Cultivos Tropicales, 2015, vol. 36, no. 1, pp. 53-62 January-March

ISSN print: 0258-5936
ISSN online: 1819-4087

Ministry of Higher Education. Cuba
National Institute of Agricultural Sciences
http://ediciones.inca.edu.cu

MANAGEMENT AND USE OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) AND EARTH WORM HUMUS IN TOMATO (*Solanum lycopersicum* L.) UNDER PROTECTED SYSTEM

Uso y manejo de hongos micorrízicos arbusculares (HMA) y humus de lombriz en tomate (*Solanum lycopersicum* L.), bajo sistema protegido

Nelson J. Charles✉ and Nelson J. Martín Alonso

ABSTRACT. The production of tomato under greenhouse system in Cuba is subjected to a high use of mineral fertilizers that resulted to high yield but it affects the nutritional quality of tomato fruits and this has brought great concern at a national level to obtain productions with a high ecological quality. In line with this, two experiments were carried out at the ¨Los 3 Picos¨ farm, in Managua Cuba, on a Feralitic Yellow Glay soil, under a protected cultivating system, with the objective to determine the effects of the arbuscular mycorrhizal fungi (AMF) of Cubense strain and earth worm´s humus, used individually or combined, as substitute to the mineral fertilizers at different dosages in tomato crop hybrid (HA 3108 Hazera). The beneficial effects of the AMF applied in the commercial biofertilizer Ecomic®, produced by the National Institute of Agricultural Sciences (INCA) and the earth worm´s humus were evaluated in the plant, using the variables: height, dry matter content of leaves, final yield and internal fruit quality of fruits. The results shown that the application of AMF, was more efficient than the earth worm´s humus at a 25 % dose of the mineral fertilizer and without any relevant effects when applied in combination with earth worm´s humus but with no significant influences on its biological parameters. Nevertheless, when increasing the mineral fertilizer by 50 %, could appreciate a synergy effects between the two products, being most efficient when applied in combination than applied separately, obtaining a higher production to that brought by the dosage of the mineral fertilizer considered to be optimum. The combination of the AMF and earth worm´s humus improves the internal quality of tomato fruits with respect to the parameters of soluble total solids and vitamin C.

Key words: HMA, protected cultivation, mineral fertilizer, tomato

RESUMEN. La producción de tomate bajo cultivo protegido, en Cuba, está siendo sometida a una fertilización mineral muy intensa, que conlleva a un alto rendimiento, pero una baja calidad bromatológica, lo que ha conllevado, a nivel nacional, un gran interés para obtener producciones con una alta calidad ecológica. Para ello, se desarrollaron dos experimentos en la Granja "Los 3 Picos", en Managua, Cuba, sobre un suelo Ferralítico Amarillento gleyzado, en un sistema de cultivo protegido, con el objetivo de determinar los efectos de hongos micorrízicos arbusculares (HMA), cepa Cubense y humus de lombriz, solos y combinados, como sustitutos de la fertilización mineral en el cultivo de tomate (hibrido HA 3108 Hazera) a diferentes dosis. El efecto benéfico del HMA aplicado a través del biofertilizante comercial Ecomic®, producido por el Instituto Nacional de Ciencias Agrícolas (INCA) y el humus de lombriz, fue evaluado en la planta, a través de las variables: altura, materia seca de las hojas, rendimiento final, y la calidad bromatológica de los frutos. Los resultados mostraron que la aplicación de HMA, fue más eficiente que el humus de lombriz al 25 % de la dosis de fertilizante mineral y sin efectos relevantes cuando se aplican combinados con el humus de lombriz, pero su influencia en los parámetros biológicos no fue significativa. No obstante, al incrementarse la fertilización mineral al 50 %, se pudo apreciar sinergismo entre ambos productos, al ser más eficiente la aplicación combinada, obteniéndose una producción superior a la que aportó la dosis del fertilizante mineral considerada óptima. La aplicación combinada de los HMA y humus de lombriz, mejoraron la calidad bromatológica de los frutos de tomate con respecto a los parámetros de sólidos solubles totales (°Brix) y vitamina C.

Palabras clave: HMA, cultivo protegido, fertilización mineral, tomate

Universidad Agraria de La Habana, Cuba.

✉ nelson78@enet.cu; nelsonm@unah.edu.cu

INTRODUCTION

The excessive use of mineral fertilizers has brought about an imbalance in the soil-plant system by reducing microbial activities and the productive potential of harvests (1); on the other hand, the use of agrochemicals in vegetables are of great concern to the consumer, mainly because of its residues (2). Such problem has led to the rescue of organic alternatives that turn out to be ideal inputs to guarantee healthy commodities and improve the chemical, physical, and biological properties of soils to preserve their productive capacity.

Among nutrient alternatives, biofertilizers and arbuscular mycorrhizal fungi (AMF) have been very efficient as substitutes of mineral fertilizers (3, 4, 5), its combination with organic matter is attributed a higher effectiveness and a synergic effect (6, 7).

In Cuba, the use of organic manure (8, 9, 10), and biofertilizers (11, 12) has been promoted with praiseworthy results (13); however, no conclusive data are available on the efficiency of AMF and earthworm humus applied alone or combined for tomato growing through fertigation and under protected conditions. With the results of this research, a reduction in the use of mineral fertilizers and better chemical and biological properties of the soil are expected without affecting production and the biological quality of the fruits.

MATERIALS AND METHODS

The trial was conducted in protected crop houses of the Tropical A-12 model, with a planted area of 540 m^2, located at “Los 3 Picos” farm, Boyeros municipality, Havana City, at 107 m a. s. l. The trial was established in two periods: from December 2009 to May 2010 and from December 2010 to May 2011.

Tomato seeds used come from hybrid HA 3108-Hazera and seedling were produced in rootball using trays with a mixture of 25% earthworm humus, 25 % rice husk, 25 % of soil and 25 % of cow manure as substrate, according to records from the Ministry of Agriculture. Seedlings were transplanted 23 days after sowing and were planted on a Yellow Gley Ferralitic Soil (14).

Table I shows the chemical characteristics of the soil at the beginning of the trial. There is a evident low fertility which is expressed by the low contents of organic matter (OM) and by the low capacity of base exchange which, under such conditions, makes the efficiency of AMF biofertilizer higher (15, 16).

The experimental design used was the subdivided plot in a random block design with four replicates and trifactorial arrangement; being factor A- different rates of nutrient carriers in fertigation; factor B- the use of earthworm humus and factor C the application of AMF.

Subplots dimensions were 1,0 m width and 5,0 m long, for a total surface of 5,0 m², the spacing used was 0,2 m by 0,9 m for tomato, where 25 plants per treatment were used in each subplot and for the sub-sub plot it was 1,0 m width by 2,5 m long, with a total area of 2,5 m^2, for a total of 12 plants per each sub-sub plot.

Nutrient carriers used in research

Mineral fertilizers (Factor A). Fertilizers used in fertigation were *Magnesium nitrate* (11-0-0-0-15); *Calcium nitrate* (15-5-0-26-0), *Ortophosphoric acid* H_3P0_4 (85 %), *Potassium nitrate* (12-0-45-0-0), *Ammonium nitrate* (34-0-0-0-0) (17) and the four rates of mineral fertilizers were a_0 (0 %), a_1 (25 %), a_2 (50 %) and a_3 (100 %).

Earthworm humus (Factor B). Earthworm humus levels that represent sub sub plots were b0 (without earthworm humus) and b. (with earthworm humus), rates of 1 kg m^{-2}. This organic compound had a pH of 7,1; organic matter of 57,10 and 50,1 % humidity; its composition N, P and K and the carbon / nitrogen ratio are shown in Table II.

Table I. Chemical characteristcs of the soil, existing in the protected cultivation no use

Depth (cm)	Ca^{2+}	Mg^{2+}	K^+ cmol(+)kg^{-1}	Na^+	CIB	P_2O_5 (kg ha^{-1})	MO (%)	pH
0-30	0,41	0,11	4,80	1,49	6,81	492,23	1,75	7,7

Table II. Chemical composition of the earthworm humus

Components	Values (%)	Contributions (t ha^{-1})
Nitrogen (N)	1,75	150
Phosphorus (P)	0,24	24
Potassium (K)	0,15	1,5
Carbon/Nitrogen ratio (C/N)	10,4 % dry basis	

AMF Glomus cubense (Y. Rodr. y Dalpé) (Factor C). AMF inoculation that represents sub-sub plots was a certified inoculum of *Glomus cubense* (Y. Rodr. and Dalpé) (25 spores g^{-1}), commercial ecological product (Ecomic®), produced by the National Research Institute on Agricultural Sciences (INCA). Ten percent of the seed weight to be treated was applied (18). The two levels of factor C, namely, c_0 (without AMF inoculation) and c_1 (with AMF inoculation).

Table III shows the 16 treatments from combinations of factors A, B and C -rates of fertilizers, earthworm humus and AMF-.

Types and nutrient concentration of used fertilizers in the different phenological stages of the crop (Table IV) were applied according to current standards (17).

Five plants taken at random in each sub-sub plot were sampled. They were evaluated as to height (cm), at 30 and 45 days post-transplanting, leaves dry mass (g), at the end of the trial. Yield (t ha^{-1}) was calculated from the mass weight of all fruits in each treatment; plants response to mycorrhizal colonization was also evaluated (19), at the end of the vegetative cycle of the crop, for which the root dying system was used (20).

The evaluation of harvest quality took into account a random sample of 15 fruits per treatment which were evaluated as to: bromatological quality, total soluble solids (°Brix), through refractometry, titrable acidity (citric acid %), by using NaOH, 0.1 N, and phenolphtalein as indicator, pH of the macerated pulp through the potential metric method, vitamin C (mg 100 g^{-1}), by extracting ascorbic acid with 2,6 dichlorophenol indophenol.

All data were processed by a multifactorial analysis of variance. These statistical analyses were done with Statgraphics 5.1.

Tabla III. Treatments applied to the experimental area from combinations of Factors A (mineral fertilizer: a_0 a_1 a_2 and a_3), B (earthworm humus: b_0 and b_1) and C (AMF: c_0 and c_1)

No.	Treatments	Combinations
1	$a_0b_1c_1$	0 % Mineral fertilizer + Humus + AMF
2	$a_0b_0c_1$	0 % Mineral fertilizer + Without humus + AMF
3	$a_0b_1c_0$	0 % Mineral fertilizer + Humus + Without AMF
4	$a_0b_0c_0$	0 % Mineral fertilizer + Without humus + Without AMF
5	$a_1b_1c_1$	25 % Mineral fertilizer + Humus + HMA
6	$a_1b_0c_1$	25 % Mineral fertilizer + Without humus + AMF
7	$a_1b_1c_0$	25 % Mineral fertilizer + Humus + Without AMF
8	$a_1b_0c_0$	25 % Mineral fertilizer + Without humus + Without AMF
9	$a_2b_1c_1$	50 % Mineral fertilizer + Humus + AMF
10	$a_2b_0c_1$	50 % Mineral fertilizer + Without humus + AMF
11	$a_2b_1c_0$	50 % Mineral fertilizer + Humus + Without AMF
12	$a_2b_0c_0$	50 % Mineral fertilizer + Without humus + Without AMF
13	$a_3b_1c_1$	100 % Mineral fertilizer + Humus + AMF
14	$a_3b_0c_1$	100 % Mineral fertilizer + Without humus + AMF
15	$a_3b_1c_0$	100 % Mineral fertilizer + Humus + Without AMF
16	$a_3b_0c_0$	100 % Mineral fertilizer + Without humus + AMF

Table IV. Fertigation standards according to the development stage of tomato

Stage	Development stage	Number of days		Daily average nutrition (g 1000 plantas^{-1})				
		Period	Total	N	P_2O_5	K_2O	CaO	MgO
1	Transplant-first flower cluster	0-20	20	20	100	40	-	-
2	First flower cluster-fruit setting of the third cluster	21-44	23	77	77	77	94	23
3	Fruit setting third cluster – harvest start	45-65	20	160	110	350	210	50
4	Harvest start – production	66-110	44	522	154	630	420	98
5	Production – final production	111-140	29	160	110	350	210	50

RESULTS AND DISCUSSION

Among the growth and development variables of tomato, plant height is considered among the most influential one on yield (21, 22). There is a direct positive relationship with yield when observations were made 45 days after transplanting, there is also a balance in this period between vegetative growth and reproductive growth (23); moreover, it is assured that the highest daily production of biomass for leaves, stem and roots of tomato, take place from 41 to 55 days post-transplanting (24), time that coincides with the period of maximum vegetative growth and the beginning of the harvest.

Figure 1, shows the results of tomato plants height 30 and 45 days after transplanting. When evaluating plant height 30 days after transplanting it was found that treatments 9 (50 % mineral fertilizer + humus + AMF), 13 (100 % mineral fertilizer + humus + AMF), 14 (100 % mineral fertilizer + without humus + AMF), and 15 (100 % mineral fertilizer + humus + without AMF), did not have significant differences among them, due to the effect produced by AMF to nutrients availability. In this stage humus is not completely available to the plant which coincides with the criteria of other authors (25), stating that earthworm humus takes at least four weeks or more to make effect on plants growth.

However, 45 days after transplanting, there is a positive effect of earthworm humus and AMF on this indicator as compared to mineral fertilizer, its effect at the rate of 50 % and humus application does not show significant differences with treatments 13 (100 % mineral fertilizer + humus + AMF), 14 (100 % mineral fertilizer + without humus + AMF), and 15 (100 % mineral fertilizer + humus + with AMF). It proves that the mineral fertilizer rate can be reduced keeping the contribution of earthworm humus and AMF (Figure 1).

The incorporation of earthworm humus has beneficial effects on soil properties and it seems that together with the positive effect of mycorrhizal fungi on nutrients uptake, its assimilation is improved (26, 27).

Mean with common equal letters do not differ significantly according to Fisher's procedure of lower significant differences (LSD), p<0,05.

This response is a good choice to reduce the use of chemicals and achieve a more sustainable production (28). The low capacity of base exchange (CBE), on the soil 6,81 cmol kg^{-1} and the scarce organic matter content (1,75 %), according to Martín, 2000 quoted by other authors (29), should be the cause of the favorable effect of combining products that for their nature, improve the physical, chemicals and microbiological properties of soils; as AMF and earthworm humus. Some authors (30) say that earthworm humus produces substances able to influence on plant growth by increasing enzimatic activity, disease suppression, and producing plant growth regulating substances or PGRS Plant Growth Regulating Substances (30).

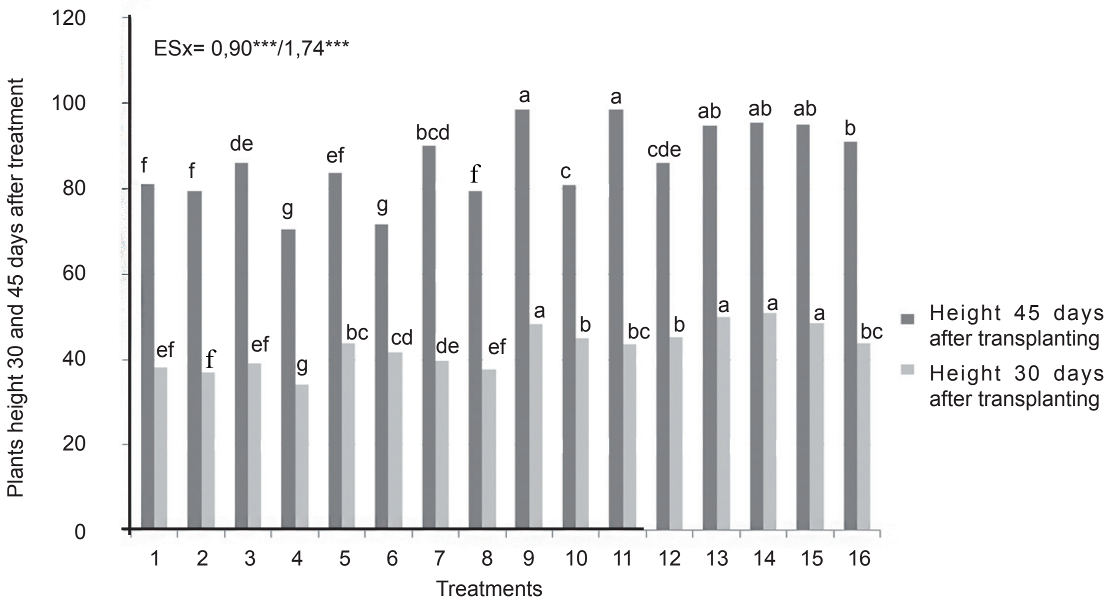

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Figure 1. Effect of earthworm humus application, AMF and rates of mineral fertilizers on the height of tomato plants under protected conditions

The fact that tomato plants reached a lower height at lower mineral fertilizer rates, confirms the efficiency of propositions for an optimum fertilization 50 % of mineral fertilizer + earthworm humus + AMF done for this crop which showed the highest height and the best yield of the research (31, 32, 33).

When dry mass was evaluated (Figure 2), a greater effect of AMF at higher mineral fertilizer rates, both combined with earthworm humus and without it, was observed. It can be seen in treatments 9 (50 % mineral fertilizer + earthworm humus + AMF), 10 (50 % of mineral fertilizer + AMF) and 13 (100 % mineral fertilizer + humus + AMF), without differences among them. The high biomass production attained in treatment 10 seems to be related with arbuscular mycorrhizal fungi that positively influenced on the increased concentration of growth hormones as indol 3-acetic acid and gibberelic acid (GA3), on the plant which improved vigor and cell growth. It brought about a higher number of leaves and greater foliar area (34). In addition, the ratio of calcium/magnesium that is of 3.73:1 (Table I), is evaluated as normal which coincides with other investigations (35), and also promotes mineral fertilizer levels as confirmed by other authors (36, 37).

Moreover, the fungus-plant symbiosis is typically mutualist since fungus depends on the plant to uptake photoassimilates and the plant in return, receives different benefits that increases its growth and improves water relations (38).

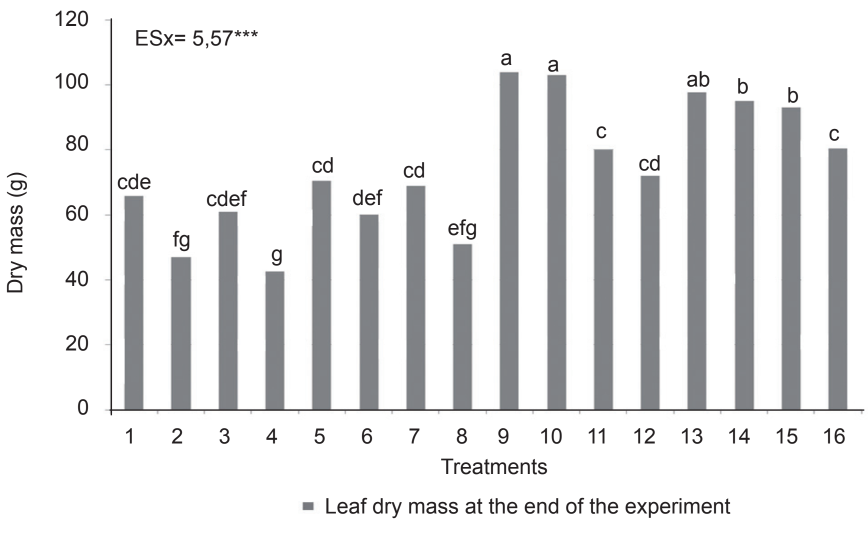
Greenhouse studies shows that symbiotic association of mycorrhizal fungi on plant roots produces different changes and modifications at the physiological level, namely, increased photosynthetic activity due to a high capacity of C02 fixation and consequently increased growth rates and biomass (39).

The capacity of base exchange on this soil (6,81 cmol kg^{-1}), is extremely low according to Martín (2000), quoted by other authors (29) provided that earthworm humus is applied, the availability of nutrients to the plant is improved and in turn, if AMF are present, the assimilation of these nutrients by the crop is more favored.

Figure 3 shows mycorrhizal colonization of plant roots at the end of the vegetative cycle of the crop.

Maximum colonization (26 %) was reached where neither mineral fertilizers nor earthworm humus were applied (treatment 2). This behavior was due to the fact that AMF showed better colonization when the base exchange capacity and organic carbon dioxide in the soil were low. Such performance was confirmed by the results of other trials (40). Under such conditions, even with a high AMF colonization it would be insufficient to reach efficient productions, something that is reflected on the biomass production of treatment 2 where the rate of mineral fertilizers did not exceed 25 % of crop needs.

Colonization percentage in treatment 9 (50 % mineral fertilizer + earthworm humus + AMF) was 17 % lower than treatment 1 (0 % mineral fertilizer + humus + AMF) by reaching a yield of 15.88 t ha^{-1} vs 45,02 t ha^{-1} respectively. Such result shows that high colonization levels do not necessarily mean increased yields, but also that nutrients required by the plant were supplied. If the colonization reached with treatment 9 (50 % mineral fertilizer + humus + AMF) and 13 (100 % mineral fertilizer + humus + AMF), of 17 and 16 % respectively, are compared, similar results will be seen as to plant height, dry mass and yield. Therefore, it means an excess of chemical fertilizers since with 50 % similar results were attained in relation with that of 100 %.



Mean with common equal letters do not differ significantly according to Fisher's procedure of lower significant differences (LSD), p<0,05

Figure 2. Effect of the earthworm humus application, AMF and rates of mineral fertilizers over the dry mass of leaves, in tomato plants (expressed in grams)

Also in treatments (3, 4, 7, 8, 11, 12, 15, 16), where only plants with soil native AMF were colonized, lower results were attained as compared to the plants colonized with AMF commercial product (Ecomic®) which proves the non-systematic use of these fungi in the greenhouse.

Colonization evaluated at the end of the trial showed values above 20 % in treatments where Ecomic® was applied which coincides with the results from previous studies (41, 42) where solid mycorrhizal inoculants have been used with mycorrhization values from 20 to 45 % of colonization.

Figure 4 shows the effect of earthworm humus application, arbuscular mycorrhizal fungi and the rates of mineral fertilizers on tomato yield expressed in t ha^{-1}.

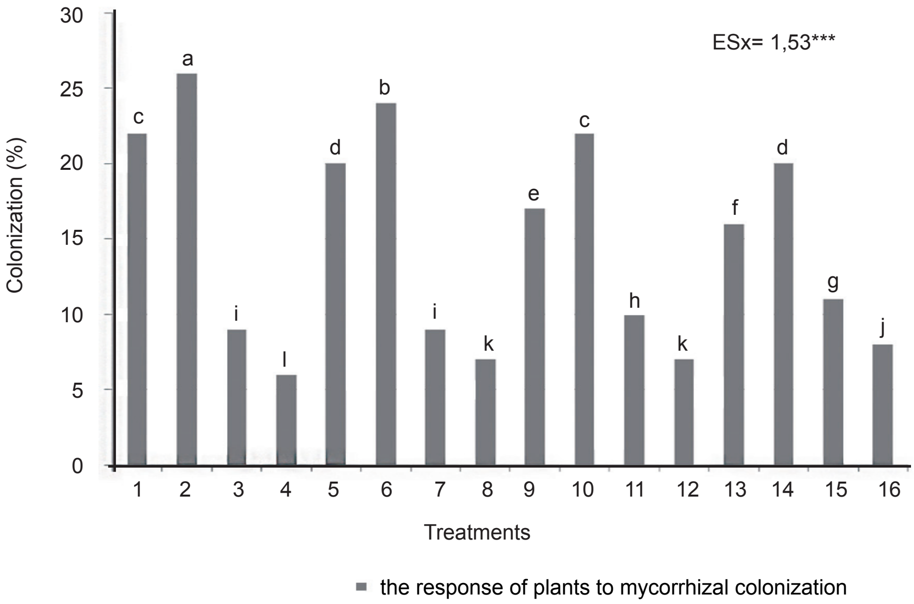


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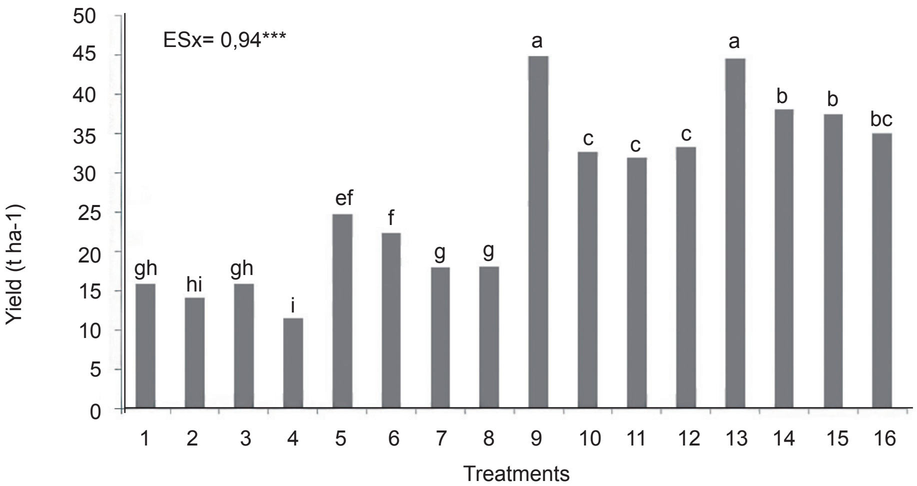
Figure 3. Plants response to mycorrhizal colonization at the end of the vegetative cycle of the crop



Mean with common equal letters do not differ significantly according to Fisher's procedure of lower significant differences (LSD), p<0.05

Figure 4. Effect of earthworm humus, AMF and rates of mineral fertilizers on the yield expressed in t ha^{-1} in tomato

It was found that with 50 % less nutrients in the irrigation water, AMF make available to the plants nutrients present on the soil, being this one the best rate to achieve an economic production. It was shown by treatments 9 (50 % mineral fertilizer + humus + AMF) with a yield of 45,02 t ha^{-1} and treatment 13 (100 % mineral fertilizer + humus + AMF) that reached a production of 44,58 t ha^{-1}, without significant differences among them. It coincides with the results from other authors (43, 44), being also similar to results in strawberries by applying AMF and vermicompost (45) and also in guava, where the combination of 75 % mineral fertilizer with biofertilizers and FitoMas-E reduced mineral fertilization in 25 % (46).

Table V shows the results of the bromatological quality evaluation of tomato fruits from different treatments. Vitamin C, ranges from 16,93 to 19,56 mg 100 g^{-1}, considering them as adequate according to established parameters (47, 48). It is important to notice that in treatments (7, 9, 11, 13 and 15) where earthworm humus was applied with mineral fertilizer, vitamin C did not show significant differences on treatments on Total Soluble Solids (TSS), (9, 11 and 15), they showed the same value range probably due to the presence of similar structures to gibberellins in the composition of earthworm humus and the action of humic substances together to the possible effect of aminoacids (49).

Titrable acidity and pH were considered normal based on the established parameters (50). Therefore, the use of these ecological products improved the bromatological quality of the fruits as compared to total soluble solids parameters (°Brix) and Vitamin C.

CONCLUSIONS

- ♦ According to results, the combination of AMF applied through the biofertilizer Ecomic® (25 spores g^{-1}) + earthworm humus (1 kg m^{-2}) at 50 % of the mineral fertilization in tomato under protected conditions, has a positive effect on the growth, development, and yield of the crop.
- ♦ As to bromatological quality, the total soluble solids content (°Brix) and vitamin C significantly increased when earthworm humus combined with mineral fertilizers was used. It showed the feasibility of using mycorrhizal inoculation and the contribution of earthworm humus to reduce mineral fertilizers and improve the biological quality of tomato.

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Table V. Behavior of the bromatological quality before different treatments applied

Treat.	Acidity (%)	TSS (°Brix)	Vit, C (mg 100 g^{-1})	pH	Treat.	Acidity (%)	TSS (°Brix)	Vit, C (mg 100 g^{-1})	pH
T1	0,40 d	5,05 c	19,34 c	4,21 a	T9	0,42 abc	5,28 a	19,53 ab	4,20 a
T2	0,37 f	5,15 bc	17,50 e	4,20 a	T10	0,40 de	4,78 d	17,57 d	4,20 a
T3	0,42 abc	4,78 d	19,48 b	4,21 a	T11	0,41 cd	5,24 ab	19,56 a	4,21 a
T4	0,36 g	4,29 g	16,96 f	4,22 a	T12	0,39 ef	4,4 ef	16,95 f	4,21 a
T5	0,43 ab	5,16 b	19,29 c	4,21 a	T13	0,41 bcd	5,16 b	19,50 ab	4,21 a
T6	0,38 f	4,82 d	17,48 e	4,20 a	T14	0,43 a	4,87 d	17,48 e	4,20 a
T7	0,42 abc	5,1 6b	19,50 ab	4,20 a	T15	0,41 cd	5,18 ab	19,56 a	4,22 a
T8	0,37 fg	4,32 fg	16,97 f	4,21 a	T16	0,40 de	4,46 e	16,93 f	4,20 a
ESx	0,003**	0,043**	0,144**	0,003**	ESx	0,003**	0,043**	0,144**	0,0001**

TSS: Total soluble solids Acidez: Titrable acidity Vit,C: Vitamin C Tratam: Treatment
Mean with common equal letters do not differ significantly according to Fisher's procedure of lower significant differences (LSD), $p<0.05$

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Received: February 7th, 2014
Accepted: April 16th, 2014