



## Effect of three sargassum extracts on bean grain production and quality

### Efecto de tres extractos de sargazo en la producción y calidad de granos de frijol

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**ABSTRACT:** *Sargassum* washouts have become a significant problem in the coastal areas of the Caribbean Sea. In Cuba, this phenomenon has occurred in the northwestern and south-central regions, making it urgent to utilize the biomass accumulated on coasts and beaches. Furthermore, macroalgae have gained interest in agriculture because they contain growth regulators and micro- and macronutrients that increase crop yields. Their extracts are considered biostimulants for organic agriculture due to their safety and biodegradability. Therefore, the objective of this study was to determine the effects of foliar spraying with three different *Sargassum* extracts on bean production and quality. An experiment was conducted under field conditions using the cultivar CUL-156. Three foliar sprays were tested with doses equivalent to 2% of three *Sargassum* extracts obtained using different methodologies. Grain yield and quality components (protein, fat, moisture, and ash) were evaluated. The results showed that foliar sprays with the aqueous extract of kelp obtained by maceration (EAS1) were the most effective, increasing the number of pulses, the number of grains, and the grain mass per plant. It also increased protein and ash.

**Key words:** macroalgae, number and mass of grains, sprinkles.

**RESUMEN:** Las arribazones de sargazo se han convertido en una problemática significativa en las zonas costeras del Mar Caribe. En Cuba se ha dado este fenómeno en la zona norte occidental y en el centro sur por lo que urge darle utilización a la biomasa acumulada en las costas y playas. Por otra parte, las macroalgas han cobrado interés en la agricultura ya que poseen reguladores del crecimiento, micro y macronutrientes que incrementan los rendimientos de los cultivos. Sus extractos son considerados bioestimulantes para la agricultura orgánica por su inocuidad y biodegradabilidad. Por tal motivo, el objetivo del presente trabajo fue determinar los efectos que la aspersión foliar con tres extractos diferentes de sargazo ejerce en la producción y calidad de granos de frijol. Se ejecutó un experimento en condiciones de campo donde se utilizó el cultivar CUL-156. Se probaron tres aspersiones foliares con dosis equivalentes a 2 % de tres extractos de sargazo obtenidos con diferentes metodologías. Se realizaron evaluaciones de los componentes del rendimiento y la calidad de los granos (proteínas, grasas, humedad y cenizas). Los resultados demostraron que las aspersiones foliares con el extracto acuoso de sargazo obtenido por maceración (EAS1), fueron las más efectivas, ya que incrementaron el número de legumbres, el número de granos y la masa de granos por planta. Además, provocó un incremento en las proteínas y las cenizas.

**Palabras clave:** macroalgas, número de granos, masa de granos, aspersiones.

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**Methodology:** Anaysa Gutierrez Almeida, Lisbel Martínez González, and Miriam de la C. Núñez Vázquez. **Supervision:** Yanelis Reyes Guerrero and Miriam de la C. Núñez Vázquez. **Writing - original draft, Writing - review & editing, and Data curation:** Yanelis Reyes Guerrero, Anaysa Gutierrez Almeida, and Miriam de la C. Núñez Vázquez.

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## INTRODUCTION

*Sargassum* is a genus of planktonic macroalgae belonging to the class Phaeophyceae (brown algae) in the order Fucales. Within this genus, the species *Sargassum fluitans* and *Sargassum natans* are recognized as the only two holopelagic species, since they originate from the Sargasso Sea and reproduce vegetatively without ever requiring a substrate (1). In the last decade, unprecedented amounts of holopelagic sargassum have been reported arriving on the coasts of the Atlantic Ocean (2). In Cuba, strandings have been reported on the south-central coast (3) and on the northwestern coast (4). This biomass accumulated on coasts and beaches can affect biodiversity by reducing light and oxygen, impact human health through the production of hydrogen sulfide during decomposition, and consequently influence the tourism and fishing industries. One alternative for the use of this biomass would be its application in agriculture. Scientific evidence demonstrates the stimulating action of sargassum extracts on crop yield and nutritional quality (5). For example, in soybean cultivation, treatment with an extract of *Sargassum crassifolium* was found to promote plant growth and yield (6). Likewise, it has been reported that an extract of *Sargassum polycystum* improved the germination of *Sesamum indicum* and stimulated the vegetative growth of *Vigna radiata*; however, this extract did not affect the growth of *Phaseolus vulgaris* seedlings (7). In Cuba, there is very little evidence of the use of sargassum biomass for agricultural purposes. Therefore, the objective of this study was to evaluate the effect of three sargassum extracts, obtained through different methodologies, on yield components and grain quality of beans (*Phaseolus vulgaris* L.) cv. CUL-156.

## MATERIALS AND METHODS

The experiment was conducted at Las Papas Farm, belonging to the Agricultural Services Department of the National Institute of Agricultural Sciences, on agrogenic Leached Red Ferrallitic soil (8). Seeds of the cultivar CUL-156 were used, which were inoculated with Azofert® prior to sowing, at a rate of  $200 \times 10^{-3}$  L of inoculum per 46 kg of seed. The sowing distance used was  $0.7 \times 0.07$  m.

Foliar sprays on the plants with the three *Sargassum* extracts at 2 % and with water in the case of the control treatment were applied at 26, 40, and 53 days after sowing (DAS), corresponding to the phenological stages of vegetative growth, flowering, and grain formation, respectively.

The extracts used were:

1. Aqueous extract of *Sargassum* obtained from fresh material in a 1:6 m:v ratio by maceration at room temperature for three months (EAS1).
2. Aqueous extract of *Sargassum* obtained from dried and ground material in a 1:25 m:v ratio, boiled for 2 h (EAS2).
3. Hydroalcoholic extract (EtOH 50 %) of *Sargassum* obtained from dried and ground material in a 1:20 m:v ratio, heated at 50 °C for 15 min (EES).

A randomized block design with four treatments and four replicates was used. 16 plots consisted of three rows, each 8 m long, with an area of 16.8 m<sup>2</sup>.

Crop management practices were carried out according to the Technical Guide of the crop (9), except that the area did not receive any mineral fertilization and irrigation was performed with a central pivot machine at intervals of approximately five days.

Evaluations were carried out at harvest, corresponding to 81 days after sowing. Twenty-five plants per plot were selected from the three central rows of each experimental unit. For each plant, the following parameters were recorded: number of pods per plant, number of grains per pod (calculated from counts of five pods selected per plant), total number of grains per plant, grain mass per plant (expressed in grams), and 100-grain mass (determined by weighing five samples of 100 grains collected per plot).

After verifying that the data met the theoretical assumptions of normality and homogeneity of variance, a two-way ANOVA was performed. In cases where significant differences between treatments were found, a comparison of means was conducted using Tukey's Multiple Range Test with  $p \leq 0.05$ , employing the statistical program SPSS v.21.

In addition, four samples of bean grains per treatment were analyzed to determine protein, ash, fat, and moisture content using a SpectraAnalyzer NIR Zeutec device. The data obtained were expressed as percentage variation with respect to the control treatment according to the following formula:

$$\text{Percentage of variation}(\%) = \frac{\text{treatment} - \text{control}}{\text{control}} \times 100$$

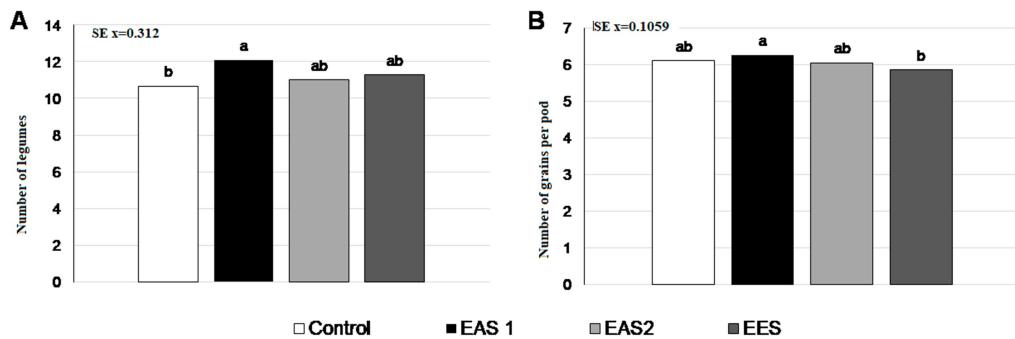
For the statistical processing, means, standard deviations, and confidence intervals were calculated at  $\alpha = 0.05$ .

## RESULTS AND DISCUSSION

Figure 1 shows the influence of the three *Sargassum* extracts on the performance of the number of pods and grains per pod in beans cv. CUL-156. It can be observed that the aqueous extract obtained by maceration (EAS1) was the only one that showed significant differences compared to the control in the number of pods, although it did not differ from the other extracts (Figure 1A). In the case of the number of grains per pod (Figure 1B), EAS1 did not differ significantly from the control, but it did differ from the 50 % hydroalcoholic extract (EES).

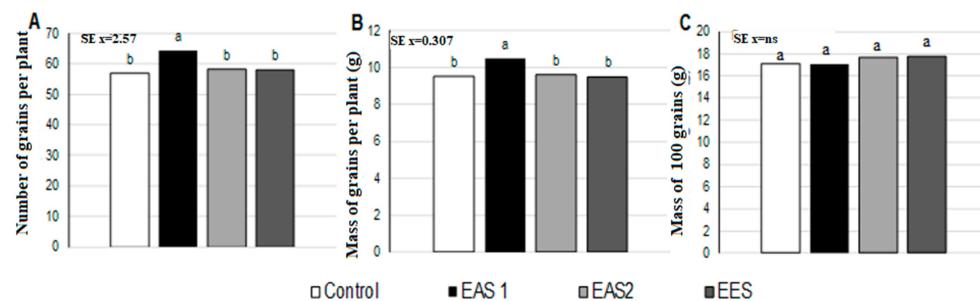
Regarding the number (Figure 2A) and grain mass (Figure 2B) per plant, it can be observed that EAS1 was the only treatment that significantly increased both indicators, which was not the case for the 100-grain mass (Figure 2C).

The different response found could be related to the composition of the extracts, since it is known that the aqueous extract obtained from dried and ground powder has a higher concentration of proteins, carbohydrates,



EAS1: Aqueous extract from fresh material in a 1:6 mass:solvent ratio. EAS2: Aqueous extract from dried and ground material in a 1:25 ratio. EES: Hydroalcoholic extract (50%) from dried and ground material in a 1:20 ratio. Means with common letters do not differ significantly according to Tukey's Multiple Range Test ( $p \leq 0.05$ ),  $n = 100$

**Figure 1.** Influence of three foliar sprays with different Sargassum extracts on the number of pods (A) and the number of grains per pod (B) of beans cv. CUL-156



EAS1: Aqueous extract from fresh material in a 1:6 mass:solvent ratio. EAS2: Aqueous extract from dried and ground material in a 1:25 ratio. EES: Hydroalcoholic extract (50 %) from dried and ground material in a 1:20 ratio. Means with common letters do not differ significantly according to Tukey's Multiple Range Test ( $p \leq 0.05$ ),  $n = 100$

**Figure 2.** Influence of three foliar sprays with different Sargassum extracts on the number of grains per plant (A), grain mass per plant (B), and 100-grain mass (C) of beans cv. CUL-156

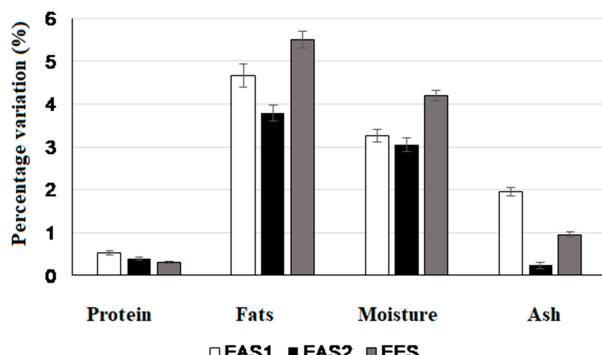
total phenols and flavonoids, and pigments than that obtained from fresh material (10); however, the latter showed the best response, which indicates that it is necessary to test more diluted solutions in the case of the extract prepared with dried powder, since in this study the same concentration was used in all three extracts. The response of the plants to the ethanolic extract was similar to that of the aqueous extract, despite the differences in solvent polarity, which lead to differences in composition.

In a study dedicated to drying methods and the use of different solvents (methanol, ethanol 70 %, acetone, chloroform, and hexane) for the preparation of *Sargassum polycystum* extracts, it was found that the ethanolic extract showed the highest amount of total phenols and also exhibited greater antibacterial activity; whereas the methanolic extract showed the highest antioxidant activity (11). On the other hand, in another study where four solvents (hexane, dichloromethane, methanol, and water) were used to prepare extracts of *Sargassum* sp., it was found that dichloromethane was the most effective solvent in promoting tomato hypocotyl growth; while in *Capsicum annuum*, hexane was superior to the other solvents in promoting seedling development (12).

Furthermore, when using extract of *Sargassum wightii* Greville (concentration of 1%) with and without *Rhizobium* in cowpea plants, it was found that both treatments stimulated vegetative growth (shoot and root dry weight, number of lateral roots, and total leaf area), biochemical indicators (total chlorophyll, carotenoids, proteins, lipids, total sugars, and amino acids), and yield and its components (number of capsules, length, weight, number of seeds per pod, and 100-seed weight); with the combination of the macroalga and the bacterium standing out.

Figure 3 shows the percentage of variation with respect to the control of some indicators of bean grain quality sprayed with different Sargassum extracts. Spraying with extract EAS1 increased protein content by 0.52 %, while EAS2 increased it by 0.39 % and EES by 0.30 %. Regarding fats, the alcoholic extract (EES) caused the greatest increase with 5.5 %, followed by EAS1 with 4.7 % and EAS2 with 3.8 %. Moisture behaved similarly, being higher in grains from plants sprayed with EES, while EAS1 caused, compared to the other extracts, a higher ash percentage (1.2 %) with respect to the control.

The results found in this study are positive and show that foliar spraying with the aqueous extract of *Sargassum* obtained by maceration of fresh material (EAS1)



EAS1: Aqueous extract from fresh material in a 1:6 mass:solvent ratio. EAS2: Aqueous extract from dried and ground material in a 1:25 ratio. EES: Hydroalcoholic extract (50 %) from dried and ground material in a 1:20 ratio

**Figure 3.** Influence of three foliar sprays with different *Sargassum* extracts on the percentage of variation with respect to the control in quality indicators of bean grains cv. CUL-156

had a favorable effect on the production of beans cv. CUL-156. This extract significantly increased the number of pods, the number of grains per plant, and the grain mass per plant, indicating an increase in crop yield. In addition, the effect on grain quality was also beneficial, as an increase in protein and ash content was observed, improving the nutritional value of beans. Although the other extracts also showed effects, the EAS1 extract was the most effective, suggesting that its composition has a more favorable impact.

These results are encouraging for considering this strategy as a good future option to improve agricultural production sustainably, taking advantage of *Sargassum* biomass, an abundant and renewable resource. However, the study also points to the need for further research and adjustment of concentrations to optimize the use of other types of extracts.

Several studies have demonstrated that marine algae such as *Padina gymnospora*, *Gracilaria edulis*, and *Ulva fasciata* increased protein content in *Capsicum annuum* (14). Likewise, in three soybean varieties, the application of a commercial biostimulant based on *Ascophyllum nodosum* increased protein and fat content, with the lowest concentrations used being the most effective (15). Foliar application of 0.5 % of the commercial extract of *A. nodosum* significantly increased bulb diameter, neck diameter, and yield in four onion cultivars. In addition, it improved total soluble solids, ascorbic acid, N, K, and P content (16). In the case of *Sargassum*, it has been found that an alcoholic extract of *Sargassum* spp. increased protein content, total phenols, flavonoids, and antioxidant capacity (ABTS) in tomato seedlings (17). When evaluating the potential of *Sargassum wightii*, *Turbinaria ornata*, and *Caulerpa racemosa* on the biochemical indicators of *Ocimum sanctum*, a considerable increase in starch, glucose, protein, and chlorophyll content was obtained in treated plants (18). The application of an extract of *Sargassum swartzii* (SSE) significantly improved phenolic content, flavonoids, proteins, and antioxidant capacity in *Vigna unguiculata* (19).

When evaluating the effect of an extract of *Sargassum polycystum* on some biochemical indicators of *Vigna radiata* and *Vigna mungo*, an increase in photosynthetic pigments, proteins, reducing sugars, total sugars, and amino acids was obtained (20). However, no reports have been found referring to the increase of these indicators in bean grains through the use of *Sargassum* spp. as a biostimulant

## CONCLUSION

The aqueous extract from fresh *Sargassum* (EAS1) showed the greatest potential as a biostimulant in beans cv. CUL-156, significantly increasing yield and grain quality, whereas the aqueous extract from dried and ground material (EAS2) and the hydroalcoholic extract (EES) had lesser effects, with EES standing out for increasing the fat content in the grains.

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