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Bioferlizing and Nematodes Control Potentials of Four Native Isolates of Arbuscular Mycorrhizal Fungi on Sweet Pepper (*Capsicum annuum)* in Togo

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Authors' contributions

This work was carried out in collaboration among all authors. Author TA designed the study, performed the statistical analysis, wrote the final version of the protocol, and wrote the last version of the manuscript. Authors MEB and ON are those who conducted the experiment at the field as bachelor student. They collected data at the field site and some laboratory work. Author KP managed the literature searches and wrote the first draft of the protocol and the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the study was to investigate the effectiveness of four isolates of the native Arbuscular Mycorrhizal Fungus (AMF) in improving pepper plant yield as well as to assess their potential for the control of nematodes.
Study Design: The experimental set up consisted of a Completely Randomized Block Design with 5 treatments corresponding to the four isolates of AMF– BEN10, GM142, 472, and WA330 – tested in comparison with the untreated plants as a control treatment.

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Place and Duration of Study: The study was conducted in 2020 from June to September « at the Research Station of the Faculty of Agronomy, University of Lomé, Togo».
Methodology: The seeds of pepper were inoculated with the four AMF isolates in the nursery. Six weeks after, the seedlings were transplanted on 3mx6m beds. Several growth parameters such as the number of leaves and branches, plant height, number of flower buds and fruit weight were recorded each three weeks after transplantation. Nematode density was assessed four times (before transplanting, at the flowering, during fruiting, and after the last harvest of pepper fruits).
Results: The AMF increased significantly the marketable pepper fruit weight by 39%, and reduced significantly the root nematode density by 20-34%. A positive correlation ($P<0.0001$; $r = 0.816$) was observed between the mycorrhization frequency and mycorrhization intensity, the former was varied from 70% to 91% and the latter from 60% to 85%. The plant height, the number of leaves, the branches and the number of flower buds were not affected by AMF inoculation.
Conclusion: The present study showed the potential of AMF to be considered alternative candidate to chemical fertilizers and pesticides for sustainable production of sweet pepper in Togo.

Keywords: Arbuscular mycorrhizal fungi; mycorrhization; nematodes; reduction; yield.

1. INTRODUCTION

The sweet pepper, *Capsicum annuum*, is a fresh dietary vegetable fruit rich in vitamin C and provitamin A (carotene) [1]. The sweet pepper is an excellent source of many nutrients and important secondary metabolites for human health (potassium, flavonoids, antioxidants), and which are known to reduce the risk of many diseases such as cancer and cardiovascular diseases [2]. This crop is very popular crop that contributes to the income of the smallholder famers, with high international export potential [2].

In Togo, the sweet pepper is grown mainly in the urban and peri-urban areas. Although these production areas increase each year to respond to the increase in the market demand at the national and international levels, the yield remains very low [3]. This low productivity of pepper plants is attributed to several factors among which the soil fertility, coupled with the impact of some biotic components including insect pests, diseases and roots feeders. Among roots feeders, nematodes are the most important pests [3,4,5], the key damaging one being *Meloidogyne* spp. [6,7]. Currently, the management of nematode pests on vegetable crops including sweet pepper is done mainly using the synthetic chemical nematicides [8,1]. However, their highly hazardous nature has led to many of these products being removed from the market and their use discontinued [9]. Other nematode management practices such as botanical [10,11], organic fertilizers [12,13] or cultural control [14] have been explored for vegetables crops, with some success. There is therefore a need to find alternatives to chemicals for the sustainable production.

Recently, many studies established that Arbuscular mycorrhizal (AM) fungi favor plant growth by improving nutrient acquisition [15], but also by increasing their resistance against abiotic and biotic stress, including nematodes [16-18]. Thus, 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 have mutualistic relationships with more than 80% of terrestrial plant species [19]. This symbiotic relationship is ancient and would have had important roles in establishment of plants on land [19]. In this symbiosis, the fungus provides the host plant with mineral nutrients, especially phosphate, receiving in turn carbohydrates [19,20]. In this way, the association with AMF can improve the provision of poorly mobile nutrients, especially phosphorus (P), but also ammonium, copper, zinc and other micronutrients [21]. AMF seems to generate a smaller carbon cost per absorption area unit than roots, and they also allow a higher exploration of soil not accessed by roots [19]. Moreover, other potential benefits of arbuscular mycorrhizal symbiosis have been mentioned, such as improved plant water relations and reduction of pathogenic infections [16], promotion of soil aggregation [21] as well as synergistic effects with other microorganisms [22,23]. We hypothesize that AMF can constitute a viable alternative to the use of chemical fertilizers and pesticides for sustainable sweet pepper production without adverse effects on the environment and human health in Togo. Therefore, the present study was conducted to evaluate the potential benefits of native AMF as biofertilizer and nematodes biocontrol agent on sweet pepper under sustainable field conditions.

2. MATERIALS AND METHODS

2.1 Experimental Site

The field experiment was conducted at the Research Station of the Faculty of Agronomy, 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 with two dry seasons in between. The soil of the experimental site is classified as a ferralsol soil [24] with the following characteristics: organic matter (OM) 1.87%; total N 0.15%; pH 6.50; available Phosphorus (P_2O_5) 0.5 mg/kg; Potassium (K_2O) 0.46 mg/kg and Magnesium (MgO) 0.01 mg/kg [24].

2.2 AMF Inoculum Used

Four pure isolates (Ben 10, 472, WA330, GM142 were isolated from yam belt from different crops and different agroecological zones in Benin [25] and maintained at the “Laboratoire des Sciences Agronomiques et de Biologie Appliquée”, (La.S.A.B.A.-University of Kara).

2.3 Nursery and Inoculation of Seedling

The nursery is done in greenhouse. The substrate used for the nursery consisted the mixture (w/w, 1:2) of the arable soil collected at the agronomy research station and the beach sand. The soil was collected from a depth of 0–25 cm and passed through a 1 mm aperture sieve to remove roots and debris. The beach sand was thoroughly washed with tap water to remove salt. The substrate mixture was sterilized at 80^{o}Cfor 72 h. Substrate pH (H2O) was 7.7, the organic carbon 20 g C kg−1, and the total N and available P (P-Brayl) were 3.4 g N kg−1 and 19 mg P kg−1, respectively, analyzed at the ITRA (Institut Togolais de la Recherche Agronomique).

The four AMF inocula (Ben 10, 472, WA330, GM142 at a dose of 6000 spores) were used to inoculate pepper seeds in plastic tanks? (50×40×20cm) during nursery period in the greenhouse. The tank filled with sterilized substrate was watered and three stripes were made in the length direction of the plastic tank as a seedbed. Thereafter, 50g of corresponding isolates 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, but sterilized substrate used for inocula production. One plastic tank was used for each inoculum making in total, five plastic tanks.

2.4 Experimental Design

The treatments were arranged in a completely randomized block design with five treatments and with four replicates. Completely randomized block design was used because the experimental units were homogeneous and also because this allows every experimental unit (plot) to have an equal probability of receiving a treatment. Each block consisted of 3 m x 6 m plots, separated by one-meter space, while blocks were separated by 2 meters. Six-weeks old plants from the nursery were transplanted at 25 cm x 25 cm in each plot, making a total number of 120 plants per plot. The plots were regularly watered and weeded until harvest.

2.5 Assessment of AMF Root Colonization

Root colonization by AMF was assessed two months after transplanting in the field. Roots were extracted by wet sieving [25]. AMF root colonization was determined according to [26], using trypan blue to stain mycorrhizal structures. A 1.0g subsample of the roots excised from the five plants, to assess the percentage of AMF colonization. At 90^{o}con a hot plate, the root samples were cleared in KOH (100g/l) for 1 h and stained with trypan blue (0.5g/l) in lactoglycerol [26] at 90^{o}c for 30 min. Percentage colonization of sweet pepper roots was estimated by visual observations of stained root segments mounted in lactoglycerol by the grid-line intercept method [27].

2.6 Assessment of Growth and Yield Sweet Pepper

Starting from the third week after transplanting and at a frequency of three weeks, twenty plants per treatment (five per plot unit) were examined and parameters such as plant height (from crown to apex), number of leaves, number of branches were assessed.

Yield estimation: A total of five fruits harvests have been done, the first harvest was done two months after the transplantation of plants, and subsequently at 15-days intervals until the end of harvest, leading to a total of five harvests for a total harvest period that lasted two months. The pepper fruits collected at each harvest time on

each elementary plot were weighted to determine the pepper fresh fruit weight (Kg) per plot per harvest. The fruit weights of the five harvests were then summed up to determine the total weight of harvested pepper fruits per elementary plot. The mean fresh fruit weight (kg/18 m^2) for each treatment was calculated by adding the total weight from the four replicates and divided the result by four. Gain in yield was calculated using the following formula:

$$\mathrm{G}\ (\%) = \frac{\text{Mean yield of specific treatment} - \text{Mean yield of control}}{\text{Mean yield of control}} \times 100).$$

2.7 Sampling, Extraction and Evaluation of the Density of Nematodes

The soil was collected from up to a depth of 15-20 cm from different treatments plots and 1cm from the selected plant to be sampled. In each plot, three soil samples were collected by boring at different locations randomly selected, and mixed according to the method described by [25] to form a representative sample of about 600g per plot. In the laboratory, pepper plant roots contained in each soil sample were removed and used to determine the density of the root nematodes, after crushing the roots with moulinex. Subsequently, nematodes density in the root-free soil was also determined. Extraction of the nematodes from soil/root sample was done using a modified Baermann plate method [28]. For both purpose, 100 g of soil or 5 g of roots were weighed in the laboratory. Roots were previously cleaned and crushed using a moulinex. Each sample is weighed into a sieve lined inside by the toilet paper for filter and the whole is placed in a plastic basin. Each sample is scattered in the screen using tweezers. Then water was added until the sample was lightly covered, thus promoting the migration of nematodes to the water which is the extraction medium.

After 24 hours incubation for the soil samples and 48 hours for the roots, the sieve containing filter paper on which is deposited the sample is gently removed from the basin. The water from the bowl containing the nematodes is collected in graduated tubes and allowed to settle for 30 minutes. Then, the volume of the extract medium was then reduced to 100 ml which was used as the final extract medium for the observation of nematodes. 10ml is removed from the 100 ml extract medium using a pipette in a petri dish and nematodes were counted with a Leica Wild M3C microscope. This operation is repeated three times for each sample.

2.8 Statistical Analysis

Data were subjected to analysis of variance by the Generalized Linear Model (GLM) procedure using SPSS 25 (Statistical Package for the Socials Sciences) version 2018. The GLM was used because the data collected were normal distributed. Data on density and percentages were log- or arsin-transformed, respectively, before being subjected to statistical analysis [29]. In the case of significant differences, means were discriminated using the Student-Newmann-Keuls) multiple range test.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Sweet pepper root colonization by AMF

The presence of AM structures was detected in all the plants at the three stages of assessment. The mycorrhization was observed in all the plants included the control plant (Fig. 1) and the intensity of root mycorrhization was found to increase with the age of the plants. Overall, the AMF colonization frequency was significantly higher (*P* <.0001) for the AMF-treated plants compared to the untreated plants (Fig. 2), with the isolates GM 142 and WA472, recording the highest frequencies. A positive correlation was found between mycorrhization frequency and mycorrhization intensity (P<0001; r = 0.816) (Fig. 3).

3.1.2 Effect of AMF isolates on height, number of branches, number of leaves and number of flower buds

Data on plant height, number of branches, number of leaves and number of flower buds are shown in Fig. 4, Table 1, Table 2 and Table 3, respectively. These results show that the supply of AMF did have any effect on these parameters, although the AMF plants showed a slight increase in vivacity compared to the control in the range of 1.51% to 4.22% in week 9. Only the mean number of flower buds was significantly different in week 11. Plants inoculated with GM142 and 472 had 17.44% and 22.14% respectively more flower buds compared to the control.

3.1.3 Effects of AMF isolates on sweet pepper fruit weight

The fresh fruit weight of sweet pepper is shown in Table 4. The results showed a significant

effect of AMF isolates on pepper fresh fruit weight. The highest weight was obtained with the 472 with an average of 8.73 kg/18 m^2 It increases the fruit fresh pepper by 39.45% compared to that obtained for untreated plants.

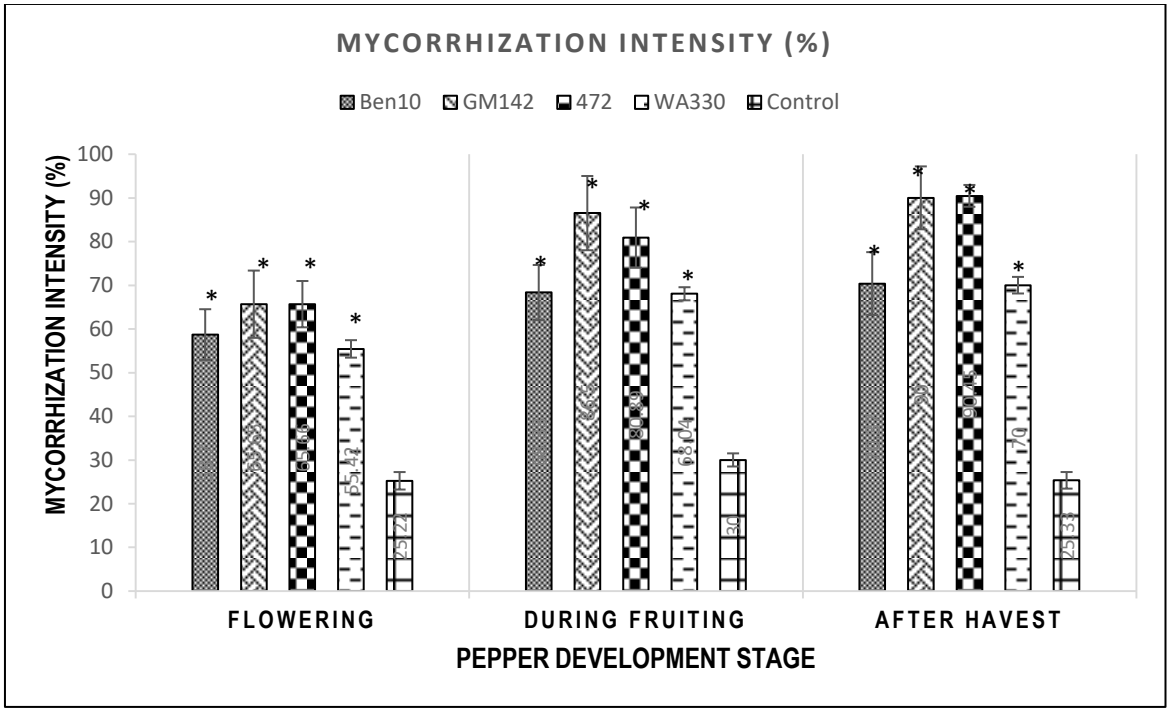

Fig. 1. Mycorrhization intensity in the roots of sweet pepper plants inoculated or not with four AMF isolates (Ben10, GM142, 472 and WA330)

*Test: significant from normal control *P <0.05%. , Average mean ± SE = Standard error*

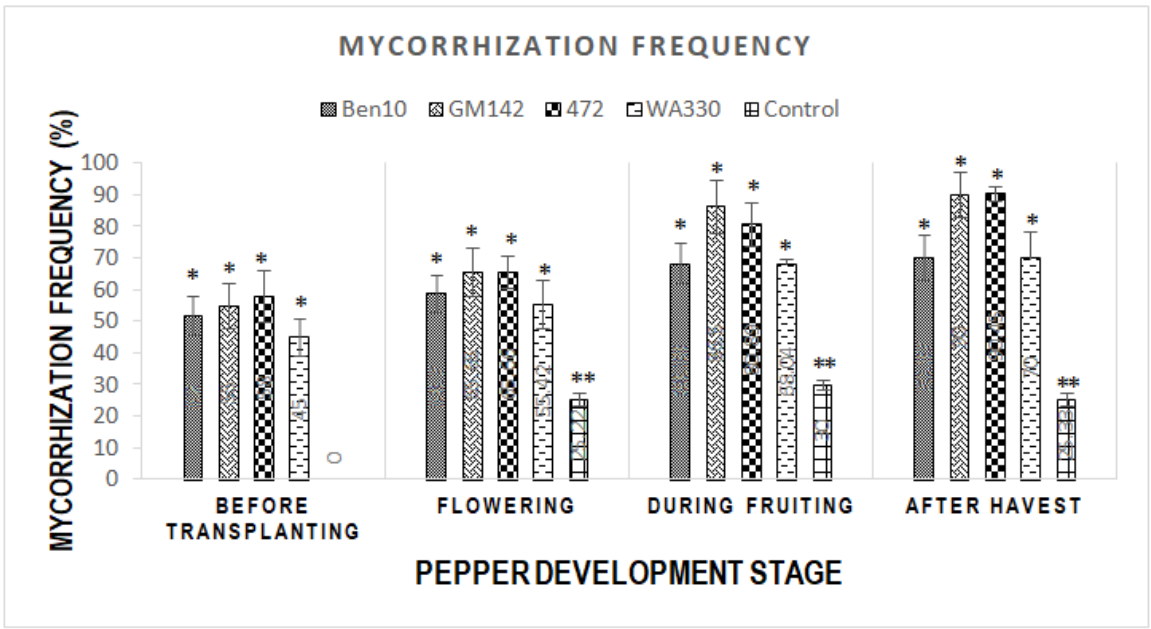

Fig. 2. Mycorrhization frequency in the roots of sweet pepper plants inoculated or not with AMF isolates (Ben10, GM142, 472 and WA330)

*Test: significant from normal control *P <0.05%. , Average mean ± SE = Standard error*

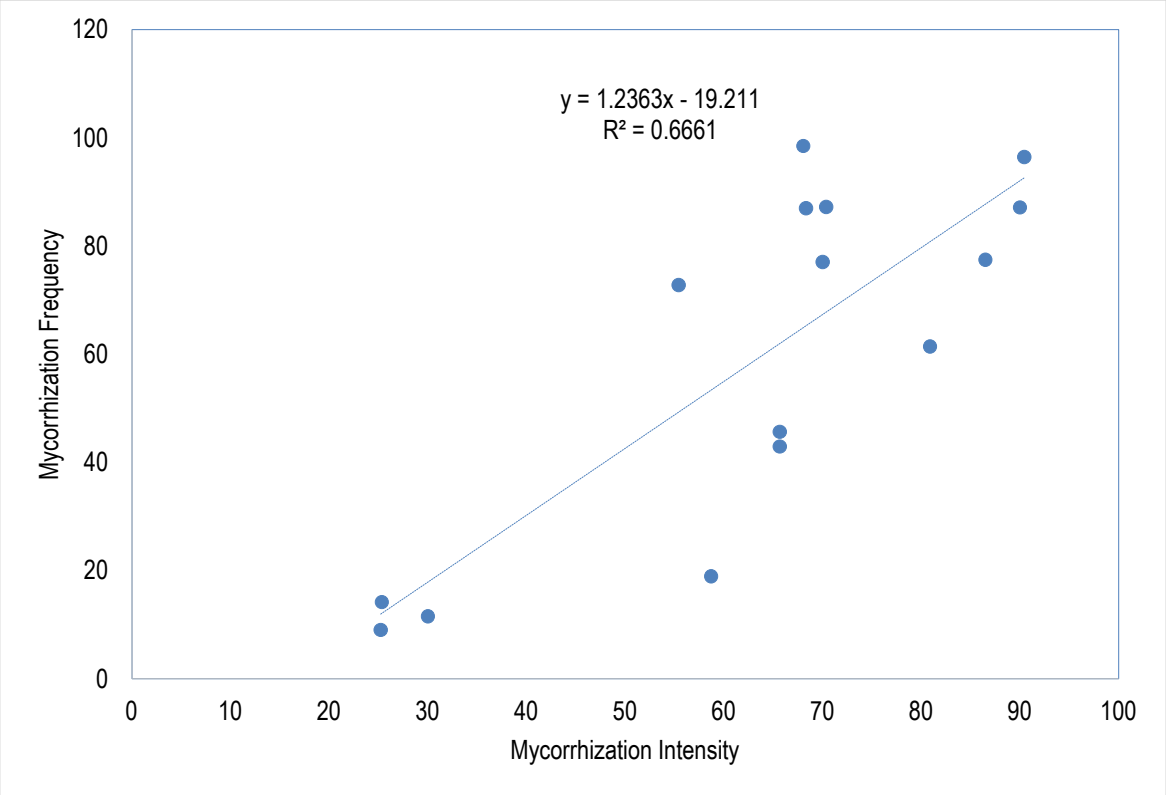

Fig. 3. Relationship between mycorrhization frequency and mycorhization intensity from the root of sweet pepper inoculated with four AMF isolates (Ben10, GM142, 472 and WA330)

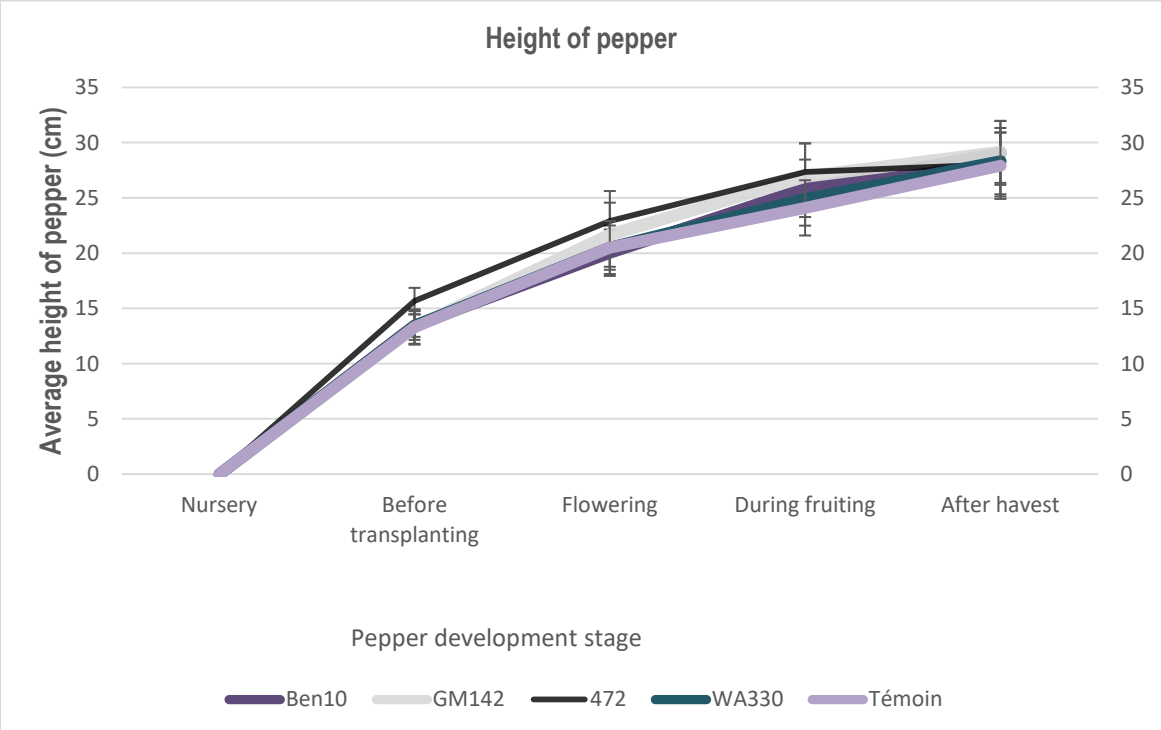

Fig. 4. Effects of four AMF isolates (Ben10, GM142, 472 and WA330) on the height of sweet pepper plant at 4 growing stages

Table 1. Effects of four AMF isolates (Ben10, GM142, 472 and WA330) on the number of branches of sweet pepper plant at four interval times of growing stage

AMF Isolates	Number of branches per plant (mean ± SE)			
	Week 2	Week 5	Week 7	Week 9
BEN10	0.00±0.00a	4.35±0.48a	10.70±0.58a	13.40±0.37a
GM142	0.00±0.00a	6.00±3.92a	12.45±0.98a	14.65±0.76a
472	0.00±0.00a	6.25±1.20a	12.80±1.23a	16.75±0.96a
WA330	0.00±0.00a	4.95±0.70a	11.45±0.76a	13.75±0.49a
Control	0.00±0.00a	5.90±0.63a	12.45±0.65a	13.60±0.53a
cv%		**9.6**	**18.6**	**15.67**
p		**0.42**	**0.41**	**0.52**

NB: Comparisons are made by column. Means in the same column followed by the same lower-case letter are not statistically different (Student-Newman-Keuls, P < 0.05). SE = Standard error.

Table 2. Effect of AMF isolates (Ben10, GM142, 472 and WA330) on the average number of leaves on sweet pepper plant at different times of growing stage

AMF Isolates	Average number of leaves per plant (mean ± SE)			
	Week 2	Week 5	Week 7	Week 9
BEN10	7.20±0.46a	34.15±3.24a	59.09±2.63a	68.45±2.09a
GM142	7.35±0.37a	41.40±5.49a	62.10±4.04a	70.30±3.17a
Strain 472	7.15±0.35a	40.00±3.25a	57.15±2.24a	67.60±2.64a
WA330	7.20±0.31a	43.25±5.11a	59.50±4.07a	68.05±3.46a
Witness	7.40±0.32a	44.65±4.46a	59.15±3.78a	66.35±2.55a
cv%	**15.4**	**11.4**	**14.9**	**15.7**
p	**0.97**	**0.49**	**0.88**	**0.90**

NB: Comparisons are made by column. Means in the same column followed by the same lowercase letter are not statistically different (Student-Newman-Keuls, P < 0.05). SE = Standard error

Table 3. Effect of AMF isolates (Ben10, GM142, 472 and WA330) on the average number of flower buds of sweet pepper at four different times of growing stage

AMF isolates	Average number of flower buds (mean ± SE)			
	Week 5	Week 7	Week 9	Week 11
BEN10	0.95±0.23a	9.05±1.46a	17.70±0.77a	16.90±0.78ab
GM142	0.50±0.50b	8.40±1.33a	17.65±1.02a	17.50±0.52a
472	0.35±0.22b	11.85±1.96a	19.10±0.80a	18.20±0.80a
WA330	0.25±0.12b	8.85±1.51a	16.35±0.66a	15.40±0.49b
control	0.20±0.09b	10.60±1.88a	16.30±0.90a	14.90±0.75b
cv%	14.9	9.75	11.6	14.2
p	0.02	0.55	0.12	0.04

NB: Comparisons are made by column. Means in the same column followed by the same lower-case letter are not statistically different (Student-Newman-Keuls, P < 0.05). SE = Standard error.

Table 4. Effects of four AMF isolates (Ben10, GM142, 472 and WA330) on the sweet pepper fresh fruit weight and average fresh fruit weight gain

AMF isolates	Fruit yield over the whole period in kg/18 m^2 (average ± SE)	
	Yield	Gain (%) in weight
BEN10	6.99±1.48b	11,66b
GM142	6.89±1.51b	10,06b
472	8.73±1.01a	39,45a
WA330	7.12±1.35ab	13,73b
Control	6.26±1.49b	-
cv%	13.2	13.6
p	0.02	0.0 3

NB: Comparisons are made by column. Means of the yield in the same column followed by the same lower-case letter are not statistically different (Student-Newman-Keuls, P < 0.05). SE = Standard error. While gain in pourcentage in the same column followed by the same lower-case letter are not statistically different (Student-Newman-Keuls, P < 0.05)

3.1.4 Effects of AMF isolates on nematode

Before transplanting, the result shows that the soil was heavily infected with nematodes (Table 5). No significant differences between plots are observed (P= 0.09). The same trend was observed between treatments regarding nematode's density in the soil at the flowering, the fruiting and at the harvest (P = 0.08, P = 0,78 and P= 0.09 respectively).

Concerning the nematode density in the roots, a progressive evolution of the number of nematodes in the roots of sweet pepper has been noted from the transplanting to the harvest for all treatments (Table 5). The analysis of the variance shows that the reduction of nematodes density by each of the four AMF isolats used is statistically significant compared to control at the flowering and at the harvest ($P \leq 0,04$). However, comparison within the different isolates of AMF used reveals similar actions.

The correlation analysis (Fig. 5) shows a negative linear relationship between mycorrhization intensity and nematode population density ($P<0.05$; $r = - 0.48$). Indeed, it was found that nematode density decreased with plant age while mycorrhization intensity did not increase.

3.2 Discussion

This study on the effect of four local AMF isolates on growth parameters, fruit yield and nematodes pressure of sweet pepper is one of the first carried out in the vegetable crops in Togo. The use of spores as inoculum to inoculate sweet pepper plants must meet two main criteria; on the one hand, the fungus must be able to intensively colonize the sweet pepper roots and on the other hand, this colonization must promote yield increase [18,20] and the reinforcement of the plants' immune system for protection against nematode pests [30,18,31].

From the experiment, it was found that most of the sweet pepper plants inoculated with the different isolates of AMF showed mycorrhizal structures in their root cortex, even the control plants. Our result suggests sweet pepper to be a mycorrhizal plant that is compatible for a symbiosis relationship with AMF [32]. The mycorrhization of the control plants a few weeks after transplanting could be due to the presence of native mycorrhizal strains in the garden soil [19]. The frequency and intensity of mycorrhization of inoculated plants varied slightly according to the isolates used [33]. In most cases, the beneficial effect of arbuscular mycorrhizae is due to an improvement of the mineral nutrition of the host plant, especially with regard to the elements that are not very mobile, such as P, Zn, Cu, and does not exclude nitrogenous nutrition [34] and also the photosynthesis activities from the plant [34,35]. More the mycorrhizal growth is increased, the more the number and the leaf surface are accentuated [36,37]. In the present study, yet no significant difference was observed during the trial on the growth parameters (height, number of leaves, branching) of the plants, regardless of the treatment. Indeed, [38] reported that the growth response of plants due to AMF infection could be positive, negative or neutral depending on many factors (edaphic, environmental, mycorrhizal and/or plant) [39,40] showed in a similar study that infection of wheat with

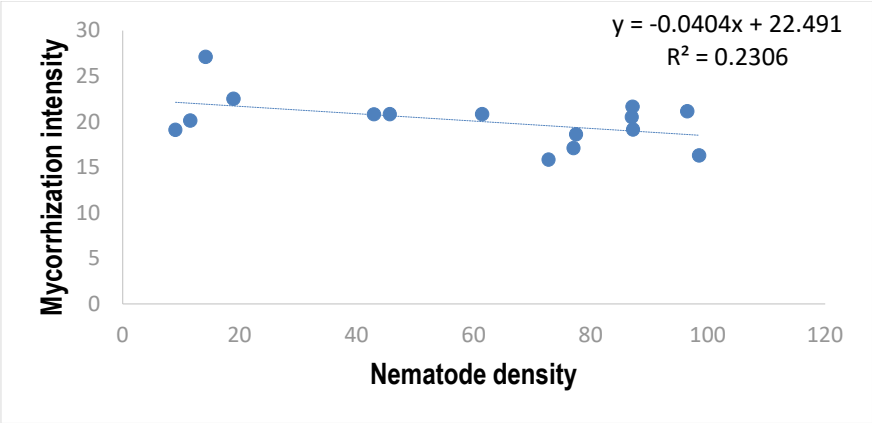

Fig. 5. Relationship between mycorrhization intensity from four AMF isolates (Ben10, GM142, 472 and WA330) and nematode density in the root of sweet pepper

Table 5. Effet of four AMF isolates (Ben10, GM142, 472 and WA330) on average number of nematodes (Mean± SE) extracted from soil and roots of sweet pepper, before transplanting, at flowering, during fruiting and at harvest

Mycorrhizae strains	Density of nematodes in soil and roots (mean ± SE)						
	Before transplanting	Beginning of flowering		During fruiting		After harvest	
	Soil	Soil	Root	Soil	Root	Soil	Root
BEN10	129.00 ±17.43a	161.6 ±15.91a	19.51 ±3.22a	182.53 ±12.11a	20.5 ±3.21a	128.31 ±13.15a	19.13 ±6.83b
GM142	129.10 ±17.11a	183.3 ±20.96a	20.81 ±3.21a	168.34 ±10.51a	18.61 ±3.12a	139.15 ±7.14a	21.65 ±6.62b
472	129.12 ±18.64a	180.8 ±54.60a	20.83 ±3.93a	170.00 ±42.41a	20.82 ±3.91a	108.37 ±12.92a	21.14 ±5.91b
WA330	129.10 ±18.31a	283.3 ±43.66a	15.84 ±2.21b	198.36 ±18.83a	16.3 ±2.21a	165.00 ±2.26a	17.10 ±7.00b
Control	129.1 ±11.10a	272.51 ±42.10a	22.10 ±3.32a	248.31 ±22.72a	20.12 ±3.33a	170.84 ±14.32a	27.11 ±9.11a
cv%	14.9	22.7	17.1	11.7	16.2	21.8	11.2
P	0.09	0.08	0.04	0.78	0.89	0.09	0.04

NB: Comparisons are made by column. Means in the same column followed by the same lower-case letter are not statistically different (Student-Newman-Keuls, P < 0.05). SE = Standard Error

Glomus falciculatus increased its drought resistance and helped the plant to grow, whereas *Glomus mosseae* had no effect. Therefore, it would be appropriate to look for the most suitable isolates in terms of their effectiveness on the growth parameters of sweet pepper. The contribution of AMF isolates seems rather beneficial in improving the fruit yield of sweet pepper. However, the yields obtained in our study (8.73 kg/18 m^2) by isolates 472 are below those obtained in Egypt 14.9 t/ha [41] and in the sub-region. This suggests other yield parameters including intrinsic and extrinsic characteristics (climate, soil, pests, diseases, etc.) of the crop [41,42]. It is also possible to think of combination fertilizer with AMF to increase yields, because, according to [42] the humus factor is of capital importance for demanding crops such as Solanaceae.

The presence of nematodes in soil before transplanting can be explained by the humectation of superior layer of soil by watering dragging their migration toward the superior layer and due to the presence of the grasses which are the natural host of nematodes [43,44].

Concerning the nematodes density in the soil, the significant difference were observed between inoculated plants and control plants at flowering and after harvest. These results are in line with many other studies which have reviewed the effects of AMF on plant growth and their interactions [41,42]. A general conclusion from these reviews suggests that AMF increase resistance to nematode infestation by slowing down nematode development. [43] established that the efficiency of the arbuscular mycorrhizal fungi native to Benin were as well very efficient in greenhouse and in the field to reduce the rate of nematodes (*Meloidogyne* spp) density in the soil and in the roots of the tomato. 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 in the roots of *C. annuum* is not linear, which can be explained by the fact that the effect of AMF inoculation was not constant during the experiment [45,46]. 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 [47,48].

4. CONCLUSION

This study showed AMF as alternatives to chemical fertilizers for sustainable production of sweet pepper in Togo. Each isolate of AMF tested was able to reduce population density of nematodes on roots and promote increase in pepper fruits yield. The identification of indigenos AMF for nematodes control would be an important step towards the quantitative and qualitative improvement of sweet pepper yield in Togo

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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