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Role of native arbuscular mycorrhizal fungi on maize (*Zea mays*) growth and nutrient uptake in acidic soils under controlled conditions

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Abstract

Indigenous arbuscular mycorrhizae Fungi (AMF) have a potential to boost maize (*Zea mays*) growth and increase the P and Zn uptake through the symbiotic association they form with the plant, even in acidic soils conditions. Five AMF inoculums produced from the most abundant and ubiquitous morphotypes isolated from field soils in maize fields in South Kivu (DRC) were assessed. A greenhouse experiment was conducted to determine the role of these AMF on nutrients uptake in a Nitisol and a Ferralsol. Eight treatments namely inoculums named AMF1 (*Gigaspora gigantea*), AMF2 (*Gigaspora* sp.), AMF3 (*Gigaspora margarita*), AMF4 (*Rhizophagus intraradices*) AMF5 (*Acaulospora reducta*), mineral phosphorus fertilizers (Pi), commercial biofertilizer Rhizatech and a Control were laid in a randomized complete block design. In the Ferralsol, Pi application, Rhizatech and AMF2 produced the highest height. Pi application resulted in the best shoot biomass. No difference was observed for the P content, but for the Zn content, AMF2 was the highest. Roots colonization did not vary among treatments. In the Nitisol, AMF4 produced the highest plant height and AMF1 the highiest chlorophyll content. AMF4 and Rhizatech colonized highly the roots. AMF3 gave the highest P however, Zinc content was equal in all treatments and the controls yielded the lowest results. Spores densities in both inoculums produced and experimental soils were low compared to the commercial inoculum but growth and roots colonisation was influenced by fertilization and soils types. The performance of efficient AMF inoculums of *Gipaspora gigantea*, *Gigaspora* sp., *Rhizophagus intraradices* and *Acaulospora reducta* applied with high densities spores and multispecies inoculums should be assessed.

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Introduction

Maize (*Zea mays*) is the second produced crop in the world and in Sub Saharan Afrcica its cultivation area has increased by 60% in the last 10 years (Santpoort, 2020). It is the third staple food after cassava and plantain in the Democratic Republic of the Congo (DRC), playing an important role in food security and income generation for many smallholder families in South Kivu province in DRC (Mushagalusa *et al.*, 2017; Anonymous, 2018). Maize yield is still very low in DRC (0.77 tons ha^{-1}), while the average yields in neighbouring countries with the same agroecological conditions are 1.574 tons ha^{-1} and 2.31 tons ha^{-1} in Rwanda and Uganda respectively (FAOSTAT, 2018). The maize actual yield is only 22% of the potential which is estimated to 3.5 tons ha^{-1} in DRC (Badibanga, 2013). This high yield gap is a result of dominant acidic soils with low soil fertility, low inputs, lack of appropriate tailored specific technologies and poor farm management practices (Tittonell and Giller, 2012).

Ferralsols and Nitisols, are among the soil orders dominant in the agricultural fields in SSA, especially in South Kivu (Ngongo *et al.*, 2009; Jones *et al.*, 2013; Bashagaluke, 2014; Bagula *et al.*, 2014). They are acidic, with low available phosphorus (P) content (Batjes, 2011), generally below 10 $mgkg^{-1}$ of P Olsen (Nziguheba *et al.*, 2016). The available inorganic P present in the soil is readily sequestered by Aluminium (Al), iron (Fe) and Manganese (Mn) in acidic soils. The mobility of sequestered P is reduced (Bucher, 2007), while it is one of the most important determinants in maize growth (Gomes *et al.*, 2015). Maize is sensitive to P and Zinc (Zn) availability as the deficiency of these elements has a detrimental effect on maize production (Ortas *et al.*, 2011). Frequently applied to agricultural soils, P is required to maintain high crop productivity but it is both expensive for poor farmers, scarce and environmentally undesirable if applied in an uncontrolled manner (Nziguheba *et al.*, 2016). Therefore, to sustain crops productivity, it is crucial to develop technologies for improving efficiency of P utilization in agriculture and reduce the rate of fossil P reserves depletion (Séry *et al.*, 2016).

AMF inoculation, is one such technology, and has been applied worldwide for decades to improve plants production (Berruti *et al.*, 2016). AMF belong to the phylum Glomeromycota and form a multifunctional symbiosis with almost 80% of land plants (Redecker *et al.*, 2013). AMF are natural biofertilizers providing water and protection against pathogen and abiotic stresses (Augé, 2001, Symanczik *et al.*, 2018) and in extreme environments (Zabinski and Bunn, 2014). With the extension of the hyphal network beyond roots zone, AMF supply to the host the low mobile nutrients such as P, Zn and other nutrients like Nitrogen (N) and Cupper (Cu) (Smith and Smith, 2011; Solaiman *et al.*, 2014; Gomes *et al.*; 2015; Crespo, 2015) and thus AMF not only contribute to reduce the rates of fertilizer requirements, but also to improve the nutrients use efficiency (Solaiman *et al.*, 2014).

Maize is highly mycorrhizal and there is evidence from different studies that AMF play an important role in increasing maize productivity (Nwaga *et al.*, 2003; Gomes *et al.*, 2015; Cozzolino *et al.*, 2016; Symanczik *et al.*, 2018) in acidic soils (Mukhongo *et al.*, 2017; Sery *et al.*, 2016). This functional ability of the AMF has led to the development of mycorrhizal inoculants as biofertilizers in agriculture but because of the complexities and specificities of agriculture in SSA (Tittonell and Giller, 2012), the native AMF can outcompete the commercial ones in specific soils conditions in term of nutrients uptake and plant growth (Kouadio *et al.*, 2017; Faye *et al.*, 2013). The bioinoculation of crops with native AMF from South Kivu's soils have not yet been extensively studied despite maize being very responsive to AMF inoculation. This study was therefore carried to assess the role played by native AMF biofertilizer from maize cropping fields from South Kivu on maize growth, nutrients uptake and roots colonization in two acidic (Ferralsol and Nitisol), agricultural soils dominant in the South Kivu, eastern DRC.

Materials and methods

Production of native AM fungal inoculum

Sixty soil samples were collected from maize cropping fields on transects set in maize growing fields (Brundrett *et al.* 1996) in South Kivu and trap

cultures established under greenhouse in Kabete field station. The methods of producing AMF inoculum, described by Brundrett *et al.* (1996) and modified in Ingleby (2007) were followed. Single strains of AMF (of at least 50 spores) were selected and used to grow soil "crude" inoculum individually in pots in the greenhouse in a sterile substrate made of soil and sand mixed at a proportion of 1:1 (w/w), using sorghum as a trap plant (Habte and Osioro, 2001; Berutti *et al.*, 2016). The soil used had a pH of 6.12 and available P content of 4.6kg P ha^{-1}. It was sterilized in an autoclave at 120^{o}C for 30 minutes.

The containers, pots of 500ml capacity pots were filled with the mixed substrate and pierced with drainage holes. Three healthy pregerminated seeds of sorghum were sown in each pot before topping with a thin layer of approximately 1cm of sterile sand over to prevent cross contamination. The nutrient solution for mycorrhizal plants, adapted from Ingelstad nutrient solution (Ingleby, 2007) was used mixed with water. Watering was done 2 to 3 times per week to field capacity.

At 4 months after planting, fine roots were collected and assessed for colonization and 50g of soil from each selected treatment was used to assess the spores densities. The roots colonization was determined using the slide method and the density of viable spores determined by the number of spores extracted in each sample from each treatment (Mcgonigle *et al.*, 1990; Brundrett *et al.,* 1996). Five strains that produced a high spore density and high roots colonization were selected for assessment of effectiveness and were named AMF1, AMF2, AMF3, AMF4 and AMF5 as they represented respectively strains of *Gipaspora gigantea*, *Gigaspora* sp.*, Gigaspora margarita*, *Rhizophagus intraradices* and *Acaulospora reducta*.

Soil preparation, experiment establishment and growth conditions

An experiment was set in the greenhouse at Kabete field station, at the faculty of Agriculture of University of Nairobi, to test the effects of the native AMF inoculums and the commercial AMF inoculant on plant growth, P and Zn uptake and root colonization.

Soils preparation

Two different agricultural soils were used. The first, locally called Kalongo, is reddish, acidic and less productive, classified as Ferralsol (FAO, 2015), was collected from the Walungu territory in South Kivu (Bagula *et al.*, 2014) and the second was less reddish, less acidic to neutral, but also less productive; classified as a Nitisol (FAO, 2015; Jones *et al.*, 2013; Karuku *et al.*, 2012), was collected from the field station in Kabete. Soils were subject to physical and chemical analyses. They were potted two weeks prior to planting, and each pot of 5L capacity was filled with 5kg of soil.

Experimental establishment

The experiment was carried out in a Randomized Complete Block Design with two factors ((i) fertilization and (ii) soil type, replicated three times. In total 8 treatments made of the 5 native AMF inoculums, Rhizatech biofertilizer, mineral P application and a control. Rhizatech is the commercial AMF biofertilzier obtained from Dudutech Company Ltd, Kenya. The blocks made thus of 8 treatments were replicated three times and thus the total number of treatments were 24 per soil type and 48 in total (Fig. 1).

	In Ferralsol							
Rep1	AMF4	Ctrl+P	AMF3	AMF2	Rhizatec	AMF1	Ctrl-P	AMF5
Rep2	AMF3	AMF2	AMF1	AMF5	Ctrl-P	AMF4	Ctrl+P	Rhizatec
Rep3	AMF1	Ctrl-P	Rhizatec	Ctrl+P	AMF4	AMF5	AMF3	AMF2
	In Nitisol							
Rep1	Ctrl-P	AMF4	AMF2	AMF3	Rhizatec	AMF1	AMF5	Ctrl+P
Rep2	AMF2	AMF5	Ctrl-P	AMF3	Ctrl+P	Rhizatec	AMF4	AMF1
Rep3	Rhizatec	Ctrl+P	AMF1	AMF4	Ctrl-P	AMF3	AMF2	AMF5

Fig. 1. Experimental design

The table 1 presents the treatments, species compositions, there and forms of application and the locations coordinates of the fields where these strains were collected.

Table 1. Description of the treatments applied in the experiment.

Treatment	Strain Code Origin*	Density (Spores/g)	Roots Colonization frequency	Rate per pot	Form of application	Strain origin location	Strain composition
1. AMF1	Kkash1	2.12	46.66(%)	100g	Crude inoculum	28° 51' 17"E; 2° 9' 5.76"S	*G. gigantea*
2. AMF2	Kkash5	2.32	40(%)	100g	Crude inoculum	28° 51' 22.5E; 2° 10' 5.6"S	*Gigaspora sp.*
3. AMF3	Wmul1a	2.6	50(%)	100g	Crude inoculum	28° 34' 39.3"E; 2° 42' 3.0"S	*G. margarita*
4. AMF4	Wmul6	2.82	43.33(%)	100g	Crude inoculum	28° 34' 5.5"E; 2° 41' 48.6S	*R. intraradices*
5. AMF5	Pkam7b	1.94	40(%)	100g	Crude inoculum	29° 0' 1.07"E; 2° 42' 19.3"S	*A. reducta*
6. Rhizatech	Rhizatech	4		50g	Crude inoculum		*G. mosseae, G. intraradices, G. etunicatum,* and *G. aggregatum*
7. Control				-	-	-	-
8. Control+P				45kg P ha^{-1}	DAP	-	

*Codes used to represent the AMF strains represents the territories and villages of origin and the number correspond to the number of the field sampled in the specific village in our database: Kkash=Kalehe Kasheke, WMul=Walungu Mulamba, Pkam = Plaine Kamaniola

N and K were applied at blanket rates of 100kg N ha^{-1} and 60kg K ha^{-1} in form of Urea and KCl (muriate potash) in order to balance the plants nutrition. Urea was applied twice, at sowing (50kg ha^{-1}) and at 4 weeks after sowing (50kg ha^{-1}) (Zingore *et al.*, 2014). The maize HD 02, a hybrid variety was used. Maize plant inoculation was done at the sowing day. For the native inoculum, each plant was inoculated with 100g of soil inoculums containing at least 2 spores g^{-1} and sorghum chopped roots (Table 1). This inoculum was mixed with the soil before transplantation. The seeds of maize were surface sterilized and pregerminated one week before transplanting two per pot. The Ingelstad nutrient solution (Ingleby, 2007) was applied to plants once a week and the remaining days in the week, plants were watered with tap water. Plants were allowed to grow up to 14 weeks, corresponding to the growing cycle of the variety used. The greenhouse temperature varied between 20 and 33°C.

Data collection

Plant growth, height, chlorophyll content, fresh shoot biomass and root colonization were observed. The height was assessed at 7 weeks and 11 weeks after sowing using a tape measure, and consisted of the height from the collar to the top of the youngest fully developed leaf. The chlorophyll content was recorded one at 11 weeks after sowing using a SPAD chlorophyll meter (SPAD 502 Plus) (Gekas *et al.*, 2013), following the instruction of the manufacturer. Shoot biomass was measured using a balance at the end of the experiment. Each observation was done in duplicate in each treatment. Dried leaves samples were ground in a bowl mill and analyzed for total P and Zn.

The concentration of total P was assessed after wet digestion of air-dried ground plant samples with a mixture of concentrated sulphuric acid (H_2SO_4) and selenium powder and salicylic acid and measured using a spectrophotometer (Spectronic 1001), while Zn was assessed using atomic absorption spectrophotometer (SBUCK 210 VGP) preceded by digestion (Okalebo *et al.*, 2002).

To assess roots colonization, fine roots were sampled at the end of the experiment with two replicates per treatment. Roots were rinsed, cleared by boiling in 2.5% (w/v) KOH for 15 minutes in autoclave, bleached acidified and stained with Trypan blue in lactoglycerol following the method modified by Koske and Gemma (1989).

Thirty (30) pieces of 1cm long roots fragments per treatment were mounted on slides and coverslip in PVLG, then placed under a compound microscope for observation of the colonization frequency recorded as the number of root fragments infected with AMF (Brundrett *et al.,* 1996).

The hyphal phosphorus contribution was calculated as follow (Heijden and Kuyper, cited by Gai *et al.*, 2006):

$$\text{Hyphal P contribution}(\%) = \left[\frac{\text{P uptake of mycorrhizal plant} - \text{P uptake of non} - \text{mycorrhizal plant}}{\text{P uptake of mycorrhizal plant}}\right] \times 100$$

Statistical analysis

Maize growth, chlorophyll content, nutrient concentration and root colonization in individual soil type were subjected to analysis of variance (ANOVA) using XLSTAT software. Pre-ANOVA assessment included the test of normality of the data. Means were separated using the Fisher LSD test at 0.05% level of significance.

Results

Physical and chemical properties of the soils used

The physical and chemical properties of the Ferralsol and Nitisol used in the experiment are presented in the Table 2.

Table 2. Physical and chemical properties of the soils used.

Soil property	Units	Ferralsol (Mean±SD)	Rating	Nitisol (Mean±SD)	Rating
pH H_2O		5.77±0.12	Moderate acidity	6.3±0.07	Low acidity
Available P	$mgkg^{-1}$	15.75±1.9	Moderate	27.65±1.6	Moderate
Total N	%	0.12±0.01	Low	0.17±0.02	Medium
CEC	$cmolkg^{-1}$	10.5±2	Low	21.2±3.7	Moderate
Organic C	%	0.78±0.52	Low	1.89±0.14	Moderate
Sand	%	29±1		40±2	
Silt	%	26±3		22±1	
Clay	%	45±2		38±1	
Texture class		Clay		Sandy clay loam	
AMF population	Spores /g soil	1.23±0.15		0.87±007	

The analyses were done in duplicate and the ratings done according to Okalebo *et al.*, (2002); Jones Jr (2001) and Hazelton and Murphy (2016).

All the soils used were acidic. The available P was moderate with an average of $15.75mgkg^{-1}$ in Ferralsol and high with average of $27.65mgkg^{-1}$ in Nitisol, considering the moderate range of $13\text{-}27mgkg^{-1}$ (Jones Jr, 2001). The total nitrogen was low and medium, same as for the organic carbon in the Ferralsol and the Nitisol respectively.

The CEC varied between the two soil types and was low ($10.5cmolkg^{-1}$) in the Ferralsol than in the Nitisol ($21.2cmolkg^{-1}$). There was a low density of AMF spores in both soils, comparable to the reported spore densities in the biofertilizers (4 spores g^{-1} of product) used in this study.

Growth, P and Zn uptake

The table 3 presents the plants height, chlorophyll content and the shoot dry biomass in the Ferralsol. Significant differences were observed ($p<0.001$) for the height of plants with the Pi treatment having the best height at both 7 and 11 weeks after sowing (124 and 141cm respectively). It was followed by Rhizatech, AMF2, AMF4 amd AMF5. The control gave the least height (68.8cm at 11 weeks after sowing).

Table 3. Maize height, chlorophyll content and shoot biomass at 7 and 11 weeks after sowing, in Ferralsol.

Treatment	Height at 7 weeks (cm)	Height at 11 weeks (cm)	Chlorophyl(7 weeks)	Chlorophyl(11 weeks)	Shoot biomass (g)
AMF1	84.66^{b}±5.55	92c±8.16	35.33^{bc}±1.078	44.03^{b}±0.85	7.316^{d}±1.56
AMF2	91^{b}±13.49	131.5^{ab}±19.18	40.66^{ab}±3.68	43.9^{b}±2.45	17.45^{b}±0.80
AMF3	67^{c}±2.94	69^{d}±4.54	42.33^{ab}±2.29	41.83^{b}±3.20	5.037^{d}±0.37
AMF4	95.33^{b}±7.40	121^{b}±2.44	39.4^{ab}±1.59	48.4^{a}±1.88	12.57^{c}±1.46
AMF5	91.33^{b}±9.84	124^{b}±14.69	36.06^{bc}±4.16	35.3^{c}±0.57	12.05^{c}±1.18
Control+P	124.33^{a}±4.78	141.5^{a}±2.85	42.76^{a}±2.08	41.13^{b}±3.27	39.27^{a}±0.96
Control-P	63.33^{c}±2.35	68.83^{d}±5.10	32.61^{c}±1.94	34.6^{c}±0.43	12.05^{c}±2.85
Rhizatech	90^{b}±8.16	134.33^{ab}±6.54	38.16^{b}±0.84	43.86^{b}±0.61	11.58^{c}±2.38
Pvalue	<0.001	<0.001	0.009	<0.001	<.0001
$LSD_{0.05}$	13.11	16.63	4.22	3.43	3
CV%	22.1	27.04	11.03	11.75	66

The chlorophyll content level varied among treatments. The Pi fertilization application significantly increased the chlorophyll content at 7 weeks after planting (p=0.009) and was equal with the treatment AMF2, AMF3 and AMF4. Rhizatech, AMF1 and Control recorded the low est concentration of P at the 7th week. After 11 weeks, some treatments increased significantly (p<0.001) their cholorophyll content and the highest value was recorded in AMF4 with the SPAD meter reading of 48.4. The AMF1, AMF2, Rhizatech, AMF3, and Control +P, followed with 44.03, 43.9, 43.8, 41.8 and 41.1 values respecitively. The Control had the lowest Chlorophyll content. Overall, the Pi application increased the dry biomass and recorded the highiest biomass (39.2g; p<0.0001) over all the other treatments, was followed by the treatment AMF4 (12.5g) and AMF5 (12.05g).

Table 4 presents the plants heights and chlorophyll content recorded at 7 and 11 weeks after sowing, and the shoot dry biomass at the end of the experiment in the Nitisol.

Table 4. Maize height, chlorophyll content and shoot biomass at 7 and 11 weeks after sowing, in Nitisol.

Treatment	Height (7weeks) (cm)	Height (11 weeks,cm)	Chlorophyll (7 weeks)	Chlorophyll (11 weeks)	Shoot biomass (g)
AMF1	107.33^{cd}±7.40	170^{b}±9.79	43.63^{a}±1.96	42.66^{a}±1.22	31.08^{b}±0.77
AMF2	91.33^{de}±5.43	159^{bc}±1.63	40.2^{a}±4.42	44.96^{a}±4.38	20.72^{d}±2.87
AMF3	98^{de}±5.88	160^{bc}±0.81	43.3^{a}±2.40	44.2^{a}±3.10	28.19^{c}±1.20
AMF4	117^{b}±5.71	189^{a}±13.88	42.3^{a}±2.69	44.4^{a}±2.12	44.06^{a}±1.92
AMF5	98.33^{d}±5.79	174.5^{ab}±11.02	40.46^{a}±0.75	40.43^{b}±2.36	17.47^{de}±0.67
Control+P	130.66^{a}±8.73	152^{c}±1.63	42.26^{a}±1.48	36.9^{b}±1.41	47.72^{a}±1.74
Control-P	93^{de}±1.41	126^{d}±11.22	32.06^{b}±2.28	40^{b}±1.63	17.47^{de}±2.22
Rhizatech	85.33^{de}±13.07	111.5^{d}±10.20	34.06^{b}±1.71	40.2^{b}±1.14	14.96^{e}±1.50
P value	0.002	<0.001	0.001	0.047	<.0001
LSD	12.62	15.39	4.17	4.12	5.55
CV	15.77	16.75	12.17	8.71	45.5

The Pi application increased significantly the height rapidly at 7 weeks (130.6cm) but at 11 weeks, the AMF4 and AMF5 had the best heights with 189 and 174.5cm respectively (p<0.001). The chlorophyll content varied between treatments at 7 weeks (p=0.001) and at 11 weeks (p=0.047). At 7 weeks after sowing, the best results were obtained from AMF1, AMF2, AMF3, AMF4, AMF5 and Pi application, and the last from Rhizatech and Control-Pi. At 11 weeks, the treatments AMF1, AMF2, AMF3, AMF4 still gave the highest chlorophyll content followed by Rhizatech, AMF5 and +Pi, and Control. The shoots dry biomass varied between treatments (p<.0001) and the highest biomass was from the control +Pi (47.7g) and AMF4 (44g). They were followed by AMF1 (31g), then by AMF3 (28.1g) and lastly AMF2, AMF5, Rhizatech and Control-P.

The P and Zinc concentrations in the maize tissue and the hyphal P contribution are presented in Table 5.

Table 5. Plant P, hyphal P contribution and Zn concentrations.

Treatments	Plant P (g/kg)		Hyphal P contribution (%)		Plant Zn (mg/kg)	
	Ferralsol	Nitisol	Ferralsol	Nitisol	Ferralsol	Nitisol
AMF1	1.12	1.70^{ab}	46.21	48.87	11.78^{ab}	10.56
AMF2	0.90	1.65^{ab}	33.33	47.16	13.70^{a}	8.51
AMF3	1.24	1.78^{a}	51.43	40.94	9^{bc}	6.39
AMF4	1.09	1.21^{b}	44.73	28.11	9.33^{b}	7.87
AMF5	0.78	1.66^{ab}	23.41	47.56	11.13^{ab}	11.15
Control+Pi	0.96	1.31^{ab}			10.71^{ab}	8.90
Control-Pi	0.60	0.87^{c}	0	0	5.84^{c}	8.13
Rhizatech	1.25	1.59^{ab}	51.82	45.16	6.83^{bc}	7.91
P-value	0.195	0.015			0.007	0.414
$LSD_{0.05}$	-	0.4			3.18	-
CV%	30.2	20.8			27.5	24

Means followed by the same letter in a column were not significant at p=0.05; LSD = Least Significant Difference; CV = Coefficient of variation.

In the Ferralsol, there was no significant difference between treatments (p=0.195). In the Nitiso, the difference was observed (p=0.015) and treatments were grouped into three groups, with the Pi fertilization giving the high P content along with AMF1, AMF2, AMF3, AMF5 and Rhizatech. AMF4 and Control were the second and last group respectively. Olant Zn content differed in the maize grown in Ferralsol (0.007). AMF2, AMF1, AMF5 and Pi fertilization gave thebhighiest contents followed by AMF3 and Rhizatech. The control treatment resulted in the lowest Zn content. However, in the Nitisol, no significant difference was observed among treatments (p=0.414).

The Hyphal P contribution, being the difference between the P uptake in the mycorrhizal inoculant and the control, varied between 23.41 and 51.82 in the Ferralsol; with the Rhizatech and AMF3 having the highest hyphal P contribution. In the Nitisol, it varied between 28 .11 and 48.87.

Maize inoculation and roots colonization

The native AMF inoculation presented functional diversity in term of roots colonization. No significant difference was observed in the Ferralsol (p=0.252) but differences were observed in the Nitisol (p=0.005) for the roots colonization. AMF4, Rhizatech and AMF5 treatments colonized the most of roots, with respectively 31.6%, 28.3% and 20% of roots colonized. The control appeared to have colonized 11.6% of roots in the Ferralsol but none in the Nitisol (Table 6).

Table 6. Maize roots colonization frequency (in%).

Treatments	Ferralsol	Nitisol
AMF1	23.33	11.66^{bc}
AMF2	18.33	$10b^{c}$
AMF3	36.66	16.66^{b}
AMF4	25	31.66^{a}
AMF5	35	20^{ab}
Control+Pi	11.66	0^{c}
Control-Pi	6.66	13.33^{b}
Rhizatech	45	28.33^{a}
P-value	0.252	0.005
$LSD_{0.05}$	-	12
CV%	45	54.2

*All the analyses were done in duplicate. Values followed by the same letters are statistically equal.

Discussion

AMF inoculum production

The failure of some strains to produce a high potential soil inoculum might have resulted from the no suitability of the specific strain to the environmental conditions and supports that AMF inocula are not always successful and in some cases the real benefits are not always positive (Corkidi *et al.*, 2004; Fayé *et al.*, 2013). The crude monospecie inoculum had low spores densities; the five selected native AMF's densities varied between 1.94 and 2.82 spores g^{-1}. The subsequent inoculation had high chances of failing to produce high mycorrhizal crop and to induce important improvement in the growth of crops. Séry *et al.* (2016) found that the dual inoculation of Yam with AMF of *A. colombiana* and *A. appendicula* contributed significantly to growth and production comparing to the single inoculation. Besides that, many AMF inoculants manufactures' products contain more than one specie; case of the Rhizatech inoculant which contains up to 4 species (*G. mosseae, G. intraradices, G. etunicatum,* and *G. aggregatum*) which are more generalist species, to ensure the product can adapt to a wide range of crops and environments (Mukhongo *et al.*, 2016; Séry *et al.* 2016).

The best alternative way to explore AMF inoculantion is to use the soil from a rhizosphere of a plant hosting AMF as inoculum (Berruti at el., 2016; Mukhongo *et al.*, 2016; Ndonda, 2018; Alexpander, 2017). The mass production method of inoculum production can enhance the AMF biodiversity and establishment and is therefore worth to be tested since it has been proved to sustain the persistence of AMF community (Mukhongo *et al.*, 2016) if a AMF-friendly management, such as fall cover cropping (Lehman *et al.*, 2012) and conservation tillage (Säle *et al.*, 2015; Alexander, 2017).

Physical and chemical properties of Ferralsol and Nitisol used

Studies of Okalebo *et al.* (2007), Karuku *et al.* (2002), and Achieng *et al.* (2010) have shown that P is one of the most limiting nutrients in Nitisols and Ferralsols, and its deficiency is often accompanied by very low use efficiency in acid soils.

The AMF densities and colonisation have been proved to be significantly influenced by the soils properties in which they are growing. AMF have been shown to be more abundant and effective in acidic soils, with the neutral soils tending to suppress the mycorrhizae association (Smith and Read, 2010; Solaiman *et al.*, 2014). This may be one of the reasons why the native AMF root colonization increased to equal the Rhizatech in the Ferralsol (Table 6). High concentration of available P reduces also the mycorrhizae formation (Lambert *et al.*, 1979; Smith and Smith, 2011); meaning that in the Nitisol with moderate rate of P (27mgkg^{-1}) and a slight acidity (pH 6.3) than the Ferralsol (pH 5.8), the colonisation could have not been very effective. The two acidic soils used had different amount of organic carbon and CEC, with the moderate values (1.89%) for Nitisol; meaning that the later had a high water retention capacity and high capacity of supplying P and Zn (Brady and Weil, 2002) to maize plants as proved in a similar study by Mukhongo *et al.* (2017).

Growth, P and Zn uptake

Different AMF showed functional diversity in terms of maize growth, P and Zinc uptake. The P application increased significantly the growth of maize in both Ferralsols and Nitisols because all are acidic soils, with low available P content. Other studies have also found that the application of mineral P in acidic soils increases its availability and its uptake (Onwonga *et al.*, 2013; Templer *et al.*, 2017). The high available P in soils tends to suppress the mycorrizae formation (Lambert *et al.*, 1979) and this could explain why the P treatment ranked among the highest height and biomass in both Ferralsol and Nitisol.

The Chlorophyll Meter Readings have been positively correlated to maize growth and yield (Gekas *et al.*, 2013). In the Ferralsol, at 7 weeks after sowing, the P application treatment showed more greenness than all the other treatments but at 11 weeks after sowing, the strains AMF4, AMF1 and Rhizatech presented more greenness ($p<0.001$). In the Nitisol, AMF1, AMF2, AMF3 and AMF4 presented the highest chlorophyll content at 11 weeks ($p=0.047$). This confirms the quick availability of nutrients in P applied treatments and means that with time, towards the end of the season, more nutrients were mobilized and more resistance to drought might have been conferred to plants by these strains.

Efficiency of AMF species is influenced differently by their development and activity of the external hyphae, hyphal transport rates, and solute interchange at the arbuscule-host root cell interface. In the Ferralsol, the lack of difference in the P content could be attributed to the constraint posed by the soil that might have sequestered the P applied since its initial level was low. AMF1, AMF2, AMF3, AMF5 and Rhizatech were as effective as P fertilization in plants P content in Nitisol. The significant effect of biofertilizers on increasing P in Nitisol ($p= 0.015$) and Zn in Ferralsol ($p=0.007$) was attributed to the functioning of AMF and probably to the plant growth promoting bacteria that were present in the soil and are known to be abundant in the neutral to slightly acidic soils and intervene in improving root growth and development (Kavoo Mwangi *et al.*, 2013), but which were not determined. They might have helped increased nutrient uptake. Plants grown on the Nitisol had higher P than the ones on Ferralsols and thatis reflected in the soils initial available P (27 and 15mgkg^{-1}, respectively).

The significant effect of P fertilizer treatments on P and Zn concentration in maize shoot and their subsequent effect on growth were due to the increased availability of the nutrients in the soil solution (Schröder *et al.*, 2011). Similar results were obtained by Onwonga *et al.* (2013) and Templer *et al.* (2017), when assessing the P concentration in maize after application of P fertilizers in acidic soils.

The hyphal P contribution was low since it is believed that up to 85% of the P can be uptaken through hyphae but here it varied between 23.1% - 51.4% in Ferralsol and 28.1% - 48.7% in Nitisol. This could be due to the low spores densities or the low diversity (monospecie inoculum) or the long time required by some AMF strains to establish the mycorrhizae association with the host and spread their extraradical hyphae in the roots. It has been proved

that increasing the AMF diversity in maize roots through co-inoculation leads to higher colonization, expression of mineral P transporters enzymes, as well as P uptake in maize shoots (Tian *et al.*, 2013).

Mycorrhizae increased the uptake of Zn in maize and this result is in line with the findings of Lambert *et al.* (1979) who concluded that mycorrhizal inoculation alone increases the concentration of P and Zn in the plant. When working on phosphate transport in maize under AMF colonization, Tian *et al.* (2013) found that co-inoculation with different AMF species (*Glomus deserticola, Glomus intraradices, Glomus mosseae* and *Gigaspora gigantea*) resulted in the highest expression level of phosphate transporter gene *ZE Ama: Pht1;6* as well as the highest P uptake; suggesting a high diversity of AM colonization may transfer more P to the intra-radical hyphae in maize roots.

Maize inoculation and roots colonization

AMF have functional diversity in term of roots colonization and induction of nutrients uptake (Feddrmann *et al.*, 2010; Smith and Read, 2010; Tian *et al.*, 2013). Generally, the colonization was low in both soils. It varied between 0 and 45%. In the Ferralsol, there was no significant difference but a high coefficient of variation meant high variations between treatments. This could be due either to the low performance of all the AMF strains in the Ferralsol which presented many chemical constraints to their development, since Lambert *et al.* (1989) proved that AMF perform very well in slightly acidic soils; or the low concentration of the propagules of the crude inoculum used. Low colonization levels were also observed by Aguk (2013), contrarily to other studies that found colonization levels varying between 41-73% and 48-68% reported by Ngakou *et al.* (2006) and Džafić *et al.* (2009) on maize. The Gigasporaceae and Glomearaceae have different colonization strategies (Feddermann *et al.,* 2010). The lower degree of colonization found for *Gigaspora margarita* and *Gigaspora gigantea* reflected the low colonization strategy of fungi belonging to Gigasporaceae as found by Feddermann *et al.* (2010). The AMF2, AMF4 and AMF5 gave a relatively higher level of rootcolonization, statistically equal to the Rhizatech in the Nitisol. These findings confirm the results obtained by other researchers that concluded that the Glomeraceae have a high affinity with the colonization of maize roots (Tian *et al.*, 2013; Gomes *et al.*, 2015) but also the Acaulosporaceae could have a high colonization of roots than the Gigasporaceae (Sery *et al.*, 2016). This is probably why the commercial inoculant was made of species exclusively from Glomeraceae family (*G. mosseae, G. intraradices, G. etunicatum, and G. aggregatum*).

AMF biofertilizer effects on root colonization, nutrient uptake and, growth and yield was not always significant in this research possibly due to the shorter period of 3.5 months that the crop needed to grow, and/or due to the low available P in the Ferralsol and its acidity and in the Nitisol the high P detected might have hindered the mycorrhizal formation to take place. According to Mukhongo *et al.* (2016), the low response to mycorrhizal treatments in growth of annual crops could be attributed to the fact that colonization starts after hyphal formation and penetration in the root.

This depends on the state of propagules (spores, hyphae) which may take long period to germinate and infect the plant. Spores persist longer in the soil but they are slow to colonize host plants as compared to hyphae and root fragments (Marin, 2006; Mukhongo *et al.*, 2016). Furthermore, Kavoo Mwangi *et al.* (2013) reported a lack of immediate expression of Rhizatech in growth of tissue culture banana plantlets for 22 weeks under nursery conditions, but they gave significant results when the inoculated plantlets were allowed to grow under field conditions for 7 months. Contrarily, Aguk (2013) and Kundu (2012) reported positive effects of AMF inoculation on potatoes after only four months of cultivation.

The low level of roots colonization in all the treatments and the less significant difference among native AMF strains could be due to the low spores densities in both crude inoculum, as they were monospecie based with densities varying between 1.94 and 2.82 spores g^{-1}; the low spores densities in

both experimental soils (1.23 spores g^{-1} for Ferralsol and 0.87 spores g^{-1} for Nitisol) compared to the 7 spores g^{-1} used by Séry *et al.* (2016) or 50 propagules g^{-1} used by Kavoo Mwangi *et al.* (2013).

Conclusion

AMF soil inoculums of five from native AMF strains of *Gipaspora gigantea*, *Gigaspora* sp., *Gigaspora margarita*, *Rhizophagus intraradices* and *Acaulospora reducta* were produced and resulted in a lower concentration of propagules compared to the concentrations in the commercial inoculum. There is a need to experiment other methods of AMF inoculum production like the massive production, or the stimulation of naturally occurring AMF species in the fields for the soil to be used as inoculum. In both Ferralsol and Nitisol, the AMF inoculum produced influenced the growth, the chlorophyll concentration, P and Zn uptake and the roots colonization differently; with some treatments equalizing the Pi application on the shoot biomass and P uptake and also equalizing with the Rhizatech in the root colonization. Evaluation for efficient AMF strains from these five species, using multispecie and high propagules densities inoculums and test their effects on maize growth should be done.

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