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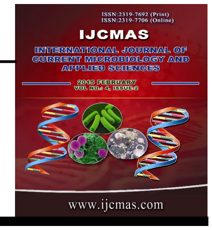

Original Research Article

Occurrence of Arbuscular Mycorrhizal Fungi in Chilli peppers (*Capsicum annuum* L.) Grown in Sahelian Soil

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A B S T R A C T

Keywords

Fungi, Colonization, Rhizosphere, Irrigation, *Capsicum annuum*

Mycorrhizal association of chilli pepper (*Capsicum annuum* L.) was carried out under sahelian irrigation condition in Bade District of Yobe State in north eastern Nigeria. The rhizosphere soil samples together with feeder root of the plant were manually collected at 5–30cm depth from twenty selected farmlands across the district that were under irrigation condition during 2012/2013 irrigation season. Spores were extracted from the soil samples and analysed for the presence and identification of mycorrhizae while the roots were analysed for mycorrhizal infection and colonization. Physico-chemical properties of the soil were also determined. The results of the study indicated the presence of mycorrhizae. All the plants sampled were found to be endomycotrophic and showing varied degree of AMF colonization in the roots which could be attributed to the physicochemical properties of the soil. The spores extracted from the soil were identified to belong to the *Glomus* and *Gigaspora* species, thereby establishing the incidence of AMF association with the test crop in the sahelian soil irrigation agro system.

Introduction

Mycorrhiza is a structural and functional association between specific fungus and the roots of higher plants. The plant (macro symbiont) gains increased exploration of the soil with the intricate net of hyphae that increases the uptake of water and nutrients from the soil interphase. The fungus (micro symbiont)) uses the carbon provided by the plant for its physiological functions, growth and development (Davies, 2011). According to Ferna'de and Fontenl, (2010), Arbuscular Mycorrhizae Fungi (AMF) influence the growth, morphology and fitness of the plant species they colonize. It has been reported by Garmendia *et al.* (2005), that AMF can be more beneficial for plant growth and physiology under dry conditions than when soil moisture is plentiful. The fine roots that perform most of the uptake process are symbiotically associated with the fungi which improve nutrient uptake and drought tolerance, and too, protect the plants against pathogens. The fungi withdraw glucose from plant roots and act as a significant sink for carbohydrates (Kottke, 2002). The effect of (AMF) and drought on fruit quality of *Capsicum annuum* was evaluated in Mexico in which the plants were exposed to 26 days drought cycle. It was observed that, the fruits of the AMF treatment subjected to drought showed similar yield to the non-drought treated subjects, with the same colour intensity and chlorophyll contents, with an increased carotenoid content of about 1.4 times compared to those non-AMF not subjected to drought condition (Mena-Violante *et al*., 2006).

Chilli pepper (*Capsicum annum* L.) belongs to the family *Solanaceae*, which is believed to be a native of Mexico and Central America, but can be cultivated in most warm regions of the world. It is a universal spice that is widely cultivated throughout temperate, tropical and subtropical countries and serves as an important crop of great commercial value which finds diverse use as vegetable, culinary and medicinal purposes. Chilli pepper has two important quality parameters which gives it great commercial attraction; the red colour due to pigment capsanthin and pungency attributed by capsaicin (Tian *et al*., 2014).

Chilli pepper is considered one of the major sources of vitamin to human, due to increasing usage among the world population. The antioxidants, vitamin C and E, and provitamin A, are all present in high concentration in this pepper species. It also constitutes a good source of carotenoids and xanthophyll (Bosland and Volova, 2002).

The aim of this work is to investigate the occurrence of Arbuscular Mycorrhizal Fungi in the roots and rhizosphere of Chilli pepper in sahelian soil, under Irrigation condition in north eastern Nigeria, and attempt to identify these fungi.

Materials and Methods

Mycorrhizal investigation was initiated on some *capsicum annuum* irrigated farms in Bade district of Yobe State in north eastern Nigeria, located within latitude $12^0 53' 10''$N, longitude $10^0 52' 38''$E and altitude 370m with a sandy-clayey soil, under Sahelian type condition, by collecting soil samples containing feeder roots of *capsicum annuum* within the rhizospheres, from the farms surveyed.

The mixture of the roots and rhizosphere soils were collected from 5 – 30 cm deep. The tools used to collect the mixture are small garden shovels with graduations. This is to enable recording of precise depth of root-soil samples collected. The farms were sampled in a randomised complete block design with five replications. The material collected were subsequently placed in plastic bags and transferred to the laboratory for analysis.

Root preparation and examination

The modified method of Phillips and Hayman (1970) was adopted for the clearing and staining of roots for examination of mycorrhizal infection or otherwise. The cleared roots segments (1–1.5 cm) earlier treated with 10% KOH, are heated in same solution at 90^0C for 1 hour, then rinsed with tap water. This is followed by addition of 20% HCl, so as to neutralise the initial KOH to allow effective binding of the fungal hyphae to stain. This was decanted after 30 minutes. The root segment were then transferred to a clean bottle containing 0.02% chlorozole black-E stain and steamed in a water bath for a period of 1 hour at

90^0C. The roots were removed from the steaming bath and left at room temperature to cool for another 30 minutes, then transferred to a bottle containing glycerol until required for microscopic examination, which was done using the Nikon research microscope for the presence or absence of arbuscules and/or vesicles in the root cells which indicates mycorrhizal infection. The percentage AMF colonisation was determined by magnified intersection method of McGoningole *et al*., 1990 in which ten slides were prepared and replicated five times. The percentage infection was then calculated using the formula, thus

%AMF = No. of intersection with AMF/Total number of intersections viewed.

Extraction and identification of spores

Mycorrhizal spores were extracted from the rhizosphere soil samples collected, using the wet sieving and decanting method described by Gerdenmann and Nicolson, (1963) and the density gradient centrifugation technique by Furlan *et al*., (1980) due to presence of organic materials. The spore suspension were then observed under a dissecting microscope, after which the spores are picked with fine tweezers and mounted in a drop of Polyvinyl alcohol-lactic acid glycerol (PVLG) and Melzer's reagent (1:1 v/v) on slides, Koske and Tessier (1983) and observed under the microscope for morphological characteristics.

The slides are placed in an incubator for 24 hours to clean the spores and the mounting medium from oil droplets and air bubbles. Also to decrease the space between the lower surface of the cover slip and the upper surface of the microscope slide so as to make it easier to take pictures of the structures of the spores.

Soil analysis

The rhizosphere soil samples were analysed for pH, Organic matter, nitrogen, phosphorus and potassium being major parameters in mycorrhizal colonization as reported by (Verinumbe, 1997).

The pH of the soil samples were determined by using the Jenway, 3020 meter, UK. The Readings recorded directly from the instrument after insertion in the soil suspension.

The available phosphorus in the soil samples were determined using the calorimetric method, while the exchangeable potassium was analysed using the atomic absorption spectrophotometer according to Thomas, (1982). Nitrogen analysis was carried out by adopting the AOAC Approved Kjeldahl method for the determination of nitrogen (AOAC International, 1999) and the wet oxidation method was adopted for the determination of the organic matter contents of the soil.

Results and Discussion

Physicochemical properties of soil

From the results of this study, table 1 gives the physicochemical properties of the rhizosphere soil of the experimental sites which includes the percentage Nitrogen, Organic matter, Potassium and the available Phosphorus content. Also included are the soil texture contents of sand, loam and clay. According to Verinumbe, 1997, mycorrhizal abundance in the soil can be attributed to the availability or otherwise of its nutrient content which is attributed to the amount of Nitrogen, Phosphorus and Potassium among others. Hopper (1983) reported that AMF colonization is influenced by the mineral salt level in the soil.

Table.1 Physicochemical properties of rhizosphere soils from individual study farms (A – E)

	A	B	C	D	E
pH	6.85	6.97	7.02	7.05	7.01
Organic matter	0.43	0.48	0.48	0.42	0.63
Nitrogen (%)	0.142	0.158	0.147	0.145	0.199
Available P (ppm)	11.5	17.1	17.0	16.5	19.5
Exchangeable K (%)	0.07	0.09	0.08	0.08	0.09
Sand (%)	46.4	45.8	46.2	45.8	45.2
Silt (%)	21.0	21.6	21.4	21.0	21.3
Clay (%)	32.6	32.6	32.4	33.2	33.5

Figure.1 showed the percentage mycorrhizal colonization of different capsicum annuum irrigated farms studied which were designated A–E with error bars.

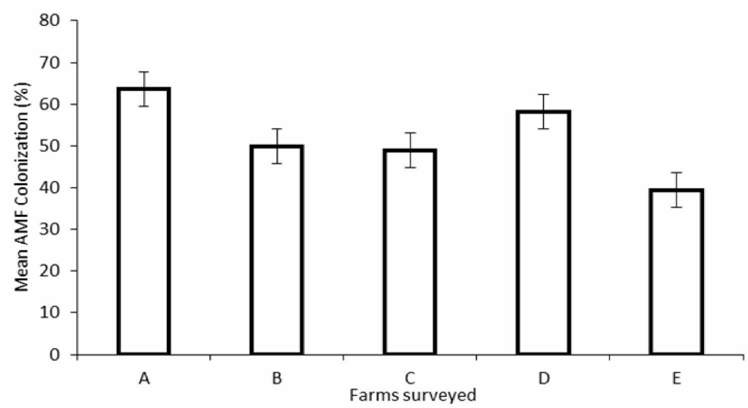


Fig.1. Mean percentage AMF colonization in different *Capsicum annuum* farms

Figure.2 Extra radical hyphae in the roots of Capsicum annuum

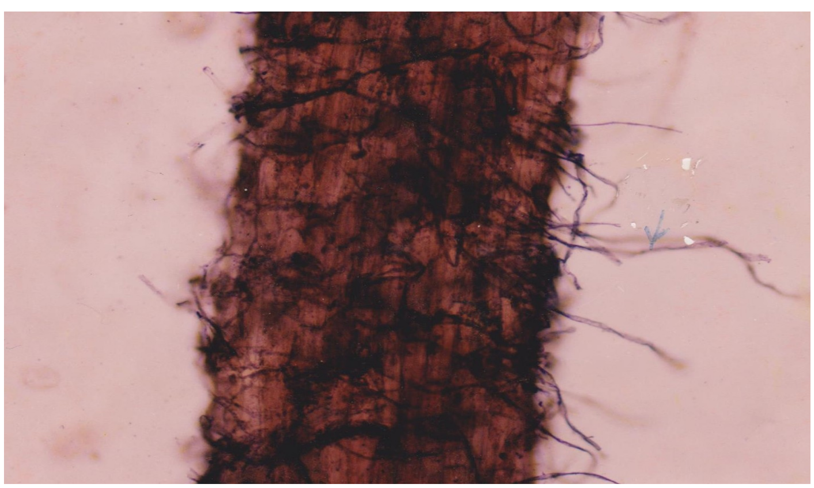


Fig.3 A young Glomus spp. mounted on PVLG X40

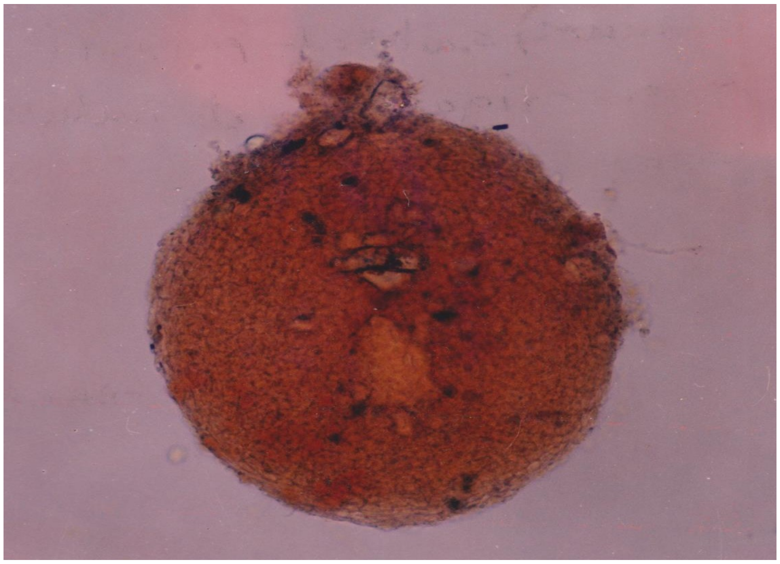
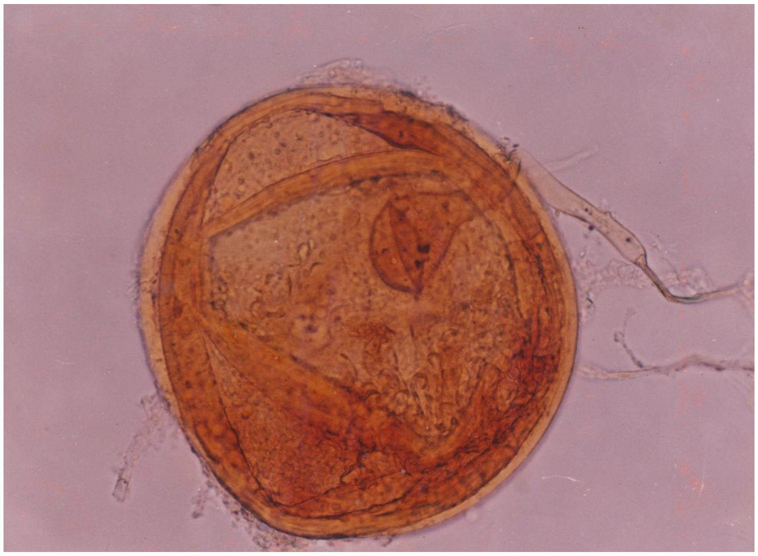


Fig.4 Mycorrhizal spore with outer yellowish and inner dark laminate walls, with bulbous hyphal Attachment (Gigaspora spp. X40)



The pH of the study area all falls between 6.86 and 7.05 indicating that the soil is almost neutral in nature, a condition that is believed to support AMF colonization. Sieverding (1991), reported that, the most common mycorrhizal fungi in neutral to alkaline soils belongs to the *Glomus* and *Gigaspora* spp. and are not seen in soils with pH readings of less than 5.5. The sandy-clayey nature of the soil will allow for easy penetration of the fungal hyphae into the soil to obtain the required minerals outside the root zone.

AMF Colonization

The presence of AMF colonization was observed in all the farms studied with varied level of establishment ranging between 35 and 65% in individual crop. It was also observed that 95% of the stands studied formed mycorrhizae at different levels. This finding agrees with the observations of Ifeoma and Ene-Obong (2010), in their studies of mycorrhizal development in *Pinus caribaea* plants.

Identification of spores

In Figure 2, the extension of extra radical hyphae from roots of *Capsicum annum* to the rhizosphre (soil) was observed. AMF are known to colonize up to 80% of the roots of terrestial plant, there by increasing their drought resistance as well as improving their nutrition, growth and disease tolerance (Elsen, *et al*., 2008). Also according to Ndiaye, *et al*., (2011), the association of AMF with plants enhances their growth under water deficient condition.

The different spore types observed during the studies were presented in figures 3 and 4. Figure 3 showed the first spore type, identified as a young *Glomus* spp mounted on Polyvinyl alcohol Lactic acid Glycerol (PVLG), by adopting the method of (Koske and Tessier, 1983). This spore is found in the soil suspension, with colours which variy from brown to yellowish with attached hyphae. It is globose in shape and variable in size, with an inner brown laminate wall which cracks under light pressure. This fits the description of *Glomus etunicatum* by Becker and Gerdenman (1977). It was also verified from the flora of british fungi colour identification chart and identified as a *Glomus* species

The second spore type obtained was brownish in colour and has a bulbous suspensor attached to long slender hyphae and presented in figure 5. Gardenmann and Nicolson, (1968) reported that only *Gigaspora* species produce a bulbous attachment among all AM Fungi, thus, this spore was identified as a *Gigaspora* species, However, this was not extended to species level.

This study confirm that Chilli peppers (*Capsicum annuum*) under Sahelian soil irrigation condition associate with Arbuscular Mycorrhizal Fungi which are clearly endomycohhyzal in nature as indicated by the extraradical hyphae of AMF observed on the root segments of the studied plant specimens. The results also revealed that, the dominant AMF species in the study area are those of the *Glomus* and *Gigaspora* spp. as indicated by the presence of enomous number of spores of these species in all the studied sites.

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