

Effect of Biological Biostimulator on Green bean Root Biomass

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Abstract. The world population continues to grow, there is an urgent need to intensify efforts and innovate to sustainably increase agricultural production. Legumes such as green beans are an excellent source of vegetable protein. In Morocco, the cultivated area of green beans increased from 6,000 hectares in 2017 to 8,000 hectares in 2020, while agricultural production reached more than 200,000 tonnes. This is due to the integration of new agricultural strategies and the use of new monitoring programs. The use of biostimulants as organic fertilizers is proposed to improve crop quality and yield while ensuring greater sustainability of green bean cropping systems. To this end, we proposed to study the efficacy of a biological rooting biostimulant. The results indicate an average increase of 57.32% in root weight from the 90-day growth control and a 59.19% improvement in root length from the 90-day control. This biostimulant shows a very original efficacy in the development of the root system of the green bean plant

Keywords : biomass, biostimulant, green bean, integration, rendement, root sustainability .

1 Introduction

The aim of the new strategies of modern agriculture is to seek sustainable ecological ways to reduce crop dependence on chemical fertilizers [1] and to ensure better yield and quality of food and crops. These chemical fertilizers are non-economic and damaging to the environment and human health [2]. Moreover, the production of organic fertilizer reagents has become a major necessity of agriculture. A biostimulant is a fertilizer that stimulates plant nutrition processes independently of the nutrients. They contain for the sole purpose of improving the agronomic or rhizospheric characteristics of the plant [3]. The global marketplace for plant biostimulants reached \$2 billion in 2019 [4]. However, an estimated 1.5 million hectares of land are at risk and by 2050, 50 percent of arable land is expected to be lost [5]. In recent years, the use of biostimulants as organic fertilizers has become a priority to improve both crop quality and yields while ensuring better sustainability [6, 7]. In addition, biostimulants are applied to directly treat seeds, leaves and other aerial organs as well as soil preparation [8]. In addition, the action of biostimulants is associated with different factors such as: environmental conditions, genetic factor, soil nature and others [9, 10].

In Morocco, the cultivation of pulses occupies a primordial place thanks to their agro-economic and environmental interests. Taking into account the current socio-economic context, the need to maintain or even increase production of agricultural and agri-food products, in order to be able to meet the demands that are increasing on these products, is currently, imposed. Primary sources of biostimulants include humic acid, fulvic acid, protein hydrolysates, algae extracts, chitosan, inorganic compounds, beneficial fungi and beneficial

bacteria [11,12]. The present work is directed towards the establishment of a treatment based on the use of biostimulants and biofertilizers from a biological process of treatment of agri-food by-products and their valorization in the agricultural field, in green beans (*Phaseolus vulgaris*).

2 Material and methods

2.1 Field Preparation

- *Soil analysis*

The soil of the experimental plot is Clay (45%) -limino (20%)-sandy (35%) with no physical constraints for the cultivation of green beans. We have chosen as parameters pH, nitrogen, matter organic, potassium, phosphorus.

- *Soil preparation*

For the preparation of the seed bed, a tillage of 25 to 30 cm was carried out using the toothed tools followed by two cross-passes with the cover crop in order to break the clods, aerate the soil and ensure the thickest layer of loose soil with weed removal,

- *Type of irrigation*

The irrigation system adopted is the drip irrigation sheath, with three irrigations per week of a flow of 2 L/h.

2.2 Plant materials

Our choice has been on the cultivation of green beans (*Phaseolus vulgaris*), belonging to the family of legumes whose part consumed is the unripe pod, because of its richness in protein, vitamins A and C and mineral salts Ca, Mg, P and K. Sowing was done in March in the field.

2.3 Biostimulants

Rooting Biostimulant: it contains extracts of fermented and stabilized products, rich in free amino acids from hydrolysate obtained by the transformation and stabilization of by-products. However, the presence of polysaccharides, phosphorus and other essential trace elements in combination with amino acids allows having a well-developed root system. It is applied drip every 7 days at a rate of 5 litres/ha. The composition (indicated on the packaging of the product) is as follows:

Extract Algae (30%); Carbohydrate Matter (20%); Total Nitrogen (12%); Ammoniacal Nitrogen (5%); Phosphorus Anhydrous (P₂O₅) (5%); Potassium Oxide (K₂O) (15%); Total Amino Acids (5%); Zinc (0.5%); Manganese (0.5%); Boron (0.35%) and Molybdenum (0.2%).

2.4 Statistical measurement and analysis

Monitoring and evaluation of the effect of biostimulants throughout the culture cycle was carried out on measurements every 15 days using 10 samples per sampling. The parameters taken into consideration for the roots are their weight and length.

The SPSS software carried out the statistical analysis. A descriptive analysis based on the SD mean and a comparison of the mean by the t student test.

3 Results

3.1 Physicochemical and microelement analysis

Table 1 presents the results of the physical-chemical and microelement analysis of the soil selected for the study. The parameters showing very high measurements (exceeding the range) are the basic trend PH (8), Oxidizable organic matter (3.36%), Phosphorus available

Olsen (77 mg/kg), Calcium available meq/100 g (22.1g), Magnesium available meq/100g (5.34g), Potassium available meq/100g (0.99) and Manganese mg/kg (9.34 mg/kg) on the other hand low levels were recorded for Active Limestone %CaCO₃, Iron mg/kg and Zinc mg/kg.

Table 1: Physicochemical characteristics and soil microelements

Parameters	Results	Standard
pH	8	Very high
Electrical conductivity Us/cm at 20°C	286	normal
Oxidizable organic matter %	3,36	Very high
Nitrogen Dumas mg/kg	2,18	haut
Phosphorus available Olsen mg/kg	77	Very high
Active limestone %CaCO ₃	2	stockings
Available calcium meq/100 g	22,1	Very high
Magnesium available meq/100g	5,34	Very high
Potassium available meq/100g	0,99	Very high
Available sodium meq/100g	0,76	Haut
Boron mg/kg	0,90	Normal
Iron mg/kg	<4	Low
Manganese mg/kg	9,34	Very high
Copper mg/kg	0,94	Normal
Zinc mg/kg	0,91	Low

3.2 Rooting biostimulant effect on root biomass

Table 2 shows the results of the comparison of the mean root weights between the control and the treated over time. In addition, the differences between the mean control and treaty weights appear to be highly significant for all time intervals ($p < 0.000$). Figure 1 presents the results of the evolution in the weight of the green bean root biomass during its vegetative cycle. This evolution appears to increase with time in the control group with a correlation coefficient of ($r = 0.99$; $p < 0.000$) and in the biostimulant group ($r = 0.99$; $p < 0.000$). In fact, the root weights at 15 days of growth in the control and the treated are respectively 1.002 ± 0.02 g and 1.494 ± 0.02 g, this difference between the two average weights was very highly significant ($t = -59.278$; $p < 0.000$). After 90 days of growth, the control and the treated weights are 20.954 ± 0.06 g and 32.969 ± 0.15 g respectively. Moreover, a marked improvement in root weight is observed under the effect of this biostimulants. Figures 1a and 1b show the density of roots in the green bean plant.

Table 2 Comparison of control green bean root weight averages and Time Treated by the student test

days	Group	Mean±SD Average root weight	Test levene	p-value	Test « student »	p-value
J 15	control plant	1,002±0,02	0,32	0,578	-59,278	p<0,000***
	treated plant	1,494±0,02				
J30	control plant	1,5±0,01	2,09	0,16	-635,05	p<0,000***
	treated plant	8,508±0,03				
J45	control plant	8,05±0,10	0,59	0,45	-119,83	p<0,000***
	treated plant	14,98±0,15				
J 60	control plant	12,99±0,11	0	1	-203,59	p<0,000***
	treated plant	23,01±0,11				
J 75	control plant	17±0,11	0,76	0,39	-238,23	p<0,000***
	treated plant	28,958±0,12				
J 90	control plant	20,954±0,06	2,11	0,16	-234,46	p<0,000***
	treated plant	32,969±0,15				

J: day; **N**: effective; **Mean**: average; **SD**: standard deviation; **Levene test** on the equality of variances; **Test “t”** for equal averages and *******: very highly significant difference.

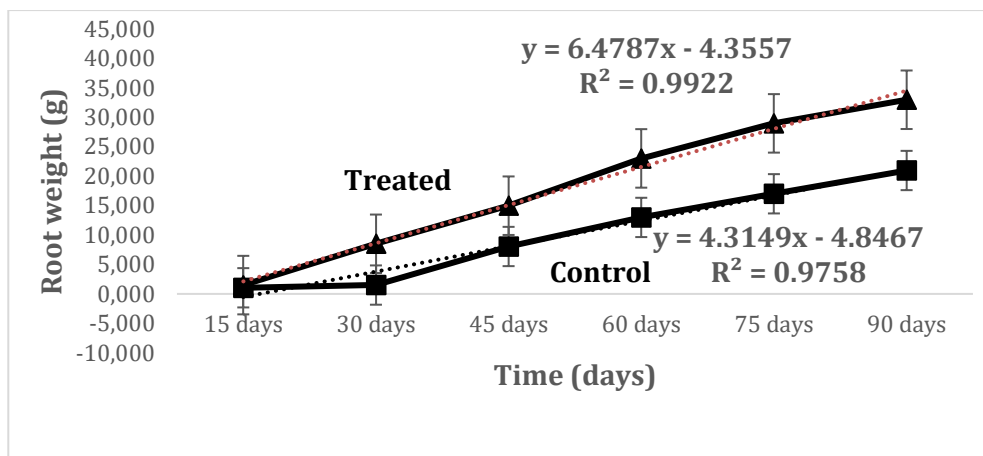
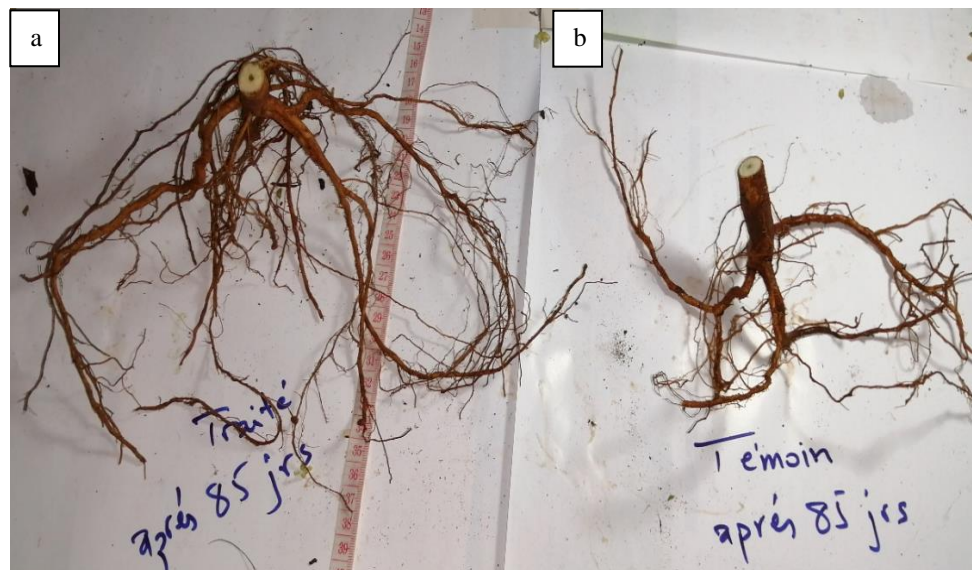


Figure 1 Evolution of the weight of green bean root biomass during its vegetative cycle



3.3 Rooting biostimulant effect on root elongation

The results of the comparison of the average length of the roots are presented in Table 3. There is a very significant difference between the mean length of the control and the treatment as a function of time, and this is apparent during all the sampling intervals of measure ($p < 0.000$). Figure 2 shows the results of the evolution of the length of the green bean root during its vegetative cycle. This evolution appears to increase with time in the control group with a correlation coefficient of ($r = 0.98$; $p < 0.000$) and in the biostimulant group ($r = 0.97$; $p < 0.000$). Indeed, in the control the average length of the roots increases from 5.03 ± 0.05 cm on the 15th day of growth to 30.80 ± 0.05 cm on the 90th day to 49.03 ± 0.05 cm on the 90th day. In addition, the mean treatment length significantly exceeds that of the control (see photo a and b).

Table 3 comparison of root-length means of the green bean between the control and the treated over time by the “student” test

Days	Group	Mean \pm SD Average root length	Test levene	p-value	Test « student »	p-value
J 15	Témoins	$5,03 \pm 0,05$	0,004	0,950	-177,419	$p < 0,000^{***}$
	Traité	$9,03 \pm 0,05$				
J30	Témoins	$8,03 \pm 0,06$	0,900	0,355	-499,69	$p < 0,000^{***}$
	Traité	$21,02 \pm 0,04$				
J45	Témoins	$12,01 \pm 0,03$	0,002	0,982	-1171,23	$p < 0,000^{***}$
	Traité	$30,00 \pm 0,03$				
J 60	Témoins	$19,03 \pm 0,05$	1,51	0,238	-526,68	$p < 0,000^{***}$
	Traité	$37,03 \pm 0,09$				
J 75	Témoins	$26,02 \pm 0,04$	1,65	0,24	-866,36	$p < 0,000^{***}$
	Traité	$45,05 \pm 0,05$				

J 90	Témoïn	30,80±0,05	1,11	0,16	-102,89	p<0,000***
	Traité	49,03±0,05				

J: day; **N**: effective; **Mean**: average; **SD**: standard deviation; **Levene test** on the equality of variances; **Test “t”** for equal averages and *******: very highly significant difference.

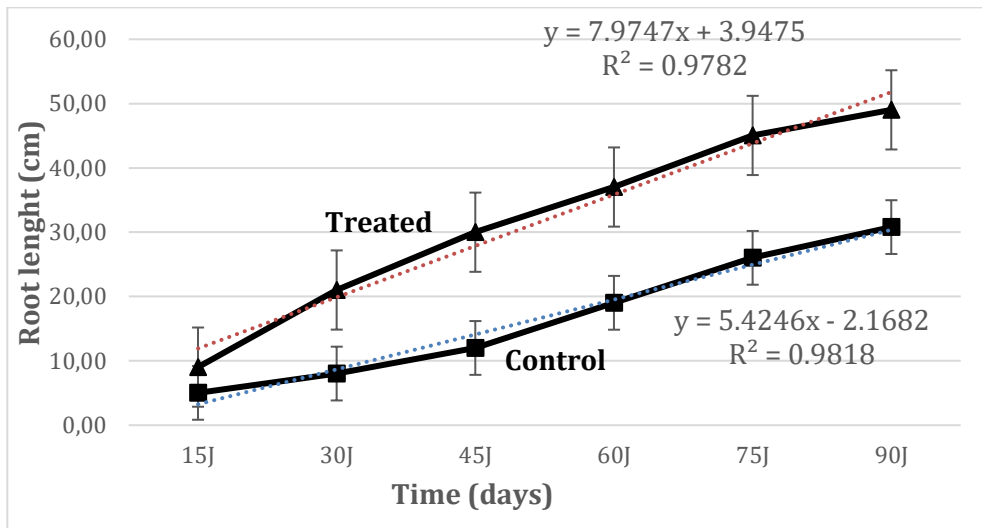
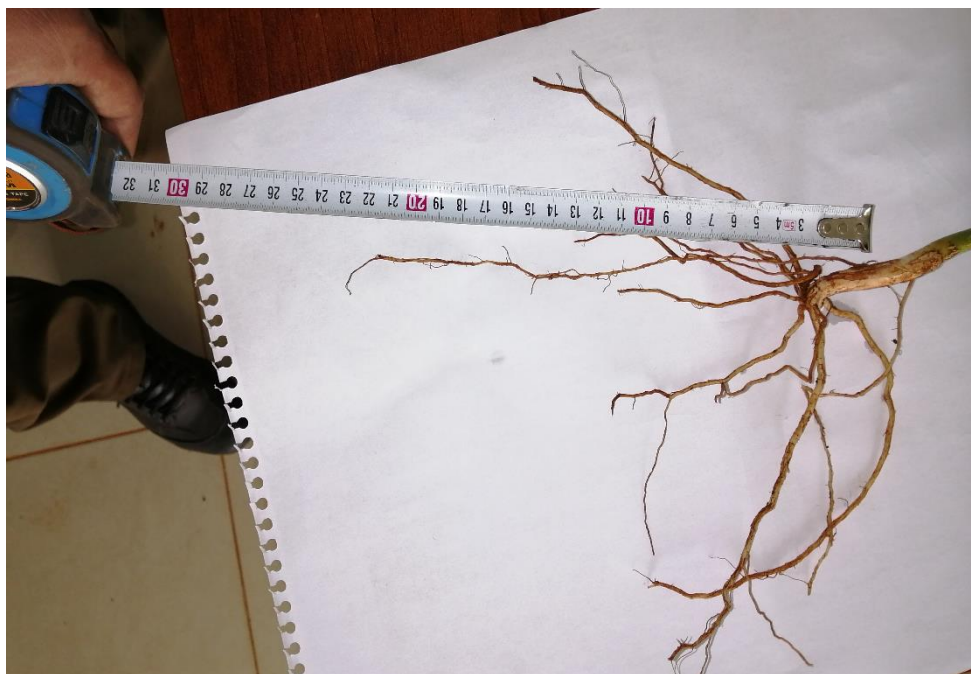


Figure 2 Evolution of the length of green bean roots during its vegetative cycled



4. Discussion

In the face of environmental constraints, plants are able to modify their root systems, rhizosphere, either directly or indirectly by establishing links with other soil organisms [13]. Improving biofertilizers has become a priority to meet increased demand for organic products. There are formulations of these biostimulators that can promote and sensitize plant defences and resistance against various environmental stresses. In our study, we conducted field experiments to study the effect of a rooting biostimulant. Results show a significant increase in root weight per plant. These results are consistent with previous work on other biostimulants (humic acid, fulvic acids) Specifying that the perception at the root level of a nutrient concentration in the medium gives rise to the induction of signals leading to local changes in root and foliar architecture. These localized responses can result in increases in root growth and root diameter as well as increases in secondary root numbers [14, 15]. This improvement is mainly due to algae extracts that improve nutrient uptake. In particular, they allow the plant to better tolerate nutrient deficiencies in nitrogen by promoting the expression and/or activity of nitrate reductase through certain compounds (mannitol) [16, 17]. The expression of root phosphatases involved in phosphate absorption can also be stimulated by certain algae extracts [18]. Work of [19] showed that the use of *Jatropha curcas* extracts or powder as a bio-stimulator on green bean crops affects crop growth and yield and showed that the leaves of *J. curcas* would contain nitrogen and mineral salts that have improved green bean growth and yield. Our results are comparable to those obtained by [20] on the development of green beans treated with liquid extracts of algae. They can be explained by the fact that aqueous extracts of algae contain water-soluble substances such as polysaccharides, phytohormones such as auxin minerals [21] these produce better overall plant growth and yield. Several biostimulants have been used that improve vegetative growth as in chilli [22, 23]

5. Conclusion

The beneficial effect of applying biostimulants can be explained by the direct action of biostimulant components on plant nutrition by acting either on the plant or on the soil. The results obtained during this work showed a significant evolution of treated green beans compared to the control especially the root biomass.

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