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Abstract: Effects of plant-based biostimulants, used alone or in combination, on yield and quality of rocket plants

The climatic conditions over the last few decades were estimated by the Intergovernmental Panel on Climate Change (IPCC) to be the warmest of any previous decade. Climate change refers to anomalous, intense, and catastrophic climatic events directly linked to the increase in temperature on Earth. These are hurricanes, floods, melting glaciers, etc. In this context, agriculture is subject to strong abiotic stresses that compromise food safety. It is therefore necessary to resort to agricultural practices that reduce the impact of agriculture on the environment, and guarantee crops production. An important answer to this problem comes from the use of biostimulants in agriculture. These are microorganisms and molecules of natural origin able to increase fertilizers effectiveness, by limiting their use. In this study, two different plant-based biostimulants were used alone and in combination to test their effectiveness on production, mineral content, and some quality parameters of greenhouse-grown rocket plants. Biostimulant treatments showed an average increase of 48.1% of the total yield and 37.2% of dry biomass of the plants, compared to control plants, without significant differences among treatments. An increase in chlorophyll, calcium, magnesium, and potassium was detected in the presence of the two biostimulants, too. Vitamin C content increased, as compared to the control when the two biostimulants were combined. This study focused on biostimulants as ecosustainable products able to increase the yield and quality of such crops as rocket.

Keywords: Plant biostimulants; eco-friendly practices; Vitamin C; Minerals; synergistic interactionons

Graphycal Abstract

Effects of plant-based biostimulants, used alone or in combination, on yield and quality of rocket plants

Origin of biostimulants

Leather



Fish



Plants



Protein hydrolysates :

Application:





The Current State of the Climate





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What is climate change?

Melting glaciers

Floods

Hurricanes





Fires





Prolunged drought





What is living agriculture in this scenario?



Drought stress

Main abiotic stresses



Salinity stress



Heat stress



What solutions are suggesting scientists to mitigate plant stress?

We have ONE WORLD



We have NO TIME



We have to decide



Development of eco-sustainable practices:

- Crop rotation
- Selection of sowing and planting date and harvesting times
- Selection of tolerant cultivars
- Appropriate irrigation techniques and planting density
- Use of mulching films
- Use of wild species resistant to various abiotic stress in breeding programs

- Grafting
- Use of plant biostimulants

Definition by European Biostimulant Industry Council (EBIC):

Biostimulants are not fertilizers or pesticides

Compounds:

Humic acids

Protein hydrolysates and amino acids

Seaweed and plant extracts

Chitosan and other polysaccharides

Inorganic compounds



Microorganisms:

Mycorrhizal fungi Plant growth-promoting rhizobacteria (*Azospirillum*, *Azotobacter*, *Rhizobium* spp.)





What is the rule of biostimulants in agriculture?

- Enhance chemical fertilizers effectiveness
- Increase the growth and productivity of crops
- Improve the resistance of plants to abiotic and biotic stresses



Protein hydrolysates: amino acids, oligo and polypeptides Extraction:

Origin:

Leather



Fish

Plants

Chemical hydrolysis: Acids or alkali at temperature up to 138 °C Enzymatic hydrolysis: Protease at temperature up to 60°C

Protein hydrolysates:

- They act directly on the enzymes of nitrogen and carbon metabolism (NR, NiR, GS, GOCAT, citrate synthase, malate and isocitrate dehydrogenase).
- They have auxin and gibberellin-like activities
- They stimulate antioxidant enzymes and the synthesis of pigments and secondary metabolites.
- They positively influence the root system development



Plants can respond differently to different biostimulants at different concentrations

Interactions between different category of biostimulants:

- Additive: each biostimulant maintains the same action as when used alone and therefore each one of them adds its own action to the overall effect of the mixture
- **Synergistic**: the overall effect of the mixture is greater than the sum of the effects of each biostimulant applied individually
 - Antagonistic: the overall effect of the mixture is lower when biostimulants are used individually



Aim of the study

To assess the effects of two vegetal-base plant biostimulants alone or in combination:

A protein hydrolysate (PH)	A tropical plant extract (PE)					
75% free amino acids and peptides 22% carbohydrates 3% mineral nutrients.	54%free amino acids and peptides23%mineral nutrients17%carbohydrates6%vitamins and0.22%phytohormones					

The treatments were compared in terms of:

- Yield and growth parameters
- Mineral composition
- Leaf quality traits

of Rocket plants



Experimental design

4 Biostimulant treatments:

- Control (non-treated)
- PH (4 ml/L)
- PE (1ml/L)
- PH + PE (4 + 1 ml/L)



IECPS

2021

Treatments were arranged in a randomized complete block design with three replicates

Results and Discussion Yield and Dry Biomass Production

		Yield (g m ⁻²)			Dry	y Biomass (g 1		
Biostimulant Treatments	Harvest	Harvest II	Harvest III	Total yYield (g m ⁻²)	Harvest 1	Harvest II	Harvest III	 Total Dry Biomass (g m⁻²)
Control	462.8 ± 30.7	832.7 ± 15 b	403.7 ± 36 b	1699.1 ± 20 b	55.6 ± 1.5	67.3 ± 1.5 b	30.0 ± 2.9 b	153.0 ± 4.5 b
РН	493.0 ± 47.6	1246.6 ± 109 a	821.4 ± 111 a	2561.1 ± 174 a	53.6 ± 4.9	102.4 ± 13.5 a	61.1 ± 8.5 a	217.0 ± 16.5 a
PE	476.8 ± 59.8	1252.2 ± 27 a	726.8 ± 73 a	2455.7 ± 87 a	49.0 ± 4.8	99.1 ± 3.5 a	50.9 ± 3.0 a	198.9 ± 3.1 a
PH + PE	542.7 ± 63.9	1176.4 ± 111 a	816.8 ± 53 a	2535.9 ± 19 a	57.3 ± 5.8	95.1 ± 14.2 a	61.2 ± 5.5 a	213.5 ± 10.3 a
Significance	NS	*	* •	***	NS	*	*	**
				+48.1%			+	37.2%
	+47	[.] 1% +9	95.2 ['] %		+4	l6.9%		IECPS

Results and Discussion Soil Plant Analysis Development (SPAD) index and International Commission on Illumination

Biostimulant Treatments		Harves	st II					
	SPAD	L *	a*	b*	SPAD	L*	a*	b*
Control	34.3 ± 1.20 b	39.7 ± 0.26	13.8 ± 0.34	19.8 ± 0.48	32.0 ± 1.53 b	40.4 ± 0.15	13.6 ± 0.39	19.6 ± 0.64
РН	38.7 ± 0.67 a	38.3 ± 0.57	13.3 ± 0.26	19.4 ± 0.43	37.7 ± 1.45 a	39.3 ± 0.77	14.1 ± 0.28	20.9 ± 0.64
РЕ	37.7 ± 0.88 a	39.0 ± 0.85	13.7 ± 0.52	20.0 ± 0.73	39.7 ± 1.45 a	39.8 ± 0.49	14.5 ± 0.14	21.3 ± 0.19
PH + PE	38.7 ± 1.76 a	39.1 ± 0.59	13.6 ± 0.34	19.5 ± 0.79	39.7 ± 1.33 a	39.5 ± 0.19	14.0 ± 0.31	20.3 ± 0.63
Significance	*	NS	NS	NS	*	NS	NS	NS

+22.4%

+21.8%

Results and Discussion

Mineral content

Biostimul ant	N (g k	N (g kg ⁻¹ dw)		P (g kg ⁻¹ dw)		K (g kg ⁻¹ dw)		Ca (g kg ⁻¹ dw)		Mg (g kg⁻¹ dw)		Na (g kg ⁻¹ dw)	
Treatmen ts	Harves t II	Harvest III	Harvest II	Harvest III	Harvest II	Harvest III	Harvest II	Harvest III	Harvest II	Harvest III	Harvest II	Harvest III	
Control	4.85 ± 0.10	5.03 ± 0.10	2.83 ± 0.19	2.93 ± 0.16	44.5 ± 0.51 b	37.5 ± 1.43 b	18.1 ± 0.10 b	26.2 ± 0.20 b	3.03 ± 0.24	2.90 ± 0.21 b	2.96 ± 0.10	2.64 ± 0.78	
РН	5.01 ± 0.14	4.99 ± 0.18	3.20 ± 0.05	3.02 ± 0.14	48.1 ± 0.64 b	54.3 ± 1.34 a	23.8 + 0.38 a	34.0 + 0.54 a	3.13 ± 0.12	4.16 ± 0.35 a	2.16 ± 0.43	4.03 ± 0.14	
PE	4.95 ± 0.18	4.71 ± 0.16	3.14 ± 0.13	3.08 ± 0.06	52.9 ± 2.26 a	53.9 ± 2.19 a	25.0 ± 0.56 a	30.0 ± 2.10 ab	3.31 ± 0.34	3.87 ± 0.24 a	2.57 ± 0.66	3.47 ± 0.67	
PH + PE	4.89 ± 0.16	4.80 ± 0.15	2.87 ± 0.10	3.10 ± 0.17	48.3 ± 1.28 b	56.6 ± 1.61 a	25.1 ± 0.34 a	32.4 ± 1.47 a	3.32 ± 0.32	4.02 ± 0.10 a	2.02 ± 0.87	4.42 ± 0.22	
Significa nce	NS	NS	NS	NS	*	***	***	*	NS	*	NS	NS	
+31.7% +29.3% +37.9% IE									[%] IE	CPS			
$\begin{array}{c} +11.5\% \\ +44.3\% \\ +37.2\% \\ +23.2\% \end{array} \qquad \begin{array}{c} +38.9\% \\ +37.2\% \\ \end{array} \qquad \begin{array}{c} 2021 \\ \end{array}$									021				

Results and Discussion

Quality parameters

	Chlor	ophyll	Nit	rate	Total I	Phenols	Total Ascorbic Acid		
Biostimulant Treatments	(mg g ⁻¹ fw)		$(\mathbf{mg} \ \mathbf{g}^{-1} \mathbf{f} \mathbf{w}) \qquad (\mathbf{mg} \ \mathbf{kg}^{-1} \mathbf{f} \mathbf{w})$			llic acid ⁻¹ dw)	$(mg g^{-1} fw)$		
	Harvest II	Harvest III	Harvest II	Harvest III	Harvest II Harvest III		Harvest II	Harvest III	
Control	0.96 ± 0.14	0.88 ± 0.09 b	4630 ± 644	4428 ± 446	$\textbf{3.98} \pm \textbf{0.07}$	4.71 ± 0.11	17.3 ± 3.22 c	14.9 ± 2.11 b	
РН	1.34 ± 0.17	1.03 ± 0.06 ab	4558 ± 375	5611 ± 377	3.95 ± 0.38	5.03 ± 0.67	23.6 ± 1.11 bc	22.0 ± 2.08 b	
PE	1.22 ± 0.14	1.11 ± 0.05 a	4634 ± 211	5897 ± 489	3.46 ± 0.20	4.32 ± 0.14	28.0 ± 0.53 b	31.3 ± 2.29 a	
PH + PE	1.43 ± 0.18	1.22 ± 0.04 a	4772 ± 396	4380 ± 631	$\textbf{3.84} \pm \textbf{0.06}$	$\textbf{4.46} \pm \textbf{0.10}$	37.0 ± 1.79 a	36.3 ± 3.25 a	
Significance	NS	*	NS	NS	NS	NS	***	***	
						+	-62.8%	+109.4%	
+22	. %	+33	.3%			+	-115%	+143.6%	

Results andDiscussionPrincipal Component Analysis (PCA)



Conclusions

- Auxym® (PE) and Trainer® (PH) application increased the total yield and total dry biomass of greenhouse-grown rocket plants, 48% and 37% on average, compared to untreated plants.
- The two biostimulants resulted in a higher mineral status of potassium, calcium and magnesium, as well as chlorophyll content in treated plants

A synergistic effect of the two biostimulants was seen in the case of total ascorbic acid content

Mixing these two types of biostimulants resulted in a synergistic effect on the quality of the produce with a half dose of each stimulant applied, which was shown to improve plant performance in terms of growth and quality

Thank you for your attention



