ISSN impreso: 0258-5936
ISSN digital: 1819-4087

Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas
http://ediciones.inca.edu.cu

EFFECT OF LEBAME ON GERMINATION OF TOMATO (*Solanum lycopersicum* L.) SEEDS

Efecto del LEBAME en la germinación de semillas de tomate (Solanum lycopersicum L.)

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ABSTRACT. The inoculation of efficient microorganisms to the ecosystem improves the soil quality and the plant growth, as well as, it helps the decomposition process of organic materials. The aim of this study was to evaluate the effectiveness of bioproduct LEBAME, determining dilutions as well as imbibition times on tomato germination of seeds. The study was carried out as a randomized complete bifactorial experimental design and treatments were combinations of three imbibition times (15, 30 y 60 minutes), four dilutions of LEBAME (2,5; 5; 10; 15 mL L^{-1}) and one control imbibed with water (0 mL L^{-1}). Data were processed through a factorial ANOVA using the IBM SPSS (version 19) program. Results showed a positive response of LEBAME on germination of tomato seeds. The dilutions studied were statistically different between them; those of 5, 10 and 15 mL L-^{1} were significantly higher. Although with only 5 mL L-^{1} it was enough to get the greater stimulation and an increase in length of the radicle and the hypocotyl, reaching values of 21 and 32 % respectively, compared to the control imbibed with water. Taking into account the results obtained in this research, the positive effect of the product LEBAME by stimulating the germination process was demonstrated, so it can be considered a promising product for Cuban agriculture.

Key words: growth, stimulation, vegetables, microorganisms

RESUMEN. La inoculación de microorganismos eficientes al ecosistema mejora la calidad del suelo y el crecimiento de las plantas; así como, ayuda al proceso de descomposición de materiales orgánicos. El presente trabajo tuvo como objetivo evaluar la efectividad del bioproducto LEBAME determinando las diluciones y los tiempos de imbibición en la germinación de las semillas de tomate. El experimento se llevó a cabo mediante un diseño completamente al azar con arreglo factorial. Los tratamientos fueron combinaciones de tres niveles de tiempos de imbibición (15, 30 y 60 minutos), con cuatro diluciones de LEBAME (2,5; 5; 10; 15 mL L^{-1}) y un control embebido en agua (0 mL L^{-1}). Los datos fueron procesados a través de un ANOVA factorial utilizando el programa IBM SPSS Statistics (versión 19). Los resultados mostraron una respuesta positiva del LEBAME sobre la germinación de las semillas de tomate. Las diluciones estudiadas difirieron estadísticamente entre ellas; las de 5, 10 y 15 mL L^{-1} fueron significativamente superiores, aunque con solo 5 mL L^{-1} fue suficiente para obtener el mayor estímulo y un incremento de la longitud de la radícula y del hipocotilo alcanzando valores con respecto al control embebido en agua de 21 y 32 % respectivamente. Teniendo en cuenta los resultados obtenidos en esta investigación se demostró, el efecto positivo del bioproducto LEBAME al estimular el proceso de germinación, considerándose un producto promisorio para la agricultura cubana.

Palabras clave: crecimiento, estimulación, hortalizas, microorganismos

INTRODUCTION

The tomato is the most widely disseminated vegetable in the world and the one with the highest economic value. It is the second most important species in the genus *Solanum spp.*, for its role in the eating habits of a large part of the world population[A].

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[A] Foolad M. Genome mapping and molecular breeding of tomato. International Journal of Plant Genomics. 2007;1-52. doi: 10.1155/2007/64358

In Cuba, in 2014 the planted area of this crop occupied 44,885 hectares, which represents 22,2 % of the area sown with vegetables, with a production level of 454 112 tons and an average yield of 10,12 t ha^{-1}. Compared to the year 2013, there was a decrease in the area harvested, the production obtained and the last indicator mentioned above in 17,32; 33,03 and 18,98 % respectively[B].

In this sense, as the years go by, the damage caused by the indiscriminate and unconscious use of fungicides, pesticides and mineral fertilizers becomes more evident, causing negative and cumulative effects on soil, plants, animals and man. The agricultural practices carried out during decades have led to an imbalance in the microbial ecology of the soil, which manifests itself in its physical, chemical and biological aspects (1).

Biofertilizers are products based on microorganisms that normally live in the soil, although in low populations, by increasing them through artificial inoculation, they are able to make available to plants, through their biological activity, a significant part of the substances nutrients they need for their development; as well as, supplying hormonal substances or growth promoters (2). The importance of these bioproducts lies in their ability to supplement or mobilize nutrients with minimal use of non-renewable resources, which can be applied in small units to solve local problems and that do not pollute the environment (3).

Plant growth promoting rhizobacteria (RPCV, according its acronyms in Spanish) influence plants through a multitude of different mechanisms and there are extensive reviews in the literature that describe them (4).

One of the bioproducts successfully used in agriculture are the efficient microorganisms (ME) which were developed in the 70s, by the Japanese professor Teruo Higa. Theoretically, this commercial product is essentially made up of three different types of organisms: yeasts, lactic acid bacteria and photosynthetic bacteria (5), finding that the success of its enhancing effect was in its mixture; that is why it is said that the sum of the three has a greater effect than each one separately[C].

Initially, the MEs were developed for the improvement of soils and the treatment of agricultural residues as a way of nutrition for plants. In recent times and in light of contemporary demands characterized by the global demand for a healthier production, interest in the use of biofertilizers has been increasing progressively. On the other hand, microorganisms manage the stability and productivity of agroecosystems and several investigations are directed at the benefit they bring to agricultural productivity (6,7).

According to this background, the objective of this work was to evaluate the effectiveness of the LEBAME bioproduct by determining the dilutions and imbibition times with the greatest effect on the germination of tomato seeds.

MATERIALS AND METHODS

The experimental work was carried out in December 2013, at the National Institute of Agricultural Sciences (INCA), located in San José de las Lajas, Mayabeque province, in the laboratory of the department of plant breeding. The indicator crop was tomato (*Solanum lycopersicum* L.), cultivar 'Mara' (8).

The bioproduct studied was LEBAME, obtained by the Cuban Institute of Investigations of Sugar Cane Derivatives (ICIDCA, according its acronyms in Spanish), which is presented in liquid form and it is composed of a combination of efficient microorganisms of the genera *Bacillus subtilis, Lactobacillus bulgaricum* and *Saccharomyces cervisiae*, with a title of 10^6 ufc mL^{-1}.

To study the germinative response of tomato seeds to the application of different dilutions and imbibition times in LEBAME, the experiment was carried out with a completely randomized design with a factorial arrangement. The treatments were made up of level combinations of two factors (A: imbibition time and B: dilutions); three different times (A: 15, 30 and 60 minutes) were combined with four dilutions (B: 2.5; 5; 10; 15 mL L^{-1}) and a control imbibed in water; giving rise to 15 treatments with five repetitions for a total of 80 petri dishes. The combinations of the treatments studied are shown in Table I.

The tomato seeds were placed in glass jars containing 100 mL of the bioproduct. Seeding of 20 seeds per plate was performed using filter paper as a humidified substrate with 5 mL of distilled water, remaining in the dark. The number of germinated seeds was evaluated daily on the five plates of each treatment, with the emission of the radicle as germination criterion, from which the days and germination percentages were determined. These data were transformed by the formula arcos√; In addition, the germination rate index (IVG) was calculated using the formula:

$$IVG=\Sigma(ni/ti) \quad (9).$$

where:
IVG - Germination speed index
ni - number of germinated seeds
ti - time necessary to reach the highest percentage of germination

[B] ONEI. Agricultura, Ganadería, Silvicultura y Pesca. In: Anuario estadístico de Cuba 2014; 2015. 33 p.

[C] Higa T. Una revolución para salvar la tierra [Internet]. EM Res Organ Okinawa; 1993 [cited 2015 Dec 10]. Available from: http: //www.biopunto.cl/

Table I. Description of the treatments studied

No	Treatments	
	D	TI (minuts)
1		15
2	2,5 mL L^{-1}	30
3		60
4		15
5	5 mL L^{-1}	30
6		60
7		15
8	10 mL L^{-1}	30
9		60
10		15
11	15 mL L^{-1}	30
12		60
13	0 mL L^{-1} (agua)	15
14		30
15		60

D: dilutions; IT: imbibition time

Four days after planting, the lengths of radicles and hypocotyls (cm) of four seedlings per plate were measured, for a total of 20 seedlings per treatments. They are indicators of growth that allowed us to calculate later the length ratio of the hypocotyl and the radicle (LR/LH).

The data were processed by means of a factorial ANOVA and the means were compared by the Duncan test (10) for 5 % significance, after verifying that they fulfilled the adjustment of normal distribution and homogeneity of variance, all this through the IBM program (11).

RESULTS AND DISCUSSION

In Table II it can be seen that there was no significant interaction between the variables studied. The effect manifested by the dilutions for most of the variables does not constitute an additive response of the imbibition times. Considering the results obtained, the effects of each of them were separated.

No significant differences were found between the three levels of imbibition time. Therefore, from the practical point of view, the imbibition of tomato seeds in LEBAME for 15 minutes (less time), becomes the best proposal studied.

The imbibition of the seeds during 15 minutes was also investigated by other authors. The results obtained with this time of imbibition, showed a significant increase with respect to the control, regarding the size of the postures, the stem and the length of the roots of two solanaceae (eggplant and chili), to be embedded in a biopreparation of the bacterium *Brevibacillus borstelensis* B65 (12).

Table III shows the effect of different dilutions and imbibition time of the LEBAME bioproduct in the germination of tomato seeds. When analyzing each factor independently, it could be observed that the germination percentage variable did not differ statistically between the treatments, showing very similar results for all. Similarly, values close to 100 % were evidenced, which is considered a high germinative power.

Figures 1 and 2 show the results of the combination analysis of dilution effect for being the only factor that was significant for the rest of the variables studied. The imbibition of the tomato seeds in the dilutions 5, 10 and 15 mL L^{-1} of LEBAME, caused a decrease in the days to the germination (Figure 1A). Practically the total of germinated seeds (99-100 %) was one day in advance with respect to those of the control treatment (97-98 %), which constituted an increase in the speed of the treatment in 26,9 % (Figure 1B). However, in relation to the treatments absorbed in the 2,5 mL L^{-1} dilution and the control in water, the results were statistically similar.

The studied dilutions differed statistically among them, the lowest reached less effectiveness; however, dilutions 5, 10 and 15 mL L^{-1} were significantly higher and did not differ from each other (Figure 2A). In this way, it is evident that with only 5 mL L^{-1} it is sufficient to obtain the greatest stimulus in the growth from an increase in the length of the radicle and the hypocotyl, reaching higher values with respect to the control embedded in water of 21 and 32 % respectively.

In Figure 2B, it can be observed that the relationship (LH/LR), showed a similar behavior between the dilutions studied; although, it showed statistical differences with respect to the control exceeding it by 10 % which differences could be given by the increase in the radicle length, providing advantages in terms of increasing the area of water absorption with respect to the aerial part.

Table II. Evidence of the effects between the factors for the variables studied

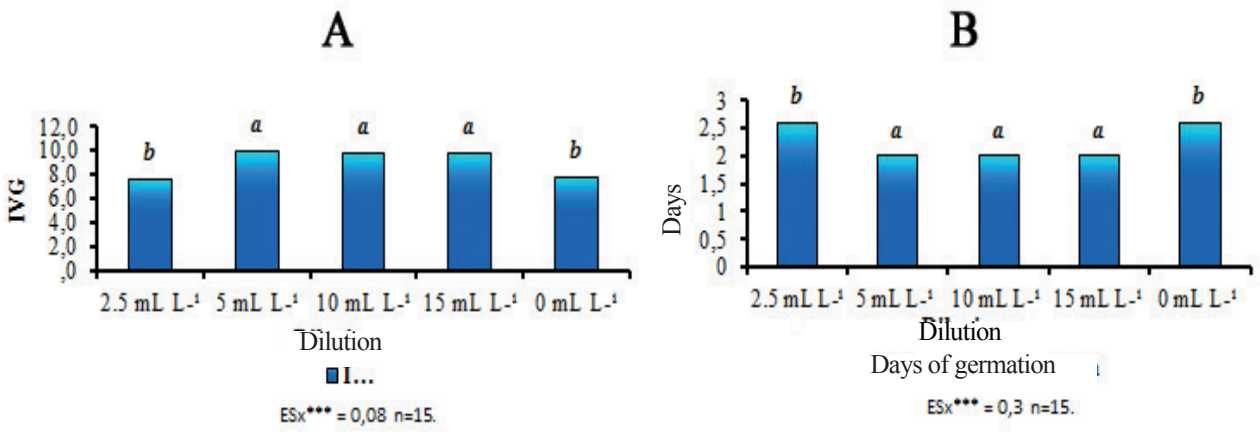
Studied variables	% Days of germination		IVG	Length		LR/LH
				Hipocotilo	Radícula	
Dilutions	0,243	0,000	0,000	0,000	0,000	0,000
Time	0,152	0,889	0,923	0,498	0,889	0,663
Dilutions*time	0,779	0,998	0,999	0,633	0,494	0,565

Value of p≤0,05 significant effect

Table III. Effect of LEBAME dose on the germination of tomato seeds

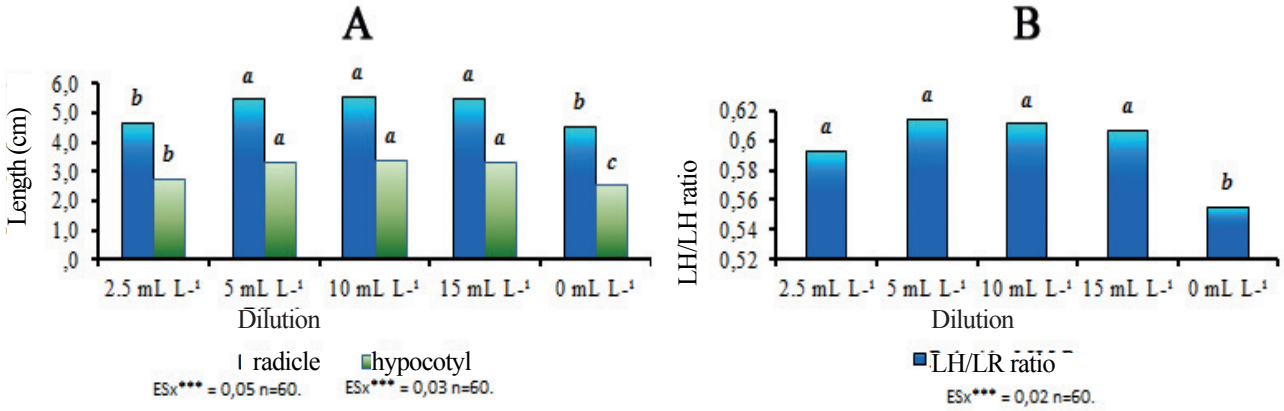
Treatments D	TI (minuts)	No	Germination (%)	arcos√
	15	1	97	1,44
2,5 mL L^{-1}	30	2	99	1,53
	60	3	98	1,48
	15	4	98	1,48
5 mL L^{-1}	30	5	100	1,57
	60	6	100	1,57
	15	7	97	1,44
10 mL L^{-1}	30	8	99	1,53
	60	9	100	1,57
	15	10	98	1,48
15 mL L^{-1}	30	11	99	1,53
	60	12	99	1,53
	15	13	98	1,48
0 mL L^{-1} (agua)	30	14	97	1,44
	60	15	97	1,46
	ESx***			0,04 NS

Means with common letters do not differ significantly according to Duncan for p≤0.05



IVG: germination rate index
Means with common letters do not differ significantly according to Duncan's test (p≤0,05)

Figure 1. Effect of LEBAME dilutions in the IVG (A) and days to germination (B)



A: length of the radicle and hypocotyl; B: hypocotyl / radicle length ratio (LH/LR)
Means with common letters do not differ significantly according to Duncan's test (p≤0,05)

Figure 2. Effect of LEBAME dilutions on some variables of tomato seedling growth

On the other hand, also the increase of the radicle lengths could be attributed to the need of the seedlings to absorb water. Similar values were obtained when evaluating the effect of a mixture of phospholipids of natural origin, on the *in vitro* germination of seeds of three tomato cultivars. An inductor effect was observed on the root growth of the seedlings, given by significantly higher radicle lengths with respect to the controls (13). Phospholipids are sources of organic phosphorus, by the action of phospholipases, with direct involvement in all phases of development and especially in the initial phase of seed germination and formation of seedlings (14).

According to other investigations (15), it is also attributable to *Bacillus* sp, the solubilization capacity of phosphates. Up to 56.0 mg L^{-1} of soluble phosphorus ($P\text{-}PO_4$), comparable to that obtained by other rhizospheric bacteria, was detected in culture media. In addition, the trial showed that they have biochemical and physiological properties related to the promotion of plant growth. The strain *Bacillus subtilis* BEB13-bs does not stand out for improving germination, while it increases the biomass in seedlings, presenting the best effect on vigor in both tomato and pepper seedlings. It also improves its root system causing a significant increase in dry mass and root length of 18-26 % and 13-15 % respectively, compared to the control treatment.

The germination depends on the viability of the embryo and the breaking of the lethargy. In the latter case, the bacteria that promote plant growth are affected; It has been explained that the reduction in ethylene levels due to the effect of the enzyme desaminase of 1-aminociclopropane-1-carboxylic acid (ACC) in the seed, would increase its germination, along with the production of indoleacetic acid (AIA) that would stimulate cell division, in order to favor the start of embryo growth (16).

Regarding efficient microorganisms (ME), it is suggested that they can coexist in mixed cultures and that they are physiologically compatible with each other and that they also have the capacity to develop beneficial effects in soils and plants (17).

Respecto a los microorganismos eficientes (ME) se plantea que pueden coexistir en culturas mixtas y que son fisiológicamente compatibles unos con otros y que, además, tienen la capacidad de desarrollar efectos beneficiosos en suelos y plantas (17).

On the other hand, work carried out in nursery conditions, showed that ME exercised the functions of increasing the speed and the percentage of seed germination due to its hormonal effect, similar to that of gibberellic acid. It also increases the vigor and growth of the stem and the roots, from the germination to the emergence of the seedlings, for its effect as RPCV; the chances of survival of the seedlings increase, as well as, they ensure a better germination and development (18).

The responses to inoculation can be variable and the microorganisms present can colonize and remain in the rhizosphere; on the other hand, increases in biomass production should be considered of ecological relevance (19).

CONCLUSIONS

- The positive effect of the LEBAME bioproduct is demonstrated by stimulating the germination process of tomato seeds.
- There were no differences between the imbibition times, which shows that, with only 15 minutes, it is enough to stimulate the variables studied.
- The effect of the 5, 10 and 15 mL L^{-1} dilutions was significant, the IVG variables, the length of the radicle, the hypocotyl and the LH/LR ratio exceeded the control by 27, 21, 32 and 10 % respectively.

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Received: June 3rd, 2016
Accepted: February 6th, 2017