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Original article

Increased nutrient supply to plants in vermicompost amended gley soil

Andy Bernal-Fundora[1*]

Juan A. Cabrera-Rodríguez[1]

Pedro J. González-Cañizares[1]

Alberto Hernández-Jiménez[1]

[1]Instituto Nacional de Ciencias Agrícolas (INCA), carretera San José-Tapaste, km 3½, Gaveta Postal 1, San José de las Lajas, Mayabeque, Cuba. CP 32 700

[*]Author for correspondence: andy@inca.edu.cu

ABSTRACT

The need to seek alternatives to improve crop nutrition given the low fertility of agricultural soils and the shortage of fertilizers is becoming increasingly important. One of these alternatives is the application of organic fertilizers and inoculation with arbuscular mycorrhizal fungi (AMF). The objective of the present work was to investigate the effect of vermicompost applications and the inoculation of a mycorrhizal biofertilizer on the nutrient supply of a Gley Nodular Ferruginous soil for pearl millet plants (*Panicum italicum* L.). Two experiments were carried out under mesocosmic conditions at the National Institute of Agricultural Sciences (INCA), four proportions of soil-vermicompost were studied, with and without mycorrhizal inoculation, in a completely randomized design with factorial structure and three repetitions. The height, the dry mass of the aerial biomass and the concentration and quantity of nutrients in plants, the frequency and intensity of colonization and the number of spores in the soil were evaluated. The application of vermicompost increased the availability of soil nutrients and it was reflected in the increase of the concentration and quantity of nutrients in the plants, which led to greater growth and development of these; in the presence of the pearl millet, the vermicompost application decreased the frequency and intensity of mycorrhization, which inhibited.

Key words: vermicompost, arbuscular mycorrhizae, *Glomus*, gleysols

INTRODUCTION

In agricultural systems, when exploitation techniques are put into practice where measures are not taken for the conservation and improvement of soils, fertility indicators are gradually affected [1,2], which make it impossible to obtain high yields of the crops. That is why it is necessary to introduce technologies to improve their productivity, by increasing the availability of nutrients for plants, which is of great importance in soils destined for livestock, where the levels of fertilizers that are destined for this branch are not sufficient to meet the nutrient demand of crops and nutrient recycling is poor.

Among the technologies for the recovery and conservation of soils is the application of organic fertilizers. It has have positive effects on the increase in organic carbon content (C), incorporation of mineral elements and intervene in the structure formation [3], such is the case of vermicompost, an organic compound that offers various qualities as a soil improver and acts as a source of nutrients for plants [4].

Other alternatives for a more efficient use of nutrients by plants is the use of biofertilizers based on arbuscular mycorrhizal fungi (AMF), whose microorganisms form symbiosis with approximately 90 % of terrestrial plants [5], facilitating the absorption of nutrients and water by them, among other advantages [6]. The pearl millet is not exempt from this interaction, because it is a culture that shows marked mycorrhizal dependence [7] and, on occasions, high mycorrhizal colonization, even when inoculated with different AMF species [8].

Previous studies have shown how the use of efficient AMF species allows reducing doses of mineral fertilizers or organic fertilizers, without affecting the agricultural yields of crops [9–11].

In an investigation carried out in a Gley Nodular Ferruginous soil, which had pasture cultivation as a precedent, it was shown that the combined use of cattle manure, as a source of organic fertilizer and mycorrhizal biofertilizer, contributed to soil fertility improvement, by increased productivity and nutritional value of forage grass species [12].

The present work aimed to investigate the effect of vermicompost applications and the inoculation of a mycorrhizal biofertilizer on the nutrient supply of a Gley Nodular Ferruginous soil for pearl millet plants (*Panicum italicum* L.).

MATERIALS AND METHODS

Two experiments were carried out under mesocosmic conditions in the greenhouse area of the Biofertilizers and Plant Nutrition department, of the National Institute of Agricultural Sciences (INCA), San José de las Lajas, Mayabeque, between months of March to May, during the years 2014 and 2015.

The soil classified as Gley Nodular Ferruginous Agrogenic [13], from the area of the Municipal Directorate of Flora and Fauna from Boyeros municipality, occupies 38.28 % of the total surface, whose extension is 897.22 ha and at the time of starting the research, it had been under exploitation for more than 12 years, with pastures and forages for livestock, planning to change its use by promoting the cultivation of moringa (*Moringa oleifera*); previously he was dedicated to the cultivation of sugar cane.

The soil was characterized from the physical and chemical point of view [13] and it was shown that it had a deficient supply of nutrients for plants, highlighting the low phosphoric and potassium availability and the low content of organic matter (Table 1).

Table 1. Characterization of the cultivable horizon of the Gley Nodular Ferruginous Agrogenic soil of Municipal Directorate of Flora and Fauna from Boyeros municipality

Parameters	**pH**	**P_2O_5**	**Ca^{2+}**	**Mg^{2+}**	**Na^+**	**K^+**	**CIB**	**C**
	-log [H^+]	**mg 100 g^{-1}**	**---------------cmol$_c$kg^{-1}---------------**					**g kg^{-1}**
Mean	6.9	1.83	22.5	16	0.17	0.21	38.87	12.3
CV (%)	4.35	4.92	3.56	5.0	3.46	2.79	4.13	48.8
IC	±0.75	±0.22	±1.99	±1.99	±0.01	±0.01	±3.99	±1.5

pH: soil: water 1:2.5 ratio potentiometry. P_2O_5: extraction with H_2SO_4 0.05 mol L^{-1} soil: 1:25 solution ratio and colorimetric determination by the development of the blue color. Exchangeable cations: extraction with ammonium acetate 1 mol L^{-1} pH 7, determination of Na and K by flame spectrophotometry and of Ca and Mg by volumetry; CIB: sum of exchangeable cations; C: organic carbon by the Walkley-Black method. CV: Coefficient of Variation; CI: Confidence Intervals

Black polyethylene bags of 4 kg capacity were filled with the soil, taking the cultivable horizon (0-20 cm), which after air drying, was sieved through a 5 mm mesh, with the objective of achieving a size of uniform aggregate and mixed with the vermicompost to make up different proportions. The vermicompost used was produced at the "Orlando López González" CCS, located in La Lisa municipality, Havana, made from cow manure and harvest residues (Table 2) and it was found in the vermicompost of 2015, lower content of nutrients, with the exception of Mg, higher humidity, a broader C: N ratio and a pH closer to neutrality.

Table 2. Characterization on a dry basis of vermicompost from CCS "Orlando López González" made from cow manure and crop residues

Years	C	N	P	K	Ca	Mg	Humidity	pH	C: N ratio
				(g kg^{-1})				-log [H$^+$]	dimensionless
2014	218.7	17.8	21.5	13.0	36.7	5.3	413	7.4	12:1
2015	208.2	14.8	19.9	11.9	35.3	6.2	441	7.1	14:1

C: organic carbon by the Walkley-Black method; digestion of vermicompost with H_2SO_4 + Se and determination of N colorimetrically by Nessler, P by the development of the blue color, K by flame spectrophotometry, Ca and Mg by volumetry; pH: potentiometric determination, vermicompost: water 1:2.5 ratio

Pearl millet (*Panicum italicum* L.) was used as an indicator culture and 10 seeds were sown in each bag; when plants reached between 10 and 15 cm in height, six plants were left per bag. From sowing and during the first 15 days, a daily irrigation was applied until the bags began to drain; later it was watered every two days with equal consideration, keeping the bags free of weeds, by manual cleaning.

The certified inoculum formulated with the species *Glomus cubense*, strain INCAM 4, produced at INCA [(14)], with a concentration of 28 spores g^{-1} of inoculant, was used, which was applied to the seeds by the coating method [(15)].

The soil was counted for resident spores in both years, at the time of setting up the experiments (Table 3).

Table 3. Quantity of spores in the Gley Nodular Ferruginous agrogenic soil of Municipal Directorate of Flora and Fauna from Boyeros municipality at the investigation beginning

Year	Number of spores Spores 50 g of soil^{-1}	Confidence interval
2014	68	25.21
2015	47	28.58
Average	57.5	27.00

Eight treatments were studied (Table 4), in a completely randomized design, with a factorial structure and three repetitions, the factors to be studied were the soil/vermicompost ratios with four levels and mycorrhizal inoculation with two levels.

Table 4. Description of the treatments used to evaluate the effect of vermicompost applications and the inoculation of a mycorrhizal biofertilizer on the nutrient supply of an agrogenic Ferruginous Gley Nodular soil

Treatments	Soil-vermicompost ratio (m/m)		Mycorrhizal
	Soil	Vermicompost	inoculant
1	4	0	with AMF
2			with AMF
3	3	1	without AMF
4			with AMF
5	5	1	without AMF
6			with AMF
7	7	1	without AMF
8			with AMF

The soil came from Municipal Directorate of Flora and Fauna from Boyeros municipality

The growth, the aerial biomass yield and the concentration and content of N, P and K in it, were determined 50 days after emergence, when the plants were in the semi-hard grain phase with yellow spikes.

Plant height (cm): using a tape measure, from the stem base to the last visible dewlap.

Dry mass of aerial biomass (g plant^{-1}): the cut material was taken to an air circulation oven at 70 ºC until reaching a constant mass.

Nutrient concentration (g kg^{-1}): an aliquot of the oven-dried material was oxidized with a mixture of concentrated H_2SO4 + Se; in the extract obtained after dilution, N concentration was determined by the Nessler method, that of P by the development of the phosphoric molybdo blue color and that of K by flame spectrophotometry.

Nutrient content (mg plant^{-1}): it was calculated from the dry biomass of aerial part and the respective concentrations of each element:

Nutrient (mg plant^{-1}) = [biomass (mg plant^{-1}) x concentration (g kg^{-1})/10]

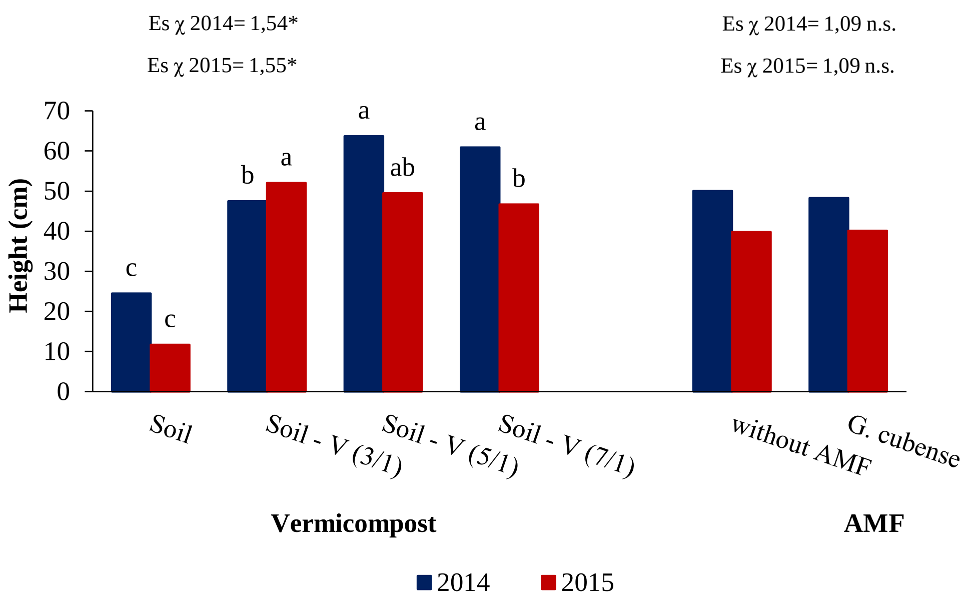
Mycorrhizal variables: plant roots were treated as described in the literature (16). The frequency and intensity of mycorrhizal colonization was determined by the intercept method (17). To determine the number of spores in each bag, the extraction method was used (18).

A bifactorial analysis of variance was performed for variables evaluated when significant differences were found between treatments, the means were compared according to Duncan's Multiple Ranges test ($p < 0.10$). The confidence interval was determined for the mean in the variables of the chemical analysis and in the count of initial resident spores. The Statgraphics Centurion XV Version 15.2.14 program was used.

RESULTS AND DISCUSSION

During the research, only the vermicompost application effect was manifested and there was no interaction between the two factors investigated in the following variables: plant height, dry aerial biomass, frequency and mycorrhization intensity and concentration and content of nutrients; while the quantity of spores present in soil was not affected with any of the treatments.

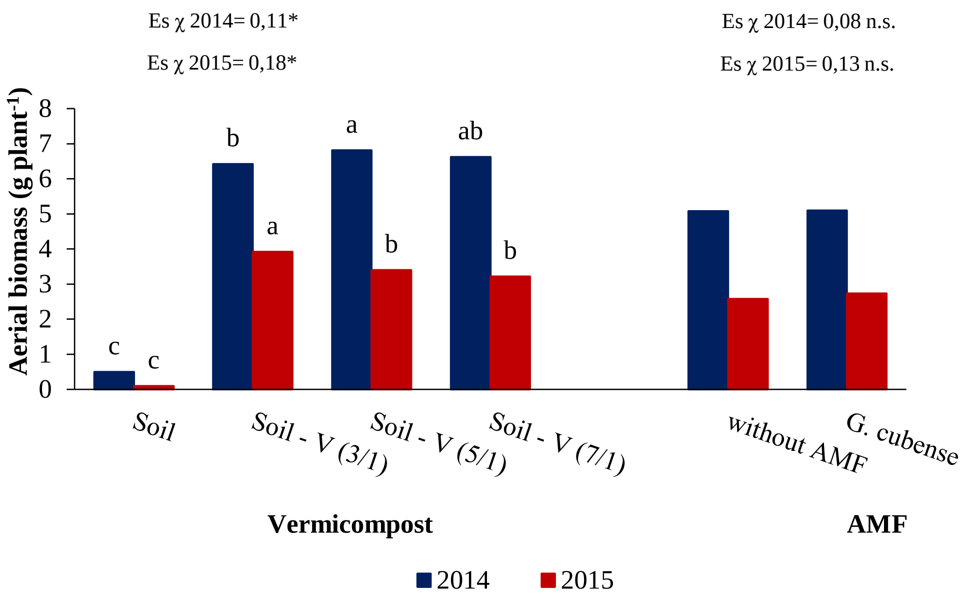
The application of vermicompost increased plant height (Figure 1) differently in each year. In 2014, with the soil/vermicompost ratios 5/1 and 7/1, plants reached greater height; while, in 2015, this manifestation was achieved with the 3/1 and 5/1 ratios, with a tendency to decrease the effect as ratio widened.



V 3/1: soil-vermicompost ratio 3/1, V 5/1: soil-vermicompost ratio 5/1, V 7/1: soil-vermicompost ratio 7/1.
Means with the same letters for each factor and year do not differ from each other according to Duncan's test (p <0.10)

Figure 1. Effect of vermicompost and inoculation with AMF on the growth of pearl millet plants. Agrogenic Ferruginous Nodular Gley Soil

Something similar was found when analyzing the dry mass yield of the aerial biomass (Figure 2), but for the year 2015, the yield decrease with the broader soil/vermicompost ratios was defined.



V 3/1: soil-vermicompost ratio 3/1, V 5/1: soil-vermicompost ratio 5/1, V 7/1: soil-vermicompost ratio 7/1.

Means with the same letters for each factor and year do not differ from each other according to Duncan's test ($p < 0.10$)

Figure 2. Effect of vermicompost and inoculation with AMF on dry mass of the aerial biomass of pearl millet plants. Agrogenic Ferruginous Nodular Gley Soil

The results corroborate those achieved by various researchers in other latitudes and who worked with different soils from the one used in this research. Evaluating the effect of different doses of vermicompost on the pearl millet growth, both plant height (19,20), as well as biomass accumulation (19), were increased by the organic fertilizer application. Also through the application of different organic fertilizer types, an increase in the aerial biomass of pearl millet (21) was obtained, which reflects crop response to organic amendment application.

It has been reported that vermicompost, from humic substances, favors the phenological development of crops by exerting a biostimulatory action on plant growth, through the incidence of phytohormones produced by this organic compound that stimulate biomass production (22.23).

On the other hand and in Cuban conditions, with other crops it has also been possible to verify the positive effect of vermicompost on growth, in such a way that working for two years in soil similar to that of this investigation. Higher biomass values were recorded in plants of *Panicum maximum* and *Brachiaria decumbens*, fertilized with this fertilizer, effects that authors attributed to the improvement of the physical, chemical and biological properties of soil (24).

In previous studies, it was shown that vermicompost has the characteristic of providing macro and micronutrients, of increasing organic carbon content and improving soil pH [25,26], which influenced optimal plant growth, since it is a compound rich in mineral elements, which have already undergone a process of decomposition and they are found in forms available to plants [27].

In correspondence with what was mentioned in the previous paragraph, results referring to the concentration and content of nutrients in millet plants, indicated that vermicompost application improved the supply of nutrients for plants (Table 5 and 6).

In the case of N and in both years, both in the concentration and in the nutrient content in the dry aerial biomass, all the soil/vermicompost ratios had the same effect and always exceeded the soil alone. The P in 2014, behaved similarly to the N; however, in 2015, the highest concentration and quantity of the nutrient was found in plants grown in the substrate with the 3/1 ratio, in correspondence with the behavior of the dry mass yield and an increase in the content of this element in plants, with mycorrhizal inoculation. For K and in 2014, both nutrition indicators were manifested in greater magnitude with the 3/1 relationship; while, in 2015, K concentration was not affected by vermicompost application, but the content was higher when the ratio was closer (3/1).

N was the nutrient that was not sensitive to changes as a function of the soil/vermicompost ratio, nor of the composition of the vermicompost, at least with those used in this research; while P and K showed some dependence on both. In this sense, the results indicated that the closer the relationship, the greater the availability of P and K for the plants.

N was the nutrient that was not sensitive to changes as a function of the soil / vermicompost ratio, nor of the composition of the vermicompost, at least with those used in this research; while P and K showed some dependence on both. In this sense, results indicated that the closer the relationship, the greater the availability of P and K for the plants.

Differences found between years were presumably due to vermicompost composition. In the one used in 2015, a lower amount of nutrients was found, except for Mg, higher humidity and a pH closer to neutrality (Table 2), requiring a greater amount of vermicompost to satisfy the needs of the plants.

Table 5. Concentration and nutrient content of pearl millet harvested 50 days after emergence (semi-hard grain phase). Year 2014

Treatment soil/vermicompost	N	P	K	N	P	K
	----------g kg^{-1}----------			-----mg plant^{-1}-----		
4/0	13.42 b	1.83 b	17.98 c	6.63 b	0.90 b	8.98 c
3/1	22.77 a	2.58 a	37.27 a	146.92 a	16.65 a	240.17 a
5/1	22.20 a	2.52 a	31.0 b	151.85 a	17.12 a	211.19 b
7/1	22.38 a	2.37 a	30.22 b	147.54 a	15.68 a	199.81 b
Se χ	2.42*	0.12*	1.57*	16.40*	0.57*	10.57*
Mycorrhizal inoculation						
- AMF	19.34	2.28	28.23	108.32	12.15	158.69
+ AMF	21.04	2.38	30.01	118.15	13.03	171.38
Se χ	1.17 n.s.	0.09 n.s.	1.11 n.s.	11.60 n.s.	0.40 n.s.	7.47 n.s.

Soil: Agrogenic Ferruginous Nodular Gley of Municipal Directorate of Flora and Fauna from Boyeros municipality; AMF: certified inoculum formulated with the species *Glomus cubense*, INCAM 4 strain produced at INCA; n.s .: no significant differences;

*: Significant differences at 10%

*: diferencias significativas al 10 %

Table 6. Concentration and nutrient content of pearl millet harvested 50 days after emergence (semi-hard grain phase). Year 2015

Treatment soil/vermicompost	N	P	K	N	P	K
	----------g kg^{-1}----------			-----mg plant^{-1}-----		
4/0	15.43 b	1.58 b	37.87	1.48 b	0.15 c	3.68 c
3/1	22.85 a	2.82 a	38.92	90.05 a	11.11 a	153.02 a
5/1	21.98 a	2.65 a	36.88	75.23 a	9.03 b	125.46 b
7/1	23.03 a	2.65 a	37.55	73.67 a	8.57 b	120.27 b
Se χ	1.55*	0.11*	1.22 n.s.	7.34*	0.66*	7.63*
Mycorrhizal inoculation						
- AMF	19.70	2.36	37.49	53.90	6.64 b	95.28
+ AMF	21.95	2.49	38.12	66.32	7.80 a	105.93
Se χ	1.09 n.s.	0.08 n.s.	0.86 n.s.	5.19 n.s.	0.47*	5.40 n.s.

Soil: Agrogenic Ferruginous Nodular Gley of Municipal Directorate of Flora and Fauna from Boyeros municipality; AMF: certified inoculum formulated with the species Glomus cubense, INCAM 4 strain produced at INCA; NS: no significant differences;

*: significant differences at 10%

The increase in concentrations and nutrient contents in plants fertilized with earthworm humus can be attributed both to the contribution of nutrients by organic fertilizer and to the increase in the availability of nutrients from the soil due to the effect of organic fertilization.

In previous works, it was demonstrated how by applying different doses of vermicompost, a greater absorption of these nutrients was achieved in the aerial organs, in the pearl millet culture, in relation to the control treatment, which influenced an increase in production dry biomass and crop yield [(28)]. Likewise, in studies carried out in other crops, the increase in the absorption of N, P and K and the growth of plants was verified, as the doses of earthworm humus applied were increased [(29)]. This same effect, in terms of nutrient absorption, was reflected when applying different doses of humic acids extracted from vermicompost, in mangosteen plants (*Garcinia mangostana*. L) [(30)].

In the two years, vermicompost depressed the frequency and intensity of mycorrhization and as the soil/vermicompost relationship became broader, the trend found was directed towards an increase in the variables evaluated (Figure 3).

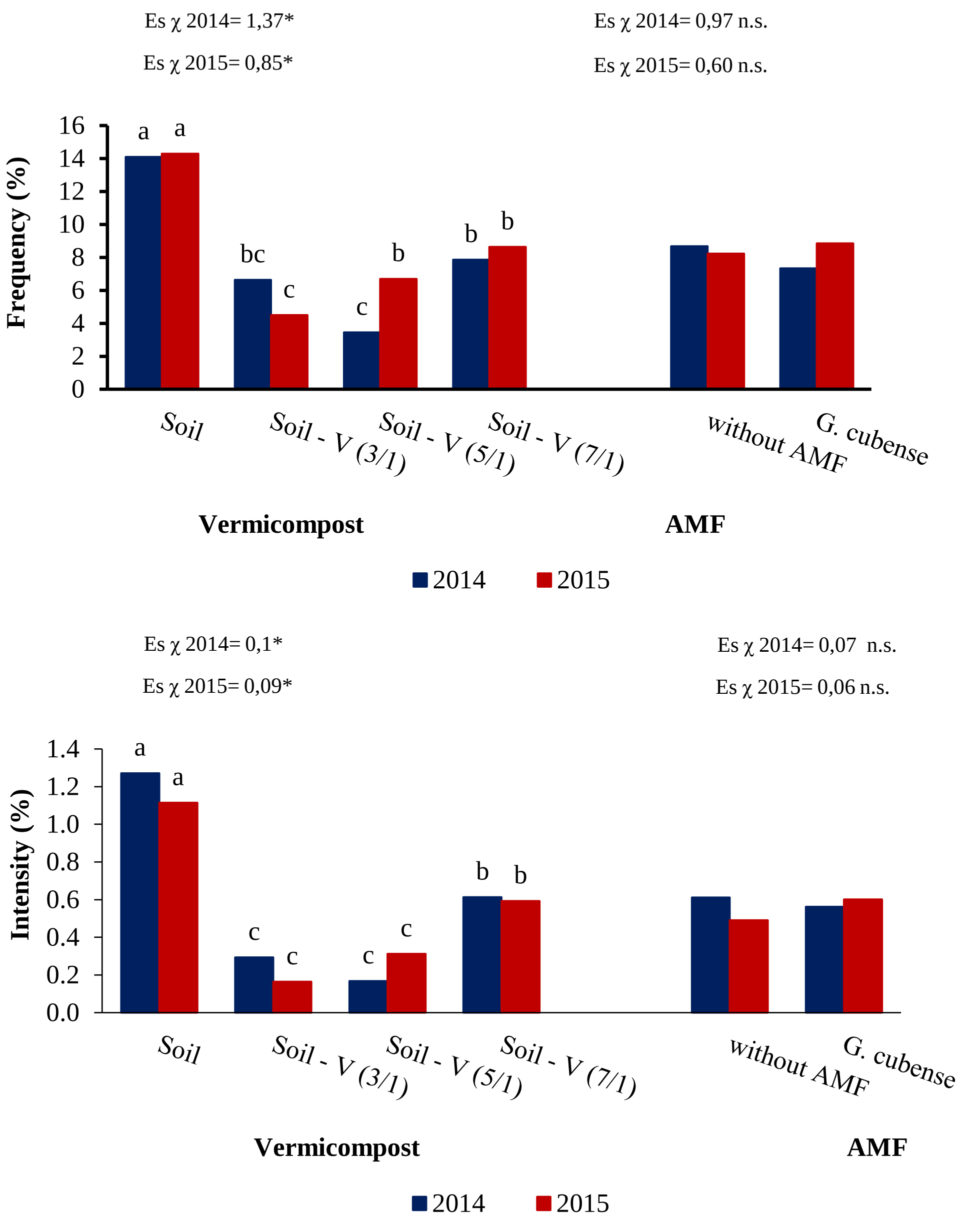


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Figure 3. Effect of vermicompost and AMF inoculation on the frequency and intensity of mycorrhizal colonization in pearl millet plants. Agrogenic Ferruginous Nodular Gley Soil

The aforementioned can be attributed to the fact that plants had a greater quantity of nutrients, since in soils enriched with organic fertilizers. It has been proven that nutrient availability controls the growth of intra- and extra-radical mycorrhizal structures; in such a way that, when plants have been sufficiently fertilized, the distribution of the structures is reduced, since the delivery of nutrients to the host plant, through AMF, loses effect [31].

These results coincide with those obtained in other investigations in which different organic sources and mineral fertilizer were applied in different crops and it was shown that when the availability of nutrients was sufficient in the soil, the nutritional status of crops was guaranteed and it was not enhanced mycorrhizal colonization [32,33].

Regarding the number of spores and in years evaluated, the treatments fertilized with vermicompost did not show differences, in relation to the treatments with soil alone, (Figure 4).

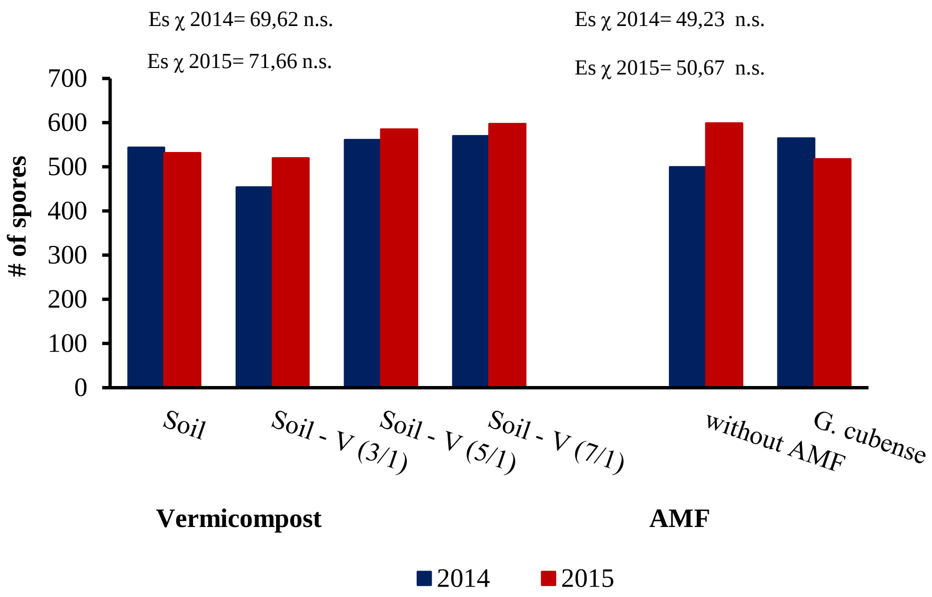

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Figure 4. Effect of vermicompost and AMF inoculation on the number of spores in 50 g of soil. Agrogenic Ferruginous Gley Nodular Soil and pearl millet as indicator culture

At the beginning of both experiments, the initial quantity of AMF spores resident in the soil was less than 70 spores per 50 g of soil (Table 3), while at evaluation time, this indicator reached figures around 550 spores in 50 g of soil (Figure 4); that is, 9.5 times more. Those amounts were similar and even higher than those observed in fieldwork carried out on the pearl millet, inoculated with mycorrhizal species of the genus *Glomus* [34] and other species of the *Poaceae* family, with a cycle of greater growth and cultivated in field conditions [35].

The fact that the number of spores obtained in the treatment without inoculation was similar to that observed in which the mycorrhizal inoculant was applied, indicated that the pearl millet played a relevant role in mycorrhizal reproduction, due to the fact that the radical architecture of this culture gives it a high capacity to reproduce AMF spores [36]. It enhances, the production of resident AMF spores and competing these with the inoculum applied, a criterion that coincides with that proposed by other authors, referring to the fact that the symbiosis between plants and resident AMF species, they are sometimes more efficient and competitive than those established with the inoculated collection species [37].

Consequently, and as has been asserted, the success of the inoculation not only depends on the infectivity and efficiency of the species to be applied, but it is also related to the amount and types of propagules resident in the soil [38].

CONCLUSIONS

- The application of vermicompost increases the availability of nutrients in the soil and is reflected in the increase in the concentration and quantity of nutrients in the plants, which causes greater growth and development of these.
- The incorporation of organic fertilizer reduces the frequency and intensity of mycorrhization, inhibiting the effect of inoculation with arbuscular mycorrhizal fungi on plants and without affecting the production of spores in the soil.

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