Research article

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Revista Facultad Nacional de Agronomía

UNIVERSIDAD NACIONAL DE COLOMBIA

Improvement of the agricultural productivity of lettuce and radish by using efficient microorganisms

Mejoramiento de la productividad agrícola de la lechuga y el rábano con el uso de microorganismos eficientes

doi: 10.15446/rfnam.v72n3.76967

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ABSTRACT

Keywords:
Biofertilizer
Lactuca sativa
Organoponic garden
Raphanus sativus

The aim of this work was to evaluate the effect of the application of efficient microorganisms (EM) on the productive performance of lettuce and radish. The experiment was carried out in an organoponic culture in the municipality of Matanzas, Cuba, from December 2017 to January 2018. Five treatments were studied: control (0), EM 8 mL m^{-2} at 0 days after the transplant (DAT) (EM 8-0), EM 8 mL m^{-2} at 15 DAT (EM 8-15), EM 10 mL m^{-2} at 0 DAT (EM 10-0), and EM 10 mL m^{-2} at 15 DAT (EM 10-15). A randomized complete block design was set with four replications per treatment. An ANOVA was applied to perform the statistical data analysis, and the Duncan's Multiple Range Test (P<0.05) was used for the comparison of means. The statistic program used was STATISTICA, version 6.0 over Windows. The parameters evaluated in lettuce were the total number of leaves, number of commercial leaves, the diameter of leaf rosette, and yield. The evaluated parameters for radish were fleshy root's diameter and weight, and yield. The results indicated a positive effect on growth-response with the application of the bio-product, which can be considered a promissory alternative for vegetable production in organoponic garden conditions. The application of EM 10 mL m^{-2} at 0 and 15 DAT showed the best productive behavior for both crops.

RESUMEN

Palabras clave:
Biofertilizante
Lactuca sativa
Jardín organopónico
Raphanus sativus

El objetivo de este trabajo fue evaluar el efecto de la aplicación de microorganismos eficientes (EM) en el rendimiento productivo de cultivos de lechuga y rábano. El experimento se llevó a cabo en un cultivo organopónico en el municipio de Matanzas, Cuba, desde diciembre de 2017 hasta enero de 2018. Se estudiaron cinco tratamientos: control (0), EM 8 mL m^{-2} a los 0 días después del trasplante (DAT) (EM 8-0), EM 8 mL m^{-2} a 15 DAT (EM 8-15), EM 10 mL m^{-2} a 0 DAT (EM 10-0) y EM 10 mL m^{-2} a 15 DAT (EM 10-15). Se estableció un diseño de bloques completos al azar con cuatro repeticiones por tratamiento. Se aplicó un ANOVA para realizar el análisis estadístico de datos, y se utilizó la prueba de rango múltiple de Duncan (P<0.05) para comparar las medias. El programa estadístico utilizado fue STATISTICA, versión 6.0 en Windows. Los parámetros evaluados en lechuga fueron el número total de hojas, el número de hojas comerciales, el diámetro de la roseta y el rendimiento. Los parámetros evaluados para el rábano fueron el diámetro y peso de la raíz carnosa y el rendimiento. Los resultados indicaron un efecto positivo en la respuesta de crecimiento con la aplicación del bio-producto, que puede considerarse una alternativa promisoria para la producción de vegetales en condiciones organopónicas de jardín. La aplicación de EM 10 mL m^{-2} a 0 y 15 DAT mostró el mejor comportamiento productivo para ambos cultivos.

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Received: December 18, 2018; Accepted: July 15, 2019
Rev. Fac. Nac. Agron. Medellín 72(3): 8937-8943. 2019 ISSN 0304-2847 / e-ISSN 2248-7026

The use of agrochemicals has grown considerably since the "Green Revolution," which has caused a reduction in the productivity of soils due to several degrading processes, such as soil compaction, salinization, desertification, and contamination with heavy metal. It provokes severe environmental problems such as eutrophication of water bodies and diseases in humans and animals (Ge *et al*., 2016; Katiyar *et al*., 2016). Therefore, the excessive production and application of non-organic synthetic fertilizers cannot be the solution to satisfy the growing need for food to supply the world population.

Several researchers have been focused on friendly, sustainable, and organic agricultural practices; which allow to reducing the costs of production, contributing to the sustainability of agricultural systems and maintaining stable the yield and crop quality (Mesa, 2016). In Cuba, at the end of the 80s, the vegetable production began to develop on a large scale in urban areas, which grew every year to reach 4,200 t in 1994, 480,000 t in 1998 and more than 1,158,452 t in 2015, without considering the productions in gardens and plots, mainly for family consumption (GNAUS, 2015).

This intensive method for vegetable production on an organic substrate has favored obtaining high yields of crops, but at the same time, it requires adequate technological discipline, where the exploitation and management of the substrates result in a vital issue. It is because plants constantly take nutrients from the soil, being removed after harvest; therefore, the productivity of the agricultural systems depend on the different initial nutrient organic resources to guarantee high yields and multiple harvests, at least for two years. At longer periods, nutrients become limited and in consequence, yield and quality decrease.

In this context, organic fertilization should be complemented with biological products based on beneficial microorganisms (the so-called plant probiotics), which stimulate growth and development of plants throughout different mechanisms such as: the synthesis of bioactive compounds like auxin, cytokinin and gibberellin (Khatab *et al*., 2015; Damam *et al*., 2016); the solubilization of phosphates (Basu *et al*., 2017; Ramírez-Gil, 2019), the production of siderophores (Stamenković *et al*., 2018), the production of lytic enzymes and antibiotics, which play an important role in the organic matter decomposition and bio-control of phytopathogens (Sabaté *et al*., 2018; Thakur *et al*. 2017).

Those biofertilizers have been pointed out as a viable alternative for achieving an agriculture development with ecological sustainability, based on low costs of production and a minimal impact on the environment (Menendez and Garcia-Fraile, 2017). For this reason, more investigations need to be done in order to study the effect of natural products on crops' growth. The aim of the present work was to evaluate the effect of the application of efficient microorganisms on the productive response of lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* L.) at organoponic conditions.

MATERIAL AND METHODS

Study area and EM applied

The experiment was carried out in an organoponic system at the municipality of Matanzas, Cuba, from December 2017 to January 2018. Lettuce (*Lactuca sativa* L. variety Riza-15) and radish (*Raphanus sativus* L. variety PS-9) were used in association. In the case of lettuce, the transplanting was used as sowing method, with six rows on the plot at a distance of 15 cm between rows and 15 cm between each plant. Direct sowing method was used for radish, which was planted in one row in the center of the bed with 3 cm between each plant. The average temperature during the experiment (dry season) was 21.7 °C, the relative humidity was 80.9%, and the average rain precipitation was 7.6 mm.

The efficient microorganisms (bio-product) was obtained from the bio-pesticide production laboratory "LABIOFAM" at the province of Matanzas. The microbiological composition analysis of the EM bio-product used was conducted at the Laboratory of Microbiology of the Faculty of Agronomy Sciences of Universidad de Matanzas, Cuba. The isolation of the microbial groups (bacteria, fungi, and yeast) from EM bio-preparation was carried out using 1 mL of the sample following the serial dilutions methodology (Stanier, 1996). Dilutions up to 10^{-6} were prepared in a sterile saline solution (0.9% of NaCl). 0.5 mL of the dilutions was inoculated on Nutrient Agar Medium for bacteria (10^{-6}), Saborout Agar Medium for fungi (10^{-5}) and Potato Dextrose Agar Medium for yeast (10^{-4}). Petri dishes for bacteria were incubated at 37 °C for 24 h, whereas fungi and yeast were grown at 30 °C for 72 h. Table 1 shows the concentration of the main microbial groups found in the initial EM preparation.

Tabla 1. Microbiological composition of EM bio-product.

Microbial groups	Concentration (CFU mL^{-1})
Bacteria	13×10^{8}
Fungi	18×10^{5}
Yeast	21×10^{6}

Experimental design

The experiment was conducted in a randomized complete block design with four replications; each one consists of an organoponic bed of 30.0 m length and 1.20 m width. With five treatments, the experimental units were of 6.0 m^2. The substrate consisted of 50% soil and 50% organic matter.

The treatments studied were:
0 = Control (without EM application).
EM 8-0 = EM (8 mL m^{-2}) at 0 days of transplanting.
EM 8-15 = EM (10 mL m^{-2}) at 0 days of transplanting.
EM 10-0 = EM (8 mL m^{-2}) at 0 and 15 days of transplanting.
EM 10-15 = EM (10 mL m^{-2}) at 0 and 15 days of transplanting.

The application of the EM bio-product was carried out early in the morning (7:00-7:30 am) using manual fumigation equipment (MATABI) of 16 liters of capacity. The application on radish was subordinated to the main crop. Insects, diseases, and weeds were intensively controlled, according to GNAUS (2007). At harvesting (28 days after seed germination in radish and 45 days after transplantation in the case of lettuce), 25 plants were randomly hand-taken in each experimental plot, and it was recorded the following morphometric and yield parameters in each crop:

Lettuce: Number of leaves per plant (by direct counting), number of commercial leaves per plant, rejecting those not suitable for commercialization; leaf rosette diameter using a measuring tape (morphometric parameters) and the yield (kg m^{-2}). Plants were weighted on with scale (Sartorius, ALC-110.4).

Radish: Fleshy root diameter (using a Vernier caliper), the weight of fleshy root and the yield, being the latter represented as kilogram per linear meter (kg m^{-1}) because of its sowing location.

Statistical analysis

The data were statistically evaluated by the Kolmogorov-Smirnov's and Bartlett's Test to verify normality and variance homogeneity, respectively. An Analysis of Variance (ANOVA) and Duncan's Multiple Ranges Test (P<0.05) (Duncan, 1955) were used for the comparison of means. STATISTICA program version 6.0 was utilized to process the experimental data.

RESULTS AND DISCUSSION

Effect the EM in lettuce

The application of EM increased the number of total leaves and the number of commercial leaves of lettuce (Table 2). Regarding the total leaves number, all the treatments with the application of efficient microorganisms displayed higher values compared with the control. Similarly, the number of commercial leaves in 0 (control) was lower than the variants with different dose of EM and times of application.

Table 2. Effect of the application of efficient microorganisms on the number of total and commercial leaves of lettuce variety Riza-15.

Treatments	Total leaves per plant	Commercial leaves per plant
0	13.29 b	12.31 b
EM 8-0	17.31 a	16.23 a
EM 8-15	17.59 a	16.59 a
EM 10-0	17.72 a	16.74 a
EM 10-15	18.05 a	17.16 a
± SE x	0.015	0.018

Different letters mean differences among treatments for each parameter (P<0.05) (Duncan, 1955). ± SE x: Standard Error of the Mean.

The higher production of commercial leaves in lettuce after EM inoculation should be positively influenced by growth parameters of the crop, which in turn determined the achieved yield. The beneficial effect of EM on the development of plant morphometric and productivity characters was also reported by several authors in different plant species such as *Spinacia oleracea* L. (Hauka *et al.*, 2016), *Phaseolus vulgaris* L. (Estrada *et al.*, 2017), *Oryza sativa* L. (Ghaffari *et al.*, 2018) and *Rubus glaucus* Benth. cv. Thornless (Robledo-Buriticá *et al.*, 2018).

The EM application increased the diameter of leaf rosette and yield (Table 3). The treatment with EM inoculation of 10 mL m^{-2} at 0 and 15 days after transplanting recorded the maximum diameter among the different variants of EM applications, which values ranged between 29.33 up to 36.56 cm. The diameter of leaf rosette in plants control was lower in comparison with all the assayed treatments.

Regarding the yield parameter in the lettuce, the treatments with the higher dose of EM (10 mL m^{-2}) and the variant EM 8 mL m^{-2} at both 0 and 15 days of transplanting promoted higher yield concerning the control; whereas the variant EM 8 mL m^{-2} at 0 days of transplanting (2.29 kg m^{-2}) showed no statistical difference with the control (1.85 kg m^{-2}).

Table 3. Effect of the application of efficient microorganisms on the diameter of leaf rosette and yield of lettuce variety Riza-15.

Treatments	Diameter of leaf rosette (cm)	Yield (kg m^{-2})
0	29.33 c	1.85 b
EM 8-0	33.08 b	2.29 ab
EM 8-15	33.43 b	2.58 a
EM 10-0	34.11 b	2.61 a
EM 10-15	36.56 a	2.67 a
± SE x	0.58	0.31

Different letters mean differences among treatments for each parameter ($P<0.05$) (Duncan, 1955). ± SE x: Standard Error of the Mean.

The positive response observed on lettuce yield might be related with the increment of the microbiota diversity in the substrate after the application of beneficial microorganisms, which in turn could improve several physiological processes such as photosynthetic activity, growth and productivity of the crops (Pedarza *et al.*, 2010). They pointed out that efficient microorganisms consist of beneficial microorganism mixture (mainly acid lactic-producer and photosynthetic bacteria, yeast, actinomycetes, and fermenting fungi), which can be applied as an inoculant to increase the microbial diversity of soils, followed by an improvement of their quality and health. This EM application allows to enhancing growth, quality, and yield of the crops.

Several researchers have reported good results in plant growth, biochemical parameters, and crop productivity by using efficient microorganisms. Arismendi (2010) observed an enhancement of the weight in lettuce variety Great Lakes 659 after the inoculation with EM. Similarly, the application of fermented organic matter, along with commercial EM on the lettuce var. Iceberg, improved the plant height, weight, and diameter of the rosette (Pomboza-Tamaquiza *et al.*, 2016). Szczech *et al.* (2016) found a better behavior of germination, fresh mass of transplants, and nitrogen content in lettuce after the application of a combination of beneficial bacterial strains.

Effect the EM on radish

The effect of the inoculation of efficient microorganisms on the diameter, weight, and yield of radish fleshy root are shown in Table 4. The application of EM revealed significant root growth-promoting effects, increasing productivity. Regarding the diameter of root and yield, all the treatments with EM showed higher results than the control without statistical difference among them. Regarding the root weight, EM 10-0 (3.89 g) and EM 10-15 (3.95 g) showed the best results in comparison with EM 8-0 and EM 8-15. The control registered the minimum yield with an average of 0.51 kg m^{-1}. The obtained yield with the application of EM was higher than the reported by the National Group of Urban Agricultural in 2007 ranged between 0.5 to 0.8 kg m^{-1}.

Table 4. Effect of the application of efficient microorganisms on diameter, fresh weight and yield of feshy root of radish variety PS-9.

Treatments	Diameter of the root (cm)	Weight of root (g)	Yield (kg m^{-1})
0	2.41 b	2.01 c	0.51 b
EM 8-0	3.24 a	2.38 b	1.16 a
EM 8-15	3.29 a	2.41 b	1.31 a
EM 10-0	3.64 a	3.89 a	1.40 a
EM 10-15	3.68 a	3.95 a	1.49 a
± SE x	0.029	0.017	0.024

Different letters mean differences among treatments for each parameter (P<0.05) (Duncan, 1955). ± SE x: Standard Error of the Mean.

The results observed in the present investigation are in agreement with those reported by Mali *et al*. (2018), who recorded an increment in root weight and yield of radish cv. Japanese white, when applied a combination of organic manure and biofertilizer. Similar trials with *Raphanus sativus* L. reported better results on leaf area, root length, fresh and dry weight, and yield; after supplying vermicompost 12.5 t ha^{-1} and a microbial consortium (Pathak *et al*., 2017). In addition, the positive effect of native microorganisms on vegetable yield have also been reported by Núñez *et al*. (2017) in carrot (*Daucus carota* L.) under organoponic conditions. Those authors observed a good response in yield and its attributes with the inoculation of a biofertilizer, mainly with a dose of 10 mL m^{-2} which produced an increment of 0.72 kg m^{-2}.

These findings could be explained due to the effect of the efficient microorganisms on plant growth in different ways, for example throughout the production of phytohormones such as auxin and cytokine-like compounds (Berger *et al*., 2015; Nghia *et al*., 2017), the solubilization of minerals such as phosphate and nitrogen (Changas-Junior *et al*., 2015) and indirectly by the production of substances with antibiotic activity which reduce the number of phytopathogen microorganisms (Grosu *et al*., 2015). Moreover, when EM interact with the organic matter, other beneficial compounds are also released, such as vitamins, organic acids, minerals, and antioxidants (IICA, 2013).

The inoculation of the substrates with efficiency microorganisms may have enhanced the soil microbiological status and the rate of decomposition of the organic matter as well, which in turn could improve the physicochemical and biological properties of soil and the content of humus (Navia-Cuetia *et al*., 2013; Campo-Martínez *et al.* 2014). These processes release minerals available for plant nutrition, which may stimulate the cellular metabolism, photosynthesis, growth, and development of crops (Kumar *et al*., 2016; Mani and Anburani, 2018).

CONCLUSIONS

The application of EM in lettuce-radish association under organoponic conditions improved morphometric parameters and yield. The dose of 10 mL m^{-2} at 0 and 15 days of transplanting, showed the best productive performance in both crops. The results obtained indicate the potential of this technology to increase the yield and quality of lettuce and radish.

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