



Effect of arbuscular mycorrhizal fungi and poultry manure on growth and nutrients contents of maize in different soil type

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ABSTRACTS

Public concerned about food safety and quality have raised interest in manipulating soil nutrients management strategies that could reduce potential threat on environment and sustain food production. Recently, the application of arbuscular mycorrhizal fungi (AMF) as bio-fertilizer has gained recognition especially, in low-input agriculture. The fungi are known to improve plant nutrition and growth. However, this effect may differ according to soil properties and nutrients concentration. A greenhouse experiment was conducted to investigate the effect of AMF and poultry manure (PM) on growth and nutrients contents in maize compared to chemical fertilizer; and to determine the effect of soil properties on colonization potential of AMF. The experiment consists of 13 treatments combinations in 2 soil types (loam and peat), viz; 6 application rates of composted PM in tones (t) ha⁻¹ (0, 4, 6, 8, 10 and 12) and 2 levels of AMF; inoculated (+AMF) and un-inoculated (-AMF) plus recommended dose of NPK (RD NPK). Un-inoculated plants showed no symptoms of root colonization and recorded no AMF spore under both soils. Addition of PM stimulated AMF colonization and sporulation, the highest root colonization (RC %) and spore counts were recorded at 8 t PM+AMF under loam and 12 t PM+AMF in peat soils. Shoot dry biomass at 8 and 12 t PM+AMF under loam and peat were comparable to RD NPK. Applying 8 and 12 t PM+AMF in loam and peat recorded the highest N & K comparable to RD NPK. However, P content in shoot were statistically higher at 8, 10 & 12 t PM+AMF in loam and at 12 t PM+AMF in peat compared to RD NPK. Application of 10 & 12 t PM+AMF in loam significantly decreased plant growth, lowered AMF RC%, and nutrient content (N & K). There was a strong positive correlation between shoot dry biomass and RC % in loam ($R^2 = 0.740$ $P < 0.01$) and peat ($R^2 = 0.884$ $P < 0.01$). From the results of this study, it could be concluded that AMF have increased the efficiency use of PM and their integration have the potential to improve plant growth due to enhanced nutrients uptake and stimulated RC% in both soils. Results also indicated significantly higher shoot dry biomass, nutrients content (N, P, & K), spore counts and RC % in loam soil compared to peat, indicating that soil properties has a significant influence on effectiveness AMF.

Keywords: AMF inoculation; poultry manure (PM); nutrients content; NPK fertilizer; maize growth; soil properties.

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1. INTRODUCTION

For optimum maize growth and yield, high fertilizer application is essential. Fertilizer sources varied from organic, chemical and bio-fertilizers. Of all the fertilizers, chemical fertilizer is the most accepted and widely used by farmers due to fast and direct effect on plant growth. However, consequences from long term usage have degraded soil from its productive quality thereby reducing yield (Loveland and Webb, 2003; Phelan, 2004; Khan et al., 2008) due to poor soil structure, low soil organic matter content (Fließbach et al., 2007; Ball, 2006; Roldán et al., 2005) and imbalance in soil microbial communities, affecting agro-ecosystem functioning (Griffiths et al., 2001; Gunapala and Scow, 1998). As a result of those negative impacts, huge interest is recently geared towards promoting soil nutrients management techniques that could strengthen sustainable agricultural structure. Application of organic amendments and microbial inoculants (bio-fertilizer) is a good agronomic practice that could maintain fertility status of soil, improve plant nutrients uptake and minimize the use of chemical fertilizer. Applying poultry manure (PM) to agricultural farms has increased recently as best means for its disposal (Erich et al., 2002; McDowell and Sharpley, 2004; Toor et al., 2006). It is the most excellent of all animal manures due to high content of N, P, and K (Ma et al., 2009; Nahm et al., 2003). PM supplies nutrients more readily than other organic source (Garg and Bahla, 2008; Chandrashekara et al., 2000), improves soil fertility (Eriksen, 2005), motivate microbial growth (Suresh et al., 2004), improve soil structure which increases infiltration rate and soil water holding capacity (Deksissa et al., 2008; Belay et al., 2001). Addition of organic amendments to soil enhances microbial community and activities. Important group among the microorganisms are arbuscular mycorrhizal fungi (AMF) which form symbiotic association with majority of higher plants aiding nutrients and water uptake limited for plant growth; in exchange, fungus obtains fixed carbon from plant host. Plants colonized by AMF benefit more from enhanced water and nutrients uptake, especially, N, P, Cu, and Zn. Thus, improve plant growth and yield, ensured plant health (Lingua et al., 2002; Pozo et al., 2002) and resistance to environmental stress (Auge, 2001; Koske and Gemma, 2005) compared to un-colonize plants.

PM and AMF could serve as sustainable nutrient alternative to chemical fertilizer. This is due to the potential of the fungi to utilize unavailable organic nutrients and increase the efficiency use of nutrients through enhanced nutrients uptake (Mamatha et al., 2002; Atimanav and Adholeya, 2002; Joner, 2000), which might improve plant growth and yield (Jackson et al., 2002; Martin and Stutz, 2004; Ortas et al., 2011). Earlier workers have clearly revealed the positive effect of organic amendments in combination with AMF (Caravaca et al., 2003; Douds et al., 2010; Tanwar et al., 2013) on plant growth and improve nutrients uptake. However, excessive application of poultry manure might be undesirable due to risk of soil and water pollution (Sharpley et al., 1999; Herpperly et al., 2009) and most particularly, the high content of P in the manure could affect AMF colonization potential (Jordan et al., 2000; Varga et al., 2004). In this context, it could be interesting to understand the optimum application rate of PM that could enhance maximum benefits from AMF symbiosis. The aim of this research is to determine the optimum application rate of PM that could stimulate AMF-plant symbiosis and provide nutrients for optimum growth of maize compared to chemical fertilizer and to investigate the effect of soil properties on colonization potential of AMF.

2. MATERIALS AND METHODS

Experimental site: The experiment was conducted at the Forestry Research Garden green house (East Campus), University Malaysia Sarawak, Kota Samarahan. Located at 0°50' and 5° N and 109°36' and 115°40'E, having average rainfall of 247 days per annum with annual mean precipitation between 2,500 and 5,000, and a monthly minimum rainfall recorded around June or July but exceeded 100 mm (Andriessse, 1968). The temperatures ranges between 23 °C (73 °F) and 33°C (91° F) in the early hours of the morning and during mid-afternoon respectively with heat index reaching 42 °C (108 °F) during dry season due to humidity reaching to about 85%.

AMF inoculum: AM inoculum was a mixture of *Gl. mossea*, *Gl. geosporum* and *Gl. etunicatum* from trap cultures of *Cymbopogon citratus*, containing spores, soil with infected root fragments and hyphae.

Source of poultry manure: Composted poultry manure was procured from a commercial poultry farm around Kota Samarahan. Prior to use, the nitrogen, phosphorus and potassium composition of manure were analyzed using Kjeldahl method (A.O.A.C., 1970) and tri-acid mixture for phosphorus and potassium as outlined by Jackson, (1973). Chemical analysis of the manure indicated a pH of 8.3; N, 2.3%; P, 1.3% and K, 1.6%.

Treatments and experimental design: The experiments consist of 6 levels of poultry manure in tones (t) ha⁻¹ (0, 4, 6, 8, 10, & 12) × 2 levels of AM fungi, inoculated (+AMF) and un-inoculated (-AMF) + 1 recommended dose of NPK (RD NPK) chemical fertilizer making 13 treatment combinations × 2 (soil types) laid out in a completely randomized block design (CRBD). The following scheme was used for categorizing the treatment combinations; **Un-inoculated (-AMF):** 0 t PM-AMF (Control), 4 t PM-AMF, 6 t PM-AMF, 8 t PM-AMF, 10 t PM-AMF, 12 t PM-AMF; **Inoculated (+AMF):** 0 t PM+AMF (AMF+ only), 4 t PM+AMF, 6 t PM+AMF, 8 t PM+AMF, 10 t PM+AMF, 12 t PM+AMF and RD NPK.

Collection of soil samples and preparation of pots: Composite soil samples at 0-15 cm depth of loam and peat soils were sampled from farms around Universiti Malaysia Sarawak. Prior to experiment, the physical and chemical properties of soils were determined using standard methods. Characteristics of soil are presented in table 1. Soil samples were passed through 4.0 mm sieve and steam sterilized at 121°C for 2 h to kill all indigenous microorganisms including AM fungi. Three kilogram (3 kg) of each soil was weighed into individual plastic pot of 15cm (diameter) x 20cm (height) capacity. Soils were mixed thoroughly with poultry manure according to treatments a week before sowing. Inoculated pots received 10 g pot⁻¹ of AM inoculum placed 3 cm below pot surface and 10 g pot⁻¹ of sterilized soil for un-inoculated pots. RD NPK at 130kg N, 130kg P, and 67kg K (Department of Agriculture, 2003) was applied to appropriate pots in form of



urea, triple super phosphate (TSP) and muriate of potash (MOP) and a control without fertilizer. TSP, MOP and half dose of urea fertilizer were applied as basal nutrient and remaining half of the urea fertilizer was applied three weeks after sowing. Four viable and sterilized maize seeds (Sweet corn) were sown to individual pot. Pots were irrigated regularly and maintained at 60% field capacity. Seedlings were later thinned to one pot⁻¹ arranged within six blocks of inoculated and un-inoculated treatments on a greenhouse bench with 12 h photoperiod. The plants were harvested six weeks after sowing (WAS).

Table 1: Initial physical and chemical characteristics of soils used for greenhouse study

Properties	Soil 1	Soil 2
pH (1:5 Soil: H ₂ O)	4.9	3.4
EC (dS m ⁻¹)	0.52	0.26
Texture	Loam	Peat
Organic matter %	0.98	38.6
NO ₃ -N (mg kg ⁻¹)	6.3	4.8
Available P (mg kg ⁻¹)	5.2	2.7
Available K (mg kg ⁻¹)	120.3	78.6

Data collection

Plant growth characteristics: Plant growth parameters viz, plant height, root volume, leaf area, shoot and root biomass were assessed.

Plant height (cm): The plant height was measured from plant base to upper tip of the tallest leaf (Fageria et al., 2006) using a meter rule.

Leaf area (cm² plant⁻¹): Leaf area was measured as described by Saxena and Singh (1965) using the formula: Leaf length (cm) x leaf width (cm) x 0.75

Root volume (cm³ plant⁻¹): Root volume was determined by non-destructive volume displacement analysis as described by Brundette, (1979).

Root and shoot dry biomass (g plant⁻¹): Plant dry biomass was determined after oven drying at 80°C until constant weight was attained.

Mycorrhizal assessment

Percent root colonization (%): Root colonization was determined as described by Phillips and Hayman (1970).

$$\% \text{ colonization} = \frac{\text{No. of colonized root}}{\text{Total root No.}} \times 100$$

Soil spore count: AM spores were isolated by wet-sieving and decanting method as outlined by Gerdeman and Nicolson (1963). Isolated spores were counted under stereo binocular microscope using a counter.

Plant shoots nutrients content: The oven dried shoots were ground to fine powder and analyzed for N, P and K. Nitrogen was determined by colorimetric nitrate method using salicylic acid as described by Cataldo *et al.*, (1975). Phosphorous and potassium were determined, by acetic acid extraction (Prokopy, 1995) and measured with spectrophotometer and flame photometer as outlined by Johnson and Ulrich, (1959) and Knudsen *et al.*, (1982) for P and K respectively.

Data analysis: All data collected were subjected to one-way and two-way analysis of variance (ANOVA) under general linear model (SPSS version 19), to determine the effect of main factors and interactions. To assess the AMF effectiveness between two soil types, mean comparison based on shoot dry biomass and RC%, were regressed. Correlation coefficient between root colonization and shoot dry biomass were assessed using Pearson correlations and differences between means were separated using Duncan's LSD P<0.05.

3. RESULTS AND DISCUSSION

Effects of different rates of poultry manure on root colonization and spore counts of AMF: Results on mycorrhizal root colonization of maize and spore density under loam and peat soils indicated no evidence of mycorrhizal colonization in both soils; therefore results were not shown (Table 2). Integration of poultry manure and AMF recorded higher spore density and RC % compared to AMF without amendment (0 t PM+AMF) in both soils. Except at the highest application rate (12 t PM+AMF) under loam soil where value did not differ significantly to 0 t PM+AMF. Applying 8 and 12 t PM+AMF recorded the highest spore counts and RC % under loam and peat soils respectively. Enhanced RC % and



sporulation due to poultry manure addition as recorded in this study is in agreement with the findings of some workers (Oehl et al., 2004; Verbruggen et al., 2010; Posada and Sieverding, 2014; Gryndler et al., 2006; Vaidya et al., 2008).

Table 2: Effect of poultry manure application on AMF spore counts and root colonization % in maize six WAS in different soil type.

Treatments	Root colonization (%)		Spore counts (g ⁻¹ soil)	
	Loam	Peat	Loam	Peat
Inoculated (+AMF)				
0 t PM+AMF (AMF only)	30.67a±3.499	22.83a±2.330	9.33a±0.803	7.67a±0.558
4 t PM+AMF	43.83b±3.027	29.17b±1.352	15.17b±1.302	7.83a±0.946
6 t PM+AMF	47.00b±0.966	34.67c±0.882	16.00b±1.065	10.00c±1.065
8 t PM+AMF	67.17c±1.887	40.00d±0.577	18.17c±0.872	12.33d±1.054
10 t PM+AMF	58.67d±2.060	43.17e±0.601	16.17b±0.703	14.67e±1.406
12 t PM-AMF	33.83a±3.124	46.50ed±0.428	11.00a±0.365	16.67ed±1.022

Values are the mean of replicates with ± SE. Means in the same row followed with different alphabet are significantly different at P<0.05 according to DMRT

They justified the effect of organic amendments to have stimulated AMF community, increase their diversity and colonization potential. Srivastava et al., (2001) observed increased AMF colonization in maize due to application of farmyard manure. As revealed in this study, high application rates of PM (10 and 12 t PM ha⁻¹) under loam soil lowered degree of RC % and spore counts (Table 2), depressed plant growth (Table 3), and N & K content in shoot (Table 4). This could be attributed to the influence of nutrients concentration on AMF symbiosis (Martin et al., 2002; Smith and Read, 2008; Talbot et al., 2008; Cheeke et al., 2011; Johnson et al., 2003). It is suggested that optimum benefit from AMF symbiosis tends to be higher at low and moderate fertilizer input (Tawaraya et al., 2012; Wang et al., 2006; Alguacil et al., 2009) compared to high rates. This was further clarified by Cheeke et al., (2012), who reported high degree of AMF colonization in *Bt* and non-*Bt* maize grown under low or no fertilizer application compared to high fertilizer treatments. On the contrary, peat recorded the highest RC% and spore counts (Table 2) with maximum shoot dry biomass (Table 3) at high application rate of poultry manure (12 t PM ha⁻¹). AMF symbiosis in peat is not clearly understood (Linderman and Davies, 2003). The fact that soil properties impact great influences on AMF colonization has been revealed (Liu and Li, 2000; Linderman and Davies, 2003; Carrenho et al., 2007). Soil P concentration, type or origin of peat and microbial composition (Ponton et al., 1990; Calvet et al., 1992; Linderman and Davies, 2004) could have negative (Wang et al., 1993) or positive impact on AMF (Biermann and Linderman, 1983). Similar to the finding of this study, Graham and Timmer (1984) reported lower root colonization by *Glomus intraradices* in *Citrus jambhiri* grown in peat compared to soil. Estaun et al., (1999) observed lower degree of root colonization of *Prunus* plantlets by AMF after three months growing period in peat (17%) compared to 60% in sandy loam and 45% in peat mixed with compost. Mitysiak and Falkowski, (2010) reported increased trend in RC % due to high application rate of compost in peat.

Effects of different rates of poultry manure and AMF on plant growth: Result on plant growth biometrics viz; plant height, leaf area, root volume, shoot, and root dry weight biomass of maize as affected by AM fungi inoculation and PM are presented in table3 and figure1 for loam and peat soils. Plant growth biometrics varied significantly between the treatments in loam and peat soil. Inoculated and RD NPK fertilizer produced taller plants (Figure 1A & B), larger leaf area, higher root volume, shoot and root biomass (Table 3) compared to un-inoculated plants. Plant growth qualities increased with increase in application of PM in un-inoculated and inoculated plants under both soils. However, a gradual decrease at 10 and 12 t PM+AMF were noted for all growth biometrics under loam soil (figure 1A & table 2). Applying 8 t PM+AMF produced the tallest plant (112.50±2.265 cm) that unequalled all the treatments in loam. While, applying 10 t PM+AMF produced the tallest plant (92.00±3.706 cm) that did not differ significantly to RD NPK (90.00±2.309 cm) and 12 t PM+AMF (89.83±1.721 cm) in peat soil (figure 1B).

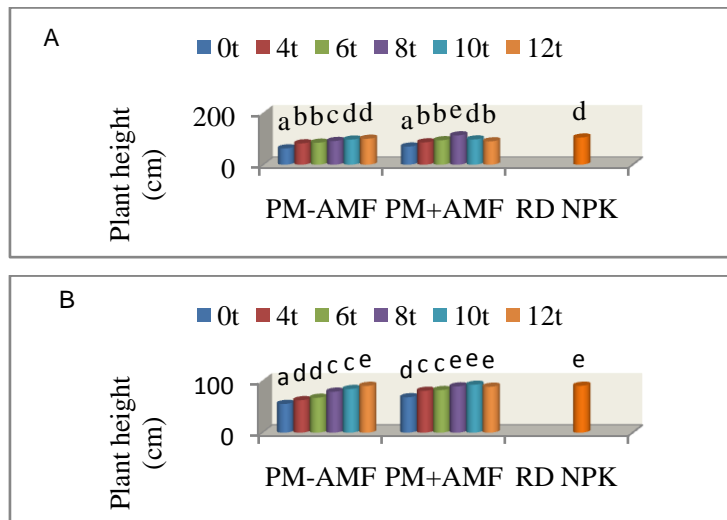


Fig. 1: Effect of AMF and poultry manure on maize plant height six WAS in (A): loam soil (B): peat

Legend: PM-AMF; poultry manure un-inoculated, PM+AMF; poultry manure inoculated; NPK chemical fertilizer

8 t PM+AMF produced shoot and root dry weight (25.33 ± 0.494 & 3.25 g) that were statistically comparable to RD NPK (25.50 ± 1.232 , 3.33 ± 0.211 g) under loam soil. Highest shoot and root dry weight were recorded in RD NPK (21.00 ± 0.365 , 3.50 ± 0.183 g) that were statistically similar to 12 t PM+AMF (19.55 ± 0.134 , 3.33 ± 0.211 g) under peat soil. Addition of poultry manure has promoted plant growth in inoculated and un-inoculated plants. However, integrating AMF and poultry manure proved to be more effective compared to sole application of either of the fertilizers. This could be attributed to the high nutrients value in PM which increases soil fertility making nutrients available for plant growth (Toor et al., 2006; Guo and Song, 2009; Zhong et al., 2010; Blaise et al., 2006; Utkhede, 2006). On the other hand, AMF inoculated plants could have benefitted more due to enhance nutrients uptake by extraradical mycelium which is lacking in un-inoculated plants (Talbot et al., 2008; Muthukumar and Udaiyan, 2000). Combination of biofertilizer and organic/inorganic fertilizers were proven to be similar or almost superior to exclusive application of recommended dose of inorganic fertilizers (Campos, 2006; Galbiatti et al., 2011; Abdullahi and Sheriff, 2013; El-Kholy and Gomaa, 2000).

Table 3: Effect of AMF and poultry manure on leaf area, root volume, shoot and root dry biomass of maize plant six WAS in different soil type

Treatments	Leaf area (cm ²)		Root vol. (cm ³)		Shoot dry biomass (g)		Root dry biomass (g)	
	Loam	Peat	Loam	Peat	Loam	Peat	Loam	Peat
Un-inoculated (-AMF)								
0 t PM-AMF (control)	120.68a	121.08a	0.94a	1.20a	3.10a	2.33a	1.33a	0.74a
4 t PM-AMF	188.85b	166.50b	1.27a	1.25ab	7.17b	4.42b	1.67a	1.48b
6 t PM-AMF	220.67c	196.88b	1.95b	1.27ab	9.00bc	4.75b	2.00ab	1.43b
8 t PM-AMF	259.92e	197.98b	2.44c	1.92c	9.50bc	8.35cb	2.58ab	1.75b
10 t PM-AMF	262.25e	193.75b	2.75c	2.30d	12.00cd	9.77c	2.33ab	1.98bc
12 t PM-AMF	283.75e	237.02cb	2.71c	2.50c	13.67cd	11.07c	2.50ab	2.00bc
Inoculated (+AMF)								
0 t PM+AMF (AMF only)	120.97a	137.38a	1.13a	1.28ab	7.00b	3.33a	1.67a	1.13a
4 t PM+AMF	211.50b	197.35b	1.78b	1.53b	11.17cd	6.83d	2.00a	1.58b
6 t PM+AMF	242.05f	225.71cb	3.28cd	1.97c	17.67d	9.50c	2.33ab	1.83b
8 t PM+AMF	320.30d	240.42cb	3.50d	2.41d	25.33e	16.33e	3.25c	3.00c
10 t PM+AMF	298.67d	249.17cb	3.09cd	2.70ef	19.33d	17.00e	3.00c	2.83c
12 t PM-AMF	228.67c	252.00cb	2.42c	2.92e	16.58d	19.55f	2.62ab	3.33d
RD NPK	324.63d	255.63cb	3.60d	3.08e	25.50e	21.00f	3.33c	3.50d

Values are the mean of six replicates. Means in the same row followed with different alphabet are significantly different at $P < 0.05$ according to DMRT.

**Effects of different rates of poultry manure and AMF on shoot nutrients**

concentration: Inoculated and RD NPK plants recorded higher shoot nutrients content (N, P, and K) compared to un-inoculated ones. N, P, and K concentration in shoots increases with increase in addition of PM in un-inoculated plants and inoculated plants in both soils. However, a gradual decrease in N and K shoot content were noted at 10 & 12 t PM+AMF (Table 4) under loam soil. N and K concentration at 8 & 12 t PM+AMF were comparable to RD NPK under loam and peat respectively (Table 4). Conversely, significantly higher P shoots contents were recorded at 8, 10 & 12 t PM+AMF in loam and at 12 t PM+AMF in peat compared to all treatments.

Table 4: Effect of AMF and PM on shoot nutrients concentration in maize plant six WAS under different soil type

Treatments	Concentration (%)					
	N		P		K	
	Loam	Peat	Loam	Peat	Loam	Peat
Un-inoculated(-AMF)						
0 t PM-AMF (control)	1.28a	1.06a	0.16a	0.13a	1.14a	1.06a
4 t PM-AMF	1.83b	1.28a	0.24bc	0.20b	1.45b	1.27b
6 t PM-AMF	2.45c	2.83b	0.27c	0.21b	1.78c	1.44c
8 t PM-AMF	2.52c	1.95bc	0.29c	0.25c	1.98d	1.71d
10 t PM-AMF	2.75c	2.30c	0.30c	0.28ab	2.13e	1.80d
12 t PM-AMF	2.93ac	2.73b	0.32cb	0.32cb	2.25cb	2.05e
Inoculated (+AMF)						
0 t PM+AMF (AMF only)	1.72b	1.57d	0.23b	0.24c	1.75c	1.36c
4 t PM+AMF	2.42c	2.12c	0.28c	0.28ab	2.07d	1.73d
6 t PM+AMF	3.23cd	2.50cb	0.34cb	0.29ab	2.57ac	2.18f
8 t PM+AMF	3.45cd	2.85b	0.44de	0.33cb	2.90bc	2.22f
10 t PM+AMF	3.12cd	3.03e	0.49e	0.36e	2.88bc	2.34cb
12 t PM-AMF	2.50c	3.13e	0.55cd	0.38ed	2.85bc	2.84bc
RD NPK	3.43cd	3.18e	0.40d	0.35e	2.87bc	2.81bc

Values are the mean of six replicates. Means in the same row followed with different alphabet are significantly different at $P < 0.05$ according to DMRT.

Liu et al., (2002) reported high plant biomass, K, Ca and Mg accumulation in tissue and their uptake by maize due to AMF symbiosis at low soil nutrients concentration. Jadhav and Patil, (1996) and Marshner and Dell, (1994) have demonstrated increased plant dry biomass, grain yield and improved accumulation of N, P and K in several plant species due to AMF symbiosis. Negative effects from high dosages of different organic amendments on AMF were revealed by some researchers (Tanwar et al., 2010; Tanwar and Angarawal, 2013). It was documented that under high nutrients availability particularly, P, AMF-plant interaction may become C sink leading to reduction in growth of plant host (Graham et al., 1997; Jifon et al., 2002). Beside saturation of soil nutrients (McArthur and Knowles, 1992; Amijee et al., 1989), P concentration in plant tissue could evidently be a critical factor influencing AMF colonization (Ratnayake et al., 1978; Braunberger et al., 1991). Azcón et al., (2003) also reported reduction in uptake of (N, P, K, Mn and Zn) and low degree of AMF colonization when high rate of N and P fertilizer were applied to lettuce plant.

Conclusion: From this study, it could be concluded that, maize responded positively to application of AMF and poultry manure. AMF have increased the efficiency use of PM by providing nutrients which enhanced maize growth over un-inoculated plants. 8 and 12 t PM ha⁻¹+AMF enhanced tissue nutrients concentration and plant growth equivalent to RD NPK in loam and peat soil respectively. Integration of PM and AMF could be considered for maize production, however; applying PM at 10 and 12 t ha⁻¹ in combination with AMF should further be investigated due to antagonistic effect on plant growth and development of AMF in loam soil as demonstrated in this study.

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