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Original article

Effect of algal extracts on the bean (*Phaseolus vulgaris* L.) production

Efecto de extractos de algas en la producción de frijol (*Phaseolus vulgaris* L.)

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ABSTRACT: In Cuba, bean production has been decreasing in recent years; therefore, the current challenge for producers is to increase it using environmentally friendly technologies. For this reason, the objective of this research was to determine the effect that applications with algae extracts have on the bean grain production. For this, an experiment was carried out in the Central Area of INCA (National Institute of Agricultural Sciences), where seeds from two lines that are in the registration phase were used, one with red grains (C-8) and another with white grains (C-13), which were sown on January 22, 2023. Three foliar sprays were carried out (25, 39 and 53 days after sowing); in the case of line C-8, three treatments were evaluated: 1) control, 2) two sprays with spirulina extract 20 mg ha^{-1} and one with sargassum extract 2 % and 3) two sprays with extract of spirulina 40 mg ha^{-1} and another with 20 mg ha^{-1}. In line C-13, the effect of three sprays with sargassum extract was evaluated, the first with 0.3 % and the other two with 2 %. The results showed that two foliar sprays with spirulina extract 20 mg ha^{-1} and one with sargassum extract 2 %; as well as three foliar sprays with sargassum extract were effective increasing the grain weight of lines C-8 and C-13 by 30 and 48.6 %, respectively.

Key words: *Arthrospira platensis*, Sargassum, yield, grains.

RESUMEN: En los últimos años, la producción de frijol en Cuba ha venido decreciendo; por lo que el reto actual de los productores es lograr incrementarla usando tecnologías amigables con el medio ambiente. Por esta razón, el objetivo del presente trabajo fue determinar el efecto que las aplicaciones con extractos de algas ejercen en la producción de granos del frijol. Para esto, se ejecutó un experimento en el Área Central del INCA (Instituto Nacional de Ciencias Agrícolas), donde se utilizaron semillas de dos líneas de frijol que están en fase de registro, una de granos de color rojo (C-8) y la otra de granos de color blanco (C-13), las cuales se sembraron el 22 de enero de 2023. Se efectuaron tres aspersiones foliares (25, 39 y 53 días después de la siembra); en el caso de la línea C-8 se evaluaron tres tratamientos: 1) control, 2) dos aspersiones con extracto de espirulina 20 mg ha^{-1} y una con extracto de sargazo 2 % y 3) dos aspersiones con extracto de espirulina 40 mg ha^{-1} y otra con 20 mg ha^{-1}. En la línea C-13 se evaluó el efecto de tres aspersiones con extracto de sargazo, la primera con 0,3 % y las otras dos con 2 %. Los resultados demostraron que dos aspersiones foliares con extracto de espirulina 20 mg ha^{-1} y una con extracto de sargazo 2 %; así como tres aspersiones foliares con extracto de sargazo fueron efectivas, incrementando la masa de granos de las líneas C-8 y C-13 en 30 y 48,6 %, respectivamente.

Palabras clave: *Arthrospira platensis*, Sargassum, rendimiento, granos.

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.), in Cuba, is considered a strategic food and constitutes, together with rice, the basic diet of the population, because this grain contains about 20 % protein, which places it in an advantageous position compared to other foods (1). In recent years, the production of this crop has been decreasing due to various factors, such as smaller sowing area, lack of inputs, the incidence of some pests, among others, so that in 2021, it was 57 642 t with an average yield of 0.86 t ha^{-1} (2). This national production does not meet the consumption needs of the population; for this reason, Cuban agriculture currently has as one of its main priorities to increase the production of this crop using environmentally friendly technologies.

Seaweed extracts represent more than 33 % of the global market for agricultural biostimulants (3). However, macroalgae are normally harvested at sea, making it difficult to standardize the raw material (4), so the use of microalgae and cyanobacteria becomes a promising alternative to solve this situation (5), although they are cultivated, generally, under artificial conditions, which makes the biomass source more expensive for the production of biostimulants (6).

Scientific evidence demonstrates the stimulating action of seaweed extracts, in particular those of the genus *Sargassum*, on crop yield and nutritional quality (7, 8), abiotic stress tolerance (9) and protection against pests and diseases (10). However, in Cuba, despite the arrival of sargassum on the coasts in recent years (11), the potential use of its extracts as agricultural biostimulants has not yet been exploited.

Cyanobacteria, within which spirulina is found (*Arthrospira platensis*, originally included in the genus Spirulina, hence the common name spirulina), has been shown to stimulate seed germination (12, 13), crop growth, yield and quality (14-16); as well as tolerance to certain abiotic stresses (17-19). In Cuba, there is no history of the use of spirulina extracts for agricultural purposes; hence the need to carry out research related to the application of spirulina extracts in our agriculture, with a view to reducing the use of agrochemicals, cost to the country's environment and economy (20).

Therefore, the objective of this study was to determine the effects that leaf spraying with sargassum and spirulina extracts have on grain production in bean cultivation.

MATERIALS AND METHODS

The experiment was carried out in an area of beans sown on January 22, 2023 in soil Ferrallitic Red Agrogenic leaching (21) in the Central Area of the National Institute of Agricultural Sciences (INCA), located in San José de las Lajas municipality, Mayabeque province. It was used two lines of beans that are in the registration phase, one red grain (C-8) and another white grain (C-13). The area had 42 rows of 90 m in length of red grain genotype and 16 of white grain genotype and the sowing distance used was 0.7 x 0.07 m.

Extracts used were: 1) Spirulina alcohol extract (EASp), prepared from dry spirulina powder marketed by Génix, LABIOFAM, S.A., dissolved in 70 % EtOH with a ratio m/v (1:20), macerated at room temperature for 10 days, Filtered and preserved at 4 ºC. 2) Aqueous extract of sargassum (EAS) prepared from the mixture of *Sargassum fluitans* and *Sargassum natans* collected on the coast of eastern beaches, Guanabo, washed several times and macerated in running water with a ratio m/v (1:6) for three months, filtered and stored at room temperature.

Foliar sprays to plants of both lines were performed at 25, 39 and 53 days after sowing (DAS), coinciding with the phases of vegetative growth, flowering and grain formation, respectively. In the case of line C-8 three treatments were evaluated: 1) control without foliar spraying, 2) the first two sprays with EASp at 20 mg ha^{-1} and the third one with EAS 2 % and 3) the first two sprays with EASp at 40 mg ha^{-1} and a third one with EASp at 20 mg ha^{-1}. In the case of line C-13 only two treatments were evaluated, a control treatment without foliar spraying and a treatment where it was used in the first spraying EAS 0.3 % and in the other two EAS 2 %.

In all cases, spraying was carried out ten rows per treatment leaving the rest as a control. The cultural attention was carried out in accordance with the Technical Guide to Cultivation (22), highlighting that no mineral or organic fertilizers were applied and that cleaning work was done manually.

At 79 DAS, harvesting was carried out and 80 plants were randomly selected for evaluation by treatment. The performance indicators evaluated were: number of legumes per plant, number of grains per vegetable and per plant, mass of grains per plant (g) and mass of 100 grains (g).

A sample design was used, so the data were processed by calculating means, standard deviations and confidence intervals at α=0.05.

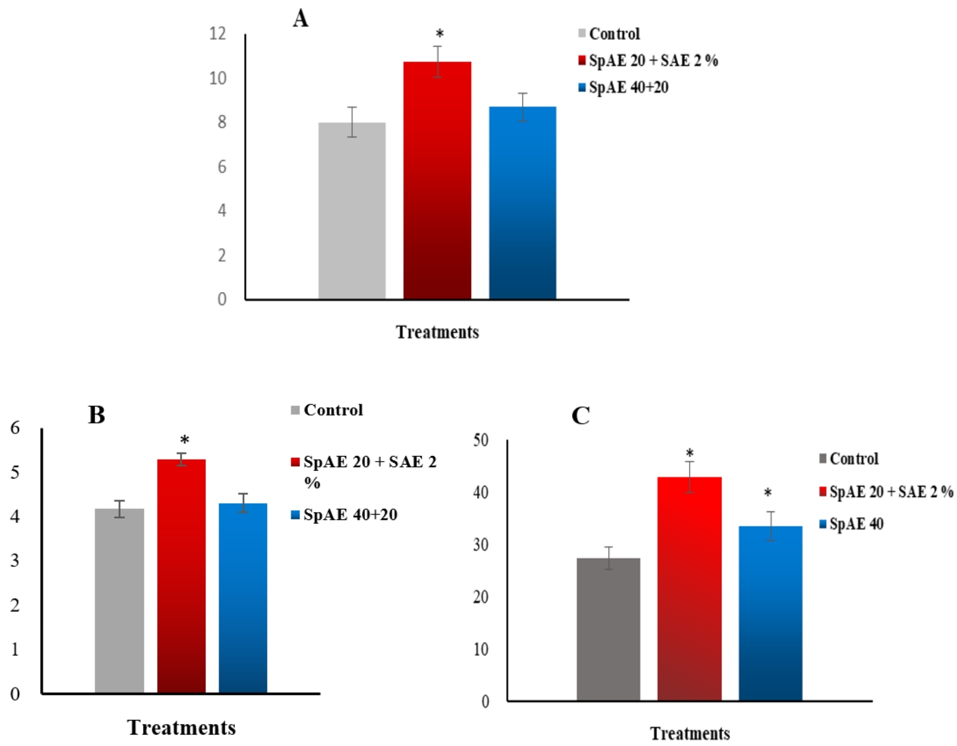
RESULTS AND DISCUSSION

Figure 1 shows application results of treatments in number of pulses (Figure 1A) and in number of grains per pulse (Figure 1B) and per plant (Figure 1C).

The treatment consisting of two foliar sprays, one in the vegetative phase and another at the beginning of flowering, 25 and 39 days after sowing, respectively, with an alcoholic extract of spirulina at a rate of 20 mg ha^{-1} and a third foliar spray in the development phase of the legumes, that is, 53 days after sowing with an aqueous extract of sargassum at 2 % was the best performance, since it significantly stimulated the three evaluated indicators.

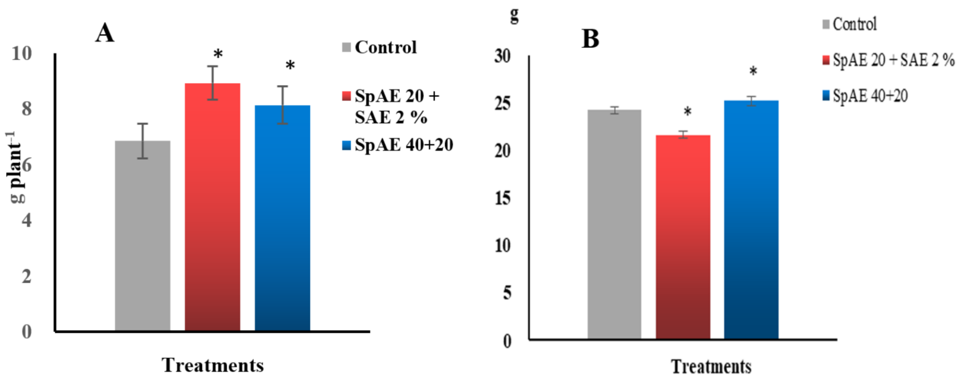
It should be noted that foliar sprays with spirulina extract at 40, 40 and 20 mg ha^{-1}, while not significantly stimulating the number of legumes and the number of grains per legume, did increase the total number of grains per plant.

Figure 2 shows the behavior of the mass of grains per plant (Figure 2A) and the mass of 100 grains (Figure 2B). It should be noted that the treatment where the sprays were made with the two extracts showed a decrease in the size of the grains, since the mass of 100 grains was significantly lower than the other treatments. However, this response did not prevent this treatment from significantly increasing the mass of grains per plant by 30 %, which is related to the increase in the number of pulses and grains per bean and per plant caused by this treatment (Figure 1).



Bars represent confidence intervals at α=0.05 and the asterisks represent treatments which differ significantly from the control treatment

Figure 1. Effect of three foliar sprays with algae extracts on the behavior of the number of legumes (A), the number of grains per legume (B) and the number of grains per plant (C) beans, line C-8



Bars represent confidence intervals at α=0.05 and the asterisks represent treatments which differ significantly from the control treatment

Figure 2. Effect of foliar sprays with algae extracts on the mass of grains per plant (A) and on the mass of 100 grains (B) of bean plants line C-8

It should be noted that, although this sowing was carried out at the end of January and the plantation received no fertilization, the number of seeds per legume, as well as the mass of 100 seeds of the plants in the control treatment reached values similar to those reported for some bean cultivars with red grains sown in October and chemically fertilized (23). However, the difference was in the number of legumes per plant that was almost 50 % lower than reported by the above-mentioned authors. However, it has been argued (24) that eight pulses per plant and an effective population density of 250,000 plants per hectare can guarantee a yield of more than 1.5 t ha^{-1}, although this will be related to the fresh mass of the grains and the number of grains per bean.

In the present work, the sowing distance used does not allow to reach this population density; however, plants that were sprayed with extracts of algae reached a number of legumes greater than 8, while, the number of seeds per legume and the average mass of grains was significantly higher in the treatments that received two sprays with EASp and one with EAS and in those that received three sprays with EASp, respectively. This indicates that, in the absence of fertilizers, the use of these extracts could become a viable alternative for bean production.

The results of the yield component evaluations of C-13 plants, at harvest time, are presented in Table 1.

It was observed that the plants of the control treatment of this line presented a number of legumes per plant and grains per vegetable similar to the C-line8; however, the mass of 100 grains reached a value much lower than those reported for commercial cultivars of white grain grown in the country (25), although it was similar to that reported for a line from CIAT, Colombia, introduced in the country (26).

Foliar sprays with the aqueous extract of sargassum at 25, 39 and 53 days after sowing did not change the mass of 100 grains but significantly increased the number of legumes per plant (32.5 %), the number of grains per legume (21.4 %) and per plant (44.8 %), as well as the mass of grains per plant (48,6 %) of that line. It should be borne in mind that this effect occurred even when a lower concentration of the extract (0.3 %) was used on the first spray.

Plant responses to applications of different types of algae extracts are due not only to the various metabolites present in their composition, but also to the stimulation of the activity of beneficial microorganisms present in the rhizosphere (27, 28). Thus, the potential of macroalgae, microalgae and cyanobacteria extracts, when used as biofertilizers and biostimulants, has been reviewed by several authors (10, 29-32).

In legumes it has been shown that the application of spirulina extracts stimulates the germination of the seeds of peanuts (13), improves germination, vigor and prevents the deterioration of the seeds of *Vigna mungo* (33), while leaf sprays stimulated the growth and yield of *Lupinus luteus* (14).

On the other hand, it was found that applications every ten days of an aqueous extract of *Sargassum polycystum* in *Vigna radiata* and *Vigna mungo* stimulated plant growth and increased concentrations of photosynthetic pigments, soluble proteins, reducing and total sugars and amino acids; response associated with the presence in the extract of plant hormones, macro and microelements (34).

Favorable results in plant growth and yield of *Vigna unguiculata* L. were obtained when plants were sprayed with an extract from Sargassum crassifolium (20 %) in combination with inorganic fertilizer (35) and subsequently, it was reported that the application of an extract from *Sargassum wightii* 1%, together with biofertilization with *Rhizobium* exhibited the best results in accelerating the growth and yield of plants of this species (36).

In beans, five foliar sprays with an extract of *Sargassum wightii* have been reported to stimulate plant growth and yield indicators (37). Recently, it was found that the application of spirulina extracts to soil, together with foliar spraying at 15, 30 and 45 days after sowing, stimulated the growth and production of plants grown in a saline soil contaminated with heavy metals, counteracting the effects of stress and minimizing grain contamination (18).

In addition, some authors have used foliar spraying at three points in the crop cycle with seaweed extract, spirulina extract and a combination of both and found that although all treatments were superior to control, the best results were obtained by applying a combination of seaweed extracts and spirulina (38).

The results obtained in this work confirm the biostimulating activity of the extracts of spirulina and sargassum, despite the differences that exist regarding the solvent and the method of preparation used to obtain them. For example, in this research 70 % ethanol was used to obtain the spirulina extract, while other authors have used solvents such as pure ethanol, methanol, acetone:methanol (1:1), water, among others (14, 34, 39).

The polarity influence of the solvent on the composition of the extracts is known. Thus, it has been reported that there is a significant change in the antioxidant activity and phytonutrient content of spirulina ethanolic extract compared to aqueous extract (40).

In relation to sargassum, it must be meant that the extract used presented a low concentration of phenols, flavonoids, proteins and soluble carbohydrates (data not published), compared with what was reported at international level. However, the foliar aspersions of it in the bean crop were able to stimulate grain production.

These results, although they must be confirmed, are of great scientific and economic importance for the country, because it demonstrated the benefit of carrying out three foliar sprays with extracts of spirulina and sargassum, relatively easy to prepare, both alone and combined, in bean cultivation, mainly in areas where no fertilization is available.

CONCLUSIONS

Foliar sprays with spirulina and sargassum extracts, one during the vegetative phase (25 DAS) and two during the reproductive stage (39 and 53 DAS), increase grain production in the bean crop, especially at 25 and 39 DAS with the spirulina extract (20 mg ha^{-1}) and 53 DAS with 2 % sargassum extract; as well as the three sprays with an aqueous sargassum extract.

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Table 1. Effect of foliar spraying with an aqueous sargassum extract on the behaviour of bean yield components, line C-13

Treatments	No. of legumes/plant	No. of grains/vegetable	No. of grains/plant	Mass of grains/plant (g)	Mass of 100 grains (g)
Control	7.7 ± 0.8	4.2 ± 0.2	29.2 ± 3.1	3.955 ± 0.41	13.81 ± 0.31
EAS	10.2 ± 0.9*	5.1 ± 0.2*	43.3 ± 3.6*	5.877 ± 0.52*	13.80 ± 0.36

*Represents the treatment averages that differ significantly from the control according to confidence intervals a α=0.05

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