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RESEARCH ARTICLE

PLANT CROP YIELD ENHANCEMENT POTENTIALS OF THREE INDIGENOUS ARBUSCULAR MYCORRHIZAL FUNGI ISOLATED FROM ILE-IFE, SOUTHWEST NIGERIA

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ABSTRACT

Food security in developing countries still remains a global concern requiring effective and sustainable solutions. This study investigated the effect of three indigenous arbuscular mycorrhiza (AM) fungal inocula namely*; Funneliformis mosseae, Claroideoglomus luteum* and *Glomus viscosum* on the growth and yield of a tomato cultivar (*Lycopersicum* sp.). The spores of the fungi were isolated from a fallow land in Ile-Ife, Nigeria and were propagated separately by maize pot culture. The obtained pure culture spores were used to infect tomato seedlings singly and in different combinations in a sterile soil culture. A control un-inoculated treatment consisted of sterile soil only. There were variations in the extent at which pots inoculated with AM inocula improved plant growth rate, fruit yield and fruit size after harvest. For the single inoculation treatments, *Funneliformis mosseae* had the best gross fresh fruit weight/pot (191.47 g), mean fruit weight (27.35 g), mean fruit size (85.47 cm^2) and gross fruit weight per plant (68.83 g); the worst was *G. viscosum* which had gross fresh fruit weight/pot (191.47 g), mean fruit weight (27.35 g), mean fruit size (85.47 cm^2) and gross fruit weight per plant (68.83 g). However, not all combinations of the AM inocula were effective in improving plant crop yield. The best combined AM species treatment (*Funneliformis mosseae _Claroideoglomus luteum*) had mean fruit weight (22.54 g) and mean fruit size (88.43 cm^2), while *F. mosseae_C. luteum_G. viscosum* combination had the least mean fruit weight (9.33 g) and mean fruit size (51.28 cm^2). On the other hand, the combination treatments were generally better in post-harvest residual spore yield in the soil than the single treatments. There was no association between fruit yield and residual spore densities. This study has shown that these indigenous AM inoculants have the potentials to individually enhance fruit yield compared to the simulated background combined effect was found in the fallow land. We conclude that adequate knowledge of AM interactions with their host plants will be required for proper composition of AM fungi inocula for optimal application in agriculture to enhance plant crop yield.

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INTRODUCTION

Food insecurity is one of the challenges facing the teeming population of many developing countries of the world (FAO 2015). Low plant crop yields due to infertile soils used for cultivation of crops threatens access to safe, abundant and nutritious food to meet the dietary needs of people (Tilman *et al.,* 2002). While fertilizer applications have been the main backbone of the successful strategy for food security in the developed countries the same does not hold for developing countries where the commodity is generally expensive and most often not readily available to farmers.

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The adverse impact of inorganic fertilizer applications on rivers and streams is also a call for concern. The beneficial relationship between mycorrhizae and plants has been fully established but remains to be exploited maximally in agriculture, presumably because we still have a lot to learn for sustainable application in the enhancement of food crop plant productivity. As arbuscular mycorrhizal (AM) fungi are gradually gaining acceptance and popularity in modern agriculture (Berruti *et al.,* 2015), and a number of food crops, e.g. tomato and maize are known to be favourably associated with them, a lot more still need to be known about the interactions among the various AM fungi that have been isolated and identified and so far tested in the field. Some pieces of research information are emerging in this regard. For instance, some AM fungi have been reported to provide

protection against their host plant root pathogens (Momotaz *et al.,* 2015), resistance to diseases (Song *et al.,* 2015; Kapoor 2008) and stress (Balliu *et al.,* 2015); thereby enhancing the growth and yields of the plants. Some studies have also shown that AM fungi could enhance specific nutrient uptake for improved crop yield (Kowalska *et al.,* 2015; Osillos and Nagpala 2014). AM fungi have also been shown to contribute to the enhancement of the physical quality and chemical composition of tomato fruits (Michalojc *et al.,* 2015; Nzanza *et al.,* 2012). The details of the effects of the interactions among the different AM fungi on the one hand, and with their hosts on the other hand still need to be studied globally, especially on species variations and combinations. Unlike their ecto-mychorhiza counterparts that are generally non-specific for host plants, environmental and fungi species variations, it has been observed that AM fungi might have selective responses to these factors. Therefore, a lot more still needs to be investigated in this regard. For optimal exploitation of the natural process of AM fungi association with their host plants, we desire to contribute to research knowledge and understanding of the effect of the interactions of the mycorhizae in respect of their co-habitation with the host plants for enhanced plant productivity. We believe that such pieces of basic scientific information should help in the formulation of effective inocula for application as bio-fertilizer. We investigated tomato plant crop yield-enhancement effect of inocula compositions of three indigenous arbuscular mycorrhizal fungi isolated from a fallow land in Ile-Ife, southwest of Nigeria.

MATERIALS AND METHODS

Arbuscular mycorrhiza inocula used in this study: The three AM inocula, *Funneliformis mosseae, Claroideoglomus luteum and G. Viscosum* used in this study were developed from indigenous AM fungi spores obtained from a more than 20-year fallow field located at Obafemi Awolowo University Teaching and Research Farm, Ile-Ife, Nigeria (7° 58 33' N, 4° 32' E). At the time of sampling, the land was habited by dense grass vegetation. The AM spores were first separated, presumptively identified by standard methods and were later propagated in pure maize plant trap culture (single sporetype) in pots for four months in greenhouse. The plants were left to dry up after which the pot cultures were harvested for AM spores, checked for purity and identified by standard methods. They were stored in plastic bags at 4 ^{o}C until use. The Soil type used in the simulated trial experiment was obtained from the same field from which the spores were isolated. It was sterilized twice in 500 g batches at 121 °C for 15 min to ensure a proper kill of all native organisms. Autoclaving option was used to conserve the organic components to a considerable extent. It was a sandy-loamy soil and used as is, except for the experimental conditions as designed for this study.

Sowing, transplanting, stacking and harvesting of the tomato cultivar: Roma tomato seeds, obtained from the Nigerian Institute of Horticultural Research and Training (NIHORT), Ibadan, Oyo State, Nigeria, were sown in a nursery tray containing sterilized soil. Seedlings were raised for 42 days, after which those of approximately equal height (5 cm) and equal numbers of leaves (9) were transplanted into 5-litre plastic pots each containing 4 kg sterilized soil (each pot had a seedling). Staking of growing tomato plant was done at 77 days after planting (DAP). Harvesting was initiated at the 15th week post-planting and lasted a period of six (6) weeks. Watering was done on a daily basis each pot receiving 250 ml of normal tap water per day without consideration for differences in physical development (simulated field condition for water supply).

Simulated AM inoculum experimental design: The experiment was conducted as a completely randomized design in a greenhouse at Obafemi Awolowo University, Ile-Ife, Nigeria. Each test was done in a four (4) kg sterile culture in a pot. Applying the banding method, each pot was treated with a calculated estimated measure of soil inoculum as shown in Table 1. The double and triple combination treatments received half and one-third of the different single AM fungi inoculums respectively. A negative control was similarly setup with the same sterile soil in pot but without AM inoculation. The experiment was performed for each treatment in triplicates (limited by unavailability of space).

Growth rate: The stem height was used and was measured as the distance between the base of the stem and the base of the petiole of the youngest leaf at weekly interval. This was used to calculate the growth rate of the plant using the following expression: Growth rate = Stem height (cm) / Days of planting. Number of matured fruits Matured fruits were defined as fruits that were more than 90% ripe (red). The number of mature fruits on each plant was counted at the time of each harvest. The gross number of fruits was expressed as the sums of the number of fruits harvested at the end of the experiment. Fresh weights of mature fruits and crop yield: The gross fresh weight of mature fruits on each plant for each treatment was defined as the sum of the weights of all fruits that were more than 90% ripe at the final harvest of all the harvests. The crop yield was estimated as the gross fresh weight of fruits per plant for each group treatment. The size of the fresh fruit was calculated as the product of the vertical and horizontal median lengths of the fruit at the time of harvest. Assessment of arbuscular mycorrhizal fungal spore density in the soil after fruit harvest

Fungal spore yield: The wet sieving method was employed in assessing the AM fungi spores present in the pot soil culture after fruit harvest. Precisely 100 g of the soil sample was mixed with 300 ml distilled water in a beaker, stirred vigorously for 15 min, allowed to settle for about 10 min and decanted through a series of three laboratory sieves (Stevenston Ayrshire, Scotland) arranged in decreasing order of mesh size (250 µm, 75 µm and 38 µm) thrice. The second and third sieve retentates were washed into McCartney bottles with sterile distilled water and centrifuged in sucrose solution (40% (w/v) at 3000 rpm for 5 min (Salami 2007). After centrifuging, the interface between the water and sucrose solution was collected using a sterile syringe, dispensed into the 38 µm sieve, and washed with distilled water to remove any trace of sucrose solution. The sieve retentates were then back-washed into a petri dish with grid lines and observed under stereomicroscope (Nikon, SMZ745T, USA) for presence of mycorrhizal spores. The post harvest spore yield was estimated as a percentage increase (%PI) and which was calculated as follows: (%PI) = ((Yt – Yo)/Yo) x 100 where Yo = Quantity of spores inoculated at the start of the experiment per gram and Yt = Quantity of spores/g of soil after harvest Estimation of AM fungi combination effect index on spores yield. The concept of combination effect of antibiotics adapted from Athamna *et al.* (2005) was used to investigate the combination effect index (CEI) of AM fungi spore yield in the

multiple combined treatments relative to the single inoculation treatments. For the treatments that received double AM fungi treatment, the CEI was calculated as:

CEI = A2/A1 + B2/B1 where

A1 = % increase of A in single treatment; A2=% increase of A in combination treatment

B1 = % increase of B in single treatment; B2=% increase of B in combination treatment

For the treatment with triple AM treatment

CEI was calculated as

CEI = A2/A1 + B2/B1+C2/C1 where
A1, A2, B1 and B2 are as previously defined for double treatment above

C1 = % increase of C in single treatment; C2=% increase of C2 in combination treatment

The A, B and C represent the different AM fungi used.

Determination of percentage colonization of the tomato cultivar roots after final harvest: Arbuscular mycorrhizal root colonization was evaluated by the clearing and staining technique as previously described (Philips and Hayman 1970). Briefly, the root specimens were washed thoroughly under running tap water and placed in a beaker containing pre-boiled 10% (w/v) KOH solution for about 15 - 30 min to clear the root tissue. After this, the KOH solution was poured off and the roots were properly rinsed in a beaker until no brown colour appeared in the rinse water. The roots were later dipped in 6% (w/v) alkaline H_2O_2 at room temperature until the roots were bleached. The bleached roots were rinsed thoroughly to remove the H_2O_2 and thereafter soaked in 1% (v/v) HCl for 3 - 4 min, incubated with staining solution (0.05% (w/v) tryphan blue in lactophenol) and kept overnight. The stained root specimens were viewed under the X40 magnification (Olymphus CH30, China) and checked for the presence of mycorrhiza infection in a grid layout. At least 10 random square grid units per ocular view were observed and scored. Percentage root colonization was calculated as: (Number of grid units colonized /Total number of grid units observed) x 100

Chemical and mineral analysis of soil samples: The pH, organic carbon, nitrogen, and mineral composition (magnesium, potassium, sodium, phosphorus,copper and manganese) of the soils were determined using standard protocols (AOAC 2016).

Determination of soil pH: Twenty-five millilitres of distilled water was added to 10 g of air-dried sample in a 50 ml beaker and stirred for about 5 min. The pH reading was taken with a calibrated pH metre electrode (Hanna, HI96107, Italy) after stirring.

Determination of soil organic carbon and organic matter: Organic carbon of substrates was determined using Walkley-Black wet oxidation method. One gram of soil sample was weighed into 250 ml conical flask and 10 ml of 0.167 M K_2Cr_2O7 was added.

Twenty milliliters (20ml) of concentrated H_2SO4 was rapidly added and the content was swirled gently until the substrate and reagents were properly mixed for about 1 min. The flask was allowed to stand for 30 min on a sheet of asbestos for about 30 after which 1000 ml of distilled water was added followed by 3 – 4 drops of ferroin indicator. The content of the conical flask was titrated with 0.5 M iron (II) sulfate until the color changed sharply from green to brown red. Percentage organic carbon (%OC) was expressed as:

% OC = (B – T) x M x 0.003 x 1.33 x 100 where,
B = blank titre value (contain no soil sample),
T = sample titre value (contain appropriate amount of soil sample),

M = molarity of $(NH_4)_2Fe(SO4)_2.6H_2O$.

Also, the percentage (%) organic matter = % organic carbon x 1.724.

Determination of total nitrogen: The three-step method of acid digestion, distillation and titration by Kjeldahl was employed using 500 g of the soil. The concentrated H_2SO_4 digest was made up to 50 ml using distilled water in a standard volumetric flask and then poured into a clean sample bottle. About 10 ml of the digests and 20 ml of 40 % NaOH were added into the distillation flask and then distilled into 5 ml boric acid in a 250 ml conical flask using the distillation unit (Kjeltec (TM) 2100, FOSS, Sweden). The distillate was titrated with standard 0.1M HCl using 50 ml digital dispenser (Solarus (R), Hirschmann) until the end point was reached indicated by the color of the distillate changing to pink. Percentage nitrogen (%N) was estimated as:

% N = (Vs –Vb) x M (HCl) x 1 x 14.007 (W x 10) where,
Vs = volume of HCl needed to titrate sample
Vb = volume of HCl needed for the blank test,
M (HCl) = Molarity of HCl,the acidity factor, = 1
14.007 = molecular weight of nitrogen,
Conversion from mg/g to %, = 10
W = weight of the sample (g).

Determination of manganese, magnesium, copper, sodium, potassium and phosphorus: Spectrophotometric method using atomic absorption spectrophometric machine (Buck Scientific, 210VGP, UK) was used for the determination of manganese, magnesium and copper while sodium and potassium were determined using flame photometry (Jenway, PFP7, UK) accordance to the manufacturer's instruction. Spectrophotometric method using Yellow Vanado Molybdate was used in the determination of phosphorus. The acid digestion extraction method was used as previously described above.

Estimation of residual soil nutrient

The percentage residual soil nutrient was calculated as:
(NCt – NCo)/ NCo x 100 where
NCt = concentration of element in pot soil after harvest and
NCo = concentration of element in pot soil before planting.

Data analysis: Data analysis was performed using GraphPad Prism software version 6.01. Data were recorded as means of triplicate treatments or measurement.

ANOVA and other column statistics were used to investigate the significance of the differences between the treatment groups and factors.

RESULTS

Plant growth and fruit yield of the ROMA tomato cultivar
Growth Rate: The growth rate, expressed by the stem height of the tomato cultivar, ranged between 0.003947/week (lowest) and 0.007376 cm/week (highest) for the single *C. luteum* and *F. mosseae_G. viscosum* double treatments respectively (Table 2). Among the single treatments, *F. mosseae* had the highest (0.006435 cm/week) and *C. luteum* the lowest (0.003947 cm/week). Similarly, the *F.* mosseae_*G. viscosum* and *C. luteum_G. viscosum* double combination treatments were the highest and lowest respectively. The triple combination was the second best (0.007151 cm/week) of all the combination treatments. The coefficient of determination (R^2) for each treatment was highly reliable and significant while the differences between the group treatments were highly significant ($P < 0.0001$) (Table 2).

Yield of tomato fruits: Yield was derived from gross fresh fruit weight and size at the end of harvest. The gross fresh fruit yield per treatment ranged between 72.08 g (lowest) for the uninoculated and 191.47 g (highest) for the *F. mosseae* treatment (Table 3). For the single treatments, *F. mosseae* had the highest yield and *G. viscosum* the lowest yield (132.76 g). Except for the *G. viscosum* all the single treatments had better yields than any of the combination treatments (73.90 g – 168.24 g), while the double combination treatments were better than the triple combination treatment. Similar trends were observed in the fruit size and the differences in values were highly significant (Anova $F = 27.56$ (3, 28); $p<0.0001$) (Fig. 1). Tukey's multiple analysis also showed that the differences observed correlated significantly among the fruit parameters investigated except for between the fruit weight per plant and each of the mean fruit weight and the mean fruit size (Fig. 2).

Arbuscular mycorrhiza fungal spore yield (% increase) after harvest: The spore yield (% increase) for single treatments ranged between 3289 for *G. viscosum*) and 4122 for *C. luteum*, $P<0.003$; and between 2083 for *F. Mosseae in a triple combination* and 5311 for *G. viscosum* in a double combination treatments, $P<0.0003$ (Fig. 3). The percentage yield for *G. viscosum* was significantly better in a combination treatment than the single treatment ($P < 0.002$). On the other hand, the percentage yields for *F. mosseae* and *C. luteum* were not significantly different between the treatments except where it was significantly reduced for *F. mosseae* in the triple combination treatment. The overall percentage spore yield was better in combination treatments than the single treatments, $P<0.0001$. The uninoculated control did not yield spores (Figure 4).

Percentage root colonized in the tomato cultivar roots at the end of the fruit harvest: More than 85% AM root colonization was observed for all the treatments (Table. 5). The triple *F. mosseae_C. luteum_G. viscosum* combination treatment had the highest percentage root colonization (98.9 ± 1.9%) while the lowest (87.9 ± 1.9) was obtained for the *C. luteum_G. viscosum* treatment. The differences were significantly different ($P < 0.02$, Wilcoxon Rank).

The observed root colonization correlated with the spore yield ($r = 0.936$) and CEI ($r = 0.667$) but only that for spore yield was significant ($P < 0.01$).

Residual soil nutrient in pot soil after harvest: Compared to the concentration before planting, there was either an increase or decrease in soil nutrient after planting depending on the treatment and the nutrient in view. Decreases were noted in manganese and magnesium (except for *F. mosseae*, 20.91 % increase). On the other hand, increases in phosphorus (range 1.55 - 42.16%), nitrogen (range 2.27 – 93.18%), potassium (range 15.15 – 72.73%) and copper (range 545.45 - 1018.18%) were observed in all the nine treatments after harvest (Table 5), and most of them were significant compared with the uninoculated control ($P < 0.001$, one way ANOVA). Except for Mg+ and K+, differences among the different combination treatments were also significant ($P < 0.008$, Wilcoxon Sign-Rank test).

Association between fruit yield and post-harvest parameters: Pearson correlation analysis showed association between some post-harvest parameters measured as shown in Table 6. The number of fruits per plant positively correlated with fresh weight of fruits per plant ($r = 0.664$) and copper ($r = 0.595$), and was negatively correlated with potassium ($r = -0.656$) but not significant. The mean size of fruit correlated positively with percentage root colonization ($r = 0.536$), magnesium ($r = 0.583$), and negatively with phosphorus ($r = -0.591$) but these were not significant. However, the mean size of fruits correlated positively and significantly with potassium ($r = 0.797$; $P < 00.5$). Also, the spore yield after harvest correlated negatively with phosphorus ($r = -0.635$) and positively correlated with percentage root colonization ($r = 0.966$). The percentage root colonization correlated positively with sodium ($r = 0.512$) and negatively with phosphorus ($r= -0.686$)

DISCUSSION

This study assessed the possible effect of three Nigerian indigenous AM fungal inocula, applied singly and in combinations, on the growth and fruit yield of tomato plants, and post-harvest soil nutrient in pot cultures. The three AM spores that were isolated from a fallow land were identified as *F. mosseae*, *C. luteum*, and *G. viscosum* in the population density ratio of 4:1:1. We were successful in propagating the spores in pure pot-cultures cultivated with maize plant and obtained spore turnover ranging between more than 34,000 and 45,000 % within four months, equivalent to a yield of about 7 – 9 spores/g of pot culture soil (Unpublished data). Similar observation has been reported by Chaurasia and Khare (2005) with *Hordeum vulgare* as the host and Selvakumar *et al*. (2016) with maize and sudan grass as host plants, but with much lower yields than in our case, and over more than one cycle of crop planting. For the tomato simulated pot-culture infection, the post-harvest spore yield was consistently higher when the spores were cultivated in multiple species combinations than in single species. In a similar vein, the root colonization was significantly enhanced in mixed than in pure (single) cultures and was almost complete (98.9 %) for the triple combination. Significant correlation ($r = 0.966$; $P < 0.001$) was established between spore yield and root colonization. By these observations, we find it reasonable to suggest that multiple-species inoculum favored propagation of AM spores in pot cultures.

Table 1. Treatment group and estimated experimental content per 4 kg of sterile soil

Treatment group	*Average number of spores/g soil in fallow field	**Calculated weight of experimental soil (CES) inoculums (g/kg pot culture)	Weight of soil inoculum per 4 kg experimental pot culture
F. mosseae	9	33	132
C. luteum	7	43	172
G. viscosum	7	43	172
F. mosseae_C. luteum	4.5/3.5	16.5/21.5	66.25/86
F. Mosseae_G. viscosum	4.5/3.5	16.5/21.5	66.25/86
C. luteum_G. viscosum	3.5/3.5	21.5/21.5	86.0/86.0
F.mosseae_C. Luteum_G. viscosum	3/2.3/2.3	11/14.5/14.5	40.3/57.3/57.3
Uninoculated control	-	-	-

The uninoculated served as a negative containing the same soil as the experimental but sterile.
* Spore densities as found in the fallow field** Calculated according to the following formula
CES = (X /Y) x Z, Where
X = Adopted Standard number of spores/g of soil inoculum = 6 (six)
Y = Number of spores/g of experimental soil inoculum
Z = Standard amount of soil inoculum per kilogram of pot soil culture = 50 g

Table 2. The growth rate of the stem of the tomato cultivar in response to the different AM inocula treatments

Treatment Group	*Growth rate (cm/week)	**R2	P - value
F. mosseae	0.006435	0.9031	0.001
C. luteum	0.003947	0.9475	0.0003
G. viscosum	0.005641	0.9036	0.0001
F. mosseae_C. luteum	0.004410	0.9418	0.0003
F. Mosseae_G. viscosum	0.007376	0.9910	0.0001
C. luteum_G. viscosum	0.005485	0.9157	0.0026
F.mosseae_C. Luteum_G. viscosum	0.007151	0.9067	0.0001
Uninoculated control	0.005759	0.9948	0.0001

* Growth was estimated as weekly measurement of the stem height up to the final day of harvest of fruits. The values used were averages for the plants in each treatment **Regression analysis was assumed to be largely linear for each group treatment in relation to weekly measurement (time).

Table 3. Yield of tomato fruits in response to combinations of AM spore treatment of tomato cultivars.

Treatment Group*	Yield of Observed Parameters			
	Gross Fresh Fruit Weight (g)	Mean Fruit Weight (g)	Mean Fruit Size (cm^2)	Fruit Weight per Plant (g)
F. mosseae	191.47	27.35	85.47	68.83
C. luteum	171.15	12.23	55.87	57.05
G. viscosum	132.76	14.75	56.48	44.25
F. mosseae_C. Luteum	112.70	22.54	88.43	37.56
F. Mosseae_G. viscosum	73.90	12.32	47.38	24.63
C. luteum_G. viscosum	168.24	10.52	51.46	56.08
F.mosseae_C. Luteum_G. viscosum	102.62	9.33	51.28	34.20
Uninoculated Control	72.08	12.01	40.86	24.03

*There were three plants per pot per treatment group and each treatment was in triplicates. (3 plants per pot x 3 replicates = nine plants per treatment group)

Table 4. Percentage root colonization of tomato crop plant by AM fungi as observed after harvest

Mean Root colonization	Species Treatment combination						
	F. mosseae	*C. luteum*	*G. viscosum*	*F. mosseae + C. luteum*	*F. mosseae + G. viscosum*	*C. luteum + G. viscosum*	*F. mosseae + C. luteum + G. viscosum*
(%)	96.7	90.1	94.5	90.1	93.4	87.9	98.9
SD	3.3	3.3	1.9	3.3	3.3	1.9	1.9

Arbuscular mycorrhizal fungi have been isolated and formulated into inocula in different forms, and applied in some agricultural systems for enhanced plant productivity (Koch *et al*. 1997; Ijdo *et al*. 2011; Li *et al*. 2011; Wang *et al*. 2011; Hernádi *et al.* 2012; Vosátka *et al*. 2013; van der Heijden *et al*. 2015; Cely *et al*. 2016). But few studies have evaluated the absolute effectiveness of the formulations. This study has revealed that AM fungi inoculated singly or in different combinations produced desirable enhancement of plant productivity differently. Our findings have shown that single AM amendment of the soil was better for enhanced tomato fruit yield in weight and size than the combined treatments. Furthermore, the enhanced performance for the single treatments varied significantly among the species. Our *F. mossae* strain was found to be significantly better than each of the *C. luteum and G. viscosum*. Similar observations have been reported for different species of AM fungi and in respect of different plant growth parameters (Banla *et al*., 2015; Rizvi *et al*., 2015). It is important to note here that whereas spore production and root colonization were stimulated better in combined species treatments, the reverse was observed for fruit yield and size. We are, therefore, inclined to infer that the AM combination treatment probably induced competition for nutrients and space in the rhizosphere with the attendant stress

Table 5. Percentage difference in mineral content of the soil pot culture after the last harvest of the fruits

Treatment combination	*Mean Initial soil nutrient concentration						
	Mg (cmol/kg)	K (cmol/kg)	Na (cmol/kg)	P (cmol/kg)	N (%)	Cu (mg/kg)	Mn (mg/kg)
	240.66	0.33	0.07	4.53	0.44	0.11	92.36
	**Mean percentage difference after fruit harvest						
F. mosseae	20.91a	72.73a	71.43ab	7.06b	61.36a	745.45bc	-36.62cde
C. luteum	-52.22de	0.00c	42.86ab	1.55b	88.64a	1018.18a	-20.16a
G. viscosum	-35.67cd	15.15c	42.86ab	36.42ab	2.27a	845.45b	-44.00e
F. mosseae_ C. luteum	-31.97bcd	51.52b	14.29ab	14.35b	20.45a	636.36dc	-36.92cde
F. mosseae_C. viscosum	-40.27de	15.15c	71.43ab	29.58b	93.18a	690.91bcd	-41.76de
C. luteum_G. viscosum	-41.12de	-6.06c	57.14ab	42.16ab	72.73a	545.45d	-31.72abc
G.mosseae_G. luteum_G. viscosum	-20.52b	9.09c	85.71a	15.89b	88.64a	863.64ab	-29.49cb
Uninoculated (Negative control)	-22.53bc	27.27b	57.14ab	34.66b	143.18a	581.82cd	-21.46ab

* Concentrations as obtained from the sterilized soil before AM inoculation and planting
** Percentage difference between post harvest values compared to the pre-planting values
Means with different letters in each column are significantly different from each other at $P < 0.05$.

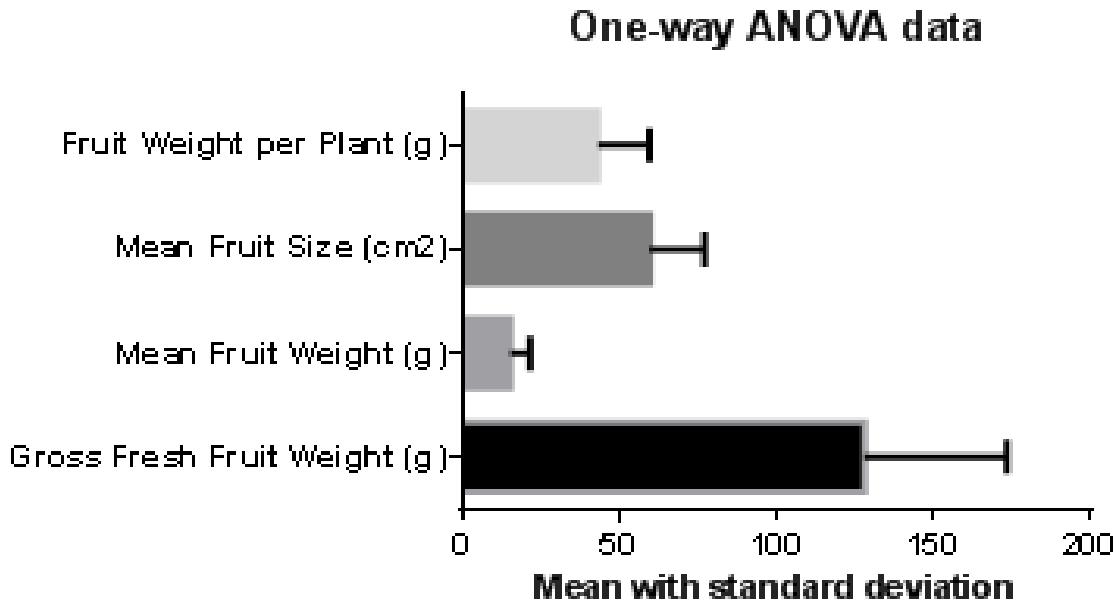

Figure 1 One way ANOVA data with standard deviation for the fruit parameters that were investigated. F = 27.56 (3, 28); P < 0.0001

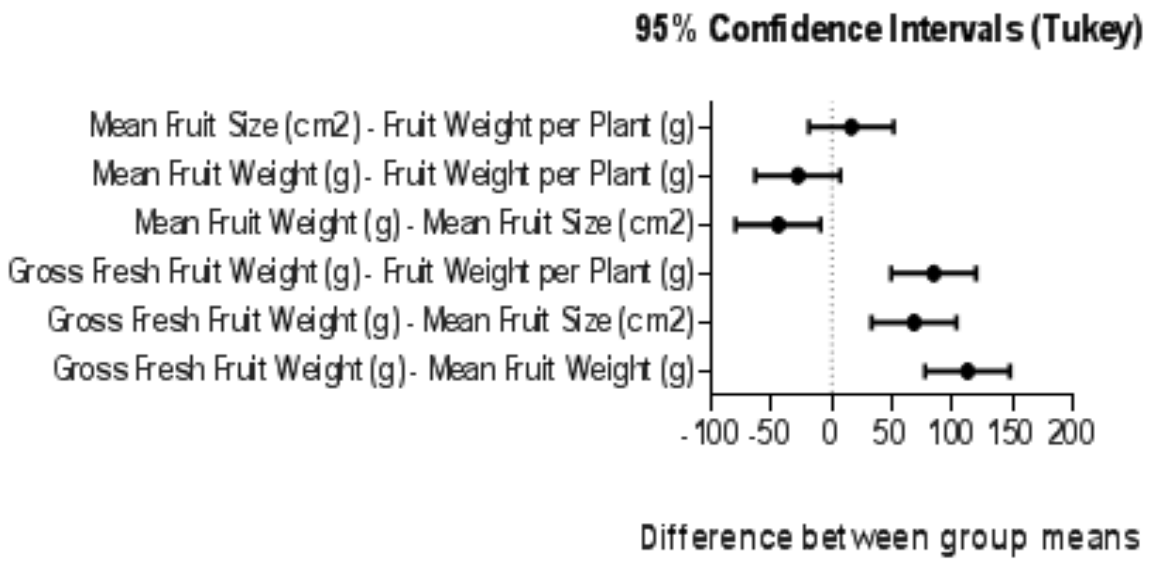

Figure 2. The Tukey's multiple analyses of significance of correlation matrix among the different fruit parameters investigated. Significant relationships were established (q value: 4.846 – 12.350, 28 df.) except between the fruit weight per plant and the mean fruit weight or the fruit mean size (q value: 3.082 and 1.784 respectively)

Table 6. Correlation analysis of plant growth rate, fruit harvest and post harvest parameters measured (n = 10)

	Number of fruits per plant	Fresh weight of fruits per plant	Mean size of fruits	Spore yield after harvest	Percentage root colonization	Mg2+	K+	Na+	P	N	Cu2+	Mn2+	Growth rate
Number of fruits per plant	1												
Fresh weight of fruits per plant	0.664[a]	1											
Mean size of fruits	-0.304	0.435	1										
Spore yield after harvest	0.171	0.433	0.451	1									
Percentage root colonization	0.144	0.483	0.536a	0.966b	1								
Mg2+	-0.488	0.012	0.583[a]	0.294	0.409	1							
K+	-0.656[a]	0.028	0.797c	0.292	0.394	0.881b	1						
Na+	-0.142	-0.145	-0.006	0.487	0.512	0.682c	0.391	1					
P	0.047	-0.402	-0.591[a]	-0.635[a]	-0.686c	-0.647[a]	-0.685c	-0.578	1				
N	-0.123	-0.484	-0.483	-0.229	-0.328	0.149	-0.043	0.504a	-0.155	1			
Cu2+	0.595[a]	0.662a	0.035	0.297	0.398	-0.040	-0.094	0.133	-0.457	-0.128	1		
Mn2+	0.398	0.093	-0.346	-0.383	-0.455	-0.165	-0.271	-0.023	-0.102	0.670c	0.151	1	
Growth rate	-0.014	-0.399	-0.443	-0.555[a]	-0.536[a]	-0.396	-0.511[a]	-0.356	0.775[a]	-0.061	-0.180	-0.132	1

n = Number of rows in working data

a = Correlation is not significantb = Correlation is significant at the 0.01 level (2-tailed)

c = Correlation is significant at the 0.05 level (1-tailed)

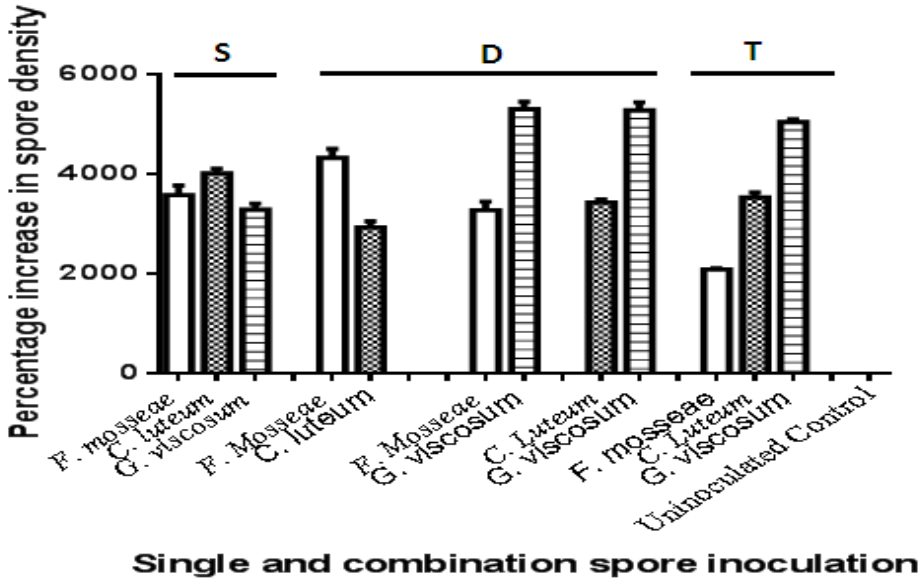

Figure 3 Post-harvest percentage increases in the spore densities of the soil cultures of the tomato cultivars in response to the single and triple combination AM spore treatments. Each treatment pot was inoculated with a total of 300 spores either in single(S) or equal proportions (150 each for double(D) and 100 each for triple(T)) combinations. Except for the *C. luteum*, percentage increase in individual species spore density was higher in the combination treatments than the corresponding single treatments, and *G. viscosum* was consistently the best. Differences in the means were significant (t = 8.955, 12 df; P < 0.0001) among the treatment.

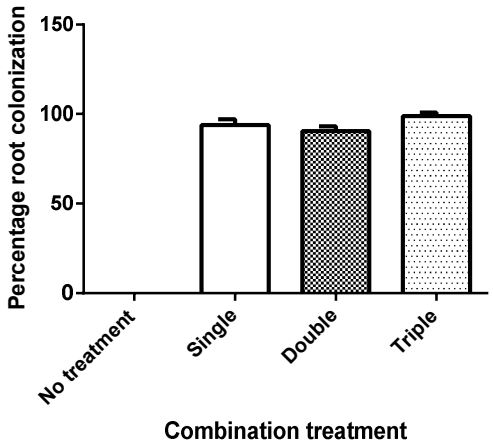

Figure 4 Combination treatment effect on percentage root colonization of the tomato crop plant by AM fungi after the last harvest of fruits. No root colonization was observed in the uninoculated control. The triple species treatment was better than the single or double species treatments. Differences between the treatment means were significant (P < 0.0007).

effects and hence the tendency towards sporulation, while they contribute collectively to the development and enhanced productivity of the plant. Similar observation has been made by Berruti *et al*. (2015) who suggested that AM fungi might be specific in their response to factors such as compatibility with the target environment, the degree of spatial competition with other soil organisms and the time of inoculation. It is thus becoming clearer that AM composition specificity and diversity would probably influence their interactions in different directions of plant productivity. Wagg *et al*. (2015) have suggested that the AM fungi composition rather than the diversity of species involved in mycorrhizal association could be more influential in determining how the species function. Gosling *et al.* (2016) have also argued that under controlled conditions, not all fungal species would adequately respond to the stress encountered to provide maximal benefit to the host plants. Similar observations of the effects by environmentally-induced stress (Dasgan *et al*., 2008) and species inocula variations (Burni *et al*., 2013) on AM fungi association with plants have been reported. Several studies have established a general increase in the uptake of soil nutrient in AM fungi-plant association (Smith *et al*. 2003; 2009; 2011; Beltrano *et al*.). However, information on soil nutrient enrichment by mycorrhizal fungi association has been scarcely reported. Our findings in this study revealed that residuals of some essential nutrients were observed in the pot culture of the AM fungi treatments after harvest and were significantly higher than in the uninoculated control, especially potassium and copper. Fu *et al*. (2015) have suggested that the structural and physiological property of plants could influence the balance between copper uptake by the plant and retention in the soil. Marschner and Timonen (2004) have also noted that the mycorrhizal fungi release of exudates and degeneration of the hyphal - network could contribute a considerable amount of nutrient to the rhizosphere. However, we noted lower levels of residual nitrogen for the treatments compared to the uninoculated control in contrast to the observation of Yeasmin *et al*. (2007) who observed 3% increase in post-harvest soil nitrogen concentration. It is possible that the comparatively low level of nitrogen may have been compensated for in the observed enhanced fruit yield. We are unable to explain the excessive depletion of magnesium and manganese but we would like to suggest a possible role in the development of the fruits whose sizes for the treatments were significantly better than those of the uninoculated control.

Conclusion

This study has shown that three Nigerian AM fungi strains of *F. mosseae*, *C. luteum*, and *G. Viscosum* spore-amendment of the soil improved crop yield of tomato cultivar used as a test plant compared to the untreated control plants. The enhanced yields were, however, better in single amendments than any of the double or triple multi-species combinations. The enhancement effects varied significantly among the single AM fungal species. On the other hand, multiple AM fungi species amendments favored enhanced spore yield. There was no correlation between fruit yield and spore yield, thus suggesting that these observations are two distinct possible outcomes of AM fungi interaction with their host plants with each compensating for the other. It would thus appear that the formulation of pure species cultures of AM fungal spores, in the practical applications for amendment of soil for enhanced crop productivity, would require a knowledge and understanding of species performance specificity and differences among other considerations, for varying environmental factors. We intend to work further in this direction.

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