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Research Article

Screening For Native Arbuscular Mycorhizal Fungi to Promote *Solanum Macrocarpon* L. Yield and Meloidogyne Spp. Suppression in the Field Conditions

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ABSTRACT
The present study aims at evaluating the effect of native arbuscular mycorrhizal fungi (AMF) inoculation at the nursery stage on the leaves yield and on damage severity of RKNs on *S. macrocarpon* under field conditions. Seedlings were grown in the greenhouse on sterilized soil with or without AMF inoculation from which three were pure isolates (*Funneliformis mosseae, Glomus Clarium, G. etunicatum*) and five were mixture of indigenous AMF spores collected from *S. macrocarpon* field (Dapaong, Kara, Sokodé, Kpalimé and Baguida). Six-week-old seedlings were transplanted to the field utilizing a completely randomized design with nine treatments replicated four times. Results showed that mycorrhizal colonization of *S. macrocarpum* inoculated and sporulation remained higher than those not inoculated. Inoculated plants produced higher biomass and the yield was 14-35% higher than those not inoculated. Each AMF inoculum resulted a significant reduction of the population density of nematodes and severity of root galls and increased leaves production of S. *macrocarpum* compared to control. But, better yield has been obtained with mixture inoculum from Dapaong, Kara and Baguida. We conclude that AMF inoculation can be used for sustainable production of *S. macrocarpum*.

KEYWORDS: Arbuscular mycorrhizal fungi; *S. macrocarpum*; nematode; leaves yield

INTRODUCTION

Solanum macrocarpon L. is a leaf vegetable plant of the family of the Solanaceae [1] as tomatoes, peppers and eggplant. The leaves of *S. macrocarpum* are used traditionally as a vegetable in many countries in West Africa included Benin, Ivory Coast, Nigeria and Togo [2–4]. It is also used for its therapeutic properties. As others many vegetables, *S. marocarpon* is grown by gardeners in the country. It is produced by almost 95% of gardeners [2]. Commonly called "Gboma" in the country, it leaves are rich in protein, fiber, calcium, iron, potassium, magnesium,

phosphorus and sodium [3,5]. In addition, Gboma is used in traditional medicine for the treatment of several diseases including: obesity, asthma, skin infections, rheumatism, gastro-esophageal disease, constipation and diabetes [6]. The consumption per capita and per year is estimated to 10 kg and that the nutritionists counsel a daily consumption included between 50 and 100g per capita [5]. Despite the importance of the species in Africa, the yield of this plant is decreasing in the last ten years doing to soil fertility, insect feeders, diseases and roots feeders among which nematodes are the most important pests. The mean nematodes damaging root of *S. macrocarpon* is *Meloidogyne* spp. [7,8].

Current nematodes management on vegetable crops such as *S. macrocarpum* was based mainly on synthetic chemical nematicides [9]. However, their highly hazardous nature has led to many of these products being removed from the market and their use discontinued [10]. Other nematode management practices such as botanical [11,12], organic fertilizers [13,14] or cultural control [15] have been explored for vegetables crops, with some success.

Recently, biological control of the nematodes using mutualistic micro-organisms such as Arbuscular mycorrhizal fungi (AMF) has been suggested as a potential alternative to chemical control [16] . AMF species colonize belonging to over 80% of all plant genera [17] and are considered to be the most widespread symbionts in plants, which generally benefit from this AMF association through increased plant nutrient uptake, plant growth and survival rates (e.g. [16,18,19]. They were known to enhance plant uptake of phosphate (P) and other mineral nutrients under certain conditions [20–22]. The AMF association may also increase host plant resistance/ tolerance against biotic [14,19,23–26] and abiotic stresses, including salinity and drought [27–30]. With a live network, they stimulate the soil structure, which usually induces greater protection against erosion, infiltration and storage of the best water [31]. The tight network of hyphae in the soil also prevents leaching of nutrients. However, several studies have indicated that sometimes arbuscular mycorrhiza can have harmful effects depending on the pests involved and other factors [32,33]. Thereafter, there is no report regarding a tripartite interaction including AMF, *Meloidogyne* spp. and *S. macrocarpon* at the field site. The objective was to compare the Colonisation efficiency of different forms of AMF inocula via toward the root knot nematode control on *S. macrocarpon* in the field. Therefore, the objective of this study is to examine the levels of nematodes and *S. macrocarpon* leaves yield that can be achieved by using some indigenous AMF inocula in S. *macrocarpum* culture.

MATERIALS AND METHODS

Experiment site description

This field experiment was conducted at the research farm of the Faculty of Agronomy at the University of Lomé, Togo (6°10.563N and 1°12.782E). The site is characterized by Guinean climate with two rainy seasons, April to July and September to November and two dry seasons in between. The soil of the experimental site is classified as a ferralsol soil with the following surface (0–15 cm) soil properties were done by Laboratory of Soil and Chemistry of Agronomy Faculty of University of Lomé as: organic matter (OM) 1. 87%; total N, 0.15%; pH 6.50; available Phosphorus (P_2O_5) 0.5 mg kg^{-1}, Potassium (K_2O) 0.46 mg kg^{-1} and Magnésium (MgO), 0.01 mg kg^{-1} .

Arbuscular Mycorrhizal Fungi (AMF) inoculum

A total of eight inocula were used from which three (*Funneliformis mosseae, Glomus Clarium* and *G. etunicatum)* were isolated and identified as single spore culture at University of basel, Botanical Institut while five were mixed spores inocula collected from the *Solanum* sp. field (Dapaong, Kara, Sokodé, Kpalimé and Baguida) in Togo. The spores morphotypes were mainly of *Glomus* sp.; *Funneliformis* sp., and *Acaulospora* sp. and the inoculum spores density was 256/25g (Dapaong); 201/25g (Kara); 301/25g (Sokodé); 269/25g (Kpalimé); 354/25g (Baguida). The spores density of three pure culture inocula 479/25g (*Funneliformis mosseae*), 300/25g (*Glomus clarium*) and 544/25g (*G. etunicatum*). The inocula were maintained in pot culture with *Sorghum bicolor* L at International Institute of Tropical Agriculture (IITA), Benin Station for six months before used for seeds inoculation. The inoculum consisted of substrate containing spores, hyphae, and mycorrhizal root fragments

with 82-85% of colonisation, and was adjusted to contain 300 mycorrhizal propagules g^{-1} by mixing with sterile sand.

***Solanum macrocarpon* seed inoculation with Arbuscular Mycorrhizal Fungi (AMF) inocula**

Seeds inoculation was done during nursery period in plastic tanks (50×40×20 cm) in the greenhouse. The substrate used for nursery consisted of soil from the arable land from the Faculty of Agriculture experimental station and Lomé beach sand (w/w, 2:1). The soil was collected from a depth of 0-25cm and passed through a 1mm aperture sieve to remove roots and debris. The marine sand was thoroughly washed with tap water to remove salt. The substrate mixture was oven sterilized at 80°C for 72 h.

The tank filled with sterilized soil was watered and three stripes were made in the length direction of the plastic tank about 1cm deep as a seedbed. Thereafter, 50g of corresponding strain inoculum was spread in each stripe before putting the seeds and closed it with the sterilized marine sand. The control plastic tank had not received any AMF strain inoculum but sterilized substrate used for inocula production. One plastic tank was used for each inoculum making in total, nine plastics tanks. The nursey was conducted in the greenhouse for six weeks before transplanting at the field.

Experimental design at the field

The treatments were arranged in a completely randomized block design were conducted in nine treatments, and four repetitions were tested for each treatment. Each replicate consisted of one plot of 3×6 m (18 m^2). Six weeks old plants from the nursery were transplanted at 25×25 cm in each plot. The plant density was 220 plants per plot. Every plot was separated by one-meter space as an edge. The plots were regularly watered and weeded until harvest. AMF roots colonization, nematodes density and root galling symptoms and leaves yield were assessed.

Assessment of AMF root colonization and spores density

AMF root colonization and spores density were assessed one month after transplanting at field. The roots of five plants per treatment of each plot were randomly sampled, cleaned with tap water and conserved in the vials (capacity 48 mL**)** containing mineral water for the analysis. The AMF spores were isolated by wet sieving and sucrose density gradient centrifugation [34]. For this purpose, 25 g air-dried soil samples from each treatment plot were suspended in 300 ml of water using a 500 ml beaker. The soil suspension was passed through 1000-, 500-, 125-, 80- and 32-µm sieves to discriminate particles. The 1000- and 500- µm sieves were checked for sporocarps, spore clusters and large spores adjacent to or inside roots. The contents of the 125-, 80- and 32-µm were layered onto a water-sucrose solution (70% ‹wt/vol›) gradient and centrifuged at 2000 tours/min for 2 minutes. After centrifugation, the supernatant was passed through the 32-µm sieve, washed with tap water, and transferred to Petri dishes. Spores, spore clusters, and sporocarps obtained from all sieves were transferred into Petri dishes, counted for each sample using a dissection microscope (Olympus SZ12) at up to x 90 magnification. The abundance of spores (= spore density) in a soil sample was expressed as the number of AMF spores g^{-1} of soil. AMF root colonization was determined according to Brundrett et al. [35]. A 1.0g subsample of the roots was excised from the five plants, to assess the percentage of AMF colonization. The root samples were cleared in KOH (100 $g.L^{-1}$) solution at 90~ on a hot plate for 1 h and stained with trypan blue (0.5 $g.L^{-1}$) in lactoglycerol [36] at 90~ for 30 min. Percentage colonization of host plant roots was estimated by visual observations of stained root segments mounted in lactoglycerol by the grid-line intercept method [37]. The number of spore was counted using a stereo microscope, a Leica Wild M3C.

Sampling, extraction and assessment of nematode density and root galling symptoms

The soil was collected from up to a depth of 15-20 cm from different treatments plots. From each plot, 3 samples were collected by borer at different locations randomly; giving in total 200g average of soil. These three samples were mixed according to method described by Barker and Niblack [38] using a plastic bag to form a representative sample of plot. The roots contained in the soil sample have been selected using tweezers that has been used for the extraction of nematodes on the roots. In the

laboratory, nematodes were extracted from the soil of each plot sample by using a modified Baermann plate method [39]. *Meloidogyne* spp. was also extracted from roots using the same technique after cleaning and crushing with moulinex. Nematodes were counted with a Leica Wild M3C microscope. Roots scoring were assessed at the end of harvest by removing every plant and counting number of galls per root gram.

Leaves yield evaluation

The harvest started two months after the transplantation of plants to the field and subsequently at 15 days intervals until the end of harvest. A total of four leaves harvests have been done. The leaves yield and increased yield by inoculum were calculated as follow:

$$Y(T/ha) = \sum_{i=1}^{n} yi$$

$$I_y(\%) = \left[\frac{T_y - C_y}{T_y}\right] \times 100$$

Y = yield; yi = yield in ith harvest; n = number of harvests; I_y = Increased yield; T_y = yield in inoculated treatment plots; C_y = yield in control plots.

Statistical analysis of the data

The data collected were analyzed with the Statistical Package for Social Science (SPSS version 20.0, GLM procedure) by analysis of variance (ANOVA) in which significant differences were recorded between treatments whenever the 95% confidence limits failed to overlap, and the means were discriminated using the Student-Newman-Keuls test. Densities data and percentages were respectively transformed into x' = log10 (x + 1) and Arcsinus√ (percent x / 100) before analysis for data standardize, x is the number of nematodes counted [40].

RESULTS

Root rate colonization and spore density of mycorrhizal fungus

Roots mycorrhization has been observed at the all treatments (fig. 1) but, rate colonization of the AMF was significantly higher ($P \leq 0.0001$) in the inoculated plants roots compared to the control plants. Except strain of Kpalime which had lower rate of AMF colonization statistically, all other strains, mixed or pure had comparable rate of roots mycorrhization. However, the higher rates were obtained with *Funneliformis mosseae* and strain of Kara. The spore density was higher significantly ($P = 0.002$) in the AMF treatments compared with control. Only spore density of *Glomus Clarium* treatment differ to the other treatments whose plants had been inoculated in the nursery.

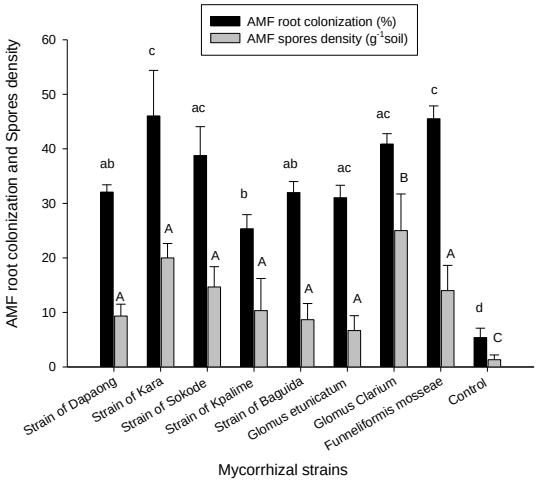

Fig. 1 AMF root colonization presented as percentage of root colonized collected from *S. macrocarpum* and AMF spores density presented as spore numbers g^{-1} of soil sampled. Nonsignificant differences between AMF roots colonizations are shown by identical letters in lowercase and between spores density in uppercase, determined using Student Newman and Keuls (SNK) at the 5% level following one-way ANOVA.

Effects of different strains of AMF on parasitic nematodes population in the soil and in the roots of *S. macrocarpum*

The nematodes counting in soil and in the roots of *S. macrocarpum* for the different treatments permitted to have the following results:

In the soil, the Table 1 showed the evolution of nematodes density from all treatments of the experiment at different periods of sampling. At the transplanting and one month after transplantation of the young plants, the analysis of the variance showed that there was no significant difference between the densities of nematodes on the plots of each treatments ($P = 0.759$ $P = 0.971$). From harvest phase, each strain of AMF applied reduced significantly

average number of nematodes in soil compared to the control (P = 0.01). At the end of harvest, the discrimination of the averages with Student-Newman-Keuls test 5% revealed that the strain of Sokode and Baguida have statistically identic effects to those of the pure strains (*G. clarium*, *F. mosseae* and *G. etunicatum*) where the reduction action of nematodes density in soil was more pronounced and defer statistically to this one of the other AMF strains ($P \leq 0.0001$).

In the roots, a progressive evolution of the number of nematodes has been noted to transplanting at harvest for all treatments (Table 2). The variance analysis showed that the reduction of nematodes density by each of the eight AMF strains used was statistically significant compared to control one month after transplanting ($P \leq 0.0001$), during the harvest (P = 0.001) and at the end of harvest (P = 0.001). Galls scoring were significantly high ($P \leq 0.0001$) in control plots (Fig 2).

Table 1: Population dynamic of the parasitic nematodes of plants in soil at different period of sample (/100g of soil).

Mycorrhizal strains	To the transplanting	One month after transplanting	During harvest	After harvest
Strain of Dapaong	50.33 ± 12.50 **a**	58.33 ± 8.33 **a**	68.33 ± 7.26 **a**	65.00 ± 10.40 **a**
Strain of Kara	47.22 ± 13.46 **a**	58.33 ± 30.04 **a**	75.00 ± 12.88 **a**	85.00 ± 22.88 **a**
Strain of Sokode	46.67 ± 10.20 **a**	58.33 ± 8.33 **a**	55.00 ± 10.40 **a**	60.00 ± 5.00 **ab**
Strain of Kpalime	38.89 ± 11.11 **a**	50.00 ± 14.43 **a**	58.33 ± 8.33 **a**	85.00 ± 2.88 **a**
Strain of Baguida	49.00 ± 13.17 **a**	50.00 ± 14.43 **a**	66.66 ± 8.81 **a**	56.66 ± 10.13 **ab**
Glomus etunicatum	50.00 ± 13.18 **a**	75.00 ± 14.43 **a**	58.33 ± 8.81 **a**	53.33 ± 7.26 **ab**
Glomus clarium	44.44 ± 14.29 **a**	58.33 ± 30.04 **a**	68.33 ± 24.40 **a**	51.66 ± 27.40 **b**
Funneliformis mosseae	30.00 ± 12.80 **a**	50.00 ± 28.86 **a**	56.66 ± 6.66 **a**	33.33 ± 8.81 **b**
Control	42.77 ± 13.46 **a**	78.33 ± 11.66 **a**	103.33 ± 8.81 **b**	116.66 ± 6.66 **c**
P	0.759	0.971	0.010	≤ 0.0001
F	0.620	0.261	3.742	12.146

Means in the same column followed by the same letters are not significantly different (Student-NewmaneKeuls, P < 0.05).

Table 2: Population dynamic of the parasitic nematodes of plants in *S. macrocarpum* root at different period of sample (/100g of root).

Mycorrhizal strains	One month after transplanting	During harvest	After harvest
Strain of Dapaong	150.00 ± 28.86 **a**	233.33 ± 60.09 **a**	248.33 ± 60.10 **a**
Strain of Kara	151.00 ± 50.00 **a**	200.00 ± 28.86 **a**	215.00 ± 28.86 **a**
Strain of Sokode	150.00 ± 28.86 **a**	266.66 ± 44.09 **a**	281.67 ± 43.73 **a**
Strain of Kpalime	183.33 ± 44.09 **a**	333.33 ± 44.09 **a**	348.33 ± 44.10 **a**
Strain of Baguida	150.00 ± 28.87 **a**	300.00 ± 57.73 **a**	320.00 ± 59.46 **a**
Glomus etunicatum	183.33 ± 16.66 **a**	300.00 ± 58.75 **a**	315.00 ± 57.73 **a**
Glomus clarium	216.66 ± 60.09 **a**	300.00 ± 76.37 **a**	317.00 ± 76.37 **a**
Funneliformis mosseae	183.33 ± 16.66 **a**	200.00 ± 50.00 **a**	215.00 ± 50.00 **a**
Control	450.00 ± 28.86 **b**	600.00 ± 28.86 **b**	616.66 ± 30.59 **b**
P	≤ 0.0001	0.001	0.001
F	6.930	5.425	5.410

Means in the same column followed by the same letters are not significantly different (Student-NewmaneKeuls, P < 0.05

Leaves yield evaluation

Yield evaluation was presented in Fig.3. The variance analysis showed significant difference between different treatments yield (P = 0.003). The Student-Newman-Keuls test resulted that the strains of Dapaong, Kara and Baguida were strains that given better yield. However, the yield gains were obtained from every AMF strain plot compared to control yield.

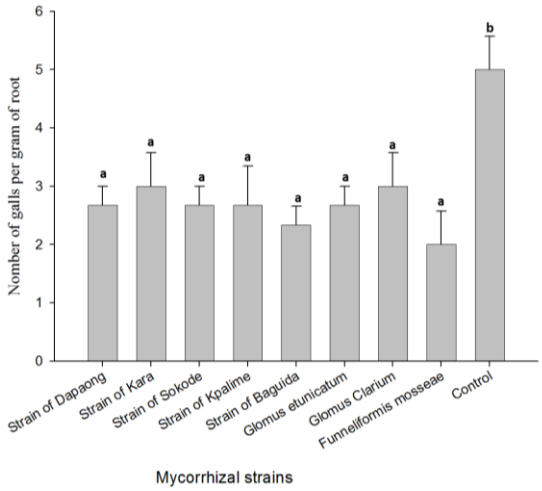

Fig. 2: Galls scoring presented as gall numbers g^{-1} in root samples collected from *S. macrocarpum*. Nonsignificant differences between sites are shown by identical letters, determined using Student Newman and Keuls (SNK) at the 5% level following one-way ANOVA.

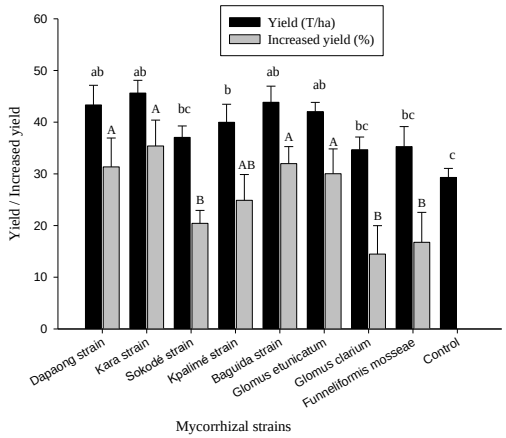

Fig. 3: *S. macrocarpum* leaves yield evaluation. Nonsignificant differences between yields of different treatments are shown by identical letters in lowercase and between yields increasing in uppercase, determined using Student Newman and Keuls (SNK) at the 5% level following one-way ANOVA.

Correlation between different parameters

The correlation matrix (Table 3) showed that the intensity of arbuscular mycorrhizal colonization was positively correlated with spores number (r = 0.878, $P < 0.001$) and negatively correlated with nematodes density in the soil (r = 0.705, $P = 0.01$) and in the roots (r = 0.591, $P = 0.02$) and with galls scoring (r = 0.645, $P = 0.01$). Galls scoring was positively correlated with nematodes density in the soil (r = 0.480, $P = 0.04$) and in the roots (r = 0.603, $P = 0.01$). Leaves yield was negatively correlated with root nematodes density (r = 0.912, $P < 0.001$) and galls scoring (r = 0.776, $P = 0.03$).

Table 3: Pearson coefficients correlation matrix between AMF and different parameters of *S. macrocarpum*

	Soil nematodes	Root nematodes	Galls scoring	Yield	AMF rate colonization	AMF spores density
Soil nematodes	1					
Root nematodes	0.602**	1				
Galls scoring	0.480*	0.603**	1			
Yield	- 0.309ns	- 0.912**	- 0.776**	1		
AMF rate colonization	- 0.705**	- 0.591**	- 0.645**	0.198ns	1	
AMF spores density	- 0.303ns	- 0.307ns	- 0.197ns	0.040ns	0.378ns	1

** Correlation is significant at the 0.01 level; *. Correlation is significant at the 0.05 level; ns. Correlation is not significant.

DISCUSSION

The results of this study show that all CMA strains tested reduce significantly parasitic nematodes density in soil and in the roots of *S. macrocarpum*. Therefore, the positive effect of AMF application on *S. macrocarpum* under nematode pest challenged conditions is resulted on this study. These results agreed many other

studies which have been reviewed the effects of both these organisms on plant growth and their interaction [23,41,42]. A general conclusion from these reviews suggests that AMF increase resistance to nematode infestation by slowing down nematode development. Affokpon and al. [24] established the efficiency of the arbuscular mycorrhizal fungi native to Benin and found that these mycorrhizal fungi were as well very efficient in greenhouse that to the field to reduce the rate of *Meloidogyne* spp in the soil of culture and in the roots of the tomato.

At transplanting, 64 nematodes per 100g soil on average has been found. This presence of nematodes can be explained by the humectation of superior layer of soil by watering dragging their migration toward the superior layer. From the transplanting to the harvest, the number of nematodes increased progressively in soil and in the roots of *S. macrocarpum* for all treatments. The increasing of these nematodes is due to their multiplication and to the migration of those of the depths toward the top soil. The evolution of density of nematodes in the roots according to the roots mycorhization indicates probable correlation between these two factors [24,43]. In other hand, nematodes density evolution indicated that the effect of AMF did not block the multiplication of nematodes but reduced their multiplication rate and the action would not be direct but rather indirect [44]. However, the reduction level of nematodes density as well in soil that in the roots of *S. macrocarpum* is not linear, which can be explained that the effect of AMF inoculation was not constant during the experiment. The lack of effectiveness consistency may be attributed to several factors, including slight variation in experimental set up, but more possibly different feeding styles of nematodes assessed. It was proposed by Johnson et al. [45] that mycorrhizal association could be considered as symbioses, but the functional range along a continuum of parasitism to mutualism according to environmental conditions (climate, temperature, abundance of soil nutrients, presence or absence of pathogens, etc.) and host plants genotype [46].

For galls scoring generally caused by sedentary endo-parasitic nematodes, the results of our study have shown that AMF can suppress galling damage symptoms caused by *Meloidogyne* spp. Many previous studies supported these results [47,48]. Ryan et al. [49] reported that the population of potato cyst nematodes (sedentary nematode) was lower for *Globodera rostochiensis* and *Globodera pallida* on potato plants inoculated with Vaminoc (commercial product with combination of three *Glomus* spp.), compared to non-inoculated plants.

Concerning leaves yield of *S. macrocarpum* in this study, *S. macrocarpum* plants inoculated with AMF species had good leaves yield in compared to non-AMF plants in the presence of nematode pests which can be explained by the higher rate of roots colonization [24,50]. General observation from these results was that, AMF inoculation significantly increased fresh weight of leaves, but that degree of effectiveness depends on AMF species. It was high convincing for strains of Dapaong, Kara and Baguida than others. Similar results were reported by Tchabi [50] where yam plants inoculated with AMF species yielded heavier tubers (microtubers) compared to non-AMF plantlets in the presence or absence of nematode pests (*S. bradys* or *Meloidogyne* spp.). The analysis of our results showed also a clear, significant correlation between mycorrhization and nematodes density as well as in soil and in the root and leaves yield of *S. macrocarpum*. Their negative correlation with nematodes density show the potentiality of these AMF strains to reduce significantly nematodes density. The positive correlation with leaves yield can be explained by not only the lower density of nematodes in the treated plots but also by the important roles of the AMF symbiotic organisms when they influence soil structure, fertility and therefore facilitate the development of vegetation [51].

CONCLUSION

This study showed that the inoculation of theses strains of AMF as alternative to chemical control was potential solution of nematodes management of *S. macrocarpum*. Each strain of AMF used reduced population density of nematodes, severity of galls on roots and increased leaves production.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in this research article.

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