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Article

Effects of Nitrogen, Azoxystrobin and a Biostimulant Based on Brown Algae and Yeast on Wild Rocket Features at Harvest and During Storage

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Abstract: Recently, the use of biostimulant substances of different origins has been affirmed. They act differently on the physiological processes of the plant, helping to improve its productive response and resistance to biotic and abiotic stress. Therefore, the response of the wild rocket to two substances known to have biostimulating activity (Azoxystrobin, and a fluid extract of brown algae and yeast), was evaluated. Two experimental trials (Exp 1 and Exp 2) were carried out in the greenhouse. The collected product, in addition to being evaluated from a qualitative point of view, was used for evaluation of shelf life. Exp 1 involved the comparison of two N levels with two Azoxystrobin levels (treated–Azo+, and untreated control). Exp 2 involved the comparison of two N levels, and two biostimulating substances based on Azoxystrobin (Azo+) and on fluid extracts of yeast and brown algae (YBA+), in addition to untreated control. A split-plot experimental design with three replications was used. Azo+ increased marketable yield of wild rocket by 16.8% and enhanced some qualitative features at harvest as the increase in chlorophyll (+17.8%) and carotenoids (+13.5%), and decrease in nitrates (−10.6%), regardless of the nitrogen level. Furthermore, Azo+ increased the shelf life (+2.5 days) of wild rocket stored at 3.5 °C. In particular, Azo+ slowed the loss of chlorophyll (yellowing) and the worsening of odor and visual appearance. As Azoxystrobin is a fungicide effective for the control of some diseases of wild rocket, its use should be promoted as it would offer not only the benefit of disease control but also improved production and shelf life. YBA+ caused an increase in the chlorophyll content (+12.5%) at harvest of wild rocket, but reduced its antioxidant activity (−40%). YBA+ did not cause substantial variations in shelf life with the exception of a slowdown in the degradation of carotenoids. Further research is desirable to evaluate other variables such as the dose and time of application.

Keywords: *Diplotaxis tenuifolia*; postharvest; strobilurins; qualitative characteristics; shelf life

1. Introduction

Wild rocket (*Diplotaxis tenuifolia* L. DC) represents a worldwide species of the Brassicaceae family that is well known for its spicy flavor and has a continuously increasing demand [1,2]. This is mainly because it represents a basic ingredient of ready-to-eat salads in combination with other leafy vegetables, such as lettuce, lamb's lettuce and spinach. Moreover, in recent years, the use of this species as baby leaf has become widespread [3].

Wild rocket is sold washed or unwashed packaged in plastic trays wrapped in polypropylene film or in pouches to create modified atmosphere packages. Sanitizing and drying is a stressful process for such delicate leaves [4,5], and avoiding these processes may result in higher product quality and longer shelf-life. For this, often wild rocket is sold unwashed, packaged in trays wrapped in film to keep physical damage of leaves to a minimum and to prevent wilting of leaves from loss of water [6].

The ready-to-use market requires a product of high quality and long shelf life which, on the one hand, has a low content of compounds harmful to health such as nitrates and on the other a high content of beneficial compounds such as antioxidants.

The quality of the produce is the result of the interaction of numerous factors (i.e., genetic, environmental, agronomic). The agronomic factors that can significantly influence the quality of leafy vegetables include the availability of nutrients and the application of substances with a biostimulating action [7]. Nitrogen is the fertilizing element that can influence mostly some quality parameters of leafy vegetables (i.e., content of nitrates, chlorophyll, tenderness, chromatic characteristics, etc.). For wild rocket, considered a nitrate hyperaccumulating species, particular attention must be paid to the nitrogen fertilization management to avoid the risk of exceeding the nitrate limits imposed by the Regulations of the European Commission (EU No 1258/2011) [8].

On the other hand, the literature reports examples concerning the improvement of yield and quality performances following the application of substances with a vigor effect or biostimulating action. These include strobilurins, substances of natural origin whose structural variants (i.e., Pyraclostrobin, Azoxystrobin, Kresoxim-methyl) are marketed as broad-spectrum fungicides, with no harmful effects on humans and the environment, and whose complementary positive effects on plant physiology, yield and quality are emphasized [9–11].

Based on the previous statement, agronomic techniques that can reduce the nitrate content in the product are certainly welcome. These include the use of products with a biostimulating action which, by promoting nitrogen metabolism, could limit its accumulation in nitric form into plant organs. Positive results in this sense concern, for example, the application of strobilurins which, by increasing the nitrate reductase activity, limit the accumulation of nitrates in wild rocket leaves [7] as well as in lettuce and spinach [12,13].

Among the biostimulating substances used in agriculture, there is a growing spread of products based on algae and yeast extracts, which have been found to have a biostimulating effect on various species. Algae extracts, even at low concentrations, are able to induce a series of physiological responses of the plant, such as increasing growth, flowering, yield, polyphenol content, shelf life, tolerance to abiotic stress of different origins (salinity, water stress, heat and frost) [14–16]. Considering the need of having high quality plant material for fresh-cut products and the high perishability of wild rocket, it is important to implement all the agronomic strategies that improve yield and qualitative characteristics together with an adequately long shelf life.

According to the literature, some agronomic practices such as the availability of nitrogen and the application of biostimulating substances can improve the quality of the product at harvest and the shelf life of some vegetables [15,17]. It has been speculated that this could also be the case of the wild rocket. To the best of our knowledge, while there is some information on the effects of nitrogen and Azoxystrobin on the harvest quality of this species [7], information regarding the effects on shelf life and evolution of some nutrients during storage is lacking. To demonstrate our hypothesis, two trials were carried out to evaluate the shelf life of the wild rocket collected in two experimental field trials in which were studied the effects on yield and some qualitative characteristics (i) of N and Azoxystrobin level, (ii) of N level and the application of Azoxystrobin or an yeast and brown algae extract based biostimulant.

2. Materials and Methods

2.1. Description of the Experimental Field Trial

2.1.1. Characteristics of the Experimental Site

The field trials were carried out in the period November 2014–February 2015 (Exp 1) and November 2015–March 2016 (Exp 2) at the Troyli company's farm in the countryside of Policoro (MT) (40°22′ N, 16°62′ E; 150 m asl), in an unheated greenhouse, covered with ethylene vinyl acetate (EVA) film with a thickness of 200 μm. The soil type, according to USDA, is 'Typic Haploxeralfs fine loamy, mixed, superactive, thermic' [18], very fertile and over 1 m deep. The climate, according to De Martonne's classification, is subhumid [19].

2.1.2. Treatments, Experimental Design and Crop Management

Exp 1 involved the comparison of two N levels (24 and 84 kg ha^{-1}, indicated respectively N_L and N_H) with two Azoxystrobin levels (Ortiva®, Syngenta, Milan, Italy) (treated and untreated control, indicated Azo+ and C respectively). A split-plot experimental design with three replications was used, in which the N level occupied the plot and Azoxystrobin levels the subplot (experimental unit, EU). Where expected, two foliar applications of Ortiva® (1.0 L ha^{-1}) were carried out, twenty and ten days before harvesting.

Exp 2 involved the comparison of two N levels (32 and 112 kg ha^{-1}, indicated respectively with N_L and N_H), and the application of two biostimulating substances (BS) based on Azoxystrobin (Ortiva®, Syngenta) and a biostimulant based on fluid extracts of yeast and brown algae (Bioproject SM23 Foliar-BioKimia® International Srl, Castel San Pietro Terme, Bologna, Italy), indicated respectively Azo+ and YBA+, in addition to untreated control (C). A split-plot experimental design with three replications was used by arranging N levels in the plots and BS in the sub-plots (EU). Where foreseen by the experimental plan, foliar applications of BS (Ortiva®, 1.0 L ha^{-1}; Bio Project SM 23 Foliar, 1.5 L ha^{-1}), considering also that Ortiva® has a withdrawal period of 7 days, were performed twenty and ten days before harvest.

In both experiments, soil P-K fertilizations were carried out with 100 kg ha^{-1} of P_2O_5 (superphosphate) and 100 kg ha^{-1} of K_2O (potassium sulfate). Nitrogen, as ammonium nitrate, was applied following the experimental plan. Disease and pest were controlled by applying the IPM disciplinary of Basilicata Region (Regione Basilicata, 2014). Weeds were controlled both by the false sowing method and hand removal. The water supply of the crop was optimal throughout the crop cycles, irrigating by sprinkler. In order to exclude the fungicidal effect, compared to the 'growth promoting' one, the observations that excluded the presence of fungal diseases in all the treatments compared were appropriately performed.

2.2. Yield Assessment

The collections were made by machine in a sampling area of 2 m^2 for each subplot, on 27 February 2015 and 7 March 2016, cutting the plants at a height of about 3 cm above the collar. Leaves and stems of the sampling area were used to determine the marketable yield.

2.3. Shelf Life Assessment

The shelf life was assessed on the marketable product, setting up two experimental trials as described below.

About thirty minutes after harvesting, unwashed wild rocket samples of about 100 g were packaged on a commercial packaging line (at the Troyli company, Tursi – MT, Italy) in polyethylene terephthalate (PET) trays (185 mm × 145 mm × 70 mm) and wrapped with laser perforated, oriented polypropylene film (OPP) with an O_2 transmission rate (OTR) of 17.8 pmol s^{-1}m^{-2} kPa^{-1}, at 23 °C and 50% relative humidity. The packages were immediately (about 1 h) transported to the laboratories by means of a refrigerated van at 4 °C.

Just after arriving at the laboratory, for each EU, 18 packs were tagged and stored in a dark controlled temperature chamber, set at constant temperature of 3.5 °C. On the film of three trays for each EU, small (ϕ10 mm) rubber caps were applied, through which, every 2–3 days, the concentrations of O_2 and CO_2 were monitored by means of a small needle inserted into the package headspace, connected to a gas analyzer (Check-Point, MPIM Srl, San Giovanni Teatino-CH, Italy).

The trials lasted 14 (Exp 1) and 16 (Exp 2) days, and for each trial, surveys were carried out every 2–3 days. At the beginning of the conservation trial on the fresh unpackaged wild rocket and at each survey date for the packaged stored wild rocket, the following qualitative parameters were assessed: dry matter (DM), total phenols (TP), total antioxidant activity (TAA), nitrates (Ni), total chlorophyll (TCh), total carotenoids (TC), odor (Od), visual appearance (VA), color characteristics of the leaves (L*, a*, b*, yellow index).

2.4. Quality Features Measurement

Dry matter. For the determination of the DM, a marketable product sample of approximately 150 g was used. The plant material, after weighing, was placed in a thermoventilated oven at a temperature of 60 °C until reaching the constant weight (about 48 h). The DM was expressed as a percentage of fresh.

Total phenols, total antioxidant activity. For each EU, about 20 g of fresh marketable product were homogenized and subjected to reflux extraction on a water bath (two times in one hour), with methanol (1:5 *w*/*v*). After filtration through a Whatmann filter paper, the methanolic extract was concentrated under vacuum and, therefore, used for the determination of TP and TAA. The TP were determined by spectrophotometry with the Folin–Ciocalteu method, as reported by Sergio et al. [20], and expressed as mg CAE (caffeic acid equivalent) g^{-1} DM. This method exploits the redox reaction in a basic environment between phenolic compounds and the Folin–Ciocalteau reagent. From this reaction, blue complexes of oxides of W_8O_{23} and Mo_8O_{23} are formed. They have a maximum absorption in the visible at about 750 nm. The TAA was determined by means of the analysis of the radical cations ABTS (2,2'-azino-bis-3-ethylbenzothiazolin-6-sulfonic) [21] and expressed as g Trolox 100 g^{-1} DM.

Nitrates. To carry out the analysis of the Ni (NO^{3-}), a sample of about 10 g of dry biomass was used which was finely ground by means of a mill (IKA, Labortechnik, Staufen, Germany) equipped with a sieve 1 mm. Analyses were performed on a 0.5 g sub-sample, with ion chromatography (Dionex DX120; Dionex Corporation, Sunnyvale, CA, USA), with a conductivity detector, using an IonPack AG14 pre-column and an IonPack separation column AS14 [22]. The Ni concentration was determined based on standards prepared with sodium nitrate ($NaNO_3$).

Total chlorophyll, total carotenoids. For each EU, a sample of about 30 g of leaf blades was finely chopped in a mortar after the addition of liquid nitrogen and, then, stored at −20 °C until use. To carry out the extraction, this material was homogenized with 80% acetone (0.2 g mL^{-1}), kept in a dark cold chamber at 4 °C for 24 h and then centrifuged for 10 min at 14,000 rpm. Three spectrophotometric readings (at 662, 646 and 470 nm) were performed on the recovered supernatant. The method is based on the property of chlorophyll to absorb light in the red zone of the visible spectrum. The absorbance values obtained were then used to calculate TCh and TC concentrations according to Wellburn [23]. All extraction procedures were performed in low light conditions.

Odor and visual appearance. Odor (Od) and visual appearance (VA) were determined by a panel of six judges (three female and three male), aged between 30 and 60, and adequately trained. The judges were selected among 10 potential panelists based on their interest in vegetables and their experience in sensory evaluation. The intensity of the evaluated attributes were quantified on a scale of 1 to 5 [24] where 5 = excellent, without defects; 4 = very good, slight defects; 3 = medium, moderate defects; 2 = poor, serious defects; 1 = not edible. The value of 3 was considered as the marketability limit while 2 was considered as the edibility limit. The assessment was performed in a room with individual booths under normal lighting conditions (ISO/DIS 8589). The samples of each EU and each repetition were subjected to panel analysis in a randomized order.

Chromatic characteristics. On 10 leaves randomly selected for each EU, the chromatic parameters L*, a*, b*, and yellow index (YI) were measured. L* represents the brightness, and the values are in the range 0–100 where 0 = black and 100 = white. a* and b* represents the chromaticity coordinates indicating, respectively, the red-green and yellow-blue components [25]. The YI was obtained by the following equation:

$$YI = (142.86\ b^*)/L^* \quad (1)$$

The measurements were carried out with a colorimeter (CR-400, Konica Minolta, Osaka, Japan; using the Spectra Magic NX software), in the apical part of the upper surface of the leaf blade, avoiding the midbird.

2.5. Statistical Analysis

The data collected at each sampling date were subjected to the analysis of variance (ANOVA) according to the split-plot experimental design, using the SPSS 17 software. The comparative analysis between means were based on the F test. Mean values were separated with the Student–Newman–Keuls (SNK) test (P = 0.05).

3. Results

3.1. Effect of Preharvest Treatments on Rocket's Yield and Quality at Harvest

In Exp 1, a positive effect of the higher N amount on the marketable yield was obtained. In fact, this parameter in N_H was on average 11.6% higher than in N_L. The application of Azoxystrobin increased marketable yield by 10.1%.

No effect of N and Azo+ treatments on DM and Ni content were observed. The higher N input increased TCh content by 12.8%; on the other hand, TC, TP and TAA decreased on average by 15.4, 11.0 and 18.2%, respectively. With the application of Azo+, TCh and TC, increased by 22.3 and 15.6%, respectively. Azo+ did not affect the phenolic content and antioxidant activity, parameters related to each other, since the latter is predominantly determined by phenols (Table 1).

Table 1. Effects of N level (NL) and Azoxystrobin (Azo) application on marketable yield (MY), leaf dry matter (DM), nitrate (Ni), total phenols (TP), total antioxidant activity (TAA), total chlorophyll (TCh) and total carotenoids (TC) of wild rocked at harvest time. C = control without Azoxystrobin, Azo+ = with Azoxystrobin; N_L = 24 kg ha^{-1} of N, N_H = 84 kg ha^{-1} of N; FW = fresh weight.

Treatments	MY	DM	Ni	TP	TAA	TCh	TC
	(kg m^{-2})	(g 100 g^{-1} FW)	(mg kg^{-1} FW)	(mg CAE g^{-1} DM)	(g Trolox 100 g^{-1} DM)	(μg g^{-1} DM)	(μg g^{-1} DM)
N level (NL)	*	ns	ns	*	*	*	**
N_L	1.47	8.6	4398.0	14.5	0.43	825.6	175.2
N_H	1.64	8.6	4374.4	12.9	0.35	931.8	147.7
Azoxystrobin (Azo)	*	ns	ns	ns	ns	*	**
C	1.48	8.5	4438.1	13.6	0.40	790.7	149.8
Azo+	1.63	8.7	4334.7	13.8	0.38	966.7	173.1
NL x Azo	ns	ns	ns	ns	ns	ns	ns

ns, * and ** indicate F test not significant, significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively.

In Exp 2, as in Exp 1, the highest marketable yield was obtained in the highest N level (+25.7%). Also in this case, Azo+ improved the yield (+ 23.4%), while no effects of YBA+ were manifested.

No effect of N, Azo+ and YBA+ treatments on DM content was observed. The higher N input increased Ni and TCh content by 37.5 and 15.4%, respectively; on the other hand, TC and TAA decreased on average by 7.8 and 26.4%, respectively, as observed in Exp 1. In respect to the control, Azo+ reduced Ni by 17.1% and improved TCh and TC by 15.6 and 10.9%, respectively. On the other hand YBA+ improved TCh by 11.7%, but reduced TAA by about 35.3% (Table 2).

Table 2. Effects of N level (NL) and biostimulant (BS) (Azoxystrobin, Azo+; extracts of yeast and brown algae, YBA+) application on marketable yield (MY), leaf dry matter (DM), nitrate (Ni), total phenols (TP), total antioxidant activity (TAA), total chlorophyll (TCh) and total carotenoids (TC) of wild rocket at harvest time. C = control without biostimulant, Azo+ = with Azoxystrobin; YBA+ = with extracts of yeast and brown algae; N_L = 32 kg ha^{-1} of N, N_H = 112 kg ha^{-1} of N; FW = fresh weight.

Treatments	MY	DM	Ni	TP	TAA	TCh	TC
	(kg m^{-2})	(g 100 g^{-1} FW)	(mg kg^{-1} FW)	(mg CAE g^{-1} DM)	(g Trolox 100 g^{-1} DM)	(µg g^{-1} DM)	(µg g^{-1} DM)
N level (NL)	*	ns	**	ns	*	*	*
N_L	1.36	8.7	3200.1	14.8	0.53	779.8	192.3
N_H	1.71	8.2	4399.9	13.9	0.39	900.2	176.7
Biostimulants (BS)	**	ns	*	ns	*	*	*
C	1.41 b	8.7	4100.0 a	14.2	0.51 a	771.2 b	175.4 b
Azo+	1.74 a	8.4	3400.5 b	14.9	0.54 a	889.4 a	194.0 a
YBA+	1.46 b	8.5	3899.5 a	14.0	0.33 b	859.2 a	184.6 ab
NL x BS	ns	ns	ns	ns	ns	ns	ns

ns, * and ** indicate *F* test not significant, significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively. Mean separation within columns by SNK test ($P = 0.05$).

3.2. Effect of Preharvest Treatments on Rocket's Characteristics during the Shelf-Life

In Exp 1, during the storage period, no significant differences between the nitrogen levels for all the parameters examined were observed. Therefore, the effects of preharvest application of Azoxystrobin will be described below, considering the average values of the two nitrogen levels.

The concentration of oxygen (O_2) inside the packages during the storage of the wild rocket was always higher in the samples treated with Azoxystrobin than in the control (Figure 1a) and, correspondingly, the carbon dioxide (CO_2) was always lower (Figure 1b).

From the analysis of the qualitative attributes, a positive effect of Azoxystrobin on freshness indicators (VA, Od, YI) is generally highlighted. The VA and Od progressively worsen during storage in both treatments, but with less intensity in Azo+ (Figure 2a,b).

The nonmarketable values of these two indicators are reached 10 days after storage (DAS) in C and 12 DAS in Azo+. The YI also increased more rapidly in the control (Figure 2c). This parameter, in fact, from the beginning of storage to the end, was reduced by 11.0 and 24.1%, respectively for Azo+ and C. TC decreased during storage by 19.1 and 23.3%, respectively for Azo+ and C (Figure 3b).

No significant effects of Azoxystrobin were observed on TP, TAA (Figure 4a,b) and Ni (Figure 5a) during storage. Ni remained unchanged while TP and TAA tended to a slight reduction and a slight increase during storage, respectively.

In Exp 2, similarly to the results of Exp 1, no significant differences were observed in all the parameters examined between the two nitrogen levels during storage. Therefore, the effects of the preharvest application of biostimulants based on Azoxystrobin and the fluid extracts of yeast and brown algae, will be described below, considering the average values of the two nitrogen levels.

From Figure 1c,d, which shows the trend of the O_2 and CO_2 concentration inside the packages during the wild rocket storage, it can be observed that, as observed in Exp 1, in the Azo+ samples O_2 is always maintained higher than in C and, correspondingly, the CO_2 is always lower. The biostimulant based on yeast and brown algae extract, on the other hand, did not have any significant effects on the concentration of the two gases (Figure 1c,d).

From the analysis of the qualitative attributes, as in Exp 1, a positive effect of Azoxystrobin on freshness indicators (VA, Od, YI) occurred. The VA and Od progressively worsen during storage in the three treatments. However, the rate of deterioration is lower in Azo+ and higher in YBA+ (Figure 2d,e).

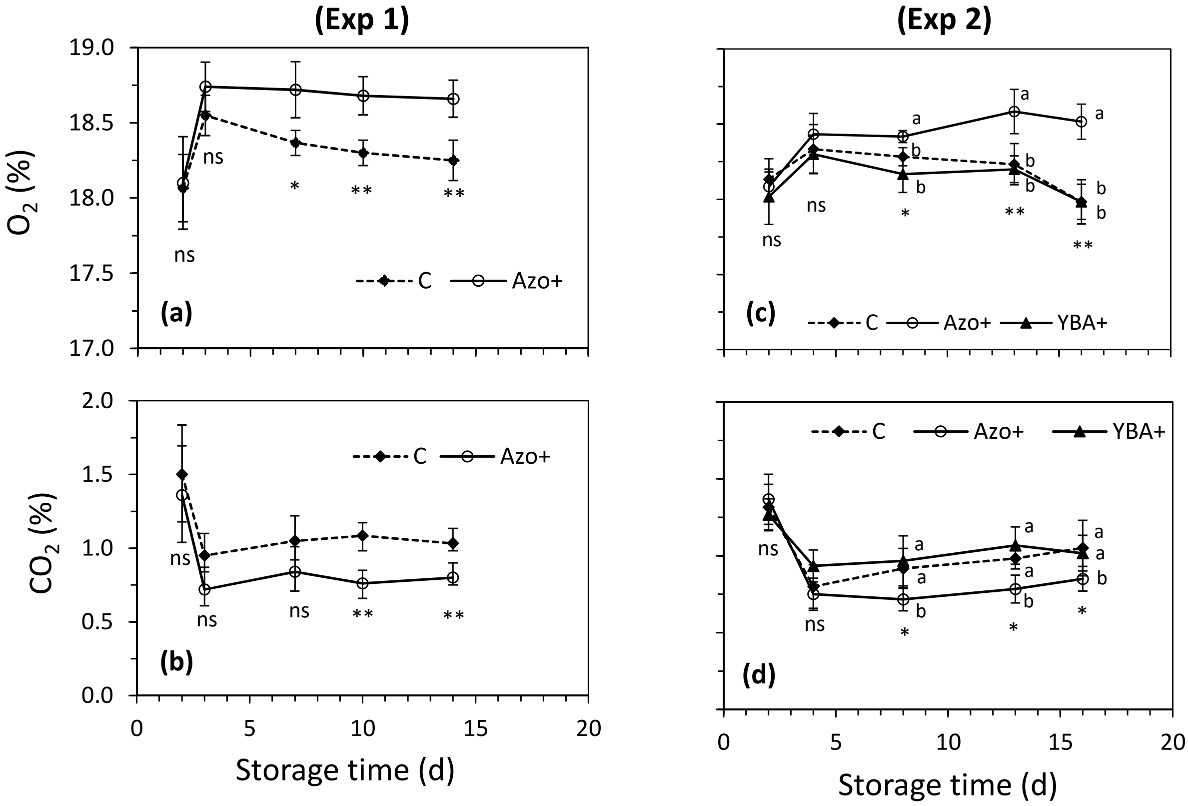

Figure 1. And carbon dioxide (CO_2) concentration trend inside the packages during the storage of the wild rocket treated with Azoxystrobin (Azo+) at preharvest in Exp 1 (**a**,**b**), and with Azoxystrobin or an algae-based biostimulant (YBA+) in Exp 2 (**c**,**d**), and of that relating to the untreated control (C). Vertical bars indicate SD (n = 3). ns, * and ** indicate *F* test not significant, significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively. Separation of mean within the days from the start of storage by SNK test ($P = 0.05$). Different lower case letter denotes significant differences.

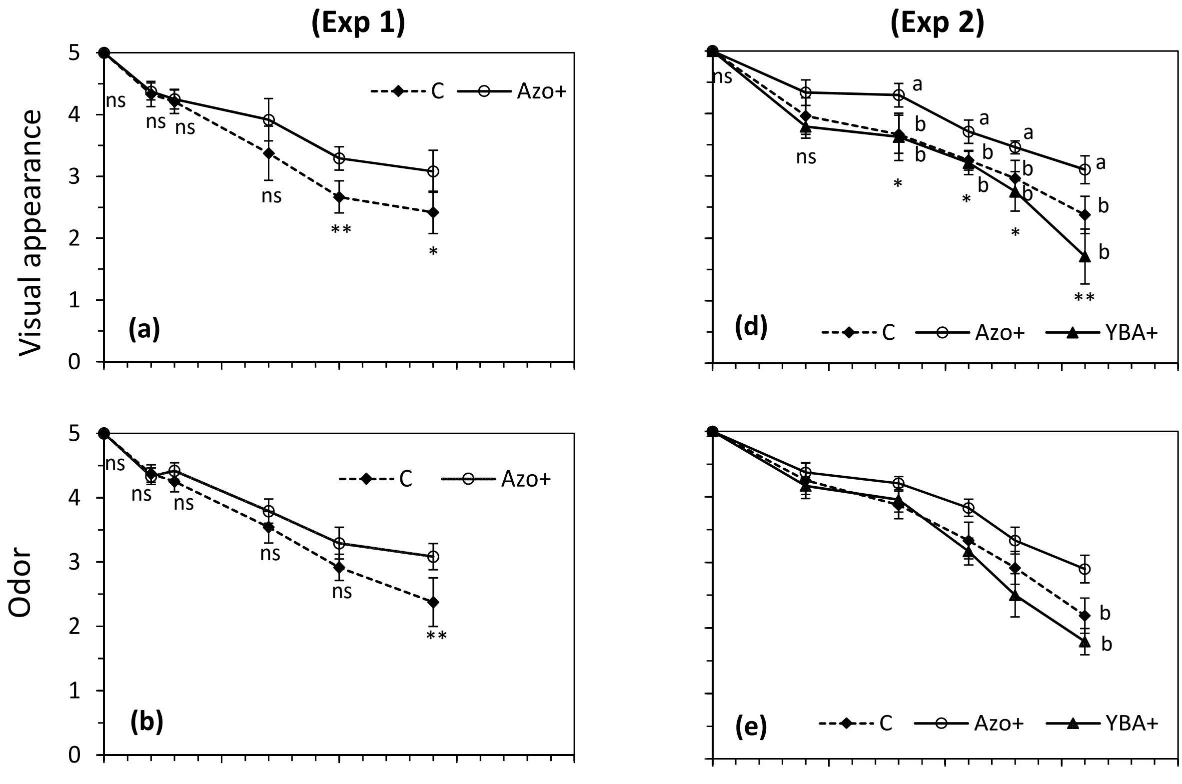

Figure 2. *Cont.*

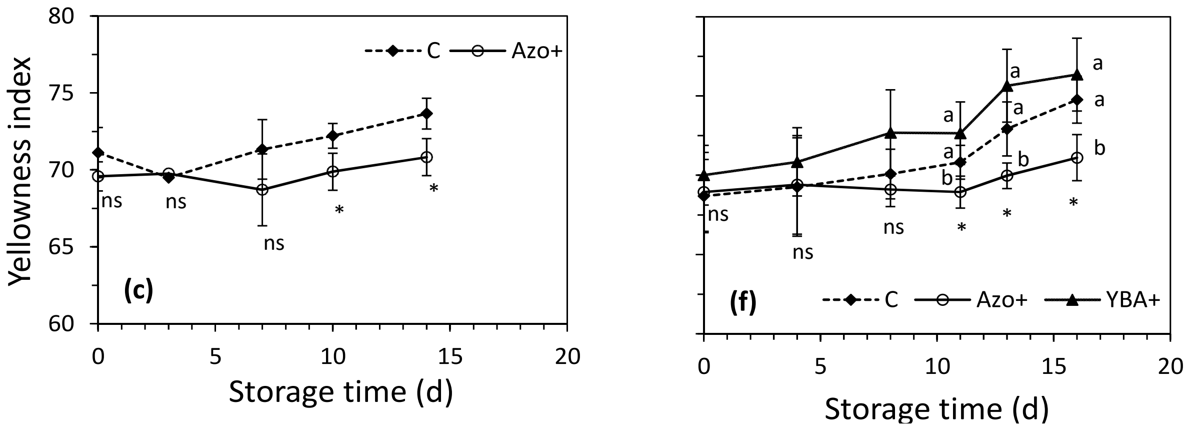

Figure 2. Trend of the visual appearance, odor and yellow index during the storage of the wild rocket treated with Azoxystrobin (Azo +) at preharvest in Exp 1 (**a**–**c**), and with Azoxystrobin or an algae-based biostimulant (YBA+) in Exp 2 (**d**–**f**), and of that relating to the untreated control (C). Vertical bars indicate SD (n = 3). ns, * and ** indicate *F* test not significant, significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively. Separation of mean within the days from the start of storage by SNK test (P = 0.05). Different lower case letter denotes significant differences.

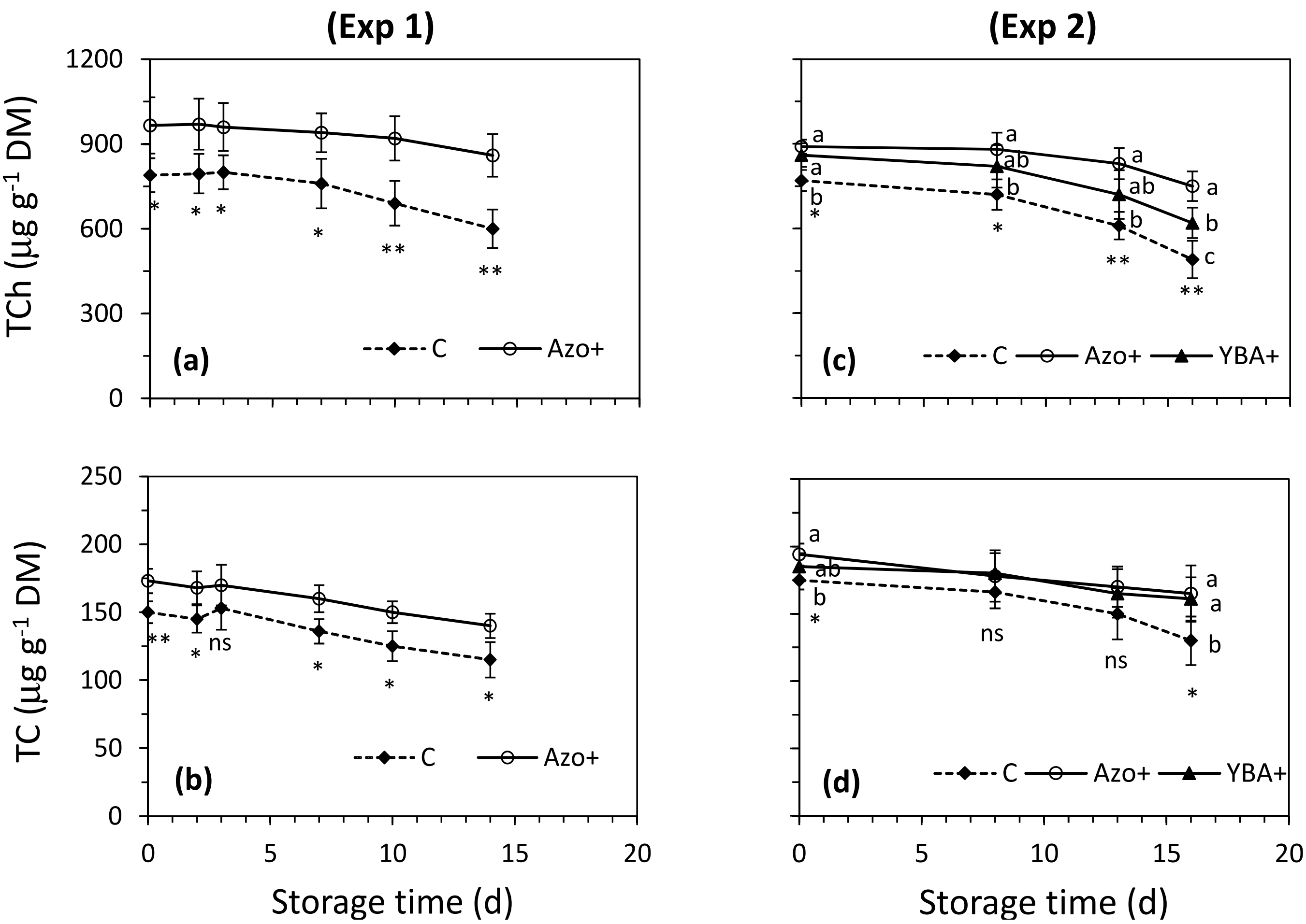

Figure 3. Trend of total chlorophyll (TCh) and total carotenoid (TC) content during the storage of the wild rocket treated with Azoxystrobin (Azo +) at preharvest in Exp 1 (**a**,**b**), and with Azoxystrobin or an algae-based biostimulant (YBA+) in Exp 2 (**c**,**d**), and of that relating to the untreated control (C). DM = dry matter. Vertical bars indicate SD (n = 3). ns, * and ** indicate *F* test not significant, significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively. Separation of mean within the days from the start of storage by SNK test (P = 0.05). Different lower case letter denotes significant differences.

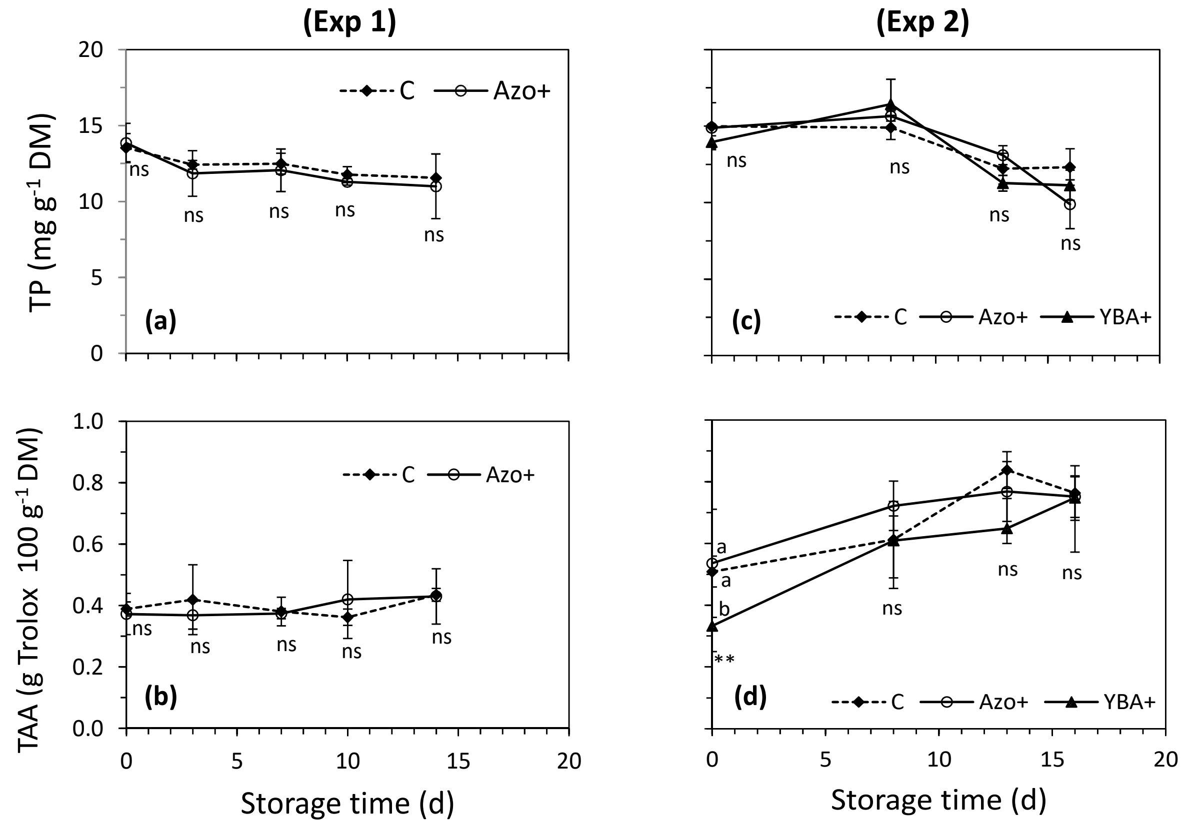

Figure 4. Trend of the nitrate content (Ni), total phenols (TP) and antioxidant activity (TAA) during the storage of the wild rocket (**a**,**b**) and with Azoxystrobin or an algae-based biostimulant (YBA+) in Exp 2 (**c**,**d**), and of that relating to the untreated control (C). DM = dry matter. Vertical bars indicate SD (n = 3). ns, ** indicate F test not significant, significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively. Separation of mean within the days from the start of storage by SNK test ($P = 0.05$). Different lower case letter denotes significant differences.

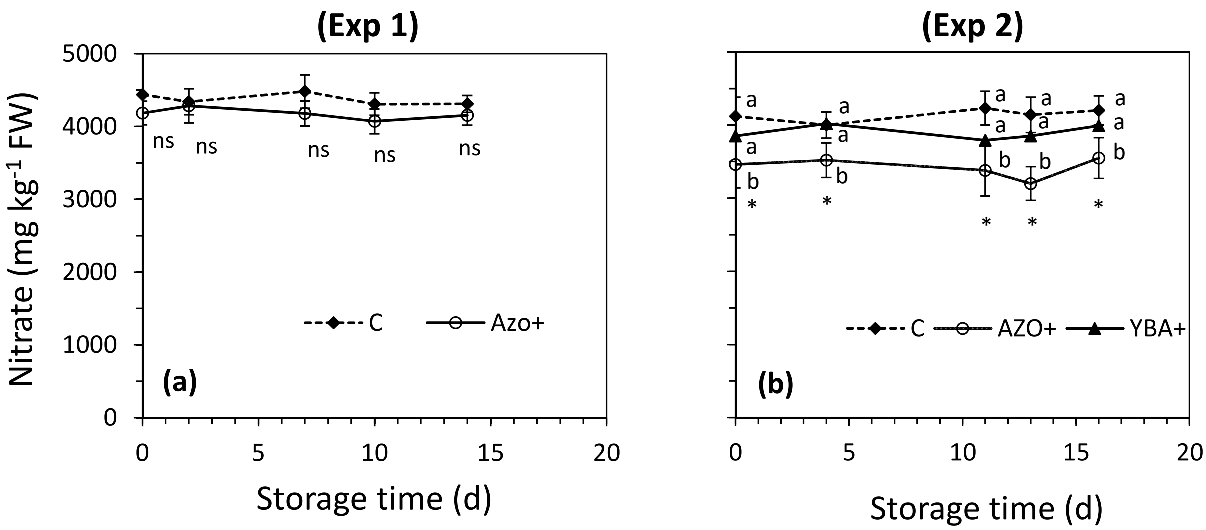

Figure 5. Trend of the nitrate content during the storage of the wild rocket treated with Azoxystrobin (Azo +) at preharvest in Exp 1 (**a**), and with Azoxystrobin or an algae-based biostimulant (YBA+) in Exp 2 (**b**), and of that relating to the untreated control (C). FW = fresh weight. Vertical bars indicate SD (n = 3). ns, * indicate *F* test not significant, significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively. Separation of mean within the days from the start of storage by SNK test (Taiz, L.; Zeiger, E. *Plant Physiology*; The Benjamin/Cummings Publishing Company, Inc., 2002; 675p = 0.05). Different lower case letter denotes significant differences.

The nonmarketable values of these two indicators reached 13 DAS in C and YBA+ and 16 DAS in Azo+. The YI also increased more rapidly in C and YBA+ (Figure 2f), probably due to the more rapid degradation of chlorophyll. In fact, between the start of storage and the end, the chlorophyll content decreased by 15.7, 27.9 and 36.4% respectively for Azo+, YBA+ and C (Figure 3c). TC also decreased between the beginning and the end of storage period of 14.9, 13.0 and 25.7%, respectively, for Azo+, YBA+ and C (Figure 3d).

During storage, the Ni remained unchanged (Figure 5b) while TP and TAA showed a trend to decrease and increase, respectively (Figure 4c,d). Furthermore, during storage, no significant effects of Azo+ and YBA+ were observed on TP, TAA (Figure 4c,d) and Ni (Figure 5b).

4. Discussion

4.1. Effect of Preharvest Treatments on Wild Rocket's Yield and Quality at Harvest

For both trials, for all evaluated features the interaction between N levels and use of biostimulating substances was not significant. Regarding the main effects of treatments on rocket's quality at harvest the results show that total chlorophylls content was higher by using the higher N level as well as by using the biostimulants (Tables 1 and 2). The yield rose with the increase in the nitrogen dose. These results agree with those obtained on wild rocket [26] and other leafy vegetables [27–29]. Azo+ has made a significant contribution to increasing the marketable yield. In addition to the normal fungicidal activity, a biostimulating action of this substance has also been observed in the literature. In support of this, a positive effect of strobilurins on cell proliferation was observed which would favor plant growth, probably linked to the transport of nitrates, iron and sugar [30]. Other authors, on the other hand, have attributed improvement in yield to the increase in chlorophyll [10,31].

The increase in chlorophyll in relation to the rise in the nitrogen level is consistent with the results obtained on wild rocket [7,26], lettuce [12] and spinach [13], as one would expect, since nitrogen is one of the main constituents of the pigment [32].

The application of Azoxystrobin and fluid extracts of yeast and brown algae improved TCh in agreement with others authors who attribute the biostimulating action of strobilurins to the rise in chlorophyll [12,13,31,33], whose biosynthesis is favored by the higher content of cytokinins [34], and by the lower synthesis of ethylene. In fact, the latter hormone is involved in the degradation of pigments and of senescence processes [35]. The application of Azoxystrobin and fluid extracts of yeast and brown algae improved TCh according to numerous authors who attribute the biostimulating action of these substances to the increase in chlorophyll [12,13,31,33], in turn determined by the increase of cytokinins, involved in its biosynthesis [34], and by the reduction of the synthesis of ethylene, a hormone known to be involved in the processes of senescence and degradation of pigments [35].

The phenol content and antioxidant activity decreased with the increase in the amount of N as also observed by others [7,26,36,37].

The increase in the nitrogen level has favored a greater accumulation of nitrates in wild rocket (Exp 2), consistent with the literature data according to which the higher soil N availability it increases the removal by the plant and, therefore, the accumulation of nitrates in the various organs [38–40]. This result partially contrasts with the data obtained in Exp 1 which show no effects of the N level on the Ni. This can be explained by the different climatic conditions that occurred before harvesting: during the 3 days prior to the harvest, the weather had been sunny in Exp 1 and cloudy in Exp 2. In fact, the accumulation of nitrates in vegetables can be influenced by various factors including the availability of the nitrogen in the soil, climatic variables, genotype, etc. [2]. The application of Azoxystrobin reduced nitrates in wild rocket plants, consistent with the experimental evidence that Azoxystrobin has a stimulating action on the activity of nitrate reductase, an enzyme involved in nitrogen metabolism [12,13,41]. The results of our study highlight that, although the agronomic management of fertilization has made it possible to obtain a product with a much lower nitrate content than the limits set by the EU regulation (7000 mg/kg FW) [8], Azo+ has made it possible to further reduce the presence of this anion, allowing to have a qualitatively better product, considering that the intake of high levels of nitrate can have negative effects on human health. Azo+ had no effect on phenol content, as also observed on lettuce [12]. Instead, Conversa et al. [13] observed an increase in these compounds in baby leaf spinach.

The increase in chlorophyll determined by YBA+ confirms the findings of other authors, according to which these compounds favor the biosynthesis of chlorophyll and delay its degradation [42,43]. On the contrary, the reduction of TAA and no variation of phenols, due to the effect of YBA+, contrasts with the literature [14].

4.2. Effect of Preharvest Treatments on Rocket's Characteristics during the Shelf-Life

In both experiments the nitrogen level did not significantly affect the shelf life of the rocket stored in polyethylene packages at 3.5 °C. Bonasia et al. [12] and Conversa et al. [13] instead observed a positive effect of a higher amount of nitrogen on the shelf life of lettuce and spinach. In particular, these authors report that the positive effects caused by the increased availability of N on the chlorophyll content and on the general appearance of the product affect the maintenance of these parameters at satisfactory levels during storage. The preharvest application of Azoxystrobin, in addition to improving the quality characteristics of the wild rocket at the time of harvest, favored a significant extension of the shelf life of the packaged and stored product at 3.5 °C. In particular, during storage, a higher O_2 content was found in the Azo+ packages than in C, which corresponded to a lower CO_2 content. This suggests that the wild rocket treated with Azoxystrobin had less respiratory activity. The parameter that best highlights the positive effect of Azoxystrobin on the slowing of the senescence process is represented by the chlorophyll, which underwent a slower degradation than the control. This is attributable to the inhibitory effect of ethylene biosynthesis by Azoxystrobin [35], a hormone known to promote the degradation of chlorophyll and the senescence process. The nitrate content did not change during storage in accordance with the results obtained on lettuce [12,44,45]. Although the phenols did not undergo significant changes, the antioxidant activity showed a slight increasing trend. Other authors, on the other hand, report an increase in phenols especially in the last phase of the conservation period [13,21,46]. This increase could be related to the stress caused by storage and wound-like response, thus indicating as such bioactives are the main responsible for antioxidant properties.

As regards the use of biostimulants, our study shows that the use of Azoxystrobin allowed to obtain highest and lowest scores respectively of visual appearance and yellowness index during the shelf life in both experiments (Figure 2a,c,d,f). This, could be due to higher chlorophyll content in samples treated with Azoxystrobin with respect to the control during the shelf life (Figure 3a–c). In this context it is important to highlight that chlorophyll in vegetables is important for the visual appearance of the products [47] if considering that its decrease is associated with cellular degradation and/or senescence, and it is often used to estimate quality loss of green vegetables [48,49]. Indeed, strong relation of chlorophyll content with overall visual quality of vegetables has been reported [50]. Moreover, from a nutritional point of view, it could be considered that chlorophyll is important for its health benefits, since its dietary naturally occurring derivatives showed antioxidant and antimutagenic activity [51–53]. Therefore, results of our study suggest that the preharvest application of Azoxystrobin is effective to enhance the quality of wild rocket during storage.

5. Conclusions

The effect of preharvest application of N levels and biostimulating substances based on Azoxystrobin or yeast and algae extract was evaluated in two trials carried out in Southern Italy on wild rocket. The main results showed that rising N availability increased yield and chlorophyll content, while it reduced TC, TP and TAA. Furthermore, the increase in N level led to a greater accumulation of nitrates in the leaves of the wild rocket only when the harvest was preceded by cloudy days. The N level did not affect quality characteristics of the wild rocket during storage.

Azoxystrobin increases yield and improves some qualitative characteristics, such as the increase in chlorophyll and carotenoids, and decrease in nitrates content of wild rocket at harvest, regardless of nitrogen level. In addition, Azoxystrobin increases the shelf life of unwashed wild rocket, packaged in PET trays and wrapped with OPP film, and stored at 3.5 °C. In particular, Azoxystrobin slowed the loss of chlorophyll (yellowing) and the worsening of odor and visual appearance. Therefore, although Azoxystrobin is not included among biostimulants, but is thought of as a fungicide effective for the control of some diseases of wild rocket, its introduction in the phytosanitary defense plan should be promoted, not only for effective disease control but also for the positive complementary physiological, productive, qualitative and shelf life effects.

The biostimulant based on yeast and algae extracts did not affect marketable yield, improved the chlorophyll content at harvest of wild rocket, but reduced its antioxidant activity. It also caused no substantial changes in shelf life except for a slowdown in carotenoids degradation. In consideration of the numerous positive effects of this type of biostimulant on various crops, reported in the literature, further research is desirable to evaluate other variables such as dose and time of application.

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