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Full Length Research Paper

The potential of endomycorrhizal fungi in controlling tomato bacterial wilt *Ralstonia solanacearum* under glasshouse conditions

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The impact of colonization by three mycorrhizal fungi on tomato bacterial wilt caused by *Ralstonia solanaceraum* was investigated. Three species of arbuscular mycorrhizal fungal (AMF) were tested (*Glomus mosseae*, *Scutellospora* sp. and *Gigaspora margarita*). Siginificant differences in tomato growth based on plant hieght was recorded between *G. mosseae* (125.25 cm) and all treatments. The combination of *G. mosseae* and *R. solanacearum* resulted in significantly taller tomato plants than *G. margarita* + *R. solanacearum* and *Scutelospora* sp.+ *R. solanacearum*. Shoot fresh and dry weight was higher in *G. mosseae* inoculated plants. No disease symptoms were observed in the combination treatment of *G. mosseae* and *R. solanacearum.* The plants treated with *Scutellospora* sp. showed low incidence of infection (105, 15%) at 15 and 20 days after inoculation, respectively. The combination of *G. mosseae* and *R. solanacearum* resulted in more increase in root morphology (root tips (434.75), root length (267.00 cm), root surface area (149.31 cm^2), root volume (3.77 cm^3), root fresh weight (4.75 g) and root dry weight (2.5 g). The treatment of *G. mosseae* + *R. solanacearum* was different significantly when compared to *G. margarita* and *Scutellospora* sp. + R. *solanacearum* treatments in all parameters considered. The highest number of AMF spores was recorded in *G. mosseae* treatment followed by *Scutellospora* sp. The concentration of N, P and K in *G. mosseae* + *R. solanacearum* treatment was significantly higher (N: 1.69; P: 0.51 and K: 1.65%) compared to *G. margarita* (N: 1.06 ; P: 0.11 and K: 1.02%) and *Scutellospora* sp., treatment (N: 1.48; P: 0.44 and K: 1.47%). Generally, the current findings has provided an evedance about the ability of AMF species to control bacterial wilt causal agents with significant differences between the species used.

Key words: Bio-control, wilt disease, tomato, *Glomus mosseae.*

INTRODUCTION

Vegetable crops are highly prone to root and soil borne diseases causing great losses in yield and quality (Sharma et al., 2004). The limiting factor for tomato (*Lycopersicon esculentum* Mill) production in many parts of the world is due to plant diseases. It has been estimated that there are around 200 known tomato diseases (Jones, 2008). Bacterial wilt disease caused by *Ralstonia solanacearum* (*Pseudomonas solanacearum*, E. F. Smith) (Gianinzzi-Pearson ,1988) is a major problem and constraint for vegetable growers especially tomato farmers in the lowland, highland tropics and worldwide (Abdullah, 1988; Hayward, 1991; Tahat et al., 2010). Estimates of yield loss due to the disease range from 15 to 95% (Kelman, 1997).

R. solanacearum is a complex species with a large heterogeneous group of related strains; it has been subdivided into five races based on host range and five biovars based on the biochemical properties (Hayward,

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Abbreviation: AMF, Arbuscular mycorrhizal fungal.

1991). Many studies on the resistance of plants to soil-borne pathogens were well reported on *Phytophthora parastica* (Dassi et al., 1998; Vigo et al., 2000) and *Fusarium solani* (Abdalla and Abdell-Fattah, 2000). The use of resistant, or tolerant varieties, cultural practices, chemical control and biological control are commonly employed methods to control bacterial wilt disease (Persley, 1986; Dalal et al., 1999). Biological control of soil borne pathogen is gaining much attention in recent years due to the effect of many chemicals used to control the disease (Haas and Défago, 2005). Among the potential microorganisms, attention has been given to the arbuscular mycorrhizal fungi (AMF) (Sharam and Johri 2002; Tahat et al., 2010a). AMF are associated with most agricultural crops and provide protection against soil-borne pathogens (Sharma et al., 2004). AMF are a group of microorganisms which effectively reduce the root diseases caused by a number of soil-brone pathogen (Liderman, 1992, 1994; Trotta et al., 1996). The interactions between mycorrhizal fungi and plant pathogens study began in early 1970's, as inhibitory factor on plant pathogens and in reducing the disease influence (Baath and Hayman, 1984; Azcon-Aguilar and Barea, 1996). Number of mechanisms are involved in controlling and suppression of the pathogen by mycorrhizal fungi roots such as exclusion of pathogen, changed P nutrition, lignifications of cell wall, and exudation of low molecular weight compounds. (Sharma et al., 2004; Tahat et al., 2011, 2009).

Different species of AMF lead to different plant responses for the colonization and different effects on pathogens (Pankova et al., 2011; Tahat et al., 2010b). The main objective of the present work was to determine the efficiency of a few mycorrhizal fungi species [*Glomus mosseae* (Nicol and Gerd.) Gerd and Trappe, *Scutellospora* sp. (Walker and Sanders), and *Gigaspora margarita* (Becker and Hall)] in controlling or suppressing tomato bacterial wilt disease.

MATERIALS AND METHODS

Microorganisms

The primary inoculum of *R. solanacearum* race 2 biovar 3 was obtained from the Laboratory of Microbiology, Faculty of Agriculture, Universiti Putra Malaysia. The bacterial inocula was confirmed as race 2 biovar 3 by applying the molecular technique described by Khakvar et al. (2008).

Pathogenicity test

Two weeks old tomato (red rock) seedlings were inoculated with bacterial suspension at the concentration of (10^7 ML^{-1}) colony forming unit (CFU). Stem puncture technique described by Jenkins and Kelman (1976) was followed. The seedlings were covered with plastic bags to increase the relative humidity, and it was kept in glasshouse conditions (25 to 30°C) for 24 h. Bacterial wilt symptoms were observed starting from three days after inoculation. Disease progress was evaluated for the plant six times starting from three days after inoculation until 15 days of incubation. The development of the disease symptoms was rated by using the scale of Kempe and Sequeira (1983) (0, no disease symptoms; 1, 1 to 25% of plant leaves wilted; 2, 26 to 50% of plant leaves wilted except the top two or three leaves; 3, 51 to 75% of plant leaves wilted; 4, plant died).

Based on the following formula, the percentage of disease severity was calculated:

$$\text{Disease severity (\%)} = \frac{X_1 + X_2 + \ldots + X_n}{Y \times \text{Maximum rating scale}} \times 100 \quad (1)$$

where, X = score of disease severity of each seedling, and Y = total number of tested seedlings

Bacterial identification

Streaming test

An infected plant was tested by taking sections from the lower part of tomato stem and placed in a test tube filled with distilled water. The presence of oozing milky exudates from the cut stem section showed that the pathogen surly was of *R. solanacearum* (Goszczynska et al., 2000).

Solubility test

A few drops of potassium hydroxide (KOH) were placed on a clean slide. Smeer of bacterial colony grown on media was taken and mixed well with KOH. Mucoid thread was seen clearly when loop from the media was taken indicating that *R. solanacearum* was Gram negative (Goszczynska et al., 2000).

Bacterial isolation

Casamino acid peptone glucose (CPG) agar media was prepared (Cuppels et al., 1978) Tissues from infected tomato plant were sterilized using Clorox (0.5%) for a few seconds, and then transferred to the CPG media plates. The plates were incubated at 28°C ± 2 for 24 h. Typical colonies (white, fluid, irregular and round) of *R. solanacearum* were observed. On tetrazolium media (TZC), *R. solanacearum* colonies appeared as pink diffused colour (Kelman and Person, 1961). This is another indicator that the pathogen is *R. solanacearum*. Stock culture of *R. solanacearum* was preserved in sterilized distilled water and stored at 4°C in screw cap bottle for two months.

Glasshouse experiment

Serdang series soil

Serdang series soil show fairly consistent morphological properties. The textures range from fine sandy loam, sandy loam, to fine sandy

clay loams. The soil is fine loamy, siliceous, isohyperthermic and red-yellow. This soils have a deep kandic horizon with between 18 and 35% clay content. The Serdang series can be classified as Haplic Nitisols (Paramananthan, 2000).

Soil preparation

The soil used in this experiment was Serdang series soil collected from the campus of Universiti Putra Malaysia. The soil was sieved using 5 mm pore sieve. The sieved soil was mixed with sand at the ratio of 3:1 (v/v soil: sand). Nutrients analysis of soil showed the following results:

pH, 5.5; N, 0.13%; P, 0.023%; K, 0.30%; Ca, 0.063%; Mg, 0.034%; S, 0.063%; Fe, 1.52%; Mn, 0.0034%; Zn, 0.0057%; Mo, 0.00064%; B, 0.0003%; Cu, 0.0015% (Sharifuddin, 1981). The soil mixture was autoclaved at 121°C for 1 h and 15 lb/inch^2 (1.055 kg/cm). The soil mixture was filled in clean plastic pots (25 cm diameter).

AMF preparation and growth conditions

G. mosseae and *Scutellospora* sp. were local species taken from the Laboratory of Soil Microbiology, Faculty of Agriculture, Universiti Putra Malaysia (Tahat et al., 2008). *G. margarita* was introduced from Indonesia. These three species were cultured in pots using corn (*Zea mays*) culture in glasshouse for three months and stored under laboratory conditions at 15 to 20°C. Wet sieves technique was used to isolate and purify the AMF spores (Phillips and Hayman, 1970). Mature and healthy spores were isolated and collected from the pot culture. 100 spores/100 gm dry soil were added to the pots and mixed well with the soil before planting of tomato.

A commercially, recommended, and certified tomato seeds (red rock) was used. The seeds were surface sterilized with 90% ethyl alcohol for 10 s, and washed with sterile distilled water. Three seeds were sowned directly into each pot. Two weeks later, the seedlings were thinned to one seedling/pot. Plants were kept under glasshouse conditions at around 25 to 30°C ± 2. *R. solanacearum* was re-cultured from stock culture. CPG media described by Cuppels et al. (1978) and TZC media described by Kelman and Person (1961) were used. Suspension of *R. solanacearum* was prepared at concentration of $10^7 ML^{-1}$ CFU and inoculated to tomato roots 30 days after planting.

Evaluation of plant development and root architecture

Plant height was recoreded weekly starting from the fifth week until the 10 week of plant growth. Disease progress was calculated for the seedlings every five days up to 20 days of incubation. The development of disease symptoms was rated using the scale described by Kempe and Sequeira (1983). Root and shoot fresh weight was recorded directly using accurate balance. Roots dry weights were measured at the end of this experiment. Root dry weight was measured for each single plant after oven drying (70°C) for 24 h. The root image analyser (Model Epson Expression 1680) was used for root morphology studies which included: root length, total root volume, root tips, and total root surface area. Root colonization percentage was assessed for adventitious and lateral root colonization according to Phillips and Hayman (1970). The roots were cleaned in 10% KOH and stained with 0.05% trypan blue in lacto phenol. The percentage of root colonization was measured using the techniques designed by Giovannetti and Mosse (1980).

Shoots nutrients analysis

Wet ashing method described by Sharifuddin (1981) was followed. Dried plant shoot (0.25 g) were grinded in a conical flask. 5 ml of 100% sulfuric acid (H_2SO_4) were added to each sample. The samples were heated on a hot plate at (450°C for 15 min) for sample digestion. 10 ml of concentrated H_2O_2 were added to each sample and heated at (450°C for seven minutes). The N, P, and K contents were measured using auto analyzer machine.

Soil spores number

AMF spores were collected from the soil by wet sieving and decanting technique (Gerdemann and Nicolson, 1963) and the spores were counted visually under binocular-microscope (INVAM, 2000).

Experimental design

Eight treatments were arranged in randomized complete block design (RCBD) with pots for each treatment. *G. mosseae* (GM), *Scutellospora* sp. (SS), G*. margarita* (GIM), Control (C), *Ralstonia solanacearum* (RS), *G. margarita* + *R. solanacearum* , *Glomus mosseae* + *R. Solanacearum* and *Scutellospora* sp. + *R. solanacearum*.

Data analysis

The data were subjected to an analysis of variance using SPSS 15.0 software (SPSS Inc. Chicago, USA).

RESULTS

Tomato roots were well colonized to produce a huge number of spores in the soil (Figure 1). *G. mosseae* and *Scutellospora* sp. produced significant number of spores compared to *G. margarita*, *G mosseae* + *R. solanacearum* and *Scutellospora* sp. + *R. solanacearum* (Figure 1).

G. mosseae and *Scutellospora* sp. can colonize tomato roots better than *G. margarita* (Figure 2). In the presence of *R. solanacearum*, root colonization by *G. mosseae* was significantly reduced. However, root colonized with by *G. margarita* was not affected by the presence of *R. solanacearum*.

Plant height

Significant differences in tomato plant height were recorded between *G. mosseae*, *G. mosseae* + *R. solanacearum* and *Scutellospora* sp. After six weeks of

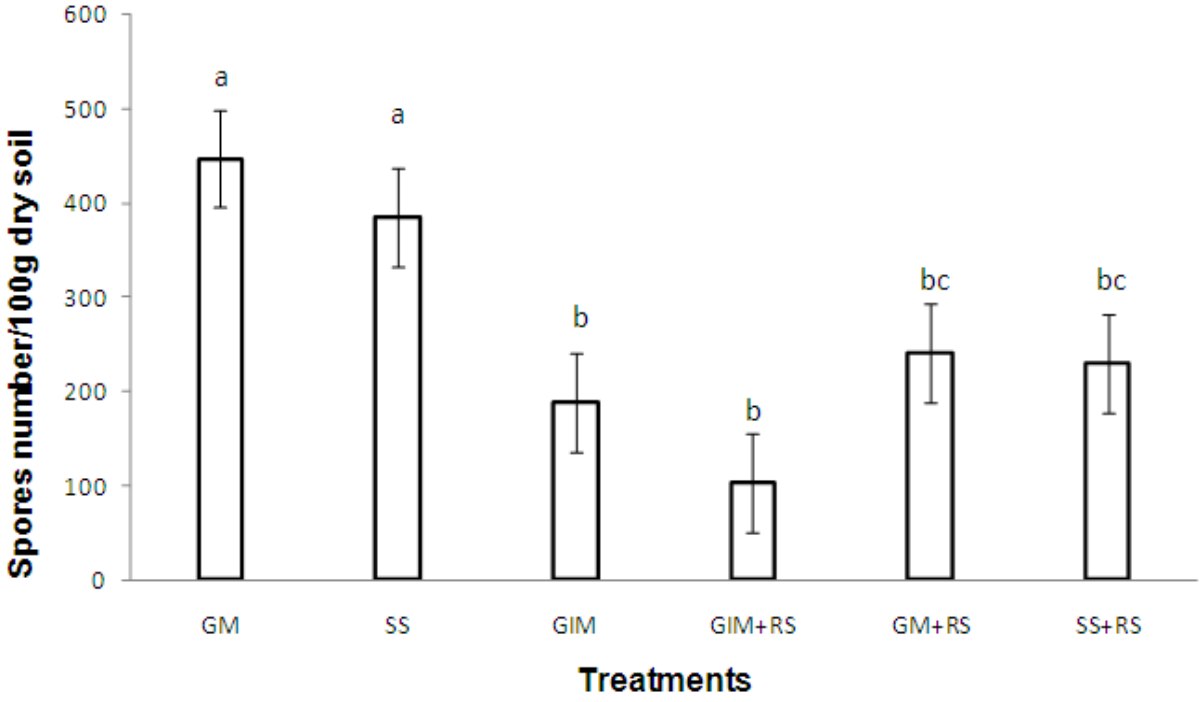

Figure 1. Effects of *R. solanacearum* on the spore production of GM, *G. mosseae*; SS, *Scutellospora* sp.; GIM, *G. margarita* 10 days after inoculation with initial inoculums (100 spore/100 g dry soil). Means followed by the same letter are not significantly different at P>0.05 level according to the Tukey honest significant difference (HSD).

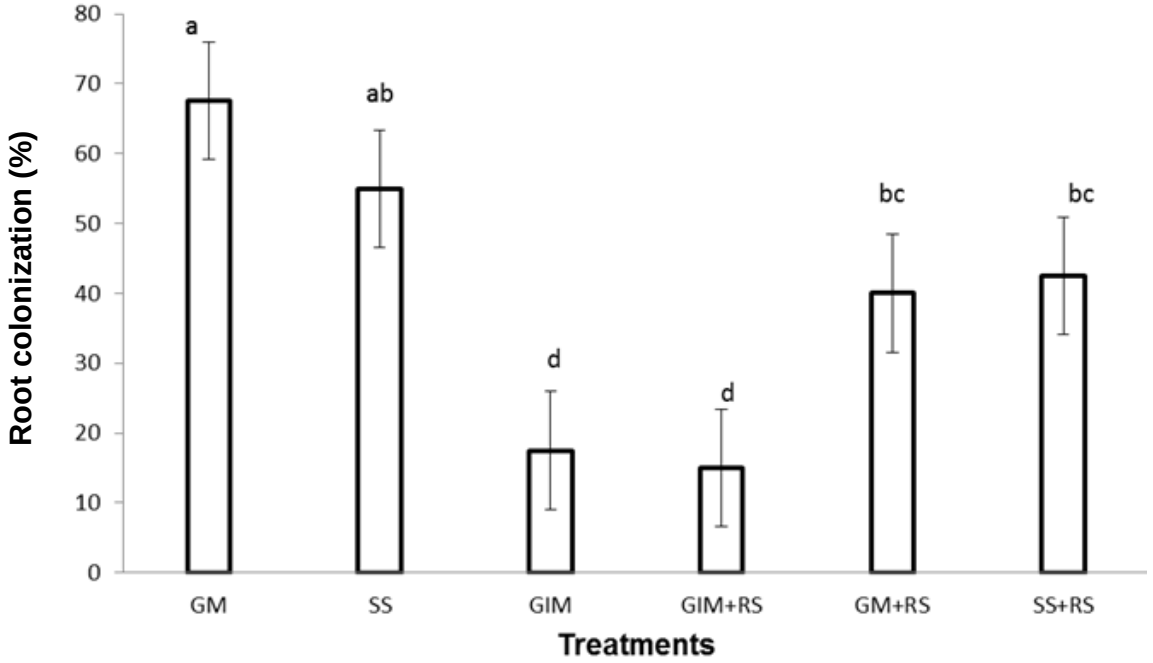

Figure 2. Effects of *R. solanacearum* (RS) on the tomato root colonization percentage inoculated by GM, *G. mosseae*; SS, *Scutellospora* sp.; GIM, *G. margarita* after 10 weeks of inoculation; C, control. The means followed by the same letter are not significantly different at P>0.05 level according to the Tukey honest significant difference (HSD).

Table 1. Effect of AMF and R. solanacearum inoculation on tomato plant height within 7 weeks of growth started from 4th to 10th week.

Treatment	Plant height (cm)						
	4th week	5th week	6th week	7th week	8th week	9th week	10th week
GM	25.25	36.5^{a}	51.25^{a}	68.25^{a}	88.50^{a}	113.5^{a}	125.25^{a}
SS	23.75	28.75^{b}	34.0^{b}	54.25^{b}	69.25^{b}	89.25^{b}	96.25^{b}
GIM	28.0	32.75^{ab}	37.50^{ab}	44.25^{c}	56.25^{cde}	71.00^{c}	83.5^{c}
C	27.75	33.75^{ab}	39.75^{ab}	45.25^{c}	58.0^{bcd}	72.25^{c}	82.0^{c}
RS	23.5	30.50^{ab}	34.25^{b}	38.0^{d}	45.5^{ef}	na0.00^{e}	0.00^{e}
GIM + RS	26.0	30.50^{ab}	34.25^{b}	38.75^{d}	43.25^{f}	na0.00^{e}	0.00^{e}
GM + RS	25.0	30.0^{b}	37.75^{ab}	47.75^{bc}	63.25^{bc}	82.75^{ab}	94.25^{b}
SS+ RS	26.25	29.25^{b}	34.00^{b}	43.75^{c}	50.25^{def}	61.25^{d}	72.0^{d}

Means within columns followed by the same letter are not significantly different at P≤0.05 level using Tukey test honest significant difference (HSD). GM, *G. mosseae*; SS, *Scutellospora* sp.; GIM, *G. margarita*; C, control; RS, *Ralstonia solanacearum.*

Table 2. Disease severity on tomato inoculated with *R. solanacearum*, following inoculation with AMF after 5, 10, 15, and 20 days.

Treatment	5 day	10 day	15 day	20 day
RS	0.00	15.00^{a}	27.50^{a}	55.00^{a}
GIM + RS	0.00	0.00^{b}	22.50^{ab}	40.00^{b}
GM + RS	0.00	0.00^{b}	0.00^{b}	0.00^{c}
SS + RS	0.00	0.00^{b}	10.00^{b}	16.25^{d}

Means within columns followed by the same letter are not significantly different at P>0.05 level using Tukey test honest significant difference (HSD). GM, *G. mosseae*; SS, *Scutellospora* sp.; GIM, *G. margarita*; RS, *Ralstonia solanacearum*.

inoculation, *G. mosseae* treated plants showed significant (51.25 cm) differences in plant growth compared to *Scutellospora* sp. (34 cm), *R. solanacearum* (34.25 cm), *G. margarita* + *R. solanacearum* (34.25 cm) and *Scutellospora* + *R. solanacearum* (34 cm). After seven weeks, plants inoculated with *G. mosseae* were growing faster than all other treatments (Table 1).

After eight weeks, the plants treated with *G. mosseae* exhibited the most vigorous growth (88.50 cm). Plants inoculated with *G. mosseae* + *R. solanacearum* gave faster (63.25 cm) plant growth compared to plants treated with *R. solanacearum* (45.5 cm) and other dual treatments. After nine weeks, *G. mosseae* showed significant (113.5 cm) plant growth compared with *R. solanacearum* which inhibited the plant growth statistically (82.75 cm). Plants treated with *R. solanacearum* were completely wilted (Table 1).

10 weeks after inoculation, *G. mosseae* plants where statistically the tallest (125.25 cm) among the treatments. Similarly, plants inoculated with *Scuttelspora* sp. also gave statistically (96.25 cm) better plant growth compared to the control. *G. margarita* did not inhibit the infection by *R. solanacearum* as the plants wilted after nine weeks (Table 1).

Disease severity

Tomato inoculated with *R. solanacearum* showed disease severity of about 15, 27, and 55% after 10, 15, and 20 days, respectively (Table 2). However, no disease symptoms were observed in *G. mosseae* + *R. solanacearum* treated plants. Plant treated with *Scutellospora* sp. + R*. solanacearum* did not show any disease infection after 10 days of inoculation. Plant treated with *G. maragarita* showed 0, 22 and 40% disease severity at 10, 15 and 20 days after inoculation, respectively. *G. mosseae* was able to prevent infection by *R. solanacearum* (Table 2).

Root morphology

Root growth parameters were significantly better in *G. mosseae* plants compared to plants inoculated with *G. margarita*, *Scuttelspora* sp., and the control (Table 3).

Table 3. Effect of AMF and *R. solanacearum* on tomato roots growth characteristics after 10 weeks.

Treatment	RT (No.)	RL (cm)	RSA (cm^2)	RV (cm^3)
GM	2258.25^{a} ±111.16	1187.50^{a} ± 151.29	435.65^{a} ± 23.35	16.19^{a} ± 1.58
SS	1594.00^{b} ± 285.93	822.96^{b} ± 94.3	372.72^{ab} ± 33.84	14.26^{a} ± 1.55
GIM	1122.25^{c} ± 99.58	337.61^{cde} ± 19.72	227.75^{dc} ± 25.82	10.60^{a} ± 1.75
C	1105.50^{c} ± 139.87	354.42^{cde} ± 48.53	250.92^{c} ± 52.67	11.36^{a} ± 2.68
RS	214.00^{d} ± 51.21	193.34^{e} ± 91.33	96.61^{e} ± 35.51	3.48^{b} ± 0.88
GIM + RS	434.75^{d} ± 150.17	267.00^{de} ± 48.11	149.31^{e} ± 17.33	3.77^{b} ± 0.67
GM + RS	1020.50^{c} ± 355.19	452.17^{c} ± 49.75	303.76^{bc} ± 33.18	11.83^{a} ± 2.52
SS + RS	960.00^{c} ± 197.65	412.47^{dc} ± 41.65	275.90^{c} ± 50.79	10.53^{a} ± 2.48

Means within columns followed by the same letter are not significantly different at P > 0.05 level using Tukey honest significant difference (HSD). GM, *G. mosseae*; SS, *Scutellospora* sp.; GIM, *G. margarita*; RS, *Ralstonia solanacearum*. RT, root tips; RL, root length; RSA, root surface area; RV, root volume.

Table 4. Effect of AMF and *R. solanacearum* on tomato after 10 weeks of planting.

Treatment	RFW g/plant	RDW g/plant	SFW g/plant	SDW g/plant
GM	22.19^{a}	2.84^{d}	31.68^{d}	7.25^{a}
SS	19.33^{a}	2.16^{cd}	28.63^{cd}	4.95^{b}
GIM	11.14^{b}	1.71^{bc}	19.57^{b}	3.00^{c}
C	10.71^{b}	1.49^{bc}	18.77^{ab}	3.57^{c}
RS	2.40^{c}	0.28^{a}	8.10^{a}	1.07^{d}
GIM + RS	4.75^{c}	0.94^{ab}	12.19^{a}	1.65^{d}
GM + RS	13.55^{a}	2.53^{d}	23.401^{b}	3.75^{c}
SS + RS	11.75^{b}	1.89^{bc}	20.05^{b}	2.82^{c}

Means within columns followed by the same letter are not significantly different at P>0.05 level using Tukey honest significant difference (HSD). GM, *G. mosseae*; SS, *Scutellospora* sp.; GIM, *G. margarita*; RS, *Ralstonia solanacearum*. RFW, Root fresh weight; RDW, root dry weight; SFW, shoot fresh weight; SDW, shoot dry weight; AMF, arbuscular mycorrhizal fungal.

G. mosseae inoculated plants had significantly higher number of root tips compared to *Scutellospora* sp., *G. margarita* and control (Table 3). Root length was also significantly longer in the *G. mosseae* treatment compared with *G. margarita*, *Scuttelspora* sp. and the control treatments. The root surface area was also significantly larger in *G. mosseae* inoculated plant than those inoculated with *G. margarita* and the control. However, the root volume parameter did not show any significant differences between the three AMF and the control.

Tomato root inoculated with *R. solanacearum* showed a significant reduction in root tips, root surface area and root volume compared to the control (Table 3). *G. mosseae* and *Scutellospora* sp. significantly reduced the effect of the disease based on root morphology recorded in the combination treatments (*G. mosseae* + *R. solanacearum* and *Scutellospora* sp.+ *R. solanacearum*). However, *G. margarita* was not able to reduce the wilt effect of *R. solanacearum* (Table 3).

Plant biomass

Shoot fresh and dry weight were higher in *G. mosseae* compared to other treatments (Table 4). Shoot fresh weight was higher in *G. mosseae* + *R. solanacearum* treatment compared with *G. margarita* + *R. solanacearum* and *Scutellospora* + *R. solanacearum*, but it was not different significantly. On the other hand, shoot dry weight was more in *G. mosseae* + *R. solanacearum* and it was varied significantly compared with *G. margarita* + *R. solanacearum* and *Scutellospora* sp., + *R. solanacearum*) treatment plants (Table 4).

Plants inoculated with *G. mosseae* + *R. solanacearum* and *Scutellospora* were not significantly different in root fresh weight and root dry weight (Table 4). The treatment of *G. mosseae* + *R. solanacearum* and *Scutellospora* sp., + *R. solanacearum* was significantly different in fresh root weight compared to *G. margarita* + *R. solanacearum*, *R. solanacearum* and the control (Table 4). Finally, tomato plants treated with *G. margarita* + *R. solanacearum*,

Table 5. Effect of AMF and *R. solanacearum* on the macro and micro nutrients contents of tomato shoots after 10 weeks of plant growth

Treatment	N%	P%	K%	Fe mg/kg^{-1}	Zn mg/kg^{-1}
GM	1.94^{a} ± 0.25	0.99^{a} ± 0.35	2.09^{a} ± 0.17	96.72^{a} ± 9.24	51.47^{a} ± 4.20
SS	1.59^{bc} ± 0.07	0.73^{ab} ± 0.09	1.65^{b} ± 0.05	77.10^{b} ± 5.28	38.42^{bc} ± 3.86
GIM	1.39^{bc} ± 0.07	0.46^{bc} ± 0.08	1.35^{c} ± 0.05	64.68^{b} ± 5.39	33.25^{d} ± 2.28
C	1.37^{c} ± 0.13	0.42^{bc} ± 0.11	1.40^{c} ± 0.10	66.96^{b} ± 4.77	38.58^{bc} ± 4.43
RS	0.92^{e} ± 0.14	0.11^{c} ± 0.04	0.78^{d} ± 0.11	38.14^{c} ± 7.03	26.07^{de} ± 3.44
GIM + RS	1.06^{de} ± 0.14	0.11^{c} ± 0.05	1.02^{d} ± 0.35	47.27^{c} ± 7.81	22.66^{e} ± 4.56
GM + RS	1.69^{ab} ± 0.04	0.51^{b} ± 0.18	1.65^{b} ± 0.05	65.69^{b} ± 8.06	46.12^{ab} ± 3.97
SS + RS	1.48^{bc} ± 0.06	0.44^{bc} ± 0.18	1.47^{bc} ± 0.47	63.95^{b} ± 4.86	36.48^{c} ± 9.63

Means within columns followed by the same letter are not significantly different at *P>0.05* level using Tukey honest significant difference (HSD). GM, *G. mosseae*; SS, *Scutellospora* sp.; GIM, *G. margarita*; C, control; RS, *R. solanacearum*.

G. mosseae + *R. solanacearum, R. solanacearum* and *Scutellospora* sp. + R. *solanacearum* were not significantly different in tomato root dry weight.

Minerals concentration

N, P and K concentration in plant shoots was statistically increased due to mycorrhizal colonization (Table 5). The highest amount of N (1.94%) was recorded in *G. mosseae* treated plant but it was significantly different when compared with *Scutellospora* sp. (1.59%) and *G. margarita* (1.39%). The plants colonized by complex inoculums *G. mosseae* + pathogen were not different statistically when compared with *Scutellospora* sp., and *G. margarita* treated plants.

Plants treated with the pathogen were unable to absorb N in a significant amount compared to mycorrhizal plant treatments. The inoculation with *G. mosseae* and *Scutellospora* sp., led to significant concentrations of P in plant shoot compard to all other treatments (Table 5). The P content in the plant shoot infected by *R. solanacearum*, *G. margarita* + *R. solanacearum*, control, *G. margarita* and *Scutellospora* sp. + R. *solanacearum* was not different statistically. In the tomato shoots, *R. solanacearum* treated plant had lower K concentration in comparison with all other treatments except *G. margarita* + *R. solanacearum* (Table 5).

Shoot micronutrients (Fe and Zn) contents were the highest in *G. mosseae* treatment (96.72 and 51.47 mg/kg, respectively). Fe contents in *R. solanacearum* treated shoots were the lowest (38.14 mg/kg) but not significantly different from *G. margarita* + *R. Solanacearum*. *G. mosseae* and *Scutellospora* sp treatments were the highest in Fe availability both were different significantly. Shoot analaysis of *R. solanacearum* and the complex treatment of *G. margarita* + *R. solanacearum* were contain the lowest concentration of Zn but both treatments were not significantly different from *G. margarita* treatment (Table 5).

DISCUSSION

Our results agree with those reported by Karaginnidis et al. (2002) and Tahat et al. (2008). They documented that tomato plant responded positively to AMF inoculation. Mycorrhizal inoculation before the pathogen attack has been reported to provide plant biological protection (Hwang et al., 1992). The plant height was suppressed by the pathogen in the treatments inoculated with *G. margarita* but in complex inoculation treatments, *G. mosseae* + *R. solanacearum* and *Scutellospora* sp. + R. *solanacrearum*, the inhibition were less significant. Karaginnidis et al. (2002) reported that the inoculation of tomato and eggplant seedlings with the AMF *G. mosseae* significantly increased shoot fresh and dry weight, and it was also found that the combination of the AMF and the pathogenic fungus significantly reduced the height and fresh weight in eggplant and tomato.

Root morphology system change induced by AMF makes root less susceptible to infection by the pathogen or inhibiting its spread. In the presence of *G. mosseae,* the pathogen was inhibited. Similar results reported that using *Glomus versiforme* (Zhu and Yao, 2004) resulted in a significant reduction of *R. solanacearum* infection. *G. mosseae* was documented to provide a bio-protection from *Phytophthora parasitica* in tomato plants (Cordier et al., 1998). Plants colonized by the AMF increased the number of root branching (Tahat et al., 2008).

The effect of AMF in plant shoot was reported in by many researchers (Dehne, 1982; Olsson et al., 1999, Ryan and Graham, 2002). The significance different between treatments on shoot weights can be explained

by the ability of mycorrhizal plant to uptake more essential nutrient from the soil. The availability of the pathogen in plant xylem is the reason behind the weak growth of shoot in pathogen and pathogen + *G. margarita* treatments. The imported mycorrhizal species was unable to adapt with Malaysian undisturbed conditions, so the growth of plant shoot and root was not well compared to the local adapted species used. The study results are in conflict with other researchers' finding (Maherali and Klironomos, 2007), they demonstrated that AMF under semi-arid conditions is very efficient in establishment plant on disturbed soil (Jamil et al., 2003).

The fast growth of AMF plant can be explained by the ability of mycorrhizal plant to increase the uptake of nutrients such as N, P and K from the soil. The AMF enhanced the resistance of tomato against the pathogen in *G. mosseae* + *R. solanacearum* and *Scutellospora* sp. + R. *solanacearum* treatments. The current results agree with the results published by Dehne (1982), he found that the growth of the plant inoculated with the AMF and the pathogens were more resistant to the pathogen due to the more nutrients absorbed from the soil. One of the most acceptable mechanisms proposed to explain thebio-control by AMF is the enhancement of the crop nutrient uptake (Smith and Gianinzzi-Pearson, 1988; Clark and Zeto, 2000; Karaginnidis et al., 2002; Harrier and Waston, 2004). *G. margarita* was unable to suppress the symptoms of the wilt disease. The ability of AMF to control or suppress bacterial wilt causal agent can be measured in term of disease progress. *G. mosseae* suppressed totally the pathogen symptoms, so no symptoms were observed, in contrast, the plants inoculated with *G. margarita* and the pathogen was severely infected showing wilt disease symptoms. In tomato shoots, the uptake of N was statistically higher in the treatments inoculated by *G. mosseae*. P concentration was more in *G. mosseae* and *Scutellospora* sp. treated plant. The combination *G. mosseae* and *R. solanacearum* did not influence the amount of N (1.69%) compared to *G.mosseeae* (1.94%).

High concentration of K was detected in the shoot of *G. mosseae* treated plant and a significant amount was found in combination treatments (*G. mosseae* + *R. solanacearum* and *Scutellospora* sp. + R. *solanacearum*). Tarafdar and Marschner (1995) detected a significant improvement of K absorption by AMF in wheat plant. P uptake by plant was reported by Karaginnidis et al. (2002). It is illustrated that *G. mosseae* can accumulate more Fe in the shoot relative to the all other treatments. *G. mosseae* was able to increase the amount of Zn in tomato shoots significantly compared to *Scutellospora* sp., *G. margarita* + *R. solanacearum* and *Scutellospora* sp., + *R. solanacearum* . The present result confirms the findings by Cuenca and Azcon (1994) who reported that an overall improvement in uptake of all micronutrients following AM inoculation, other authors found that the AM plants reduced the uptake of Zn, Fe, Mn and Cu (Weissennorn et al., 1995).

Trotta et al. (1996) demonstrated that the increased P nutrient uptake by AMF might have contributed to reduce damage by *P. parastica* in tomato. High P fertilizer increased the vascular disease incidence on both mycorrhizal and non-mycorrhizal plant (Dehne, 1982). During the early stage of colonization by *Glomus intraradies,* suppression of defence-related properties is associated with the successful establishment of AM symbiosis (Guenoune et al., 2001).

Zhu and Yao (2004) provided another evidence for the reduction of *R. solanaceraum* in the plant xylem. They demonstrated that the population of *R. solanaceraum* was inhibited by *G. versiforme* inoculation so that no symptoms were observed. In current study, the inoculum density from the three species used (100 spores/100 g soil) was sufficient to increase the nutrient absorption surface area which resulted in strengthened root system through the positive effect on the host root colonization. The time of inoculation was a critical point to achieve significant results for bio-control of plant against *R. solanaceaum.* Throughout the present study, the AMF was applied before the pathogen to give the AMF time to colonize the root system, so that the plant can be more resistant against the pathogen (Bartschi et al., 1981). AMF induced plant resistance to the pathogen seems to depend on the time elapsed between the inoculation of the AMF and of the pathogen (Hwang et al. (1992).

Another important mechanism involving *R. solanacearum* bio-control of studies was the root morphological changes. The number of root tips, root length, root surface area and root volume were significantly higher in *G. mosseae* + *R. solanacearum* treatment compared to *G. margarita* + *R. solanacearum* which was not different from *R. solanacearum* treatment.

Spores count in both *G. mosseae* and *Scutellospora* sp. increased statistically in relation to the other treatments (*G. margarita*, *G. mosseae* + *R. solancearum* and *Scutellospora* sp. + R*. solancearum*). *G. mosseae* treated plants had high spore number, significantly increased root colonization percentage and enhanced the P uptake. The findings in this study are different from the field study which has been reported by Ryan and Angus (2003); they found that high colonization percentage did not correspond with the greatest nutrient growth P, K, Ca, Cu, and S uptake. Zn concentration was positively correlated with colonization percentage by AMF. The variation among AMF species in minerals concentration and all other parameters mentioned in this study could be related to the phylogeny of AMF community. This results was explained by Maherali et al. (2007) who reported that plant growth parameters increased when the two putatively complementary AMF families (Glomeraceae

and Gigasporaceae) were present in the community, but plant biomass was not stimulated by adding the third, putatively no complementary AMF family (Acaulosporaceae).

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