APPLICATION TIMES OF AN *Azospirillum* BIOPRODUCT IN TOMATO GROWTH, DEVELOPMENT AND YIELD

Elein Terry✉, María de los A. Pino and N. Medina

ABSTRACT. A field experiment was conducted in a compacted Red Ferralitic soil for two years, with the objective of studying the application times of a bioproduct based on *Azospirillum brasilense* Sp-7 in tomato crop. The work was carried out in two stages: nursery phase and growth-development phase, having a total of five treatments with a randomized complete design at the nursery phase and a randomized block design at transplanting phase. Results showed a positive answer to this rhizobacterial inoculation combined with nitrogen fertilizer as observed by plant vigor and yield. From the point of view of handling viability of the product as well as its economic feasibility, inoculation at sowing time turned out to be the most advantageous.

Key words: tomato, *Azospirillum brasilense*, plant growth stimulants

RESUMEN. Con el objetivo de estudiar diferentes momentos de aplicación de un bioproducto a base de *Azospirillum brasilense* Sp-7 en el cultivo del tomate, se desarrolló un experimento de campo durante dos años sobre un suelo Ferralítico Rojo compactado, realizándose el trabajo en dos etapas: fase de semillero y fase de floración-fructificación, teniéndose en cuenta un total de cinco tratamientos distribuidos completamente aleatorizados en la fase de semillero y bloques al azar en la fase de trasplante. Los resultados mostraron una respuesta positiva a la inoculación de esta rizobacteria en combinación con el fertilizante nitrogenado, manifestado a través del vigor y rendimiento alcanzado por las plantas. Desde el punto de vista de la viabilidad en el manejo y la factibilidad económica, la inoculación en el momento de la siembra resultó ser la más ventajosa.

Palabras clave: tomate, *Azospirillum brasilense*, sustancias de crecimiento vegetal

INTRODUCTION

Soil microorganisms have among other functions, the property of nitrogen fixation; they increase nutrient uptake and availability through root system, so that they are able to produce physiologically active substances by interacting with plants. Finally, they unchain a higher activation of the vegetable metabolism and consequently they increase plant development as well as yields (1).

Crop biofertilization is an important strategy in order to improve the biological, chemical and physical conditions of the soil, acquiring each time greater importance not only for the yields usually reached but also for the economic application and contribution to environment preservation (2).

Some variability in the response to *Azospirillum* inoculation recorded may be attributed, among others, to the lack of a methodology of inoculation. However, the results in this sense and doubts about the work method in Cuba, are the same as in the international literature (3).

It is suggested that *Azospirillum* inoculation makes plants absorb ions more efficiently from the soil (the mechanisms of this process are not deeply known). Also, it has been suggested that an increment of mineral absorption is due to a general growth of root system rather than to a more effective mechanism of ion absorption (4). It seems that the promotion of water and mineral absorption plays a vital role in *Azospirillum*-plant association.

Keeping in mind the bell-shape effect of the bioproduct application, where the stimulation begins with an increase, later it is stable and finally it decreases for competition with the native flora from the soil, then it is necessary to know which is the advisable time to be applied (5). Therefore, the present work was directed to evaluate the effect of different application times of a bioproduct based on an *Azospirillum brasilense* strain in tomato crop.

MATERIALS AND METHODS

The present work was conducted in the experimental areas of the National Institute of Agricultural Sciences (INCA) using a compacted Red Ferralitic soil (6); its chemical characteristics can be observed in Table I. Soil organic matter was determined by Walkley and Black method, phosphorus by Oniani method whereas Ca and Mg through EDTA. Sowing period was August-September, 1997 and 1998; some climatic conditions of this period can be observed in Table II.

M.Sc. Elein Terry and María de los A. Pino, Junior Researchers from the Agronomy Department and Dr. N. Medina, Senior Researcher from the Biofertilizers and Plant Nutrition Department, Instituto Nacional de Ciencias Agrícolas, Gaveta Postal 1, San José de las Lajas, Havana, Cuba.
✉ Corresponding author: e-mail: terry@inca.edu.cu

Table I. Soil chemical characteristics

Depth (cm)	Ph (H_2O)	Organic matter (%)	P_2O_5 (ppm)	K^+ (cmol.kg^{-1})	Ca^{++} (cmol.kg^{-1})	Mg^{++} (cmol.kg^{-1})
0 - 20	6.3	2.6	298	0.51	10.2	2.3

Table II. Climatic conditions

Months	Average temperature (°C)		Rainfall (mm)		Relative humidity (%)	
	1997	1998	1997	1998	1997	1998
August	26.6	26.7	179.2	153.8	82.0	85.0
September	25.6	25.7	242.1	248.8	86.0	87.0
October	24.5	24.7	150.3	398.9	83.0	86.0
November	23.8	22.1	170.3	265.1	86.0	85.0

The tomato cultivar used was INCA-17 recommended for early season (5). The bioproduct based on *Azospirillum brasilense* Sp-7 was applied at different phenological phases of the crop (nursery and three days after transplanting) according to the treatments studied (Table III), with a liquid inoculant of title 2,7x10^8 cfu.mL^{-1} of culture medium at a dose of 20 L.ha^{-1}. The technique of dilution in order to know the most probable number (MNP) according to Mc Cardy was used. Urea was applied as a nitrogen fertilizer, but P_2O_5 and K_2O were not applied because their contents in the soil were adequate for the crop.

Table III. Treatments

Treatments	Nursery	Three days after transplanting	One month after transplanting
1	Az.+ 30 kg N.ha^{-1}	45 kg N.ha^{-1}	45 kg N.ha^{-1}
2	Az. +30 kg N.ha^{-1}	45 kg N.ha^{-1}	*Azospirillum*
3	30 kg N.ha^{-1}	Az. +45 kg N.ha^{-1}	45 kg N.ha^{-1}
4	30 kg N.ha^{-1}	*Azospirillum*	45 kg N.ha^{-1}
5	30 kg N.ha^{-1}	45 Kg N.ha^{-1}	45 kg N.ha^{-1}

Az.: *Azospirillum* sp

Transplanting was carried out 25 days after seed germination in 28m^2 plots at a planting distance of 1.40x0.30 m. In order to verify rhizobacterial effectiveness as well as crop answer, the following variables were measured in 10 plants per treatment at transplanting: plant height (cm), shoot diameter (cm), root length as well as fresh and dry weights were evaluated; besides, the following measurements were recorded in 14 plants per treatment at the development stage: plant height (cm), shoot diameter (mm), number of leaves, flowers, bunches and fruits; finally, agricultural yield and average fruit weight were determined.

The experimental design employed was randomized block with five treatments and four replications at transplanting, evaluating the effects on the nursery phase through a randomized complete design. For the statistical processing of data, a single and double classification variance analysis was applied, using Duncan's Multiple Range Test to detect differences among treatments.

RESULTS AND DISCUSSION

Effects of the bioproduct on seedling vigor in nursery. Azospirillum sp is a microorganism able to produce a root-bacterium association, where the number of root hairs increases, provoking a larger root surface and therefore water and nutrients become more available too; on the other hand, it promotes the growth of its host plants through producing hormones, one of them is auxin, which plays an important role due to cellular lengthening and division (8) caused in inoculated plants, which can be shown in seedling height (Table IV).

Also, it is appreciated that plants inoculated differ statistically from those just receiving mineral fertilization. With regard to the remaining parameters of vigor evaluated: plant height, shoot diameter, root length and fresh and dry weights of plants, there is a tendency to increase when inoculating this rhizobacterium. Thus, the marked promoting effect on seedling vigor seems to be related to bacterial capacity to secreting substances around the roots that are able of inducing root hair branching and bending, it permiting the plants to achieve a better use of the mineral fertilizer applied.

On the other hand, seedling behavior was similar in both years; maybe the average temperature that was similar in both years (Table II) was the main factor contributing to this result, because it influenced carbon assimilation and cellular division and, therefore, plant growth rate.

The efficient population of *Azospirillum* cells after inoculation is essential to obtain a plant answer to bacterial presence; then, before starting to work with a microorganism as a biofertilizer, it is necessary to know the strain chosen with that objective is able to colonize crop roots.

The behavior of total populations of *Azospirillum* in tomato rhizosphere is shown in Table V. In the treatment 1, where inoculation was made, the bacterial concentration was higher than in treatment 3 where the native *Azospirillum* was present. This result was directly related to seedling vigor because it was higher in plants inoculated with *Azospirillum*; it shows the positive relation between plant and microorganism.

Taking into account these aspects, it is possible to establish that there exists a greater potentiality of the plant-microorganism system to express their association, that together with a good agronomic management permits a better plant physiologic stage from the early ages and, therefore, a greater vigor of them.

Influence of PGPR on the growth, development and yield of tomato crop. Some authors (9) indicated that still without knowing the precise mechanisms through which bacteria promote plant growth, there is literally a hundred of studies demonstrating that effect.

Table IV. Influence of bioproduct on different vigor parameters in tested plants

Treatments	Height (cm)		Shoot diameter (cm)		Root length (cm)		Fresh weight (g)		Dry weight (g)	
	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
1	20.15 a	20.02 a	0.32 a	0.30 a	15.11 a	15.22 a	5.12 a	5.10 a	2.12 a	2.08 a
3	17.32 b	17.50 b	0.25 b	0.24 b	13.32 b	13.41 b	3.20 b	3.32 b	1.20 b	1.25 b
SEx	0.80***	0.78***	0.02***	0.03***	0.75***	0.72***	0.55***	0.50***	0.12***	0.10***

Same letters do not differ significantly for p< 0.001

Table V. *Azospirillum* population in tomato rhizosphere 30 days after sowing

Year	Treatments	cfu.mL^{-1}
1997	1	2.2 x 10^{8}
	3	0.8 x 10^{5}
1998	1	2.5 x 10^{8}
	3	1.2 x 10^{5}

As a part of this mechanism, the restrictions to pathogenic growth and occurrence of changes in the hormonal balance of inoculated plants are observed, it causing a more vigorous plant growth.

Table VI shows the promoting effect of rhizobacteria on some tomato growth parameters 80 days after sowing, appreciating that treatments differed from each other, obtaining the best behavior those plants inoculated at sowing time (T1) and three days after transplanting (T3) with urea as complementary fertilization. This result is corroborated by some works fulfilled with inoculations of *Azospirillum* sp in tomato seedlings (10).

Table VI. Rhizobacterial effect on tomato growth 80 days after sowing

Treatments	Height (cm)		Shoot diameter (mm)		Leaf number/plant	
	1997	1998	1997	1998	1997	1998
1	71.3 a	71.4 a	12.7 a	13.3 a	18 a	20 a
2	67.2 b	68.4 b	10.7 b	11.2 b	16 b	17 b
3	70.2 a	71.1 a	12.4 a	13.1 a	17 ab	19 a
4	65.4 c	66.3 c	9.5 b	10.0 b	14 c	13 c
5	68.7 b	69.2 b	10.4 b	11.4 b	16 b	17 b
SEx	1.4***	1.5***	0.2***		0.20***	0.21***

Same letters do not differ significantly for p< 0.001

The lowest results recorded in treatment 4, where rhizobacteria was inoculated three days after transplanting without nitrogen fertilizer, could cause microorganism death, due to the lack of available source to perform their functions effectively.

Plant weakness is added to this effect because it does not have the mineral fertilization required at transplanting, when the volumes of mineral extraction are higher; however, the levels present in the soil are not enough where experiments were carried out. Therefore, it shows that inoculation of this growth-promoting rhizobacteria when transplanting is not able to replace crop nutritional demands; similar results were obtained in experiments with other doses of a bioproduct at different application times in onion (11).

On the other hand, differences related to the treatment whith mineral fertilization only (T5) show the positive effect of this bacterium, as the bacterial concentration enhanced root growth, placing the plants in an advantageous position in the search of water and nutrients; may be, it is the greatest effect obtained with *Azospirillum* (12).

According to what is previously discussed, the products helping the formation of a vigorous plant should be applied at sowing time, to let them have a better use of its functional and photosynthetic possibilities to obtain more nutritional substances.

Growth promotion is observed by an increase of plant development and productivity (Table VII); the results are shown on yield and its components.

In correspondence with the results recorded when plant vigor was evaluated, a similar behavior was appreciated between plants inoculated at sowing (T1) and those inoculated three days after transplanting (T3).

Also, differences between years were not recorded, because temperature, that is the most limiting factor in tomato production, almost did not vary during the experimental period. The same occurred with relative humidity in relation with rainfall; only in the last month they were higher in 1998 than in 1997; nevertheless, it did not negatively influence plant response to yield.

Therefore, for a better handling of the product, inoculation at sowing time is more viable, keeping in mind that the inoculant was used in a liquid support; on the other hand, it is strategical to carry out inoculation at the early stage of plant development, since after seed germination and root emergence the colonization and multiplication of these bacteria start until reaching the limit of their ecological niche; therefore, a late application will affect colonization by competitiveness with the microflora present in the soil.

Also, the application of this bioproduct at sowing time is economically feasible due to the small amount of inoculum used. Similar results were obtained in works of doses and application times of a growth promoter on garlic crop (13).

Table VII. Influence of bioproduct on yield and its compounds

Treatments	Yield ($t.ha^{-1}$) 1997	Yield ($t.ha^{-1}$) 1998	Number of flowers/plant 1997	Number of flowers/plant 1998	Number of bunches/plant 1997	Number of bunches/plant 1998	Number of fruits/plant 1997	Number of fruits/plant 1998	Average fruit weight (g) 1997	Average fruit weight (g) 1998
1	23.35 a	23.24 a	7.10 a	8.09 a	5.85 a	6.14 a	14.53 a	15.12 a	65.62 b	66.25 b
2	20.12 b	19.68 b	6.00 b	5.96 b	4.50 b	4.55 b	12.42 b	12.39 b	73.12 a	72.08 a
3	23.08 a	22.52 a	7.05 a	7.89 a	5.70 a	6.07 a	14.50 a	15.47 a	65.30 b	66.15 b
4	17.14 c	16.85 c	4.25 c	3.98 c	3.13 c	3.10 c	10.12 c	10.07 c	67.14 b	66.34 b
5	20.02 b	19.42 b	6.02 b	5.87 b	4.57 b	5.03 b	12.15 b	11.86 b	73.05 a	72.13 a
SEx	0.22***	0.20***	0.19***	0.18***	0.13***	0.14***	0.20***	0.22***	0.75***	0.77***

Same letters do not differ significantly for $p < 0.001$

These results suggest it had a positive effect of the rhizobacteria on tomato crop, which is corroborated with the results of inoculating with *Azospirillum* sp in several crops including tomato (14). Inoculation at sowing time had significant changes in several growth parameters, it expressing an agricultural yield increment.

REFERENCES

1. Okon, Y., Bloemberg, G.V and Lutenberg, B. J. J. Biotechnology of biofertilization and phytostimulation. Agricultural biotechnology. New York Marcel Dekker, 1998, p. 327-349.
2. Hernández, Y., García, O. A., and Ramori, M. Biofertilizantes, estado actual y estrategia de uso de sus factores microbiológicos. Revisión Bibliográfica. La Habana. ICA, 1999.
3. Hernández, Y., Sarmiento, M. and García, O. Influencia del método de inoculación con *Azospirillum* en el comportamiento de gramíneas de pastos. *Revista Cubana de Ciencias Agrícolas*. 1996, vol. 30, no. 2, p. 225.
4. Bashan, Y., Holguin, G. and Ferrera-Cerrtao, R. Interacciones entre plantas y microorganismos benéficos. I. Azospirillum. *Terra*. 1996, vol. 14, no. 2, p. 159-192.
5. Bashan, Y. *Azospirillum*; plant growth-promoting strains are nonpathogenic on tomato, pepper, cotton and wheat. *Can. Journ of Microb*, 1998, vol. 44, no. 2, p. 168-174.
6. Hernández, A and Jaimez, E. Génesis y clasificación de suelos (aplicado a los suelos de Cuba). 120 p. 1997.
7. Alvarez, M. Variedades de tomate INCA-17. *Cultivos Tropicales,* 1996, vol. 17, no. 2, p. 81-83.
8. Bashan, Y., Holguin, G. and Ferrera-Cerrato, R. Interacciones entre plantas y microorganismos benéficos. II.Bacterias asociativas de la rizosfera. *Terra,* 1996, vol. 14, no. 2, p. 195-209.
9. Edathil-Thomson, T., Manian, S and Udaiyan, K. Effect of interactions of three growth-promoting microorganims on VAM colonization, spore density, plant growth and nutrient accumulation in tomato (*Lycopersicon esculentum*) seedlings. *Pertanika Journal of Tropical Agricultural Sciences*. 1995, vol. 18, no. 3, p. 187-191.
10. Ravelo, R., Cuñarro, R., Almenares, J. C., Zamora, J., Martín, A. and Rodríguez, F. Influencia de dosis y momentos de la aplicación de Azotobacter en el rendimiento y calidad del cultivo de la cebolla. In: Libro Resumen. Evento Científico de producción de cultivos en condiciones tropicales. Liliana Dimitrova (3:1997:La Habana).
11. Baldini, J., Caroso, L., Baldani, V.L.D and Dobereiner, J. Recent advance in BNF with non-legume plants. *Soil Biology and Biochemistry*. 1997, vol. 29, no. 5-6, p. 911-922.
12. Dommelon, A-Van, Keijers, V., Vanderleynden, J. and Zamaroczy, M. *Journal of Bacteriology*. 1998, vol. 18, no. 10, p. 2552-2559.
13. Lewis, L. Comportamiento del cultivo del ajo (*Allium sativum* L) ante los efectos de dosis, momentos y forma de aplicación de *Azotobacter chroococcum* como estimulador del crecimiento y desarrollo. [Tesis de maestría]. La Habana : ISCAH, 1997
14. Bashan, Y. Azpospirillum plant-promoting strains are nonpathogenic on tomato, pepper, cotton and wheat. *Canadian Journal of Microbiology*. 1998, vol. 44, no. 2, p. 168-174.

Received: March 15, 2000
Accepted: June 6, 2000