

# Arbuscular Mycorrhizal Fungi Inoculation and Intercropping Combine to Control Nematodes in Bananas

## ABSTRACT

**Aims:** To investigate the combined effect of arbuscular mycorrhizal fungi (AMF) and intercropping on the control of the nematode *R. similis* in banana.

**Study design:** The study involved an experiment in which banana-banana monocrops alongside banana-groundnut and banana-sweet potato intercrops were inoculated with arbuscular mycorrhizal fungi to control the nematode *R. similis*.

**Place and Duration of Study:** Centre Africain de Recherches sur les Bananiers et plantains (CARBAP), from september 2016 to April 2017

**Methodology:** An experiment was conducted under greenhouse conditions (photoperiod 12 h. average temperature 24 - 28°C and 70 - 80% relative humidity) and AMF were tested against *R. similis* with banana intercropped with either groundnut, sweet potato or banana itself. The plants were cultivated in boxes (30 x 15 x 10 cm) as an intercropping system with the following plant combinations: banana-banana, banana-groundnut and banana-sweet potato. The experimental set-up was a completely randomized design comprising four treatments and six replicates: (1): Nematode (Nem), (2) AMF, (3) AMF + Nema and (4) control without nematode and without AMF. The ratio of banana: intercrop was 1:1 in the intercropping treatments. A total of 72 boxes was considered in the experimental set-up.

**Results:** AMF root colonization of banana was clearly affected by intercropping with about 25% increment observed in banana co-cultivated with groundnut or sweet potato than banana-banana combination. Positive effects of AMF expressed as an increase of banana biomass compared to the control treatment was observed in root fresh as well as in shoot dry weights. However, the impact of AMF colonization in intercropping systems on *R. similis* did not confirm its bioprotective effect. Intercropping had significant ( $P < .05$ ) effect on *R. similis* and sweet potato has been shown to be more effective in controlling *R. similis* with 62% reduction compared to groundnut (24% reduction). Contrarily, banana plant growth decreased in the banana / sweet potato combination.

**Conclusion:** Findings in this study indicate that *R. similis* biological control in the banana intercropping system is mostly dependent on the intercrop species than AMF

*Keywords:* AMF; Banana; Groundnut; Intercropping; *Radopholus similis*; Sweet potato

## 1. INTRODUCTION

Banana (*Musa* spp.) is the main fruit in international trade and plays a key role in the economics of many developing countries. It is also the major staple food for millions of people in the sub-Saharan Africa. However, its production faces many constraints among which are nematodes. Nematodes have been reported as a major constraint in banana production [1]. They destroy roots and corm tissues, reducing the capacity of the plant to absorb water and nutrients from the soil. Secondary infection of damaged tissues by fungi and bacteria is possible resulting to the extension of the vegetative growth cycle, production of small bunches, reduced life span of the production unit and toppling of plants particularly during windstorms and heavy rain periods [2,3]. Amongst nematodes, *Radopholus similis*, the burrowing nematode, is the most devastating pest of banana around the world [4].

Presently, nematodes are mostly controlled with nematicides. However, several of these chemicals have been withdrawn from the market in the last few decades due to environmental and human health concerns [5]. Moreover, the widespread

use of chemical pesticides against nematodes and other pathogens increased their resistance to pesticides, whereas their natural enemies have been killed in large numbers [6]. The consumer demand for safer food has forced farmers to reduce the use of pesticides. This has encouraged the development of alternative control measures such as corm paring, fallow, the use of resistant cultivars and the use of clean planting material [7]. The application of biocontrol microorganisms such as arbuscular mycorrhizal fungi (AMF) is another option that gave promising results. The positive effects of AMF in controlling crop pests have been demonstrated in several studies [8,9]. Regarding banana nematodes, several studies have reported a decrease in population of nematodes in the presence of AMF [10-14]. In addition, AMF improved plant resistance to abiotic stresses and plant nutrition [15].

Another mechanism involved in disease control is allelopathic suppression of soil-borne pathogens mediated by root exudates [16,17]. Therefore, intercropping with special emphasis on root exudate-mediated effects can be one strategy to control nematodes in sustainable agriculture [18]. Many authors have reported the beneficial effect of intercropping for pest management and particularly for the control of nematodes. Intercropping banana with cover plants such as *Crotalaria* sp (Fabaceae), *Tagetes* sp (Asteraceae), *Mucuna* sp (Fabaceae) have often been reported to reduce nematode populations by their ability to exude nematotoxic substances (deshydrolyzidine, alpha-thienyl, L-3,4-dihydroxyphenylalanine, respectively) into the soil [19,20]. Much of the banana production is done by smallholder subsistence farmers, with up to 87% of the world bananas being produced in these farming systems and consumed locally [21]. However, due to declining land sizes and food security needs, intercropping banana with non-food crops is not beneficial for small scale farmers with respect to nematode control. These farmers mostly intercrop banana with food crops and in the case of Cameroon, groundnut and sweet potato are regularly found as intercrops in most of the banana-based cropping systems. Thus, there is need to investigate the impact of these crops on nematode control in banana intercropping systems.

Cultural and biological treatments tested in controlled or field environments are not always conclusive for the control of plant parasitic nematodes. However, when used in combination, they may contribute to the management of nematodes under a more environmental friendly strategy [22]. The combination of intercropping system and AMF inoculation could thus represent an interesting option to control nematodes in banana. Our work is focused on *Musa accuminata* Colla cv. Grande Naine intercropped with sweet potato (*Ipomoea batatas* (L) Lam cv TiB1) and groundnut (*Arachis hypogaea* L. cv A26) in combination with an AMF (*Rhizophagus irregularis* (Blasz. Wubet. Renker & Buscot) C. Walker & A. Schüssler comb. Nov. MUCCL 41833) inoculation. We hypothesised that the intercrop species can have negative, positive or neutral effects on AMF and *Radopholus similis*.

The study aims at investigating the combination of an AMF with intercropping on *R. similis* infestation of banana (Grande Naine) plantlets grown under greenhouse conditions.

## 2. MATERIAL AND METHODS

### 2.1 Planting Material

*Musa accuminata* cv. Grande Naine was used in the trial and the planting material was obtained by *in vivo* macropropagation using the method described by [23]. Sweet potato (*Ipomoea batatas* (L) Lam cv TiB1) tubers and groundnut (*Arachis hypogaea* L. cv A26) seeds were provided by the National Institute of Agronomic Research for Development (IRAD), Njombe. These cultivars were chosen based on their adaptation to most of the agro-ecological zones of Cameroon. The tubers were surface sterilized with 70% ethanol, rinsed several times with sterilized distilled water and germinated on sterilized (121°C for 60 min) sand. Plantlets were obtained after 8 weeks of culture. They were then separated from the growing tubers and their height homogenized for about 10 cm by cutting the plantlet apex. Healthy peanut seeds were carefully rinsed under running water, then surface sterilized with 65% sodium hypochlorite solution for 10 minutes. They were then rinsed in sterilized (121°C for 15 min) distilled water and further pre-germinated in 90 mm Petri dishes on humidified blotting paper.

### 2.2 Microbial Material

The AMF strain used was *Rhizophagus irregularis* (Blasz. Wubet. Renker & Buscot) C. Walker & A. Schüssler comb. Nov. MUCCL 41833 (<http://www.mycorrhiza.be/ginco-bell/index.php>) and was provided by GINCO. The inoculum consisted of a mixture of spores, hyphae and colonized leek roots (0.5 cm length). The concentration was about 120 propagules.g<sup>-1</sup> of inoculum.

The population of nematodes *Radopholus similis* used in this study were originally isolated from banana cv. Grande Naine roots from plantations at Centre Africain de Recherche sur les Bananiers et Plantains (CARBAP) station Njombe.

Nematode extraction from infested roots was performed according to the methods described by [24]. The inoculum multiplication was performed on banana cv. Grande Naine plants obtained from *in vivo* macropropagation [23]. The plants were transplanted to pots containing 3 kg of sterile (2x1hour at 121°C) sand/coffee ash (proportion 1:2 v/v) substrate after one month of acclimatization. For inoculation, three holes were perforated into the substrate close to the plant roots and 2000 (juveniles and adults) *R. similis* were inoculated per plant. The inoculated plants were grown for three months in the greenhouse. After this period, the nematodes were extracted from the banana roots according to the method described by [24]. One ml tubes were prepared with 200 nematodes in sterile distilled water.

## 2.3 Plant Bioassay

The experiment was conducted under greenhouse conditions (photoperiod 12 h, average temperature 24 - 28°C and 70 - 80% relative humidity) in boxes (30 x 15 x 10 cm). The plants were cultivated as an intercropping culture system with the following plant combinations: banana-banana, banana-groundnut and banana-sweet potato (fig. 1). The experimental set-up was a completely randomized design comprising four treatments and six replicates: (1) Nematode (Nema), (2) AMF, (3) AMF+Nema and (4) control without nematode and without AMF. The number of banana plants was 2 in monoculture. The ration of banana: intercrop was 1:1 in the intercropping treatment. The plants were separated with a distance of 20 cm in each dual system giving a total of 72 boxes in the experimental set-up.

Banana plantlets were weaned in 125 ml pots containing sterilized (2x1hour at 121°C) sand/coffee ash substrate (proportion 1:2 v/v). Two weeks after weaning plants were transferred into boxes and inoculated with AMF. For the AMF treatments, 24 grams of inoculum (2880 propagules) were spread as a layer between two layers of sterilized substrate in each box before plants were transferred from pots into the boxes. At the same time, pre-germinated seeds of groundnut and potato vines were sown.

Four weeks after AMF inoculation, nematodes were introduced in the holes (2cm depth) made between the two plants in nematodes treatments. 5 ml aliquot that contains 1000 nematodes (juveniles and adults) were inoculated per plant combination with a syringe. Plants were not watered for 24 h to ensure that the nematodes are not washed away. The plants were fertilized with 2g of NPK (10-11-18) per box at two weeks after transplanting and were watered when needed.



Fig. 1: Plant combination in different cropping systems: A: Banana-Banana; Banana-Groundnut; C: Banana-Sweet potato.

## 2.4 Data Collection

After sixteen weeks of growth in boxes (Twelve weeks after nematode inoculation), the plants were gently removed from the substrate and washed thoroughly under tap water. For banana-banana combination one plant was used for variable assessment.

### 2.4.1 Growth variables

Root fresh weight was determined after sampling. Also, the shoot dry weight was determined after drying the leaves, pseudostem and the corm for 72 hours at 70°C in an oven.

### 2.4.2 AMF root colonization

Mycorrhizal root colonization was evaluated twice on root subsamples: (1) 4 weeks following transplant into boxes (that is at the time of nematode inoculation) and (2) at harvest (16 weeks after transplant into boxes). Roots were soaked overnight in 10% KOH at room temperature. They were washed several times with deionized water and soaked in alkaline (3.5 % H<sub>2</sub>O<sub>2</sub>) for 30 minutes [25]. The roots were subsequently stained at room temperature for 45 min with a solution of blue ink (Parker<sup>P</sup> Quink®) diluted in 1 % HCl at 1:50 proportions [26]. Root colonization was assessed according to the method of [27] to determine the percentage of arbuscules, vesicles/ spores and hyphae.

#### **2.4.3 Nematodes assessment**

Root necrosis index (RNI) was assessed using five randomly selected functional primary roots from each sample as described by [24]. Nematode extraction was done by the maceration and sieving method [24] of 50 g sub-sample. *R. similis* counts were determined as the number of nematodes (juveniles and adults) per 50 g root fresh weight.

#### **2.4.4 Data analysis**

Data were normalized prior to statistical analysis. The data for nematodes were log(x + 1) transformed, while root colonization by the AMF (% arbuscules, % vesicles and % hyphae) were arcsine (x /100) transformed. Data were analyzed by two way ANOVA and the Tuckey's test was used to identify significant differences (P < 0.05) between treatments. All statistical analyses were performed with STATISTICA (Stat soft. 2001) software.

### **3. RESULTS AND DISCUSSION**

#### **3.1 Results**

##### **3.1.1 AMF root colonization of banana plants and intercrops**

The cropping system significantly influenced the AMF colonization levels of banana plants ( $P < .05$ ) (Fig. 2). Banana plants intercropped with groundnut and sweet potato showed higher root colonization levels than banana monocropping. The hyphae colonization was 37 % and 39% higher respectively for banana-groundnut and banana-sweet potato combinations compared to Banana monocropping. Similarly, the increments of arbuscule colonization were 50% and 48% respectively for banana-groundnut and banana-sweet potato combinations compared to Banana monocropping. Vesicle colonization followed the same trend. However no significant difference was observed between banana-groundnut and banana-sweet potato combinations. The presence of nematodes in banana plants inoculated with AMF did not affect hyphae, arbuscules and vesicles/spores colonization. However, no significant difference was observed between AMF and AMF+Nema treatments (Fig. 2). Plants of the control and Nema treatments were also checked for AM colonization but did not show any presence of AMF.

Root colonization of groundnut and sweet potato was noticed in presence as well as in absence of nematodes (Table 1). However, no significant differences were observed in the hyphae, arbuscules and vesicles between the treatments and between the intercrops (Table 1).

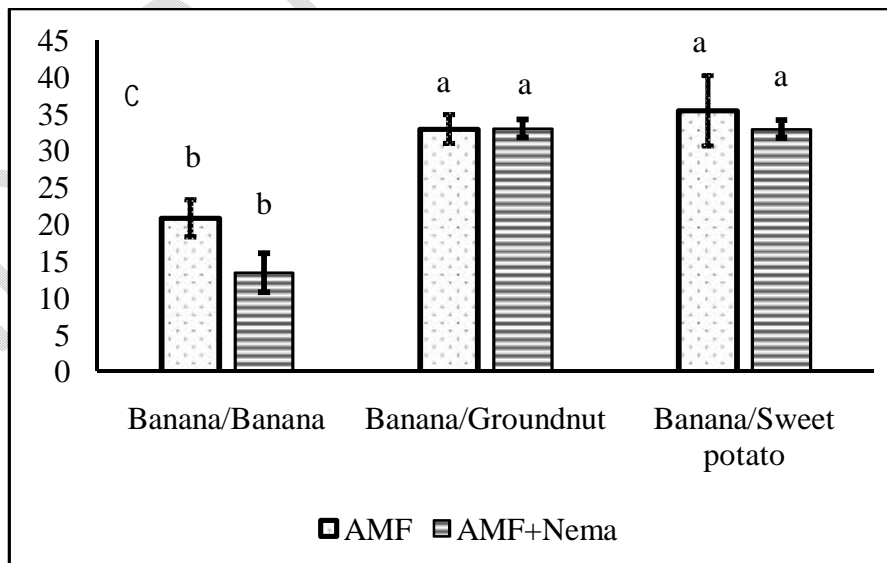
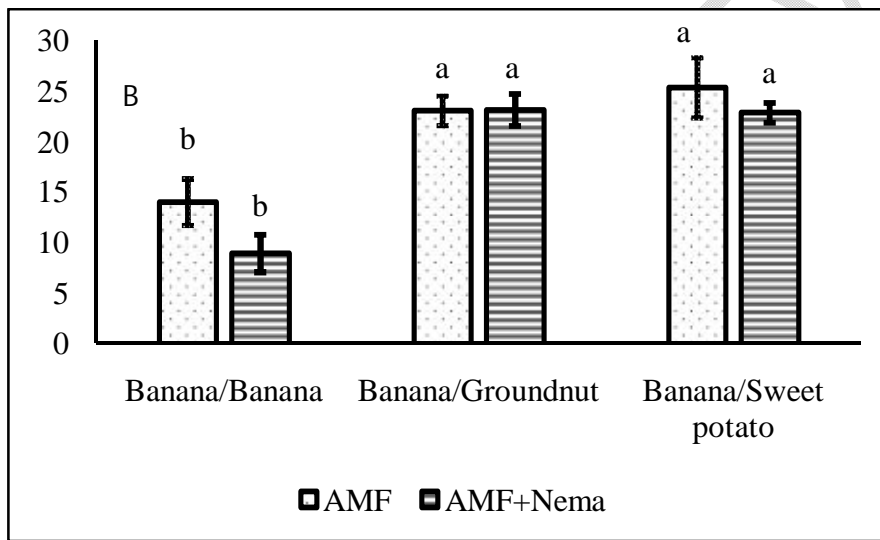
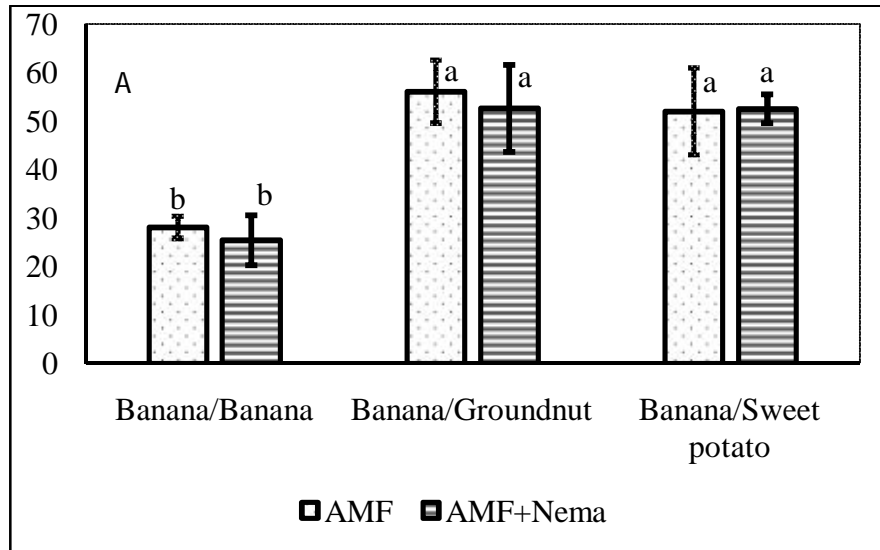


Fig. 2: AMF root colonization (%) of banana plants under different intercropping systems: A: Hyphae. B: Arbuscules. C: Vesicles/spores. AMF: Arbuscular mycorrhiza fungi, Nema: Nematode. Values (mean±S.E.) followed by the same letter are not significantly different according to Tukey test at a probability threshold of 5%.

**Table 1: AMF root colonization (%) of groundnut and sweet potato under different intercropping systems.**

Intercrops	AMF root colonization (%)					
	Hyphae		Arbuscules		Vesicles/spores	
	AMF	AMF+Nema	AMF	AMF+Nema	AMF	AMF+Nema
Banana-Groundnut	52.66±2.64a	50.33±1.78a	33.66±1.90a	30.00±9.00a	26.66±8.47a	20.67±5.66a
Banana-Sweet potato	46.22±1.54a	43.66± 7.11a	30.10±2.08a	27.55± 5.07a	22.88±2.70a	20.33±7.11a

AMF: Arbuscular mycorrhiza fungi, Nema: Nematode. Values (mean±S.E.) followed by the same letter in the same column are not significantly different according to Tukey test at a probability threshold of 5%.

### 3.1.2 Nematode infestation of banana plants and intercrops

Nematode infestation (the number of nematodes in 50g fresh root) and root necrosis index (RNI) are presented in Table 2. Nematode population density and RNI were reduced by AMF inoculation. The number of nematodes was 2880±352 and 2267±159 respectively for Nema and AMF+ Nema treatments in the banana monocropping, 2103±271 and 1833±74 respectively for Nema and AMF + Nema treatments in the banana-groundnut combination and 1052±131 and 888±203 respectively for Nema and AMF + Nema treatments in the banana-sweet potato combination. However, the post-hoc analysis (Tukey Test) revealed that this reduction was not significant. RNI was also decreased by AMF inoculation although the reduction was not significant ( $P > .05$ ) (Table 2).

The cropping system significantly affects nematode infestation of banana plants. The number of nematodes was 24% and 62% less respectively for banana-groundnut and banana-sweet potato compared to banana-banana cropping system. With respect to RNI, the reduction was 24% and 63% respectively in banana-groundnut and banana-sweet potato compared to banana-banana cropping system. Although banana-groundnut system reduces the nematode infestation compared to banana-banana system, no significant difference was observed ( $P > .05$ ). Contrarily, the banana-sweet potato system significantly lowered nematode number and RNI compared to banana-banana and banana-groundnut systems (Table 2).

*R. similis* was found in the roots of groundnut and sweet potato in the intercropping systems in the presence or absence of AMF inoculation (Table 3) suggesting that these crops are possible host plants. The number of nematodes in groundnut roots was 687±111 and 590±85 respectively for Nema and AMF + Nema treatments whereas in sweet potato it was 93±40 and 38±22 respectively for Nema and AMF + Nema treatments. AMF inoculation did not significantly influence the number of nematodes in the two intercrops. On the contrary the intercrop species showed significant difference in number of nematodes with the less colonized roots of sweet potato. (Table 3).

**Table 2: Root necrosis index (RNI) and nematode population density of banana under different intercropping systems.**

Culture combination	Nematode population density		RNI (%)	
	Nema	AMF+Nema	Nem	AMF+Nema
Banana-Banana	2880±352a (A)	2267±159a (A)	17.97	14.10
Banana-Groundnut	2103±271a (A)	1833±74a (A)	13.12	11.20
Banana-Sweet potato	1052±131a (B)	888±203a (B)	6.56	5.25

AMF: Arbuscular mycorrhiza fungi, Nema: Nematode. Values (mean±S.E.) followed by the same letter are not significantly different according to Tukey test at a probability threshold of 5%; capital letters in the same column and small letters in the same line.

**Table 3: Nematode population density of intercrops under different intercropping systems**

Intercrops	Nematode population density (per 50g fresh root)	
	Treatments	
	Nema	AMF+Nema
Groundnut	687±111a (A)	590±85a (A)
Sweet potato	93±40a (B)	38±22a (B)

AMF: Arbuscular mycorrhiza fungi, Nema: Nematode. Values (mean±S.E.) followed by the same letter are not significantly different according to Tukey test at a probability threshold of 5%; capital letters in the same column and small letters in the same line.

### 3.1.3 Plant growth parameters

Root fresh weight (RFW) of banana plants from the different intercropping combinations are shown in Table 4. RFW was significantly affected by treatments and intercropping combination ( $P < .05$ ). The treatment had in each plant combination a significant influence on root fresh weight. AMF and AMF+Nema treatments significantly increased RFW (27.5% and 23.5% increment, respectively) compared to the control and Nema treatments independent of plant combination. Contrarily, Nema did not affect RFW compared to the control. However, no difference was observed between AMF and AMF+Nem. The plant combination had in each treatment a significant effect on banana RFW. The highest RFW was obtained in banana-groundnut combination with  $76.16 \pm 7.60$ ,  $117.50 \pm 8.35$ ,  $90.61 \pm 9.66$  and  $107.40 \pm 6.44$  for control, AMF, Nema and AMF+Nema treatments, respectively (Table 4).

Shoot dry weight (SDW) of banana plants from the different intercropping combinations are shown on Table 5. SDW of the banana plants was significantly increased in AMF and AMF+Nema treatments while no effect was noticed in Nema treatment compared to the control. Banana-sweet potato intercropping significantly decreased the SDW of banana plants than banana-banana and banana-groundnut combinations independent of the treatments. The highest SDW was observed in banana-groundnut intercropping.

**Table 4: Root fresh weight (g) of banana plants grown under different intercropping systems.**

Intercropping systems	Treatments				P-value
	Control	AMF	Nema	AMF+Nema	
Banana-Banana	$70.16 \pm 4.56$ ab (AB)	$80.30 \pm 5.47$ a (B)	$58.19 \pm 3.06$ b (AB)	$76.11 \pm 4.34$ a (B)	$P = 0.006$
Banana-Groundnut	$90.61 \pm 9.66$ ab (A)	$117.50 \pm 8.35$ a (A)	$76.16 \pm 7.60$ b (A)	$107.40 \pm 6.44$ a (A)	$P = 0.004$
Banana-Sweet potato	$56.04 \pm 5.75$ ab (B)	$75.84 \pm 7.00$ a (B)	$47.40 \pm 3.65$ b (B)	$75.22 \pm 5.48$ a (B)	$P = 0.001$
P-value	$P = 0.005$	$P = 0.0002$	$P = 0.004$	$P = 0.0001$	

AMF: Arbuscular mycorrhiza fungi, Nema: Nematode. Values (mean $\pm$ S.E.) followed by the same letter are not significantly different according to Tukey test at a probability threshold of 5%; capital letters in the same column and small letters in the same line.

**Table 5: Shoot dry weights (in grams) of banana plants grown under different intercropping systems.**

Intercropping systems	Treatment				P-value
	Control	AMF	Nema	AMF+Nema	
Banana-Banana	$21.77 \pm 1.45$ b (A)	$36.78 \pm 2.59$ a (A)	$19.79 \pm 1.32$ b (A)	$30.63 \pm 2.27$ a (A)	$p = 0.0001$
Banana-Groundnut	$25.41 \pm 1.62$ b (A)	$38.28 \pm 1.51$ a (A)	$23.10 \pm 1.47$ b (A)	$34.29 \pm 1.63$ a (A)	$p = 0.0001$
Banana-Sweet potato	$16.31 \pm 0.92$ b (B)	$27.63 \pm 1.45$ a (B)	$14.83 \pm 0.84$ b (B)	$20.03 \pm 2.18$ b (B)	$p = 0.0001$
P-value	$p = 0.0001$	$p = 0.0006$	$p = 0.0001$	$p = 0.0001$	

AMF: Arbuscular mycorrhiza fungi, Nema: Nematode. Values (mean $\pm$ S.E.) followed by the same letter are not significantly different according to Tukey test at a probability threshold of 5%; capital letters in the same column and small letters in the

### 3.2 Discussion

AMF symbiosis with its bio-fertilizing and bio-pesticidal aspects is of great interest especially in the context of sustainable agriculture. The present study investigated the interaction of intercropping and AMF inoculation to control nematodes and enhance the growth of banana in a greenhouse. As far as root colonization of banana is concerned, this was clearly affected by the intercropping. An increase in the colonization rate of about 25% was observed in banana co-cultivated with groundnut or sweet potato than banana-banana combination. This increase is probably due to the rapid establishment of the mycelial network. Indeed, groundnut and sweet potato being annual crops with life cycles varying between 3 and 4 months easily develop their rooting system during the first month. Also, these intercrops are mycotrophic and therefore contribute effectively to the rapid development of mycelial networks which apart from spores and root fragments, the mycelial is of high significance in AMF colonization of the newly developed roots [28]. The well-established AM symbiosis in groundnut and sweet potato thus stimulated the colonization of banana plant in the intercropping systems. Similar findings were obtained by [29] on wheat intercropped with faba bean. The presence of *R. similis* does not influence root colonization by AMF in banana as well as in intercrops. Thus, no significant reduction in hyphae, arbuscules and vesicles was observed in mycorrhized banana plants and intercrops. Similar results have been reported on banana plants mycorrhized in the presence of *Meloidogyne incognita*, *Pratylenchus coffeae*, *Pratylenchus goodeyi* and *R. similis* *in vitro* and in the greenhouse [10,12,11,13]. Although *R. irregularis* and *R. similis* cohabit the same ecological niche, they may not have the same infection site. *R. irregularis* infects very fine roots (tertiary and rarely primary and secondary) while *R. similis* prefers large primary roots [30]. Contrastingly, [31]'s meta-analysis in 2005 showed that AMF colonization was reduced by ectoparasitic, migratory endoparasitic and sedentary endoparasitic nematodes. [32] concluded that AMF colonization might be suppressed by plant parasitic nematodes depending on the AMF species.

The assessment of the impact of AMF colonization in intercropping systems on *R. similis* did not confirm its bioprotective effect. Reduction of *R. similis* in banana roots was not significant ( $P < 0.05$ ) whatever the plant combination. Our results were not concordant with those reported by [20] and [13]. These authors used the nylon mesh to separate the belowground part of each plant in the intercropping system to avoid root interactions. Only haphae was able to spread from one compartment to the other. In our case, no nylon mesh was used as in the natural environment allowing the strong interaction of the two root systems. This might facilitate the infection of newly formed roots by *R. similis* from old infested roots, thus AMF was not able to significantly reduce *R. similis* in the intercropping system. However, intercropping has a significant effect on nematodes the roots of banana. This effect is a function of the intercrop species. Sweet potato has been shown to be more effective in controlling *R. similis* compared to groundnut. Sweet potato could therefore be considered as a poor host and the groundnut as an intermediate host [20]. The reduction of *R. similis* in the presence of sweet potato would evoke the phenomenon of allelopathy.

On the other hand, in groundnuts the phenomenon of diversification of food resources of the pathogen may be the cause of the reduction of *R. similis* in the roots of the banana plant. Previous studies on the value of intercropping for sustainable pest control have found mixed and sometimes conflicting results reflecting the complex and variable nature of associational resistance and susceptibility [18]. [33] observed that the bio-protective effects of AMF were clearly present in the good and intermediate hosts of *R. similis* with moderate to high relative mycorrhizal dependency but absent in sweet potato which is an intermediate of *R. similis* with a negative relative mycorrhizal dependency as well as in a poor and non-host of *R. similis*. They concluded that the bioprotective effect of AMF for *R. similis* depends on the host level and relative mycorrhizal dependency level (low, moderate or high).

RFW and SDW of banana were impacted by AMF as well as intercrop species. Banana SDW was increased when associated to AMF in absence as well as in the presence of the nematodes independent of the intercropping combinations. The increase in SDW has been reported in many studies and often attributed to the higher supply of water, phosphorus and other nutrients with low mobility such as ammonium, potassium, copper, iron, sulfur, molybdenum or zinc [34]. In addition, AMF is able to extend the absorbing network beyond the nutrient depletion zones of the rhizosphere allowing access to a larger volume of soil [35]. However, the increase in SDW was not followed by an increase in RFW. RFW of the banana plants was significantly decreased in NEMA treatment compared to control independent of the intercropping combination. This is probably due to the feeding of nematodes on the roots reducing their growth and biomass production [36,7]. Interestingly, an increment of 23.28%, 37% and 37% in RFW for banana-banana, banana-groundnut and banana-sweet potato combination (respectively) was obtained when AMF was co-inoculated with NEM.

The most striking was the reduction of banana plant growth in the banana-sweet potato combination by 28% and 11% for shoot and root loss respectively compared to the banana-banana system. [37] stated that intercropping using species with high biomass production reduced crop productivity. In addition, banana and sweet potato are highly demand potassium and nitrogen and they may compete for these elements [38]. Contrarily, groundnut shows no negative effects on the growth of banana plants in the banana-groundnut system compared to banana-banana combinations. In recent years, legume intercropping has been documented as an advantageous crop production system and several legume-based intercropping systems are known to be very productive and efficient evoking its facilitative effect in the system. An important example in relation to intercropping involves facilitative interactions between legumes and non-legumes that can contribute to higher nutrient uptake in intercrops as compared to sole crops resulting to high land equivalent ratio [39].

In a wide view, intercropping is dependent upon crop competition for light, water and nutrients or allopathic effects that may affect yield. In order to limit this problem, crop species should be selected in such a way that they show complementarity or mutual sharing of nutrients, light and water in order to record advantages of intercropping [40,41].

#### 4. CONCLUSION

In this study, we demonstrated that banana intercropped with different species shows beneficial effects on AMF root colonization which is of great interest in plant communities. However, the control of *R. similis* in the intercropping system is mostly dependent on the intercrop species than AMF inoculation. Although sweet potato has efficiently control *R. similis* in banana-sweet potato intercropping, no benefits were observed in the banana plant growth. This suggests that this species could be used in a crop rotation system to control nematodes in the soil than intercropping system with this species.

#### REFERENCES

1. Sarah JL. Banana nematodes and their control in Africa. *Nematropica*. 1989; 19:199-216.

2. Sarah JL. Burrowing nematode. In: Jones DR, editor. Diseases of banana, abaca and enset. CAB International: Wallingford; 2000.
3. Gowen SC, Quénéhervé P, Fogain R. Nematode parasites of bananas and plantains, In: Luc M, Sikora RA, Bridge J, editors. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. 2nd ed. CABI Publishing: Wallingford; 2005.
4. Hölscher D, Dhakshinamoorthy S, Alexandrov T, Becker M, Bretschneider T, Buerkert A et al. Phenalenone-type phytoalexins mediate resistance of banana plants (*Musa* spp.) to the burrowing nematode *Radopholus similis*. Proc. Natl. Acad. Sci. U. S. A. 2014; 1: 105–110.
5. Rich JR, Dunn R, Noling J. Nematicides: Past and present uses. In: Nematology: Advances and Perspectives, Vol 2. Nematode Management and Utilization (Eds.) Chen ZX, Chen SY, D. Dickson W, editors. CABI Publishing: Wallingford; 2004.
6. Weng W, Yan J, Zhou M, Yao X, Gao A, Ma C. Roles of Arbuscular mycorrhizal Fungi as a Biocontrol Agent in the Control of Plant Diseases Microorganisms. 2022; 10(7): 1266.
7. Queneherve P. 2009. Integrated Management of banana nematodes. In: Ciancio, Aurelio, Mukerji KG, editors. Integrated Management of Fruits Crops and Forest Nematodes, Chapter: Integrated Management of Banana Nematodes. Springer; 2009.
8. Azcon-Aguilar C, Barea JM. Arbuscular Mycorrhizal and biological control of soilborne plant pathogens-An overview of the mechanism involved. Mycorrhiza. 1996; 6: 457-464.
9. Harrier LA, Waston CA. The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. Pest Management Science. 2004; 60: 149-157.
10. Elsen A, Gervacio D, Swennen R, De Waele D. AMF-induced biocontrol against plant parasitic nematodes in *Musa* sp.: a systemic effect. Mycorrhiza. 2008; 18, 251–256.
11. Vos C, Tesfahun AN, Panis B, De Waele D, Elsen A. Arbuscular mycorrhizal fungi induce systemic resistance in tomato against the sedentary nematode *Meloidogyne incognita* and the migratory nematode *Pratylenchus penetrans*. Appl. Soil Ecol. 2012b; 61: 1–6.
12. Koffi MC, Vos C, Draye X, Declerck S. Effects of *Rhizophagus irregularis* MUCL 41833 on the reproduction of *Radopholus similis* in banana plantlets grown under in vitro culture conditions. Mycorrhiza. 2013; 23: 279–288.
13. Anene AA, Declerck S. Combination of *Crotalaria spectabilis* with *Rhizophagus irregularis* MUCL 41833 decreases the impact of *Radopholus similis* in banana. Applied Soil Ecology. 2016; 106: 11–17.
14. Rodrigues E, Silva MT, Calandrelli A, Miamoto A, Rinaldi LK, Moreno BP, et al. Pre-inoculation with arbuscular mycorrhizal fungi affects essential oil quality and the reproduction of root lesion nematode in *Cymbopogon citratus*. Mycorrhiza. 2021; 31:613–623.
15. Smith SE, Read DJ, editors. "Mineral nutrition, toxic element accumulation and water relations of arbuscular mycorrhizal plants," in Mycorrhizal Symbiosis, 3rd ed. London: Academic Press; 2008.
16. Hao W, Ren LX, Ran W, Shen QR. Allelopathic effects of root exudates from watermelon and rice plants on *Fusarium oxysporum* f.sp. *niveum*. Plant Soil. 2010; 336:485–497.
17. Ratnadass A, Fernandes P, Avelino J, Habib R. Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. Agronomy for Sustainable Development. 2012; 32: 273–303.

18. Chadfield VGA, Hartley SE, Redeker KR. Associational resistance through intercropping reduces yield losses to soil-borne pests and diseases. *New Phytologist*. 2022; 235: 2393–2405.
19. Chitwood DJ. Phytochemical based strategies for nematode control. *Annual Review of Phytopathology*. 2002; 40: 221-49.
20. Van der Veken L, Win PP, Elsen A, Swennen R, De Waele D. Susceptibility of banana intercrops for rhizobacteria, arbuscular mycorrhizal fungi and the burrowing nematode *Radopholus similis*. *Applied Soil Ecology*. 2008; 40: 283- 90.
21. Frison EA, Escalant JV, Sharrock S. The global Musa genomic consortium: A boost of banana improvement. In: Jain MS, Swennen R, editors. *Banana improvement cellular, molecular biology and induced mutations. Proceedings from a Meeting held in Leuven, Belgium, 2001 Sep 24–28*. NH (USA): Science Publishers; 2004.
22. Aktar MA. Current option in integrated management of plant-parasitic nematodes. *Integrated Pest Management Review*. 1997; 2: 187-197.
23. Kwa M. Activation de bourgeons latents et utilisation de fragments de tige du bananier pour la propagation en masse de plants en conditions horticoles in vivo. *Fruits*. 2003; 58: 315-328.
24. Speijer PR, De Waele D. Screening of Musa germplasm for resistance and tolerance to nematodes. INIBAP Technical Guidelines. International Plant Genetic Resources Institute, 1997.
25. Koske RE, Gemma JN. A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research*. 1989 ; 92: 486-505.
26. Vierheilig H, Coughlan AP, Wyss U, Piché Y. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Appl Environ Microbiol*. 1998; 64:5004–5007
27. McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. A new method which gives an objective measure of colonization of roots by vesicular- arbuscular mycorrhizal fungi. *New Phytol*. 1990; 115: 495–501.
28. Mandou MMS, Mvondo Ze A, Etoa FX, Declerck S. Effects of extraradical mycelium network of an arbuscular mycorrhizal fungus on the growth of banana plantlets. *Journal of Plant Biology Research*. 2015; 4(1): 22-32.
29. Ingraffia R, Amato G, Frenda A S, Giambalvo D. Impacts of arbuscular mycorrhizal fungi on nutrient uptake, N<sub>2</sub> fixation, N transfer, and growth in a wheat/fababean intercropping system *PLoS ONE*. 2019; 14 e0213672.
30. Stoffelen R. Early screening of *Eumusa* and *Australimusa* bananas against root-lesion and root-knot nematodes. *Dissertationes de Agricultura No 426*, Katholieke Universiteit Leuven, Belgium; 2000.
31. Hol GWH, Cook R. An overview of arbuscular mycorrhizal fungi–nematode interactions. *Basic and Applied Ecology*. 2005; 6: 489-503.
32. Schouteden N, De Waele D, Panis B, Vos C. Arbuscular Mycorrhizal Fungi for the Biocontrol of Plant-Parasitic Nematodes: A Review of the Mechanisms Involved. *Front. Microbiol*. 2015; 6:1280.
33. Van der Veken L, Cabasan MTN, Elsen A, Swennen R, De Waele D. Effect of single or dual inoculation of the arbuscular mycorrhizal fungus *Glomus mosseae* and root-nodulating rhizobacteria on reproduction of the burrowing nematode *Radopholus similis* on non-leguminous and leguminous banana intercrops. *Journal of Plant Diseases and Protection*. 2021; 128: 961–971.
34. Poveda J, Abril-Urias P, Escobar C. Biological Control of Plant-Parasitic Nematodes by Filamentous Fungi Inducers of Resistance: Trichoderma, Mycorrhizal and Endophytic Fungi. *Front. Microbiol*. 2020; 11:992.
35. Parihar P, Bora M. Effect of mycorrhiza (*Glomus mosseae*) on morphological and biochemical properties of Ashwagandha (*Withania somnifera*) (L.) Dunal. *J. Appl. Nat. Sci*. 2018; 10: 1115–1123.

36. Guedira A, Évaluation de la résistance à deux nématodes : *Radopholus similis* et *Meloidogyne* spp. chez quatre géotypes de bananier au Maroc. *Comptes-Rendus Biologie*. 2004 ; **327** : 745-751.
37. Pypers P, Sanginga JN, Kasereka B, Walangu-lulu M, Vanlauwe B. Increased productivity through integrated soil fertility management in cassava-legume intercropping systems in the highlands of Sud Kivu DR Congo. *Field Crops Research*. 2011; 120: 76-85.
38. Mandou MS, Adamou S, Nwaga D, Etoa FX. Intercrops Influence Mycorrhizal Symbiosis Development, Growth and Nutrient Uptake of Banana. *Int. J. Curr. Microbiol. App. Sci*. 2016; 5(12): 84-94.
39. Zhang F, Li L. Using competitive and facilitative interactions in intercropping systems enhances crop productivity and nutrient-use efficiency. *Plant and Soil*. 2003; 248: 305–312.
40. Gebru HA. Review on the Comparative Advantages of Intercropping to Mono-Cropping System. *J. Biol. Agric. Healthc*. 2015; 5: 1–13.
41. Bybee-Finley KA, Ryan MR. Advancing intercropping research and practices in industrialized agricultural landscapes. *Agriculture*. 2018; 8: 80.

UNDER PEER REVIEW