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Effect of Arbuscular Mycorrhizal Fungi and Chemical Fertilizer on Growth and shoot nutrients content of Onion under Field Condition in Northern Sudan Savanna of Nigeria

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Abstract: *Field experiment was conducted to determine if arbuscular mycorrhizal fungi (AMF) could reduce the excessive amount of chemical fertilizer used in cultivation of onion. Inoculated and un-inoculated onion plant were grown with varying levels of N and P fertilizer (00-00, 40-20, 60-30, 80-40, 100-50 and 120-60 kg ha^{-1} N and P respectively), K was constant at 50 kg ha^{-1} laid out in randomized complete block design with 3 replications. Mycorrhizal colonization (%), plant height (cm), number of leaves per plant, fresh and dry shoot biomass (g), and N, P, and K concentrations in plant were determined. The results showed no significant difference in plant height and number of leaves per plant between inoculated and un-inoculated plants at 4 weeks after transplant (WAT) for all treatments. Significant ($P < 0.05$) differences in plant growth response were observed among treatments at 8 WAT. Plant growth characteristics and nutrients concentration in plant tissue of un-inoculated plants increases with increase in N and P fertilizer application. Inoculated plants with 60-30-50 kg ha^{-1} NPK produced plants with highest growth parameters (38.63 cm, 13.66, 27.80g and 3.74 g) for plant height, number of leaves, fresh shoot and dry biomass respectively as compared to un-inoculated plants with high dosages (120-60-50 kg ha^{-1} NPK) of fertilizer. Reduction in plant growth response and nutrients concentration of inoculated plants were observed with increase in fertilizer application from, 80-40, 100-50 and 120-60 kg ha^{-1} N and P. Root colonization by AMF occurred in all treatments including un-inoculated plants. Colonization potential of AMF decreases with increase in fertilizer application. Root colonization level of Un-inoculated plants ranges from 11.2% in control plants to 2.4% in plants treated with 120-60-50 kg ha^{-1} NPK. Applying fertilizer at 60-30-50 kg ha^{-1} NPK recorded the highest colonization level (39.7%) followed by (31.9%) in plants treated with (40-20-50 kg ha^{-1} NPK) and values were statistically different compared to all treatments. Mycorrhizal inoculation influenced early growth and concentrations of N, P and K at 60-30-50 kg ha^{-1} NPK fertilizer application rate. From this study, it can be concluded that using AMF could reduced the amount of excessive chemical fertilizer needed to produce onion.*
Key words: *Plant growth, mycorrhiza, NPK fertilizer, onion plant, root colonization.*

I. Introduction

Onion (*Allium cepa* L.) is a high value vegetable crop for its popularity in many spicy dishes use as matured bulbs or as green vegetables, when harvested earlier (Barzegar et al., 2008; Mahanthesh et al., 2008) and has medicinal uses (Corzo-Martìnez and Villamiel, 2007). The crop is cultivated in the semi-arid northern region of the country, where the soil is characterized with poor nutrients concentration and low organic matter (Amans et al., 1996). Onion is widely grown during rainy and dry season. However, higher yields are being obtained in dry season due to lower incidence of pest and diseases. The average bulb yield in Nigeria has stagnated and is low, around 15 tons ha^{-1} compared to other countries (FAOSTAT, 2006). This is mainly due to declining soil fertility largely as a consequence of intensive farming with low nutrient inputs. Onion has high demand for nutrients especially, nitrogen (Vachhani and Patel, 1993; Drost and Koening, 2002) consequently much emphasis is being given on sufficient fertilization to ensure high yields with acceptable bulb quality. Depletion of native soil fertility coupled with high cost and incorrect application of chemical fertilizer are major constraints to onion production. Moreover, concerns with environmental pollution and high risks of health hazards from excessive NO_3-N leaching are becoming major setbacks to the use of chemical fertilizers. Verily, an effort to minimize high application rate of chemical fertilizer use becomes imperative.

Root colonization with arbuscular micorrhizal fungi (AMF) have enhanced the uptake of nutrients, especially, P, N, and other nutrients and improve plant growth (Smith and Read, 1997; Gerdemann, 1975), reduced the amount of fertilizer required by plant (Miyasa et al., 2003; Robson et al., 1981; Joubert and Archer, 2000) and reclaim degraded soil. The interactions of onion with AMF under field conditions were well documented (Hayman and Mosse, 1971; Mosse and Hayman, 1971; Mosse, 1973). The fungi form a symbiotic association with host plant thereby improving the plants growth through acquisition of soil nutrients via their extramatrical hyphae. Other benefits of AMF for sustainable crop production are, resistance to environmental

stress and biological control of root pathogens (Gianinazzi and Vosátka, 2004; Vosátka and Albrechtova, 2008). Furthermore, complementary effect of AM fungi as an alternative for reducing fertilizer need of major crop species were reported (Mosse, 1981; Lindermann and Davies, 2004). The objectives of this work was to study the effect of AM fungal species *Glomus intraradices* in combination with varying application rate of N and P fertilizer on colonization percentage, growth and nutrients concentration in onion plant at early growth period under field conditions.

II. Materials And Methods

Experimental site

The experiment was conducted at Horticultural Garden, Mohamet Lawan College of Agriculture, Maiduguri, during 2008/2009 cool dry season from October to January. Maiduguri is located at $11^0$15'N, $13^0$15'E latitude, which lies in the semi-arid region in northeastern Nigeria, characterized by a short rainy season of 3-4 months (June – September) with an annual rainfall varying from 300mm to 650mm and a long term mean of 503mm (Grema et al., 1995). The basic physico-chemical properties of soil indicated that soil of the study was sandy loam (57% sand, 23.4% silt and 19.7% clay), neutral (pH 6.8), with EC (1.5 dS m^{-1}). The soil had low nitrogen (0.17%), organic carbon (0.86%), available soil P (5.8 mg kg^{-1}), and exchangeable K (0.32 meq/100 g soil).

Spore isolation and mycorrhiza inoculum

AMF species Glomus intraradices used for this study was isolated under actively growing Mangifera indica tree in a farm around the college premises, using the wet sieving and decanting method as outlined by Gerdemann and Nicolson (1963). The spores were mass produced using corn as trap plant grown on 3 kg of sterilized sand soil (2:1) as substrate for 3 months in 20 cm (diameter) x 10 cm (height) pot in a greenhouse. Substrate from pot was a mixture of hyphae, infected corn root fragments and sandy soil containing approximately, 115 spores per 10 g with 82 percent root colonization used as inoculum.

Onion seedling production

Onion seedlings (Bama red) local variety were produced from seeds grown on a sand-soil-compost (5:2:1 v/v) growth medium. Prior to sowing, the substrate was steam sterilized at 121^{o}C to kill all indigenous microbes including AMF. Seedlings were raised in a seed box for four weeks, grown under greenhouse condition with natural light interception, and watered regularly.

Treatments and experimental design

The experiment was a two-way factorial combination of six levels of N and P fertilizer application rates (00-00, 40-20, 60-30, 80-40, 100-50 and 120-60 kg ha^{-1} N and P), K was constant at 50 kg ha^{-1} application rate with two levels of AM inoculums, inoculated (M_1) and un-inoculated (M_0) laid out in randomized complete block design with 3 replications. Fertilizer used were, 46% urea for N, single super phosphate for P and muriate of potash for K. The treatments combination were: T_1M_0: (00-00-00 kg ha^{-1} NPK) Absolute control, T_2M_0: (40-20-50 kg ha^{-1} NPK), T_3M_0: (60-30-50 kg ha^{-1} NPK), T_4M_0: (80-40-50 kg ha^{-1} NPK), T_5M_0: (100-50-50 kg ha^{-1} NPK), T_6M_0: (120-60-50 kg ha^{-1} NPK), T_1M_1: (00-00-00 kg ha^{-1} NPK + AMF), T_2M_1: (40-20-50 kg ha^{-1} NPK + AMF), T_3M_1: (60-30-50 kg ha^{-1} NPK + AMF), T_4M_1: (80-40-50 kg ha^{-1} NPK + AMF), T_5M_1: (100-50-50 kg ha^{-1} NPK + AMF), T_6M_1: (120-60-50 kg ha^{-1} NPK + AMF).

Healthy seedlings of equal height and number of leaves were sorted for transplants. Before transplant, P and K fertilizer were incorporated into soil as basal nutrients while nitrogen fertilizer was applied in two splits at two and six weeks after transplanting according to treatments combination. 10 g of AMF inoculums were placed with seedlings into each planting hole for inoculated plots. Plot size were kept at 2m x 2m for each treatment with one seedling per hole and transplanted at spacing of 15 x 20 cm within and between rows. Standard agronomic management and irrigation were maintained uniformly for all treatments. Plants were harvested 8 weeks after transplanting (WAT).

III. Data Collected

Ten plants were randomly selected from each plot and the following parameters were measured; plant height (cm), number of leaves, shoot fresh and dry matter yield (g kg^{-1}), concentration (%) of N,P, and K, in plant shoot, and mycorrhiza root colonization (%). Plant height and number of leaves were recorded at 4 and 8 WAT. Plant height was measured from plant base to upper tip of the tallest leaf (Fageria et al., 2006) using a meter rule, number of leaves was done by visual counting. Fresh shoot and dry weight biomass (g/plant). Dry weight was determined after oven drying at 75^{o}C until constant weight is attained. Nutrients concentration; N, P, and K in plant shoot were analyzed using Kjeldahl apparatus (Nelson and Sommers, 1973) for nitrogen, while 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.

AMF colonization percentage was estimated using trypan blue staining method as outlined by Phillips and Hayman (1970). Briefly, wash root thoroughly to be free from soil. Cut root into approximately 1cm, add 10% KOH to clear root path. Wash root and boil in 2N HCl at 65^{o}C in a water bath and stained with 0.1% trypan blue. Root pieces will be observed under the microscope at 40x magnification using the grid line intersect method (Giovanetti and Mosse, 1980).

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

IV. Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA). Differences between treatments were separated using Fisher's least significant difference (LSD) at 5%.

V. Results

Plant growth response

Data for plant growth parameters in this study are presented, for plant height (Fig: 1) and (Table 1) for biomass. There was no significant difference in plant height and number of leaves per plant between inoculated and un-inoculated plants at 4 WAT for all treatments (Data not shown). Significant ($P< 0.05$) difference in plant growth response was observed among treatments at 8 WAT. Definite trends in plant growth characteristics of un-inoculated plants were observed. Plant growth characteristics increases with increase in N and P fertilizer application. Inoculated plants with 60-30-50 kg ha^{-1} NPK (T_3M_1) produced plants with highest growth parameters (38.63 cm, 13.66, 27.80 and 3.74 g) for plant height, number of leaves, fresh shoot and dry biomass respectively as compared to treatments with high dosages (120-60-50 kg ha^{-1} NPK) of fertilizer.

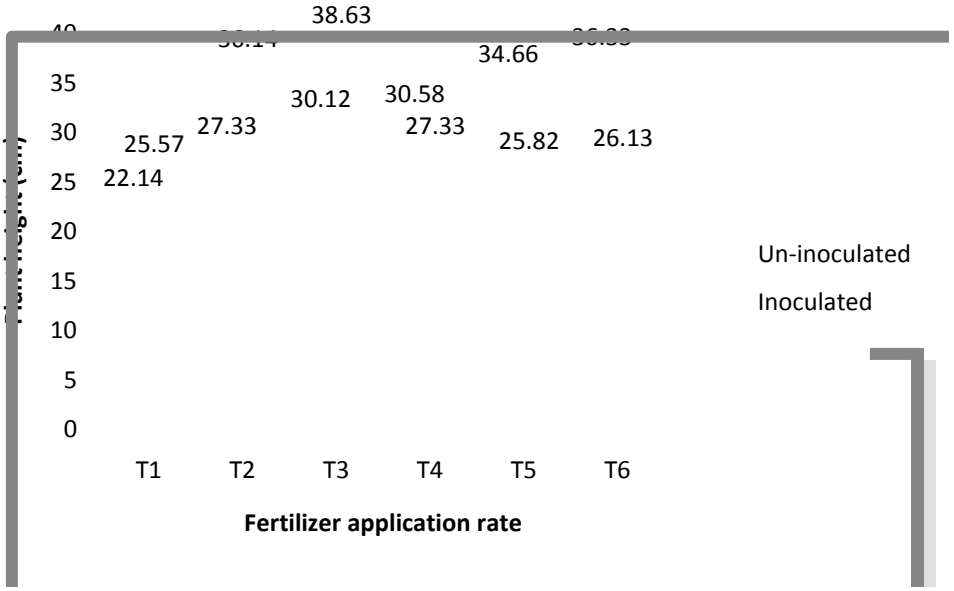

Fig. 1: Effect of AMF and N and P fertilizer on plant height at 8 WAT

Legend: T_1: (00-00-00 kg ha^{-1} NPK) Absolute control, T_2: (40-20-50 kg ha^{-1} NPK), T_3: (60-30-50 kg ha^{-1} NPK), T_4: (80-40-50 kg ha^{-1} NPK), T_5: (100-50-50 kg ha^{-1} NPK), T_6: (120-60-50 kg ha^{-1} NPK)

Table 1: Effect of AMF and N and P fertilizer on number of leaves, shoot fresh and dry weight at 8 WAT

NPK application (kg/ha)		Shoot fresh weight (g)		Shoot Dry weight (g)		Number of leaves per plant	
		M_0	M_1	M_0	M_1	M_0	M_1
T1	00-00- 00	12.24a	22.89a	1.76a	2.46a	7.45a	8.33a
T2	40-20- 50	21.02b	24.57a	2.11a	3.31ab	9.23b	12.33b
T3	60-30-50	23.25b	27.80b	2.96a	3.74ab	9.33b	13.66b
T4	80-40-50	24.85b	23.58a	3.36ab	2.29a	12.66bc	8.00c
T5	100-50-50	27.67bc	23.57a	3.68ab	2.29a	13.33bc	9.33c
T6	120-60-50	27.71bc	22.80a	3.71ab	2.44a	13.66bc	9.66c

Values followed by the same alphabet are not significantly ($P<0.05$) different according to Fischer's LSD test

Concentration of N, P, and K in plant shoot

Increase shoot nutrients content were observed with increase in fertilizer application of un-inoculated plants (Table 2).

Table 2: Effect of AMF and N and P fertilizer on nutrients concentration of onion plant shoot at 8 WAT

NPK application (kg/ha)		Nutrient concentration (%)					
		N		P		K	
		M_0	M_1	M_0	M_1	M_0	M_1
T1	00-00- 00	0.14a	0.32a	0.10a	0.76a	1.12a	1.11a
T2	40-20- 50	1.41b	4.31b	0.22b	1.31b	1.14a	2.17b
T3	60-30-50	2.62c	4.46c	1.07c	1.34b	2.73b	3.18c
T4	80-40-50	3.23d	2.10d	1.12c	0.77a	2.82c	2.13b
T5	100-50-50	4.32e	2.13d	1.31d	0.75a	3.15ab	1.14a
T6	120-60-50	4.44f	2.09e	1.32d	0.76a	3.17ab	1.13a

Values followed by the same alphabet are not significantly ($P<0.05$) different according to Fischer's LSD test

However, those values were statistically not significant to inoculated plants that were treated with, 40-20-50 and 60-30-50 NPK kg ha^{-1} Reduction in plant growth response and nutrients concentration of inoculated plants were observed with increase in fertilizer application from, 80-40, 100-50 and 120-60 kg ha^{-1} N and P.

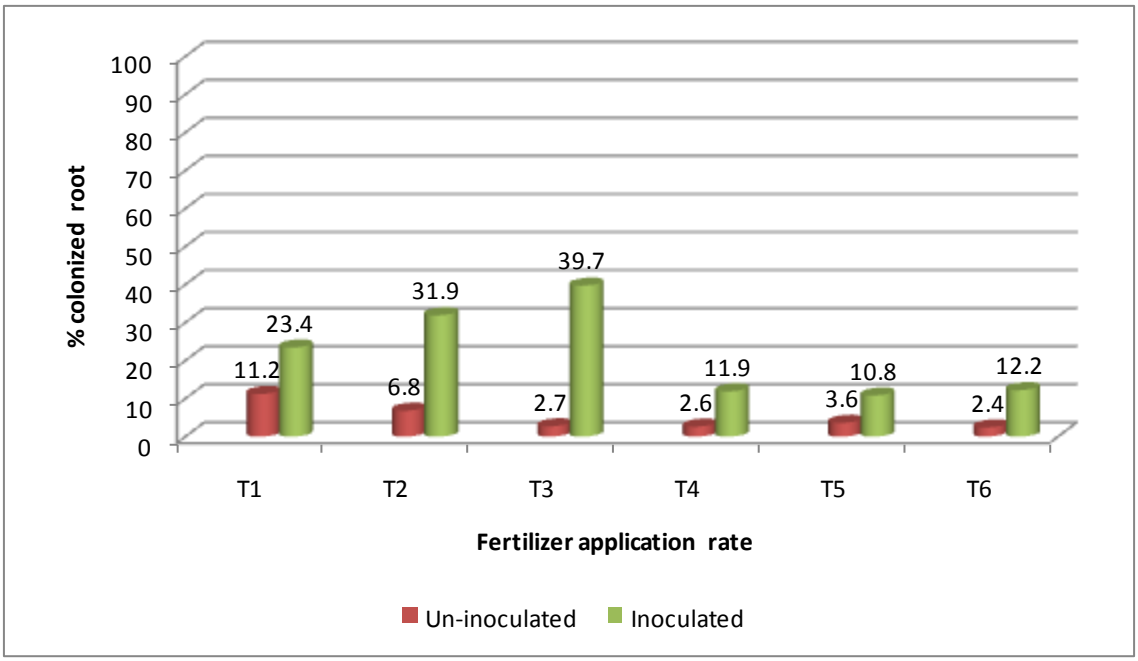

Fig. 2: The AMF root colonization on onion plant root as affected by N and P fertilizer application at 8 WAT

Legend: T_1: (00-00-00 kg ha^{-1} NPK) Absolute control, T_2: (40-20-50 kg ha^{-1} NPK), T_3: (60-30-50 kg ha^{-1} NPK), T_4: (80-40-50 kg ha^{-1} NPK), T_5: (100-50-50 kg ha^{-1} NPK), T_6: (120-60-50 kg ha^{-1} NPK)

Root colonization

Root colonization by AMF occurred in all treatments including un-inoculated plants (Fig. 2). Colonization potential of AMF decreases with increase in fertilizer application. Root colonization level of Un-inoculated plants ranges from 11.2% in control plants to 2.4% in plants treated with 120-60-50 kg ha^{-1} NPK. Root % colonization were affected with increase in fertilizer application in all the treatments. Applying N and P fertilizer at 60-30-50 kg ha^{-1} NPK recorded highest colonization level (39.7%) followed by (31.9%) in plants treated with (40-20-5050 kg ha^{-1} NPK) and values were statistically different compared to all treatments.

VI. Discussion

The results in this study revealed increased plant growth parameters, nutrients concentration in plant shoot and root % colonization when 60-30-50 kg ha^{-1} NPK was applied to inoculated plants and this was comparable to un-inoculated plants treated with high dosages of fertilizer. The influence of AM fungi in

reducing fertilizer need of major crop species were reported by Mosse, (1981) and Lindermann and Davies, (2004). It is assumed that AMF have the potential to reduce the high application rate of fertilizer needed to produce high onion yield (Smith and Read, 1997; Gerdemann, 1968). Moreover, onion plant benefits positively to AM symbiosis (De Melo, 2003; Stribley, 1996; Plenchette et al., 1983), it makes little growth without mycorrhiza unless heavily fertilized (Smith and Read, 1997; Gerdemann, 1968). Growth response of un-inoculated plant under high nitrogen fertilizer application rate of, 100 and 120 kg ha^{-1} NPK as recorded in this study was also reported by Islam et al (1999) and Singh et al (2000).

Result also revealed low level of colonization under un-inoculated plants. This implies the ubiquitous nature of the fungi, occurring naturally in most agricultural soils (Cabello, 1999; Caimey et al., 1999; Franco-Ramirez et al., 2007) and, the poor root colonization by the native AMF compared to the applied inoculums as observed in this study could be attributed to, reduction in population of the native AMF due to different soil management practices in the research field. Reduction in plant growth characteristics, shoot nutrient content and colonization % level of inoculated plant with increase in fertilizer application is in agreement with the fact that excessive chemical fertilizers have negative effect on AMF colonization (Gryndler et al., 2005a, b; Valentine et al., 2001).

VII. Conclusion

Mycorrhizal inoculation influenced early growth and nutrients uptake of N, P and K at 60-30-50 kg ha^{-1} NPK fertilizer application level. From this study, it can be concluded that using AMF could reduced the amount of fertilizer needed to produce onion since increased plant growth parameters, nutrients concentration in plant shoot and root % colonization were obtained when 60-30-50 kg ha^{-1} NPK was applied to inoculated plants and this was comparable to un-inoculated plants treated with high (120-60-50 kg ha^{-1} NPK) dosages of chemical fertilizer .

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