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AGRONOMIC EFFECT OF THE BIOSOLID IN TOMATO CULTIVATION (*Solanum lycopersicum*): BIOLOGICAL CONTROL OF *Rhizoctonia solani*

Efecto agronómico del biosólido en cultivo de tomate (*Solanum lycopersicum*): control biológico de *Rhizoctonia solani*

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ABSTRACT. In this paper the agronomic effect of effluent from the anaerobic digestion of pig manure in cultivating tomato (*Solanum lycopersicum*) and its effect as a biological control of plant pathogen *Rhizoctonia solani* are determined. For this, a study was conducted compared with bioproducts and recognized antagonists (vermicompost leachate, efficient microorganisms, *Trichoderma harzianum* and *Lecanicillium lecanii*). Experiments to determine the effect agronomic of the effluent were performed on a laboratory scale and field. The properties of the effluent as biological control were tested *in vitro* using both crude effluents as filtering. Also in PDA culture medium, superficially treated at different concentrations of the effluent, restrictive activity of possible metabolites on the development of plant pathogen *Rhizoctonia solani* was analyzed. The results showed a significant effect of effluent on soil and development of tomato plants, both laboratory scale and field (greater foliage, increase in the number of flowers, fruits and plant height), especially with application of the effluent 5 %. Regarding the effect on plant pathogens *Rhizoctonia solani* a significant reduction in the diameter of the colony, compared to the control and the other antagonists used was obtained. The surface treatment of the culture medium with concentrations digested 15, 20 and 25 % reduced the development of phytopathogen to over 70 % in only 24 h.

Key words: biocontrol, biofertilizers, Biogas, vegetables

RESUMEN. En este trabajo se determinó el efecto agronómico del efluente de la digestión anaerobia de estiércol porcino en el cultivo de tomate (*Solanum lycopersicum*), y su efecto como control biológico del fitopatógeno *Rhizoctonia solani*. Para ello se realizó un estudio comparado con bioproductos y antagonistas reconocidos (lixiviado de humus de lombriz, microorganismos eficientes, *Trichoderma harzianum* y *Lecanicillium lecanii*). Los experimentos para determinar el efecto agronómico del efluente se realizaron a escala de laboratorio y de campo. Las propiedades del efluente como control bilógico fueron evaluadas *in vitro* utilizando tanto el efluente crudo como filtrado. Además en medio de cultivo PDA tratado superficialmente a diferentes concentraciones del efluente, se analizó la actividad restrictiva de posibles metabolitos, sobre el desarrollo del fitopatógeno *Rhizoctonia solani*. Los resultados mostraron un efecto significativo del efluente sobre el suelo y el desarrollo de las plantas de tomate, tanto a escala de laboratorio como de campo (mayor follaje, aumento en el número de flores, frutos y altura de la planta), sobre todo con la aplicación del efluente al 5 %. Respecto al efecto sobre el fitopatógeno *Rhizoctonia solani* se obtuvo una reducción significativa del diámetro de la colonia, respecto al testigo y a los demás antagonistas utilizados. El tratamiento de la superficie del medio de cultivo con efluente a concentraciones de 15, 20 y 25 % redujo el desarrollo del fitopatógeno en más de un 70 % en 24 h.

Palabras clave: biocontrol, biofertilizantes, Biogás, hortalizas

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INTRODUCTION

The use of large-scale chemical fertilizers has contributed to the loss of soil quality, leading to eutrophication, heavy metal contamination, and generally a negative environmental effect (1, 2). Therefore, the use of biofertilizers is an essential strategy to improve soil quality, increase yields and quality of agricultural products (3), thus contributing to environmental quality, animal and human health.

In this sense, effluents from the anaerobic digestion of organic waste (agricultural, forestry and agricultural) constitute an alternative for the use of these as biofertilizers, whose properties have been demonstrated by several authors. These effluents from anaerobic digestion contain microbial biomass, semi-degraded organic matter and inorganic compounds, which can be used as soil conditioners on farmland (4). The composition of most effluents is, on average, 8,5 % organic matter, 2,6 % nitrogen, 1,5 % phosphorus, 1,0 % potassium and a pH of 7,5 (5). The use of effluent as a biofertilizer for sustainable agriculture would be one of the most important components of integrated nutrient management, as they are more profitable and are a renewable source of plant nutrients (6).

The effluent usually contains microorganisms such as *Pseudomonas, Klebsiella*, *Samonella, Penicillium, Shigella, Bacteriodes, Aspergillus* and *Bacillus* that enhance their agronomic properties. *Klebsiella* and *Clostridium spp*., are nitrogen releasers and fixers, while *Bacillus* and *Pseudomonas* spp., are phosphate solubilizers (7). These microorganisms accelerate microbial processes in the soil and increase the availability of nutrients assimilable by plants (3). Other authors have evaluated the biofertilizer quality of the effluent from the digestion of cow manure and chicken manure and from guinea pig manure (4,7,8).

On the other hand, the biological control of diseases is much less generalized than pests one and in particular that of foliar diseases (9), so the consumption of fungicides remains very high in some crops such as tomatoes in Cuba. For example, *Trichoderma* species have been used for the management of multiple diseases caused by soil fungi (10) and have been involved in the management of foliar diseases with promising results (10, 11). *Lecanicillium* (*Verticillium*) *lecanii* is one of the biological control agents that has the greatest potential for sustainable agriculture, due to the diversity of organisms it can regulate (10). In addition, other organic products with potential for disease management have been applied, for example worm humus or vermicompost, LIPLANT, liquid extract obtained from solid humus (9), compost, compost tea and efficient microorganisms (ME).

In Cuba, increasing pig production in a decentralized manner has favored the excessive generation of manures where anaerobic digestion has played an important role as a treatment technology, with the use of biogas energy and the obtaining of effluent, which in the majority of The cases are underused (12). Anaerobic biodigesters are used on a small scale (small producers), in the treatment of manures (pork, beef, goat, sheep, etc.) for the production of biogas, mostly used in cooking food, but there are few experiences on Use of effluents (13). In addition, studies on the application of the effluent beneficial properties and their agronomic effect on national crops have been limited (12). On the other hand, these effluents can also contribute to the biological control, especially of soil fungi, given their content of secondary metabolites. Studies on this have not been reported to date.

Therefore, the objective of this work was to evaluate the effect of effluent from the digestion of porcine manure on the growth and development of the tomato crop (*Solanum lycopersicum*), as well as its biological control effect of *Rhizoctonia solani*.

MATERIALS AND METHODS

Physical-chemical characteristics of biofertilizers and soils used

The methods described in the 2012 Standard Methods (14) were used to characterize the initial physicochemical composition of the biofertilizers used (porcine manure effluent and worm humus leachate) and the soils used in the studies. The analyzes performed in the characterization were: the percentage of dry matter (DM %) and organic matter (OM %) by the gravimetric method and Calcium (Ca^{2+}) and magnesium (Mg^{2+}) by the EDTA titration method. Phosphorus as orthophosphate (PO_4^{3-}) by colorimetry, and ammoniacal nitrogen ($N\text{-}NH_4^+$) by the Kjendalh method, potassium (K) and sodium (Na) by flame photometry and acidity (pH) by potentiometry.

Application of effluent in tomato plants to laboratory

The experiment of the effluent application in tomato plants at laboratory scale was carried out in the Biogas laboratory of the Sancti Spíritus University. 30 Rilia variety seeds were sowing by treatment in germination trays (36x36x50). Two soil types were used; soil 1: sialitic brown (fluffy and calcium) and soil 2: sialitic brown (stratigraphic and humic), according to the classification of Cuban soils 2015 (15), representative of this region.

For each soil, five treatments were used (control, anaerobic effluent of 5 and 10 % porcine manure and Californian red worm humus leachate with 5 and 10 % goat manure, based on a factorial design 22 (16).) Five treatments were applied dose of biofertilizers by foliar spray during the evaluated vegetative phase (until onset of flowering), every 7 days. Each treatment was tested in triplicate and the main indicators to evaluate were plant height and leaf number.

Application of the effluent in tomato plants to Field Scale

The research was carried out on the farm of a producer of CCS "Celia Sánchez" located in Banao town, Sancti Spíritus province. A random block experimental design was used in a brown soil with carbonate differentiation. It consisted of seven treatments and three replicates with plots of three square meters and a space between blocks of one square meter, for a total area of 0,023 ha, where the Rilia variety was planted at a distance of 90 cm of ridge for 35 cm between plants. The preparation of the soil was done with the following tasks: breaking, crossing, fluffy, furrowed, against furrowed and furrowed for planting. The humidity was maintained using the irrigation (superficial by furrows and infiltration). In addition, cleaning work was carried out according to the need of the cultivation and clotting (manual) of the plants during the experimentation. The treatments used were: A, B and C with weekly foliar applications of effluent at 5, 10 and 15 % respectively and D control (water). The main indicators to be evaluated were: plant height, number of leaves, and number of flowers, agricultural yield and components and fruit weight. Thirty plants were measured by treatments until the beginning of the harvest.

Analysis of effluent activity as biological control

For the analysis of the effluent activity as biological control, two experiments were carried out in which the capacity of the soil fungus *Rhizoctonia* solani (*R. solani*), considered phytopathogenic for the tomato, was evaluated. The filtered effluent (by 0,2 μm biological filter) and crude effluent were used in comparison with recognized antagonist bioproducts (*Trichoderma harzianum*, *Lecanicillium lecanii*) and also efficient microorganisms (EM), made in the laboratory of biofertilizers of the Sancti Spíritus University, using as inoculum the microbial consortium IH-plus commercialized by the Station of Pastures and Forage "Indio Hatuey".

Experiment 1: Evaluation of the ability of the filtrates of several liquid bioproducts in the reduction of phytopathogen growth, when mixed at 25 % (v/v) in potato dextrose agar medium (PDA). The evaluated bioproducts were: effluent, *Trichoderma harzianum A-34* liquid with conidia (without preservatives), *Trichoderma harzianum* A-34 liquid fermented to mycelial phase without conidia (without preservatives), *Lecanicillium lecanii* strain LV-5 liquid with conidia (without preservatives) and ME. A strain of *Rhizoctonia solani* was used as control. A 5 mm disk of the phytopathogen *Rhizoctonia solani* was seeded in the center of the Petri dishes filled with the PDA and 25 % (v/v) of the different filtrates respectively and incubated at 26±1 °C for three days. Five replicates were used for each bioproduct evaluated and the diameter of *R. solani* colony was measured daily in cm.

Experiment 2: evaluation of the developmental capacity or invasion of the phytopathogen on the surface of the culture medium treated with the complete (unfiltered) bioproducts. The effect of the effluent and EM at concentrations of 5, 10, 15, 20 and 25 % (v/v) in sterile water applied over the entire surface of the PDA medium in Petri dishes was evaluated. For this purpose, five replicates were used and the phytopathogen was planted in the center of each plate, placed in incubation at 26±1 °C for 48 h. The diameter of the fungus colony (cm) was measured at 24 and 48 h.

Statistical analysis

The experimental data were processed to verify normality using the nonparametric tests of Kolmogorov-Smirnov (17) and Kruskal-Wallis with the Mann-Whitney test. In cases where the ANOVA premises were not fulfilled, the appropriate transformations were made to the variable analyzed. If the two previous premises were verified, a simple classification variance and bifactorial ANOVA analysis was used for the completely randomized design and Duncan's Multiple Range Test (18) for a 5 % error probability level, using the package SPSS version 18 (19). In addition, the coefficient of variability and the standard error for the described variables were determined.

RESULTS AND DISCUSSION

Results of the physico-chemical characterization of biofertilizers and soils used

In Table I, the initial characteristics of the materials used in the test of the biofertilizer capacity of the effluent of anaerobic digestion and leachate of worm humus in two types of soils can be observed.

Table I. Initial composition (average three samples) of the biofertilizers and soils used

Sample	OM (%)	DM (%)	pH	NH_4^+-N (mg*L^{-1})	PO_4^{3-} (mg*L^{-1})	Na (mg*L^{-1})	K (mg*L^{-1})	Ca^{2+} (10^3 mg*L^{-1})	Mg^{2+} (10^3 mg*L^{-1})
Soil 1	4,6	82,6	7,8	100,0	11,4	26,0	1,0	4,9	0,0
Soil 2	7,8	79,8	7,6	0,0	6,5	7,0	3,0	0,8	1,9
Leachate	22,8	0,3	6,5	0,0	44,4	45,0	16,0	13,1	1,9
digested	56,9	1,5	7,5	300,0	1,4	45,0	16,0	21,3	2,4

According to the analytical procedures, the soils used in the study showed variability in their composition, with soil 1 being poorer in organic matter than soil 2 and slightly more alkaline, characteristic of this type of soil. In general soil 1 has a higher content of nitrogen, phosphorus, favorable to crop growth, sodium and calcium, showing a clayier texture than soil 2, only in the potassium elements (generally low for both, this being element of the essential elements for plants) and magnesium, soil 2 surpassed the soil 1. It can be said that both soils are not rich in nutrients (macro elements) and require the incorporation of the same, in order to enhance their agricultural capacities.

In the case of biofertilizers chosen for the study, variability in results is also observed. As for the pH, the effluent presents slightly alkaline values, which may not be favorable for some cultures, which prefer slightly acidic pH, as present in the leachate. With the exception of the phosphorus content, the effluent had values higher or equal in the other parameters evaluated, especially in assimilable nitrogen content, thus presenting better biofertilizer characteristics than the earthworm humus leachate.

After applying the biofertilizers and finishing the experiment, the soil of each treatment was analyzed to see in which they benefited. Table II shows the values of the parameters evaluated to the soils, observing that in the soil 1 the application of the sludge and the leachate in minimum doses did not represent any impact on organic matter with respect to the control. In the case of soil 2 with fewer doses, organic matter was maintained above the control. The pH value for both soils treated with biofertilizers remained close to neutrality.

The determination of inorganic phosphates showed that the mud had a significant contribution, which was to be expected due to its high initial phosphate concentration. Regarding the determination of sodium and potassium, a higher contribution was obtained from leachate in soil 1. For soil 2, the Na concentration contributed by the effluent was significant, and both biofertilizers increased their concentration in K. As for calcium, a significant contribution of biofertilizers was not observed, although a significant contribution of Magnesium by the effluent in the soil 2 was noted.

Table II. Final composition (average three samples) of the soils used after the application of biofertilizers

	OM (%)	DM (%)	pH	PO_4^{3-} (mg L^{-1})	Na (mg L^{-1})	K (mg L^{-1})	Ca^{2+} (10^3 mg L^{-1})	Mg^{2+} (10^3 mg L^{-1})
Control soil 1	2,3	84,0	6,1	9,9	3,0	0,0	16,4	0,0
Effluent (5 %)	3,7	84,3	6,9	33,8	5,0	1,0	10,7	0,0
Effluent (10 %)	7,0	77,3	6,5	113,0	2,0	2,0	13,1	0,0
Leachate (5 %)	2,9	82,5	7,4	14,4	9,0	2,0	8,2	1,0
Leachate (10%)	3,2	81,1	6,9	18,6	13,0	7,0	11,5	0,0
Control soil 2	9,5	56,8	7,8	34,1	9,0	11,0	9,8	7,8
Effluent (5 %)	14,7	62,9	7,5	29,4	14,0	6,0	8,2	4,9
Effluent (10 %)	7,7	64,4	7,6	113,0	12,0	10,0	9,8	2,9
Leachate (5%)	8,5	66,2	7,7	11,6	10,0	4,0	9,8	1,9
Leachate (10%)	7,8	61,0	7,6	0,4	10,0	10,0	8,2	0,0

Effect of effluent application on tomato plants to laboratory scale

As for the germination of tomato seeds, 75-98 % of germination was obtained in both types of soil and no significant differences were observed regarding the control.

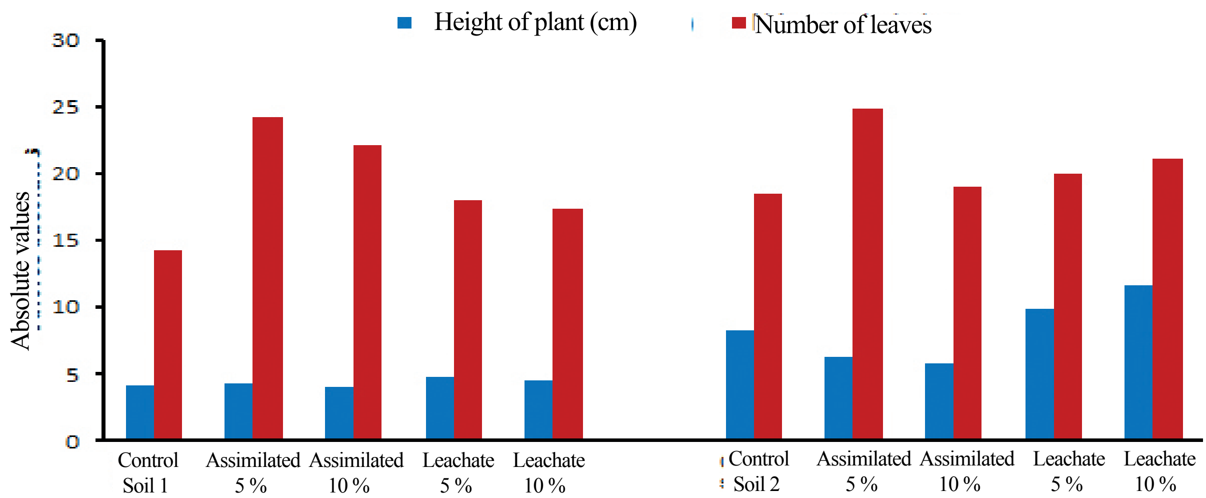
Regarding the height of the plants and the number of leaves, it was obtained that at the 19 days of germination there were still no differences regarding the control; however, after 61 days this difference was significant (Figure 1). In soil 1 there were no significant differences in plant height, but in the number of leaves, where the application of biofertilizers favored this parameter with respect to the control, being the most favored the plants fertilized with the effluent (5 and 10 %), A result that may be caused by the greater presence of nutrients in the effluent than in the leachate. In soil 2, significant differences were observed in plant height, with the most favorable being those fertilized with leachate and lower results were obtained for plants fertilized with effluent. Regarding the number of leaves, this difference is significant only for the minimum effluent dose (5 %), the value being higher.

In general, the number of leaves per plant for all treatments was greater than the control, highlighting that the effluent 5 % showed the best results, thus confirming the foliar benefit in plants that are treated with this type of biofertilizers (20, 21). These results are in agreement with those published by other authors (13), who used two doses of effluent that marked statistical differences for height after 32 days of establishing the bean (*Phaseolus vulgaris* L.), reaching the highest height (0,65 m) and the highest growth rate (0,27 m) in the last period, a result that also corresponds to the increase in plant growth caused by the application of the effluent to the soil (2).

At 64 days (end of experimentation) the number of roots was better with the application of effluent 5 % in both soils, which is why these plants are considered to be more robust (resistant) and adaptable to environmental and nutritional changes. In general, for both soils, the root length did not show a significant difference. In addition, partial flowering was observed at 64 days in the case of soil treatment 2 with 5 % leachate and complete flowering with 5 % effluent. This suggests that there is a positive (hormonal) influence in this vegetative stage of the plant with the application of biofertilizers.

Effect of effluent application to tomato plants (field field)

Table III shows the results of the chemical composition of the soil and the biofertilizer used in the experiment, observing that in the main parameters of agricultural interest, the soil presents values, lower than the effluent. In general, the effluent will contribute organic matter (OM), nitrogen (N), phosphorus (PO_4^{3-}), potassium (K) and sodium (Na), which favor and increase the agroproductive capacities of the soil, raising production values.



A simple classification variance analysis was used with the Duncan Multiple Rank Test for a 5% error probability level and the non-parametric Mann-Whitney test

Figure 1. Mean values of plant height and number of leaves for each fertilization alternative, at the end of the experiment (64 days)

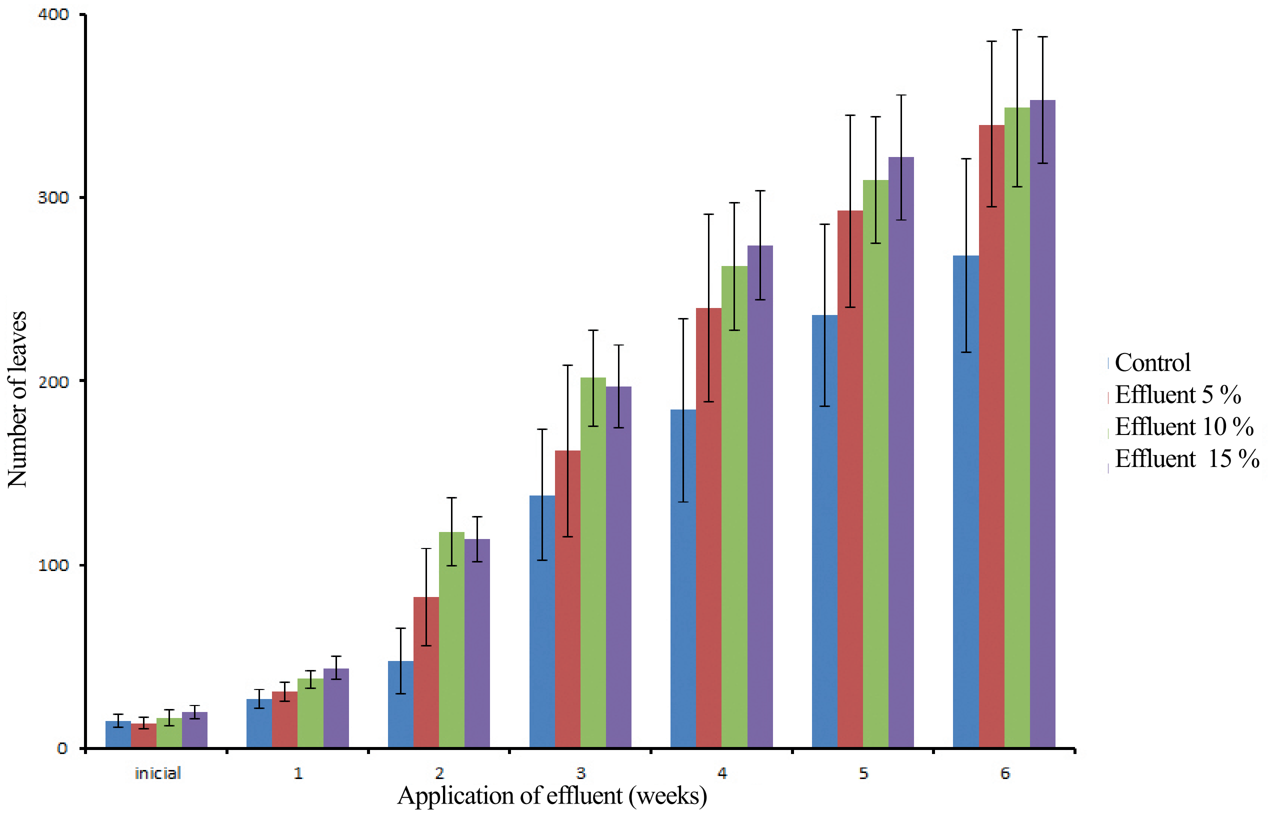
Due to the liquid nature of the effluent it is logical to find values of dry and fixed matter (DM and FM) higher in the soil than in the effluent, as well as pH values, being slightly acidic for this type of soil, and slightly basic for the effluent due to the production process of the same.

Similar to laboratory experiments, with respect to plant foliage (number of leaves), from the second application significant differences were observed with respect to the control, but not among the different effluent concentrations tested (Figure 2).

The tomato plants were significantly higher from the second application of the effluent (Figure 3). Regarding the number of fruits per plant, after the fifth application, significant differences were observed with respect to the control, where the 5 % effluent showed the best results (Figure 4). Similar results (22, 23) showed an increase in the average number of fruits per plant with the use of FitoMas-E® (commercial organic fertilizer). The number of fruits per plants is a direct indicator of crop yield (22, 23).

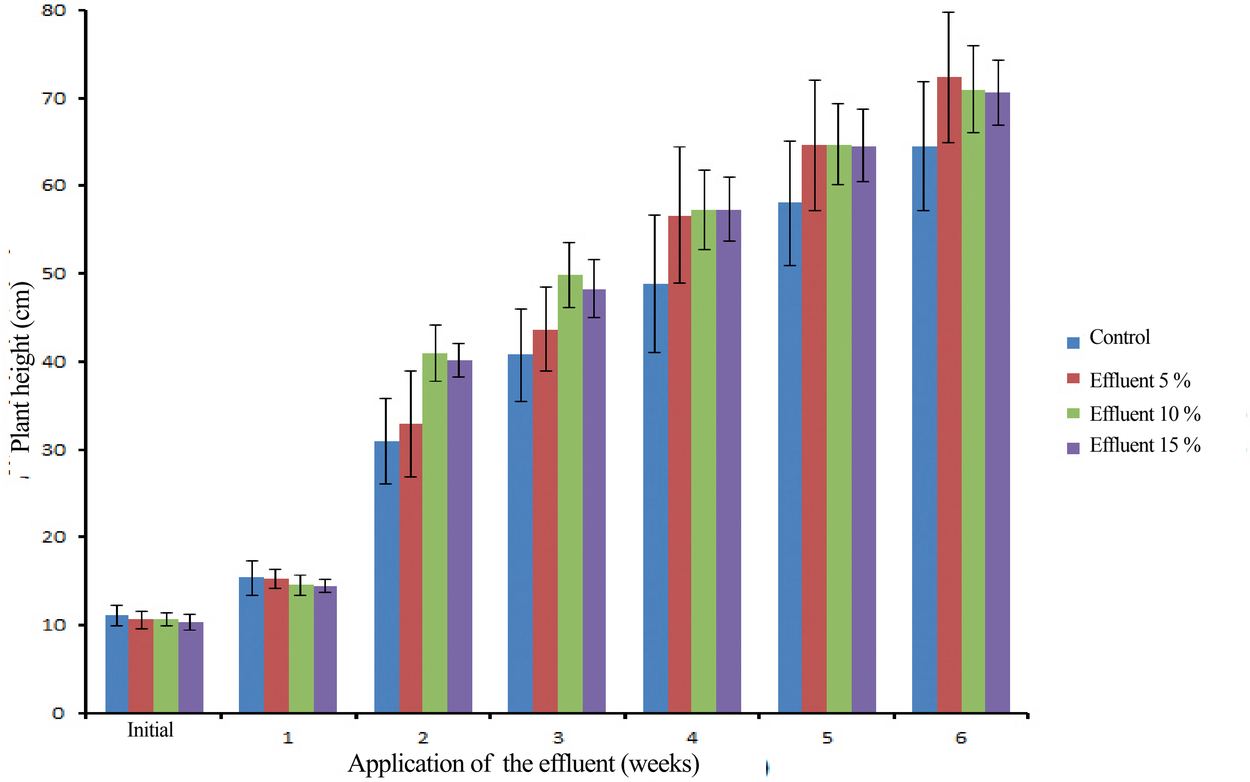
Table III. Initial composition (average of three samples) of the biofertilizer and soil used in the experiment

Sample	MS (%)	MO (%)	FM (%)	pH	$N\text{-}NH_4^+$ (mg L^{-1})	K (mg L^{-1})	Na (mg L^{-1})	PO_4^{3-} (10^{-3} mg L^{-1})
Soil	94,2	0,9	93,1	5,7	10,0	1,0	2,0	26,0
Effluent	1,0	1,7	0,5	7,6	540,0	16,0	44,0	33,0



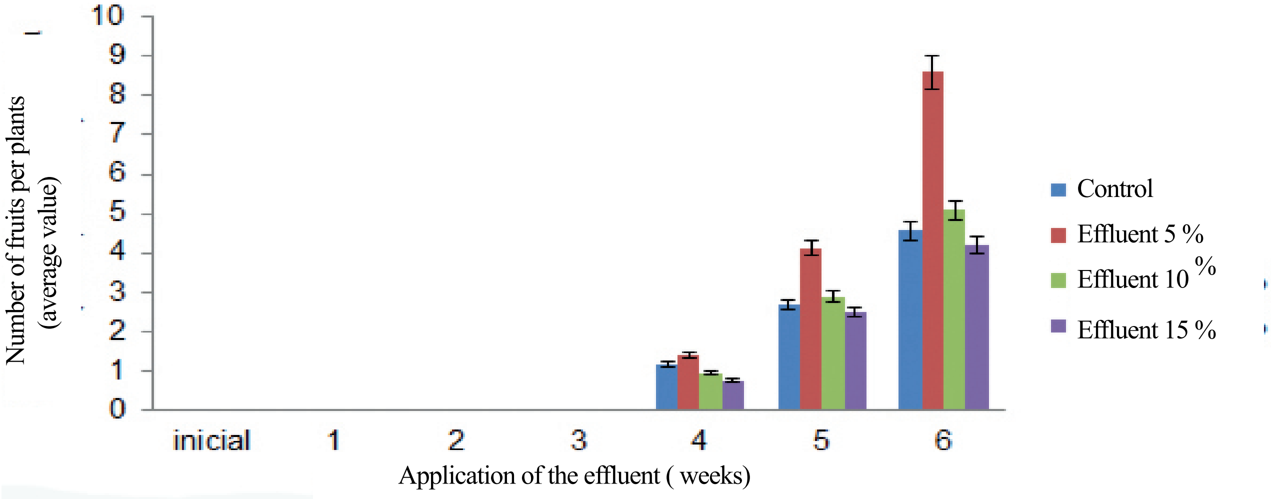
The bars indicate the standard deviation of the sample where 30 plants per treatment were analyzed as sample size. It was used a simple classification variance analysis and the Duncan Multiple Rank Test for an error probability level of 5 %

Figure 2. Average number of leaves at different times of effluent application at concentrations of 5, 10 and 15 %



The bars indicate the standard deviation of the sample where 30 plants per treatment were analyzed as sample size. It is used a simple classification variance analysis and the Duncan Multiple Rank Test for an error probability level of 5%

Figure 3. Average height of the plants at different times of effluent application at concentrations of 5, 10 and 15 %



It is used a simple classification variance analysis and the Duncan Multiple Rank Test for an error probability level of 5%

Figure 4. Average number of fruits at different times of effluent application at concentrations of 5, 10 and 15 %

Table IV shows the production of each of the variants in the different evaluations made with statistical differences among these and from these with the control from week five of sampling, where the best variant was the 5 % effluent with a mean of 33,06 fruits per plant which exceeded the control in more than 12 fruits, the treatment where the effluent was applied to 10 % reached an average superior to the control in more than two average fruits per plants. However, in general, these values are slightly lower than those reported by different researchers (23), where an average of 40,55 fruits per plant was achieved with the application of FitoMas-E®.

Table IV. Average number of fruits per plant in Rilia tomato variety

Treatments	Weeks of sampling				Total of harvested fruits
	4	5	6	7*	
Control	1,40[ab]	2,90[b]	4,90[bc]	11,67[de]	20,87[bc]
Effluent 5 %	1,73[a]	4,03[a]	8,13[a]	19,17[a]	33,06[a]
Effluent 10 %	1,37[ab]	2,90[b]	5,30[b]	13,70[bc]	23,27[b]
Effluent 15 %	1,33[ab]	2,57[b]	4,20[cd]	11,23[de]	20,33[c]
CV (%)	*5,64*	*10,26*	*13,91*	*12,89*	*12,15*
ES	*0,054*	*0,090*	*0,140*	*0,266*	*0,114*

CV: Coefficient of variation. ES: Standard error. * In week seven the harvest was realized
Non-common letters in the same column indicate significant differences between treatments evaluated by the Duncan Multiple Rank Test for a 5 % error probability level (p≤0.05)

Effect of effluent biofungicity

Experiment No. 1: With the exception of the *T. harzianum* A-34 filtrate with conidia where the pathogen showed a higher development than the untreated control, all remaining filtrates at 25 % concentration in PDA medium reduced the growth of colonies of *R. solani*, similar to the results obtained in several investigations (24), but with the *fusarium* phytopathogen. The application of effluent was the one that had the best antagonistic effect, since it reduced the growth of *R. solani* colonies in less than 24 h (Figure 5), followed by *L. lecanii* and DM and finally *T. harzianum* without conidia. The *T. harzianum* with conidia showed no difference with the control of *R. solani*. In a similar investigation (25), the PDA medium was amended with 50 % of a filtrate of a liquid product of *T. harzianum* A-34 with conidia by static route obtaining a reduction of 28,4 % of the diameter of the colony of *R. solani*.

The effluent and ME filtration, whose use is being initiated in Cuba, reduced the development of *R. solani* by 84,9 % and 57,8 %, respectively, demonstrating its potential for its use in the management of this fungus. In this way, it is shown how some publications indicate the microorganisms present in the effluent and ME if they have an antagonistic effect on phytopathogens (26). On the other hand, the filtrate of *L. lecanii* LV-5, used in Cuba as an entomopathogenic fungus, reduced the development of *R. solani* to 73,4 %, indicating that although it is not an antagonist with marked competitive activity with phytopathogens produces metabolites that showed a much more marked activity than *T. harzianum* A-34 and could be evaluated the possible use of liquid products and indeed their metabolites in the management of diseases.

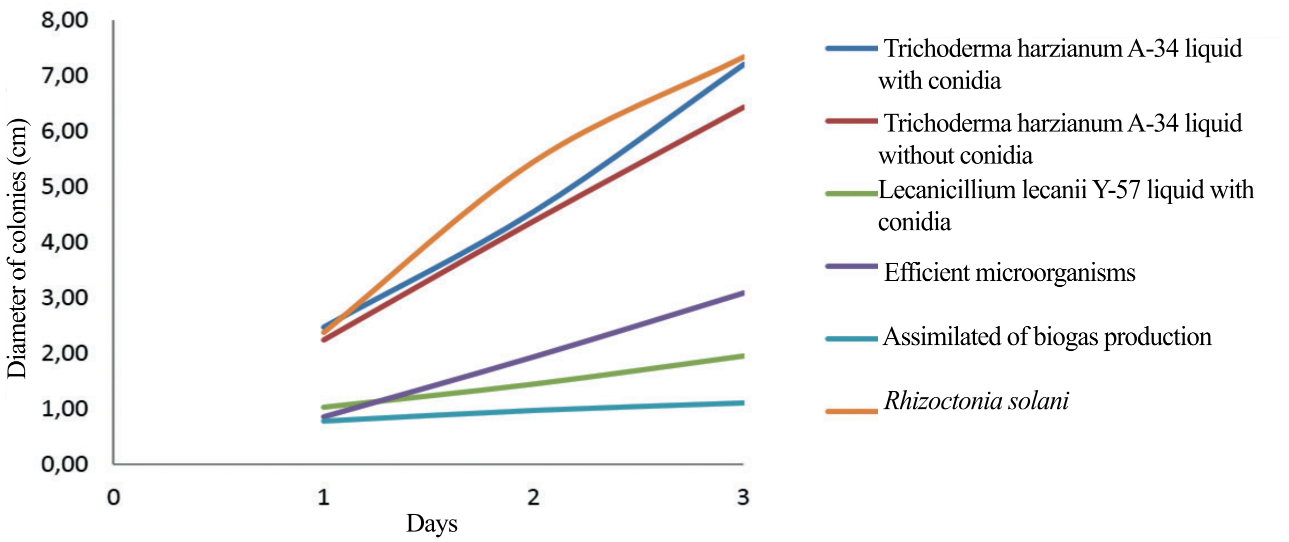


Figure 5. Growth of *Rhizoctonia solani* on potato dextrose agar (PDA) treated with the filtrates of liquid bioproducts

Experiment No. 2: The second experiment consisted in applying the effluent and efficient microorganisms (EM) as complete products, that is, unfiltered, to the plates with PDA medium, where the *R. solani* phytopathogen was subsequently planted. In this case, the growth of phytopathogen (its invasiveness) was quantified in the plates with different concentrations of both bioproducts (Table V). The results showed that, when EM was applied at concentrations of 5 to 25 %, the growth of phytopathogen colonies was significantly reduced; reaching values higher than 50 % at concentrations of 10 to 20 % EM and above 70 % at 25 % give me. When the effluent addition was evaluated, similar results were obtained, except that with this bioproduct, although at the concentration of 25 % a similar reduction is achieved than with EM, in the order of 70 %, at concentrations below 15 and 20 %, Even higher reductions are achieved and do not differ significantly from the values obtained with effluent at 25 %, but in relation to the lowest of 5 and 10 %. It is noteworthy that in these experiments with the complete bioproducts, the development of microorganisms in the surface of the environment was observed, so that not only had the effect of the metabolites but also the competition effect of the microorganisms coming from the bioproducts that contributed to limit the development of the phytopathogen.

CONCLUSIONS

The comparative study of the anaerobic digestion effluent of porcine manure and leachate of worm humus made it possible to discern its positive effect on tomato cultivation in different soils, where these were improved in terms of availability of organic matter and micronutrients. Better results were obtained with the effluent at low concentrations (5 %) when sialitic (fluffy and calcium) soils are available, but effluent or leachate can be used when sialytic (humic and stratigraphic) soils are available. At field scale, a positive effect of effluent application was also obtained, as it favored an increase in the number of fruits per plant with only 5 % of the effluent. The effluent also had biofungicidal properties for the biological control of the phytopathogen *R. solani*, reducing by more than 70 % at concentrations of 15 to 25 % the growth of its colonies in 24 h. These results open a field of research on its use in the treatment of seeds, for the application to soil of seedlings or plantations, as well as in its evaluation for the management of other diseases caused by soil microorganisms and particularly for the management of foliar pathogens so necessary in Cuba for the reduction of the use of chemical fungicides.

Table V. Development of *Rhizoctonia solani* on PDA agar medium treated surface with effluent at different concentrations

Treatments		Diameter of colony (cm) in each tiem of incubation		Reduction of colony size (in %) i each treatment	
	24 h	48 h	24 h	48 h	
Control (*R. solani*)		3,84 [a]	4,18 [a]		
Efficient microrganisms (in %)	5	2,58 [b]	2,80 [b]	32,81	33,01
	10	1,78 [c]	1,82 [c]	53,65	56,46
	15	1,60 [c]	1,70 [c]	58,33	59,33
	20	1,74 [c]	1,88 [c]	54,69	55,02
	25	1,08 [d]	1,16 [d]	71,86	72,25
CV (%)		*11,07*	*11,71*		
ES		*0,15*	*0,17*		
Effluent (in %)	5	2,04 [b]	2,22 [b]	46,88	46,89
	10	1,22 [c]	1,50 [c]	68,23	64,11
	15	0,98 [c]	1,48 [c]	74,48	64,59
	20	0,96 [c]	1,26 [c]	75,00	69,86
	25	1,06 [c]	1,10 [c]	72,40	73,68
CV (%)		*27,21*	*25,1*		
ES		*0,29*	*0,31*		

Figures followed by non-common letters represent significant difference according to the Duncan Multiple Rank test ($p < 0.05$)
CV: coefficient of variation. ES: Standard error

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