

*Full Length Research Paper*

# **An indigenous and an exotic AMF strains improve *Dioscorea alata* growth and induce tolerance to *Meloidogyne* spp.**

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## **Abstract**

Yam (*Dioscorea* spp.) is susceptible to a host of pests including root-knot-nematodes (RKN). Considering that arbuscular mycorrhizal fungi (AMF) improve plant growth and enhance resistance or tolerance to nematodes, there is interest in studying AMF-RKN interactions on yam plants. In this perspective, a study was undertaken to assess the potential of some AMF in growth enhancement and yam protection against *Meloidogyne* spp. For that purpose, two pot experiments performed on *Dioscorea alata* (cultivar *Bètè-bètè*) were carried out for comparing the efficiency of two AMF based inocula, an indigenous strain of *Rhizophagus irregularis* selected in yam rhizosphere in Cote d'Ivoire and a commercial inoculum of *R. intraradices*. Results showed that both AMF inocula were efficient in improving plant growth either in the absence or in the presence of *Meloidogyne* spp. and plant tolerance to nematode infection was achieved through root biomass increase. Indigenous *R. irregularis* was the best inoculum in plant growth enhancement while commercial *R. intraradices* was the most effective inoculum in the RKN control. Comprehensively, indigenous *R. irregularis* was the most promising strain because it showed a better mycorrhizal capacity either in the absence or the presence of nematode and seems to be more compatible to yam plants.

**Keywords:** indigenous AMF, commercial AMF inoculum, root-knot-nematodes, *Dioscorea alata*, pot experiment

## **Introduction**

Yam (*Dioscorea* spp.) is one of the most important staple food crops widely cultivated in west Africa considered as the yam belt (FAO, 2016). Tubers of *Dioscorea alata* L. are an important source of carbohydrates for millions of people throughout the tropics (Marcos *et al.*, 2011). Globally, yam is the second most important root and tuber crop after cassava, in terms of production and in West and Central Africa production zone (Tchabi *et al.*, 2016b). Due to land pressure, yam crops are increasingly cultivated on low fertile soils (Yasuoka, 2009) which is a major constraint that limits yam productivity and causes progressively yam yield decline. Yam is challenging

various attacks from different pathogens. Indeed, yam is susceptible to a host of pests and diseases, including plant-parasitic nematodes, which further affect productivity, tuber quality and storability (Mudiope *et al.*, 2012). Among the nematodes that affect yam productivity in West Africa, *Scutellonema bradys* and *Meloidogyne* spp. are reported as the most important (Bridge *et al.*, 2005; Coyne *et al.*, 2006). Pesticides can be used for nematode control, but they are expensive, unavailable or highly toxic for both the user and the environment. In addition to this, most effective nematicides do not comply with current environmental demands and will have to be replaced by other means of nematode control with less impact on non-target organisms (Hol and Cook, 2005). Plant-parasitic

nematodes, including endoparasitic nematodes and arbuscular mycorrhizal fungi (AMF) occur together in the rhizosphere and colonize the same area of roots of host plants and, therefore, interact with each other. AMF effects are well-documented on the enhancement of plant nutrient uptake (Biricolti *et al.*, 1997; Goussous and Mohammad, 2009; Halder *et al.*, 2015; Nikolaou *et al.*, 2002; Sieverding *et al.*, 1991), plant drought and salinity tolerance (Augé, 2001, 2004; Augé *et al.*, 2015; Porcel *et al.*, 2011) and disease resistance (Pozo and Azcón-Aguilar, 2007; Tchabi *et al.*, 2016a). Considerable attention has also been paid to the potential role of mycorrhiza as biocontrol agents (Diedhiou *et al.*, 2003; Hussey and Roncadori, 1982; Jothi and Sundarababu, 1998). Various plants have been studied (Alban *et al.*, 2013; Banuelos *et al.*, 2014; Campos *et al.*, 2013; Cooper and Gordon, 1987; Elsen *et al.*, 2003) among which yam (Tchabi *et al.*, 2016a, 2016b). (Tchabi *et al.*, 2016b) highlighted the fact in their study AMF products commercially produced in Europe were used due to their availability, may not be the most suitable or compatible for tropical conditions. They also mentioned yam as a suitable candidate for bio-enhancing with AMF and the necessity to perform studies in order to determine more precisely appropriate genotype x AMF strain combinations. We try to address this issue selecting indigenous yam rhizosphere AMF strain and assessing his potential as a biocontrol agent against root-knot-nematodes *Meloidogyne* spp., comparing it to a widely commercial AMF using *Dioscorea alata* in greenhouse conditions.

## Materials and methods

### Plant material

A local cultivar of *Dioscorea alata* commonly called “Bètè-bètè” was used for it is the second most important yam species grown in Côte d’Ivoire (Doumbia, 1995; Doumbia *et al.*, 2004). “Bètè-bètè” is also the first main yam varieties cultivated in Yamoussoukro region a yam production area in Côte d’Ivoire (Digbeu *et al.*, 2009). Harvest time is characterized by wilting of the aerial parts of the plant. Tubers were submitted to a hot water treatment (50°C for 20 minutes) (Coyne *et al.*, 2010) to disinfest the tubers of nematodes and other tuber-borne pests and diseases. The disinfested tubers were thereafter cut into mini-setts weighing 30-45g and soaked in a liquide mixture containing mancozeb (80%) and wood ash. Finally, they were air-dried for 24 hours. Mini-setts were placed in sterilized sawdust beds under shade to sprout. Six weeks after, uniformly sprouted and growing setts were transplanted to 10L perforated plastics pots, at one per pot, filled with 8 kg sterilized soil and sand mix (1:1, vol:vol). At transplanting, sprouted setts were separated from the sett to maintain only roots, in order to rapidly allow mycorrhizal symbiosis to establish.

### AMF species used for inoculation

Two AMF species including an indigenous strain of *Rhizophagus irregularis* isolated from yam field in

Yamoussoukro and a commercial strain of *Rhizophagus intraradices* (formerly named *Glomus intraradices*; Myke® Pro P501) manufactured by Premier Tech Biotechnologies were used.

### Preparation of the native AMF inoculum

Yam roots were sampled from yams fields of three villages named Seman, Zambakro and Logbakro located in Yamoussoukro region. Roots were cut into 1cm pieces and used as inocula for root fragments cultures (Walker, 1999). One gram of yam roots fragments was placed in a hole with five to height onion (*Allium cepa*) seeds in the substrate of each 2-l pot filled with a local soil and sand mix (3:1, vol:vol) sterilized twice (121°C, 1h) with intervals of 24h. They were all covered with sterilized substrate. Two weeks after the emergence of the seeds, some seedlings were removed from each pot in order to acquire a planting density of two seedlings per pot. After six months of culturing in greenhouse conditions one morphotype of AMF sporulated in the pot from Zambakro with a very high density (80 spores.g<sup>-1</sup>) and spores were isolated by wet-sieving method (Gerdemann and Nicolson, 1963). Soil containing in Zambakro pot were used in pot substrate cultures (Walker, 1999) method in 10L pots as containers for mass-multiplication. Maize (*Zea mays*) was used as host plant for AMF rapid multiplication for three months with the same substrate used before. The maize plants were uprooted and roots were cut into 1 cm pieces and 10L pot soil were mixed thoroughly and used as inoculum. This inoculum was made of soil, colonized root fragments of maize, AMF spores and hyphae. Using molecular tools as (Séry *et al.*, 2016), this morphotype was identified as *Rhizophagus irregularis*.

### *Meloidogyne* spp. inoculum source

The nematode inoculum was made using a population of *Meloidogyne* spp., isolated from naturally galled tomato roots. The inoculum was prepared by finely cutting infected tomato roots that were soaked in a jar containing NaClO (0.25%) and shaken for 2 min (Hussey and Barker, 1973). Nematode eggs and juveniles were collected on a 25 µm sieve, rinsed in sterile water and counted under a 40x binocular magnifier. Yam plants were inoculated one week after transplanting by pipetting 12 ml of an aqueous suspension containing 8000 eggs and juveniles within four holes dug around the plants.

### Experimental design

Two experiments were carried out in this work. The first aimed to assess the performance of the indigenous inoculum *Rhizophagus irregularis* produced on yam plant growth in greenhouse as compared to the exotic inoculum *Rhizophagus intraradices*. This experiment involved one factor namely AMF inocula with each AMF inocula and a non-inoculated control (C). The second experiment aimed to assess the efficacy of both inocula in RKN control on yam plant. It involved two factors: AMF inocula (non-inoculated control or C, Rz1 and or

Rz2) and the period of *Meloidogyne* spp. inoculation either simultaneously with AMF inoculation or one week before AMF inoculation and no nematode inoculation. For AMF treatments, each plant received either 3000 spores of commercial *Rhizophagus intraradices* (following the manufacturer's instructions) or 500 spores of native *Rhizophagus irregularis*. Each plant inoculated with *Meloidogyne* spp. received 8000 eggs and juveniles. Both experiments involved three replicates for each treatment. Pots were arranged in a completely randomized. Plants were watered three times weekly until harvest at 4 months after transplanting.

#### Assessment of AMF root colonization, yam growth parameters and nematode infection

Plants were harvested after heavily watering them the previous day to soften the soil and enable removal of roots and tubers without damage. They were divided into leaves and vine (shoot), and roots. Each part was weighed using an OHAUS balance for collecting fresh weight. Shoot and roots were oven-dried at 70°C until constant weight and weighed. Total dry biomass production was calculated as the sum of the shoot dry and root dry biomass in the first experiment. A subsample of roots in each replicate from each treatment was collected and stained using the method of Phillips and Hayman (1970) in order to assess the percentage of mycorrhizal colonization in yam roots according to the method of (Trouvelot *et al.*, 1986). For each treatment, nematodes density per gram of root was assessed. The roots were chopped into ~2 cm pieces. The roots were thoroughly mixed before removing a 2 g sub-sample to extract nematodes using the maceration technique described above. Nematode eggs and juveniles were collected on a 25 µm sieve, rinsed in sterile water and counted under a 40x binocular magnifier. Data were reported as number of juveniles and eggs per gram of fresh roots.

#### Data analysis

All data were subjected to analysis of variance (ANOVA) using Generalized Linear Model of mean. Values were compared using least significant difference (LSD) test at 0.05 level of probability when the F-ratio was significant. All statistical analyses were carried out using the software Statistica 7.1.

#### Results

##### AMF inocula mycorrhizal capacity in the absence of *Meloidogyne* spp.

Four months after inoculating yam plants with *Rhizophagus intraradices* and *Rhizophagus irregularis*, all roots contained mycorrhizal organ (Table 1). Non-inoculated control plants were free of mycorrhizal organ. Both inocula were significantly different for the frequency ( $p=0.0096$ ) and the intensity ( $p=0.0000$ ) of mycorrhization. The indigenous *R. irregularis* showed a significant higher mycorrhizal capacity than the commercial *R. intraradices*. Plants inoculated with *R. irregularis* presented 26% of mycorrhizal roots while those inoculated with *R. intraradices* presented 17% of mycorrhizal roots. Furthermore, *R. irregularis* inoculated yam roots showed the highest root colonization degree even though the intensity was low for both inocula (<10%).

##### AMF inoculation impact on yam growth in the absence of *Meloidogyne* spp.

All growth parameters were significantly improved by AMF inoculation (Table 1) as compared to non-inoculated plants. Root and shoot dry weight and then total dry biomass were significantly ( $p=0.0001$ ;  $p=0.0000$ ) affected by AMF inoculation. The highest values of root and shoot dry weight were obtained with *R. irregularis*. Consequently, *R. irregularis* was the best inoculum for yam plant growth under greenhouse conditions.

**Table 1:** Impact of *Rhizophagus intraradices* and *R. irregularis* on yam plant growth four months after AMF inoculation in the absence of nematodes

AMF inocula	Frequency mycorrhization (%)	of Intensity of mycorrhization (%)	Root dry weight (g)	Shoot dry weight (g)	Total dry biomass (g)
Control (no AMF)	0±0.00	0±0.00	1.38±0.01b	3.05±0.16b	4.43±0.16c
Commercial <i>intraradices</i>	<i>R.</i> 17.31±1.99b	0.17±0.02b	1.70±0.22b	9.90±0.95a	11.61±1.13b
Indigenous <i>irregularis</i>	<i>R.</i> 26.77±2.06a	0.64±0.01a	3.54±0.29a	10.78±0.14a	14.31±0.17a
P-value	0.0096	0.0000	0.0001	0.0000	0.0000

(Values sharing the same letter in each column are not significantly different ( $P < 0.05$ ) according to the LSD test.)

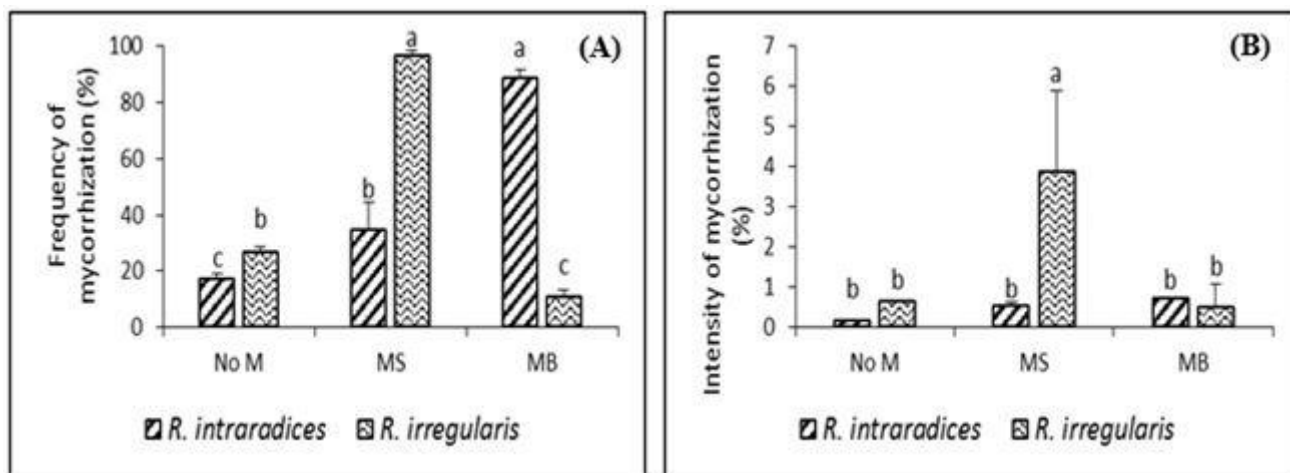
##### AMF mycorrhizal capacity in the presence of *Meloidogyne* spp.

Yam root frequency ( $p=0.000$ ) and intensity of colonization ( $p=0.008$ ) were significantly affected by the interaction between nematodes and AMF inocula. No

colonization was observed within non-inoculated plants roots. AMF mycorrhizal capacity showed different trend across the *Meloidogyne* spp. inoculation gradient (Figure 1). When yam plants were simultaneously inoculated with nematode and AMF, indigenous *R. irregularis* showed the best mycorrhizal frequency.

However, when nematode were inoculated before AMF, the better mycorrhizal capacity was obtained with the commercial *R. intraradices*. Nematode inoculation significantly increased *R. intraradices* mycorrhizal capacity. Frequency of mycorrhization noticed within roots inoculated with *R. irregularis* increased when simultaneous AMF and nematode happened and it was

depressed when nematodes were inoculated before. Nematode inoculation did not significantly affect colonization intensity induced by both AMF inocula (Figure 1). Only *R. irregularis* showed the highest value when AMF and nematode were inoculated at the same time (Figure 1).



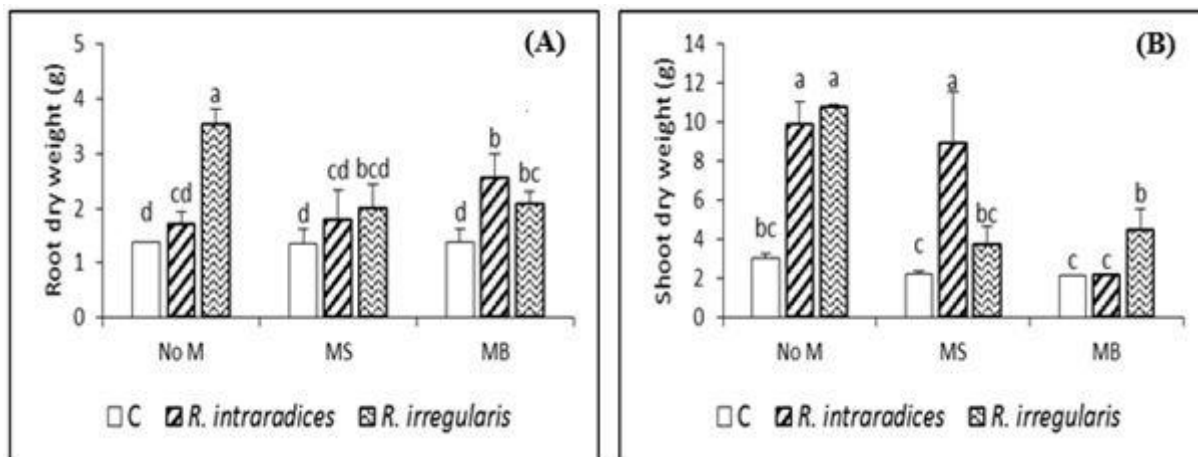
**Figure 1:** Frequency (A) and intensity of mycorrhization (B).

(Values sharing the same letter in each color are not significantly different ( $P < 0.05$ ) according to the LSD test. NoM= absence of Meloidogyne spp; MS= Meloidogyne spp inoculated at the same time with AMF; MB=inoculation of Meloidogyne spp. before AMF)

### Effect of AMF inocula and *Meloidogyne* spp. on yam plants growth

Four months after transplanting yam plants, differences in yam growth parameters were observed. *Meloidogyne* spp. inoculation did not affect control plants root dry weight while it decreased control plants shoot dry weight even though not significantly (Figure 2). When performing AMF inocula at the same time with

nematodes, no difference in root dry weight was observed between AMF inocula while the commercial inoculum allowed an increase in shoot dry weight. Significant impacts of both AMF inoculants were observed on root dry weight when performing nematode inoculant before. The significant impact of AMF inoculant on shoot dry weight was observed only with the indigenous inoculum (Figure 2).



**Figure 2:** Root dry weight (A) and shoot dry weight (B) four months after yam sett transplanting in pots

(Values sharing the same letter in each color are not significantly different ( $P < 0.05$ ) according to the LSD test. C= control, no AMF; NoM= absence of Meloidogyne spp; MS= Meloidogyne spp inoculated at the same time with AMF; MB=inoculation of Meloidogyne spp. before AMF)

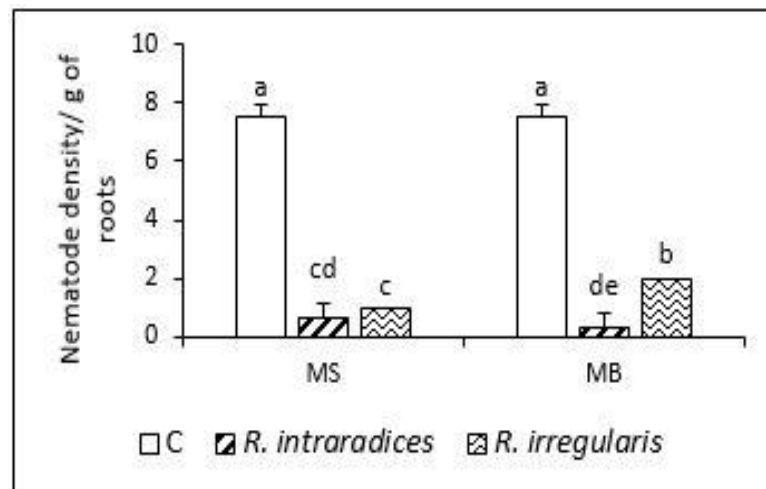
### Effect of AMF inoculation on *Meloidogyne* spp. density and damage

Four months after yam sett transplanting, AMF have affected negatively nematode population. Juveniles and

eggs density per gram of root decreased significantly when plants were inoculated by AMF (Figure 3). Nematode inoculation either before or at the same time as AMF inoculation did not affect both AMF inocula efficacy as biocontrol against *Meloidogyne* spp. Indeed,

*R. intraradices* and *R. irregularis* decreased juveniles and eggs density per gram of root as compared to AMF-free plants. AMF performance as biocontrol against nematode varies between both inocula used and the period of *Meloidogyne* spp. inoculation. *R. intraradices* and *R. irregularis* showed similar performances on

juveniles and eggs density per gram of root when AMF and nematode were simultaneously inoculated. *R. intraradices* was better than *R. irregularis* concerning juveniles and eggs density per gram of root under delayed AMF and nematode inoculation (Figure 3).



**Figure 3:** Juveniles and eggs of *Meloidogyne* spp. density per gram of roots

(Values sharing the same letter in each color are not significantly different ( $P < 0.05$ ) according to the LSD test. C= control, no AMF; MS= *Meloidogyne* spp. inoculated at the same time with AMF; MB=inoculation of *Meloidogyne* spp. before AMF)

## Discussion

This work was undertaken for assessing AMF potential, in the absence or presence of *Meloidogyne* spp. inoculated either before or simultaneously with AMF inocula on yam (*Dioscorea alata*). In this study, one of the most interesting things was the comparison of two AMF inocula including an indigenous AMF inoculum and a commercial AMF both from the same genus. These inocula showed different behaviour along the experiments. Without nematode, it was shown that both inocula colonized yam plants with similar mycorrhization frequency and *R. irregularis* did it in a greater extent (intensity of mycorrhization). This result was similar to that found by (Rodríguez *et al.*, 2012) working on different AMF genera and species having different infectivity levels where the equal infectivity at strain level was observed. They concluded comparing their results to those from others authors, that the intensity of mycorrhization is more useful to establish the infectivity differences at deeper level when many strains are examined. Thus, the indigenous *R. irregularis* revealed a better mycorrhizal capacity comparing to commercial *R. intraradices* through it highest mycorrhization intensity. When, AMF were challenged by *Meloidogyne* spp. inoculation either simultaneously or prior, *R. intraradices* and *R. irregularis* showed different behaviour. The percentage of mycorrhizal colonization can increase (Tchabi *et al.*, 2016b) or decrease (Séry *et al.*, 2016) or have no effect (Cooper and Gordon, 1987) in the presence of plant parasitic nematodes, depending on the arbuscular mycorrhizal species involved (Waceke *et al.*, 2001) or plant cultivars (Tchabi *et al.*, 2016b). *R.*

*irregularis* better infectivity (higher mycorrhization intensity) could be due to the fact that although there is no specificity between AMF and their hosts, greater compatibility can occur between some species of AMF and plants (Campos *et al.*, 2013; Cesaro *et al.*, 2008). Indeed, *R. irregularis* as an indigenous strain isolated from yam rhizosphere may be more compatible to yam plant than the commercial *R. intraradices*.

An enhancement in yam plants growth after AMF inoculation has been clearly demonstrated as it is well-known in the literature with various plants. AMF have been shown particularly important for improving various plant growth and yield (Cozzolino *et al.*, 2013; Ortas, 2010; Seema and Garampalli, 2015; Shuab *et al.*, 2014). Studies on yam plant interaction with AMF were scarce but recently, some authors have focused their work on this matter. Our results were similar to those found by (Dare *et al.*, 2010; Lu *et al.*, 2015; Tchabi *et al.*, 2008, 2009, 2010). Indeed, they noticed AMF potential to improve yam plant growth either in greenhouse and field conditions. AMF beneficial effect on plant growth is generally a consequence of an improvement of plant nutrient uptake (Aka-Kacar *et al.*, 2010; Al-Karaki *et al.*, 2004; Dare *et al.*, 2010). In the presence of *Meloidogyne* spp. inoculated at the same time as AMF inocula, no significant difference was observed between AMF-free and AMF inoculated plants. The presence of the nematode may interfere with nutrient flow between the root and the fungus, reducing AMF efficiency (Cofcewicz *et al.*, 2001). Yam plants pre-inoculated with nematode before AMF, enhanced the root dry weight as compared to AMF-free plants. This ability of mycorrhizal plant to grow well despite the infection by nematode, was also

observed by (Cooper and Gordon, 1987) . It is one of the mains effect of AMF on the interaction of host plant and plant parasitic nematode. Inside the roots, the root-knot nematodes (*Meloidogyne* spp.) create a feeding site deriving continuous nourishment from adjacent cells and producing galls that affect the plant's metabolism and resource allocation. This impairs the absorption and transport of water and nutrients which results in decreased plant development (Borowicz, 2001; Carneiro *et al.*, 2002). The presence of AMF, which compete for space and nutrients with the nematode, may reduce this effect, inducing plant development even in the presence of the pathogen, as observed in guava plants (Campos *et al.*, 2013). Both AMF inocula showed similar efficacy in root dry weight enhancement. The increment induced by *Rhizophagus intraradices* for shoot development when AMF and *Meloidogyne* spp. were simultaneously inoculated meanted that nematode inoculation did not alter *R. intraradices* performance to improve plant growth. According to (Alban *et al.*, 2013) working on coffee plants, if AMF and the nematode were simultaneously present in the soil, AMF colonized the roots before nematode infestation. Thus, coffee plants can regain the energy lost by the parasitic interaction, preventing the reduction in plant growth. The same phenomenon surely happened with *R. irregularis* and *R. intraradices* inoculated yam plants. *R. intraradices* and *R. irregularis* showed themselves faster and more competitive than *Meloidogyne* spp. *R. intraradices* could therefore express its efficacy and improve shoot growth while *R. irregularis* prevented reduction in plant growth. (Alban *et al.*, 2013) proposed also for coffee plant that if coffee seedlings are attacked by *Meloidogyne exigua* it is possible to use AM fungi in order to prevent productivity loss. This occurs because the symbiosis interaction speeds up. It aims to maintain plant growth similar to non-infected plants. This is what happen exactly with *R. intraradices* inoculated plants. In this case, *R. irregularis* was more efficient than *R. intraradices* probably because of it compatibility with yam plant as a strain isolated from yam rhizosphere.

The interaction between AMF and nematodes has been studied by several workers and it has resulted in nematode reduction (Alban *et al.*, 2013; Campos *et al.*, 2013; Castillo *et al.*, 2006; Habte *et al.*, 1999; Tchabi *et al.*, 2016a), no effect (Manandhar, 2011) or even an increase in numbers of nematodes (Atilano *et al.*, 1981). In this work, juveniles and eggs density per gram of roots was decreased when plants were inoculated with AMF. The outcome of AMF and nematodes interactions depend on several factors including host plant, AMF and nematode species and the cultivation conditions (Diedhiou *et al.*, 2003; Elsen *et al.*, 2003). The nature of interaction varied to neutral, positive or negative. Several mechanisms may operate simultaneously in the enhanced resistance or tolerance of mycorrhizal plants to RKN. AMF and RKN occur together in the rhizosphere and colonize the same area of roots of host plants. Therefore, a competition between both organisms for feeding sites and carbon substrates from host photosynthesis (Hol and Cook, 2005) can occurred. In addition to this, the presence of mycorrhiza in the host can reduce attraction to roots and juvenile penetration

and retard nematode development after penetration (Poza *et al.*, 2010) by inducing changes in root exudation into the mycorrhizosphere. This may result in a decrease of nematodes density per gram of roots. Nevertheless, in this study, nematode density per gram of root decrement was due to the increase of root biomass by AMF inoculation. *R. intraradices* showed a better potential in reducing nematode damage than *R. irregularis*.

## Conclusion

The current study has shown that both AMF inocula used were efficient in improving plant growth in the absence even in the presence of *Meloidogyne* spp. and induced yam plants tolerance to nematode infection. Tolerance to nematode infection was achieved through root biomass increase. Commercial *R. intraradices* was more effective in root-knot-nematodes control while indigenous *R. irregularis* was the best in improving yam growth. Thus inocula were both good for plant growth enhancement and tolerance to *Meloidogyne* spp. Among the AMF inocula, that with *R. irregularis* was the most promising because it not only allowed tolerance to root-knot-nematodes and was efficient in increasing plant growth, but also presented a better mycorrhizal capacity either in the absence or the presence of nematode and seems to be more compatible to yam plants. This compatibility could have been of high interest if these experiments were carried out under more real conditions with various environmental factors such as field conditions.

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