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DIVERSITY AND ABUNDANCE OF NATIVE ARBUSCULAR MYCORRHIZAL FUNGI IN THE RHIZOSPHERE OF *Allium cepa L* (ONION PLANTS) GROWN IN KANO STATE, NIGERIA

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ABSTRACT
Arbuscular mycorrhizal fungi (AMF) are soil borne fungi that form symbiotic association with plants roots and serve as soil bio-fertilizers by supplying mineral nutrients, plant growth hormones, and protection to the root against pathogens whereas the plants supplies the fungus with energy substrates most importantly carbon. The present study was undertaken to assess the diversity and abundance of native AMF species associated with onion plants at Kura local government farms which is the largest production cluster of onion in Kano, the assessment was done based on morphological aspect. The study revealed the presence of nine different AMF species belonging to five genera namely; Glomus, Gigaspora, Scutellespora Dentiscutata and Acalaspora. The dorminant genus was Glomus with Glomus botryoides having the highest relative abundance of 17.47% and Glomus etunicatum having the lowest relative abundance of 7.30%. The result of the soil analysis revealed the presence of high amount of phosphorus at all the sampling sites especially at site A which is significantly higher. The results after pot culture trapping of the AMF using the rhizospheric soil shows that four different AMF species associated with the onion plants could be cultured and used as bio-fertilizers in other to minimize the excessive usage of synthetic chemicals into the environment.
Keywords: Arbuscular, Mycorrhiza, Bio-fertilizer, Trapping, Morphology

INTRODUCTION

As a means towards developing sustainable environment, soil organisms that are considered plant bio-fertilizer have been used for applications to the environment. Arbuscular mycorrhizal fungi (AMF) are among these organisms and have been successfully used in both agronomy and environment (Berruti *et al.,* 2016). Arbuscular mycorrhizal fungi are members of the phylum Glomeromycota that form symbiotic associations with plant roots and supplies mineral nutrients, plant growth hormones, and protects the root against pathogens whereas the plants supplies the fungus with energy substrates most importantly carbon (Aktar *et al.,* 2009; Smith and Read, 2008). In addition AMF may also directly benefit crop species through increased resistance to disease (Jung *et al.,* 2012), tolerance to drought and adverse soil conditions (Augé, 2001; Daei *et al.,* 2009), competitive ability over non-mycorrhizal plants (Cameron, 2010; Veiga *et al.,* 2011) and indirectly through improved soil structure and increased soil nutrient retention (Bender *et al.,* 2015). Although these fungi are obligate symbionts they are not host specific and one species may be found to be associated with various plants in the same environment, also one host plant can support mixed populations of AM fungal species (Khade and Rodrigues, 2009).

Arbuscular mycorrhizas occur naturally in soil and form the oldest group of organisms that live symbiotically with nearly 90% of higher plant (Redecker *et al.,* 2000; Wang and Qiu, 2006).

Onion is one of the oldest and most widely used vegetable known to human beings belonging to family Liliaceae and it is cultivated throughout the world, (Kavitha and Reddy, 2018). Onion has many nutritional and medicinal values such as; rich source of phosphorus, calcium, vitamin C, protein and carbohydrates. Onion is also known to check the deposition of cholesterol in blood vessels, thus protect against heart diseases resulting from blockage of arteries (Barakade *et al.,* 2011).

Onion plants are highly mycorrhizal because it has a sparse system without root hairs which makes the crop dependent for water and nutrient acquisition on arbuscular mycorrhizal fungi (Shinde and Shinde, 2016). It has been shown previously that inoculation of onion plants with mycorrhizal fungi influenced early growth and nutrient uptake by plants (Albrechtova *et al.,* 2012; Abdullahi and Sheriff, 2013), and increased tolerance to salinity and water stress (Bolandnazar *et al.,* 2007). However, results of these studies were variable depending on AM species, soil fertility and experimental conditions.

Kano State is one of the largest-producers of onions in Nigeria. Onion production in this region involves intensive cultivation and heavy reliance on synthetic inputs such as chemical fertilizers and pesticides (Base Line Report on Horticulture, Kano, 2020).

Given the high cost of these inputs and their detrimental effect on the environment, the use of appropriate management practices is important to increase the productivity and production of the crop. In this regard the inoculation of microorganisms like AMF seems to be an attractive effort to minimize the usage of these chemicals.

The present study was hence undertaken to assess the diversity and abundance of native AMF species associated with onion plants in Kano, which could be cultured and used as bio-inoculants or bio-fertilizers in oder to minimize the excessive usage of synthetic chemicals into the environment.

MATERIALS AND METHODS

Study Site

The soil samples were collected from three sites at Kura local government agro-ecological zone. Kura is the largest onion production cluster in Kano state as it lies within the Kano river irrigation project (Base Line Report on Horticulture, Kano, 2020). Its geographical coordinates are latitude [11^0 46^I 30^{II}] North, and longtitude [8^0 25^I 49^{II}] East.

Soil Sampling

Soil samples were collected from the rhizosphere of the onion plants farmlands that had no previous history of microbial inoculation. Two hundred grams of soil (0-20 cm depth) were taken from five location points to obtain 1 kg of composite sample. The soil sample from each farm was mixed thoroughly to make a homogenous composite sample and each sample was packed independently in polythene bag, sealed, labeled and it was transported to the laboratory.

Soil Analysis

Initial soil analysis was done to reveal the soil pH, carbon (C), nitrogen (N), and phosphate (P) content. The soils were air-dried, grounded, and passed through a 2-mm sieve prior to analysis. Soil analysis was done using the method adopted by Manga and Rabiu (2020).

AMF Spore Extraction and Morphological Identification

AMF spores were extracted from the soil samples using the wet sieving and centrifugation method of Brundrett *et al.* (1996). 50 g of soil samples was mixed with 250 mL of tap water. The resulting mixture was passed through 450, 250, 120 and 45 µm sieves. Same process was repeated 3times and the residue from 120 and 45 µm each sieve was transferred into centrifuged tubes and added with 60% sucrose solution, then centrifuged at the speed of 3000rpm for 5 minutes. The supernatant solution is poured into the 45µm sieve and then rinsed with water. The filtrate was transferred into Petri plates with little distilled water and was observed under stereomicroscope. AMF spores were counted using stereomicroscope and compound microscope was used for identifications by observing diagnostic characteristics such as spore wall, colour, size and type of hyphal attachment using bibliographies (Schubler and Walker 2010; Goswami *et al.,* 2018), and the reference culture database established by INVAM.

$$\text{Relative AMF abundance} = \frac{\text{number of spores of a particular AMF}}{\text{total number of spores of all the AMF in the sample}} \times 100$$

Statistical Analysis

The data reported in this study were expressed as mean values of three replications. Differences among the mean values were tested by ANOVA at p<0.05. Pearson correlation analysis was done between the values of soil macro nutrients and total spore abundance for the three sites

RESULTS

The physico chemical parameters of the three sampling sites were represented in Table 1. Soil PH is slightly acidic to neutral (5.53-6.39), the soil organic carbon is low across all the sites, while the values of soil macro nutrients are in moderate to high level.

The morphological assessment of the AMF spores isolated from the onion rhizosphere was represented in Table 2. A total of nine different AMF species were identified morphologically from the rhizosphere, with *Glomus botryoides* having the highest relative abundance of 17.47% and *Glomus etunicatum* having the lowest abundance of 7.3% respectively. Correlation coefficient between the mean no of spores and values of N, P, and K were recorded in Table 3. The values show a strong negative correlation between Nitrogen, Phosphorus and the mean values of spores. Table 4 shows the morphological assessment of AMF species that were retain after pot culture trapping of the rhizospheric soil. A total of 4 different species appeared to be highly propagated after the pot trapping.

Table 1: Physico-Chemical Parameters of the Three Sampling Sites

S/N	Site	PH	O.C (%)	N (%)	P (mg/kg)	K(mol/kg)
1	Fegin zabi	5.56	0.58	0.385	55.79	0.112
2	Katsinawa	5.53	0.96	0.210	36.79	0.142
3	Daneji	6.39	0.66	0.140	31.81	0.081

Table 2: Morphological Diversity and Relative Abundance of AMF Spores Isolated From Onion Plants Rhizosphere at Three Sites

S/N	Color	Shape	Spore Surface	Morphological Identification	Mean no of Spores per 50g of Soil Site A	Site B	Site C	Total	%Relative Abundance of each specie
1	Green	Globose, Subglobose	Smooth	*Gigaspora Sp.*	7.33	6.00	14.33	27.66	14.79
2	Reddish Black	Globose	Smooth	*Glomus constrictum*	9.00	6.33	7.33	22.66	12.12
3	Brown	Globose, Cluster	Smooth	*Glomus Intraradices*	5.33	3.67	6.33	15.33	8.19
4	Reddish Brown	Elongated	Smooth	*Dentiscutata erythropa*	4.00	6.33	8.00	18.33	9.80
5	Black	Globose	Rough	*Glomus botryoides*	8.00	10.00	14.67	32.67	17.47
6	Light Brown	Globose	Smooth	*Acalaspora Sp.*	3.00	6.67	5.33	15.00	8.02
7	Dark brown	Globose, cluster	Grainy	*Glomus rubiforme*	7.33	5.67	7.33	20.33	10.87
8	Cream	Subglobose	Rough	*Scutellspora sp*	5.00	10.00	6.33	21.33	11.41
9	Orange	Globose	Smooth	*Glomus etunicatum*	3.00	4.33	6.33	13.66	7.30
	Total no of spores at each site				**52.0**[abc]	**59.0** [abc]	**76.0**[abc]	**187.0**	

Values were represented as a means of three replications. Total mean Values for the three sites are indicating no significant differences at P<0.05

Site A: Fegin Zabi
Site B: Katsinawa
Site C: Daneji

Table 3: Pearson Correlation between the Mean no of Spores of The Three Sites and Values of Nitrogen, Phosphorous and Potassium

Mean N0 of Spores for the sites	Nitrogen	Phosphorous	Potassium
Values of r	-0.88762	-0.84639	-0.69552

Table 4: Morphological Diversity and Distribution of AMF Spores Isolated From Trap Cultures of Onion Plants Rhizosphere

S/N	Color	Shape	Spore Surface	Morphological Identification	No of Spores per 100g of Soil F/Zabi	Katsinawa	Daneji
1.	Green	Globose, Subglobose	Smooth	*Gigaspora Sp.*	33.33	41.33	49.00
2.	Dark Brown	Globose, Cluster	Grainy	*Glomus rubiforme*	37.33	34.67	50.00
3.	Light Brown	Globose,	Smooth	*Acalaspora Sp.*	37.33	40.67	51.67
4.	Black	Gobose	Smooth	*Glomus botryoides*	41.67	43.33	56.67
	TOTAL				149.67[c]	160.00[b]	207.33[a]

Values were represented as a means of three replications. Different letters within the same row indicate that mean values are significantly different at P <0.05 using one way anova

DISCUSSION

In the present study a total of nine different AMF species belonging to five Genera were found to be associated with the rhizosphere of onion plants within the three sampling sites. The species are *Gigaspora specie, Dentiscutata erythropa, Acalaspora Sp, Scutellespora sp, Glomus constrictum, Glomus intraradices, Glomus botryoides, Glomus rubiforme*, and *Glomis etunicatum*. The Genus *Glomus* is the most abundant with a total of 5 different species. Among the AMF species observed *Glomus botryoides* is the most abundant with relative abundance of 17.47% while *Glomus etunicatum* is the least with relative abundance of 7.30% (table 2).

The number of species identified from this study is higher than the number of species observed by Rao *et al.* (2000) who recorded eight different AMF species associated with onion plants rhizosphere, and lower compared to the findings of Guillermo *et al.,* 2009 who recorded fourteen different AMF species from onion farms. However the dorminance of Glomus species in our observations is similar to the findings of both Rao *et al.* (2000) and Guillermo *et al.* (2009). It has been reported also by various researchers that Glomus species are the most abundant among AMF species in tropical areas (Mathimaran *et al.,* 2005; Hijri *et al.,* 2006), regardless of the host plant and intensity of disturbance in the study sites. The high abundance of Glomus species in fields is attributed to the abiltiy of the species to produce a relatively high number of spores within a very short period of time (Oehl *et al.,* 2003; Khade and Rodriguez, 2009), and probably as a consequence of the strong selection pressure imposed by agricultural fields leading to the predominance of fast root-colonizing AMF species (Oehl *et al.,* 2004).

The mean number of spores recorded in the present study is relatively low to moderate which is 52-76 spores per 50g of soil. The low number of spores per gram of soil is probably as a result of conventional farming practice on the sampling farms, which involved over usage of synthetic chemical fertilizers. This can be supported from the values of macro nutrients especially phosphorus and nitrogen recorded from the soil analysis of the study sites which is relatively high (table 1). Also past studies has shown that AMF abundance stongly correlate negatively with high Phosphorus and Nitrogen concentrations in the field. Mäder *et al.* (2002) reported higher AMF colonization in organic farms when comparing organic and conventional soil management systems. Similarly, Oehl *et al*. (2003) found higher AMF levels in plants grown on organic soils compared to conventional ones. Guillermo *et al.* (2009) also found higher AMF spores in onion organic farms than in conventional farms.

In the present study the mean total values of spores correlate negatively with the values of nitrogen and phosphorus (table 3), thus as the values of nitrogen and phosphorus decreases, the number of spores will increases. Similar observation has been made by Selvaraj *et al.* (2011) and Guillermo *et al.* (2009). It has been reported that native AMF populations are often reduced by soil management practices such as tillage, high-dosage use of systemic fungicides, and soluble phosphate fertilizers (Albrechtova *et al.,* 2012).

The result after pot culture trapping shows maximum propagation of four AMF species out of the nine previously observed from the field samples (table 4). Similar observations of decrease in AMF species diversity were made by Khade and Rodrigues, (2009); Morelos *et al.* (2014); Chairul *et al.* (2019) and Danesh *et al.* (2022) aftrer AMF pot culture trapping . Pot culture trapping is an important technique for obtaining abundant healthy spores of different species so as to establish mono specific cultures that can be used as AMF inoculum or bio-fertilizers which can be used for plants production (INVAM, 2021). A likely reason for failure in production of new spores by the remaining species during trapping of AM fungi may perhaps be due to the fact that spores may have not been viable when isolated from the field samples (Miller *et al.,* 1985) or the spores may have extended dormancy or their quiescence was not broken in the conditions and time span used (Khade and Rodrigues, 2009).

CONCLUSION

The findings from the present study revealed the presence of nine different AMF species associated with onion plants rhizosphere at Kura Local Government, Kano. Although the relative abundance of the species are low to moderate but the AMF can be mass produce using pot culture trapping in other to select the most viable and aggressive species that can be used as bio-inoculants for plants usage.

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