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Revista Facultad Nacional de Agronomía

Use of effective microorganisms and FitoMas-E® to increase the growth and quality of pepper (*Capsicum annuum* L.) seedlings

UNIVERSIDAD NACIONAL DE COLOMBIA

Uso de microorganismos eficientes y FitoMas-E® para aumentar el crecimiento y la calidad de plántulas de pimiento (*Capsicum annuum* L.)

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ABSTRACT

Keywords:
Bio-products
Capsicum annuum
Nursery
Organoponic

The agricultural bio-products based on effective microorganisms (EM) are a suitable alternative to reduce the use of chemical fertilizers and have a positive effect on the growth and development of plants. The aim of the present work was to evaluate the effect of EM and FitoMas-E® on the production of pepper seedlings (*Capsicum annuum* L.). The experiment was carried out in an organoponic garden in the municipality of Matanzas, Cuba. It was constituted for four treatments: control (without application of bio-products), EM (4 mL m^{-2}), FitoMas-E® (0.1 mL m^{-2}) and EM (4 mL m^{-2}) + FitoMas-E® (0.1 mL m^{-2}). A randomized block design was performed with three replications per treatment. One-way analysis of variance was performed to determine differences among treatments and Duncan´s Multiple Range Test for media comparison. Seedling height, number of leaves per seedling, stem diameter, root length, slenderness index, fresh and dry weight of leaf and root and shoot/root ratio based on dry weight data were determined. The application of EM and FitoMas-E® had a positive effect on the growth and quality of the pepper seedlings under nursery condition. The treatment 4 showed the best results regarding the morphological parameters: plant height (17.19 cm), number of leaves (6.01), stem diameter (3.98 mm), root length (8.82 cm) as well as fresh and dry weight of leaves and roots. The combined application of EM and FitoMas-E showed to be effective in promoting the growth of roots and aerial organs but maintaining a shoot/root ratio ranged from 1.28 to 2.5, which are suitable values in order to obtain quality pepper seedlings.

RESUMEN

Palabras clave:
Bioproductos
Capsicum annuum
Semillero
Organopónico

Los bioproductos agrícolas basados en microorganismos eficientes (EM) constituyen una alternativa viable para disminuir el uso de fertilizantes químicos, y tienen un efecto positivo sobre el crecimiento y desarrollo de las plantas. Este trabajo se realizó con el objetivo de evaluar el efecto de microorganismos eficientes (EM) y FitoMas-E® en la producción de plántulas de pimiento (*Capsicum annuum* L.), para lo cual se realizó un experimento en un jardín organopónico en el municipio de Matanzas, Cuba. Se estudiaron cuatro tratamientos: control (sin aplicación de los bio-productos), EM (4 mL m^{-2}), FitoMas-E® (0.1 mL m^{-2}) y EM (4 mL m^{-2}) + FitoMas-E® (0.1 mL m^{-2}). Se estableció un diseño de bloques completamente al azar con tres repeticiones por tratamiento. Se realizó un análisis de varianza para determinar diferencias entre los tratamientos y la prueba de Rangos Múltiples de Duncan para la comparación entre las medias. Se evaluaron los indicadores altura de las plántulas (cm), número de hojas por plántula, diámetro del tallo (mm), longitud de la raíz (cm), el índice de esbeltez, peso fresco y seco foliar y de la raíz (g) y la relación parte aérea/raíz con relación al peso seco. La aplicación de EM y FitoMas-E® tuvo un efecto positivo sobre el crecimiento y la calidad de las plántulas de pimiento en semillero. El tratamiento 4 mostró los mejores resultados con relación a los indicadores morfológicos: altura de la planta (17.19 cm), número de hojas (6.01), diámetro del tallo (3.98 mm), longitud de la raíz (8.82 cm), así como en el peso fresco y seco de las hojas y de las raíces. La aplicación combinada de EM y FitoMas-E fue efectiva para estimular el crecimiento de la raíz y los órganos aéreos, manteniendo una relación parte aérea/raíz entre 1.28 y 2.5, lo cual es importante en función de obtener plántulas de pimiento con calidad.

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Current crops production operates under an intensive agriculture model, characterized by the indiscriminate use of chemical fertilizers and pesticides, which increase the costs of production (Meena *et al*., 2017). The overuse of those agrochemicals has a negative impact on the environment, reducing soil fertility and microbiota diversity (Modi *et al*., 2017). Interest in the development of new plant-based products that promote microorganisms has increased, which allows reducing the application of agrochemicals while maintaining high crops yield (Akintokun *et al*., 2019).

Pepper (*Capsicum annuum* L.) is one of the most important fruit worldwide, due to its exquisite flavor and nutritional values (Guilherme *et al*., 2020). In Cuba, this fruit may be used fresh or processed, and it is extensively cultivated in all regions with a total area of 7,029 ha in 2019 and a production of 79,726 t (ONEI, 2020).

Organoponic garden technology, as a production model in urban, suburban and personal agriculture, has been developed with very good results in the production of vegetable and fruits (Terry *et al*., 2014). In this context, to obtain quality seedlings, it is necessary to apply a proper amount of organic matter that ensures soil fertility and the availability of nutrients, which satisfy the nutritional demand of the crops.

The crop nursery stage is a fundamental step in growing several crops under tropical conditions. The use of bio-products that stimulate plants growth is usually underestimated, however, it can be an key element for an optimal growth. Bio-products based on EM have been used on pepper and other crops, due to several advantages, such as low costs, simple technology, minimal impact on the environment and positive effect on growth and productivity (Pradhan *et al*., 2019). Moreover, many EM-based products have shown to be a plant root promoter, which increase seedling survival during stressful conditions of transplanting (Jochum *et al*., 2019).

FitoMas-E® is a natural bio-nutrient product based on high-energy active substances (aminoacids, nitrogenous bases and polysaccharides), as well as minerals containing nitrogen, phosphorus and potassium. It has been successfully supplied to crops to stimulate growth and development (Díaz *et al*., 2021). Recently, the combined application of FitoMas-E and EM enhanced the agronomic response in common bean, by improving morphological, physiological and reproductive parameters (Calero-Hurtado *et al*., 2019). Hence, this work aimed to evaluate the effect of Effective Microorganisms and FitoMas-E® on the production pepper seedlings.

MATERIALS AND METHODS

Plant material and nursery preparation

The experiment was carried out under organoponic conditions in the municipality of Matanzas, Cuba, from January to February 2018. Seeds of *Capsicum annuum* L., Español Liliana variety were supplied by the Liliana Dimitrova Horticultural Research Institute. Three beds were used, each divided into four plot nurseries, which were prepared by making furrows transversely to the plot, with 15 cm between rows. The substrate consisted of organic matter (filter cake, 50%) and red ferralitic soil (50%). The agro-technic management during the experiment was performed following the recommendations of Rodríguez *et al*. (2011), on intensive vegetable production in gardening and semi-covered technology (Technical Handbook for Organoponic). A daily watering was carried out by a micro-jet irrigation system in order to achieve a homogenous moisture of the substrate. After 7 days germination, the seedbed was subjected to a thinning process to obtain a proper number of seedlings m^{-1}. Weeds were manually removed during the experiment.

Bio-products

The EM were supplied by the Bio-pesticide Production Laboratory LABIOFAM, province of Matanzas, Cuba, which consisted of a mixture of bacteria (*Bacillus* spp. and *Pseudomonas* spp.) at 13×10^8 CFU mL^{-1}, fungi from the genera *Trichoderma*, *Aspergillus*, *Rhizopus*, *Mucor* and *Penicillum* (18×10^5 CFU mL^{-1}) and the yeast (*Saccharomyces* sp.) at 21×10^6 CFU mL^{-1}. FitoMas-E® was supplied by the Station of Sugarcane Research “Antonio Mesa”, province of Matanzas. A foliar application of both bio-products was performed in the morning (7:00-9:00 am), 5 and 15 days after seeds germination by using a fumigation backpack (MATABI).

Treatments and evaluated parameters

The studied treatments consisted of: control (without application of bio-products) (T1), EM at 4 mL m^{-2} (T2), FitoMas-E® at 0.1 mL m^{-2} (T3), EM at 4 mL m^{-2} + FitoMas-E® at 0.1 mL m^{-2} (T4). The concentrations of the bio-products were those recommended by Montano (2008) for FitoMas-E® and Álvarez *et al*. (2012) for EM-based bioproduct.

Plant height (cm) was determined measuring the length from the base of the root up to the shoot tip by a measuring tape; number of leaves per seedling by direct counting, stem diameter (mm) by using a caliper (Vernier) at 1 cm above the base of the stem, root length (cm) measured from the base of the stem up to the root tip using a measuring tape. Slenderness Index (SI) was calculated as the ratio between plant height (H) and diameter of the stem (D) (SI=H/D, according to Birchler *et al*., 1998). Fresh weight of leaves and roots (g) was measured by a digital balance (Sartorius, ALC-110.4). Dry weight of leaves and roots (g) was also evaluated. Samples were dehydrated using an oven at 70 °C for 72 h and dry matter was successively weighed by a Sartorius balance until a constant weight was achieved. Plant shoot/root ratio was calculated according to Romero *et al*. (2012) and expressed as the ratio between shoot dry weight and root dry weight. For this purpose, 25 seedlings of 35-days-old were randomly taken from each experimental plot to evaluate.

Experimental design and statistical analysis

The experiment was conducted in a randomized block design with three replications. The collected information was processed by Statgraphic plus 5.1 software. Normality data were first analyzed by Kolmogorov Smirnov´s and Bartlett´s Tests and subjected to one-way analysis of variance (ANOVA) and means separated by Duncan´s Multiple Range Test (P<0.05).

RESULTS AND DISCUSSION

Effect of EM and FitoMas-E® on morphological parameters

Table 1 shows the effect of both bio-products and their combination on distinct growth parameters. Plant height is an important indicator of seedling quality to achieve a successful transplant. The tallest seedlings were recorded for those treated with FitoMas-E® (T3) and the combination of EM and FitoMas-E® (T4), with no statistical difference between them; whereas T2 showed no positive response compared to the control. Similarly, T3 and T4 reported the highest number of leaves per plant and highest stem diameter among all treatments. Root length increased with the application of EM and FitoMas-E alone or with the combination of these bio-products. The highest value was observed with the combined application (T4), followed by T3 and T2.

Table 1. Effect of effective microorganisms and FitoMas-E® on morphological parameters of pepper seedlings.

Parameters	Treatments				SE
	T1	T2	T3	T4	
Plant height	15.20 b	15.93 b	16.64 a	17.19 a	0.13
Number of leaves/plant	4.72 c	5.03 bc	5.47 b	6.01 a	0.11
Stem diameter	3.36 c	3.51 bc	3.65 b	3.98 a	0.05
Root length	7.18 d	7.80 c	8.23 b	8.82 a	0.04

T1: control (without the application of bio-products), T2: EM (4 mL m^{-2}), T3: FitoMas-E® (0.1 mL m^{-2}), T4: EM (4 mL m^{-2}) + FitoMas-E® (0.1 mL m^{-2}). SE: standard error. Different letters indicate statistical differences among treatments of each row (Duncan test, P<0.05).

These results are consistent with other authors who reported an increase in plant height after application of EM on *Phaseolus vulgaris* L. (Calero *et al*., 2017) and *Daucus carota* L. (Núñez *et al*., 2017). The increase in plant height and stem diameter was also observed in tomatoes (*Solanum lycopersicum* L.) after the application of FitoMas-E® (0.7 L ha^{-1}), after 7 and 30 days of transplantation (Ricardo and Aguilar, 2015).

Santana *et al*. (2016) studied the effect of FitoMas-E® and *Trichoderma harzianum* Rifai on the germination and seedling growth of tomatoes and reported a better response on stem length after 25 days of seed germination, compared with non-treated plants. However, the combination of both bio-products did not increase height; on the contrary, an increase was observed in the stem diameter, as well as fresh and dry weight.

The increase in stem diameter after the application of EM and/or FitoMas-E® was previously reported by Lescaille *et al*. (2015), combining two strains of EcoMic® with EM. Similarly, the application of FitoMas-E® and *Trichoderma harzianum* enhanced the germination stage, seedling growth and stem diameter of tomatoes (Santana *et al*., 2016).

Díaz *et al*. (2019) reported a stimulating effect of EM on morphological, physiological and biochemical parameters of *Sorghum bicolor* L. (Moench). After seed immersion into a solution of EM-based product IHPLUS® 6%, root and stem length, seedling vigor and the activity of α-amylase were significantly promoted.

The application of FitoMas-E® (0.1 mL m^{-2}) on *Cucumis melo* L. seedlings raised plant height, stem diameter, number of leaves per plant and root length (Pérez, 2018). The ability of FitoMas-E® to stimulate the growth of roots and shoots was also demonstrated under *in vitro* conditions by Gallego (2016), who suggested the use of FitoMas-E® as an alternative for *in vitro* rooting and shoot induction of *Saccharum* spp. cultivar.

The positive effect of FitoMas-E® on pepper seedlings growth may be associated with the presence of various active components, such as amino acids and carbohydrates chelating compounds, which may act as a delivery system of those substances that can be used by the cells as energetics or in the synthesis of new polypeptides or metabolites (Batista *et al*., 2015). The rooting properties of FitoMas-E® could be explained due of the presence of tryptophan in its formulation, which is used by plants and soil microorganisms as a precursor for the biosynthesis of indole-3-acetic acid (IAA), which stimulate root growth by enhancing cell division and elongation (Jeyanthi and Kanimozhi, 2018).

EM could have contributed to the plant promoting effect observed in the present research. *Bacillus* and *Pseudomonas* species were reported to release plant regulatory metabolites such as gibberellins and IAA, which play an important role in plant biological processes such as cell differentiation, expansion and division as well as regulation of genes (Hernández-Montiel *et al*., 2017; Yousef *et al*., 2018). Moreover, both genera are able to release low molecular weight organic acids and binding to phosphate through their hydroxyl and carboxyl groups, which provoke the conversion of the insoluble phosphates to soluble forms (Florez-Márquez *et al*., 2017; Nithyapriya *et al*., 2021). This element is an essential part of the chemical structures of important macromolecules such as nucleic acids and antioxidant compounds, and it also plays a key role in several ATP-depending metabolic pathways.

Pseudomonas species are well known to produce siderophorous compounds, which commonly chelate iron in the rhizosphere forming a complex and increasing the availability of iron to plants. This element is vital for plant metabolism since it acts as a cofactor in enzymatic reactions and redox reactions involved in the processes of aerobic respiration and photosynthesis; thus, enhancing plant growth and productivity (Sharma *et al*., 2020). *Pseudomonas* genus has also been documented to control several phytopathogens, due to their ability to produce a wide range of inhibitory substances such as hydrogen cyanide (Lakshmi *et al*., 2015) and siderophores (Singh, 2018). Similarly, *Bacillus* spp. was also found to produce those substances (Chinakwe *et al*., 2019). These mechanisms allow controlling the population of pathogen microorganisms in the soil, that negatively impact plant growth and development.

The positive effect of EM-based bioproduct on the growth of pepper seedlings, may also be associated with the presence of various fungi and yeasts that promote plant growth, which have been described to have several mechanisms to enhance plant growth. The production of various types of phytohormones has been recorded in *Trichoderma* spp. (Contreras-Cornejo *et al*., 2014), *Rhizopus* spp. (Evstatieva *et al*., 2020) and *Penicillium* sp. (Khan *et al*., 2009). In addition, *Trichoderma* and *Penicillium* have been found to be a good biofertilizers (Wakelin *et al*., 2007; Zin and Badaluddin, 2020). Similarly, *Aspergillus* was reported to convert the insoluble tri-calcium phosphate into soluble forms by processes of acidification, chelation and exchange reactions (Padmavathi, 2015). On the other hand, *Saccharomyces* has shown plant growth promotion activity through similar mechanisms described above, such as IAA production and phosphate solubilization (Shih-Feng *et al*., 2017).

Fresh and dry weight

Figure 1 shows the effect of EM and FitoMas-E® application on foliar fresh weight of pepper seedlings. The combination

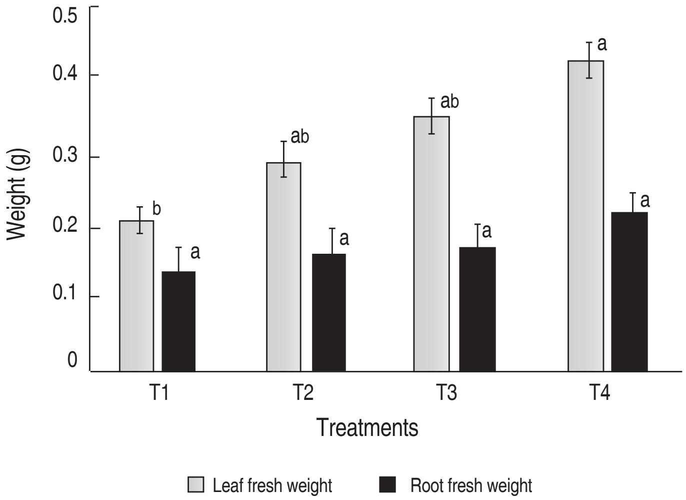

Figure 1. Effect of EM and FitoMas-E® on the foliar and root fresh weight of seedlings of *Capsicum annuum* L. T1: Control (without application of bio-products), T2: EM (4 mL m^{-2}), T3: FitoMas-E® (0.1 mL m^{-2}), T4: EM (4 mL m^{-2}) + FitoMas-E® (0.1 mL m^{-2}). Different letters indicate statistical differences among treatments for each organ (Duncan test, P<0.05).

of EM and FitoMas-E® (T4) increased the foliar fresh weight up to 0.43 g, which did not differ from the other treatments but was higher than the control. This result may be attributed to the promoted effect of combined application of EM and FitoMas-E® on the root growth, which allow the plant to absorb a higher amount of water and nutrients, that can be translocated to the upper parts of the plant and thus, increasing plant metabolism and fresh weight. The root fresh weight showed no difference among treatments and the control.

Foliar and root dry weights significantly increased after the application of EM and FitoMas-E® (Figure 2). In the case of foliar dry weight, there was no difference between T3 and T4. However, the application of EM (4 mL m^{-2}) by itself did not increase the foliar dry weight, in comparison with the control. Root dry weight showed a positive response with the combined application; however, no differences were found between control, T2 and T3.

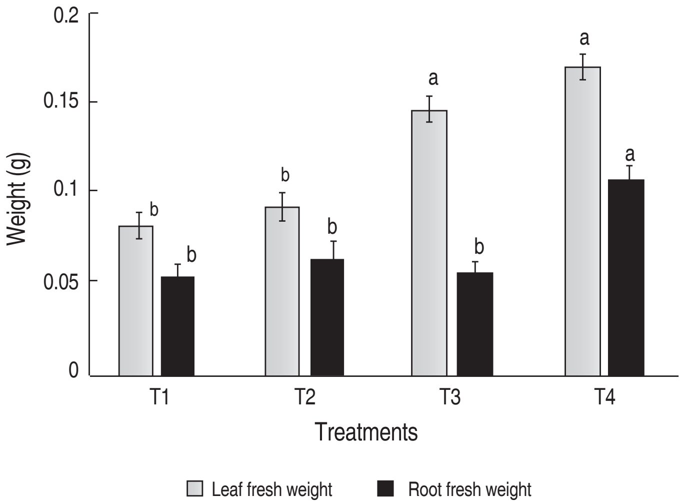

Figure 2. Effect of EM and FitoMas-E® on leaf and root dry weight of seedlings of *Capsicum annuum* L. T1: control (without application of bio-products), T2: EM (4 mL m^{-2}), T3: FitoMas-E® (0.1 mL m^{-2}), T4: EM (4 mL m^{-2}) + FitoMas-E® (0.1 mL m^{-2}). Different letters indicate statistical differences among treatments for each organ (Duncan test, P<0.05).

According to Santana *et al*. (2016), root and total fresh weight of tomatoes were improved after the application of FitoMas-E® and *T. harzianum*. Similarly, Calero *et al*. (2017) reported an increase in dry weight in *Phaseolus vulgaris* L. with the application of FitoMas-E® and the mixture of this bio-product with other bio-preparations based on effective microorganisms. The enrichment of the substrate with amino acids supplied by FitoMas-E®, may play an important role in the protein metabolism, which is essential for the accumulation of plant biomass generating a higher dry weight.

Slenderness index (SI) and shoot/root ratio

Slenderness index has been used to evaluate the seedling quality in various plant species such as *Moringa oleifera* Lam. (Castillo *et al*., 2013), *Pithecellobium dulce* (Roxb.) Benth and *Platymiscium diadelphum* S. F. Blake (Parra and Maciel, 2018). Although this parameter has not been employed for this fruit species, it was considered useful in order to assess pepper seedlings growing under organoponic conditions.

Table 2 shows the slenderness index (SI) and the shoot/root ratio (S/R) for pepper seedlings after the application of FitoMas-E® and EM. SI showed similar results for all treatments and control. The application of FitoMas-E® at 0.1 mL m^{-2} (T3) increased the shoot/root ratio, whereas this relation was similar among the rest of the treatments and control. In the present work, SI ranged from 4.32 up to 4.56 without differences among treatments with the application of the bio-products and control. The fact that FitoMas-E® and/or EM did not affect SI, may indicate a proportional increased of plant height and stem diameter, and the suitability of SI to be used to select pepper seedlings with quality.

Table 2. Effect of EM and FitoMas-E® on the Slenderness Index (SI) and shoot/root ratio (S/R) in seedlings of pepper.

Variable	Treatments				SE
	T1	T2	T3	T4	
Slenderness index (SI)	4.53 a	4.55 a	4.56 a	4.32 a	0.05
S/R ratio	1.28 b	1.41 b	2.11 a	1.66 b	0.007

T1: control (without application of bio-products), T2: EM (4 mL m^{-2}), T3: FitoMas-E® (0.1 mL m^{-2}), T4: EM (4 mL m^{-2}) + FitoMas-E® (0.1 mL m^{-2}). SE: standard error. Different letters indicate statistical differences among treatments for each parameter (Duncan test, $P<0.05$).

Terán (2014) demonstrated when working with tomatoes seedlings, that plants of high quality are those with a proportional ratio between plant height and stem width, which indicate a better support to cope with the stressful conditions created during seedling transplantation. Studies with forest species reported the highest seedling qualities for those with a low SI (short and width seedling). On the contrary, a high SI is associated with tall and thin seedlings and less possibilities of survival (Rodríguez, 2008).

The shoot/root ratio is expressed on the base of the dry weight for both organs and represents a balance between the use of water by the foliage and its absorption capacity by the roots. Cano and Cenita (2004) recommended that the ratio should not exceed the value of 2.5. According to this index, the higher quality of the plant corresponds to that of a minor ratio shoot/root. This allows to the seedlings a better probability of survival under field conditions, based on a regulation between transpiration and water absorption processes (Soriano, 2011). Literature showed few works concerning the optimal S/R ranges for different vegetable species. However, there are reports on forest species which described the optimal range between 1.5 to 2.5 for a better plant performance (Rodríguez, 2008; Mateo *et al*., 2011).

The S/R ratio in the present work ranged from 1.28 up to 2.11, which indicates a good balance between shoot transpiration and water absorption by root. It permits the aerial organs to be supplied with water and nutrients to carry out the photosynthesis and others metabolic reactions in leaves (Rodríguez, 2008). Similar results were reported in *Pinus patula* Schiede ex Schltdl. & Cham (Romero *et al*., 2012) and *Pinus montezumae* Lamb. (Hernández *et al*., 2014) on nursery conditions.

CONCLUSIONS

The application of effective microorganisms and FitoMas-E® to pepper seedlings, revealed the potential of those bio-products to improve morphological and physiological parameters, that lead the production of seedlings with quality at nursery stage and their subsequent establishment after transplantation. The combination of EM (4 mL m^{-2}) and FitoMas-E® (0.1 mL m^{-2}) showed the best results regarding plant height, number of leaves per plant, stem diameter, length of root and fresh and dry weight of leaves and roots. The results reinforce the novel approach of using those bio-products as biofertilizers, in order to replace agrochemicals and develop an effective and sustainable agriculture.

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