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Short communication

New biostimulant and its influence on the production of common beans

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ABSTRACT

The use of biostimulants has increased in agriculture in the last years, as an important agroecological practice. *Spirulina* and vinasse have properties that promote the crop growth and development as well as improve their nutritional quality. The objective of the present paper was to determine the effect of a biostimulant, composed of an aqueous suspension of *Spirulina* jelly and vinasse, applied alone or in combination with other biostimulants, in the bean grain production. For this, two experiments were performed, using 'Inqueño' cultivar. In the first, two foliar sprays were made with two doses equivalent to 3.5 and 7 L ha^{-1} of the new biostimulant and in the second, two sprays were also made. The first with the new biostimulant (2.5 L ha^{-1}) and the second one was carried out with the new biostimulant at the same dose or with Quitomax® or with the combination of both. The application of the new biostimulant, composed by *Spirulina*

and vinasse in combination with Quitomax® improved the grain and pod production in bean plants biofertilized with Azofert® and it may be an alternative for increasing the crop production.

Key words: *Phaseolus vulgaris*, chitosan, yield, *Spirulina*, vinasse

INTRODUCTION

The bean (*Phaseolus vulgaris* L.) is a legume with high nutritional values for human consumption worldwide and especially in Cuba and it is considered a source of calories, dietary fibers, minerals and vitamins [(1)]. It is a food of high popular demand, it contributes to the prevention and treatment of pathologies, such as cardiovascular diseases, diabetes and cancer, which constitute serious problems in human health [(2)]. This legume has traditionally been an important element in Latin America and in a large number of developing countries in which it is cultivated [(3)].

In Cuba, it is cultivated throughout the country; however, national production is not yet capable of meeting the population's consumption needs. Beans need high amounts of nitrogen, a basic component of proteins, enzymes, nucleic acids and vitamins. Of all the nitrogen extracted by the plant, the highest percentage is absorbed until the moment the grain is filled and the maximum extraction rate occurs in the flowering stage [(4)].

Some authors have shown that cyanobacteria under suitable conditions could be useful as a source of nitrogen in crop production [(5)]. Among them, we find *Spirulina*, a symbiotic, multicellular and filamentous green-blue microalga. It uses nitrogen from the air and contains between 46-71 % protein, 8-16 % carbohydrates and 4-9 % lipids in its dry mass. In addition, it contains minerals, essential amino acids such as leucine, isoleucine and valine; a relatively high concentration of provitamin A, vitamin B12, vitamin K and β-carotene, its fatty acids contain linolenic and γ-linolenic acid and the polyunsaturated fatty acids ω-3 and ω-6 [(6)]. This composition of *Spirulina* has made it possible to use it as a stimulator of plant growth and development [(7)], as a partial substitute for chemical fertilizers [(8)], as well as a biofortification agent [(9-11)].

On the other hand, vinasse, a residue obtained from the production of ethanol from sugar cane, beet, corn, wheat, rice, etc., has been used in several countries as a source of nutrients for crops [(12,13)] and as a medium supplement for the culture of bacteria [(14)] and microalgae [(15)]. In addition, it has been reported that it produces beneficial effects on the physical-chemical and biological properties of soils [(12,16)].

In Cuba, the company LABIOFAM, S.A. has produced a new biostimulant, which is composed of an aqueous suspension of *Spirulina* jelly and vinasse that is expected to be capable of stimulating plant growth and performance, when applied both alone and in combination with other biostimulants of national production.

Due to all of the above, the objective of this work was to determine if the application of this new product, which contains *Spirulina* and vinasse, alone or in combination with Quitomax® stimulates the production of grains in the bean crop.

MATERIALS AND METHODS

To fulfill the objective of this work, two experiments were carried out at the headquarters of the National Institute of Agricultural Sciences, located in the municipality of San José de las Lajas, Mayabeque province. In both cases, a bean production area (*Phaseolus vulgaris* L.) cv Inqueño was used. The first area was sown on December 13, 2017 and when the plants were in the flowering phase, 30 linear rows of 80 m each were selected for the execution of the experiment (1 600 plants per row), which is equivalent to to an area of 1 680 m^2.

The new biostimulant, made up of an aqueous suspension of *Spirulina* jelly and vinasse, in a proportion of 64 and 36 %, respectively, was supplied by the *Spirulina* Base Business Unit of Zaragoza, San José de las Lajas, Mayabeque; belonging to the Company LABIOFAM S.A.

Two foliar sprays were carried out on the plants; the first was carried out in the flowering phase and the second fifteen days after the first. In both cases, two doses of the biostimulant were used, equivalent to 3.5 L ha^{-1} (dose recommended by the manufacturer) and 7 L ha^{-1}. The sprays were made with a 16 L backpack at ten rows per treatment, which is equivalent to an area of 560 m^2. The cultural care was carried out in accordance with the Technical Guide for cultivation [17], except for the fertilization that was done with the complete formula (NPK 9:13:17) at a rate of 100 kg ha^{-1}.

The second production area was sown on October 3, 2018 and unlike the previous one, in this case the seeds were inoculated, prior to sowing, with Azofert® (at a rate of 200 mL 50 kg^{-1} of seed), *Rhizobium*-based biostimulant.

Ten days after sowing, the first application of the new biostimulant was made to 30 rows of 80 m long with a dose lower than those used in the previous experiment and that was equivalent to 2.5 L ha^{-1}. Ten days later, the furrows were separated into three groups of

ten furrows each and sprayed: the first with the new biostimulant at a similar dose, the second with Quitomax® (100 mg ha^{-1}), a polymer-based biostimulant of chitosan and the third with the new biostimulant (2.5 L ha^{-1}) and Quitomax® (100 mg ha^{-1}). The cultural attentions were similar to those applied in the first experiment.

At the time of harvest, in both experiments the evaluations of the components of the plant performance were carried out, such as: number of legumes per plant, number of grains per legume and per plant, mass of grains per plant and mass of 100 grains. Furthermore, in the first experiment, to estimate agricultural yield, four areas per treatment of 1 m^2 each were selected and the harvest was carried out. The plants were threshed and the grains were dried to 14 % humidity, then weighed and the yield was expressed in t ha^{-1}.

When performing the treatments by rows, a sample design was used for the evaluations, which consisted of taking four random samples of ten plants each, from the four central rows, by treatment and the data obtained were processed by calculating the means, the standard deviation and the confidence intervals at $\alpha = 0.05$; for which the Excel Program, Windows-7 was used.

RESULTS AND DISCUSSION

The two foliar sprays to bean plants cv. Inqueño, with the new biostimulant based on *Spirulina* and vinasse, in general, they did not exert a significant effect on any of the performance components analyzed, except in the number of grains per legume, which increased significantly with the two sprays of the dose of 3.5 L ha^{-1}. Regarding the estimated performance, no significant differences were found between treatments, despite the fact that the lower dose used stimulated performance by 22 % compared to the control treatment (Table 1).

Table 1. Influence of a new biostimulant based on *Spirulina* and vinasse on the production of beans from bean plants cv. Inqueño (means ± confidence intervals)

Treatments	Nu. legumes plant $^{-1}$	Nu. grains legumes^{-1}	Nu. grains plant^{-1}	Mass of grains plant^{-1} (g)	Mass of 100 grains (g)	Estimated yield t ha^{-1}
Control	12.0 ± 0.9	4.1 ± 0.2	53.1 ± 4.8	14.2 ± 1.3	27.87 ± 0.39	1.69 ± 0.15
T1 (3.5 L ha^{-1})	13.1 ± 1.1	**5.0 ± 0.1***	60.0 ± 5.6	15.9 ± 1.4	27.50 ± 0.67	2.07 ± 0.33
T2 (7.0 L ha^{-1})	12.2 ± 1.1	4.2 ± 0.2	52.7 ± 6.4	14.7 ± 1.8	28.99 ± 0.43	1.82 ± 0.21

*Representa las medias que difieren significativamente del tratamiento control según intervalo de confianza a α=0.05

Various investigations have been carried out to determine the influence of biostimulants on the production of beans. For example, some authors have used foliar spraying, at three times of the crop cycle, with extracts of seaweed, *Spirulina* extract and a combination of both and have found that, although all the treatments were superior to the control, the best results were obtained with the application of the combination of extracts of seaweed and *Spirulina* [18]. On the other hand, the stimulation of grain production in this crop has also been reported with the foliar spraying of biostimulants such as Fitomas-E®, Biobras-16®, Lebame and efficient Microorganisms [19,20]; but, in this case, foliar sprays were made from stage V3 to R5 (one spray per aspersion). In the present work, only two foliar sprays were during the entire crop cycle carried out.

These first results show the potential of this new product as a biostimulant in bean cultivation. Given the importance that the doses and times of application have in the response of plants to the application of biostimulants [21–25], it is necessary to test lower doses and other times of application of the product, in order to achieve a significant stimulation of the number and mass of grains per plant, variables closely related to crop yield.

For these reasons, in the second experiment two foliar sprays were also carried out, but both in the vegetative phase of the crop and with a lower dose (2.5 L ha^{-1}) than the one used in the first experiment.

The results of this experiment are presented in Table 2. It can be seen that only treatment T3 (spraying with biostimulant ten days after sowing, das and foliar spraying with the biostimulant and Quitomax® at 20 days) exerted a significant influence on the number of legumes and grains per plant. In a similar way, to what was observed with the yield in the previous experiment, this treatment caused an increase of almost 21 % in the mass of grains per plant; however, this increase was not significant.

From these results it is inferred that the new biostimulant applied at a rate of 2.5 L ha^{-1}, at 10 and 20 das (T1), was not adequate to stimulate the production of grains in bean plants, nor was it effective the substitution of this biostimulant by Quitomax® (T2) in the second spray. However, the application of the new biostimulant at 10 das and foliar spraying with both biostimulants at 20 das (T3) was able to stimulate significantly the number of legumes per plant, which also translated into an increase in the number of grains per plant. This shows the benefit of the combination of the two biostimulants for this crop.

Table 2. Influence of the sprays of a new biostimulant (based on *Spirulina* and vinasse) alone or in combination with Quitomax® in the production of beans from bean plants cv. Inqueño, whose seeds were inoculated with Azofert® prior to sowing (means ± confidence intervals)

Treatments	Nu. legumes plant^{-1}	Nu. grains legumes^{-1}	Nu. grains plant^{-1}	Mass of grains plant^{-1} (g)	Mass of 100 grains (g)
Control	16.6 ± 2.2	4.2 ± 0.2	69.8 ± 9.5	19.8 ± 3.1	26.9 ± 1.1
T1 (Sp+V)	15.7 ± 1.6	4.1 ± 0.2	63.7 ± 6.8	16.3 ± 1.8	25.6 ± 0.6
T2 (Quit)	19.1 ± 2.3	4.0 ± 0.2	74.9 ± 8.6	19.6 ± 2.3	26.1 ± 0.7
T3 (Sp+V+Quit)	**22.8 ± 2.3***	3.9 ± 0.1	**89.3 ± 9.2***	23.9 ± 2.7	26.7 ± 0.8

Sp + V: Biostimulant based on Spirulina and vinasse Quit: Quitomax®

*Represents the means that differ significantly from the control treatment according to the confidence interval at $\alpha = 0.05$

The Quitomax® biostimulant has been successfully applied in bean cultivation as a stimulator of agricultural yield. However, this effect has been achieved with a total dose of 400 mg ha^{-1}, applied foliarly, at two moments of the crop cycle, around 20 days after sowing and during flowering [26].

In the present work, a lower dose was used and a single application was made during the vegetative phase; which could explain the non-response found in the T2 treatment. However, the positive response found with the use of the T3 treatment suggests that the combination of these two biostimulants is capable of stimulating the production of grains, even though the spraying was carried out in the vegetative phase of the crop. This also shows the need to continue investigating the doses and times of application to optimize the combined use of this new biostimulant with Quitomax®, which will result, not only, in an increase in grain production, but could also improve the nutritional quality of the same; given the chemical composition of *Spirulina* and vinasse.

These results are the first to be reported, in Cuba, with the application of this new biostimulant, based on *Spirulina* and vinasse, in the cultivation of beans and they suggest the need to continue researching in this regard, to determine the doses and the most suitable application times to significantly stimulate grain production in this crop. On the other hand, it was shown that the combination of this new biostimulant with others of national production such as Azofert® and Quitomax® can be an efficient alternative to increase the sustainable production of beans in the country.

CONCLUSIONS

- In this work, it was demonstrated that the new biostimulant based on *Spirulina* and vinasse in combination with Quitomax® stimulated the production of legumes and grains of bean plants biofertilized with Azofert®.
- The results of this study suggest that the combined application of these biostimulants constitutes an alternative to stimulate the production of this crop.

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