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Assessment of yield and nitrate content of wild rocket grown under salinity and subjected to biostimulant application

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Highlights

- *Saline irrigation reduced wild rocket yield by 6.0%, 21.1%, and 33.7% (EC2, EC4 and EC6, respectively).*
- *Wild rocket yield increased from harvest I to VI under saline conditions other than EC6.*
- *Biostimulant application increased the SPAD index compared to untreated plants: +9 and +12% at EC4 and EC6 conditions, respectively.*
- *Together with genetic traits and biostimulant application, harvest time plays a key role in modulating plant response to salinity.*

Abstract

Soil secondary salinity due to irrigation is a condition that frequently occurs in Mediterranean areas, and negatively affects crop growth and yield. Biostimulants are proven to alleviate the detrimental effect of salinity on plant growth and production. Four increasing saline concentration levels of water irrigation reaching 6.0 dS m^{-1} (Electrical Conductivity - EC) were combined with foliar biostimulant treatments (tropical plants and a protein hydrolysate) in pots containing wild rocket. The combined effect of experimental factors improved the SPAD index with greater increases in the EC4 and EC6 plants (+9 and +12% compared to untreated, respectively) but also caused an increase in nitrate content (+48%, on average, compared to the untreated control) without exceeding the EC legal threshold. Overall, for the other parameters analyzed, the response of wild rocket both to application of both salinity and biostimulant was consistent with previous studies. Our results show that biostimulant effectiveness in alleviating the detrimental effect of salinity was not evident for all parameters analyzed. In addition, harvest time affected most parameters, showing the important role of growing conditions in modulating plant response to salinity stress when biostimulants are applied. Plant response thus seems to depend on biostimulant application (type, dose, timing), growing conditions, and genetic traits.

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Introduction

The FAO reports that globally primary soil salinization will annually make up to 1.5 million hectares of farmland in lowland areas unproductive. The annual economic loss is estimated to be around $31 million (FAO, 2021). Soil secondary salinity is a condition that frequently occurs in arid and semi-arid regions where rainfall is too low to maintain regular leaching of rainwater through the soil. When irrigation is practiced without adequate drainage (natural or artificial), salts accumulate in the root zone, negatively affecting several soil properties (Qadir *et al.*, 2014).

This phenomenon frequently occurs in Mediterranean areas when farmers are forced during summer to irrigate crops with water of poor quality because of both seasonal drought and competition between agricultural, social, or industrial uses of good quality water resources. When used for irrigation, saline water has severe consequences for soils: i) it reduces their infiltration capacity; ii) alters their structure and nutrient equilibrium; iii) makes soils overall less suitable for most crops (Qadir *et al.*, 2014).

Crops that grow in saline soils are subject to osmotic, nutritional, and toxic stresses (Vasantha *et al.*, 2010; Shahbaz and Ashraf, 2013) that result in a decrease in production (IPCC, 2019). Salinity acts on crops: i) by inhibiting plant nutrient absorption due to osmotic effect (Munns, 2002; Munns, 2005; Sergio *et al.*, 2012); ii) through specific ionic toxicity (*e.g.*, Na^+ and Cl^-) (Munns, 2002; Munns, 2005; Yeo *et al.*, 1991); iii) ionic imbalances affecting metabolic components of plant growth (Grattan and Grieve, 1999).

These stress conditions can reduce: i) net photosynthesis (Munns *et al.*, 2005; Cantore *et al.*, 2007; Munns and Tester, 2008); ii) leaf area expansion rate (Wang and Nil, 2000); iii) fresh and dry weight of leaves, stems, and roots (Hernandez *et al.*, 1995;

Chartzoulakis and Klapaki, 2000). These effects can lead to a reduction in yield, but in a variable way depending on: i) level of salinity; ii) plant genotype; iii) salt ions present; iv) climatic conditions; v) agronomic techniques (Cucci *et al.*, 2000; Cucci *et al.*, 2014; Flagella *et al.*, 2002).

One of the main goals of modern agriculture is sustainability, including the possibility of growing crops on marginal lands, such as saline soils, also thanks to the use of tools, such as biostimulants, able to mitigate saline stress.

In the latest European Regulation on fertilizers (EU2019/1009), which includes biostimulants, these are defined as innovative agronomic tools that, once applied to the plant or rhizosphere, can: i) stimulate plant growth and productivity; ii) increase tolerance to abiotic stresses; iii) improve product quality (Bulgari *et al.*, 2019, Rouphael *et al.*, 2020). As regards the relationship between salinity and biostimulants, it is widely reported that the application of biostimulants can alleviate the effects of salinity by enhancing crop tolerance (Campobenedetto *et al.*, 2022; Di Mola *et al.*, 2021, in hemp). Biostimulants can increase the concentration of proline, simple sugars, and abscisic acid, antioxidants capable of counteracting the damage caused by the accumulation of free radicals (Carillo *et al.*, 2020). It has been extensively reported that protein hydrolysates of plant origin show good results, improving tolerance to salinity in various horticultural crops, thereby increasing yield and dry matter accumulation (Moncada *et al.*, 2020; Sorrentino *et al.*, 2021). Previous work in southern Italy has shown that legume-derived protein hydrolysate (LDPH) was able to alleviate the detrimental effect of saline irrigation water on baby spinach plants. The study reported a significant improvement in the yield of plants subjected to low and moderate saline stress (3 and 6 dS m^{-1}) when treated with the biostimulant, reaching values similar to those of plants not subject to saline stress and not treated with biostimulant (El-Nakhel *et al.*, 2022). Wild rocket (*Diplotaxis tenuifolia* L.) has a high nutritional quality, a unique aroma, and pungent flavor; its leaves contain a high amount of fibers, iron, ascorbic acid, phenols, carotenoids, and glucosinolates (Di Venere *et al.*, 2000; Barillari *et al.*, 2005; D'Antuono *et al.*, 2009; Cavaiuolo and Ferrante, 2014), to which important bioactive properties are often ascribed (Ramos-Bueno *et al.*, 2016). The cultivation of wild rockets is widespread in Mediterranean areas, where it is grown both in greenhouses and open fields. In 2020, in southern Italy, 3500 hectares were used for rocket cultivation with an annual production of approximately 400,000 tons (ISTAT, 2021).

Previous research on the behavior of wild rocket grown on saline soils reported contrasting results. De Vos *et al*. (2013) reported wild rockets to be a salt-tolerant species, noting no growth reduction up to a concentration of 100 mM NaCl of the hydroponic solution. In contrast, Bonasia *et al.* (2017) reported significant reductions in the yield of wild rockets (almost 20%) when cultivated in a nutrient solution with an electrical conductivity of 3.5 dS m-1 compared to 2.5 dS m^{-1}.

An excessive content of nitrates in leaves is considered a negative trait of wild rocket quality because it can have severe consequences on human health: in the human body, nitrates can be converted into nitrites which can cause methemoglobinemia or can be used to produce the cancer-causing compounds nitrosamine and nitrosamide (Gangolli *et al.*, 1994; Walker, 2000). For this reason, the limit for nitrate content in wild rockets for human consumption is set by EU Commission Regulation 1258/2011. Nevertheless, nitrates are very important for plants because they are used for the biosynthesis of nucleic acids and proteins (Cavaiuolo *et al.*, 2014); their assimilation and translocation depend upon different genetic and environmental factors (Xu *et al.*, 2012). Among the external factors, primary or secondary salinity can affect the assimilation and accumulation of nitrates in leaf tissues. Di Mola *et al*. (2017) tested lettuce irrigated with water at increasing levels of salinity and found that the nitrate content decreased when the saline stress increased in all phenological stages: at the mature head stage, nitrate content was about 63% below the value recorded in the control plants irrigated with tap water.

Some research on the ability of biostimulants to reduce the detrimental effects of salinity has already been carried out (Dell'Aversana *et al.*, 2020; Di Mola *et al.*, 2021; El-Nakhel *et al.*, 2022). Yet few studies concerning the combined effects of salinity and biostimulants have been conducted up to now on wild rockets (Bulgari *et al.*, 2019; Franzoni *et al.*, 2020).

The current research aimed to assess the efficacy of two biostimulants (legume-derived protein hydrolysate and tropical plant extracts) in limiting the detrimental effect of irrigation with water of different salinity levels on yield and some qualitative traits of wild rockets.

Materials and Methods

Plant materials, experimental treatments, pot management, and soil salinity measurements

A pot experiment (pot size: 0.40 m diameter and 0.36 m height; surface area 0.13 m^2) was conducted in a protected environment (tunnel) on wild rocket (*Diplotaxis tenuifolia* L.) at the Experimental Station of the Department of Agricultural Sciences, University of Naples Federico II, in Portici (Southern Italy; 40 ° 48.870 'N; 14 ° 20.821' E; 70 m a.s.l.). The "Reset" cultivar (Maraldi Sementi Srl, Cesena, Italy) was used; it has green leaves with medium-sized lobes, high potential yield, great tolerance to *Fusarium,* and overall appreciable crop flexibility, making it suitable for production in any season. Each pot was filled with soil of the following characteristics: 91% sand, 4.5% silt, and 4.5% clay; 253 ppm of P_2O_5, 490 ppm of K_2O, 2.5% of organic matter, 0.101% of total nitrogen and pH 7.4. The wild rocket was cultivated over six cycles during the winter/spring of 2020-21.

According to the experimental design, four saline irrigation treatments [tap water, at electrical conductivity (EC) of 0.70 dS m^{-1}, hereafter referred to as EC0, and water at EC of 2.0, 4.0, and 6.0 dS m^{-1}, hereafter referred to as EC2, EC4, and EC6] were factorially combined with the following three foliar biostimulant treatments: i) untreated, hereafter BC; ii) treated with Auxym®, a tropical plant extract -TPE (Hello Nature Italy srl, Rivoli Veronese, Italy), hereafter BA; iii) treated with Trainer®, a hydrolysate protein derived from legumes-LDPH (Hello Nature Italy srl, Rivoli Veronese, Italy), referred to hereafter as BT.

All treatments were replicated three times for a total of 36 pots (4 salinity levels x 3 biostimulant applications x 3 replicates) placed in a complete randomized block design, with each pot accounting for a replicate.

The electrical conductivities of treatments EC2 to EC6 were obtained by adding NaCl to tap water as previously reported (Di Mola *et al.*, 2021). They were regularly checked before each watering. In order to fully restore the pot water losses, calculated by the Hargreaves formula (Hargreaves and Samani, 1985), 26 waterings were done over the six production cycles. For all treatments, the first three irrigations were done with tap water to promote seedling germination and rooting, and then saline irrigations started. A total of 28.5 L pot^{-1} was applied with 34.6, 69.1, and 103.7 g NaCl pot^{-1} in EC2, EC4, and EC6, respectively. At each harvest, soil was sampled at 0-20 cm depth and a soil water solution extraction method (1:5 dilution) was used to measure soil EC ($EC_{1:5}$; Basic 30 CRISON conductimeter; Crison Hach, Barcelona, Spain). As for biostimulant products, both types Auxym® and Trainer® were applied three times per production cycle (at a rate of 2 mL L^{-1} and 3 mL L^{-1}, respectively), starting from the emission of new leaves at each cycle. The timing of biostimulant applications is reported in Table 1. Three seedling groups per pot, accounting for a planting density of 23 plants m^{-2}, were transplanted on 8 October 2020. During each production cycle, nitrogen, as ammonium nitrate (26% N), was applied at the rate of 18 kg N ha^{-1}. The timing of nitrogen fertilization is reported in Table 1. No pesticide treatment was carried out. Plants were harvested six times, starting from 25 November 2020 until 20 May 2021. The six harvests, referred to hereafter as I, II, III, IV, V, and VI, define the production cycles whose duration was 48, 75, 35, 29, 21, and 16 days, respectively (Table 1).

The air temperature under the tunnel was monitored by a Vantage Pro2 weather station (Davis Instruments, Hayward, CA, USA) on an hourly basis. Data are reported as ten-day minimum and maximum temperatures (Figure 1).

Yield, yield components, root growth, and nitrate content

At each harvest, plants were cut at 3 cm from the ground to determine yield expressed in kg m^{-2} of fresh weight (fw). The number of leaves per pot and average leaf weight were also measured. A sample of 100 g of leaves was collected from each pot and oven-dried at 60°C until a constant weight was achieved to determine dry matter content. A sub-sample of 0.5 g per pot was then ground using an IKA mill (IKA-Werke, Staufen, Germany), sieved through a 2 mm screen, and used to measure nitrate content using a Foss FIAstar 5000 (FOSS Italia S.r.l., Padova, Italy) continuous flow Analyzer as previously reported (Di Mola *et al.*, 2022a).

At the final harvest (VI), every pot was emptied, roots were separated by plant, and soil was removed. The maximum length of roots of each plant was measured and clean roots were weighed before and after oven-drying at 60ºC.

Color parameters and soil plant analysis development index

On ten undamaged fully expanded leaves per pot, collected at each harvest, leaf color parameters L * (lightness), a * (green/red) and b * (blue/yellow) were determined by a colorimeter (Minolta CR-300 Chroma Meter, Minolta Camera Co. Ltd., Osaka, Japan), using an optical sensor of 8 mm, and reported as average. L* ranges from 0 (black, no reflection) to 100 (white, perfect diffuse reflection), a* from green (-60) to red (+60), and b* from blue (-60) to yellow (+60). In addition, the soil plant analysis development (SPAD) index (SPAD-502, Konica Minolta, Tokyo, Japan) was measured on the middle part of the same ten leaves and an average value was recorded.

Statistical analysis

All results were subjected to analyses of variance (ANOVA; SPSS software package, version 22, Chicago, IL, USA) with a 2-(root weight and root length) or 3-way. The means were separated using Tukey's Test at P≤0.05.

Results

Soil electrical conductivity

The $EC_{1:5}$ increased with the rising of water salinity. It was, on average, 0.48, 0.77, 0.98, and 1.22 dS m^{-1} under conditions EC0, EC2, EC4, and EC6, respectively.

Except for the un-salinized control (EC0), the level of salinity grew over time, from November (harvest I) to May (harvest VI)

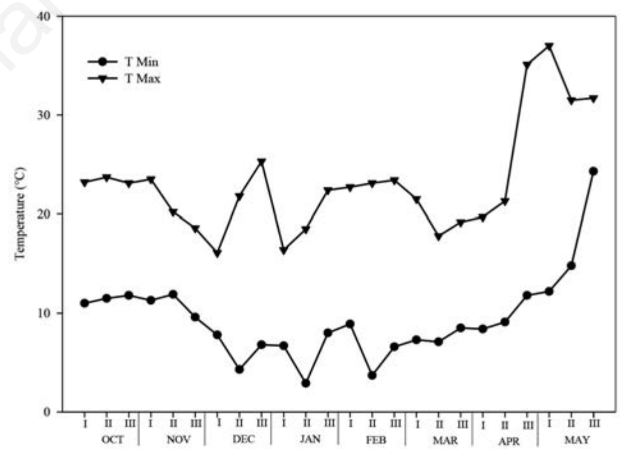

Figure 1. Maximum and minimum air temperature during the whole growing period of wild rocket. I, first ten days; II, second ten days; III, third ten days.

Table 1. Timing of fertilizer and biostimulant applications, and production cycle durations.

Cycle	Agricultural Practices (DAT/DAPH)				Harvest
	Fertilization	Biostimulant 1	Biostimulant 2	Biostimulant 3	DAT/DAPH
I	18	26	33	40	48
II	9	18	25	32	75
III	7	14	20	25	35
IV	2	8	14	21	29
V	2	6	12	17	21
VI	2	6	12		16

DAT, days after transplant (only for the first cycle); DAPH, days after previous harvest (from second cycle onwards). Biostimulants 1, 2, and 3 indicate the first, second, and third applications, respectively.

(Figure 2). Under EC2 and EC4 the highest value of $EC_{1:5}$ was already measured at harvest V (0.95 and 1.33 dS m^{-1}, respectively), and without any further significant change. Nevertheless, in EC6 pots it was recorded at harvest VI (1.78 dS m^{-1}) (Figure 2).

Marketable yield, yield components, and root growth

Yield and its components (number of leaves per pot and average leaf weight) and root growth parameters (weight and length) were significantly affected by treatments (saline irrigations, biostimulant applications, and harvests (Tables 2 and 3).

As for yield (kg fw m^{-2}), there were significant between saline irrigation and harvest interactions (Table 2 and Figure 3) and biostimulant application and harvest (Table 2 and Figure 4). Yield rapidly increased from harvest I to VI (January to May) under control and EC2 (+75 and +65%, respectively). The same occurred under EC4, albeit at a lower rate with respect to EC0 and EC2 (+28%; Figure 3). By contrast, at EC6 there was no significant yield increase along production cycles (Figure 3) but rather a slight, albeit significant, decrease in yield at harvest III with respect to I (-12%; Figure 3). In addition, the EC6 yield appeared, on average, much lower than that obtained by other saline treatments (Figure 3). As for biostimulant applications, the lowest marketable yield was given, on average, by plants of the untreated control BC (Figure 4), and the highest by plants treated with BA, whereas the yield shown by BT plants was intermediate. Under control conditions and regardless of the type of the applied product, marketable yield significantly increased with time, but the increments measured at VI with respect to I were higher in treatment BA than BT (+64 and +43%, respectively). The lowest increments along harvests (II to VI) were recorded in untreated control BC plants (+32%; Figure 4).

The salinity of the irrigation waters significantly increased leaf dry matter content (%) up to EC4 but led to no further significant increase at EC6. Nevertheless, it significantly reduced the number of leaves per pot up to EC6. By contrast, average leaf weight did not change significantly with saline irrigations (Table 4).

Application of BT significantly increased the percentage of leaf dry matter content in comparison with the untreated control BC, while the BA outcome was intermediate between them. BT and BA showed a significantly higher number of leaves per pot than BC. In addition, no effect of biostimulant treatments was recorded for average leaf weight (Table 4). Leaf dry matter content (%) increased significantly up to the third harvest, and then decreased until the final harvest. The second harvest showed the highest number of leaves per pot, although they were lighter than others. By contrast, the first harvest produced fewer leaves but a higher yield. The best combination of the number and weight of leaves was reached at the final harvest (VI; Table 4). Saline irriga-

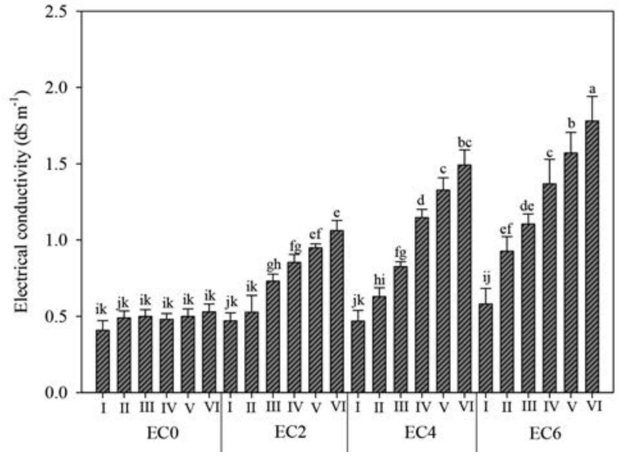

Figure 2. Effect of saline irrigation treatments (EC0=0.7 dS m^{-1}; EC2=2.0 dS m^{-1}; EC4=4.0 dS m-1; EC6=6.0 dS m^{-1}) and harvesting time (I to VI) interaction on soil electrical conductivity (EC1:5; dS m^{-1}). Different letters indicate significant differences according to the Tukey test (P<0.05). Vertical bars indicate standard error.

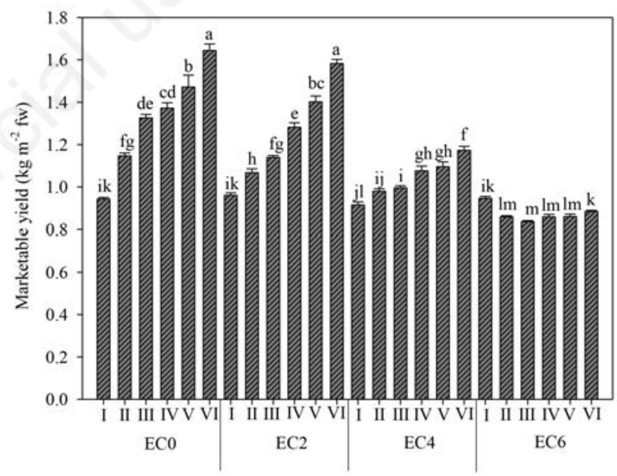

Figure 3. Wild rocket marketable yield as affected by the interaction of saline irrigation treatments (EC0=0.7 dS m^{-1}; EC2=2.0 dS m^{-1}; EC4=4.0 dS m^{-1}; EC6=6.0 dS m^{-1}) and harvesting time (I to VI). Different letters indicate significant differences according to the Tukey test (P<0.05). Vertical bars indicate standard error. Fw, fresh weight.

Table 2. Results of analysis of variance on yield and yield components.

Significance	Yield FW	Leaf DM	Leaf n°	ALW
Salinity (S)	0.001	0.01	0.001	ns
Biostimulant (B)	0.001	0.01	0.001	ns
Harvest (H)	0.001	0.001	0.001	0.001
S x B	ns	ns	ns	ns
S x H	0.001	ns	ns	ns
B x H	0.01	ns	ns	ns
S x B x H	ns	ns	ns	ns

The table reports the significance of treatments and their interactions at P<0.01 and P<0.001. FW, fresh weight; DM, dry matter; n°, number; ALW, average leaf weight; ns, not significant.

tions at EC6 significantly reduced the root weight (Table 5). Nevertheless, a positive and significant effect of salinity of the irrigation waters on root length already appeared at EC2, with values remaining stable up to treatment EC6. BA and BT significantly increased root weight whereas both reduced, by the same extent, the root length with respect to the untreated control BC (Table 5).

Color parameters, soil plant analysis development index, and nitrate content

There were significant effects of treatments (saline irrigations, biostimulant applications, and harvests) on color parameters, the SPAD index, and nitrate content (Table 6). As regards color, L* significantly decreased with salinity up to EC6 (Table 7), whereas a* was significantly higher (more negative) already at EC2 than that measured under control conditions (EC0) and without any further increase at the highest ECs (EC4 to EC6; Table 7). Finally, no effect of saline irrigation treatments was, by contrast, recorded for b* values (Table 7). As for biostimulant applications, L* was significantly lower with respect to the untreated control BC by Auxym® but not by Trainer®. Only BT produced a significant decrease of a* (less negative) with respect to the untreated control BC, while both BT and BA reduced b* with respect to BC, and the greatest reduction was determined by BT (Table 7).

Finally, L* changed among harvests, increasing as production cycles proceeded through time from November to May, as did b*. By contrast, a* increased significantly up to harvest IV but then decreased until harvest VI (Table 7). With regard to the SPAD index and nitrates, the following interactions were significant: saline irrigations x harvests (Table 6, Figures 5 and 6), biostimulant applications x harvests (Table 6, Figures 7 and 8), and saline irrigations x biostimulant applications (Table 6, Figures 9 and 10). The salinity of irrigation waters influenced SPAD values differently over time (I to VI; Figure 5): the SPAD index increased significantly up to harvest II in all conditions. Nevertheless, while in EC0 and EC2 it remained constant up to harvest IV, albeit with further significant reduction until VI (Figure 5), under conditions EC4 and EC6 it varied irregularly until the end of production cycles (Figure 5). Regardless of the type of applied product, biostimulants positively affected the SPAD index with increments of about 8%, on average, with respect to the untreated control BC. As for production cycles, the SPAD index reached the highest value at harvest II in all conditions with a further significant decrease up to

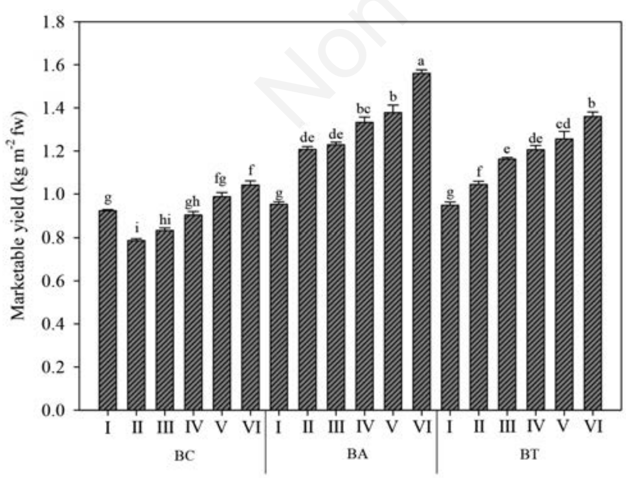

Figure 4. Wild rocket marketable yield as affected by the interaction of biostimulant treatments. BC=not treated-control; BA=treated with Auxym; BT=treated with Trainer) and harvesting time (I to VI). Different letters indicate significant differences according to the Tukey test (P<0.05). Vertical bars indicate standard error. Fw, fresh weight.

Table 3. Results of analysis of variance of root growth (root weight and root length).

Significance	Root Weight g plant^{-1}	Root Length cm plant^{-1}
Salinity (S)	0.001	0.001
Biostimulant (B)	0.01	0.01
S x B	ns	ns

The table reports the significance of treatments and their interactions at P<0.01 and P<0.001. Ns, not significant.

Table 4. Effect of saline irrigation treatments (EC0=0.7 dS m^{-1}; EC2=2.0 dS m^{-1}; EC4=4.0 dS m^{-1}; EC6=6.0 dS m^{-1}), biostimulant treatments and harvesting time (I to VI) on leaf dry matter, leaves number, and average leaf weight in wild rocket.

Treatments	DM (%)	Leaf n° m^{-2}	leaf^{-1}
Salinity			
EC0	10.7 ab	5354.3 a	0.26
EC2	10.2 b	4439.0 b	0.30
EC4	11.3 a	4157.7 bc	0.27
EC6	11.1 a	3709.4 c	0.27
Biostimulant			
BC	10.4 b	3594.7 b	0.28
BA	10.8 ab	4819.6 a	0.29
BT	11.2 a	4831.0 a	0.26
Harvest			
I	10.6 bc	3123.6 e	0.30 a
II	11.3 ab	5679.9 a	0.18 b
III	11.9 a	3839.2 d	0.28 a
IV	11.0 b	4233.2 cd	0.29 a
V	10.1 c	4562.6 bc	0.28 a
VI	10.1 c	5052.1 ab	0.31 a

BC, not treated-Control; BA, treated with Auxym; BT, treated with Trainer; EC, electrical conductivity. Different letters within each column indicate significant differences according to the Tukey test P<0.05.

Table 5. Effect of saline irrigation treatments (EC0= 0.7 dS m^{-1}; EC2= 2.0 dS m^{-1}; EC4= 4.0 dS m^{-1}; EC6= 6.0 dS m^{-1}) and biostimulant treatments on root weight (g plant^{-1}) and length (cm plant^{-1}) of wild rocket.

Treatments	Root Weight g plant^{-1}	Root Length cm plant^{-1}
Salinity		
EC0	4.32 a	17.11 b
EC2	4.87 a	28.78 a
EC4	4.84 a	26.11 a
EC6	3.69 b	28.44 a
Biostimulant		
BC	3.84 b	27.83 a
BA	4.88 a	25.31 b
BT	4.58 a	22.19 c

BC, treated-Control; BA, treated with Auxym; BT, treated with Trainer. Different letters within each column indicate significant differences according to the Tukey test P<0.05.

Table 6. Results of analysis of variance of CIELAB color parameters, soil plant analysis development index, and nitrate content.

Significance	L*	a*	b*	SPAD	Nitrate
Salinity (S)	0.001	0.001	ns	0.001	0.001
Biostimulant (B)	0.001	0.001	0.001	0.001	0.001
Harvest (H)	0.001	0.001	0.001	0.001	0.001
S x B	ns	ns	ns	0.001	0.05
S x H	ns	ns	ns	0.001	0.001
B x H	ns	ns	ns	0.001	0.001
S x B x H	ns	ns	ns	ns	ns

The table reports the significance of treatments and their interactions at P<0.01 and P<0.001. L*: brightness; a*: chroma component; b*: chroma component; ns, not significant; SPAD, soil plant analysis development.

harvest V in the untreated control BC and in BT but until harvest VI in BA (Figure 7). Finally, the biostimulant effect on the SPAD index was positive under all salinity conditions even though its values on average decreased with increasing ECs (Figure 9).

The nitrate content of the leaves varied among production cycles reaching the highest amount at harvest III in EC0, but already at harvest I in treatments EC2, EC4, and EC6 (Figure 6). On average, it decreased with increasing salinity of irrigation water: such decreases, with respect to the EC0 control, amounted to -15, -26, and -36% in EC2, EC4, and EC6, respectively. Regardless of the type of applied product, biostimulant applications increased nitrate content by about 48%, on average, with respect to the untreated control BC (Figures 8 and 10). As regards production cycles, nitrate content was the highest at III in both BA and BT, unlike the untreated control BC which showed the highest nitrate content already at harvest I (Figure 8). Moreover, in all conditions, there were further significant decreases up to harvest VI (Figure 8). Finally, nitrate content was markedly reduced by saline conditions (EC2, EC4, and EC6) with respect to the unsalinized control EC0 with or without biostimulant applications (Figure 10).

Table 7. Effect of saline irrigation treatments (EC0=0.7 dS m^{-1}; EC2=2.0 dS m^{-1}; EC4=4.0 dS m^{-1}; EC6=6.0 dS m^{-1}), biostimulant treatments and harvesting time (I to VI) on CIELAB color parameters of wild rocket leaves.

Treatments	L*	a*	b*
Salinity			
EC0	42.0 a	-6.61 a	15.7 ns
EC2	40.1 b	-6.90 b	16.0 ns
EC4	39.8 bc	-6.84 b	15.6 ns
EC6	39.6 c	-6.80 b	15.8 ns
Biostimulant			
BC	40.6 a	-6.92 b	16.2 a
BA	39.9 b	-6.82 b	15.8 b
BT	40.6 a	-6.62 a	15.3 c
Harvest			
I	39.1 e	-5.78 a	14.3 e
II	39.6 cd	-6.34 b	14.8 d
III	39.4 de	-7.08 d	15.5 c
IV	40.0 c	-7.59 e	16.1 b
V	41.5 b	-7.24 d	17.0 a
VI	42.5 a	-6.74 c	17.1 a

Different letters within each column indicate significant differences according to the Tukey test P<0.05. L*: lightness ranges from 0 (black, no reflection) to 100 (white, perfect diffuse reflection); a*: ranges from green (-60) to red (+60); b*: ranges from blue (-60) to yellow (+60); BC, not treated-Control; BA, treated with Auxym; BT, treated with Trainer.

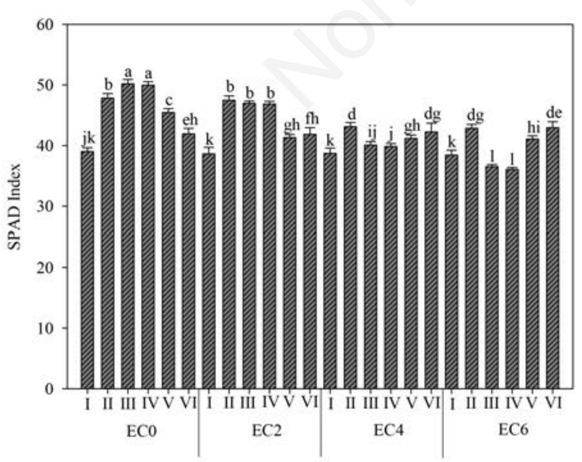

Figure 5. The soil plant analysis development index as affected by the interaction of saline irrigation treatments (EC0=0.7 dS m^{-1}; EC2=2.0 dS m^{-1}; EC4=4.0 dS m^{-1}; EC6=6.0 dS m^{-1}) and harvesting time (I to VI). Different letters indicate significant differences according to the Tukey test (P<0.05). Vertical bars indicate standard errors.

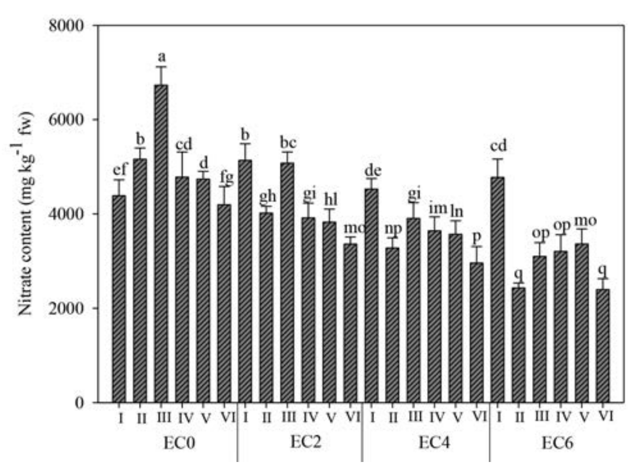

Figure 6. Nitrate content of wild rocket leaves as affected by the interaction of saline irrigation treatments (EC0=0.7 dS m^{-1}; EC2=2 dS m^{-1}; EC4=4.0 dS m^{-1}; EC6=6 dS m^{-1}) and harvesting time (I to VI). Different letters indicate significant differences according to the Tukey test (P<0.05). Vertical bars indicate standard errors. Fw, fresh weight.

Discussion

Soil and water salinity are both considered among the greatest problems affecting, at present, a large part of agricultural lands, especially those subject to irrigation. Based on the evaluation of data from 73% of global land areas, FAO (2021) estimated that more than 4.4% of topsoil (0-0.3 m) or more than 8.7% of sub-soil (0.3-1.0 m) of the total land area is salt-affected. Thus, it should be imperative to identify sustainable tools to mitigate the negative effects of salinity on crops, namely a reduction in yield and crop product quality.

The present study started from two considerations: the first was that biostimulants are reportedly able to improve tolerance to abiotic stresses including salinity in several plant species (Van Oosten *et al.*, 2017; Bulgari *et al.*, 2019; Dell'Aversana *et al.*, 2020; Abou-Sreea *et al.*, 2021; Campobenedetto *et al.*, 2021; D'amato and Del Buono, 2021; Ahmad *et al.*, 2022; El-Nakhel *et al.*, 2022); the second was that, to our knowledge, very few studies on the combined effect of salinity and biostimulant have been conducted on wild rocket (Bulgari *et al.*, 2019; Franzoni *et al.*, 2020; Di Mola *et al.*, 2023).

The results of the present experiment were somewhat singular

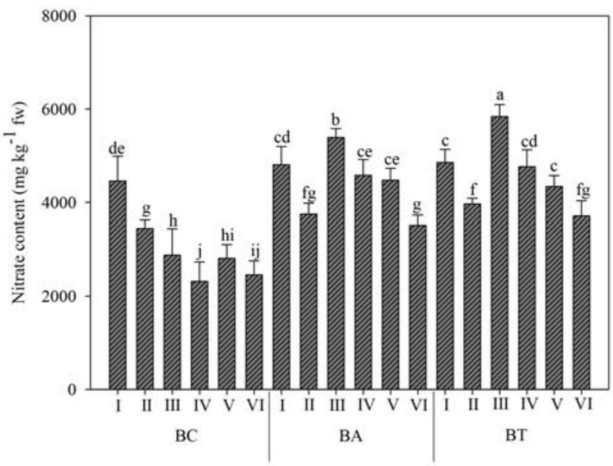

Figure 7. The soil plant analysis development index as affected by the interaction of biostimulant treatments (BC, not treated-Control; BA, treated with Auxym; BT, treated with Trainer) and harvesting time (I to VI). Different letters indicate significant differences according to the Tukey test (P<0.05). Vertical bars indicate standard errors.

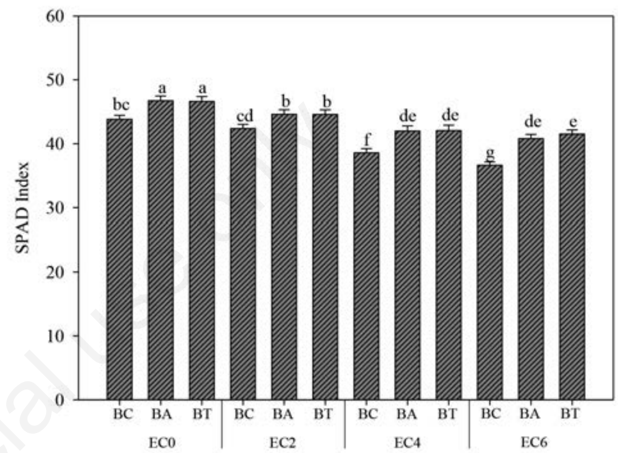

Figure 9. The soil plant analysis development index as affected by the interaction of saline irrigation treatments (EC0=0.7 dS m^{-1}; EC2=2.0 dS m^{-1}; EC4=4.0 dS m^{-1}; EC6=6.0 dS m^{-1}) and biostimulant treatments (BC, not treated-Control; BA, treated with Auxym; BT, treated with Trainer). Different letters indicate significant differences according to the Tukey test (P<0.05). Vertical bars indicate standard errors.

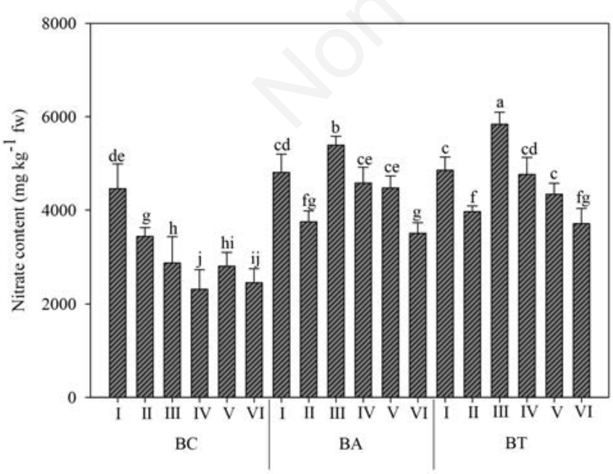

Figure 8. Nitrate content of wild rocket leaves as affected by the interaction of biostimulant treatments (BC, not treated-Control; BA, treated with Auxym; BT, treated with Trainer) x harvesting time (I to VI). Different letters indicate significant differences according to the Tukey test (P<0.05). Vertical bars indicate standard errors. Fw = fresh weight.

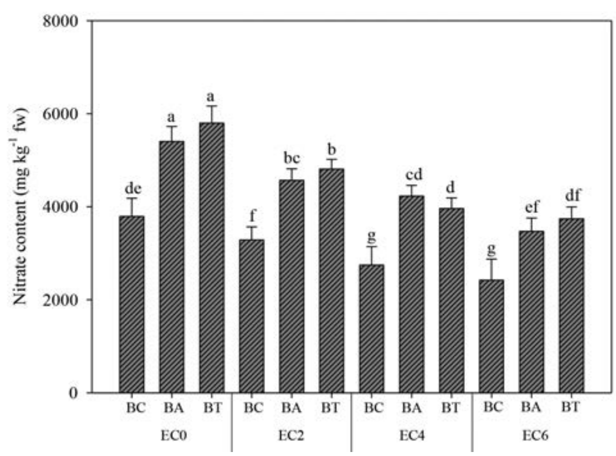

Figure 10. Nitrate content of wild rocket leaves as affected by the interaction of saline irrigation treatments (EC0=0.7 dS m^{-1}; EC2=2.0 dS m^{-1}; EC4=4.0 dS m^{-1}; EC6=6.0 dS m^{-1}) and biostimulant treatments (BC, not treated-Control; BA, treated with Auxym; BT, treated with Trainer). Different letters indicate significant differences according to the Tukey test (P<0.05). Vertical bars indicate standard errors. Fw, fresh weight.

since biostimulant application did not always appear effective in mitigating the negative effects of salinity. Indeed, the products used (tropical plant extract or legume-derived protein hydrolysate) rarely influenced, whether positively or negatively, wild rocket response to salinity since saline irrigation x biostimulant applications interacted significantly only for SPAD and nitrates. Albeit not significantly, biostimulant application also elicited an increase in the yield of salt-stressed plants: +28.8%, +47.7%, and +36.5%, for EC2, EC4, and EC6, respectively (data not shown). The SPAD index responded positively to BA and BT application: in both cases it was greater than in the untreated control BC, attesting to a greater presence of chlorophyll in leaves (better quality thanks to a high aesthetic value, better cancer protection, *etc*.) (Hedges and Lister, 2007; Limantara *et al.*, 2015). Further, the response was greater in conditions EC4 and EC6 than in EC0 and EC2 (+9 and +12% *vs.* +6 and +5%, respectively), thus resulting in an appreciable mitigation effect of salinity. However, there was a worsening effect of biostimulants (both BA and BT products) on nitrate accumulation, irrespective of salt levels, as reported also in other studies (Di Mola *et al.*, 2019; Poberezny *et al.*, 2020; Ottaiano *et al.*, 2021; Di Mola *et al.*, 2022b). In the latter case, biostimulants appeared to negatively influence the response of wild rockets to salinity but in no case was the legal threshold set by EU Commission Regulation 1258/2011 exceeded. Based on our data it may be hypothesized as being due to the direct consequence of a greater N uptake due to biostimulant application (Halpern *et al.*, 2015; Di Mola *et al.*, 2020), coupled with an altered metabolism of the same nutrient, thus accumulating as nitrates (N reserve) in the leaves. Further, it could vary with the type of products used, climatic/environmental factors, *etc*. (Ahmad *et al.*, 2022). In any case, the chemical composition of biostimulants could play an important role in N uptake and metabolism or nitrate accumulation (Rouphael and Colla, 2020). The high nitrate content in leafy vegetables for fresh consumption is negative for the deleterious effects that such substances can have on the health of consumers. According to previous findings, nitrate presence in dietary intake can be converted into methemoglobin-producing nitrite, which binds to oxygen (Greer and Shannon, 2005) and causes hypoxemia (Chan, 1996), or can cause gastric cancer (Song *et al.*, 2015). However, as stated above, in this experiment no exceedance of the legal threshold set by the EC for nitrate content in the leaves was observed.

As for the average effect of irrigation with saline water or application of biostimulants, both led to predictable results. In particular, regardless of biostimulant applications, salinity markedly i) increased the electrical conductivity of pot soils ($EC_{1:5}$ of EC6 was about 1.5-fold greater than that of EC0); ii) diminished the marketable yield by 36% (at EC6 with respect to the control EC0), reducing the number of leaves (Qados, 2011; Schiattone *et al.*, 2017; Rahneshan *et al.*, 2018; El-Nakhel *et al.*, 2022; Hannachi *et al.*, 2022) and increasing their percentage DM content (Romero-Aranda *et al.*, 2001; Vysotskaya *et al.*, 2010; Munns and Gilliham, 2015; Lucini *et al.*, 2016; Stavidrou *et al.*, 2020). Considering both our yield results and the indications of Maas and Hoffman (1977), this species could be classified as moderately sensitive to salinity (Schiattone *et al.*, 2017).

Moreover, regardless of biostimulant applications, salinity also changed the root growth dynamic (Snapp and Shennan, 1992; Shalhevet *et al.*, 1995; Kafi and Rahimi, 2011; Lovelli *et al.*, 2012; Arif *et al.*, 2019; Zou *et al.*, 2022). While root weight was negatively influenced by the highest level of salt stress (EC6), plants grown under conditions EC2 to EC4 developed longer, presumably thinner, roots than those of non-salinized control (EC0) plants. Under saline conditions, this increment in root length, presumably due more to new lateral root initiation than to the extension of the existing roots (Shalhevet *et al.*, 1995; Munns and Gilliham, 2015), should generally allow the plants to uptake more water and nutrients, useful under stress conditions (Bernstein and Kafkafi, 2002). This behavior, already found in other species (Shalhevet *et al.*, 1995; Lovelli *et al.*, 2012; Arif *et al.*, 2019), appeared as a compensatory effect for the previously reported reduction in plant root system weight, due to salinity.

On average, salinity affected quality aspects as well. First, it gave less bright leaves (L* of EC6 was reduced by 6% as compared to EC0); although the leaves appeared of a slightly more intense green overall, a* was more negative under saline conditions than in the nonsalinized control EC0 (an increase of 4 and 3% in EC2 and EC6 was observed, respectively). Thus, there was an improvement in marketable yield quality since the intensity of greenness is one of the most important qualitative parameters for leafy vegetables produced for fresh consumption like wild rocket, and consequently one of the main aims for producers.

As for the average effect of biostimulant application, as expected it enhanced marketable yield regardless of the type of product applied. The improvement was explained by the increase in leaf number and % DM. Moreover, the use of biostimulants also increased root weight and decreased root length, unlike salinity stress. Biostimulants, on average, improved the aesthetic characteristics of the wild rocket since BT applications increased greenness with respect to both BC and BA and BA and BT reduced yellowing with respect to BC.

The responses of wild rockets to both treatments (salinity and biostimulants) often changed over time, depending on the duration of saline stress (Gao *et al.*, 2015; Stavidrou *et al.*, 2017; Isayenkov and Maathuis, 2019). Early production cycles (autumn/winter) gave lower yields than those at the end of the entire experimental season (spring), in accordance with previous research findings on wild rockets (Di Mola *et al.*, 2022b). This specific response over time was particularly evident in EC0, still quite noticeable in EC2, barely present in EC4 until it completely disappeared in EC6 and was undoubtedly due to worse environmental conditions (lower air temperatures, lack of radiation, *etc.*) in autumn/winter than in spring, but also due to the above-mentioned duration of stress (Gao *et al.*, 2015; Stavidrou *et al.*, 2017; Isayenkov and Maathuis, 2019). In addition, environmental conditions affected the nitrate content of leaves (the saline irrigation x harvest interaction was significant). At least in EC0 (approximately from March harvest III to May harvest VI) and in EC2 and EC4 (approximately from November harvest I to May harvest VI), this result suggested that nitrate content decreased when the temperature (Figure 1) and radiation increased (Di Mola *et al.*, 2022b; Franzoni *et al.*, 2020), the latter being higher during spring/summer (maximum in June/July) than in autumn/winter (minimum in December; Jiang *et al.*, 2020).

Notably, the decrease in nitrate content in leaf tissue also corresponded to a decrease in the SPAD index that was about 13.3% lower in EC6 compared to EC0. A negative correlation between SPAD and salt stress has been frequently reported (El-Hendawy *et al.*, 2005; Lamian *et al.*, 2017; Shah *et al.*, 2017). Lucini *et al.* (2015) emphasized that, in different leafy vegetables, lower SPAD values are associated with an excess of Cl or NaCl concentration in the root zone.

Conclusions

The current research aimed to assess the effectiveness of applying biostimulants (legume-derived protein hydrolysate and tropical plant extracts) to alleviate the detrimental effect of salinity stress on growth, yield, and some quality traits of wild rockets. However, the combined effect of biostimulants and salinity was found only for the SPAD index (positively) and nitrate content (negatively). In all likelihood, the salt-stressed plants respond differently to treatments with biostimulants depending on the chemical composition of the latter. Most of the studied parameters were differently affected by the harvest time, highlighting the important role of growing conditions in modulating plant response to salinity stress even when biostimulants are applied. Instead, the single effect of both experimental factors confirmed what is already reported in the literature: the detrimental effect of salinity and the beneficial effect of biostimulant application. Therefore, future research is required to determine the right conditions (harvest time and biostimulant application - type, dose, timing) that allow the detrimental effects of salinity stress to be mitigated in species like *Diplotaxis tenuifolia* that are harvested more than once during the whole growth cycle.

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