Cultivos Tropicales, 2018, vol. 39, no. 3, pp. 71-79 July-September

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

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

COMBINATION OF APPLICATION FORMS OF CHITOSAN IN THE DEVELOPMENT OF BIOFERTILIZED SOYBEAN

Combinación de formas de aplicación de quitosano en el desarrollo de soya biofertilizada

Daimy Costales Menéndez✉ and Alejandro B. Falcón Rodríguez

ABSTRACT. In this work the combined application of seed addition and foliar spraying of a chitosan polymer in the development of soybean plants (*Glycine max* (L.) Merril), cultivar IS-27, inoculated with the biofertilizer Azofert-S® was evaluated. Nodulation and growth variables were determined in phases R2 and R5, as well as the components of crop yield (R8). The experiment was carried out in the winter season, in an experimental area of the National Institute of Agricultural Sciences, using a random blocks design in the field. Prior to sowing, the seeds were treated with a mixture of Azofert-S® and chitosan (0,01, 0,1 and 1 g L^{-1}). These concentrations were applied by foliar spray in phases V2 and R2 of the crop. Combination of both forms of application of chitosan benefited the nodulation and growth of the cultivar in R2 phase, specifically, the number and dry nodular mass, number of flowers, length of the stem, aerial dry mass, foliar area and the concentration of nitrogen in nodules and seeds, when applying 1 g L^{-1}. In the R5 phase of development of IS-27, only the length of the stem was benefited, while in the harvest it was the number of pods with the application of chitosan at 0,01 g L^{-1}. The rest of the components of the yield were not stimulated with the combination of the forms of chitosan application. It is possible that the separate application of both chitosan application forms, improve the productivity of the soybean, so it must be demonstrated in later studies.

Key words: biostimulants, *Glycine max*, inoculation, polymer

RESUMEN. En este trabajo se evaluó la aplicación combinada de adición a semillas y aspersión foliar de un polímero de quitosano en el desarrollo de plantas de soya (*Glycine max* (L.) Merril), cultivar IS-27, inoculadas con el biofertilizante Azofert-S®. Se determinaron variables de nodulación y de crecimiento en las fases R2 y R5, así como los componentes del rendimiento en la cosecha (R8). El experimento se realizó en época de invierno, en un área experimental del Instituto Nacional de Ciencias Agrícolas, mediante un diseño de Bloques al Azar en campo. Previo a la siembra, las semillas fueron tratadas con una mezcla de Azofert-S® y quitosano (0,01, 0,1 y 1 g L^{-1}). Estas concentraciones, se aplicaron por aspersión foliar en las fases V2 y R2 del cultivo. La combinación de ambas formas de aplicación de quitosano benefició la nodulación y el crecimiento del cultivar en la fase R2, específicamente, el número y la masa nodular seca, el número de flores, la longitud del tallo, la masa seca aérea, el área foliar y la concentración de nitrógeno en nódulos y semillas, al aplicar 1 g L^{-1}. En la fase R5 de desarrollo de IS-27, solo se benefició la longitud del tallo, mientras que en la cosecha fue el número de vainas con la aplicación de quitosano a 0,01 g L^{-1}. El resto de los componentes del rendimiento no fueron estimulados con la combinación de las formas de aplicación de quitosano, Es posible, que las formas de aplicación por separado del quitosano, mejoren la productividad de la soya, por lo que debe demostrarse en estudios posteriores.

Palabras clave: bioestimulante, *Glycine max*, inoculación, polímero

INTRODUCTION

Glycine max (L.), Merrill, is one of the crops richest in protein and oil, making it the most striking oilseed and with the best prospects in the preparation of food diversity for human and animal consumption (1). It is a crop with high demand for nitrogen (N), being the most critical nutrient for its development (2). The requirements of this nutrient can be covered from the contribution of N of the soil by the mineralization of organic N and by means of the biological fixation of atmospheric dinitrogen (FBN). The latter represents the process with the greatest impact on world

Instituto Nacional de Ciencias Agrícolas (INCA), carretera San José-Tapaste, km 3½, Gaveta Postal 1, San José de las Lajas, Mayabeque, Cuba. CP 32700

✉ daimy@inca.edu.cu

production, since legumes can be grown without nitrogen fertilization, if they are suitably inoculated with rhizobeaceae bacteria (3).

Soybeans are symbiotically associated with bacteria of the genus *Bradyrhizobium* and form nodules capable of fixing nitrogen efficiently, so that biofertilizers are developed and marketed with selected strains of rhizobia (4-6). Biofertilizers are an excellent alternative as biostimulators of growth, provide greater efficiency in nitrogen fixation and utilization, in addition to ensuring an increase in crop yields and a reduction in costs, compared with nitrogen fertilizers (3.7).

On the other hand, biostimulants made from polymers of chitosan are applied in different ways in different crops of economic interest and act as regulators of plant growth and physiology, in addition to activating defensive and protective responses against various pathogens (8,9).

Although there are results of the bioestimulant action of chitosan in experiments *in vitro* and *ex vitro* of soybean, with different concentrations and application forms (10,11), it is not known if the combination of application to the seeds with the foliar spray benefits the development of the crop in field conditions. Therefore, the objective of the work was to evaluate the combination of the aforementioned forms of chitosan application, in nodulation, growth and soybean yields (cultivar IS-27) biofertilized with the Azofert-S® inoculant.

MATERIALS AND METHODS

The research was developed in an experimental area of the National Institute of Agricultural Sciences (INCA), located in San José de las Lajas, Mayabeque province. The coordinates of this area are North Latitude (N: 353.05), East Latitude: 382, 875) and an altitude of 138 m a.s.l. The experimental area was divided by a Random Blocks design, where four blocks of five rows (5 m^2) were used and four repetitions per treatment. The technology of direct seeding was used at distances of 0.75 m between rows and 0.05 m between plants, with a dose of 50 kg ha^{-1} of seeds, to ensure at least 35 plants per square meter. The soil that predominates in this area is Ferralitic Red Leachate typical, eutric (12) and its characterization was made, from the taking of samples between 0 and 10 cm deep, which were described morphologically. The chemical characteristics were determined by analytical methods (13) and contained 3.7 % organic matter (OM), 16.3 cmol kg^{-1} of calcium (Ca), 2.1 cmol kg^{-1} of magnesium (Mg), 24.9 ppm of phosphorus (P) and pH = 7. These values of the nutritional elements evaluated in the soil are suitable for the cultivation of soy, since this is characterized by a medium fertility, with a relatively high percentage of matter organic, neutral pH and the contents of P, Ca and Mg are adequate.

Climatological characteristics

The experiment was carried out in the winter season (January-April 2015) and the climatic variables registered monthly, by the Tapaste Meteorological Station (No. 78374, located at km $31^1/_2$ road to Tapaste, Mayabeque), were : diurnal / nocturnal temperatures (28.05/16.73 °C), relative humidity diurnal / nocturnal (87.87/49.14 %) and accumulated rainfall of 3.91 mm during this period.

Effect of chitosan added to the seeds and by foliar spray on nodulation and soybean growth in two phases of development (R2 and R5)

The main physical-chemical characteristics of the chitosan polymer (Q) are its molar mass (124 kDa) and its degree of acetylation (13.7%), which were determined by viscosimetry and reading in the infrared, respectively. From a stock solution of 10 g L^{-1} of the polymer aliquots were taken corresponding to the concentrations 0.01; 0.1 and 1 g L^{-1} both for its application to seeds, and for foliar spray.

For the application to the seed, prior to sowing, a mixture of the different concentrations of chitosan and inoculum of the Azofert-S® biopreparation was prepared (No. RCF 005/13, L1, volume 1, folio 033, 2013) based of strain ICA 8001 of *B. elkanii* for the cultivation of soybeans (14). The viability of the inoculant was 8 x 109 CFU mL^{-1} and the mixture applied to the seeds corresponded to the dose per hectare. Soybean seeds (IS-27) inoculated with Azofert-S® without addition of Q were used as control treatment. Subsequently, foliar sprays were made to the crop in the growth phase (V2) and flowering (R2), in hours early in the morning with a backpack of 16 L capacity. Irrigation was carried out periodically and cultural care was carried out manually in the crop.

The treatments that were used were the following:

- Q - 0 g L^{-1} (seed inoculated with Azofert-S® (SI) without trying with chitosan)
- Q - 0,01 g L^{-1} (SI + foliar spray V2 and R2)
- Q - 0,1 g L^{-1} (SI + foliar spray V2 and R2)
- Q - 1 g L^{-1} (SI + foliar spray V2 and R2)

Ten plants per block (40 plants per treatment) were selected for the morphoagronomic evaluations related to nodulation and growth to soybean plants in phases R2 and R5 (plants with pods and seeds under development); while 20 plants were harvested per block, for a total of 80 plants per treatment, to determine the agricultural yield and its components evaluated at the time of harvest (R8).

The nodulation evaluations were: number, dry mass (g) and percentage of effectiveness (%) of the nodules per plant. The latter was expressed by the visual method of the efficiency of the nodules after a cross section of them. Also, the macronutrient concentration (%) was determined in total formed nodules, in the third pair of trifoliate leaves and in seeds for each treatment. Nitrogen (N) was determined by the Nessler Method, phosphorus (P) by the Oniani Method, by interchangeable cations by extraction with NH_4Ac 1mol L^{-1} at pH=7 and potassium (K) by flame photometry. All these techniques are described in the Laboratory Techniques Manual published by Paneque *et al*. (13)

The morphoagronomic variables related to the growth that were evaluated were the number of leaves, branches, flowers and pods per plant, besides the length of the stem (g), the dry mass of the aerial part, the relative content of total chlorophylls (SPAD units) to the third pair of trifoliate leaves (top to bottom) with a portable Minolta SPAD* 502 plus meter and the leaf area (cm^2) with the portable meter AM 300, UK, to each plant.

The color measurements, such as CIELAB coordinates (L*a*b*) and Delta-E* (ΔE*) were made by supporting the colorimeter viewer (Minolta Chroma CR-400, Japan) in the third pair of trifoliate leaves , with uniform color and outside the central rib. The color differences that are perceived as equals of the three-dimensional color space, have equal distances between them. This difference is expressed by the value ΔE*, which is the distance between the two points within the color space and requires the values L*a*b * to measure the changes in hue and density. The luminosity, L *, corresponds to the white color when its value is 100 and to the black when it is 0. The chromaticity (tone and chroma) is indicated by a* and b* together, a* represents the axis that goes from green colors (-a*) up to red colors (+a*) and b * represents the axis that evolves from blue (-b*) to yellow (+b*).

The components of soybean yield assessed were: the number of total pods; the number of grains per pod and per plant; the percentage of pods (%); the weight of 100 seeds (g) and agricultural yield (t ha^{-1}), from dry grains up to 14 % humidity.

Experimental design and statistical analysis of the data

The experiment was repeated twice between September 2014 and April 2015, with similar performance of the results. The results corresponding to the period from January to April 2015 are presented. The data obtained were submitted after the verification of the premises of the ANOVA, to a variance analysis of Simple ANOVA. The resulting means were compared with the Duncan test for p≤0.05, by means of the statistical package Statgraphics Plus, version 5. The graphs were made in SigmaPlot.

RESULTS

Effect of chitosan added to the seeds and by foliar spray on nodulation and soybean growth in two developmental phases **(**R**2 y** R**5)**

The mixture of the chitosan polymer and Azofert-S® inoculant that was applied on seeds prior to sowing, favored the nodulation process of the IS-27 variety, specifically, the number, dry mass and effectiveness of the total nodules, in the soy phase R2 (Figures 1 and 2).

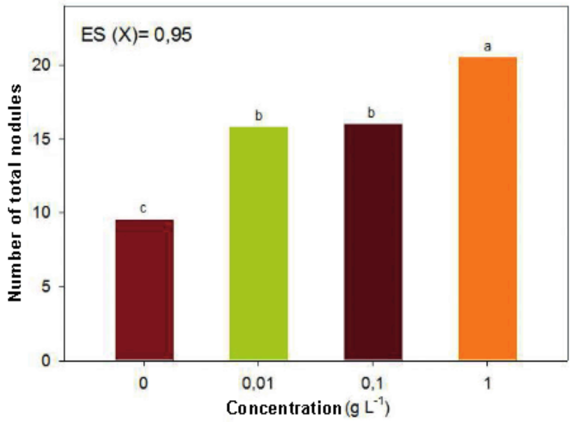

Figure 1. Effect of the chitosan polymer on the number of total nodules of soy in the R2 phase when applied on seeds and by foliar spray

The number of total nodules was stimulated with the different concentrations of chitosan, with higher and different values of the control inoculated with Azofert-S® but not treated with the bioproduct. All nodules were 100 % effective.

The concentration of chitosan that stood out the most was 1 g L^{-1} with respect to the concentrations 0.01 and 0.1 g L^{-1}, both in the number and in the dry mass of the total nodules formed in the roots of soybean (Figures 1 and 2).

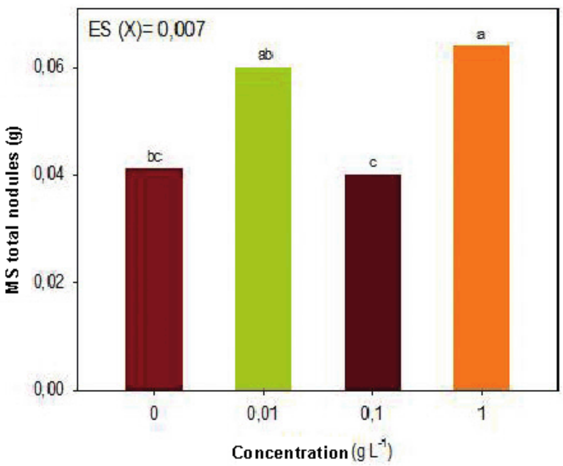

Figure 2. Effect of the chitosan polymer on the dry mass of the total nodules of soybean in the R2 phase when it was applied on seeds and by foliar spray

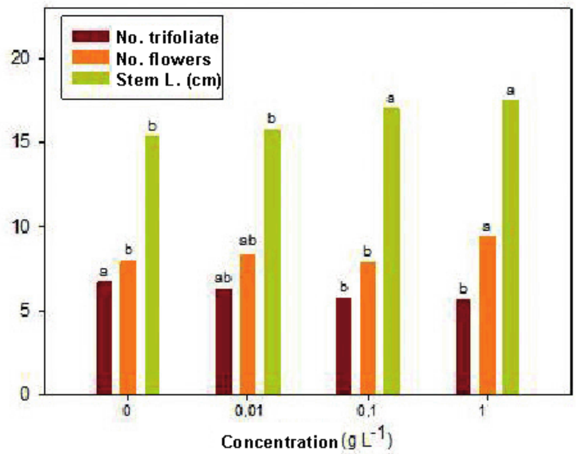

Figure 3. Effect of the chitosan polymer on the stem length, the number of trifoliate leaves and soy flowers in the R2 phase when applied on seeds and by foliar spray

Both combined forms of application of chitosan (on seeds plus foliar spray) positively affected the growth process of soybean in R2 (Figures 3, 4 and 5).

In the number of trifoliate leaves, concentrations 0.1 and 1 g L^{-1} negatively affected this variable with respect to the control not treated with chitosan, while the lower concentration did not differ from this (Figure 3).

However, the number of flowers, the length of the stem and the foliar area of the plants were stimulated with the concentration of 1 g L^{-1} of chitosan (Figures 3 and 4). In this last variable, increases were obtained with chitosan of 62.85 %, with respect to the untreated control (Figure 5).

Table 1 shows the concentration of macronutrients (N, P and K) determined in total nodules and third trifoliate leaves in phase R2, as well as in seeds (phase R8) of soybean.

The concentration of nitrogen in soybean nodules and leaves was high in all treatments, which could be due to a greater fixation of the atmospheric N2 by *B. elkanii*, contrary to what was accumulated in seeds (Table 1). All polymer concentrations differed from the inoculated control, the highest (1 g L^{-1}), which caused the highest percentage of N in the nodules, followed by 0.1 g L^{-1}.

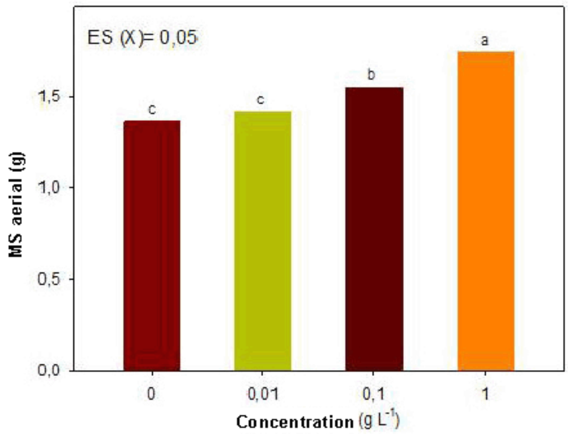

Figure 4. Effect of the chitosan polymer on the dry mass of the aerial part of soybean in the R2 phase when it was applied on seeds and by foliar spray

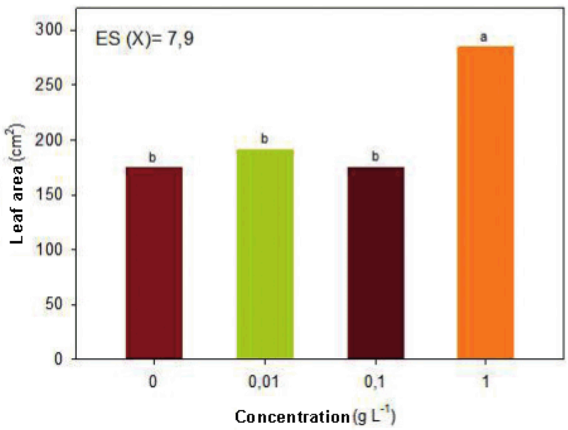

Figure 5. Effect of the chitosan polymer in the soybean area in the R2 phase when applied to seeds and by foliar spray

The percentage of nitrogen was reduced with the increase in the concentration of chitosan in soybean leaves, unlike 1 g L^{-1} of the polymer, which favored the concentration of this element in seeds (Table 1).

However, the contents of P and K showed significant differences between the treatments depending on the organ of the evaluated plant. In both leaves and seeds, the concentration of 1 g L^{-1} of chitosan was emphasized in accumulating higher percentages of K and P, respectively (Table 1).

Chitosan was evaluated in phases R2 and R5 with both forms of application and there was no effect of the same on the development variables: number of branches, flowers and pods, the content of total chlorophylls and the aerial dry mass, for not having differences between treatments (Table 2).

Despite significant differences in the number of three leaflets, none of the concentrations of the polymer differed from the inoculated control, while the length of the stem was stimulated with the concentration of 1 g L^{-1} of chitosan, above the rest of the treatments (Table 2).

According to the CIEL Chromaticity Diagram *a*b*, significant differences were observed in the color coordinates of soybean leaves, except for Delta-E * of phase R2 (Table 3). Those cultivated in R2 showed greater green tonality in their trifoliate leaves than in R5, because of the luminosity (L*) as a result of the variables a* and b*. In addition, changes in hue and density of color ΔE* were greater in R5, and they were accentuated in the plants that were applied to the polymer.

In R2, a* color values were negative (-12.30 to -12.58) while the b* values were positive (19.46 to 21.92). The lower value of a* was obtained with the 100 mg L^{-1} dose of the polymer with respect to the control, green, without differences of the concentration of 1000 mg L^{-1}. In the variable b*, the green tone is more accentuated with the control treatment without differences with the concentration of 10 mg L^{-1} of chitosan. The L* value was higher in the control plants without differences with the concentration of 1000 mg L^{-1} of chitosan. In R5, a* color values were negative (-9.10 to -9.81) and the treatments showed no significant differences. In the variable b*, the values were positive (11.97 to 14.55) and showed a light green color, where the concentration of 100 mg L^{-1} was highlighted without differences with the control. Therefore, the luminosity was higher in the control plants compared to those treated with chitosan. The plants treated with chitosan had higher Delta-E* values, with significant differences from the control plants.

The yield variables were evaluated at the time of harvest when the crop was in the R8 phase (Table 4).

Table 1. Effect of Chitosan polymer added to seeds and by foliar spray on the concentration of macronutrients (N, P, K) of total nodules and trifolioles (third pair) in R2, in addition to mature soybean seeds (R8)

Chitosan (g L^{-1})	Nodules			Leaves			Seeds		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
Q - 0	3,79 c	0,30	1,16	6,16 a	0,45	1,68 c	5,80 c	0,57 b	1,51
Q – 0,01	4,39 b	0,30	1,10	5,99 a	0,41	1,76 b	6,07 b	0,56 b	1,44
Q – 0,1	4,56 ab	0,34	1,09	5,02 b	0,45	1,76 b	6,12 b	0,57 b	1,45
Q - 1	4,99 a	0,30	1,09	5,08 b	0,45	1,87 a	6,39 a	0,64 a	1,52
ESx	0,10*	0,02 (ns)	0,02 (ns)	0,17*	0,03 (ns)	0,017*	0,022	0,007	0,08 (ns)

Equal letters do not differ statistically for p<0,05, according to Duncan's test

Table 2. Morfoagronomic soybean response (phase R5) to the application of the chitosan polymer added to sedes and by foliar spray

Chitosan ($g\ L^{-1}$)	No. branches	No. leaves	No. flowers	No. pods	Stem length (cm)	Chlorophyll (spad)	Foliar area (cm^2)	MS aerial (g)
Q - 0	12,60	22,10 ab	46,10	47,30	37,70 b	45,30	962,30	8,79
Q - 0,01	13,25	23,50 a	46,10	45,70	37,35 b	45,90	991,54	9,09
Q - 0,1	13,10	19,60 b	35,30	45,50	36,91 b	46,50	1131,34	7,92
Q - 1	13,20	24,10 a	36,80	48,90	39,63 a	45,75	1162,90	9,36
ESx	0,5 (ns)	1,04	4,45 (ns)	1,5 (ns)	0,63	0,52 (ns)	72,2 (ns)	0,8 (ns)

Equal letters do not differ statistically for p<0,05, according to Duncan's test

Table 3. Effect of the combination of addition to seeds in conjunction with the Azofert-S® inoculum plus foliar spraying of the chitosan polymer, in the physiological variable color in soybean leaves, cultivated in the phases R2 and R5

Chitosan ($g\ L^{-1}$)	Phase R2				Phase R5			
	L*	a*	b*	ΔE*	L*	a*	b*	ΔE*
Q-0	35,71 a	-13,58 b	21,92 a	4,30	28,50 a	-9,81	13,45 ab	8,49 b
Q-10	33,90 b	-13,51 b	20,73 ab	4,31	25,66 b	-9,20	11,97 b	12,31 a
Q-100	33,50 b	-12,30 a	19,99 b	4,44	25,99 b	-9,76	14,55 a	11,16 a
Q-1000	35,12 ab	-13,28 ab	19,46 b	4,09	25,62 b	-9,10	12,79 b	12,07 a
ESx	0,58*	0,37*	0,47*	0,47 (ns)	0,58*	0,32 (ns)	0,54*	0,69*

Equal letters do not differ statistically for p<0,05, according to Duncan's test

Table 4. Effect of the combination of addition to seeds in conjunction with the Azofert-S® inoculum plus foliar spraying of the chitosan polymer, in soybean yield, grown in the field (R8)

Chitosan ($g\ L^{-1}$)	No. pods	No. vain pods (%)	No. grains x pod	No. grains x plant	P 100 grains (g) x plant	Agricultural yield ($t\ ha^{-1}$)
Q-0	70,5 b	16,73 b	1,79	122,5	10,94 a	1,91
Q-10	99,0 a	20,02 a	1,85	137,6	11,06 a	1,93
Q-100	76,1 b	13,60 c	1,82	137,7	10,52 b	1,83
Q-1000	76,7 b	16,61 b	1,87	128,6	10,15 b	1,77
ESx	2,21	0,79	0,04 (ns)	4,99 (ns)	0,10	0,1 (ns)

Equal letters do not differ statistically for p<0,05, according to Duncan's test

Chitosan positively influenced the number of pods with the lowest concentration (0.01 $g\ L^{-1}$), which also produced the highest number of empty pods (20 %), in contrast to the intermediate concentration (0.1 $g\ L^{-1}$) that obtained approximately 14 %. The rest of the polymer concentrations did not differ from the inoculated control (Table 4).

The weight of 100 grains was greater with the control inoculated without differences with the concentration of 0.01 $g\ L^{-1}$, with values higher than the highest concentrations of chitosan tested. In the rest of the yield components (number of grains per pod, number of grains per plant and agricultural yield), no significant differences were observed between the treatments (Table 4).

DISCUSSION

In this work, the combination of two forms of application of chitosan in biofertilized soybean plants with Azofert-S® exerted a positive effect on the nodulation and growth processes of cultivar IS-27.

The highest concentration (1 $g\ L^{-1}$) of chitosan stimulated both nodulation and growth variables (number of flowers, stem length, aerial dry mass, leaf area, nitrogen concentration in nodules and mature seeds, as well as color (Delta-E*) in soybean leaves, in phase R2 (Figures 1, 2, 3, 4, 5 and Tables 1 and 3).

However, the second application of chitosan did not modify these variables with respect to the plants inoculated and not treated with chitosan in the R5 phase, except the length of the stem, the number of leaves (Table 2) and the yield with its components (Table 4).).

The combination of the application forms of chitosan reduced the nitrogen concentration in trifoliate soybean leaves (Table 1), in spite of obtaining higher aerial dry mass and foliar area with the concentration of 1 g L^{-1} (Figures 4 and 5). However, the concentration of nitrogen in soybean nodules and seeds was high in all chitosan treatments, which demonstrates the benefit of the polymer to the process of fixation of atmospheric N_2 by *B. elkanii* and perhaps also by the absorption of nutrients from soil. All chitosan concentrations differed from the inoculated control, with the highest concentration (1 g L^{-1}) which accumulated the highest percentage of N in the aforementioned organs (31% increase in nodules compared to the control treatment), followed by 0.1 g L^{-1} (Table 1). However, other forms of joint application of chitosan and rhizobia increased this indicator in cowpea (*Vigna unguiculata*) and corn (*Zea mays*) plants with increases of up to 40 % in nitrogen content (15,16).

The beginning of the color change is a reliable indicator of important transitions of development in different organs of the plant, due to the accumulation of pigments (chlorophylls, anthocyanins, among others). In fact, physiological changes associated with changes in source-sink relationships are identified and are related to the nitrogen and water deficit in crops (17-19).

In legumes, those morphological characteristics that mark the transition between the reproductive phases are identified, from the appearance of the first floral bud to the harvest maturity (17).

In this work, the highest values of Delta-E* were in the R5 phase, with the chitosan treatments, with respect to the control. Therefore, this could be related to an advance of the development phase of the crop, although all the treatments are in the same color chromatic zone.

There are several studies that corroborate the role of chitosan, through different forms of application in germination, growth, physiology and yields of different crops (11,20-24).

The application form and the concentration of chitosan, among other factors, are of great relevance in the stimulation of these processes (11,23,24). In IS-27, the group of bioactive products INCA, obtained increments the same growth variables evaluated in this study, as well as, increasing the percentage of nitrogen and proteins in leaves and the dry mass of the nodules when only applied the polymer to seeds at concentrations of 0.1 and 0.5 g L^{-1}, together with the inoculum of B. elkanii (11). Other authors stimulated growth and protected the crop from diseases with the same form of application, but with higher concentrations (5 to 10 g L^{-1}) than those tested in this study (25,26). All of the above demonstrates the efficacy of the application of chitosan to seeds, with a wide range of concentrations, in the development of soybeans.

Regarding the application form by foliar spray, there are a significant number of reported results in various plant processes and species (27-30). For example, the application of chitin and chitosan oligomers to 1 g L^{-1} caused variations in photosynthesis, stomatal conductance, transpiration and concentration of carbon dioxide (CO_2) in intercellular soybeans (27). Previously, improvements in nodulation, growth and pod formation were obtained in the laboratory with foliar spraying of chitosans under different conditions of cultivar IS-27 (10,11).

The higher concentration of chitosan, in spite of stimulating some variables during the phases of soybean growth, did not stimulate in the same way the yield and its components. The number of pods per plant only increased with the concentration of 0.01 mg L^{-1}, since the remaining concentrations had a negative impact on the weight of the 100 grains and the total yield with respect to the control (Table 4). For the above and the results of this work, suggest that it is possible to achieve a greater beneficial effect on the development of the cultivar, if the application forms are applied separately from the chitosan.

The agricultural yield of IS-27 was low with all the treatments (Table 4), since the expected yield with this cultivar is 3 t ha^{-1} with low inputs (31). This behavior may be due to the winter season, which causes reductions in the accumulation of aerial dry mass and in the translocation of assimilated foliage to the grain.

It is known that this cultivar has higher productivity in spring because it requires long and intense solar radiation, in addition to numerous precipitations, reason why the relationship between genotype-environment (climatological factors) is very important. In this sense, it was demonstrated that some edaphoclimatic conditions, fundamentally the sowing date, influence the obtaining of the yield of soybean cultivars in the same locality (32,33).

It would be of great interest to evaluate the effect of chitosan, in the symbiotic interaction and in the development of the cultivar inoculated with Azofert-S®, in the spring season.

CONCLUSIONS

- The combined application of chitosan to the seeds and by foliar spray of biofertilized soy, improved the development of the plants, depending on their concentration and the physiological phase of cultivar IS-27.
- The concentration of 1 g L^{-1} of chitosan in the V2 phase, favored the nodulation in addition to the concentration of 0.1 g L^{-1} in the R2 phase, which stimulated the growth of the culture, specifically, the length of the stem, the dry mass of the aerial part, the foliar area with increments of 62.85 % and the concentration of nitrogen in nodules and seeds.
- In phase R5, there was no beneficial effect of chitosan on the performance of IS-27, grown in winter time.
- More experiments must be done to arrive at a valid conclusion regarding the combined application of chitosan, to improve the productivity of soybean inoculated with Azofert®.

BIBLIOGRAPHY

1. Ainsworth E A, Yendrek CR, Skoneczka J A, Long S P. Accelerating yield potential in soybean: potential targets for biotechnological improvement: Targets to improve soybean yields. Plant, Cell & Environment. 2012;35(1):38–52. doi:10.1111/j.1365-3040.2011.02378.x
2. Xu G, Fan X, Miller A J. Plant Nitrogen Assimilation and Use Efficiency. Annual Review of Plant Biology. 2012;63(1):153–82. doi:10.1146/annurev-arplant-042811-105532
3. Callaghan M. Microbial inoculation of seed for improved crop performance: issues and opportunities. Applied Microbiology and Biotechnology. 2016;100(13):5729–46. doi:10.1007/s00253-016-7590-9
4. Nápoles MC, Gómez G, Costales D. Signals in Soybean´s Inoculants. In: Soybean - Biochemistry, Chemistry and Physiology [Internet]. INTECH Open Access Publisher; 2011. Available from: http://www.intechopen.com/articles/show/title/signals-in-soybean-s-inoculants
5. De Souza R, Ambrosini A, Passaglia L MP. Plant growth-promoting bacteria as inoculants in agricultural soils. Genetics and molecular biology. 2015;38(4):401–19. doi:10.1590/S1415-475738420150053
6. Ulzen J, Abaidoo RC, Mensah N E, Masso C, AbdelGadir AH. Bradyrhizobium inoculants enhance grain yields of soybean and cowpea in Northern Ghana. Frontiers in plant science. 2016;7:1770. doi:10.3389/fpls.2016.01770
7. De Bashan L E, Antoun H, Bashan Y. Involvement of Indole-3-Acetic Acid Produced by the Growth-Promoting Bacterium Azospirillum Spp. in Promoting Growth of Chlorella Vulgaris. Journal of Phycology. 2008;44(4):938–47.
8. Falcón AB, Costales D, González P D, Nápoles MC. Nuevos productos naturales para la agricultura: las oligosacarinas. Cultivos Tropicales. 2015;36(1):111–29.
9. Pichyangkura R, Chadchawan S. Biostimulant activity of chitosan in horticulture. Scientia Horticulturae. 2015;196(30):49–65. doi:10.1016/j.scienta.2015.09.031
10. Costales D, Falcón A B, Nápoles M C, De Winter J, Gerbaux P, Onderwater RCA, *et al*. Effect of chitosaccharides in nodulation and growth *in vitro* of inoculated soybean. American Journal of Plant Sciences. 2016;7(09):1380–91. doi:10.4236/ajps.2016.79131
11. Costales D, Nápoles MC, Falcón R A B, González Anta GFA, Rossi A. Influencia de quitosanas en la nodulación y el crecimiento vegetativo de soya (*Glycine max* L. Merrill). Cultivos Tropicales. 2017;38(1):138–46.
12. Hernández A, Morales M, Borges Y, Vargas D, Cabrera JA, Ascanio MO, *et al.* Degradación de las propiedades de los suelos Ferralíticos Rojos lixiviados de la "Llanura Roja de La Habana", por el cultivo continuado. Algunos resultados sobre su mejoramiento. La Habana, Cuba: Ediciones INCA; 2014.
13. Paneque PVM, Calaña NJM, Calderón VM, Borges BY, Hernández GTC, Caruncho CM. Manual de técnicas analíticas para análisis de suelo, foliar, abonos orgánicos y fertilizantes químicos [Internet]. 1ra ed. La Habana, Cuba: Ediciones INCA; 2010. 157 p. Available from: http://mst.ama.cu/578/
14. Nápoles MC. Medio de cultivo para Bradyrhizobium japonicum. Biopreparado resultante. OCPI; 22 797.
15. Berger LR, Stamford NP, Santos CERS, Freitas ADS, Franco LO, Stamford TCM. Plant and soil characteristics affected by biofertilizers from rocks and organic matter inoculated with diazotrophic bacteria and fungi that produce chitosan. Journal of soil science and plant nutrition. 2013;13(3):592–603. doi:10.4067/S0718-95162013005000047
16. Agbodjato NA, Noumavo PA, Adjanohoun A, Agbessi L, Baba M L. Synergistic Effects of Plant Growth Promoting Rhizobacteria and Chitosan on *In Vitro* Seeds Germination, Greenhouse Growth, and Nutrient Uptake of Maize (*Zea mays* L.). Biotechnology Research International. 2016;2016:1–11. doi:10.1155/2016/7830182
17. Clavijo M JA, Bhakta M, Gezan S A, Boote K J, Vallejos C E. From flower to seed: identifying phenological markers and reliable growth functions to model reproductive development in the common bean (*Phaseolus vulgaris* L.): Flower to seed: reproductive growth of *P . vulgaris*. Plant, Cell & Environment. 2013;36:2046–2058. doi:10.1111/pce.12114
18. Shibghatallah MHB, Khotimah S N, Suhandono S, Viridi S, Kesuma T. Measuring leaf chlorophyll concentration from its color: A way in monitoring environment change to plantations. In: AIP Conference Proceedings. AIP; 2013. p. 210–3.
19. Zhao D, Tao J. Recent advances on the development and regulation of flower color in ornamental plants. Frontiers in Plant Science. 2015;261:1–13. doi:10.3389/fpls.2015.00261
20. Mondal MMA, Malek MA, Puteh A B, Ismail MR. Foliar application of chitosan on growth and yield attributes of mungbean (*Vigna radiata* (L.) Wilczek). Bangladesh Journal of Botany. 2013;42(1):179–83.
21. Salachna P, Zawadzińska A. Effect of chitosan on plant growth, flowering and corms yield of potted freesia. Journal of Ecological Engineering. 2014;15(3):97–102.
22. Mahdavi B. Effects of Priming Treatments on Germination and Seedling Growth of Anise (*Pimpinella anisum* L.). Agriculture Science Developments. 2016;5(3):28–32. doi:10.21828/ASD-05-03-001

23. Malerba M, Cerana R. Chitosan Effects on Plant Systems. International Journal of Molecular Sciences. 2016;17(7):996. doi:10.3390/ijms17070996
24. Yin H, Du Y, Dong Z. Chitin Oligosaccharide and Chitosan Oligosaccharide: Two Similar but Different Plant Elicitors. Frontiers in Plant Science. 2016;7:522. doi:10.3389/fpls.2016.00522
25. Chookhongkha N, Miyagawa S, Jirakiattikul Y, Photchanachai S. Chili growth and seed productivity as affected by chitosan. In: Proceedings of the International Conference on Agriculture Technology and Food Sciences (ICATFS'2012), Manila, Philippines. 2012. p. 17–8.
26. Zeng D, Luo X, Tu R. Application of Bioactive Coatings Based on Chitosan for Soybean Seed Protection. International Journal of Carbohydrate Chemistry. 2012;2012:1–5. doi:10.1155/2012/104565
27. Khan W M, Prithiviraj B, Smith DL. Effect of foliar application of chitin and chitosan oligosaccharides on photosynthesis of maize and soybean. Photosynthetica. 2002;40(4):621–4. doi:10.1023/A:1024320606812
28. Rodríguez ABF, Costales D, Peña DG, Morales D, Mederos Y, Jerez E, *et al*. Chitosans of different molecular weight enhance potato (*Solanum tuberosum* L.) yield in a field trial. Spanish journal of agricultural research. 2017;15(1):25.
29. Hanafy AAH, Aboul Ella NMR, Allam HA, El Wakil AF. Effect of pre-harvest chitosan foliar application on growth, yield and chemical composition of Washington navel orange trees grown in two different regions. African Journal of Biochemistry Research. 2016;10(7):59–69. doi:10.5897/AJBR2016.0908
30. Bistgani Z E, Siadat SA, Bakhshandeh A, Pirbalouti A G, Hashemi M. Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of Thymus daenensis Celak. The Crop Journal. 2017;5(5):407–15. doi:10.1016/j.cj.2017.04.003
31. Ponce M, Fe C, Ortiz R, Moya C. Incasoy-24 E Incasoy-27: Nuevas Variedades De Soya Para Las Condiciones Climaticas De Cuba. Cultivos Tropicales. 2003;24(3):49.
32. Chacón A, Alemán R, Barreda A, Colás A, Rodríguez G, Cardoso S. Influencia de la época de siembra sobre el crecimiento y desarrollo de tres cultivares de soya [*Glycine max* (L.) Merr.]. Centro Agrícola. 2009;36(1):33–9.
33. Maqueira L, Torres W, Roján O, Pérez S, Toledo D. Respuesta del crecimiento y rendimiento de cuatro cultivares de soya (*Glycine max* (L.) Merril) durante la época de frío en la localidad de los palacios. Cultivos Tropicales. 2016;37(4):98–104. doi:10.13140/RG.2.2.17255.65447

Recibido: 1 de diciembre de 2017
Aceptado: 30 de abril de 2018