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Full Length Research Paper

Mycorrhizal root colonisation and the subsequent host plant response of soil less grown tomato plants in the presence and absence of the mycorrhizal stimulant, Mycotech

Y. Cwala[1], C. P. Laubscher[1], P. A. Ndakidemi[1]* and A. H. Meyer[2]

[1]Department of Horticultural Sciences, Faculty of Applied Sciences, Cape Peninsula University of Technology, P.O. Box 652, Cape Town 8000, South Africa.

[2]ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa.

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A hydroponic trial was carried out to assess the effect of inoculating tomato plants with AM fungi, in the presence and absence of a mycorrrhizal stimulant (Mycotech). Four treatments were applied: (1) AM inoculant, (2) mycorrhizal stimulant, (3) AM + Stimulant and (4) Stimulant alone. *Arbuscular mycorrhizal* treated plants were 14% root colonized, whilst AM + Stimulant treated plants, were 25% colonized. No colonization was observed in non -AM and non-AM + Stimulant treated plants. However, AM + Stimulant treated plants did not show any significant improvement over AM treated plants, in either the plant nutrient concentrations, except Cu, or the growth of the plants, despite a root colonization advantage. In general, there was also no improvement in colonized plants over non- colonized plants, possibly due to the abundant supply of nutrients. However, Stimulant treated plants performed better than the other plants in terms of their P, K, Ca, Mg and Mn nutrition. The causes of this increase remain unknown. This study has shown that the combined use of mycorrhizal stimulant and AM have a definite advantage for improving root colonization levels, but more research with regard to nutrient supply in the system, is required to ensure further benefits to the plants.

Key words: *Arbuscular mycorrhizal*, dry matter, hydroponics, inoculant, macronutrients, micronutrients, tomatoes.

INTRODUCTION

Hydroponics or soil less production of crops, as opposed to traditional field and greenhouse production in soil arguably represents the most efficient crop production system in terms of nutrient and water use in the world. Tomatoes are currently the biggest soil less grown vegetable crop on a worldwide scale. It is part of the daily diet in many countries, as it constitutes an important source of minerals, vitamins and antioxidants (Grierson and Kader, 1986), Due to the enormous benefits obtained from tomato production worldwide, continuous research and improvement of hydroponic systems is of uttermost importance. Past research on hydroponically grown tomatoes has mainly focused on synchronizing efficient nutrient and water uptake and supply related to yield. In comparison, very little information is available with regard to plant/micro-organism interactions in soil less culture, especially those interactions that are mutually beneficial to both the plant and microbes. The relationship between plant and arbuscular mycorrhizal (AM) fungi, in particular, seems to be of great importance in sustaining an efficient hydroponic system.

Arbuscular mycorrhizal fungi form an integral part of many of the traditional agricultural crop production systems (Baumgartner, 2003), where they colonise plant roots through natural symbiosis (Schubert and Cravero,

*Corresponding author. E-mail: ndakidemip@cput.ac.za. Tel: +2721-460-3196. Fax: +2721-460-3193.

1985), or at times artificially introduced via inoculation. This symbiosis is characterised by a bi-directional exchange of nutrients between the plant and the fungus (Smith et al., 1994), in which the host plant provides carbohydrates (sugars) to the fungus, whereas the fungus provides the host with a range of nutrients, in particular phosphorus, but also other macro- and micronutrients. The benefits rendered to the host plants primarily include enhanced water and nutrient uptake, leading to increased growth and reproduction. Inoculation with AM can also benefit plants by stimulating growth regulating substances, increasing photosynthesis, improving osmotic adjustment under drought and salinity stresses and increasing resistance to pest and disease (Al-Karaki, 2006). Most of the above-mentioned benefits were demonstrated under traditional cultivation practices in soil, although several articles have reported successful root colonisation and subsequent benefits to crops in soil less mediums (Al-Karaki, 2006; Ojala and Jarrel, 1980). However, according to Ojala and Jarrell (1980), their investigation into these reports where soil less ‘’sand cultures’’ were employed, suggests that most, if not all, have involved weekly or twice-weekly application of nutrient solutions. On the contrary, in cases where much stricter application of nutrient solution was involved, little success with regard to mycorrhizal colonization, and rarely any positive response in plants to inoculation, was obtained. This is probably due to the finding that oversupply or high P concentration has been shown to inhibit root colonisation (Brundrett et al., 1996; White and Charvat, 1999), although this has not always been proven, especially under field soil conditions (Schubert et al., 1990). Thus, the methods by which similar benefits, as for soil-based systems, may be achieved in soil less ‘’sand culture’’ systems, are not without any drawbacks, especially where more regular (daily) application of nutrient solutions are involved.

Yet, since AM root colonisation is principally triggered by chemical signals contained in the root exudates, the complimentary use of the product, Mycotech, could supersede the possible inhibitory effects of high nutrients supply, as it has been shown to stimulate AM root colonization, even in its natural habitat in nutrient-rich soils. MYCOTECH comprised of a naturally-occurring botanical compound, iso-flavanone- formononetin, which was originally isolated and identified from clover roots (C. Bender, personal communication, 2009). The added benefit of using this stimulant could possibly render further benefits in plant performance (that is increased plant growth, nutrient uptake), as a result of increased root colonization, which would make inoculation with AM fungi even more feasible under hydroponic conditions.

The aim of this investigation was to conduct a hydroponic experiment to assess the success of inoculating tomato plants with AM fungi, in the presence and absence of a mycorrhizal stimulant (Mycotech), and to quantify possible subsequent growth and nutritional benefits.

MATERIALS AND METHODS

Seedlings and application of treatments

In this experiment, involving hydroponically grown tomato plants, four treatments were applied. One treatment involved inoculation of tomato seeds (Rodade) with a mycorrhizal inoculant MINI PLUG AM SPORE® [Insect Science (Pty) Ltd, South Africa, 26A First Avenue, Tzaneen, South Africa, 0850]. The tomato seeds were supplied by Hygrotech [Hygrotech (Pty) Ltd, Strand 7139, Liquenda, Erf 991, Annandale Pad, Stellenbosch, South Africa, 7600]. The second treatment involved the application of a mycorrhizal stimulant Mycotech [Insect Science (Pty) Ltd, South Africa, 26A First Avenue, Tzaneen, South Africa, 0850] as a medium-drench when seedlings had grown for six weeks. The growth medium was Hygromix® [Hygrotech (Pty) Ltd, Strand 7139, Liquenda, Erf 991, Annandale Pad, Stellenbosch, South Africa, 7600], which was sterilised (gamma-irradiated at a minimum absorbed dose of 25 kGy per kg medium) to ensure a mycorrhiza-free growth medium. The third treatment involved the application of both Mini Plug Am Spore® and Mycotech , at seed and seedling stage, respectively, as for the first and second treatments. These treatments were applied at rates of 0.05 g mini plug AM Spore® per 25 ml size seedling tray hole and 10 ml dissolved Mycotech per seedling (0.05 g Mycotech diluted in 500 ml sterilised distilled water). The fourth treatment (control) received neither the inoculant nor the stimulant. A second dose of treatments were applied at the time when the seedlings (10 cm long) were transferred to 15 cm (upper diameter) free-draining pots (one seedling per pot), containing sterilised (gamma-irradiated at a minimum absorbed dose of 25 kGy per kg) water -rinsed CONSOL® sand [Consol (Pty) Ltd, Germiston 1400, South Africa]. The application rate of the second dose was 0.1 g MINI PLUG AM SPORE® per plant and 20 ml dissolved Mycotech® per plant (0.1 g Mycotech® diluted in 1000 ml sterilised distilled water).

Trial layout

A randomised block design was used to accommodate the four treatments. Each treatment was randomly allocated within each of five block replicates, represented by one row of four plants per block (one plant per pot), each row functioning as an experimental unit.

Hydroponic system

Pot plants were watered via a splash-free drip irrigation sand-based open hydroponic system. The hydroponic system was fitted with an automated time-indoor-controller-pump-system (Vertex VPE 50M.37KW Hunter pump; 4-station Eco-logic indoor-controller), connected to a commercially available 475 litre (600 mm wide × 2000 mm high) Slimline M Nel Tank (Nel Tank cc, Corner of Van Riebeeck and Saxenburg, Blackheath, South Africa, 7600). The nutrient solution was comprised of municipal tap water and a mixture of Hydroponic (CL-FREE) and Calcium nitrate , supplied by Hygrotech . The pH (8.3) and electrical conductivity (EC = 0.1) were measured with a conductivity meter [SM 802 pH/EC/TDS Meter (Spraytech, 34 John and Merriman Street, Bellville, South Africa, 7535)]. The nutrient solution was prepared in accordance with the EC and empirical recipes (prescribed by Hygrotech) and adjusted in accordance with the flowering stages of the plants. The initial pump cycles were set at three times a day at one minute per cycle, allowing *ca* 35 ml nutrient solution per plant. The cycles and running times were subsequently adjusted to accommodate increased growth rates and water use efficiencies of the

plants.

Growth conditions

The experiment was conducted in an environmentally controlled growth tunnel at the Nietvoorbij research farm of ARC Infruitec-Nietvoorbij in Stellenbosch, Cape Town, South Africa. The tunnel (20 × 6 × 2.5 m) was fitted with a white 70% transparent shade cloth (roof cover), a floor heating system, as well as a fan and wet wall, to permit upper limit temperature control between 25 - 27°C. Pots were placed on 30 cm high, tightly-stacked, inverted, rectangle asbestos containers. All possible precautions were taken to minimise the likelihood of contamination.

Data collection and analyses

Sampling

The experiment was terminated when the plants had reached the early fruit set stage. Sampling was carried out by means of a destructive measurement. Three out of the four plants of each replicate per treatment were sampled. Above-growth plant parts were harvested (cut off). Roots were separated from the growth medium by carefully rinsing it in tap water and collecting it with two sieves, that is 1 and 0.25 mm aperture (top to bottom) in preparation for the assessment of mycorrhizal colonisation.

Root colonisation

Roots (*ca* 5.5 g sample) were subjected to clearing and staining procedures as described by Brundrett et al. (1994). Stained root segments of 10 mm long (0.3 - 0.5 mm diameter) were mounted in Polyvinal-Lacto-Glyserol (PVC) on slides, using a fine forceps, to accommodate 25 segments per slide and to prepare four slides per sample. Mounted segments were covered with cover slips. The percentage of root colonisation by mycorrhizal fungi was subsequently calculated as a number of root segments out of 100 identified as colonised under a compound microscope as described by Brundrett et al. (1994).

Root and above-growth mass

Plant weight was measured by weighing the total root and above-growth mass (dry weight) of each plant, separately.

Plant mineral concentrations

Above-growth plant parts (leaves, petioles and stems) of each plant were slowly dried to constant mass in a fan oven at 70ºC, milled and dry ashed in a microwave furnace. The residues were taken up in acidified distilled water, diluted to 100 ml and analysed for P, K and Na using a Varian Liberty 200, inductively coupled plasma atomic emission spectrometer. Nitrogen was determined on the milled plant material using a Leco Nitrogen Determinator [FP-528 series (LECO Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085, USA)].

Statistical analysis

The data (root colonisation, leaf nutrient concentrations, leaf nutrient accumulation, root mass, above-growth plant mass, and total plant mass) were analysed using SAS version 6.12 packages (SAS, 1990). The analyses were performed on observations for four different treatments, replicated five times. The influence of these factors and their interactions were tested with an ANOVA. Student's *t*-LSD (Least Significant Difference) was calculated at the 5% significant level to compare treatment means. Shapiro-Wilks's test was performed to test for non-normality (Shapiro and Wilk, 1965).

RESULTS AND DISCUSSION

A. mycorrhizal treated plants were 14% root colonized, whilst AM + Stimulant treated plants, were 25% colonized. No colonization was observed in non-AM and non-AM + Stimulant treated plants. Although the root colonization level was relatively low in this study, it was still within the percentage range that is in agreement with similar studies involving hydroponically grown tomato plants (Dasgan et al., 2008). The overall low levels of root colonization, in all likelihood, can be due to the abundant supply of plant available nutrients, notably with regard to plant available P (dissolved P or phosphate). Several studies have drawn a link between mycorrhizal receptiveness and plant available P (Bryla and Koide, 1998; Ikiz, 2003; Rehber, 2004; Sari et al., 2001; White and Charvat, 1999) and have found that high P supply often inhibits root colonization. A concentration as low as 7 mg P kg^{-1} soil was shown to inhibit root colonization by AM fungi (Brundrett et al., 1996), although tolerance to much higher concentrations, has also been shown in soil (Plenchette et al., 1983; Schubert et al., 1990). The P level in the present study was exceedingly higher than these limits. Moreover, in soil, P is slowly diffusing and would normally be less available to plants for direct uptake, whereas in nutrient solution in soil less systems, the phosphate form of P is more readily available for easy and direct uptake by roots. Therefore, the fact that the combined use of Mycotech and Mini Plug AM Spore resulted in a significant increase in the root colonization level, suggest that the combined use of these two products could offset the inhibitory effect of high P availability in nutrient solution in soil less culture.

Despite the root colonization advantage of AM + Stimulant treated plants over AM treated plants, there was however, no significant improvement in the macro- and micro-nutrient concentrations and accumulation in the shoots, except for Cu (Tables 1 - 4), where the combined used of Mycotech and Mini Plug AM Spore contributed 25% more to Cu accumulation and concentration in the shoots than AM treated plants (Tables 2 and 4) . Generally, there was also no nutritional improvement in colonized plants over non-colonized plants, possibly due to the nutrient-rich environment to which the roots were subjected to. Due to the emphasis that is normally put on the AM fungus specific P-uptake ability in soil, the lack of differences in P concentration between colonized and non-colonized plants, in the present study, is possibly owing to the absence of diffusion limits for P in hydroponic solution (Hawkins and George, 1997), as was also concluded by Dasgan et al.

Table 1. Effect of treatments on macro-nutrient concentrations of shoots at the start of the fruit ripening stage.

Treatments	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})
AM	27.500b	6.5400b	38.273b	13.6600b	3.9200b*
AM + Mycotech	28.280ab	6.7467b	38.760b	13.5400b	4.1033ab
Mycotech	29.987a	7.3533a	43.293a	15.2867a	4.4067a
Control	27.740ab	6.5067b	38.033b	13.3667b	3.7933b
LSD (P<0.05)	2.4737	0.5983	3.0676	1.549	0.3179

AM = Mycorrhizal inoculant Mini Plug AM Spore; Mycotech = Mycorrhizal stimulant Mycotech. *Means followed by the same letter(s) are not significantly different from each other at P 0.05.

Table 2. Effect of treatments on micro-nutrient concentrations of shoots at the start of the fruit ripening stage.

Treatments	Na (mg kg^{-1})	Cu(mg kg^{-1})	Zn(mg kg^{-1})	Mn (mg kg^{-1})	Fe(mg kg^{-1})	B(mg kg^{-1})
AM	407.87a	2.9667b	59.200a	39.000ab*	147.27a	61.067a
AM + Mycotech	421.53a	3.9333a	57.600a	37.333b	131.67a	57.267a
Mycotech	431.67a	3.2667ab	59.600a	42.333a	155.33a	62.533a
Control	389.40a	3.2000ab	52.400a	36.667b	135.33a	59.333a
LSD (P 0.05)	59.328	0.806	13.086	4.2393	45.317	10.424

AM = Mycorrhizal inoculant Mini Plug AM Spore; Mycotech = Mycorrhizal stimulant Mycotech. *Means followed by the same letter(s) are not significantly different from each other at P 0.05.

Table 3. Effect of treatments on macro-nutrient accumulation in shoots at the start of the fruit ripening stage.

Treatments	N (g plant^{-1})	P (g plant^{-1})	K (g plant^{-1})	Ca (g plant^{-1})	Mg (g plant^{-1})
AM	3.2823a	0.78040ab	4.56640ab	1.62913a	0.46733ab*
AM + Mycotech	3.2291a	0.77017ab	4.42817b	1.54517a	0.46823ab
Mycotech	3.3191a	0.81367a	4.68900a	1.69767a	0.48947a
Control	3.2072a	0.75127b	4.39787b	1.54587a	0.43833b
LSD (P 0.05)	0.2259	0.0573	0.2168	0.2049	0.0406

AM = Mycorrhizal inoculant Mini Plug AM Spore; Mycotech = Mycorrhizal stimulant MYCOTECH. *Means followed by the same letter(s) are not significantly different from each other at P 0.05.

Table 4. Effect of treatments on Micro-nutrient accumulation in shoots at the start of the fruit ripening stage.

Treatments	Na (mg plant^{-1})	Cu (mg plant^{-1})	Zn (mg plant^{-1})	Mn (mg plant^{-1})	Fe (mg plant^{-1})	B (mg plant^{-1})
AM	48.516a	0.35153b*	7.0926a	4.6556a	17.538a	7.2901a
AM+Mycotech	48.103a	0.45160a	6.5727a	4.2600a	14.901a	6.5663a
Mycotech	48.723a	0.35953b	6.5888a	4.7233a	17.276a	7.0391a
Control	45.111a	0.36980ab	6.0801a	4.2343a	15.745a	6.8702a
LSD (P 0.05)	7.8907	0.0899	1.6434	0.5151	5.9831	1.464

AM = Mycorrhizal inoculant Mini Plug AM Spore; Mycotech = Mycorrhizal stimulant Mycotech. *Means followed by the same letter(s) are not significantly different from each other at P 0.05.

(2008) in their research.

Interestingly, however, Mycotech treated plants, performed generally better than the plants associated with the other treatments in terms of their N, P, K, Ca, Mg

Table 5. Effect of treatments on shoot, root and total (shoot + root) dry weight at the start of the fruit ripening stage.

Treatments	Shoot (kg)	Root (kg)	Shoot + Root (kg)
AM	0.096567a*	0.022693a	0.119260a
AM + Mycotech	0.094900ab	0.019617a	0.114517a
Mycotech	0.088767b	0.022820a	0.111587a
Control	0.094460ab	0.021567a	0.116027a
LSD ($P \leq 0.05$)	0.007400	0.006400	0.009000

AM = Mycorrhizal inoculant Mini Plug AM Spore; Mycotech = Mycorrhizal stimulant Mycotech. *Means followed by the same letter(s) are not significantly different from each other at *P* 0.05.

and Mn nutrition (Tables 1 and 3), and to a similar extent, also in terms of shoot nutrient accumulation (Tables 2 and 4). Mycotech is comprised of a naturally-occurring botanical compound, iso-flavanone-formononetin, which was originally isolated and identified from clover, roots (C. Bender, personal communication, 2009). The chemical contribution of this stimulant to plant nutrition should thus, not be underrated and possible solitary use of MYCOTECH should be considered. However, more in depth research is required to shed more light on this finding.

Dasgan et al. (2008) noted in their investigation on soil and open soil less systems with different plants species that AM root colonisation is routinely accompanied with plant growth increases. However, contrary to these reports, they found out in their investigation that, plant growth was not significantly increased in tomato plants. Likewise, in the present study there was no significant growth improvement in response to the fairly low levels of root colonization observed in colonized plants over non-colonized plants, either in terms of shoot, root or total plant weight (Table 5) . This is in all likelihood due to an abundant supply of P causing the C-costs to the host to outweigh any benefits from colonization (Ryan and Graham, 2002). Neither was there any significant growth improvement in AM + Stimulant treated plants over AM treated plants, despite having a significant root colonization advantage of 9%. Since increased uptake of P was found to be the primary reason for increased growth in colonized plants (Gianinazzi-Pearson and Gianinazzi, 1983), the general lack of positive plant growth response (plant weight) could be ascribed to the lack of differences in P concentration between AM colonized and non-colonized plants.

Furthermore, since responsiveness to mycorrhzal root colonization could potentially occur at different stages of plant development in tomatoes (Bryla and Koide, 1988), and since measurements were taken only at the early fruit ripening stage by means of destructive measurement, it is not certain whether responses to colonization, either nutrient or growth responses, could have occurred either during earlier stages or even later stages (beyond the fruit ripening stage) of plant development. However, the likelihood of this occurring in the present study is nevertheless very small.

CONCLUSION

AM root colonization of tomato plants is obtainable under sand-based hydroponic systems. The supplementary use of the mycorrhizal stimulant, Mycotech was shown to increase the level of root colonization, despite an abundant supply of nutrients, but no benefit to the plants because of AM inoculation, and no added benefit because of the addition of the stimulant, could be quantified. Combined use of Mycotech and AM inoculation under minimum-fertiliser-input in sand-based hydroponic systems seems to be a more feasible prospect. The prospect for solitary use of Mycotech, for nutritional benefits, requires further investigation. This study is preliminary in nature and due to its limited scope, further research is required.

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