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USE OF BIOGAS PLANT EFFLUENT AND EFFICIENT MICROORGANISMS AS BIOFERTILIZERS IN ONION PLANTS (*Allium cepa* L, CV. '*Caribe-71*')

Uso de efluente de planta de biogás y microorganismos eficientes como biofertilizantes en plantas de cebolla (*Allium cepa* L., cv. '*Caribe*-71')

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ABSTRACT. This work evaluated the effect of the application of biogas plant effluent and efficient microorganisms (ME), as biofertilizers in onion culture (*Allium cepa* L, cv. 'Caribe-71'). The experiment was carried out at a field scale (in plots of 1,5 x 8,0 m) under a Latin Square design, where four treatments were applied: I. mixture of effluent and ME 5 % (v/v); II. mixture of effluent and ME 10 % (v/v); III. mixture of effluent and ME 15 % (v/v); and IV. Control treatment: with chemical fertilizer (NPK complete formula). A total of 12 foliar applications of the biofertilizers were carried out (two before sowing and then every seven days). The indicators were determined: height of the main leaf, number of bulbs, diameter of pseudostem, diameter of bulb, number of bulbs and fresh mass of plants. The results showed that the foliar application of the biogas plant effluent and the efficient microorganisms in the form of a mixture had a positive effect on the onion culture compared to the chemical fertilization of this one, due to the contribution of nutrients and beneficial microbiota that improves soil conditions and stimulates the growth and development of the plant. This work demonstrates the possibility of incorporating organic fertilization during onion cultivation, in accordance with the principles of agroecology in the context of the necessary sustainable agricultural development.

Key Words: organic agriculture, agroecology, composting, vegetables

RESUMEN. En este trabajo se evaluó el efecto de la aplicación de efluente de planta de biogás y microorganismos eficientes (ME), como biofertilizantes en el cultivo de la cebolla (*Allium cepa* L, cv. 'Caribe-71'). Se realizó el experimento a escala de campo (en canteros de 1,5 x 8,0 m) bajo un diseño en Cuadrado Latino, donde se aplicaron cuatro tratamientos: I. Mezcla de efluente y ME al 5 % (v/v); II. Mezcla de efluente y ME al 10 % (v/v); III. Mezcla de efluente y ME al 15 % (v/v); y IV. Tratamiento control: con fertilizante químico (Fórmula completa NPK). Se realizaron en total 12 aplicaciones foliares de los biofertilizantes (dos antes de la siembra y posteriormente cada siete días). Se determinaron los indicadores: altura de la hoja principal, número de bulbos, diámetro del seudotallo, diámetro del bulbo, número de bulbos y masa fresca de las plantas. Los resultados mostraron que la aplicación foliar del efluente de planta de biogás y los microorganismos eficientes en forma de mezcla tuvieron un efecto positivo sobre el cultivo de la cebolla comparado con la fertilización química de esta, dado al aporte de nutrientes y microbiota benéfica que mejora las condiciones del suelo y estimula el crecimiento y desarrollo de la planta. Con este trabajo se demuestra por tanto la posibilidad de incorporar la fertilización orgánica durante el cultivo de la cebolla, en concordancia con los principios de la agroecología en el marco del necesario desarrollo agrícola sostenible.

Palabras clave: agricultura orgánica, agroecología, compostaje, hortalizas

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most demanded and important vegetables in Cuba (1). On a national level, Santi Spíritus province occupies the fourth place in the area sown with the cultivation

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of onion 'Caribe-71' whose yield has been 25 t ha^{-1} as average (2). Specifically in the Banao region, it is where the largest production area and best yields are concentrated; but the soils of this locality, are seriously damaged by erosion, due to the intensive production work that takes place there and to the cultivation technique used (irrigation of water by continuous drainage), consuming large amounts of chemicals per year to maintain the productive indices.

Onion is a plant that develops in different types of soils, preferably organic soils, light or sandy, silty and silty-sandy; as well as, climates ranging from warm, temperate and cold these included between 50 and 300 meters above sea level, but improves its production above 900 m (3).

On the other hand, fertilization of the onion crop is carried out without a prior integral diagnosis, and it is based on the empirical application of solid fertilizers based on chemical synthesis and common visual characteristics, as occurs in several regions (4). Also, the use of pre-established chemical fertilization doses is a fashionable reference for many producers, regardless of the fertility of their soils (5).

It is important to highlight that an inadequate management of synthetic fertilization, in addition to causing negative alterations in the physiological activities of the plant and in yield (6), degrades the soil, so it is necessary to integrate sustainable management methods for the preservation of the same (4). The use of biofertilizers would provide socioeconomic and ecological benefits among which stand out the improvement of soil quality, food quality and safety, human and animal health, as well as environmental quality (7).

The effluent from a biogas plant that treats agricultural waste provides semi-degraded organic matter and inorganic compounds, which can be used as soil conditioners in farmland (8). This bioproduct is a recognized high quality organic fertilizer (7,9) and contains on average: 8,5 % organic matter, 2,6 % nitrogen, 1,5 % phosphorus, 1,0 % potassium and a pH of 7,5 (10).

On the other hand, pork production in a decentralized manner in Cuba has generated a large amount of waste, where anaerobic digestion has shown an important role, as a treatment technology, with the energy use of biogas and obtaining an effluent, which in the majority of cases is underutilized (11). This effluent usually provides a group of microorganisms with liberating and nitrogen-fixing activity in soil and phosphate solubilizers (12).

The main use of anaerobic digestion in Cuba is to produce biogas, which is used as fuel in the cooking of food essentially. Studies on the beneficial properties of this effluent and its agronomic effect on national crops are still limited (9).

Another of the biofertilizers of greater use at present are the efficient microorganisms (ME) (13), which are constituted by a mixed culture of microorganisms, mainly, photosynthetic bacteria and lactobacilli yeasts, actinomycetes and fermenting fungi, which can be applied as an inoculant to increase the microbial diversity of soils. These develop beneficial effects, increasing the quality and health of the soils and plants, which in turn increase the growth, quality and yield of crops. It promotes germination, flowering, and the development of fruits and the reproduction of plants. In addition, it improves physical, chemical and biologically the soil environment, and suppresses pathogens and pests that promote diseases. The ME also increases the photosynthetic capacity of the crops and ensures a better germination and development of the plants and increases the effectiveness of the organic matter as fertilizer (14). So it was decided to use them in this work and check their capabilities in this way.

Therefore, the objective of this work was to evaluate the effect of the effluent application of biogas plant and efficient microorganisms on the cultivation of onion cv. 'Caribbean-71'.

MATERIALS AND METHODS

Initial conditions

The experiment of the onion culture (*Allium cepa* L.) cultivar 'Caribe-71' was developed under field conditions in the non-rainy period between the months of November to January, on a soil of material transported of low fertility type sialitic brown (fluffy and calcic), according to the classification of the soils in Cuba of 2015 (15), belonging to the Ornamental Plants Garden of the Cooperative of Credits and Services (CCS) "Nieves Morejón", Cabaiguán municipality, Santi Spíritus province.

Origin of bioproducts

The effluent from the biogas plant used was collected at the anaerobic waste treatment plant of "El colorado", Cabaiguán municipality. The efficient microorganisms used were obtained in the Agronomy Laboratory of the UNISS, from the stock strains of the so-called efficient microorganisms (ME), HI plus, patented by the Institute of Pastures and Forages "Indio Hatuey", Matanzas.

Characterization of soil and bioproducts

For the initial physical-chemical characterization of the bioproducts and the soil used in the study, the procedures described in the 2012 Standard Methods (16) were used and they were carried out in the Biogas and Environmental Engineering laboratory of the Santi Spíritus University. The dry matter (MS %) determined was determined as total solids, organic matter (MO %) as volatile solids and fixed matter (MF %) by the gravimetric method of ignition. The content of calcium ($Ca^{2+)}$) and magnesium ($Mg^{2+)}$) was determined by the EDTA titration method, in addition phosphorus as orthophosphate (PO_4^{3-}) by colorimetry and ammoniacal nitrogen ($N\text{-}NH^{4+}$) by the Kjendalh method. Potassium (K) and sodium (Na) were quantified by flame photometry and acidity (pH) by potentiometry.

Experimental design; cultural work and foliar application of bioproducts

The experiment was based on a statistical design Cuadrado Latino as described by Tony Crilly (17), design in which each treatment appeared only once per row and column (Table I), where the effect of the soil type is as homogeneous as possible in the statistical results; in addition, any effect that may exist, due to too much or lack of nutrient in it, can be suppressed. It was considered a main factor and a control factor or block variable that was introduced in order to eliminate its influence on the response variable and thus reduce the experimental error (18).

Table I. Scheme of experiment design, type Square Latin

Control	5 %	10 %	15 %
15 %	Control	5 %	10 %
10 %	15 %	Control	5 %
5 %	10 %	15 %	Control

The experiment was carried out in a plot of 110 m^2 ($11x10^{-3}$ ha), in which four planting beds of 1,5 m wide and 8,0 m long, with a distance between them of 1,0 m were built, being the planting area 48 m^2 ($4{,}8 x10^{-3}$ ha). Each planting bed was divided into four sub-beds of 2,0 m in length, reaching an area of 3,0 m^2 ($0{,}3 x10^{-3}$ ha), in a matrix form as described in Table I, similar to that designed by several authors (2,19,20). In these sub-beds the control was seeded, and the treatments of the effluent and ME at 5, 10 and 15 % (v / v).

The bulbs were planted at a distance between plants of 8,0 cm and between rows of 30,0 cm, planting in each sub-bed 125 bulbs and 500 bulbs per planting bed, for a total of 2000 bulbs throughout the experiment (21).

Irrigation was performed with an interval of four days and duration of 15 min per planting bed. In all the treatments the cultural attentions referring to risks, weeds, fertilization and phytosanitary attention, were carried out according to the Technical Instructions of the Cultivation of the Onion (22).

In addition to the treatment with the biogas plant effluent and the efficient microorganisms, doses of the aqueous preparation of tabaquina were applied weekly from 15 days after the bulbs were planted (23), to control part of the pests that attack this crop, as the Trips tabaci Lind, which manifests high attack intensity (82,5 %) to this cultivar (24). The tabaquina always applied after the application of the treatments.

The first application of the different effluent and ME mixtures was carried out before sowing; while, the NPK complete formula (22-10-6) was incorporated into the control soil. The following applications of the different mixtures of effluent and ME were carried out every seven days with manual fumigation backpack (foliar applications), at a volume of 1 L of them per mason for each experiment. The samples used as control were fertilized as indicated by the Technical Instructions of the Cultivation of the Onion (22).

Indicators of the growth and development of the evaluated onion

Four samplings were made at 29, 45, 61 and 75 days after sowing and before harvest. To select the seedlings to be sampled, a one meter long rod was placed in the center groove in each treatment and the following variables were measured with a vernier caliper: height of the main leaf (cm) and diameter of the pseudostem (mm), in addition to the number of children (U). In the sampling of the harvest at 90 days, the equatorial diameter of the bulbs (cm), the weight (fresh mass) of the sample of the sub planting bed by treatment (g), and the total weight of the planting bed per treatment (kg) were measured, with the help of an analytical balance. The total yield (t ha^{-1}) was calculated from the total planting bed weight of each treatment.

Statistical analysis

The experimental data were determined the normality of the distribution of these, if the level of “p” is not significant ($p \leq 0{,}05$) they were processed by simple analysis of variance (ANOVA) for a completely randomized design and the test of Duncan’s Multiple Ranges (25) for an error probability level of 5 %, using the statistical package SPSS version 18.0 (26). If the level of “p” is significant ($p \leq 0{,}05$) non-parametric tests were applied, such as the Kruskal-Wallis test, in addition the coefficient of variability and the standard error for the described variables were determined.

RESULTS AND DISCUSSION

Chemical composition of soil, effluent and efficient microorganisms

The chemical composition of macronutrients that was determined to the different mixtures of the organic fertilizers used as biofertilizers in the experiments is shown in Table II.

In the analysis of the initial characteristics of the soil it was observed that it is poor in organic matter. The soil contains low nitrogen content in ammoniacal and calcium form and it was not detected in the potassium and magnesium analysis conditions, four of the main nutrients for plants, only the phosphorus is found in higher concentrations.

As the concentration of the bioproducts in the solution is increased (from 5 to 15 %), an increase in the composition of the different chemical elements was observed, with the exception of Na. That is to say, with the mixture of effluent and ME, a more enriched product can be obtained in terms of the chemical composition of the main macro and micro elements considered indispensable (N, P, K, Ca, Mg and MO).

According to Álvarez-Hernández in the discussion of his article (3), where he refers to what was reported by a group of researchers; there is no exact relation of the NPK proportion required to obtain good productive results. This relationship varies greatly and depends on several factors (type of soil, physiological and genotypic characteristics of the species, environmental conditions, etc.), concluding that the onion is not sensitive to the addition of larger elements such as other horticultural crops.

Effect of bioproducts on growth and development indicators of onion cultivation

Regarding the diameter of the pseudostem, it was observed that the control reached an average diameter of 7,3 mm, statistically inferior to the treatments with the fertilization of the bioproducts, accentuating their difference from the 55 days of sowing (Figure 1).

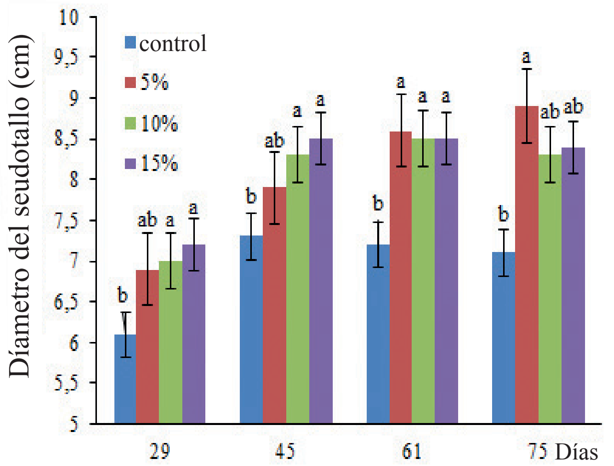
The treatment of the bioproducts at 5 %, experienced a periodic increase from the first sampling until the harvest, achieving a final pseudostem diameter of 8,9 mm. The treatment of bioproducts at 10 % reached a diameter of 8,3 mm while in the treatment of bioproducts at 15 %, an average diameter of 8,4 mm was observed. These values are similar to those obtained in an experiment carried out in Granma province (28) and slightly lower than the results reported by Estrada Prado and collaborators (29) which obtained 11,2 mm, in another type of soil.

Regarding the height of the main leaf (Figure 2), it was observed that there were differences between the fertilized plants with the bioproducts and the control from the first days of growth. After 45 days, greater growth of the main leaf was observed in those plants fertilized with the effluent and ME mixture.

Table II. Initial composition (average of three samples) of the soil and biofertilizers used in the experiment

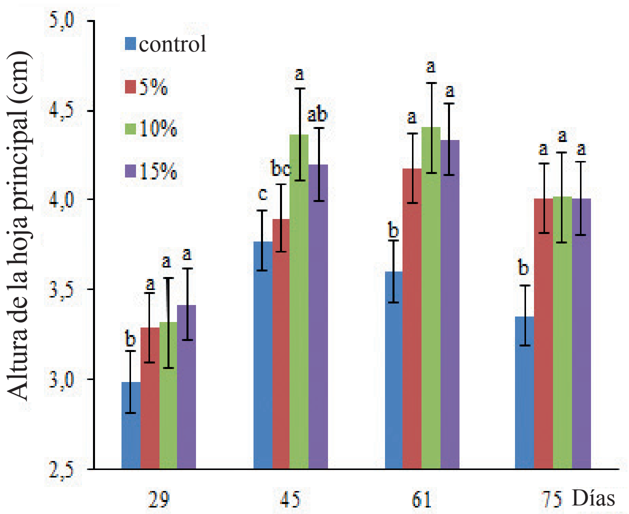
Tratamiento	pH	MS	MO	MF	$N\text{-}NH_4^+$	K^+	Na^+	PO_4^{3-}	Ca^{2+}	Mg^{2+}
			(%)				(mg L^{-1})			
Suelo	7,02	95,31	0,90	94,41	0,02	nd	0,10	11,30	4,90	nd
Efluente + ME (5 %)	7,16	0,17	0,11	0,05	0,03	0,80	4,40	0,83	24,05	4,86
Efluente + ME (10 %)	7,34	0,35	0,23	0,11	0,06	1,60	3,90	1,50	40,08	14,58
Efluente + ME (15 %)	6,61	0,53	0,68	0,32	0,08	2,00	4,00	2,17	56,11	24,30

Percentage of dry matter (DM), percentage of organic matter (OM), percentage of ashes (MF), potential of Hydrogen in water (pH), ammoniacal Nitrogen ($N\text{-}NH_4^+$), Potassium (K), Sodium (Na), Phosphorus as ortho phosphate (PO_4^{3-}), Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Not detected (nd)



Bars on the columns indicate the standard error. Equal letters do not differ significantly according to the Duncan Multiple Range Test (p≤0,05), n = 10 plants per treatment

Figure 1. Diameter of the pseudostem of the onion plant, evaluated at 29, 45, 61 and 75 days after sowing



Bars on the columns indicate the standard error. Equal letters do not differ significantly according to the Duncan Multiple Range Test (p≤0,05), n = 10 plants per treatment

Figure 2. Height of the main leaf of the onion plant, evaluated at 29, 45, 61 and 75 days after sowing

It is noteworthy that in the control plants, from the first sampling to the second, the height of the main leaf increased 78 mm, from the second to the third it decreased 27 mm and from the third to the fourth 15 mm. This was influenced by the appearance of the plague, common miner (*Liriomyza trifolii* Burgess in Comstock.), considered among the pests with the highest incidence in this variety (1). This pest was detected by observation 45 days after sowing. In these control plants, Thrips (*Thrips tabaci* Lind), better known as "onion thrips", was also detected 66 days after sowing, very common in this onion variety and season (24,27).

In the plants treated with the mixture of the 5 % bioproducts, the height of the main leaf was increased up to 61 mm from the first to the second sampling, from the second to the third 27 mm and from the third to the fourth its height was reduced by 16 mm, because it was also attacked by the aforementioned pests, but causing less damage.

In the case of plants treated with 10 % bioproducts, from the first sampling to the second, it increased 104 mm, from the second to the third it increased 4 mm and from the third to the fourth, it decreased 39 mm. The damages of the pests were greater than those observed in the plants treated with the 5 % bioproducts and lower than those observed in the control plants. In plants treated with 15 % bioproducts, from the first sample to the second, it increased 78 mm, from the second to the third 13 mm and from the third to the fourth, it decreased by 32 mm. That is, in this case there was greater affectation by pests than in the other plants analyzed with or without treatment.

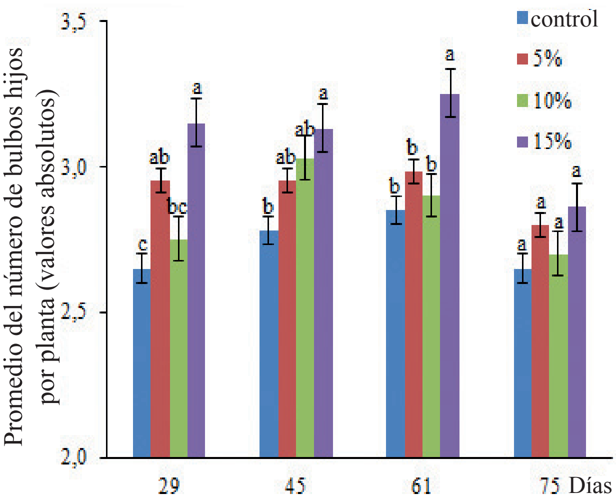
In the fourth sampling, a significant decrease in the height of the main leaf was observed, given the characteristics of the crop, since in its last stage it begins to lose the height of the leaves (3), also influenced, and the damages caused by the pests. In summary, the plants treated with the mixture of 10 % bioproducts reached the highest heights of their main leaf (44,0 cm). These values were lower than those reported for this variety grown in other regions of the country (28,29), and this is fundamentally due to the fact that soil conditions were not the most suitable for this crop, so it must emphasize the role of biofertilizers used, when they have poor soils.

Effect on yield: number of bulbs per planted bulb

In the four treatments tested, the average number of bulbs per plant or bulb planted was between 2,5 and 3,5, similar to the results obtained by other authors who obtained on average 2,5 b bulb sprouts per bulb planted, in the same variety, cultivated in the west of the country (18). Other authors have also obtained averages between 3,1 and 2,01 in other varieties, planted outside Cuba respectively, under an organic cultivation system and the same harvest time (30.31).

In general, the tendency was to increase from the first to the second sampling and to continue for the third and fourth sampling (Figure 3). Observing that the bulbs that were quantified in the first sampling were those that reached greater development and those that appeared in the second and third sampling suffered greater damages by the plagues.

The plants treated with the mixture of the 15 % bioproducts showed significant differences with respect to the control regarding the average of the number of sprouts of bulbs obtained. However, by the end of the harvest these were seriously affected by the aforementioned pests.



Bars on the columns indicate the standard error. Equal letters do not differ significantly according to the Duncan Multiple Range Test (p≤0,05), n = 10 plants per treatment

Figure 3. Number of bulbs children of the onion plant, evaluated at 29, 45, 61 and 75 days after sowing

Effect on performance: bulb diameter and weight

The measurements of the bulb diameter in the plants treated with the bioproducts at 5, 10 and 15 % had an average higher than the control, being 5% higher than the others (Table III). The result obtained was lower than the potential of this variety since studies carried out in the country reported the optimum diameter of the bulb between 4 and 5 cm (2), more recent reports report an average of 3,52 cm (19).

The other variables quantified as the weight of the quarry and the sub-canter of each treatment tested, and the equivalent yield at ha^{-1} were superior to the control, where the plants treated with the 5 % bioproducts showed the highest values, although without differences with the other concentrations of the bioproducts evaluated. Despite this, the yield values obtained are lower than those reported in the literature for yields of this variety, cultivated in other areas of the province and the country, 16-25 t ha^{-1} and average of 24,3 t ha^{-1} respectively (2,20), although these values are higher than the yields obtained in this municipality, where they do not exceed 12 t ha^{-1}, according to the reports of the Agricultural Company of Cabaiguán[A].

Therefore, it was achieved under the conditions in which the experiment was developed, increase the yields of this variety of onion in the municipality, above 14 t ha^{-1} when the bioproducts were used at 5 and 15 %, which demonstrates the effectiveness of the treatments applied, even though the characteristics of the soil where the experiment was developed were not the ideal ones, nor those required by the crop.

Organic fertilization is therefore a viable alternative, since according to some authors (32), in this crop there are producers that exceed the figure of 15 chemical treatments, only in the seedling phase and an even greater number in the transplant stage, which makes the production of onions untenable, with the consequent aggression to the environment.

Therefore, the application of effluent bioproducts from biogas plant and efficient microorganisms in the form of a mixture, stimulated the growth and development of onion plants (*Allium cepa* L. cv. 'Caribe-71'), as well as increased yield of the harvest. This result may be motivated by the content of macro and micronutrients in its composition, which has a positive effect as soil improvers and organic fertilizer in the cultivation of onions.

[A] Freñi Delgado Herrera. Especialista en Cultivos Varios. Delegación Municipal de la Agricultura, Cabaiguán. 2014.

Table III. Harvest yield of the different treatments

	Diámetro bulbo (cm)	Peso subcantero (Kg)	Peso cantero total (Kg)	Rendimiento t ha^{-1}
Control	1,89 (b)	1,385 (c)	13,372 (b)	11,14 (b)
5 %	2,14 (a)	2,064 (a)	17,584 (a)	14,65 (a)
10 %	2,01(ab)	1,884 (b)	16,762 (a)	13,90 (a)
15 %	2,08 (ab)	2,034 (a)	17,378 (a)	14,48 (a)
ES	0,09	0,13	0,85	0,71

Non-common letters in the same column indicate significant differences between the treatments evaluated according to the Multiple Range Test (p≤0,05) ES: Standard error

In addition, the application of these bioproducts improves the microbiota of the soil by stimulating the one that allows the assimilation of nutrients in each vegetative stage of the plant. This result corroborates the statements made by several authors about the potentialities of both bioproducts as high quality biofertilizers (7,8,11,13).

CONCLUSIONS

The foliar application of the biogas plant effluent and the efficient microorganisms in the form of a mixture had a positive effect on the cultivation of the onion compared to the chemical fertilization of the latter, given the contribution of nutrients and beneficial microbiota that improves soil conditions and stimulates the growth and development of the plant. This work therefore demonstrates the possibility of incorporating organic fertilization during the cultivation of onions, in accordance with the principles of agroecology within the framework of the necessary sustainable agricultural development. However, future research should focus on the technical and economic feasibility of the application of these organic fertilizers, since its relatively easy acquisition, together with the growth and development of both technologies in our country (production of efficient microorganisms and installation of biogas plants), can contribute to the formulation of new bioproducts that substitute imports of chemical fertilizers and reduce the production costs of onions without affecting their yields.

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