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The Effects of Arbuscular Mycorrhizal Fungi (AMF) as Biofertilizer on the Growth, Yield and Nutrient Uptake of Tomato (*Lycopersicon esculentum* Mill.)

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Abstract: ***This study was conducted to determine the effect of arbuscular mycorrhizal fungi (AMF) to the growth, yield and nutrient uptake of tomato. AMF from the rhizosphere soil of wild legumes and cogon were trapped using corn roots as bait then isolated through wet sieving and sucrose centrifugation methods. Isolated spores of Gigaspora and Acaulospora dominated by Acaulospora laevis and colonized corn roots having a colonization rate of 86% were used as inoculants. The experiment utilized a completely randomized design with six treatments replicated three times. Data were evaluated using Levene's test and ANOVA. Means with significant differences were compared using LSD and Games-Howell. Results showed that tomatoes inoculated with different preparations of AMF resulted to a faster increase in height, higher number of leaves and tallest plants from the 2nd week to the 10th week after inoculation. AMF application also resulted to earlier flower onset and fruit emergence. Inoculated plants had the highest yield of marketable fruits while those grown with the farmer's practice gave the highest yield of non-marketable fruits. Leaves of inoculated plants had the highest amount Phosphorus (P), lower percentage of tissue Nitrogen (N), and higher Potassium (K) content as compared to the control. AMF inoculation did not affect soil pH but decreased the soil electrical conductivity (EC), K, P and gave the lowest increase of percent organic matter. Colonization was seen in all the plants treated with AMF, with T2 (100 AMF spores + 0.25 g colonized corn roots) registering the highest rate.***

Keywords: AMF, colonized roots, biofertilizer, tomato, *Acaulospora*

1. Background of the Study

Improved and modern farming practices have been done to maximize crop production. These practices involve crop management through direct manipulation of soils, cultivars and pests (Johnson and Pfleger, 1992) which are not always advantageous to the ecosystem. The application of fertilizers contributes a lot in maintaining high crop productivity and soil quality. However, some studies have shown that continued use of inorganic fertilizers, herbicides and fungicides can diminish the quality and productivity of soils (Yang et al., 2004) by increasing soil acidity, change in soil nutrient availabilities and increased concentrations of NO^-_3-N and NH^+_4-N **(**Darusman, et al., 1991).In addition, human exposure to inorganic fertilizers and fungicides lead to a great health risk. Studies have shown that frequent exposure to these substances can cause liver and kidney damage, Blue Baby Syndrome, reduced immune response, birth defects and even cancer (Stout, 2010). The unfavorable effects of these chemicals to human health and to the environment promoted the use of beneficial microorganisms in the soil to increase crop yield and maintain soil ecological balance. Consequently, agricultural researches are now geared towards the discovery and use of microorganisms which are safer alternatives to inorganic fertilizers and chemical fungicides.

Most farmers now use biofertilizers made up of living microorganisms. Application of biofertilizers to plant surfaces or soil allows the microorganisms to colonize the rhizosphere together with the plants and thus increase the supply or availability of nutrients to the host plants (Vessey, 2003). In this way, biofertilizers have been used as alternatives to chemical fertilizers to increase soil fertility and crop production in sustainable farming (Wu et al., 2004).

The arbuscular mycorrhizal fungi (AMF) are well-known fungal symbionts that have an essential role in the establishment of plant communities, nutrient cycling and maintenance of soil structure (Miller and Jastrow, 1994 as cited by Rahman et al., 2006). They are capable of mobilizing Phosphorus, Nitrogen and serve as carbon sink in the soil (Bonfante and Genre, 2010). Their importance in sustainable agriculture is based on their role as link between plant and soil. They are agents of nutrient transfer and therefore contribute to soil conservation, soil nutrition and plant nutrition (Rai, 2006).

The high metabolic rate and strategically diffuse distribution of these fungi in the upper soil layers allow them to efficiently translocate ions from the soil to the host plant. A number of studies had demonstrated that AMF influenced the growth and nutrient uptake of some crops like chickpea and custard-apple (Kumar et al., 2002). The symbiotic association of the mycorrhizal fungus also resulted to a greater uptake of P and increased chlorophyll content in AMF treated plants as compared to the non-mycorrhizal plants (Ojha et al., 2008).

The abilities of AMF to colonize their host and facilitate the transport of nutrients from the soil to the plant make these fungi an interesting object of research to improve crop yield and soil quality.

2. Statement of the Problems

This study aims to assess the effect of the AMF isolates as a biofertilizer to tomato (*Lycopersicon esculentum* Mill.). Specifically, it seeks to answer the following questions:

1. What is the effect of AMF on the growth performance of tomatoes in terms of:

1.1 percent increase in height at 2nd, 4th, 6th, 8th and 10th week after AMF inoculation?
1.2 number of leaves at 2nd, 4th, 6th, 8th and 10th week after inoculation?
1.3 final height at 2nd, 4th, 6th, 8th and 10th week after AMF inoculation?

2. What is the effect of AMF on the yield of tomatoes in terms of:

2.1 mean weight and mean count of tomato fruits in relation to size as small, medium and large?
2.2 mean weight and mean count of marketable fruits?
2.3 mean weight and mean count of non-marketable fruits?
2.4 mean total fruit weight and mean total count?

3. What is the effect of AMF in the nutrient uptake of tomato measured in the leaves in terms of:

3.1 Nitrogen (N)?
3.2 Phosphorus (P)?
3.3 Potassium (K)?

4. What is the effect of AMF in the soil profile of the pot soil of tomato in terms of:

4.1 pH?
4.2 EC?
4.3 OM?
4.4 P?
4.5 K?

5. What is the colonization rate of the tomato roots inoculated with 100 AMF spores, 100 AMF spores + colonized tomato roots and those colonized with corn roots only?

3. Materials and Methods

The procedure on the setting up of trap culture and extraction of AMF spores were based on the protocols presented by INVAM, Brundrett et al. (1996), Brundrett (2008), PhilRice (2009) and Brown et al. (2010).

Collection of Specimens and Setting up of trap Culture

Approximately 10 to 50 g fresh soil samples together with the roots of *Calopogonium muconoides* Desv., *Centrosema pubescens* Benth., *Mimosa pudica* L. and *Imperata cylindrical (*L.) P. Beauv. were recollected randomly from the field of Don Mariano Marcos Memorial State University – South La Union Campus, Agoo, La Union during the month of May 2013. The soils were taken from the rhizosphere, (approximately 20 cm deep) to ensure that the mycorrhizal roots and extramatrical spores were collected.

The collected rhizospheresoils of *C.muconoides* Desv., *C. pubescens* Benth., *M. pudica* L. and *I. cylindrical (*L.) P. Beauv were chopped (together with their roots) into small fragments. About 750 ml of the chopped blend were mixed with sterilized coarse sand in a 1:1 (v/v) ratio. Gallon Ziploc was used for mixing to break up the small clumps of soil or roots and improve the chances of a homogeneous product. Total volumes of 1, 500 ml sand-soil mixture were transferred to a 1.5li clay pot.

Three to five corn seeds were surface sterilized with 10% household bleach (chlorox), sown and grown in pots in a screen house for three months. The plants were monitored daily to protect them from pest and diseases. Watering of the plants was stopped after three months. The pots were allowed to dry in an undisturbed shaded room with a stable temperature of 26-28 ºC for 1-2 weeks. All the trap cultures we restored in gallon Ziploc plastic bags until used.

Preparation of Treatments

Extraction of AMF Spores as Inoculum

This method is the universal technique of extracting spores from the soil (Brown et al., 2010; Brundrett et al., 1996). It involves the washing of a slurry soil through a graded series of soil sieves (no. 50, 100, 325 and 500). Ten to 50g of soil samples from the trap cultures were suspended in 500 ml to one li of tap water. The mixture was placed in a plastic pail. Soil macro-aggregates were crushed with hands or blended at high speed for approximately five seconds. After 10-30 seconds that the soil particles had settled down, the upper layer of soil suspension were poured onto the coarse sieve first (no. 50) to remove large pieces of organic matter. The liquid that passed through the sieve were collected in a series of stacked sieves arranged in this manner: no. 100, 325 and 500.

The debris from each sieve were collected and placed in 50 ml beakers, added with water then transferred into test tubes. The tubes containing the mixtures were centrifuged for 5 minutes at 2000 rpm. The supernatant together with the floating debris were discarded. The pellet were resuspended in 50% sucrose solution (500 g sugar/500 ml distilled water) then centrifuged at 2000 rpm for 1 minute. The supernatant was decanted into the finest sieve (no. 500). The material that was collected in the sieve was washed for 1-2 minutes with tap water to remove the sucrose then transferred to a Petri dish. The spores were manually collected using an extruded plastic micropipette tip to separate the spores from organic material. Once this was done, spores were rinsed in distilled water containing 0.2 mg/ml Streptomycin (De la Peña et al., 2005), picked then transferred onto a sterile filter paper for an hour until dried. A sterile needle dipped in sterilized distilled water was used in picking the spores then transferred to a sterilized container with 1 ml sterilized distilled water. The spores were stored at 4ºC in a refrigerator until it was used as inoculum.

The spores collected were dominated by *Acaulospora laevis*, *Acaulospora mellea*, *Acaulospora morrowiae*, and few *Gigaspora margarita*. Since there were two treatments (T1 and T2) inoculated with 100 AMF spores per pot, a total of 3, 000 AMF spores were isolated.

Preparation of Corn Roots as Inoculum

The colonization rate of the corn roots from the trap culture was 86%. The procedures below on the assessment of root infection by AMF were followed. After verifying the colonization rate effected by AMF, the remaining corn roots were washed thoroughly, cut into 2-cm pieces then immersed to a solution containing 0.2 mg/ml Streptomycin (for 3 minutes) as disinfectant. The roots were rinsed with sterilized distilled water then air-dried for 24 hours. About 0.25 g corn roots were applied to T2 and T3.

Recommended Rate of Fertilizer (RRF)

Before sowing, the NPK content of the pot soil were analyzed at the Bureau of Soils Fertilizer and Pesticide Analysis Laboratory at San Fernando, La Union. This served as a baseline data. The RRF was 1.03 g Nitrogen only since the soil used had sufficient amount of Phosphorus and Potassium based on soil analysis.

Application of Treatments and Inoculation of Tomatoes with AMF

The seeds of tomatoes (Marimar variety) were obtained from East-West Seeds, Philippines. Surface sterilized seeds (with 10% NaOCl, household bleach, for 5 minutes) were sown in a seedbed then allowed to germinate for two weeks. The two-week old seedlings were transplanted to pots containing 10 kg sterilized sand-soil mix. Five seedlings were transplanted in each pot. After another two weeks, some of the seedlings were uprooted so that only one seedling was left in each pot. This was also the time when AMF inoculation was done. There were three set-ups that correspond to the mycorrhizal group: set-up 1 was inoculated with 100 AMF spores (consisted of *Gigaspora* sp., and *Acauolospora* sp. dominated by *A. laevis)* /pot. The spores were suspended in one ml distilled water then manually applied to the tomato seedling roots using a dropper.

Moreover, set-up 2 was inoculated with 100 AMF spores + 0.25 g colonized corn roots/pot while set-up 3 was applied with 0.25 g colonized corn roots only/pot. The control group did not receive AMF (no AMF). The other treatments consisted of sterilized soil + recommended rate of fertilizer and the other employed farmer's practice wherein a pinch of urea was applied liberally to the pot soil.

Each treatment has five sample plants and was replicated three times. A total of 90 pots containing 10 kg soil were used in the study.

These were the treatments used in the study:

T0 = no AMF (control)
T1 = 100 AMF spores
T2 = 100 AMF spores + 0.25 g colonized corn roots
T3 = 0.25 g colonized corn roots only
T4 = recommended rate of fertilizer (RRF = 1.03 g N)
T5 = farmer's practice

Measurement of Growth Parameters

The effect of AMF as a biofertilizer were measured in terms of different growth parameters like plant height, percent increase in height, number of leaves, leaf increment, total yield in terms of mean total fruit weight and mean total fruit count, mean weight and count of small (S), medium (M) and large (L) fruits, mean weight and count of marketable and non-marketable fruits.

Plant height (cm). The height of the plant was measured using a ruler or meter stick from the border of the pot to the top of the main plant stem. The initial measurement of the height of the plants was done on the day of AMF inoculation. The succeeding measurements were done every after two weeks up to the termination of the study or until the 10^{th} week.

Number of Leaves. The number of leaves were counted every after two weeks. The initial counting was done on the day of AMF inoculation then terminated on the 10^{th} week.

Total Yield and Fruit Weight (g). The total yield of the tomatoes were measured based on the mean total fruit weight and mean total fruit count and whether they were marketable or non-marketable. The numbers of marketable as well as non-marketable fruits from each treatment were counted. Using a vernier caliper, the fruits were classified as follows: small fruits had a length of < 50 mm and a diameter of < 30 mm, medium fruits had a length equal to 50 mm and a diameter of 30 mm while large fruits had a length of >50 mm and a diameter of > 30mm. The fruits were counted then weighed immediately after harvest. Defective fruits due to insect infestation or those that dropped from the soil by any reason were also included and considered as non-marketable fruits.

The mean weight or mean count of marketable fruits is the mean of the sum of the weights or counts of the medium and large fruits while the mean weight or mean count of non-marketable fruits correspond to the mean of the sum of the small and defective fruits.

Measurement of Nutrient Content of Plant Tissue and Soil

Fifteen plants from each treatment were harvested for plant analysis. Plant samples were brought to the Bureau of Soils Fertilizer and Pesticide Analysis Laboratory at San Fernando, La Union for the analysis.

Likewise, the pH and NPK content of the soil samples from all the treatments were analyzed at the Bureau of Soils Fertilizer and Pesticide Analysis Laboratory at San Fernando, La Union.

Assessment of Root Infection by AMF

Rapid ink staining method of Vierhilig et al. (1998) was the procedure used in assessing root infection by AMF.

Tomato roots inoculated with AMF were carefully washed to remove the soil after which they were blot dried then cut to about 1 cm. The 1-cm roots were wrapped in gauze cloth then tied with cotton twine to a stirring rod. The root samples were stained in groups. The wrapped root samples were submerged in a beaker containing 10% KOH then heated in a water bath to about 85-88℃ for 1 to 4 hours. Stained roots were decolorized with KOH, poured off then replaced with a fresh 10% KOH and heated for another hour. Cleared roots were removed then rinsed twice with 5% acetic acid. The roots were covered with 5% black Parker ink in 5% acetic acid then heated in a water bath with a temperature of 85-88ºC for 5 minutes, after which the ink solution was removed. The roots were rinsed twice with 5% acetic acid. Further destaining by soaking the roots 5% acetic acid for 20 minutes was done. Stained roots were stored in a Petri dish containing 1glycerol:1 water ready for examination.

Assessment of Colonization

Fragments of roots of tomatoes inoculated with AMF were randomly selected from a stained sample. They were mounted on microscope slides in groups of five. At least 300, 1-cm root fragments were examined per treatment. One hundred (100) 1-cm root fragments correspond to one replicate. The presence or absence of infection in root samples was recorded in each of the root pieces. The result was expressed as a percentage.

Research Design and Statistical Treatment

This study employed a completely randomized design (CRD). Levene's test was employed to determine the homogeneity of variances. Those data that were found to be insignificant in the Levene statistics were analyzed directly using the classic One-way Analysis of Variance (ANOVA), otherwise they were subjected to Welch ANOVA. Post hoc analysis employed LSD ($p<0.05$) for the data variances that were homogeneous and Games-Howell was used for those that were found to be heterogeneous based on the Levene's test. SPSS version 22 statistical tool pack was used.

4. Results and Discussion

Table 1: Mean % increase in height of one-month old tomatoes at 2nd, 4th, 6th, 8th and 10th week after AMF inoculation

Treatments	**2nd week*	**4th week*	**6th week*	*8th week*	*10th week*
no AMF (control)	307.28 a	510.53 ab	574.20 a	655.06ab	704.26 a
100 AMF spores	326.05 a	520.54 ab	606.18 a	633.56ab	692.77 a
100 AMF spores + 0.25 g colonized corn roots	296.34ab	502.93 ab	570.90 a	616.42ab	632.56 a
0.25 g colonized corn roots	303.82 a	555.10 a	641.01 a	711.04 a	731.05 a
RRF (1.03 g N)	266.42bc	464.55 bc	558.56 a	595.94 b	639.70 a
Farmer's practice	234.96 c	398.63 c	437.15 b	484.20 c	500.95 b

*means are significantly different at $p<0.05$; means with different letters differed significantly.

Percent increase in height. Table 1 shows that the mean % increase in the height of the plants significantly differed from the 2nd week to the 10th week after AMF inoculation.

There was approximately more than three-folds increase in the height of the plants inoculated with AMF two weeks after application particularly T1 (100 AMF spores) having the highest mean of 326.05%. The other two treatments namely T3 (0.25 g col. corn roots only) and T2 (100 AMF spores + 0.25 g col. corn roots) grew faster as indicated by a 303.82% and 296.34% growth rates as compared to T4 (266.42%) and T5 (234.96%) but they are slower compared to the control that grew 307.28% higher than the initial height of the plants.

Results further show that T1 (100 AMF spores) did not significantly differ from T2 (100 AMF spores + col. corn roots), T3 (colonized corn roots) and the control but significantly differed from T4 (RRF) and T5 (farmer's practice) on the 2nd week after AMF inoculation. Also, T2 (100 AMF spores + 0.25 g colonized corn roots) did not significantly differ from T4 (RRF) while T5 (farmer's practice) differed from the other treatments except T4 (RRF).

Apparently, plants inoculated with AMF-colonized corn roots (T3) had the fastest growth compared to the other treatments from the 4th to the 10th week after inoculation. The increase was five times to more than seven times as shown by the height mean increase of 555.10%, 641.01%, 711.04% and 731.05% growth rate. On the other hand, plants inoculated with 100 AMF spores consistently grew faster with 520.54%, 606.18%, 633.56% and 692.77% growth rate as compared to those treated with the RRF (464.55%, 558.56%, 595.94%, 639.70%). They also grew faster than those applied with the farmer's practice (398.63%, 437.15%, 484.20%, 500.95%) from the 4th to 10th week after AMF inoculation. The plants in this treatment (T1- 100 AMF spores) also grew rapidly as compared to the control group (510.53%, 574.2%) from the 2nd week (as mentioned previously), 4th week (520.54 %) to the 6th week (606.18%) after AMF inoculation which indicates a fast growth rate attributed to these fungi during this period. However, there seems to be a rapid growth for the control group only on the 8th and 10th week after AMF inoculation as indicated by 655.06% and 704.26% compared to 633.56% and 692.77% growth rate in T1. This implies that AMF application hastened the growth of plants as compared to non mycorrhizal plants.

Meanwhile, the plants inoculated with 100 AMF spores + 0.25 g colonized corn roots (T2) grew faster during the 4th, 6th and 8th week after AMF inoculation as shown by 502.93%, 570.90% and 616.42% growth rate as compared to those in T4 (RRF = 1.03 g N) with 464.55%, 558.56% and 595.94% increase in height. Apparently, it is only on the 10th week that the plants had grown a little faster than T2 which had about 639.70% growth rate, while T2 (100 AMF spores + 0.25 g colonized corn roots) had 632.56%. On the other hand, plants in T2 consistently grew faster than those grown with the farmer's practice particularly from the 4th to the 10th week after AMF inoculation but had a slower growth than the control in the same periods.

Statistical analysis showed that T3 (0.25 g colonized corn roots only) did not significantly differ from T1 (100 AMF spores), T2 (100 AMF spores + colonized corn roots) and the control but significantly differed from T4 (RRF = 1.03 g N) and T5 (farmer's practice) on the 4th week after AMF inoculation. In addition, T4 (RRF =1.03 g N) did not significantly differ from T5 (farmer's practice), T2 (100 AMF spores + colonized corn roots), T1 (100 AMF spores) and control but differed from T3.

On the 6th week after AMF inoculation, no significant difference was seen among T3 (colonized corn roots only), T1 (100 AMF spores), T2 (100 AMF spores + colonized corn roots), T4 (RRF = 1.03 g N) and the control, but all these treatments significantly differed from T5 (farmer's practice). Same result was seen during the 10th week after AMF inoculation.

Moreover, on the 8th week after AMF inoculation, T3 (colonized corn roots only) did not significantly differ from T1 (100 AMF spores), T2 (100 AMF spores + colonized corn roots) and the control but significantly differed from T4 (RRF = 1.03 g N) and T5 (farmer's practice). Furthermore, T4 (RRF = 1.03 g N) did not significantly differ from T1 (100 AMF spores), T2 (100 AMF spores + 0.25 g colonized corn roots) and the control but significantly differed from T3 (0.25 g colonized corn roots) and T5 (farmer's practice). In contrast, T5 (farmer's practice) differed from all the treatments.

Results of this study showed that inoculation of AMF (in any form) to tomatoes one month after sowing enhanced the growth of the seedlings 14 days to 70 days after inoculation. This finding seems to be in agreement with the observation of Oseni et al. (2010) that AMF inoculation led to a detectable growth of tomato seedlings as measured by stem length, leaf number and other growth parameters at 14, 28 and 42 days after AMF inoculation although the result did not significantly differ from the control.

Jayachandran and Shetty (2003) also reported that inoculation of AMF consisting of a mixture of spores dominated by *Glomus* and *Acaulospora* together with a few species of *Gigaspora* and *Scutellospora* plus colonized roots (75% colonization rate) of Sudan grass and Pigeon pea significantly increased saw grass growth by 14% compared to the control plants in peat soil under saturated conditions.

Table 2: Mean number of leaves of tomato after AMF inoculation

Treatments	*2nd wk*	**4th wk*	**6th wk*	*8th wk**	*10th wk*
no AMF (control)	15.07	18.33d	21.47b	30.53	34.53b
100 AMF spores	16.20	19.07cd	21.13b	30.07	42.00a
100 AMF spores + 0.25 g colonized corn roots	17.13	19.40bcd	25.53a	28.53	39.27a
0.25 g colonized corn roots	16.73	22.33a	24.67a	28.4	40.40a
RRF (1.03 g N)	16.80	20.13bc	23.80ab	29.53	33.60bc
Farmer's practice	17.00	20.67b	22.40b	28.13	30.00c

*means are significantly different at $p<0.05$; means with different letters differed significantly.

Number of leaves. Table 2 illustrates that mean number of leaves of the plants significantly differed on the 4th, 6th and 10th week after AMF inoculation but no significant difference was seen during the 2nd and 8th week.

The plants inoculated with AMF propagules, 100 AMF spores + 0.25 g colonized corn roots had the highest mean number of leaves which is 17.13 two weeks after AMF inoculation. This was followed by the plants grown with the farmer's practice, those applied with RRF and T3 (0.25 g colonized corn roots only) having a mean of 17, 16.80 and 16.73 leaves respectively. On the other hand, T1 (100 AMF spores) had a mean number of leaves of 16.2 while the control has 15.07.

Furthermore, the plants inoculated with 0.25 g colonized corn roots effected the highest number of leaves four weeks after AMF inoculation with a mean of 22.3. It was again followed by the treatments that employed farmer's practice and those treated with RRF with means of 20.67 and 20.13 respectively. The other two treatments containing AMF namely, 100 AMF spores + 0.25 g colonized corn roots and T1 (100 AMF spores) having means of 19.40 and 19.07 were consistently higher than the control with a mean of 18.33. Post hoc analysis employing LSD showed that T3 (0.25 g colonized corn roots) significantly differed from all the treatments. In contrast, T5 (farmer's practice) did not significantly differ from T4 (RRF = 1.03g N) and T2 (100 AMF spores + 0.25 g colonized corn roots) but significantly differed from the other treatments. Moreover, T2 (100 AMF spores + 0.25 g colonized corn roots) did not significantly differ from the other treatments except T3 (0.25 g colonized corn roots).

Consequently, the plants inoculated with AMF propagules, 100 AMF spores + 0.25 g colonized corn roots had the highest mean number of leaves which is 25.53, six weeks after AMF inoculation. It was followed by T3 (0.25 g colonized corn roots only), T4 (RRF = 1.03 g N) and T5 (farmer's practice) with means of 24.67, 23.80 and 22.40 respectively. It is during this period that the control had a greater number of leaves as compared to T1 (100 AMF spores) having means of 21.47 and 21.13 correspondingly.

Findings of the study further show that T2 (100 AMF spores + 0.25 g colonized corn roots) and T3 (0.25 g colonized corn roots only) have comparable effects to the plants but differed significantly from the other treatments except T4 (RRF = 1.03 g N).Those plants treated with the RRF did not significantly differ from all the other treatments.

It is evident in the results that it is only during the 8th week after AMF application that the control had the highest number of leaves with a mean of 30.53 but did not significantly differ from the other treatments. This was followed by T1 (100 AMF spores), T4 (RRF = 1.03 g N) and the other two mycorrhizal groups namely, T2 (100 AMF spores + 0.25 g col. corn roots) and T3 (0.25 g colonized corn roots only) with the corresponding means of 30.07, 29.53, 28.53 and 28.4. The plants grown with the farmer's practice had the lowest mean of 28.13.

On the 10th week after AMF inoculation, the three treatments containing AMF namely, T1 (100 AMF spores), T3 (0.25 g col. corn roots only) and T2 (100 AMF spores + 0.25 g col. corn roots) effected the highest number of leaves having means of 42, 40.40 and 39.27 respectively. The other two treatments namely, T4 (RRF = 1.03 g N) and T5 (farmer's practice) had fewer leaves (33.60 and 30) at this time as compared to the control with a mean of 34.53.

Statistical analysis showed that the plants inoculated with AMF (T1, T2 and T3) did not significantly differ from each other but significantly differed from the other treatments. This shows that AMF propagules in any form can improve the growth performance of tomatoes in terms of leaf increase at this stage of plant growth. Results further show that the effect of T4 (RRF = 1.03 g N) did not significantly differ from T5 (farmer's practice) and the control. However, the effect of the farmer's practice and the control significantly differed from each other.

Favorable nutrient condition is displayed by a positive growth performance of a plant. The distance of the roots to any part of the plant influences nutrient availability particularly in the case of the less mobile nutrients. In order to maintain rapid, optimal growth, all plant tissues must have a favorable nutrient status (Taiz and Zeiger, 2010). Arbuscular mycorrhizal fungi play a vital role in plant-soil interactions because they reduce the distance traveled by nutrients from soil to the roots through their hyphae. The intraradical and extraradical structures which are unique morphological and physiological structures of AMF serve as putative nutrient uptake sites (Bago, 2000).

Findings of the present study showed that inoculation of varied species of AMF at different rates and concentrations dominated by *A. laevis* had a significant effect to growth performance of tomato as indicated by increase in plant height and leaves. Rapid increase in height and increase in the number of leaves play a significant role in seedling development and survival (Oseni et al., 2010). It can also indicate enhanced nutrient uptake. In this study, the inoculated plants particularly those in T3 (0.25 g colonized corn roots) significantly increased in height as compared to the control as early as 4 weeks after AMF inoculation and continued until the 10th week. The mycorrhizal plants (T1, T2 and T3) also had higher number of leaves on the 2nd week after AMF inoculation as compared to the control. This result implies that AMF inoculation allows plant to subsist the seedling stage hence increase its potential to survive and grow.

Similar observation has been reported by Manila and Nelson (2013) where tomatoes inoculated with *A. laevis* and *G. fasciculatum* over a period of three weeks resulted to a significant increase in plant height, 53% higher than non-mycorrhizal plants, increase in leaf number, leaf area, fresh and dry matter of shoot and root. The study of Prasad et al (2012), showed that inoculation of *Chrysanthemum indicum* L. with *A. laevis* and *P. fluorescens* at medium concentration of superphosphate showed maximum height while a combination of *G. mosseae* + *A. laevis* + *P.fluorescens* resulted to a maximum root length. Leaf area, fresh and dry shoot weights were also maximum in these combinations at low concentration of superphosphate.

Previous studies also showed that AMF enhanced the growth of cucumber (Yan et al., 2012), banana (Aggangan et al., 2013), *Helianthus annuus (*Awotoye et al., 2009), sweet basil (Zolfaghari et al., 2013), chickpea (Kumar et al., 2002) and peach (Wu et al., 2011).

The fast growth of tomatoes inoculated with different preparations of AMF may be attributed to the early mycorrhizal colonization of the plants. In general, the susceptibility of host plants to root colonization is affected by root age (Tawaraya, 2007).The study conducted by Rini et al. (2012) showed that root infection was observed when *Glomus* sp. and *Gigaspora* sp. were inoculated to one month old oil palm seedlings but no colonization was observed when the AMF were inoculated at germination stage. In this study, AMF were inoculated to tomatoes a month after they germinated. At this stage AMF were able to enhance nutrient uptake hence, allowed a rapid increase in height and number of leaves. Once the roots were colonized (Table 10), mycorrhizal associations were formed.

Table 3: Mean height of tomatoes (cm) at 2nd, 4th, 6th, 8th and 10thweek after AMF inoculation

Treatments	*2nd week*	**4th week*	**6thweek.*	*8th week*	*10th week*
no AMF (control)	34.73	57.60 c	65.00 b	74.07	79.13
100 AMF spores	36.47	57.93 bc	67.40 ab	74.40	77.07
100 AMF spores + 0.25 g colonized corn roots	36.93	62.60 ab	70.73 ab	76.40	78.47
0.25 g colonized corn roots	34.67	63.00 a	72.53 a	80.33	82.67
RRF (1.03 g N)	35.80	61.07 abc	73.13 a	77.80	83.73
Farmer's practice	33.87	56.87 c	62.27 c	69.13	71.33

*means are significantly different at $p<0.05$; means with different letters differed significantly.

Final height. The tallest plant with mean heights of 36.93 cm and 36.47 cm were noted in tomatoes inoculated with 100 AMF spores + 0.25 g colonized corn roots (T2) and tomatoes inoculated with 100 AMF spores (T1).This was followed by T4 (RRF = 1.03 g N), T0 (no AMF) and T5 (farmer's practice with the corresponding mean heights of 35.80 cm, 34.73 cm and 33. 87 cm.

Moreover, T3 (0.25 g colonized corn roots) emerged as the tallest (63 cm) on the 4th week. As compared to the control group, the plants treated with AMF namely, T2 (100 AMF spores + 0.25 g col. corn roots) and T1 (100 AMF spores) were taller with mean heights of 62.60 cm and 57.93 cm respectively while the treatment that employed farmer's practice effected the lowest mean height of 56.87 cm. ANOVA showed significant differences among the treatment means on the 4th week after AMF inoculation. Post hoc analysis using LSD revealed that T3 (0.25 g col. corn roots only), did not significantly differ from T2 (100 AMF spores + 0.25 g colonized corn roots) and T4 (RRF = 1.03 g N) but significantly differed from the control, T1 (100 AMF spores) and T5 (farmer's practice). In addition, T1 (100 AMF spores), did not significantly differ from T2 (100 AMF spores + 0.25 g colonized corn roots), T4 (RRF = 1.03 g N), control and T5 (farmer's practice).

Furthermore, while T4 (RRF = 1.03 g N) had the highest mean of 73.13 cm on the 6th week, the plants treated with AMF namely, T3 (0.25 g col. corn roots only), T2 (100 AMF spores + 0.25 g col. corn roots) and T1 (100 AMF spores) were taller than T0 (no AMF, control) and T5 (farmer's practice) six weeks after AMF inoculation with mean final heights of 72.53 cm, 70.73 cm and 67.40 cm respectively. Post hoc analysis using LSD showed that T3 (0.25 g col. corn roots only) did not significantly differ from T4 (RRF = 1.03 g N), T2 (100 AMF spores + 0.25 g col. corn roots) and T1 (100 AMF spores). Moreover, T1 (100 AMF spores) and T2 (100 AMF spores + 0.25 g col. corn roots) did not significantly differ from the control while T5 (farmer's practice) significantly differed from the other treatments.

Meanwhile, the treatments did not significantly differ from each other during the 8th and 10th week after AMF inoculation although T3 (80.33 cm) and T4 (83.73 cm) remained to be the tallest during these weeks. Apparently, T2 (100 AMF spores + 0.25 g col. corn roots) and T1 (100 AMF spores) remained to be taller than the control group (74.07 cm) and T5 (69. 13 cm) on the 8th week after AMF inoculation with mean final heights of 76.40 cm and 74.40 cm respectively.

On the 10th week after AMF inoculation, T2 (100 AMF spores +0.25 g col. corn roots) and T1 (100 AMF spores) having mean final heights of 78.47 cm and 77.07 cm respectively were taller as compared to the treatment that employed farmer's practice with a mean final height of 71.33 cm. However, they are shorter than the control with a mean final height of 79.13 cm.

This observation is in agreement with the findings of Sirichaiwetchakul et al. (2011) wherein the height of cherry tomatoes inoculated with different AMF species significantly differed at 45, 52 and 59 days after planting. However, no significant difference was seen in the growth of the plants 66 days after planting, although the tomatoes inoculated with *Glomus mosseae* emerged as the tallest.

The data also shows that there was a continuous increase in the growth of the plants despite of the fact that they were infected with diseases. This might be due to the continuous supply of nutrients to the plants as a result of mycorrhization as most studies support (Sirichaiwetchakul et al., 2011; Rai, 2006).

Table 4: Effect of AMF in the mean number of days of flower onset and emergence of tomato fruits from sowing

Treatments	*No. of Days of Flower Onset*	*No. of Days of Fruit Emergence*
No AMF (control)	52.87 a	60.80 a
100 AMF spores	49.93 b	56.33 c
100 AMF spores + 0.25 g colonized corn roots	50.13 b	56.80 c
0.25 g colonized corn roots	52.00 ab	60.00 ab
RRF	53.47 a	61.80 a
Farmer's practice	49.53 b	57.47 bc

*means are significantly different at $p<0.05$; means with different letters differed significantly.

Number of days of Flower Onset. Results of the study showed that AMF inoculation resulted to a faster onset of flowering and fruit emergence from the time the seeds were sown. Apparently, those plants inoculated with AMF flowered earlier than the uninoculated ones. Results also revealed that emergence of fruits were faster in the

mycorrhizal plants than their non-mycorrhizal counterparts particularly those applied with RRF and the control.

Table 4 shows that the plants grown with the famer's practice together with those inoculated with 100 AMF spores and a combination of spores and colonized corn roots had the shortest days of flower onset. The aforementioned treatments effected an average of 49.53, 49.93 and 50.13 days respectively. These treatments were followed by the plants inoculated with 0.25 g colonized corn roots, control and those treated with RRF which bore their flowers after 52, 52.87 and 53.47 days respectively.

Statistical analysis showed that all the plants inoculated with different AMF propagules together with the farmer's practice did not significantly differ from each other in terms of the number of days of the onset of flowers. All of these treatments differed from the control and those applied with RRF except T3 (0.25 g colonized corn roots) which had a comparable effect to these two treatments. In addition, those plants applied with RRF did not significantly differ from the control.

Number of days of Fruit Emergence. Results consistently showed that those inoculated with 100 AMF spores and a combination of spores and colonized corn roots had the shortest days of fruit emergence registering a mean of 56.33 and 56.80 respectively. This was followed by those grown with the farmer's practice, those inoculated with 0.25 g colonized corn roots, control and RRF wherein fruits emerged after 60, 60.80 and 61.80 days respectively. Statistical analysis showed that the tomatoes inoculated with 100 AMF spores and the combination of spores and colonized corn roots together with the farmer's practice had a similar effect to the plants in terms of days of fruit emergence. However, they significantly differed from the control, those inoculated with 0.25 g colonized corn roots and those plants treated with RRF. Nonetheless, inoculation with 0.25 g colonized corn roots had a similar effect to farmer's practice. In general, late flowering and fruiting may critically affect yield as these are the bases of most farmers in using fertilizers. The time of harvest has a significant impact in the overall return of investment of farmers because they have to cope with the time when the demand of valuable crops including tomatoes is high in the market.

Abundance of macronutrients like nitrogen together with water can promote fast vegetative growth (Relf, McDAniel and Morse, 2014). The association of AMF with the roots of the treated tomatoes may have facilitated the transport of these nutrients to the plant, hence stimulated rapid flowering and even the onset of fruiting. As most studies support, inoculation of different AMF species to several crops including tomatoes has a positive effect to the growth and reproductive behavior of this plant (Sirichaiwetchakul et al., 2011). Since tomato is a mycorrhizal plant, association of AMF with its roots becomes beneficial. Once the roots are colonized, these fungi facilitate the decomposition of complex organic material and increase the uptake of nitrogen from the soil (Hodge, Campbell and Fitter, 2001). The findings of Leigh et al. (2008) indicate that the uptake of organic nitrogen is essential for the plant-fungal symbiosis. This might be the reason why the mycorrhizal plants flowered earlier and bore fruits faster than the non-mycorrhizal group and the control. On the other hand, the farmer's practice of applying unmeasured fertilizers may have been a factor in the early flowering and fruiting of the plants although it does not guarantee a good fruit quality. As was shown previously, this treatment bore the highest number and weight of non-marketable fruits (see Table 7). Too much fertilizer added to the soil as some farmers do may be stressful to the plants hence, may result to unfavorable fruit yield (Too Much Fertilizer Can Cause Gardening Problems, 1997).

Table 5: Mean weight and mean count of tomato fruits categorized as small, medium and large

Treatments	*Small size*		**Medium size*		**Large size*	
	weight (g)	*count*	*weight (g)*	*count*	*weight (g)*	*count*
no AMF (control)	95.40	7.33	143.37c	5.53c	112.75a	2.87ab
100 AMF spores	109.33	8.47	182.71bc	7.33bc	172.68a	4.47a
100 AMF spores + 0.25 g colonized corn roots	91.12	7.60	227.99ab	9.13ab	171.43a	4.27a
0.25 g colonized corn roots	118.41	8.93	180.16 bc	7.33bc	136.16a	3.47a
RRF (1.03 g N)	80.29	6.40	218.13abc	8.73ab	162.07a	3.93a
Farmer's practice	217.48	17.53	290.56a	11.33a	21.97 b	0.93b

*means are significantly different at $p<0.05$; means with different letters differed significantly.

Mean weight and count of small fruits. When the fruits were categorized into small, medium and large, results showed that the plants grown with the farmer's practice bore the highest mean weight and the greatest number of small fruits amounting to 217.48 g and 17.53 respectively. This was followed by T3 (0.25 g colonized corn roots) and T1 (100 AMF spores) having small fruits weighing 118.41 g and 109.33 g respectively with corresponding mean counts of 8.93 and 8.47. The plants inoculated with 100 AMF spores + 0.25 g colonized corn roots bore small fruits weighing 91.12 g with a mean count of 7.6, lower than the control which registered a mean weight of 95.40 g and a mean count of 7.33. The plants grown with the RRF affected the lowest mean weight of 80.29 g and fewest small fruits of 6.4. Statistical analysis showed that there was no significant difference among the treatment means for both weight and counts of the small fruits.

Mean weight and count of medium fruits. Apparently, the plants grown with the farmer's practice also bore the highest mean weight and the greatest number of medium fruits registering an amount of 290.56 g and 11.33 fruits respectively. This was followed by T2 (100 AMF + 0.25 g colonized corn roots) and T4 (RRF) with mean weights and counts of 227.99 g and 9.13 fruits for T2, while 218.13 g and 8.73 fruits for T4. Moreover, T1 (100 AMF spores)

and T3 (colonized corn roots) registered mean weights corresponding to 182.71 g and 180.16 g both of which effected a mean of 7.33 fruits. The control gave the lowest mean weight of 143.37g having a mean count of 5.53.

Although the plants grown with the farmer's practice had the highest mean weight of medium-sized fruits, it did not significantly differ from T2 (100 AMF + 0.25 g colonized corn roots) and T4 (RRF) but significantly differed from T1 (100 AMF spores), T3 (0.25 g colonized corn roots) and the control. The treatments containing AMF namely, T1 (100 AMF spores), T2 (100 AMF + 0.25 g colonized corn roots) and T3 (0.25 g colonized corn roots) together with T4 (RRF) did not significantly differ from each other. Among the inoculated plants, only those applied with 100 AMF spores + 0.25 g colonized corn roots significantly differed from the control.

Furthermore, the plants grown with the farmer's practice having the highest mean count of medium-sized fruits did not significantly differ from T2 (100 AMF + 0.25 g colonized corn roots) and T4 (RRF), but differed significantly from the other treatments. Among the plants inoculated with AMF, only T2 (100 AMF + 0.25 g colonized corn roots) significantly differed from the control.

Mean weight and count of large fruits. In terms of large fruits, T1 (100 AMF spores) had the highest mean weight and count of 172.68 g and 4.47 fruits followed by T2 (100 AMF + 0.25 g colonized corn roots) with a mean weight of 171.43 g and a mean count of 4.27. In addition, the plants grown with the RRF registered a mean weight of 162.07 and a mean count of 3.93 while the fruits from T3 (0.25 g colonized corn roots) weighed 136.16 and had a mean count of 3.47. On the other hand, the control had a mean weight of 112.75 g and a mean count of 2.87 while the fruits harvested from T5 (farmer's practice) had the lowest mean fruit weight of 21.97 g and the lowest mean count of 0.93.

Statistical analysis showed that there is a significant difference among the treatment means in terms of mean weight and count of large fruits. The weight of the large fruits derived from the plants inoculated with AMF (T1, T2, T3) did not differ significantly from the control and T4 (RRF) but differed from T5 (farmer's practice). In terms of the number of large fruits, the mycorrhizal plants (T1, T2 and T3) have a comparable effect with T4 (RRF) and the control but significantly differed from the T5 (farmer's practice). Moreover, those plants grown with the farmer's practice did not significantly differ from the control in terms of their large fruit count.

As far as the results of this study is concerned, it corroborates with the findings of Sirichaiwetchakul et al (2011) where tomatoes inoculated with a particular species of AMF resulted to bigger fruits in terms of fruit weight and diameter as compared to the control. Tomatoes inoculated with *G. mosseae* had bigger fruits that weighed 21.36 g/fruit and a diameter of 31.53 mm/fruit as compared to the plants applied with other AMF species. However, no significant difference was seen on the effect *G. mosseae* with those inoculated with *Acaulospora* sp., *E. schenckii*, *S. fulgida* and another species of *Glomus* but they differed significantly from the control.

While the results of this study show that the plants grown with the farmer's practice had the highest yield in terms of mean total weight and count (Table 7), this treatment also effected the highest yield of small fruits in terms of mean weight and count (Table 5). This finding may be attributed to improper fertilization of the plants. Since most farmers are not mindful of the real and ideal nutrient content of the media they use (because they do not subject it to soil analysis), they have the tendency to oversupply the soil with nutrients by adding fertilizer to it in the form of animal manure or chemicals.

High fertilization is stressful to plants and may result to stunted growth and unfavorable fruit yield. Excessive fertilization may lead to root burn which reduces the ability of roots to absorb and transport nutrients. Too much salt in the soil leads to the outflow of water from the plant root cells leading to plant dehydration (Too Much Fertilizer Can Cause Gardening Problems, 1997).

Table 6: Mean weight (g) and count of marketable and nonmarketable fruits at harvest

Treatments	*Marketable*		*Non-marketable*	
	weight	*count*	*weight*	*count*
no AMF (control)	256.12	8.40	125.43	9.27
100 AMF spores	355.39	11.80	153.45	10.27
100 AMF spores + 0.25 g colonized corn roots	399.41	13.40	119.51	8.60
0.25 g colonized corn roots	316.33	10.80	149.01	9.93
RRF (1.03 g N)	380.21	12.60	114.24	7.80
Farmer's practice	312.53	12.00	238.79	18.87

Mean weight and count of marketable fruits. Table 6 shows that T2 (100 AMF spores + 0.25 g colonized corn roots) effected the highest mean weight and count of marketable fruits amounting to 399.41 g and 13.4 respectively. This was followed by the fruit yield of plants applied with RRF having a mean weight of 380.21 g and a mean count of 12.6. The other treatments inoculated with AMF namely, T1 (100 AMF spores) and T3 (0.25 g colonized corn roots) had mean weights corresponding to 355.39 g and 316.33 g with mean fruit counts of 11.8 and 10.8

respectively. Lastly, the control had the lowest mean weight and number of marketable fruits measuring 256.12 g and 8.4. ANOVA showed no significant difference among the treatment means in terms of fruit marketability.

Mean weight and count of non-marketable fruits. The plants grown with the farmer's practice had the highest yield of non-marketable fruits amounting to 238.79 g and a mean count of 18.87. Following this treatment were T1 (100 AMF spores) and T3 (0.25 g colonized corn roots) with mean fruit weights of 153.45 g and 149.01 g, having mean counts of 10.27 and 9.93 respectively. In addition, the plants inoculated with 100 AMF spores + 0.25 g colonized corn roots and those grown with the RRF had lower mean weights of nonmarketable fruits as compared to the control. The said treatments registered an amount of 119.51 g and 114.24 g respectively while the control yield gave a mean fruit weight of 125.43 g. Consequently, T2 (100 AMF spores + 0.25 g colonized corn roots) and T4 (RRF) effected mean fruit counts of 8.6 and 7.8 respectively, lower than the control having a mean count of 9.27. Statistical analysis showed that there was no significant difference among the treatment means.

In general, the result of this study indicates that the plants inoculated with 100 AMF spores + 0.25 g colonized corn roots performed well in yielding marketable fruits as compared to the control although the means were not significantly different. There was an increase of about 35.88% (399.41 g vs 256.12 g, see Table 6) as compared to the control.

The effect of AMF to plant nutrition is related to the efficiency of the inoculated plants to take up nutrients and so facilitate the conversion of these nutrients to fruits especially at lower level of fertilization (Nedorost and Pokluda, 2012). In this study, the soil used had sufficient nutrients of P and K except N based on soil analysis. This might be the reason why the control group and the plants applied with RRF was not at par with the mycorrhizal group (T1, T2 and T3) in terms of yield as indicated by the insignificant difference of their means. Many studies had noted that AMF facilitate nutrient uptake in soils that have poor nutrient contents or under stress conditions. Oseni et al. (2010) concluded that AMF strain (Biocult) enhanced tomato seedling growth in the vermiculite in the absence of nutrient application.

Table 7: Total fruit yield as measured by weight (g) and count

Treatments	**Mean total weight (g)*	*Mean total count*
no AMF (control)	381.54b	17.67
100 AMF spores	508.85a	22.07
100 AMF spores + 0.25 g colonized corn roots	518.92a	22.00
0.25 g colonized corn roots	465.34ab	20.73
RRF (1.03g N)	494.45a	20.40
Farmer's practice	551.31a	30.87

*means are significantly different at $p<0.05$; means with different letters differed significantly.

Total fruit yield. Table 7 shows that the plants treated with the farmer's practice had the greatest amount of yield in terms of mean total weight and count amounting to 551.31 g and 30.87. This was followed by T2 (100 AMF spores + 0.25 g colonized corn roots) and T1 (100 AMF spores) with mean total weights of 518.92 g and 508.85 g respectively, with corresponding counts of 22 and 22.07.

The plants applied with the RRF registered a mean total weight of 494.45 g having a mean count of 20.4 fruits while those inoculated with 0.25g colonized corn roots weighed 465.34 g with a mean count of 20.73. The control had the lowest mean total weight of 381.45 g and registered a mean total count of 17.67.

Statistical analysis showed that the treatments containing AMF propagules namely T1 (100 AMF spores), T2 (100 AMF spores + 0.25g colonized corn roots), T3 (0.25 g colonized corn roots only) and also the plants grown with the RRF and farmer's practice did not significantly differ from each other in terms of total fruit weight. All of these treatments differed from the control except T3 (0.25 g colonized corn roots only). However, no significant difference was seen in the mean total count of the fruits.

This result may be in agreement with the findings of Nedorost and Pokluda (2013) where the greatest yield in tomatoes under different fertilization regimes were those inoculated with *Glomus mosseae* which was 10% higher than the control. Similarly, Sirichaiwetchakul et al (2011) confirmed that tomatoes inoculated with *G. mosseae* had the highest yield of 248 g/plant although it did not significantly differ from the other mycorrhizal treatments and the control. In another study, AMF inoculation significantly increased the number of fruits and flowers together with shoot dry matter even under severe, moderate and mild drought –stressed conditions. Fruit yield due to AMF were even higher than the non-mycorrhizal plants by more or less 24.7% (Subramanian et. al., 2006).

Table 8: Nutrient content of plant tissues/ leaves at the end of the experiment

Treatments	*% Nitrogen (N)*	*% Phosphorus (P)*	*% Potassium (K)*
no AMF, control	4.54	0.15	3.47
100 AMF spores	3.47	0.18	3.62
100 AMF spores + 0.25 g colonized corn roots	3.9	0.34	3.66
0.25 g colonized corn roots	4.01	0.18	3.86
RRF (1.03 g N)	4.03	0.24	3.98
Farmer's practice	4.02	0.29	3.66

Nitrogen (N) content. Tissue analysis showed that the plants in the control set-up had the highest percentage of N content which is 4.54 followed by T4 (RRF = 1.03g N) 4.03%, T5 (farmer's practice) 4.02% and T3 (0.25 g

colonized corn roots only) 4.01%. On the other hand, T2 (100 AMF spores + 0.25 g col. corn roots) and T1 (100 AMF spores) had a nitrogen content of 3.9% and 3.47% respectively.

Nitrogen, being a macronutrient is continuously required in relatively large amounts by rapidly growing plants (Taiz and Zeiger, 2010). In tomatoes, an adequate N supply is critical because they accumulate this nutrient continuously throughout their growth. According to Wilcox (1993) as cited by Wahle and Masiunas (2003), the percentage of total N in the leaves drops steadily from the seedling stage to the start of fruit development. At the seedling stage, approximately 80% of the total N in the plant can be found in the leaves. As development progresses, there is a shift of N accumulation from the leaves to the developing fruit. At harvest, approximately 24% of the total N is in the leaves and about 69% is in the fruit. In essence, N from the leaves decrease as the plants develop and as this nutrient is mobilized for fruiting.

Since the AMF-inoculated plants have a faster growth rate, some of the absorbed nitrogen might have been used in the growth and development process. This might be the reason why AMF-inoculated plants (T1, T2 and T3) had lower N content in their leaves as compared to the control, T4 (RRF = 1.03g N), T5 (farmer's practice). In addition, it has been observed in this study that the AMF-inoculated plants bore fruit earlier than the control and the other treatments. During this process, this nutrient might have been used earlier at the onset of fruiting. Of the three major nutrients, plants require nitrogen in the largest amounts because it promotes rapid growth, increases leaf size, hastens crop maturity, promotes fruiting and seed development (Tucker, 1999). This nutrient is a component of major macromolecules like amino acids, proteins, nucleotides, nucleic acids and an integral part of chlorophyll (Heldt, 2005; Nelson and Cox, 2008).

There is also a possibility that AMF consumed some of the nitrogen in the soil. The study of Hodge et al. (2001) show that AMF can enhance decomposition of organic matter and can transfer N acquired from a patch of organic material to host plants in proportion to hyphal density in the patch. Once these fungi detect patches of organic matter, their hyphae proliferate in the patch, obtain the inorganic N and transport it in the mycelium in the form of arginine (Govindarajulu et al., 2005). The experiment conducted by Hodge and Fitter (2010) showed that AMF used the N from organic material to stimulate their own growth and meet their own nutritional demands although some of the N was transferred to the plants. Besides, the mechanism of N transfer to the plant may operate in a similar manner as the P transfer because it has recently been discovered that a plant ammonium transporter that is mycorhizza-specific is present and activated in arbusculated cells (Guether et al., 2009).

Phosphorus (P) content. It is remarkable that the plants inoculated with 100 AMF spores + 0.25 g col. corn roots had the highest amount of P in their tissues as indicated by 0.34% compared to the other treatments, T4 (RRF = 1.03g N) and T5 (farmer's practice) having 0.29% and 0.24% P content respectively. The other treatments containing AMF namely, T1 (100 AMF spores) and T3 (0.25 g colonized corn roots only) both contain 0.18% which is still higher than the control that contains 0.15% P.

The meristem region of growing plants is high in phosphorus (Tucker, 1999). Phosphorus is one of the key macronutrient required for plant growth and metabolism. It is one of the nutrients continuously required in relatively large amounts by rapidly growing plants (Taiz and Zeiger, 2010). The significance of this element to the plant has something to do with its role in energy transfer because it is an essential component of macromolecules such as nucleotides, phospholipids and sugar phosphates. However, P may be a limiting factor to plant growth because of its low availability to plant soil. This is due to the slow diffusion of P and its high fixation in soils (Shen et al., 2011). Moreover, the organic phosphates found in the soil are in unavailable forms with low solubility so that they cannot be readily utilized by the plants. Essentially, they have to undergo a variety of solubilization reactions (Selvaraj and Chellapan, 2006) and mycorrhizas play a role in this process.

In this study, it seems that the combination of 100 AMF spores and 0.25 g colonized corn roots (at a colonization rate of 86%) were efficient in promoting P uptake by the tomatoes. Results of this study are in agreement with the findings of Jayachandran and Shetty (2003) that AMF inoculation significantly increased the P uptake of saw grass. The study utilized AMF inoculants (mixture of spores and colonized roots of Sudan grass and Pigeon pea with a colonization rate of more than 75%) dominated by *Glomus* and *Acaulospora* together with a few species of *Gigaspora* and *Scutellospora*. Furthermore, the study of Farzaneh et al. (2011) showed that moderate level of AMF colonization (18–55% of roots), enhanced the P and Mn uptake of chickpea.

Mycorrhizal plants transport P more efficiently than the non-mycorrhizal forms (Selvaraj and Chellapan, 2006) due to the presence of fungal hyphae that reduce the distance traveled by phosphorus. The extraradical fungal mycelium acts as an extension of the root system, enabling more thorough exploration of the soil for nutrients not only phosphorus, but also zinc, and copper (Pfeffer et al., 1999; Sylvia et al., 2005). Their narrow diameter relative to roots also facilitate nutrient uptake (Sylvia et al., 2005). Because of these anatomical and physiological attributes, it has been found out that the presence of mycorrhizal associations in roots can increase the rate of inflow of phosphorus up to six times more than that of the root hairs (Bolan, 1991).

Studies have shown that the uptake of phosphorus by the fungus to be transported to its host is stimulated by the transfer of carbon from the plant to the fungus across the mycrorrhizal interface. An increase in the carbon (in the form of carbohydrates) supplied by the plant to the AMF also increases the uptake of phosphorus and also hastens its transfer from the fungi to the plant. When the products of photosynthesis that should be supplied to the fungi are decreased, P uptake and transfer is also lowered (Bücking and Shachar-Hill, 2005).

Potassium (K) content. Plants in the T4 (RRF = 1.03g N) had the highest amount of K as indicated by 3.98% tissue content followed by those in T3 (0.25 g colonized corn roots only) that contains 3.86%. In addition, both plants in the T2 (100 AMF spores + 0.25 g col. corn roots) and T5 (farmer's practice) had 3.66% K content whileT1 (100 AMF spores) had 3.62% which is higher than the control having 3.47% K content**.**

Although T3 (0.25 g colonized corn roots only) and T2 (100 AMF spores + 0.25 g col. corn roots) had lower % K in their leaves, they are not at par with T4 (RRF = 1.03g N). In general, mycorrhization can also lead to enhanced K absorption. However, the study of Beltrano et al. (2013) showed that K uptake of pepper in non-saline treatment was not modified by mycorrhization. In mycorrhizal plants no significant differences were determined for K content regardless of salinity and P level. In addition, Wu et al. (2011) reported that mycorrhizal efficiencies for K and Mg were higher in peach roots than in leaves, suggesting a better acquisition of these nutrients in the roots than in the leaves. They ascribed this finding to the fact that fungal hyphae extend from the root surface hence, they increase the surface areas of the root acquiring more macroelement in this area. In this study, K was measured on the topmost part of the foliage.

Table 9: Effect of AMF in the profile of the pot soil before and after planting

	pH		*EC (mS/cm)*			*% OM*			*P (ppm)*			*K (ppm)*		
Treatments	*Before*	*After*	*Before*	*After*	*% change*	*Before*	*After*	*% change*	*Before*	*After*	*% change*	*Before*	*After*	*% change*
No AMF	6.9	6.8	0.32	0.14	56.25%	1.5	3.0	100%	66	38.67	41.41%	275	108	60.73%
100 AMF spores	6.9	6.83	0.32	0.13	59.38%	1.5	3.0	100%	66	42.33	35.86%	275	120	56.36%
100 AMF spores + 0.25 g col. corn roots	6.9	6.9	0.32	0.11	65.62%	1.5	2.17	44.67%	66	38	42.42%	275	105.33	61.7%
0.25 g col. corn roots	6.9	6.9	0.32	0.12	62.5%	1.5	2.67	78%	66	23	65.15%	275	142.67	48.12%
RRF	6.9	6.9	0.32	0.13	59.38%	1.5	2.5	66.67%	66	33.33	49.5%	275	133..33	51.52%
Farmer's practice	6.9	6.9	0.32	0.13	59.38%	1.5	2.5	66.67%	66	46.33	29.8%	275	165.33	39.88%

Soil pH and electrical conductivity (EC). Table 9 reveals that there is a slight change in pH of the pot soil of the control and T1 (100 AMF spores) but no evident change was seen in the soils of the other treatments before and after AMF inoculation. On the other hand, the soil EC of the AMF-inoculated plants particularly T2 (100 AMF spores + 0.25 g colonized corn roots) and T3 (0.25 g colonized corn roots) greatly decreased by 65.62% and 62.5% respectively as compared to the initial measurement. This was followed by T1 (100 AMF spores), T4 (RRF = 1.03 g N) and T5 (farmer's practice) with 59.38% decrease in EC while the control only had 56.25% decrease.

The findings of this study is in agreement with the result of Yan et al. (2012) who reported that AMF inoculation did not affect soil pH but decreased soil EC and soil nutrients including K^+. Among the nine treatments used, the set-up containing GLa (G. aggregatum) + GLe (*G. etunicatum*) + GLm (*G. mosseae*) + GLv (*G. versiforme*) + GIm (*Gigaspora margarita*) +ACl (*A. lacunosa*) resulted in the lowest EC value while inoculation with GLe alone resulted in the highest EC value.

EC indicates the amounts of nutrients available in the soil for the crops to absorb (Capewell, n.d.). The major and minor nutrients important for plant growth in the form of cations and anions are dissolved in the soil water, thus determine the EC level of the soil.

Apparently, results of this study revealed that T2 which is a mixture of AMF spores and colonized corn roots may have promoted root growth since it had the highest colonization rate (Table 5). Colonization of the tomato roots may have resulted to a more active root system that facilitated the translocation of nutrients from the soil to the plant hence reducing ions in the soil. This observation was ascertained by Yan et al. (2012) who suggested that communities of AMF enhanced soil aggregate formation which was correlated to mycorrhizal colonization of roots. According to Borie et al. (2008) as cited by Yan et al. (2012), hyphal development due to AMF contributes to the formation and stability of soil aggregates.

Organic matter (OM).In general, the OM content of the pot soil of all the treated plants increased after planting. The treatment containing 100 AMF spores together with the control increased by 100% while T3 (0.25 g colonized corn roots only) increased by 78%. T2 (100 AMF spores + 0.25 g colonized corn roots) had the lowest increase in % OM which is 44.67% followed by T4 (RRF = 1.03 g N) and T5 (farmer's practice) both of which increased by 66.67%.

Soil organic matter is a mixture of materials like plant and animal matter in different stages of decomposition. It is a repository of nutrients especially nitrogen and potassium (Baldock, 2011). Decomposition of organic matter is a biological process that occurs naturally. Factors like soil

organisms, the physical environment and the quality of organic matter affect the rate of decomposition (Bot and Benites, 2005). Several products like carbon dioxide (CO_2), energy, water, plant nutrients and resynthesized organic carbon compounds are released during decomposition.

The organic matter present in the pot soil increased because they may have been synthesized directly from the plants. These simple substances may be sugars, amino acids and cellulose that can be readily consumed by many organisms. Conversely, in natural ecosystems, soil organic matter increases when the rate of organic matter addition is higher than the rate of decomposition (Bot and Benites, 2005).

In the present study, it appears that the treatment containing the mixture of AMF spores and colonized corn roots effected the lowest increase in %OM in contrast to the control and those inoculated with 100 AMF spores. One possible explanation for this is that the AMF that survived in T3 may have used up the organic matter in the pot soil. According to Bot and Benites (2005) soil microorganisms use the soil organic matter as food. As they break down the organic matter, any excess nutrients are released into the soil through the process of mineralization. Upon decomposition, these nutrients (N, P and S) are in forms that plants can use. Consequently, plants inoculated with AMF spores and roots may have utilized these nutrients during growth and food production. Portions of N from the soil may decrease as plants grow and as it is transported to plant tissues and converted during fruit production (Wahle and Masiunas, 2003).

The mineralization process and the uptake of nutrients like N and P may have been facilitated by the presence of AMF in the roots, resulting to a reduction in the %OM in this treatment. Hodge et al. (2001) noted that there is an enhanced decomposition and N capture from decaying grass leaves in the presence of AMF. In fact they found out that organic material is an important source of N for AMF (Hodge et. al., 2010). However, Finlay (2008) suggested that further research is still needed to distinguish between the direct capacity of AMF to mobilize organic substrates and their possible, indirect effects on decomposition and plant nutrient uptake, caused by stimulation of decomposers and subsequent uptake of their decomposition products by mycorrhizal hyphae.

In addition, the real significance of mycorrhizal fungi is that they serve as conduits between the plants and the soil nutrients needed for their growth, enabling the flow of energy-rich compounds required for nutrient mobilization while at the same time providing a means for the translocation of mobilized products back to their hosts (Finlay, 2008)

Soil Phosphorus (P) and Potassium (K).The amount of P and K in the pot soil of all the treatments decreased. In terms of P concentration, Table 7 revealed that T3 (0.25 g colonized corn roots only) decreased by 65.15% as compared to P concentration before planting. This was followed by T4 (RRF = 1.03 g N), T2 (100 AMF spores + 0.25 g colonized corn roots) and the control with a percent decrease of 49.5%, 42.42% and 41.41% respectively. On the other hand, T1 (100 AMF spores) had a reduction of 35.86% while T5 (farmer's practice) decreased by 29.8%.

In terms of K concentration, T2 (100 AMF spores + 0.25 g colonized corn roots) decreased by 61.7% as compared to the initial measurement followed by the control with a reduction of 60.73%. The treatment inoculated with 100 AMF spores decreased by 56.36% followed by T4 (RRF = 1.03 g N) which was lower by 51.52%.

As the experiment progressed, there might have been a continuous utilization of the nutrients (P and K) during peaks of growth, flowering and eventually converted to fruit mineral contents resulting to a lowering in the concentration of these nutrients together with the soil OM at the end of the experiment.

Potassium is considered a macronutrient for plants and is the most abundant cation within plant cells. In plants, it is responsible for balancing the charges of cellular anions, enzyme activation, control of the opening and closing of stomata and serving as an osmoticum for cellular growth (Morgan and Connolly, 2013).

The findings of this study may be in accordance with the study of Yan et al. (2012) who reported that inoculation of soil with AMF communities greatly decreased the concentrations of NO^{-3}, Cl^{-}, $SO2^{-4}$, Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} in the soil.

Table 10: Mean colonization rate of tomato roots by the Arbuscular Mycorrhizal Fungi

Treatments	*Colonization Rate (%)*
100 AMF spores	58.33
100 AMF spores + 0.25 g col. corn roots	75.67
0.25 g col. corn roots only	72.00

Table 10 shows that AMF were able to colonize the treated plants at different rates. The roots of tomatoes inoculated with 100 AMF spores + 0.25 g colonized corn roots had the highest mean colonization rate of 75.67% followed by those inoculated with 0.25 g colonized corn roots with a mean of 72%. In the same manner, those inoculated with 100 AMF spores also colonized the roots registering a mean rate of 58.33%. The result of this study showed that the combination of spores and colonized corn roots from trap culture showed a greater colonization rate than spores and roots alone. This may be due to the combined effect of spores and colonized corn roots in forming AMF association with the host root. Spores alone may not be as effective as the colonized roots because there is a tendency that some of the isolated spores may not be viable**.** Some spores may be quiescent due to innate period of dormancy-mechanisms that allow them survive adverse conditions (Tommerup, 1987).

Initially, when spores germinate, they give rise to hyphae that grow toward the roots of the host plant (Bharadwaj, 2007). In *Scutellospora* and *Acaulospora* species, hyphae emerge from a germination shield within the spore. Hyphae that germinate from spores have a limited capacity to grow and eventually die if they do not encounter asusceptible host root within a week.

Colonized corn roots on the other hand can be used as propagules of these fungi. These roots contain vesicles which are hyphal swellings in the root cortex that accumulate storage products like lipids and cytoplasm (Brundrett, 2008). They can develop thick walls in older roots and may function as propagules (Biermann and Linderman 1983).Hyphae may also originate from colonized root fragments in the soil (Brundrett, 2008).Work with pot cultures indicates external hyphae (fragments or attached to mycorrhizal roots) are most infective in the families Glomaceae and Acaulosporaceae and least infective in the family Gigasporaceae (INVAM).

5. Conclusions

Inoculation with 100 AMF spores effected a rapid growth rate on the tomatoes two weeks after inoculation, while inoculation with 0.25 g colonized corn roots effected the highest % increase in height from the 4th week to the 10th week after AMF inoculation.

In terms of leaf number, 100 AMF spores + 0.25 g colonized corn roots effected the highest mean number of leaves on the 2nd and 6th weeks while those inoculated with 0.25 g colonized corn roots effected the highest mean on the 4th week after AMF inoculation. Those inoculated with 100 AMF spores together with the control effected the highest mean number of leaves on the 8thweek. All the mycorrhizal group had the highest number of leaves on the 10th week after AMF inoculation.

Inoculation of 100 AMF spores + 0.25 g colonized corn roots effected the tallest plants two weeks after AMF inoculation while application with 0.25g colonized corn roots gave the highest mean height on the 4th and 8th week. Increase in height on the plants applied with the RRF was noted on the 6th and 10th week.

AMF inoculation promoted earlier flower onset and fruit emergence from the time of sowing. In terms of fruit weight and count, plants inoculated with 100 AMF spores and 100 AMF spores + colonized corn roots effected the highest weight and number of large fruits but did not significantly differ from the other treatments except farmer's practice.

The plants inoculated with 100 AMF spores + 0.25 g colonized corn roots had the highest yield of marketable fruits in terms of weight and count although no significant difference was seen among the treatment means. Those plants grown with the farmer's practice had the highest weight and count of non-marketable fruits.

The plants treated with the farmer's practice had the greatest fruit yield in terms of mean total weight and count but did not significantly differ from those treated with AMF in terms of mean weight.

Inoculation of AMF resulted to a lower percentage of N but higher K content in the tomato leaves as compared to the control, while highest Phosphorus content in leaves were recorded in the plants inoculated with 100 AMF spores + 0.25 g colonized corn roots.

AMF inoculation did not influence the soil pH. Inoculation with 100 AMF spores + 0.25 g colonized corn roots effected the lowest soil EC and K and had the lowest increase in terms of % OM while inoculation with 0.25 g colonized corn roots effected the lowest concentration of soil P as compared to the other treatments.

Colonization of AMF was seen in the plants treated with AMF in Treatments 1, 2, and 3 with plants applied with 100 AMF spores + 0.25 g colonized corn roots registering the highest rate.

6. Recommendations

Any form of AMF propagule used in the study (spore or colonized corn roots) can be utilized as biofertilizer for other high value crops.

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