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Effect of Arbuscularmycorrhizal Fungi and Poultry Manure on Growth and Nutrients Uptake by Maize under Field Condition

Rakiya Abdullahi
Department of Molecular Biology
Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia
Email: rakiyaa6@gmail.com

Samuel Lihan
Department of Molecular Biology
Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia
Email: lsamuel@frst.unimas.my

Rebicca Edward
Deptt. of Plant Science and Environmental Ecology Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia
Email: erebicca@gmail.com

***Abstract* – Field experiment was conducted to study the effect of arbuscularmycorrhizal fungi (AMF) and poultry manure (PM) on the growth and nutrients uptake by maize. The experiment was laid out in a randomized complete block design with 6 levels of PM in tones ha^{-1} (0, 4, 6, 8, 10, & 12) and 2 levels of AMF; (inoculated , +AMF and un-inoculated, -AMF) + recommended dose of NPK (RD NPK) chemical fertilizer; making 13 treatment combinations replicated 3 times. Plant growth (height, leaf area, root volume, shoot, and root dry weight biomass), root colonization (RC %) and uptakes of N, P, & K were assessed after six weeks growing period of maize. Inoculated plants and RD NPK recorded higher values for plant growth biometrics and nutrients uptake compared to un-inoculated plants.Poultry manure application enhanced RC % and AMF spore density with maximum value (43.00±38.70 %, 17.33±1.202 g^{-1} soil) at 12 t PM ha^{-1}. Applying 12 t PM+AMF produced plants with the highest shoot and root biomass weights (199.00±3.055, 9.57±0.713 g) that were comparable to RD NPK (194.33±2.404, 9.27±0.376 g). Increase in shoot dry biomass due to AMF revealed, 19.3%, 20%, 12.2%, 7.2%, 7.9%, and 15.2% over 0, 4, 6, 8, 10 & 12 t PM ha^{-1} of un-inoculated plants. Applying 12 t PM+AMF recorded higher shoot dry biomass by 2.3% over RD NPK. RC % correlated positively with shoot biomass (R^2= 0.753). Maximum N, P, & K uptake were recorded at 12 t PM+AMF compared to all the treatments. It could be concluded that PM have potentially stimulated AMF symbiosis, enhanced maize growth and nutrients uptake under normal field condition compared to chemical fertilizer, thus could be considered for maize production.**

***Keywords* – Nutrients Uptake, AMF, NPK Fertilizer, Poultry Manure, Plant Growth.**

I. INTRODUCTION

Low-input agricultural system is gaining grounds as substitute to conventional high-input crop production due to negative environmental impact, health related issues and high cost of fertilizing crops [23,62]. Contrary to chemical fertilizers, applications of organic amendments to agricultural ecosystems might enhance crop yield, improve soil quality and reduce environmental pollution [66, 16]. Organic amendments could improve soil fertility, increase water holding capacity, soil aggregate stability and stimulate microbial population and diversity [58, 67]. An important group of the soil microbial populations is arbuscularmycorrhizal fungi (AMF).

Arbuscularmycorrhizas (AM) are group of fungi that live symbiotically in association with great majority of higher plants [28]. From such association, plant host provide synthetic carbon to fungi while in exchange; the fungus provides nutritional needs of the plant. Plant roots once colonized by AMF benefit from enhanced nutrients and water uptake, protection from soil-borne pathogens improving plant health [59, 60], tolerance to heavy metals and resistance from environmental stress [24,49]. AM fungi are important components of sustainable agricultural system. Its applications in low-input farming systems have gained popularity in many developed countries due to positive effect on environment and their potential to increase efficiency use of externally added fertilizers [13, 45].

In low-input system of agriculture, compost and farmyard manure are commonly used as source of nutrients to maintain soil fertility and improve crop production. It is suggested that the fungi are most efficient in low to moderately fertilized soil thus may be crucial in sustainability of low-input farming [65, 31]. Several researchers have highlighted positive influence of organic amendmentsapplication on AM population, diversity and colonization [6, 20,40, 48,57]. However, negative impacts have also been documented [27, 51, 32]. Conflicting results could be attributed to the nature, type, application rates, concentration and availability of nutrients in the amendments [26, 21]. George [25] reported positive effects from high application rates of composted turkey litter on AMF population and diversity compared to high rate of un-composted broiler manure. Several AMF studies with organic amendments were conducted in sterilized soil under controlled conditions, revealing positive results [38, 70, 41, 56, 42]. Abdullahi et al. [1] reported enhanced plant biomass yield and nutrients accumulation in maize grown on sterilized loam and peat soil under greenhouse due to AMF inoculation in combination with composted poultry manure in the same study region. Conversely, benefits accrued from laboratory study mostly do not reflect under field condition [12]. Though, limited studies conducted under field condition by some researchers [69, 8, 44, 5] have revealed complementary results on AMF root colonization with improved plant growth and yield due to enhanced nutrients uptake. However, there is no documented data on performance of maize inoculated with indigenous AMF isolates under field condition in the study area. As such, the present study was conducted to test the efficacy of indigenous AMF isolates under normal field condition where native AMF coexist. The aim was to determine the effect of AMF in combination with poultry manure on growth and nutrients uptake by maize under field condition.

II. Materials and Methods

The experiment was conducted at Kampong Raeh. Located at (1°20'0" North and 110°29'0" E). Having average rainfall of 247 days per annum with mean annual precipitation between 2,500 and 5,000, and a monthly minimum rainfall recorded around June or July but exceeded 100 mm (Andriesse, 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%. The soil characteristics of the experimental field are presented in table 1. The experiments comprised of 6 levels of poultry manure × 2 levels of AM fungi, inoculated (+AMF) and un-inoculated (-AMF) + recommended dose of NPK chemical fertilizer making 13 treatment combinations with three replications, laid in randomized block design. The following scheme was used for categorizing the treatment combinations;

Un-inoculated(-AMF)	Inoculated (+AMF)
0 t PM-AMF (Control)	0 t PM+AMF
4 t PM-AMF	4 t PM+AMF
6 t PM-AMF	6 t PM+AMF
8 t PM-AMF	8 t PM+AMF
10 t PM-AMF	10 t PM+AMF
12 t PM-AMF	12 t PM+AMF
RD NPK chemical fertilizer (check)	

Land used for the experiment was previously cropped to maize fertilized with chemical fertilizer only. The land was cleared and demarcated to plots sizes of $2m^2$ with three replications for each treatment. Inoculated and un-inoculated plots were separated by 2 m width unplanted boarder from each other and 0.5 m between each plot. Composted Poultry manure containing 2.3% N, 1.3% P, and 1.6% K was incorporated into soil a week prior to sowing, according to treatments. The recommended dose for inorganic fertilizers was 130kg N, 130kg P, and 67kg K [19] applied to appropriate plots in form of urea, triple super phosphate (TSP) and muriate of potash (MOP). TSP, MOP and half dose of urea fertilizer were applied as basal nutrient at time of sowing and remaining half of the urea fertilizer three weeks after sowing. Two maize seeds per hill were sown at 25 cm x 75 cm. AM inoculum was a mixture of *Gl. mossea*, *Gl. geosporum* and *Gl. etunicatum* from trap cultures of *Cymbopogoncitratus*, containing spores, soil with infected root fragments and hyphae applied at rate of 10g, sown with maize seeds for inoculated plots. One plant per hill was maintained after seedling emergence. Weeds were controlled manually using hoe when necessary. Planting was done on 19th September, 2014, grown in field under rain fed condition and harvested six weeks after sowing, (2nd November, 2014). Plant growths parameters *viz*; plant height, root volume, leaf area, shoot and root biomass, mycorrhizal assessment and nutrients uptake were analyzed.

Table 1: Salient Physical and chemical properties of soil before experiment

Characteristics	Values
Soil texture	Silt loam
pH (Soil:H_2O 1:5)	5.3
EC (dS m^{-1})	0.67
N-NO_3 (mg kg^{-1})	8.7
Available Phosphorus (mg kg^{-1})	6.8
Potassium (mg kg^{-1})	113.6
Organic C %	1.01
AMSc* spore count ($10g^{-1}$ soil)	2

AMSc*: Arbuscularmycorrhizal spore count

The plant height was measured from plant base to upper tip of the tallest leaf [22] using a meter rule. Leaf area was measured as described by Saxena and Singh [53]. Root volume was determined by non-destructive volume displacement analysis as described by Brundette, [7]. Plant dry biomass was determined after oven drying at 80°C until constant weight was attained. Root colonization was determined as described by Phillips and Hayman [47].

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

Relative mycorrhizal effectiveness (RME) was calculated as the differences between the shoot dry biomass weight of mycorrhizal plant and the shoot dry weight of non-mycorrhizal plant due to symbiosis [72] using the formulae;

$$\text{RME}(\%) = \frac{Y^{AMF+} - Y^{AMF-}}{Y^{AMF+}} \times 100$$

Where; Y^{AMF+} and Y^{AMF-} are the shoot dry biomass of mycorrhizal and non-mycorrhizal plant respectively.

The oven dried shoots were ground to fine powder and analyzed for N, P and K. Nitrogen was determined by calorimetric nitrate method using salicylic acid as described by Cataldo*et al.* [11]. Phosphorous and potassium were determined, by acetic acid extraction and measured with spectrophotometer and flame photometer as outlined by Johnson and Ulrich, [34] and Knudsen *et al.* [35] for P and K respectively. Nutrients uptake were determined based on nutrient concentration in plant shoot in relation to shoot dry biomass. 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 ofmain factors and interactions. Correlation coefficient between root colonization and plant growth were assessed using Pearson correlations and differences between means were separated using Duncan's LSD P<0.05.

III. Results

Plant growth and mycorrhizal effectiveness

Result indicated superior plant height, leaf area, and root volume in RD NPK and inoculated plants compared to un-inoculated ones. RD NPK significantly produced the tallest plants (231.67±8.988 cm) that unequalled all the treatments (Fig. 1). Applying 12 t PM+AMF recorded the largest leaf area, shoot and root dry biomasses that were comparable to RD NPK (Table 2).

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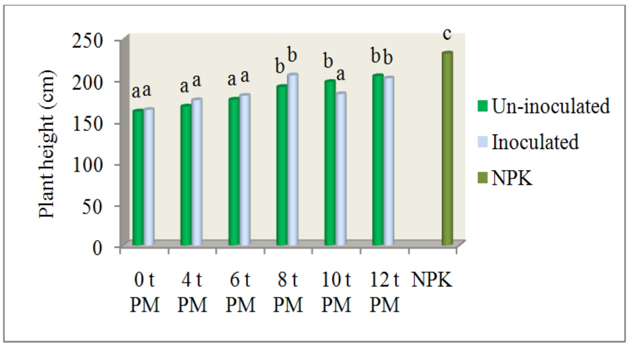

Fig.1. Effect of AMF and poultry manure on maize plant height six weeks after sowing under field condition.

Table 2: Effect of AMF and poultry manure on leaf area, root volume, shoot and root dry biomass of maize six weeks after sowing under field condition

Treatments	Leaf area (cm^2 $plant^{-1}$)	Root vol. (cm^3)	Shoot dry biomass (g)	Root dry biomass (g)
Un-inoculated (-AMF)				
0 t PM	411.27^a	4.07^a	92.00^a	3.77^a
4 t PM	493.13^b	5.97^{ad}	102.67^b	4.67^b
6 t PM	512.00^b	7.70^{dc}	144.33^c	5.43^b
8 t PM	540.90^e	9.50^d	158.67^c	6.97^b
10t PM	548.37^e	10.23^c	166.67^d	7.47^{bc}
12t PM	563.03^e	10.27^c	168.67^d	8.17^{bc}
Inoculated (+AMF)				
0 t PM	490.00^b	6.97^{ae}	114.00^b	4.77^a
4 t PM	561.53^e	8.97^{ac}	128.33^h	5.27^b
6 t PM	571.77^e	9.17^{ac}	164.33^e	6.27^b
8 t PM	579.03^e	10.30^c	171.00^e	7.43^c
10t PM	598.27^d	12.23^e	181.00^e	7.97^c
12t PM	629.40^d	13.30^d	199.00^f	9.57^d
RD NPK	617.27^d	12.53^e	194.33^f	9.27^d

Values are the mean of replicates. Means within same column followed with different superscript are significantly different at P<0.05 according to DMRT

However, root volume (13.30±0.265 cm^3) recorded at 12 t PM+AMF was incomparable to all the treatments (Table 2). Results in this study revealed maize root colonization by AMF and growth response of maize due to AMF inoculation by enhancing plant growth over un-inoculated plants. Increase in shoot dry biomass due to AMF revealed, 19.3%, 20%, 12.2%, 7.2%, 7.9%, and 15.2% over 0, 4, 6, 8, 10 & 12 t PM ha^{-1} of un-inoculated plants (Table 3).

Table 3: Effect of poultry manure application on root colonization and GRM of maize inoculated with AMF six WAS under field condition

Treatments	Root colonization (%)	Spore counts (g^{-1} soil)	RME (%)
0 t PM+AMF	20.33^a	8.33^a	19.30
4 t PM+AMF	22.33^a	10.00^b	20.00
6 t PM+ AMF	26.00^c	10.67^b	12.20
8 t PM+ AMF	27.67^c	12.00^b	7.20
10 t PM+ AMF	40.33^b	16.33^e	7.91
12 t PM+ AMF	43.00^b	17.33^e	15.24

Values are the mean of replicates. Means within same column followed with different superscript are significantly different at P<0.05 according to DMRT

Root colonization and spore counts increases with the addition of poultry manure (Table 3).

Nutrients uptake

Nutrients uptake increases with addition of PM in inoculated and un-inoculated plants. Uptakes of N, P, and K were higher in inoculated plants and RD NPK compared to un-inoculated ones (Table 4). However, applying 12 t PM+AMF recorded maximum N, P & K uptake compared to all the treatments.

Table 4: Effect of AMF and PM on shoot nutrients content & uptake by maize plant six WAS under field condition

Treatments	**Uptake (kg ha^{-1})**		
	N	**P**	**K**
Un-inoculated (-AMF)			
0 t PM	149.30^a	16.56^a	162.20^a
4 t PM	185.03^a	20.53^b	191.83^a
6 t PM	286.33^b	33.20^c	270.93^b
8 t PM	391.30^c	47.60^d	372.23^c
10t PM	509.20^{ab}	58.33^e	449.07^d
12t PM	516.80^{ab}	64.09^f	477.90^{de}
Inoculated (+AMF)			
0 t PM	215.70^b	29.64^{ab}	210.43^a
4 t PM	284.70^b	41.06^{ac}	302.90^b
6 t PM	487.83^{ab}	57.52^{cd}	448.83^d
8 t PM	522.80^{ab}	68.40^{gf}	479.47^{de}
10t PM	630.13^{ac}	79.64^{jh}	518.33^{ac}
12t PM	742.47^{ac}	97.51^{ef}	576.83^{bc}
RD NPK	692.83^{ac}	83.56^{ie}	543.87^{bc}

Values are the mean of replicates. Means within same column followed with different superscript are significantly different at P<0.05 according to DMRT

IV. Discussions

The finding of this study have clearly outlined the benefits obtained from the integration of AMF and poultry manure compared to sole application of either of the fertilizers. AMF inoculation significantly enhanced plant growth (height, leaf area, root volume, shoot and root dry biomass) and nutrient uptake over un-inoculated plants.Positive growth response of crop to AMF inoculation grown under field conditions has been reported in *Zea mays* L. [37, 39, 14], *Cucumissativus* [46], *Cicerarietinum* L. [5], *Allium fistulosum*[61] and *Zingiberofficinale*[52]. Improve plant growth due to application of organic amendments is obvious. Manure application could increase soil fertility, improve soil physical and chemical properties which might create enable condition for crop growth [71]. However, growth enhancement due to AMF inoculation could be attributed to enhance nutrients uptake by AMF hyphae. Evidence of higher plant growth and nutrients uptake in inoculated plants compared to un-inoculated plants has been

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referenced [63, 9, 68]. Enhanced nutrients uptake might have influenced larger leaf area which increased photosynthesis, [10, 2]. This could also be related to increase in the efficiency use of poultry manure by AMF and production of growth stimulating hormones [18, 3]. Increased root volume in inoculated plant and RD NPK compared to un-inoculated plants and perhaps increase in absorption of root surface area due to extension of AMF hyphae of inoculated plants might be factors that led to improved nutrients uptake and plant biomass [15, 50]. Since hyphae can exploit larger soil volume beyond normal root hairs and utilize nutrients that are bound to soil particles or from organic sources compared to un-inoculated plants [30, 36] thereby making nutrients available for plant use. Possibilities of AMF to produce organic exudates that could stimulate surrounding microbial communities capable of decomposing organic material has been proven [29, 43].

Despite the fact that the present study was conducted under normal field condition, un-inoculated plants showed no symptoms of colonization. This could be due to low native AMF population (Table 1) with poor root colonization potential. It is evident that, degree of root colonization is reflected to AMF spore counts and other infective propagules. Application of PM has enhanced spore density and root colonization of the inoculated plots. It was suggested that organic amendments might increase nutrients availability for plant growth, create better soil aeration and conditions favourable for proliferation of AMF [33, 17]. Previous researchers have justified positive influence of organic amendments to the development of AMF [54, 64, 55].

V. Conclusion

It could be concluded that AMF inoculation has enhanced plant growth and nutrients uptake compared to chemical fertilizer. Integration of AMF and poultry manure could be a suitable option for chemical fertilizer to bolster maize production, thus could be recommended.

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