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Evaluation of the Effectiveness of Arbuscular Mycorhyzian Fungi and Organic Fertilizers on Nematods (Meloidogynespp) Phytoparasites and Agronomic Performance of Chili Pepper (Capsicum Annuum L.) in South-Togo

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Abstract:- In integrated pest management, agricultural practices aimed at improving the nutritional and immune capacities of the plant are important. The aim of the present study conducted at the Station d'ExpérimentationsAgronomiques de Lomé (SEAL/UL) of the Ecole Supérieure d'Agronomie of the University of Lomé is to evaluate the effect of Arbuscular Mycorrhizal Fungi (AMF) (Ben10 and 472) and organic fertilizers (compost based on manure and AgroBio based on palm kernel cake and neem seeds) on plant-parasitic nematodes and on some agronomic performances of pepper. The trial was conducted in a randomized complete block design with two-factor split plots. Each treatment was done in three replications. The experimental unit was a 4 m long by 1.2 m wide (4.8m²) plot. Six (6) weeks after sowing, the chilli plants were transplanted to previously laid out beds following a 50cm×50cm cultural pattern. Carbofuran (Furadan) and aqueous extract of fresh Moringa leaves at the dose of 25% were used as positive controls for the selected nematode treatments.

On average, 80.74% of the inoculated plants were mycorrhized before transplanting. Three (3) months after inoculation, the root cortex of the plants was mycorrhized at a rate ranging from 12.88 to 16.12%. A strong positive correlation was observed between mycorrhization frequency and intensity (r = 0.93; p < 0.0001). Similarly, a positive significant linear relationship (r = 0.89; p < 0.0021) was observed between mycorrhizal parameters and agronomic performance of chili pepper (size, number of leaves, number of branches). The inoculation of AMFs as well as the use of Moringa aqueous extract significantly (62.14%) reduced the pressure of nematodes infested with chili compared to the control without inocula and Moringa aqueous extract. Inoculation of AMFs, spraying of Moringa aqueous extract and amendment of biofertilizers also improved the growth parameters (height, number of leaves & branching) and productivity of chilli (18-34%) compared to control plants.

***Keywords**:- Capsicum annuum, arbuscular mycorrhizal fungi, mycorrhiza, nematodes, pests, agronomic performance.*

I. INTRODUCTION

In order to meet the challenge of covering the food needs of ever-growing populations, improved crop productivity and appropriate pest control is becoming a requirement. Highly appreciated by populations around the world for its gustatory, nutritional and medicinal qualities, the world production of chili pepper is estimated in 2018 at 35 million tons, 48.7% of which is produced by China. In Togo, 43 vegetables and fruits including chili (Capsicum annuum L.) are regularly produced. The area devoted to pepper cultivation is increasing and expanding towards urban and peri-urban areas [1].

Like vegetable production, chilli cultivation is confronted with numerous constraints that lead to a decrease in yields that can reach 40% of agricultural production [2, 3].

Among the phytosanitary constraints, nematode attacks are still very little known by farmers despite their considerable agronomic impact. Indeed, because of their extreme resistance, their great physiological variability, their underground life and the polyphagia of some of their species, they remain very difficult to combat. They represent a serious phytosanitary problem, especially in the tropical world where there is a permanent climate favorable to their multiplication [4]. Faced with these different constraints, producers adopt production practices that are potentially dangerous for the health of the actors in the chilli production and consumption chain and for the environment through the excessive use of nematicides, and other pesticides and synthetic fertilizers. However, nowadays, the search for healthier alternatives for the protection of crops against pests is a major concern. Symbiotic microorganisms and botanical extracts with nematicidal and insecticidal effects are potential alternatives for sustainable control of root-knot nematodes and insect pests [5-7]. Research has demonstrated the existence of arbuscular mycorrhizal fungi (AMF) in the soil, which enter into symbiosis with plants by penetrating their root system to improve the hydromineral supply [8] and strengthen the immune system of the plants [9]. MCAs are therefore a serious candidate for a healthier, sustainable and economically more profitable agriculture. It is in this perspective that the present study was initiated to evaluate the effect of AMFs associated with organic fertilizers in the improvement of agronomic performance of chilli and the control of plant-parasitic nematodes infested with this crop in southern Togo.

II. MATERIALS AND METHODS

A. Experimental site

This study was conducted at the Station d'ExpérimentationsAgronomiques de Lomé (SEAL) of the Ecole Supérieure d'Agronomie of the University of Lomé. SEAL (06°17'N, 001°21'E) has a tropical Guinean climate with a bimodal rainfall regime with average annual rainfall ranging from 800 to 1100 mm. The soil is a well-drained ferrallitic type with a low organic matter content (< 10 g.kg-1) and a slope of less than 1% [10].

B. Material

- **Chilli:** chilli of the variety ICPN3 was used as plant material for this study. Table 1 summarizes the characteristics of the variety used.

Variety	Maintainer	fruit color	Sowing cycle maturity at 95% (days)	Potential fruit yield (t/ha)	Average height (cm)
ICPN3	ITRA	Red	90-120	7-15	35-65

Table 1: Characteristics of the ICPN3 variety pepper

- **Moringa oleifera**: Its leaves have insecticidal and nematicidal properties [7] and are used by spraying the aqueous extract on the leaves and at the foot of the chilli plants on the beds.
- **Inocula used**: Two strains of AMF of the genus Glomus: Ben10 and 472, all imported from the International Institute for Tropical Agriculture (IITA) in Benin. Different quantities of each strain corresponding to 6000 spores (Table 2) were used in this study.

Pure strains of AMF	Amount of stumps	Number of spores
Ben10	50g	6000
472	300g	6000

Table 2: Strains used as inoculum for pepper seedlings

- **Organic fertilizers**
- **AgroBio:** organic fertilizer produced from neem seeds (Azadirachta indica) and palm kernel cake by the company BioPhyto in Benin.
- **Compost:** organic manure made from household waste and animal excrement (mainly dung). The contents in N, P2O5, K2O and organic matter are higher than 1.5%, 2.5%, 0.8% and 40% respectively according to the supplier, the NGO ENPRO (Ecosystème Naturel Propre).

C. Nursery and inoculation of AMF

The pepper nursery is made in a substrate composed of a sterilized mixture of arable soil and sea sand (raw mineral soil of marine input). Ten (10) kilograms of substrate (2/3 topsoil to 1/3 sea sand) were placed in germinators. A total of seven germinators were used and arranged in two batches. The first batch was composed of four germinators each having received different quantities of each strain corresponding to 6000 spores and the second batch (three germinators) did not receive any strain of AMF. Three (3) seedling lines were drawn and a quantity of 6000 spores of inoculum was deposited on the composite substrates of the germoirs of the first batch. The chilli seeds were then sown along the lines. The seeded germinators were placed in a greenhouse. The inoculation of AMFs to the seedlings was thus done at the nursery except for the control germinators. The seedlings were reared for six (6) weeks in the nursery (Figure 1) prior to transplantation to the beds set up in the SEAL compound. Each bed received one vigorous seedling from the nursery.

Fig. 1: Nursery of inoculated and non-inoculated ICPN3 pepper plants

D. Experimental design

The experimental set-up used was a randomized complete block design with split plots and 3 replicates. The design was chosen so that the greatest precision would be sought on the levels of nematicide or nematicide repellent (Ben10, 472, Moringa 25%, Furadan, Control). Thus, the three fertilizer levels (AgroBio, Compost and Control) are placed in the main plots. The experimental unit is a 4.0m long by 1.20m wide (4.8m²) plot. Six (6) weeks after sowing, the pepper plants are transplanted following a 50cm×50cm cropping pattern. The different objects of the trial are presented in Table 3. Fifteen (15) treatments were tested. Figure 2 shows the layout of the experimental units and the distribution of the different treatments in the field.

Factor 1 (Fertilizer)	**Factor 2 (Nematicide)**	**Designation**
Composting (C)	**Ben10 (B)**	Pepper inoculated with Ben10 and transplanted on a plot amended with compost (CB)
	472 (4)	Pepper inoculated with 472 and transplanted on a plot amended with compost (C4)
	Moringa 25% (M)	Pepper not inoculated and transplanted on a plot amended with compost. The plant is treated at a frequency of 2 weeks with an aqueous extract of Moringa leaves at 25% (CM)
	Furadan (F)	Pepper not inoculated and transplanted on a plot amended with compost. The plant is treated every 2 weeks with Furadan 3 GR at a dose of 4 g/plant (CF)
	Witness (T)	Pepper not inoculated and transplanted on a plot amended with compost (CT)
AgroBio (A)	**Ben10 (B)**	Pepper inoculated with Ben10 and transplanted on an amended plot at AgroBio (AB)
	472 (4)	Pepper inoculated with 472 and transplanted on a plot amended with AgroBio (A4)
	Moringa 25% (M)	Pepper not inoculated and transplanted on an amended plot at AgroBio. The plant is treated at a frequency of 2 weeks with an aqueous extract of Moringa leaves at 25% (CM)
	Furadan (F)	Pepper not inoculated and transplanted on an amended plot at AgroBio. The plant is treated every 2 weeks with Furadan 3 GR at a dose of 4 g/plant (AF)
	Witness (T)	Pepper not inoculated and transplanted on an amended plot at AgroBio (AT)
Witness (T)	**Ben10 (B)**	Pepper inoculated with Ben10 and transplanted on a plot without any amendment (TB)
	472 (4)	Pepper inoculated with 472 and transplanted on a plot without any amendment (T4)
	Moringa 25% (M)	Pepper not inoculated, transplanted on a plot without any amendment. The plant is treated at a frequency of 2 weeks with an aqueous extract of Moringa leaves at 25% (TM)
	Furadan (F)	Pepper not inoculated, transplanted on a plot without any amendment. The plant is treated every 2 weeks with Furadan 3 GR at a dose of 4 g/plant (TF)
	Witness (T)	Pepper not inoculated and transplanted on a plot without any amendment (TT)

Table 3: Purpose of the trial

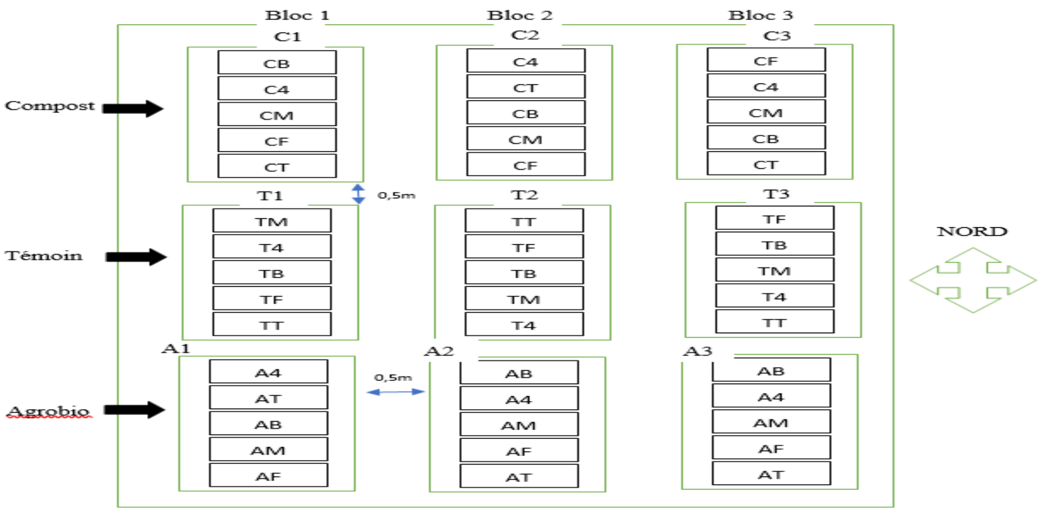

Fig. 3: Diagram of the experimental device

E. Extraction of nematodes from roots

Nematode density was assessed in the roots of chili plants following the modified method of Bearmann Pan and adapted by Coyne [11]. The method involves suspending chili roots in water. Three root samples were collected from each elemental plot. The respective samples were placed in plastic bags, tied, labeled and transported to the laboratory for nematode extraction. These samples were taken at periods corresponding to transplanting, at the beginning of flowering, during fruiting and after harvesting of chilli fruits. In the laboratory, ten (10) grams of root were suspended in 100 ml of water. After 48 hours, 10 ml of solution were collected three (3) times and subjected to nematode counting using binocular magnifying glasses (×10 magnification). The average number of nematodes thus obtained is the average of the 3 repetitions. This number is extrapolated on 100 mlnematode counting using binocular magnifying glasses (×10 magnification). The average number of nematodes thus obtained is the average of the 3 repetitions. This number is extrapolated on 100 ml.

F. Fixation and identification of nematodes

The fixation is done to have an idea on the most abundant genus in the market garden soil. It consisted in adding the suspension of nematodes to the moderately heated fixing solution in equal volume. This fixing solution is composed of: triethanolamine 2%, formalin, (35% formaldehyde), 8% and 90% distilled water [11]. The fixation solution remains stable for a very long time and the nematodes keep a close to living appearance, as they do not dry out. The identification of the genus Meloidogyne of nematodes was made from the FAO identification keys.

G. Evaluation of the level of colonization of the root cortex of chilli by AMFs

The density of AMF colonization of the root cortex of chili plants was assessed on 30 fine root fragments collected from 45 sampled plants (3 plants per treatment). Root fragments were treated in a series of reagents (KOH 10%, HCl 1% and Trypan Blue 0.05%). Mycorrhizal parameters were assessed on the basis of the proportion of root cortex colonized by arbuscules, vesicles, hyphae and appressoriums. The below rating scale proposed by [12] was followed for the evaluation. The following criteria were followed: 0 = no infection; 1= trace; 2 = less than 10%; 3 = from 10 to 50%; 4 = from 51 to 90%; 5 = more than 90%.

The frequency and intensity of mycorrhization and the shrub content of the mycorrhized portion were determined according to the following formulas:

F%= (n/N) x 100 where N= number of fragments observed and n= number of mycorrhized fragments (F% = Frequency of mycorrhization),

M% = (95n5 + 70n4 + 30n3 + 5n2 + n1) / N where n1, n2, n3, n4, n5 are the number of mycorrhized fragments noted from 1 to 5 respectively (M%= Mycorrhization intensity).

A% = (100mA3+50mA2+10mA1) / 100 where mA3, mA2, mA1 are the % of m respectively affected by the notes A3, A2, A1. With mA3= ((95n5A3+70n4A3+30n3A3+5n2A3+n1A3)/number of mycorrhizal fragments), similarly for A2 and A1.

H. Production of the aqueous extract of fresh Moringa leaves

Fresh Moringa oleifera leaves were harvested from the SEAL compound and processed as follows: Weighing the leaves with an electronic balance, crushing 1250 g of leaves in 3750 ml of distilled water in a moulinex to obtain the 25% dose, draining the crushed Moringa paste using a sieve.

Thus 5000 ml of aqueous solution of Moringa are sprayed at a frequency of two (2) weeks from the 1st week after transplanting at the foot of the pepper and on these leaves. Furadan (Carbofuran) on the other hand was applied in granulated formulation (3 Gr) at a frequency of 2 weeks starting from the 1st week after transplanting at the foot of the plants.

I. Statistical analysis

The effects of the different treatments on mycorrhizal parameters, agronomic performance and nematode density were analyzed using R software version 4.1.3. The arithmetic means of the different treatments were

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discriminated and compared using Duncan's test at the 5% threshold when a significant difference was found in the analysis of variance. Percentage data were transformed using a circular function arcsin(√(X/100)) before analysis. Nematode density and nematode root attack scores, for reasons of reducing subjective judgmental variance, were transformed according to the logarithmic function log(X+1) (X being the number of nematodes or the score index). The software R version 4.1.3 also helped to draw a Pearson correlation circle in order to show, eventually, the possibilities of linear adjustments between mycorrhizal parameters, agronomic performance and nematode density, by a principal component analysis.

III. RESULTS

A. *Effect of AMF inoculation on mycorrhization frequency*

The presence of mycorrhizal structures was observed 06 weeks after inoculation of chili plants with the pure strains of AMF (Ben10 and 472). The average frequency of mycorrhization was statistically identical, regardless of the type of fertilizer used, throughout the trial period. It ranged from 30.88 to 37.57% from transplanting to harvest. Mycorrhized plants showed high frequencies that remained more or less constant from transplanting to plant senescence (Table 4). Non-mycorrhized seedlings showed the presence of mycorrhizal structures in their root cortex as soon as they flowered. Duncan's test indicated that the average mycorrhization frequencies of inoculated seedlings were significantly identical to each other (80.73 to 88.47%) and significantly different from the other treatments (0.00% to 4.99%) throughout the trial period.

Treatments	Transplanting	Flowering	Fruiting	Harvest
Ben10	80,74 a	80,73 a	83,33 a	88,47 a
472	81,85 a	80,73 a	83,32 a	88,51 a
Moringa25%	0,00 b	4,07 b	2,59 b	2,22 b
Furadan	0,00 b	4,44 b	1,48 b	1,85 b
Control	0,00 b	0,00 b	4,99 b	4,58 b
p	$6,76.10^{-12}$	$1,09.10^{-15}$	$1,83.10^{-15}$	2.10^{-16}
CV	50,36	30.96	31,23	24,07

Table 4: Effect of treatments on mycorrhization frequency

NB: Means in the same column followed by the same lower case letter are not statistically different (Duncan, $p < 0.05$).

B. *Effect of AMF inoculation on mycorrhization intensity*

Mycorrhizal dependence represents the impact of the establishment of mycorrhizal symbiosis on growth parameters, resistance or tolerance to attack and productivity compared to that of the artificially non-mycorrhized plant. In this study the investigations focused on the level of colonization of the root cortex of chilli by mycorrhizal structures. Plants inoculated with pure strains of AMF (Ben10 and 472) had low intensities at transplanting (2.14 and 2.27%) which increased progressively with plant age to reach 12.88% at the beginning of flowering and 16.12% during fruiting. These intensities were significantly the same between inoculated plants (Table 5) and significantly different from controls. Duncan's test revealed that mycorrhization intensity at nematicides increased independently of the type of fertilizer used throughout the trial period.

Treatments	Transplanting	Flowering	Fruiting	Harvest
Ben10	2,14 a	12,86 a	16,12 a	18,47 a
472	2,27 a	12,66 a	15,00 a	17,47 a
Moringa25%	0,00 b	0,27 b	0,37 b	0,44 b
Furadan	0,00 b	0,33 b	0,53 b	0,78 b
Control	0,00 b	0,72 b	1,03 b	0,83 b
p	$6,76.10^{-12}$	$1,09.10^{-15}$	$1,83.10^{-15}$	2.10^{-16}
CV	50,36	30.96	31,23	24,07

Table 5: Effect of treatments on mycorrhization intensity

NB: The means of the same column followed by the same lowercase letter are not statistically different (Duncan, $p < 0.05$)

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- **Arbuscular content**

  The assessment although subjective of the quality of the arbuscule of the root cortex of chilli is very important in the mycorrhizal dependence on growth parameters, resistance or tolerance to pest attacks and productivity compared to that of the non-mycorrhizal plant. Shrub quality was related to the artificial inoculation of a pure strain of AMF to the plant from the nursery (Table 6). Duncan's test at the 5% threshold shows that plants inoculated with Ben10 and 472 are significantly identical in terms of shrub quality throughout the trial period. These two (2) treatments are significantly different from the others.

Treatments	Transplanting	Flowering	Fruiting	Harvest
Ben10	7,09 a	20,46 a	43,14 a	48,93 a
472	7,28 a	20,19 a	41,09a	50,21 a
Moringa25%	0,00 b	5,93 b	6,16 b	8,14 b
Furadan	0,00 b	4,43 b	4,21 b	6,64 b
Control	0,00 b	8,49 b	11,43 b	12,69 b
p	2.10^{-16}	$7{,}19.10^{-07}$	$4{,}14.10^{-10}$	$2{,}52.10^{-11}$
CV	28,42	45,64	41,85	35,14

Table 6: Effect of treatments on root cortex shrub content

NB: Means in the same column followed by the same lower case letter are not statistically different (Duncan, $p < 0.05$)

C. Effect of treatments on mean chili plant height

Data on average plant height and average number of leaves per plant per treatment are recorded in Tables 7, 8, 9, and 10. Under the trial conditions, the amendment had a beneficial effect on plant height. In fact, the plants from the plots amended with either compost or AgroBio and having been mycorrhized were taller and had more leaves than the control plants without fungal inocula and without fertilizer, from week 3 after transplanting until the senescence of the plants. Analysis of variance revealed a significant effect ($p < 0.01$) between organic fertilizer levels. Plants inoculated with Ben10, 472 and Moringa 25% were statistically identical to each other (Table 8). These three treatments showed a significant difference from the non-nematicide control and Furadan over the entire trial period. The effects of both factors are significant on the size of chilli plants from week 6 onwards.

Treatments	3th Week AR	6thWeek AR	9eSemaine AR	12eSemaine AR
AgroBio	17,06 a	29,46 a	44,33 a	46,66 b
Compost	16,20 b	31,86 a	45,60 a	49,06 a
Control	11,40 c	21,06 b	39,40 b	41,40 c
p	$3{,}06.10^{-05}$	0,0009	0,0015	0,001
CV	12,25	9,94	4,20	4.93

Table 7: Effect of organic fertilizer on the average height (cm) of chilli

NB: Means in the same column followed by the same lower case letter are not statistically different (Duncan, $p < 0.05$); AR: After transplanting

Treatments	3thWeek AR	6thWeek AR	9thWeek AR	12th Week AR
Ben10	16,00 a	31,00 a	45,22 a	47,66 a
472	16,33 a	31,00 a	45,33 a	48,11 a
Moringa25%	15,55 a	28,66 a	45,00 a	47,55 a
Furadan	13,22 b	23,66 b	40,55 b	43,66 b
Témoin	13,33 b	23,00 b	39,44 b	41,55 b
p	0.001	$2{,}92.10^{-08}$	$8{,}87.10^{-09}$	$5{,}23.10^{-06}$
CV	12,25	9,94	4,20	4,93

Table 8: Effect of treatments on the average height in centimeters (cm) of chili pepper

NB: Means in the same column followed by the same lower case letter are not statistically different (Duncan, $p < 0.05$); AR: After transplanting

D. Effect of treatments on the average number of leaves per plant

Under the conditions of the trial, the application of manure is beneficial to the plants in terms of their number of leaves. Indeed, the plants transplanted to the amended plots had more leaves than the control during the whole trial period. Similarly, inoculation with AMFs and application of the botanical extract resulted in plants bearing more leaves than their control and those treated with Furadan. Analysis of variance of the trial results revealed that the effect of the treatments was significant. Application of Duncan's test ($p < 0.001$) shows that under the trial conditions and from week 3 to week 9 after transplanting, the mean number of leaves is statistically different (Compost>AgroBio>Control) (Table 9). Plants inoculated with Ben10 and 472 and then plants treated with 25% Moringa are statistically identical. These three treatments showed a significant difference from the control plants without nematicide and Furadan (Control and Furadan were statistically identical) (Table 10).

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Treatments	3th Week AR	9thWeek AR
AgroBio	18,13 b	45,93 b
Compost	21,66 a	47,26 a
Control	12,13 c	39,66 c
p	0,001	$2{,}00.10^{-05}$
CV	15,38	1,68

Table 9: Effect of organic fertilizer on the average number of leaves of chilli

NB: Means in the same column followed by the same lower case letter are not statistically different (Duncan, $p < 0.05$); AR: After transplanting

Treatments	3th Week AR	9thWeek AR
AgroBio	18,13 b	45,93 b
Compost	21,66 a	47,26 a
Control	12,13 c	39,66 c
p	0,001	$2{,}00.10^{-05}$
CV	15,38	1,68

Table 10: Effect of treatments on the average number of pepper leaves

NB: Means in the same column followed by the same lower case letter are not statistically different (Duncan, $p < 0.05$); AR: After transplanting

E. Effect of treatments on average chili fruit yield.

Under the trial conditions, the application of compost and AgroBio as a background fertilizer had a significant effect ($p< 0.05$) on chili fruit yield (Table 11). Similarly, inoculation of AMFs and application of botanical extracts brought a significant gain in yield of 33.89%, 32.55% and 31.20% respectively for 472, Moringa 25% and Ben10 compared to the control (Duncan, $p < 0.05$) (Table 12).

Treatments	Yield (t/ha)	Yield gain over control (%)
AgroBio	3,78 a	18,40
Compost	4,04 a	26,64
Control	3,19 b	-
p	0,024	
CV	14,19	

Table 11: Effect of organic fertilizer on average fruit yield of chilli

Note: Means in the same column followed by the same lower case letter are not statistically different (Duncan, $p < 0.05$)

Treatments	Yield (t/ha)	Yield gain over control (%)
Ben10	3,91 a	31,20
472	3,99 a	33,89
Moringa25%	3,95 ab	32,55
Furadan	3,51 b	17,78
Control	2,98 c	-
p	0.0002	
CV	12,07	

Table 12: Effect of treatments on average chilli fruit yield

NB: Means in the same column followed by the same lower case letter are not statistically different (Duncan, $p < 0.05$)

F. Effect of treatments on nematode density in chilli roots

Under the conditions of the trial, the application of fertilizer had a significant effect ($p< 0.05$) on the density of nematodes found in the roots of chili pepper. During fruiting to harvest, the average number of nematodes found in the roots of transplants in the AgroBio-amended plots was significantly different from that of the Compost and Control at fruiting and harvest (Compost and Control were statistically identical) (Table 13). In addition, all levels of the nematicide factor (Ben10, 472, Moringa 25% and Furadan) are significantly identical to each other and significantly different from the Control without any nematicide (Table 14).

Treatments	Flowering	Fruiting	Harvest
AgroBio	1,00 b	1,86 b	1,13 b
Compost	1,20 b	2,66 a	3,80 a
Control	2,46 a	2,53 a	2,06 a
p	0,01	0,02	0,15
CV	49,33	21,46	64,40

Table 13: Effect of organic fertilizer on nematode density in pepper roots

NB: Means in the same column followed by the same lower case letter are not statistically different (Duncan, $p < 0.05$)

Traitements	Floraison	Fructification	Récolte
Ben10	1,22 b	1,77 b	2,55 b
472	1,11 b	1,66 b	2,44 b
Moringa25%	1,00 b	1,88 b	2,44 b
Furadan	1,33 b	1,77 b	2,33 b
Control	3,11 a	4,66 a	5,22 a
p	$2{,}23.10^{-06}$	$4{,}55.10^{-05}$	$3{,}58.10^{-05}$
CV	43,12	50,82	37,76

Table 14: Effect of treatments on nematode density in chilli roots

NB: Means in the same column followed by the same lower case letter are not statistically different (Duncan, $p < 0.05$)

G. Effect of the interaction of the different treatments on the dependent variables retained in the experiment

Axes 1 (F1) and 2 (F2) show, respectively 39.18% and 23.41% affinity between the variables retained in this experiment. Indeed, the agronomic performance of the pepper and the pressure (density and attack) of nematodes are well represented in the correlation circle and are close to each other on both sides of axis 1 (F1). Mycorrhizal parameters, on the other hand, are very close to the positive coordinate axis 2 (F2) (Figure 4).

Principal component analysis (PCA) of the variables selected in this experiment reveals a strong positive linear relationship between the frequency and intensity of mycorrhization from the beginning of flowering to intensive fruiting. All agronomic performances of chilli are also strongly positively correlated from flowering to fruiting. The correlation circle shows a positive linear relationship between mycorrhizal parameters and overall agronomic performance (As the frequency or intensity of mycorrhization increases, the pepper performs better and produces more fruit under the trial conditions). On the other hand, Figure 4 reveals the existence of a negative linear relationship between mycorrhizal parameters and nematode density counted during the experiment. The PCA also showed that as mycorrhizal parameters increased, the density and symptoms of root-knot nematode attacks decreased on the roots from week 6 to week 12 after transplanting.

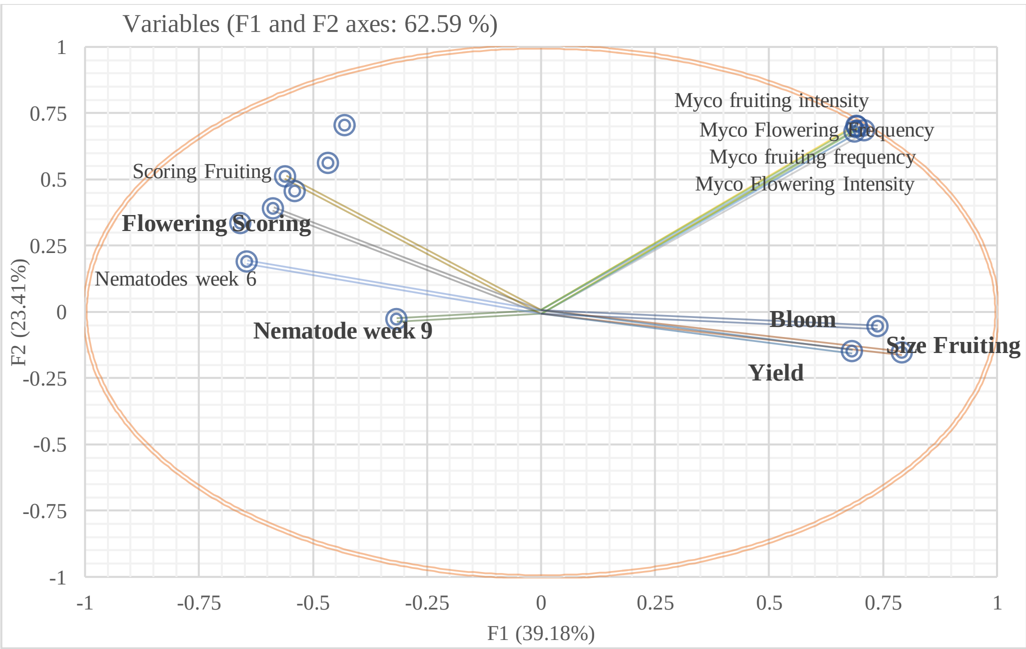

Fig. 4: Relationship between mycorrhizal parameters, root-knot nematode density and agronomic performance of chilli.

IV. DISCUSSION

The present study on the effect of inoculation of AMF strains (Ben 10 and 472), use of Moringa oleifera extract and organic manure amendment (Compost and AgroBio) on agronomic performance as well as on root-knot nematode pressure, is part of a long-term program of introduction of AMFs in sustainable vegetable production in Togo.

The use of AMF spores as inoculum to chilli plants induces both root colonization and increased chilli yields as reported [8, 13]. The different treatments used also resulted in the strengthening of the immune system of the plants thus giving the plants the ability to effectively control nematodes as reported by [8, 14-16] in their respective works. The use of aqueous extract of fresh leaves of Moringa not only favored the reduction of the pressure of pests infested to the pepper, but also stimulated the growth of the latter and finally, the use of organic manure favored the permanent availability of mineral elements for the plant as pointed out by [3, 17, 18].

From the results of this study, it is evident that chili is a highly compatible plant with the selected AMFs; even the control plants were naturally mycorrhized by native AMF strains in the soil a few weeks after transplanting. The frequency and intensity of mycorrhization of the inoculated plants varied significantly depending on the strain of AMF used, reinforcing the findings of [19] who stated that AMFs show species preferences and exhibit ecological specificity of symbiosis with the host plant.

The results showed that at the root level, there is a significant difference related to the different treatments selected (strains of AMF and aqueous extracts of Moringa) on the density of nematodes present there. In fact, the effectiveness of AMF inoculation on nematodes in roots is linked to a competitiveness for infestation sites whose occupation first by AMFs would prevent nematode penetration according to the conclusions of [20]. The use of Furadan and Moringa aqueous extract also significantly reduced nematode density in chili roots. The efficacy of Furadan is related to carbofuran, confirming the results [21] on tomato where this active ingredient reduced root gall formation. The 25% dose of aqueous extract of Moringa is effective in reducing nematode density in roots due to the nematicidal properties contained in its fresh leaves as found [7].

This study also showed, in most cases, the beneficial effect of mycorrhizae following the improvement of mineral nutrition of the host plant through the efficient mobilization of the not very mobile elements (Phosphorus, Zinc, Copper) and nitrogen by the arborescences of the AMFs as reported [16, 22]. The experimental results also showed that the contribution of AMFs is beneficial in improving the fruit yield of chilli. Ben10 and 472 strains optimized the yield compared to the control. Similar results were obtained from the work of [23] on rice and [6] on yam, which also used AMF strains of the same genus as the one used in this study.

Plots amended with Compost or AgroBio showed better growth of the seedlings transplanted to them than the controls without fertilizer. Indeed, both Compost and AgroBio are organic fertilizers and therefore, growth stimulators par excellence according to [24]. The beneficial role of organic matter on plant health and growth has also been documented by [25]. The plants treated with the aqueous extract of Moringa 25% also had a good growth as well as the mycorrhized plants. Indeed, according to [26], Moringa oleifera has a growth hormone, zeatin, which would have a growth stimulating effect.

V. CONCLUSION

This study was carried out at the Station d'Expérimentations Agronomiques de Lomé and focused on the evaluation of the efficacy of inoculation of AMF strains (Ben10 and 472) and the use of an aqueous extract of fresh leaves of Moringa oleifera in the improvement of agronomic performance of chili pepper and in the management of nematode pressure.

The study provided information on the mycorrhizal status of chilli with the two selected strains of AMF (Ben10 and 472) and on the proven efficacy of this mycorrhizal inoculation in association with organic fertilizers and an aqueous extract of fresh Moringa oleifera leaves, in improving the agronomic performance of chilli as well as in the management of nematode pressure infested with this plant.

It would be advisable to test the strains selected in this study on the vegetable crops most commonly used in local culinary principles. In addition, popularization of trapping and propagation techniques for indigenous strains of AMF would be a clear step towards the adoption of these ecological and biological practices in the agricultural practices of vegetable producers in Togo.

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