed as percents were arcsine transformed.

Results

Physical, Physico-Chemical, Chemical, and Biological Properties of Substrates and Compost Teas

Physico-chemical and chemical properties of substrates were not affected by autoclaving (Table 2). BD (0.19 to 0.70 g cm^{-3}), WHC (45 - 65% v/v), pH (5.0-6.5), and EC (≤ 2.0 dS m^{-1}) of all substrates were within limits recommended for seedling starter and transplant substrates (Ingram et al., 1993; Robbins and Evans, 2001). TOM varied from 727.40 - 739.67 g kg^{-1}, TOC from 406 - 427 g kg^{-1}, ash from 287 - 297 g kg^{-1}, and C/N 71:1 - 78:1. Compared to compost teas, the nutrient content of the substrates was low. Bacteria, the predominant microorganisms in non-autoclaved substrates, were significantly lower in autoclaved substrates (Table 2). A similar result pattern was also observed with total microbial population. In contrast, yeast population was unaffected by autoclaving and predominant in autoclaved substrates, whereas fungal populations were below the detectable limit.

As with substrates, the physico-chemical and chemical properties of compost teas were not affected by microfiltration (Table 2) and their pH were within the recommended range of 5.8 to 6.5 (Whipker, 1999) for fertigation of vegetable crops, grown in soil-less substrates. EC across compost teas was however, above the upper limit, 3 dS m^{-1}, recommended as the maximum level for fertiliser solutions (Whipker, 1999). Conversely, Cu and Zn concentrations were below the respective target ranges of < 0.2 and < 0.50 mg l^{-1}, recommended for water used to irrigate vegetable crops grown in containers (Whipker, 1999). Bacteria were the predominant microorganisms in unfiltered compost tea (UCT) and no microorganisms were detected in micro-filtered compost tea (FCT).

Seedling emergence and growth

Seedling emergence was not affected by substrate type, autoclaving, or their interaction. Neither were stem and total fresh weight, root and stem length, and number of leaves affected by secondary or tertiary interactions of substrate type, nutrient amendment, and autoclaving ($p > 0.05$). Stem fresh weight was highest in non-autoclaved substrates with UCT + F, as was stem length in non-autoclaved PM substrate with UCT + F. Total fresh weight and number of leaves were highest in substrates with UCT + F whereas average taproot length did not vary significantly across treatments.

In contrast, there was a significant substrate type by nutrient amendment interaction ($p < 0.05$) (Fig.1A-H) on root and leaf fresh weight, dry biomass (root, stem, leaf and total dry matter), stem diameter, and root/shoot ratio, which indicated that the effects of the respective nutrient amendments were not consistent across substrates. Figure

1 A-H shows that UCT + F had no significant effect on root and leaf fresh weight, dry biomass, stem diameter, and root/shoot ratio in PM, but compared to the FO treatment, it significantly increased root and leaf fresh weight, dry biomass, and stem diameter in PNM substrates. Moreover, seedlings supplied with UCT + F had similar root (Figure 1A) and leaf fresh weight (Figure 1B), dry biomass (Figure 1C - F), and stem diameter (Figure 1G) across substrates. In contrast to UCT + F, FCT +F increased root/shoot ratio in PNM substrate (Figure 1H) and resulted in leaf fresh weight (Figure 1B), stem (Figure 1D), leaf (Figure 1E), and total dry matter (Fig. ure 1F) values, which were comparable ($p > 0.05$) to that of seedlings treated with FO. As with UCT + F, FCT + F had no effect on root fresh weight, dry biomass, and stem diameter in PM substrates and values for these growth parameters were similar across substrates. Root and leaf fresh weights, dry biomass, and stem diameter of seedlings supplied with FO were significantly higher in PM compared to PNM substrates. Root/shoot ratio of seedlings supplied with FO did not differ across substrates.

Dry biomass, and root and stem diameter were also affected by a significant autoclaving by nutrient amendment interaction (Figure 2). This indicates that the effects of the respective nutrient amendments were not consistent across autoclaving (non-autoclaved and autoclaved). Figure 2 showed that UCT + F had no significant effect on dry biomass and stem diameter in non-autoclaved substrates, but compared to the FO treatment, it significantly increased stem (Figure 2B), leaf (Figure 2C), and total dry matter (Figure 2D), and stem diameter (Figure 2E) in autoclaved substrates. Moreover, seedlings supplied with UCT + F had similar root (Figure 2A), leaf (Figure 2C), and total dry matter (Figure 2D), and stem diameter (Figure 2F) across autoclaving treatments. In contrast to UCT + F, FCT +F increased root diameter in autoclaved substrates (Figure 2E) and resulted in dry biomass values, which were comparable ($p > 0.05$) to that of seedlings treated with FO. As with UCT + F, FCT + F had no effect on dry biomass (Figure 2A-D) and stem diameter (Figure 2F) in non-autoclaved substrates. However, dry biomass of seedling supplied with FCT + F was significantly higher in non-autoclaved compared to autoclaved substrates. Stem and root diameter (Figure 2E) did not differ across autoclaving treatments for seedling supplied with FCT + F. Dry biomass, and root and stem diameter of seedlings supplied with FO were significantly higher in PM compared to PNM substrates. Leaf area was affected by substrate by nutrient amendment by autoclaving interaction ($p < 0.001$) (Figure 3). Figure 3 shows that although FCT + F supplied to non-autoclaved PM substrate resulted in highest leaf area, FCT + F applied to other substrates had no positive effect on leaf area. In contrast, leaf area across UCT + F treatments was similar, and FO supplied to non-autoclaved PM resulted in a higher leaf area compared to FO supplied to non-autoclaved PNM substrates.

Root System Architecture

Network width to depth ratio was the only RSA parameter that was not affected by secondary or tertiary interactions of substrate type, nutrient amendments, and autoclaving ($p > 0.05$). Both substrate and nutrient amendment had a main effect on network width to depth ratio, with network width to depth ratio being highest in PM substrate supplied with UCT + F.

In contrast, all other RSA parameters were significantly affected by substrate by nutrient amendment interaction (Table 3). UCT + F and FCT + F had no effect on maximum number of roots, network length, surface area, and root length density of seedlings grown in PM substrates (Table 3). However, applied to PNM substrates, UCT + F and FCT + F significantly increased maximum number of roots. Moreover, UCT + F increased network solidity in PM but had no effect on network solidity, bushiness, surface area, volume, length, and length distribution in PNM substrates. In contrast, FCT + F supplied to PNM substrates increased network length and length distribution, surface area, and root length density but had no significant effect on network solidity relative to FO treatments (Table 3). For FCT + F treatments, network length and length distribution, surface area, and root length density were significantly higher in PNM compared to PM substrates. However, comparable maximum number of roots, network length and length distribution, surface area, bushiness, and root length density were recorded across UCT + F treatments applied to PNM and PM substrates ($p > 0.05$). A similar result trend was observed within FO treatments with respect to maximum number of roots, network surface area, volume, solidity and length, and root length density.

Network volume and solidity were however, higher in UCT + F supplied to PM compared to UCT + F supplied to PNM substrates (Table 3). In contrast to UCT + F results, network length distribution and bushiness were higher in FO applied to PM compared to FO applied to PNM substrates. Results showed that there was also a significant substrate by autoclaving interaction, which affected network length and surface area, and root length density ($p < 0.05$) (Table 3). Network length was similar across substrates and autoclaving, except for seedlings grown in non-autoclaved PNM substrates, which had significantly higher network lengths compared to those in PM substrates (Table 4). A similar results trend was observed with root length density. Network surface area was highest in non-autoclaved PNM substrates, lower in non-autoclaved PM, and generally lowest in autoclaved substrates (Table 4).

There was also a significant nutrient amendment by autoclaving interaction, which affected maximum number of roots, network length, and root length density (Table 5). UCT + F increased maximum number of roots in autoclaved substrates but had no effect in non-autoclaved substrates (Table 5). In contrast, FCT + F increased maximum number of roots in non-autoclaved substrates but had no positive effect on maximum number of roots in autoclaved substrates. The maximum numbers of roots for FO treatment supplied to non-autoclaved and autoclaved substrates were similar. However, within FCT + F treatments, maximum number of roots was higher in non-autoclaved compared to autoclaved substrates. Conversely, within UCT + F treatments, maximum number of roots was higher in autoclaved compared to non-autoclaved substrates (Table 5). Network length across autoclaved and non-autoclaved substrates applied to nutrient amendments was similar, except for seedlings grown in non- autoclaved substrate supplied with FCT + F, which had higher network lengths. Results trend of root length density was similar to that of network length.

Specific root length was the only factor affected by a substrate by nutrient amendment by autoclaving interaction. Figure 4 shows that specific root lengths of all the treatments, except FO applied to A-PM and UCT + F applied to A-PNM, were similar to that of FO applied to non-autoclaved PNM substrate.

Root Colonisation

Entry points of fungi were observed in a few root samples from PM substrate treated with FO (Fig. 5A). However, infection did not progress to the formation of vesicles and arbuscules therefore, mycorrhizal colonisation could not be confirmed (Figure 5B). Neither fungal entry points, vesicles nor arbuscules were observed in any of the other root samples.

Relationship of RSA Parameters to Shoot Growth Parameter and Root / Shoot Ratio

There were significant linear interdependencies between many of RSA parameters and the respective shoot growth variables (data not shown). However, many of the RSA parameters, which had significant linear independencies with growth parameters, were not completely independent from each other (Table 6). Multiple stepwise regression identified the most significant RSA parameters accounting for the greatest differential variance in seedling growth or nutrient parameters. This resulted in fewer RSA parameters being predictors of shoot growth (Table 7). Regression models for all the shoot growth parameters were significant ($p < 0.01$) (Table 7) but accounted for small variation in shoot growth. RSA parameters in the models were positively related to all shoot growth parameters. With the exception of stem width, network width to depth ratio positively affected all shoot measurements ($p < 0.01$) (Table 7). Together with root length density ($\beta = 0.26$, $p < 0.01$), taproot length ($\beta = 0.23$, $p < 0.05$), and network width to depth ratio ($\beta = 0.36$, $p < 0.001$) explained 22% of the variation of shoot fresh weight. Moreover, network width to depth ratio ($\beta = 0.35$, $p < 0.001$) and network surface area ($\beta = 0.38$, $p < 0.001$) explained 23% of variation of shoot dry weight together (Table 7). Twenty-two percent of the variation in stem length was explained by network width to depth ratio ($\beta = 0.41$, $p < 0.001$) and network surface area ($\beta = 0.25$, $p < 0.001$). In contrast, maximum number of roots was the only RSA variable, which was significantly related to stem width accounting for 12% of the variation. Specific root length ($\beta = 0.31$, $p < 0.01$) and network width to depth ratio ($\beta = 0.023$, $p < 0.05$) explained 14% of the variation of leaf area whereas root length ($\beta = 0.34$, $p < 0.01$) and network width to depth ratio ($\beta = 0.34$, $p < 0.01$) explained 19% of variation in the number of leaves. Root length density ($\beta = 0.39$, $p < 0.001$) and network bushiness ($\beta = 0.33$, $p < 0.01$) explained 20% of the variation of root/shoot ratio.

Discussion

The combinatory effects of compost tea + fertiliser treatments on seedling growth were more discernible in substrates not inoculated with *G. intraradices*. UCT + F supplied to PM substrates resulted in higher or highest seedling growth (root and shoot) compared to FO supplied to PNM or autoclaved substrates. However, comparable growth to that of seedlings cultivated in PM substrates drenched with UCT + F was observed when UCT + F was supplied to PNM or autoclaved substrates. The enhanced shoot and root growth observed in this study agree with the findings of Arancon et al. (2007) and Lazcano et al. (2010). In most cases, FCT + F, which had similar nutrient supplying capacity as UCT + F but without microorganisms, resulted in significantly lower seedling growth when applied to PNM substrates. This result pattern of lower seedling growth with FCT + F compared to

UCT + F treatment was also observed in autoclaved substrates. In contrast to findings of Hargreaves et al. (2008) and Pant et al. (2009), these results suggest that seedling growth enhancement was predominantly related to a microbial priming effect caused by UCT + F and non-autoclaved substrates treatments. More specifically, to the total fungal populations of UCT + F and non-autoclaved substrates, which were significantly higher compared to autoclaved substrates supplied with FO or FCT + F treatment. It is unlikely that the increased seedling growth was due to the direct effects of the endomycorrhizal fungus since *G. intraradices* did not colonise roots and extramatrical mycelia, which increases the absorptive surface of the plant root system (Menge, 1983), were not observed in the root samples. However, increased seedling growth may be related to other fungal taxa of non-autoclaved substrates and UCT + F, including *Trichoderma* spp., *Aspergillus* spp. and *Penicillum* spp., which have been reported as the predominant species in compost tea and peat substrates (Epstein, 1997; St. Martin et al., 2012). Samuel and Muthukkaruppan (2011) found that *Aspergillus niger* and *Penicillium* spp. exhibited three plant growth promoting (PGP) traits, including the production of indole acetic acid (IAA), ammonia, and catalase, which may directly, indirectly, or synergistically promote plant growth. Indole acetic acid (IAA) controls a wide variety of processes in plant development and growth, and plays a key role in shaping plant root architecture such as regulation of lateral root initiation, root vascular tissue differentiation, polar root hair positioning, root meristem maintenance and root gravitrophism (Aloni et al., 2006; Richardson et al., 2009). Other phytostimulators including *Trichoderma harzianum* strain T-22 and *Aspergillus fumigatus* have also been reported to produce phytohormones, most commonly auxins, cytokinins, and giberellins and to a lesser extent ethylene, which are all known to enhance seedling growth (Arshad and Frankenberger Jr, 1991; Khan et al., 2011).

It is likely that the continuous addition of UCT + F resulted in the recolonisation of autoclaved substrates with fungi, to a level, which was similar to that of non-autoclaved PM substrates supplied with any of the nutrient amendments. This may explain why seedling growth in autoclaved substrates supplied with UCT + F, was not significantly different from that of tomato planted in PM substrates supplied with any of the nutrient amendments. Numerous researchers have demonstrated that microbial recolonisation of sterilised substrates resulted in similar plant growth promoting or disease suppressive effects as its non-sterilised counterpart (Nakasaki et al., 1998; Scheuerell and Mahaffee, 2005).

According to Kuzyakov et al. (2000), microbial priming effects, which affect the homeostatic equilibrium of nutrients in the substrate (Blagodatskaya and Kuzyakov, 2008; Cleveland and Liptzin, 2007), can be positive or negative. Positive in that nutrients held in passive or recalcitrant pools are released due to an increase in microbial activity and organic matter decomposition and negative in that, microorganisms introduced into the soil or substrate can immobilise carbon or nitrogen, or both elements, resulting in less than optimum plant growth (Kuzyakov et al., 2000). The net effect of microbial inocula and/ or nutrient additives, whether positive or negative, depends on the influence of these inputs on the microbial community dynamics, particularly the population metrics of beneficial and deleterious microorganisms and their metabolite profiles.

The relatively low root/shoot ratios indicate that nutrient availability was high, and the typical plant response of proportionally higher shoot than root growth under high nutrient availability conditions (Kang and van Iersel, 2004; Reich et al., 2002) was observed in this study. Although this response may adequately describe relative root and shoot growth as influenced by nutrient availability, a sound physiological basis for this response it yet to be established because of little evidence of fine control of phloem loading in response to sink demand for photosynthates (Lynch et al., 2012; Minchin et al., 2002). The unexpectedly and relatively higher root/shoot ratio in PNM supplied with FCT + F compared to FO or UCT + F further suggest that nutrient uptake may have been affected by microbes in UCT. The increased supply of nutrients from FCT + F without complementary microbes, may have negatively affected the homeostatic equilibrium of nutrients in the substrate (Blagodatskaya and Kuzyakov, 2008; Cleveland and Liptzin, 2007), resulting in lower nutrient uptake in PNM substrates. The effects of FCT + F on seedling growth in PM substrates were not as discernible as in PNM substrates. This suggests that FCT + F had a lesser effect on the equilibrium of PM substrates, which implies that PM had a higher threshold level to the negative effects of this treatment (Blagodatskaya and Kuzyakov, 2008). The higher threshold level is likely due to a more diverse microbial profile or a greater population of specific microbial taxa with synergistic roles and functions in PM substrate (Blagodatskaya and Kuzyakov, 2008; Torsvik and Øvreås, 2002).

It is likely that the colonisation of the roots with *G. intraradices* was negatively affected by the relatively high nutrient level of substrates supplied with the nutrient amendments (Azcón et al., 2003; Liu et al., 2000). For AM fungi, P is the main element influencing colonisation of host plant roots (Linderman and Davis, 2004; Peters and Habte, 2001). Peters and Habte (2001) found that AM fungal activity and symbiotic effectiveness was maximum at a solution P concentration of 0.2 mg/l and that AM fungal colonisation tended to decrease with medium solution P concentrations of > 0.2 mg/l. The continuous application of compost teas with mean P concentration of 105.50 g kg^{-1}, to the substrates, may have resulted in P concentration above the ideal range reported to maximise root colonisation with AM. It is likely that the strain of *G. intraradices* fortified in the PNM is not acclimated to high temperatures (>30 ˚C), which are typical of tropical wet and dry climates. Therefore, the high temperatures may have also prevented or negatively affected colonisation of tomato roots with *G. intraradices*. Parke et al. (1983) reported that VA mycorrhizal root colonisation was greatly reduced or prevented at or above 29.5˚ C. In our study, substrate temperature ranged from 27 to 34 ˚C, which is higher than 21-25 ˚C range, reported to be ideal for mycorrhizal root colonisation (Liu et al., 2004; Martin and Stutz, 2004). It is however, less likely that other factors including pH, substrate moisture levels, and light would have negatively affected mycorrhizal root colonisation since these factors were all within ideal ranges described by Pozo et al. (1998).

Moreover, it is improbable that *G. intraradices* had any significant effect on RSA of tomato seedlings. However, substrate, nutrient amendments, and autoclaving had significant main and/or interaction effects on RSA parameters. As with seedling growth, the effect of nutrient amendments on RSA parameters was more discernible in PNM substrates. The results of FCT + F treated seedlings having higher network surface areas and lengths than FO and UCT + F treated seedlings, suggest

preferential photosynthate partitioning to root growth to acquire nutrients (Nielsen et al., 1998). The higher network surface area and length results are therefore congruent with those of root/shoot ratio and further support the assertion that nutrient availability, particularly N was lowest in FCT + F treated seedlings. In contrast to Hodge et al. (1998), increases in number of roots, in response to lower nutrient level, were not observed in this study. These results suggest that root initiation was foregone, to some extent, in favour of increasing network surface area and length of existing roots. Such response would have required less investment of resources and yield greater 'returns' in terms of resource acquisition compared to root initiation and the maintenance of new roots (Bloom et al., 1985). This assertion is further supported by Pregitzer et al. (1997), who reported that fine lateral roots might be the least expensive to construct but the most expensive to maintain based on an increase in N concentration. Lambers (1987) and Janssens et al. (2002) estimated that 26–34% of net primary productivity of plants was used for fine root growth and maintenance. Robinson (2001) and Hodge (2004) reported that increased root proliferation in response to fertility, particularly in nitrogen patches, was more economically beneficial and discernible under interspecific plant competition conditions, rather than in monocultures.

With reference to architectural traits, Lobet (2012) ranked root system size characteristics as the most important features in water and nutrient uptake, followed by root system shape and distribution properties. However, in this study, root system spatial distribution and shape parameters were more useful predictors of the respective growth and nutrient uptake variables than absolute root system size measurements. Root system size parameters are likely more useful predictors of water and nutrient uptake under dynamic field conditions or a less controlled environment, where root size is not restricted by the sizes of seedling containers, soil profile is deeper and more heterogeneous, and water and nutrient supply may be more limiting. As with root to shoot ratio, the various ratios, which describe the shape and distribution properties of the root system, are suggestive of functional growth equilibriums that are ideal for maximising resource acquisition, under the specific conditions. However, as is evident by the relatively low variation explained by predictors in seedling quality models, the use of root shape and distribution properties alone may not be a good proxy to infer resource acquisition by plants (Pierret et al., 2007).

The low level of variation explained by these predictors is understandable in the context of heterogeneous root behaviour (Pierret et al., 2007), with only 10 and 30% of the total root length of a given root system being effectively involved in nutrient and water uptake, respectively (Robinson, 1991). More accurate models can be developed by including variables that capture: spatial pattern of root activity, how spatial distribution of root activity varies with time and the influence of environmental on this pattern, root demographics, and ontogenetic drift (Hodge, 2004; Pierret et al., 2007; Reich et al., 2002).

With the exception of stem width, network width to depth ratio, a root system shape variable, was the most important trait, which positively affected seedling quality. Increasing network width to depth ratio values indicates an increase in horizontal relative to vertical exploration for resources, which is desirable in soil-less-container production of vegetables, particularly under fertigation. Caution must however be

taken when interpreting the effect of compost tea on network width to depth ratio, since taproot length was restricted within the container's depth. Models are therefore condition specific and are not likely to be appropriate for open-field conditions, where taproot growth is not restricted by containment.

Root length density and taproot length, which have been reported as important traits for water uptake and drought tolerance (Kashiwagi et al., 2006), were positively related to shoot fresh weight, but not related to shoot dry weight. The expected increases in shoot wet weight due in part by increases in specific root length, was not evident in this study. However, specific root length was a predictor of leaf area as was network surface area to shoot dry weight and stem length. Higher specific root length and network surface area imply thinner roots and better soil coverage, which are both important for improving resource exploitation efficiency (Fitter, 1991; Hodge, 2004). Maximum number of roots, which probably increased water uptake efficiency, was the only RSA variable that was positively related to stem width. The positive relationship between network bushiness and root length density to root/shoot ratio indicate that a more homogenous branching distribution with a precision rather than a scale effect may result in better seedling growth (Campbell et al., 1991; Topp et al., 2013).

As with rhizotron and pot studies, the effects of compost tea on RSA and the relationships of RSA with seedling quality parameters reported in this study was influenced by the width and depth of the container. Parallel runs were not done to investigate and weight and/ or partition the effect of container size on the overall effect of treatments.

Conclusions

Seedling growth and RSA, resulting from planting in commercial substrate inoculated with *G. intraradices* (PM) are comparable to non-inoculated substrate (PNM) supplied with UCT + F made from LCC. With the exception of leaf area, UCT + F or FCT + F applied to PM substrates (non-autoclaved or autoclaved) had no significant effect on growth parameters measured. Growth enhancement effect observed in the non-inoculated substrate is attributed to the activities and products of microorganisms indigenous to compost teas and substrates. *Glomus intraradices* did not colonise roots and increased P uptake was not observed in substrate inoculated with the endomycorrhizal fungi. Nutrient amendments affected RSA including traditional root traits e.g. specific root length, root surface area and volume and novel traits, such as network bushiness and solidity. The findings of this study have practical implications for growth substrate selection, fertiliser management strategy, seedling performance, and the overall profitability of vegetable seedling or production enterprises. Selection of the non-inoculated substrate and the fertiliser + unfiltered compost tea combination, means that the initial higher cost of purchasing inoculated substrate will be avoided. However, the cost, time, and labour associated with the production and application of compost tea will be incurred. Conversely, the combination of the inoculated substrate and FO means that the higher initial cost of the substrate will be incurred but the cost, time, and labour associated with the production and application of compost tea will be avoided. Studies on cost-benefits analysis between these two combinations, particularly over a crop production cycle, will prove useful in decision-making. Further studies on spatial patterns of root

activity, how spatial distributions of root activity vary with time and the influence of environmental factors on this pattern in soil-less culture, are needed to better understand the effect of RSA on plant growth.

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Figure Captions:

Fig. 1. Interaction effect of substrate and nutrient amendments on (A) root fresh weight and (B) leaf fresh weight (C) root dry matter (D) stem dry matter (E) leaf dry matter (F) total dry matter (G) stem diameter, and (H) root/shoot ratio of tomato. Error bars indicate one standard error. Means with different lowercase letters are significantly different within substrate type; those with different uppercase letters are significantly different across substrate types within the same nutrient amendment [unfiltered (UCT + F) or filtered compost tea + fertiliser (FCT + F), and fertiliser only (FO)], $P < 0.05$, least square means analysis.

Fig. 2. Interaction effect of autoclaving and nutrient amendments on (A) root dry matter and (B) stem dry matter (C) leaf dry matter (D) total dry matter (E) root diameter, and (F) stem diameter of tomato. Error bars indicate one standard error. Means with different lowercase letters are significantly different within autoclaving (autoclaved or non-autoclaved; those with different uppercase letters are significantly different across autoclaving within the same nutrient amendment [unfiltered (UCT + F) or filtered compost tea + fertiliser (FCT + F), and fertiliser only (FO)], $P < 0.05$, least square means analysis.

Fig. 3. Interaction effect of substrate, autoclaving, and nutrient amendments on leaf area of tomato. Error bars indicate one standard error. Means with different lowercase letters are significantly different within substrate type; those with different uppercase letters are significantly different across substrate types within the same nutrient amendment [unfiltered (UCT + F) or filtered compost tea + fertiliser (FCT + F), and fertiliser only (FO)], $P < 0.05$, least square means analysis.

Fig. 4. Interaction effect of substrate, autoclaving, and nutrient amendments on specific root length. Error bars indicate one standard error. Means with different lowercase letters are significantly different within substrate type; those with different uppercase letters are significantly different across substrate types within the same nutrient amendment [unfiltered (UCT + F) or filtered compost tea + fertiliser (FCT + F), and fertiliser only (FO)], $P < 0.05$, least square means analysis.

Fig. 5. A) Entry point of fungi in root sample of potting mix inoculated with *G. intraradices* (PM) and treated with fertiliser only B) Mycorrhizal colonisation of root cortex of tomato plant grown in perlite under greenhouse conditions (reference sample, which was not from this study).

Table 1. Root system architecture parameters measured in seedling growth assays.

Root system traits category	Parameter	Description	Unit
Size	Taproot length	Length of the taproot.	cm
	Network[a] length	Length of all roots in the network.	cm
	Average root diameter	Average width of all roots in the network.	cm
	Network surface area	The sum of the local surface area at each root of the network skeleton, as approximated by a tubular shape whose radius is estimated.	cm^2
	Network volume	The sum of the local volume at each root of the network skeleton, as approximated by a tubular shape whose radius is estimated.	cm^3
Shape	Network width to depth ratio	The value of network width divided by the value of network depth.	-
	Maximum number of roots	Maximum number of roots exploring a given soil horizon	-
Distribution	Specific root length	Total network length divided by network volume.	cm/cm^3
	Network length distribution	Ratio of deep to shallow root exploration relative to total network depth.	
	Root length density	total length of root system network / unit volume of substrate in which crop is planted.	cm/cm^3
	Network solidity	The total network area divided by the network convex area.	-
	Network bushiness	The ratio of the maximum to the median number of roots.	-

[a]Network refers to the whole root system.
Source: Topp et al. (2013, Supporting information)

Table 2. Physico-chemical, chemical and biological properties[a] of substrates and compost tea.

Substrate	BD (g/cm3)	WHC (%v/v)	pH	EC (dS m^{-1})	TKN	NO_3-N + NO_2^--N	P	K	TOM	TOC	Ash	C/N	Log_{10} populations (substrate: CFU g dw^{-1})			
					----------(g kg^{-1}) ----------								Bacteria	Fungi	Yeast	Total
PNM[b]	0.19a	50.2a	6.20a	1.03a	0.52a	0.08a	0.07a	0.19a	730.70a	406a	269a	77:1a	6.15a	4.03a	5.52a	15.70a
A-PNM	0.15a	49.0a	6.10a	1.04a	0.45a	0.08a	0.07a	0.20a	727.40a	416a	297a	78:1a	5.18b	Bdl[c]	5.39a	10.57b
PM	0.16a	51.3a	6.10a	1.06a	0.49a	0.07a	0.07a	0.21a	739.67a	427a	277a	76:1a	6.28a	4.50a	5.67a	16.45a
A-PM	0.14a	49.8a	5.90a	0.99a	0.51a	0.08a	0.07a	0.20a	733.29a	419a	287a	71:1a	5.25b	bdl	5.58a	10.83b
Compost tea[d]	Temp (°C)	DO (mg l^{-1})	pH	EC (dS m^{-1})	NH_4^+-N	NO_3-N + NO_2^--N	P	K	Ca	Mg	Cu	Zn	Log_{10} populations (compost tea: CFU ml^{-1})			
					---------- (mg l^{-1}) ----------								Bacteria	Fungi	Yeast	Total
UCT	23.8	2.65a	6.24a	4.63a	16.33a	353.00a	105.50a	48.86a	189.70a	174.00a	0.04a	0.08a	6.67	4.86	6.40	17.93
FCT	24.0	2.69a	6.15a	4.52a	16.02a	350.02a	104.60a	45.90a	167.00a	141.93a	0.04a	0.08a	bdl	bdl	bdl	-

Values represent means of 6 replications; means in columns followed by the same letter are not significantly different, $P > 0.05$, Tukey's test.

[a]BD - bulk density, WHC - water holding capacity, EC - electrical conductivity, TKN – total Kjeldahl nitrogen, P - phosphorus and K = potassium, TOM - total organic matter, TOC – total organic carbon, C/N –carbon to nitrogen ratio, and CFU – colony forming units.

[b]PNM- potting mix (Sunshine Professional growing mix®, Sun Gro Horticulture, British Columbia, Canada) not inoculated with *G. intraradices*, A-PNM – autoclaved potting mix (Sunshine Professional growing mix®, Sun Gro Horticulture, British Columbia, Canada) not inoculated with *G. intraradices*, PM – potting mix (PRO-MIX 'BX'/Mycorise® PRO, Premier Horticulture Ltd., Dorval, Canada) inoculated with *G. intraradices*, and A-PM – autoclaved potting mix (PRO-MIX 'BX'/Mycorise® PRO, Premier Horticulture Ltd., Dorval, Canada) inoculated with *G. intraradices*.

[c]bdl- below detection limit of log_{10} 2.3 CFU/ml of compost tea (Scheuerell and Mahaffee 2004, 1157).

[d]UCT – unfiltered compost tea, FCT – filtered compost tea

Table 3. Interaction effect of substrate by nutrient amendment on maximum number of roots, root system length, distribution, surface area, and volume.

Substrate[a]	Nutrient amendment[b]	MNR[c]	NL (cm)	RLD	NLD (cm/cm^3)	NSA (cm^2)	NV. (cm^3)	NB	NS
PNM	FO	31bA	103.71bA	1.04bA	0.56bB	211.92bA	5.94aA	2.10aB	0.25aA
	UCT + F	45aA	115.85abA	1.16abA	0.94abA	198.09bA	4.61aB	2.37aA	0.24aB
	FCT + F	44aA	151.44aA	1.51aA	1.45aA	322.68aA	9.29aA	3.00aA	0.28aA
PM	FO	42aA	96.47aA	0.97aA	1.63aA	182.90aA	5.09abA	3.15aA	0.22bA
	UCT + F	41aA	98.79aA	0.99aA	0.76abA	244.39aA	12.30aA	1.88bA	0.32aA
	FCT + F	33aA	86.76aB	0.87aB	0.77bB	179.60aB	4.17bA	2.58abA	0.25abA

Values represent means of 6 replications; means with different lowercase letters are significantly different within substrate type (PM or PNM); those with different uppercase letters are significantly across substrate type within the same nutrient amendment treatment (FO, UCT + F or FCT + F), $P < 0.05$, least square means analysis.

[a]PNM- potting mix (Sunshine Professional growing mix®, Sun Gro Horticulture, British Columbia, Canada) not inoculated with *G. intraradices*, and PM – potting mix (PRO-MIX 'BX'/Mycorise® PRO, Premier Horticulture Ltd., Dorval, Canada) inoculated with *G. intraradices.*

[b]FO – fertiliser only, UCT + F – unfiltered compost tea and fertiliser, FCT + F – filtered compost tea and fertiliser.

[c]MNR – maximum number of roots, NL- network length, RLD – root length density, NLD – network length distribution, NSA- network surface area, NV – network volume, NB - Network bushiness, and NS – Network solidity.

Table 4. Interaction effect of substrate by autoclaving on maximum number of roots, network length, root length density, and network surface area.

Substrate[a]	Autoclaving	NL[b] (cm)	RLD (cm/cm^3)	NSA (cm^2)
PNM	Non-autoclaved	155.27aA	1.55aA	332.42aA
	Autoclaved	92.06bA	0.92bA	156.03bA
PM	Non-autoclaved	95.19aB	0.95aB	248.62aB
	Autoclaved	92.82aA	0.93aA	155.96bA

Values represent means of 6 replications; means with different lowercase letters are significantly different within substrate type (PM or PNM); those with different uppercase letters are significantly across substrate type within the same autoclaving treatment (non-autoclaved or autoclaved), $P < 0.05$, least square means analysis.

[a]PNM- potting mix (Sunshine Professional growing mix®, Sun Gro Horticulture, British Columbia, Canada) not inoculated with G. intraradices, and PM – potting mix (PRO-MIX 'BX'/Mycorise® PRO, Premier Horticulture Ltd., Dorval, Canada) inoculated with *G. intraradices.*

[b]NL- network length, RLD – root length density, and NSA - network surface area.

Table 5. Interaction effect of nutrient amendment by autoclaving on maximum number of roots, root system length, distribution, surface area, and volume.

Autoclaving	Nutrient amendment[a]	MNR[b]	NL (cm)	RLD (cm/cm^3)
Non-autoclaved	FO	38aA	116.63abA	1.17abA
	UCT + F	36aB	108.51bA	1.09bA
	FCT + F	47aA	150.56aA	1.51aA
Autoclaved	FO	36bA	83.54aA	0.83aA
	UCT + F	51aA	106.14aA	1.06aA
	FCT + F	31bB	87.65aB	0.88aB

values represent means of 6 replications; means with different lowercase letters are significantly different within autoclaving treatment (non-autoclaved or autoclaved); those with different uppercase letters are significantly across autoclaving treatment within the same nutrient amendment treatment (FO, UCT + F or FCT + F), $P < 0.05$, least square means analysis.

[a]FO – fertiliser only, UCT +F – unfiltered compost tea and fertiliser, FCT + F – filtered compost tea and fertiliser.

[b]MNR – maximum number of roots, NL- network length, and RLD – root length density.

Table 6. Pearson's correlation coefficients (r) values between root system architecture parameters.

	MNR[a]	SRL	NL	NLD	NWD	NSA	NV	RLD	NB	NS	TRL	ARD
MNR	1	.377**	.671**	-.089	.495**	.186	-.228	.671**	-.042	.167	.194	-.406
SRL	.377**	1	-.157	.008	.120	-.595**	-.431**	-.157	-.042	-.513**	.058	-.546**
NL	.671**	-.157	1	-.093	.086	.756**	.128	1.000**	-.122	.456**	.262*	-.085
NLD	-.089	.008	-.093	1	-.146	-.029	.012	-.093	.631**	-.291*	-.040	.048
NWD	.495**	.120	.086	-.146	1	-.028	-.057	.086	-.186	.099	.119	-.069
NSA	.186	-.595**	.756**	-.029	-.028	1	.666**	.756**	-.091	.702**	.179	.530**
NV	-.228	-.431**	.128	.012	-.057	.666**	1	.128	-.097	.592**	.025	.946**
RLD	.671**	-.157	1.000**	-.093	.086	.756**	.128	1	-.122	.456**	.262*	-.085
NB	- .042	-.042	-.122	.631**	-.186	-.091	-.097	-.122	1	-.339**	-.153	-.033
NS	.167	-.513**	.456**	-.291*	.099	.702**	.592**	.456**	-.339**	1	-.168	.550**
TRL	.194	.058	.262*	-.040	.119	.179	.025	.262*	-.153	-.168	1	-.072
ARD	- .406	-.546**	-.085	.048	-.069	.530**	.946**	-.085	-.033	.550**	-.072	1

* Significant at 0.05 probability level
** Significant at 0.01 probability level
*** Significant at 0.001 probability level

[a]MNR – maximum number of roots, SRL – specific root length, NL- network length, NWD- network width to depth ratio, NSA- network surface area, NV – network volume, RLD – root length density, NB - network bushiness, NS – network solidity, TRL – taproot length, and ARD – average root diameter.

Table 7. Multiple linear regression analysis of shoot growth, root to shoot ratio, and root system architecture parameters.

Parameter	Regression equation	Multiple R value	Adjusted R^2 value	*f* value	Significance of *f*
Shoot fresh wt.	y = 7.43 + 12.73NWD[a] + 3.97 RLD + 0.27TRL	0.49	0.22	10.57	< 0.001
Shoot dry wt.	y = 1.09 + 0.004NSA + 2.15NWD	0.50	0.23	11.71	< 0.001
Stem length	y = 34.96 + 17.29NWD + 0.018NSA	0.47	0.22	9.87	< 0.001
Stem width	y = 4.87 + .015MNR	0.34	0.12	9.12	< 0.010
Leaf area	y = 177.91 + 0.14SRL + 154.88NWD	0.40	0.14	6.68	< 0.010
Number of leaves	y = 6.01 + 2.05NWD + 0.05TRL	0.46	0.19	9.46	< 0.001
Root/shoot ratio	y = 0.08 + 0.022RLD + 0.008NB	0.48	0.20	10.11	< 0.001

Analyses were done using SPSS Version 17.0 statistical package (SPSS Inc., 2008) for Windows with stepwise regression, probability of *f* for entry =0.01limit and probability of *f* for removal = 0.05 limit.
[a]NWD – network width to depth ratio, RLD – root length density, TRL – Taproot length, NSA –network system area, MNR – maximum number of roots, SRL – specific root length, LA – leaf area, and NB –network bushiness.

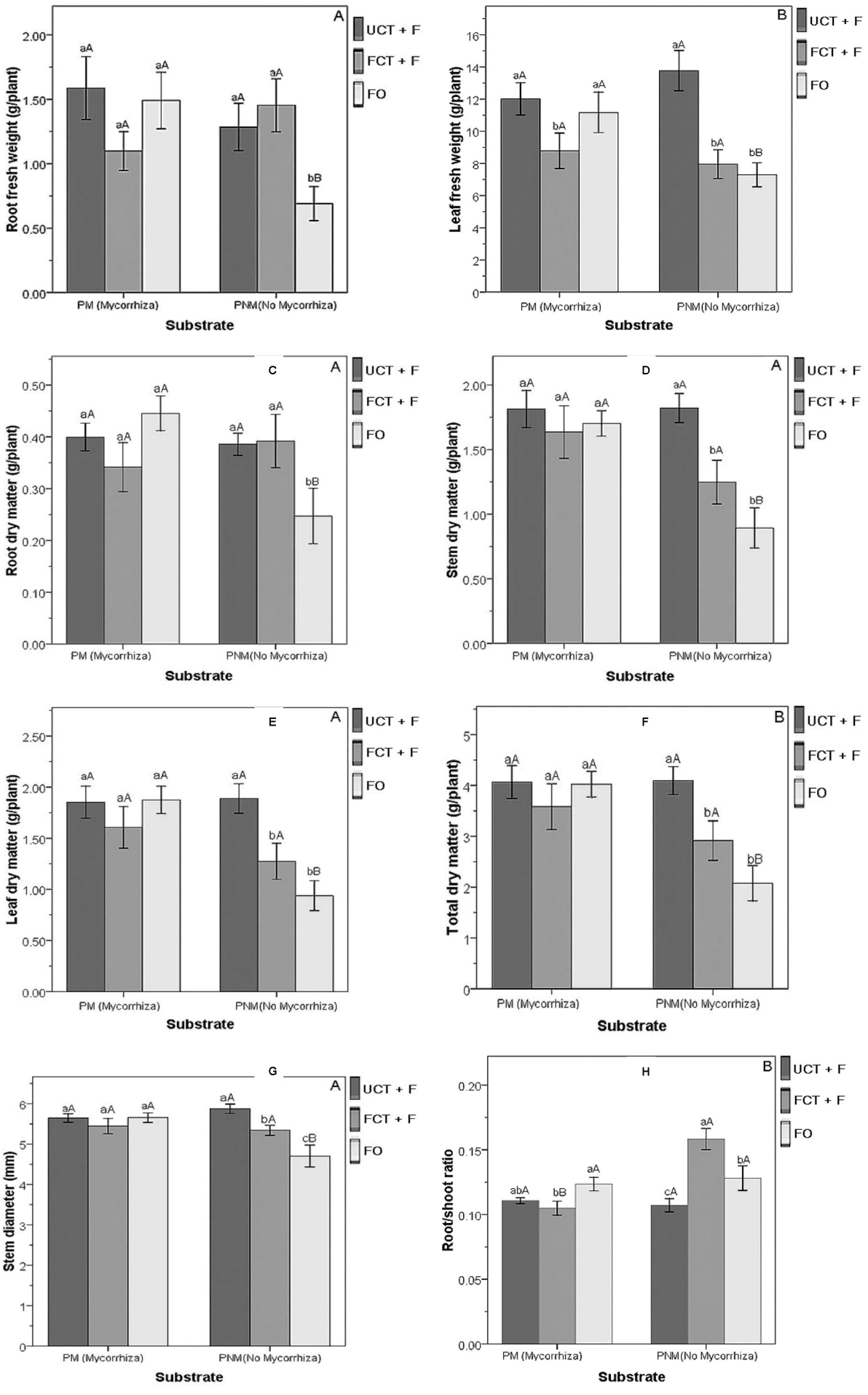
A
Root fresh weight (g/plant)
2.00
1.50
1.00
0.50
0.00
aA
aA
aA
aA
aA
bB
PM (Mycorrhiza)
PNM(No Mycorrhiza)
Substrate
UCT + F
FCT + F
FO
B
Leaf fresh weight (g/plant)
16
14
12
10
8
6
4
2
0
aA
bA
aA
aA
bA
bB
PM (Mycorrhiza)
PNM(No Mycorrhiza)
Substrate
UCT + F
FCT + F
FO
C
A
Root dry matter (g/plant)
0.50
0.40
0.30
0.20
0.10
0.00
aA
aA
aA
aA
aA
bB
PM (Mycorrhiza)
PNM(No Mycorrhiza)
Substrate
UCT + F
FCT + F
FO
D
A
Stem dry matter (g/plant)
2.00
1.50
1.00
0.50
0.00
aA
aA
aA
aA
bA
bB
PM (Mycorrhiza)
PNM(No Mycorrhiza)
Substrate
UCT + F
FCT + F
FO
E
A
Leaf dry matter (g/plant)
2.50
2.00
1.50
1.00
0.50
0.00
aA
aA
aA
aA
bA
bB
PM (Mycorrhiza)
PNM(No Mycorrhiza)
Substrate
UCT + F
FCT + F
FO
F
B
Total dry matter (g/plant)
5
4
3
2
1
0
aA
aA
aA
aA
bA
bB
PM (Mycorrhiza)
PNM(No Mycorrhiza)
Substrate
UCT + F
FCT + F
FO
G
A
Stem diameter (mm)
6
5
4
3
2
1
0
aA
aA
aA
aA
bA
cB
PM (Mycorrhiza)
PNM(No Mycorrhiza)
Substrate
UCT + F
FCT + F
FO
H
B
Root/shoot ratio
0.20
0.15
0.10
0.05
0.00
abA
bB
aA
cA
aA
bA
PM (Mycorrhiza)
PNM(No Mycorrhiza)
Substrate
UCT + F
FCT + F
FO

Figure 1

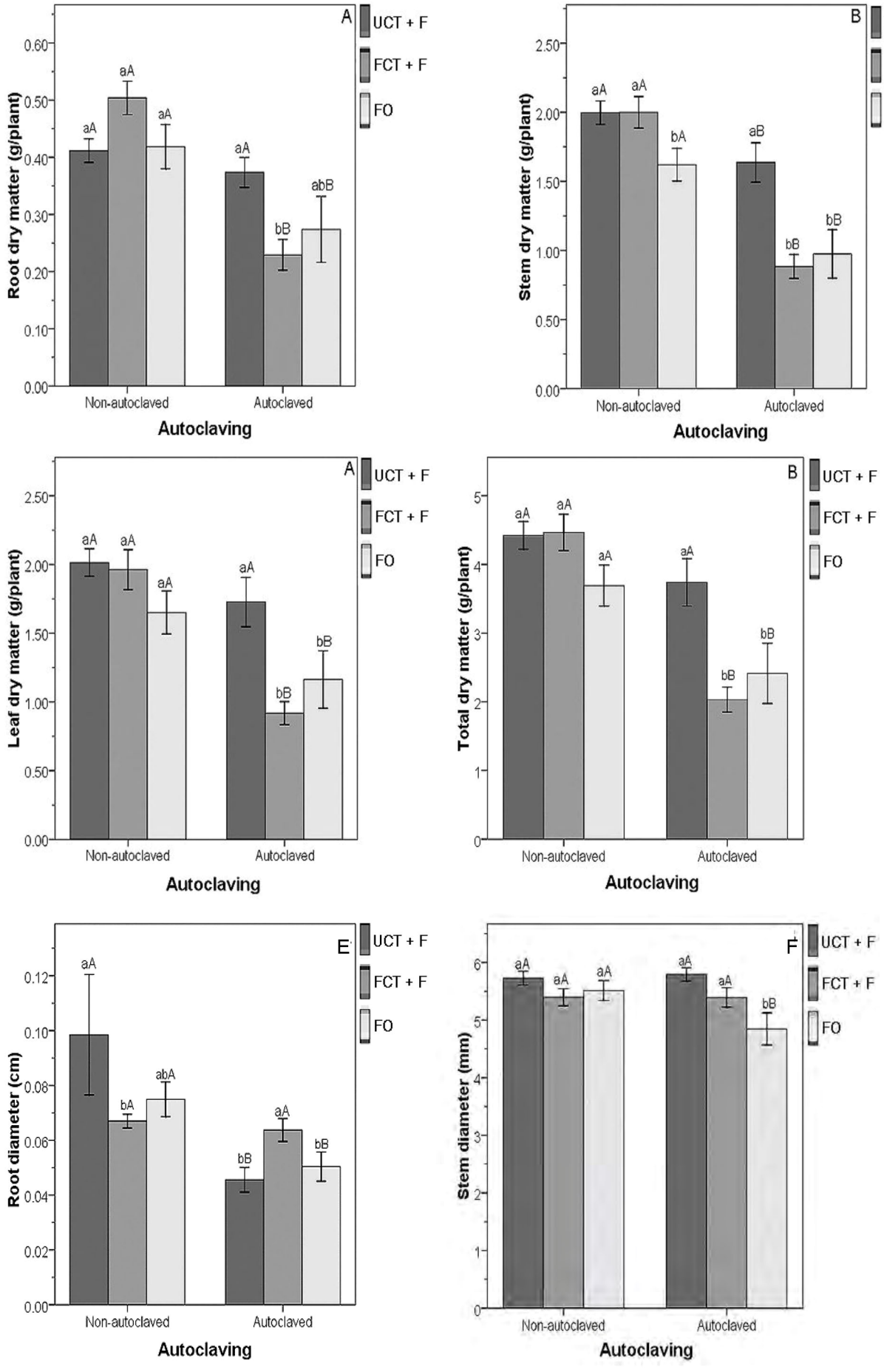
A
B
C
D
E
F
UCT + F
FCT + F
FO
Root dry matter (g/plant)
Stem dry matter (g/plant)
Leaf dry matter (g/plant)
Total dry matter (g/plant)
Root diameter (cm)
Stem diameter (mm)
Non-autoclaved
Autoclaved
Autoclaving

Figure 2

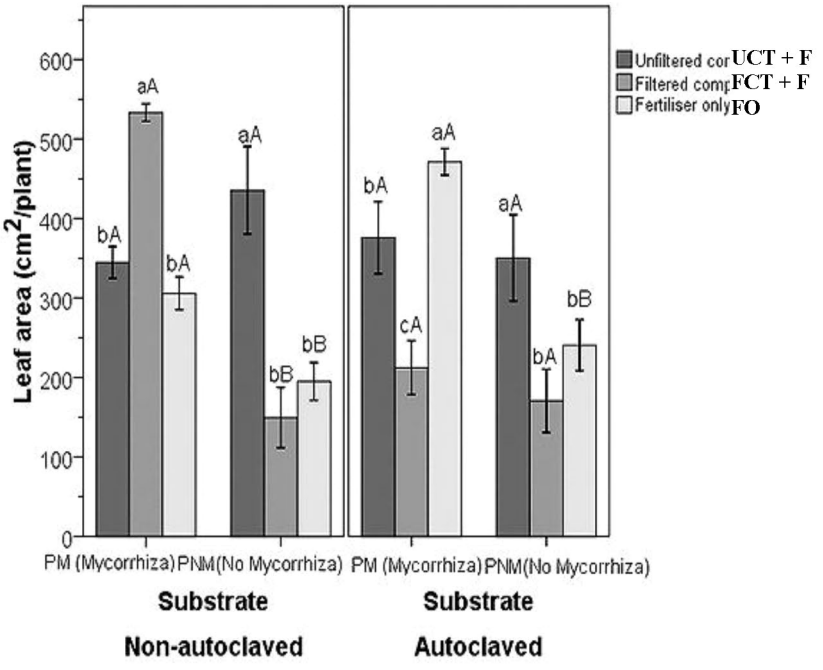

Figure 3

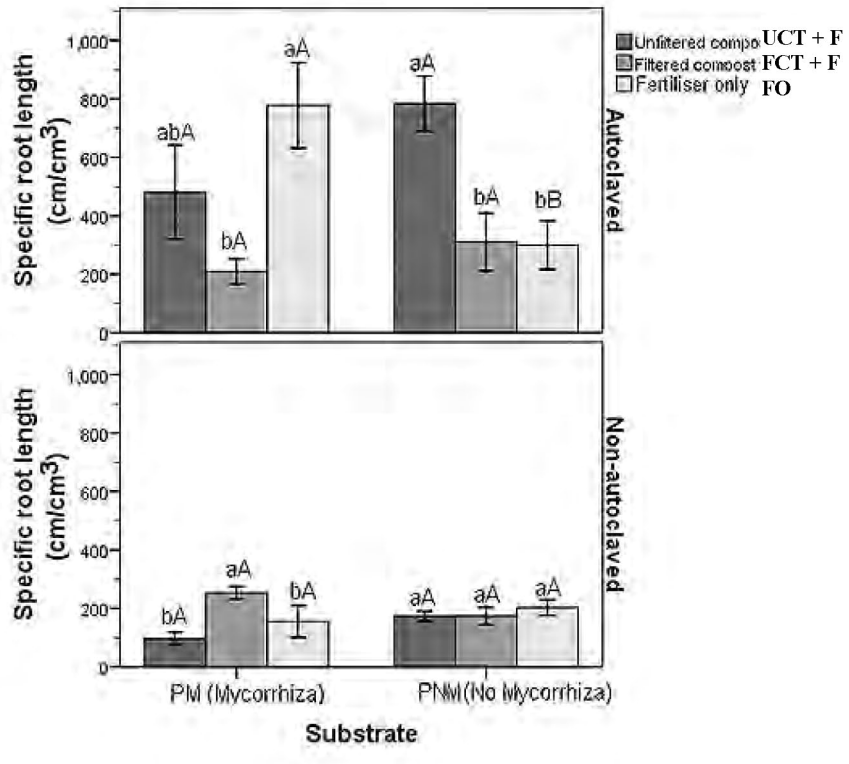

Figure 4

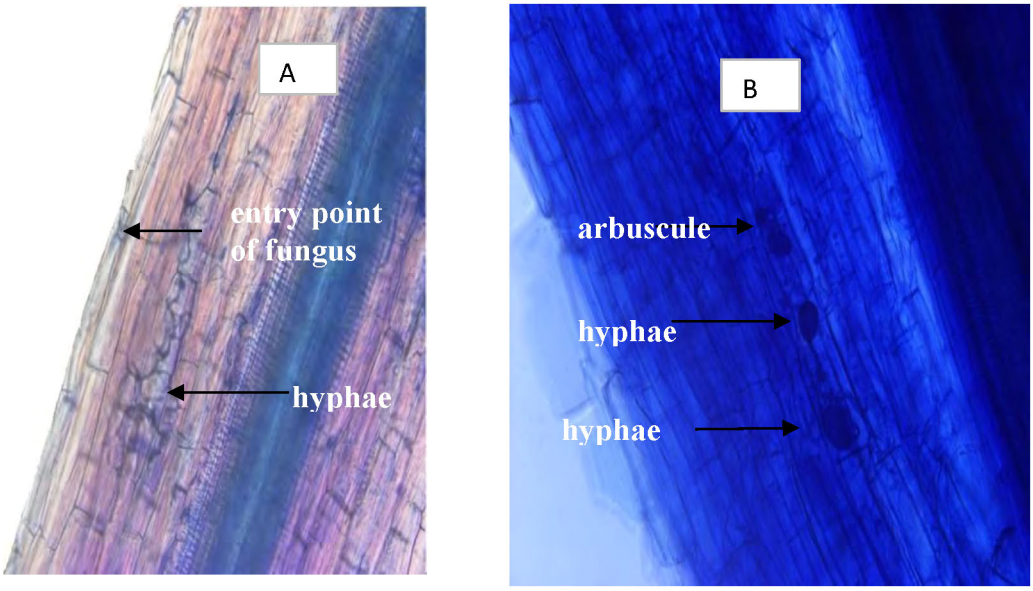

Figure 5