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An algal extract enriched with amino acids increases the content of leaf pigments but also the susceptibility to the powdery mildew of lettuce

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Abstract Lettuce (*Lactuca sativa* L.) is amongst the most cultivated and consumed vegetables in the world. This vegetable is frequently grown under greenhouse conditions which, in its turn, are highly favorable for powdery mildew (*Golovinomyces orontii*). This disease can reduce both the yield and market value of infected lettuces. Thus, this work was aimed at evaluating the effect of a commercial fertilizer (Biostimul®) composed of algal extract (*Kapaphycus alvarezii*) enriched with amino acids (AEAAs) on leaf pigments and yield of purple curly lettuce (cv. Rubinela) and the severity of powdery mildew under hydroponic conditions in greenhouse. Plants were weekly sprayed with AEAAs at 0, 0.1, 0.2, 0.4, 0.8 and 1.6 mL.L^{-1} and inoculated or not with powdery mildew at 24 days after sowing. In non-inoculated plants, the AEAAs increased the SPAD index, as well as the contents of chlorophylls, carotenoids, and anthocyanins. In inoculated ones, these variables were not affected by AEAAs treatment, except for the content of anthocyanins, which decreased with increasing doses of the product. The AEAAs increased the severity of disease in a dose-dependent manner, which was associated with an increase in sporulation rate and colony diameter. The number of leaves, leaf and root fresh weight, root volume, root length, plant height, canopy diameter, leaf and root dry weight were not affected neither by AEAAs nor by powdery mildew. Our results demonstrated that although biostimulating plant physiology, no positive effect on yield was met. In addition, as a side effect, AEAAs increased the severity of lettuce powdery mildew.

Keywords Biostimulant · Seaweed extract · *Golovinomyces orontii* · Hydroponics

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Introduction

Lettuce is frequently grown in greenhouses (Li & Kubota, 2009). However, this system favors the development of powdery mildew (Simko et al., 2014), one of the most important foliar plant diseases under these conditions (Camara et al., 2018). Although lettuce yield is in general not highly affected, plants have their visual appearance impaired reducing the market value (Camara et al., 2018).

The lettuce powdery mildew has been associated to three obligate biotrophic fungus species (Lebeda & Mieslerová, 2011), i.e., *Podosphaera fusca* (Shin et al., 2006); *Golovinomyces cichoracearum*

(synonym *Erysiphe cichoracearum*) (Lebeda & Mieslerová, 2011; Simko et al., 2014) and *Golovinomyces orontii* (synonym *Erysiphe orontii*) (Cabral et al., 2019). Nonetheless, to date only the last one has been reported occurring in Brazil (Cabral et al., 2019).

Although these pathogens can infect both leaf surfaces, white and powdery colonies are in general more noticeable on the upper side. Highly infected leaves become slightly yellow, then later brown and eventually die, what often leads to yield reduction (Simko et al., 2014). The powdery mildew severity is usually higher under warm climates and greenhouse conditions, in the absence of standing water on leaf surface (Lebeda et al., 2012).

Powdery mildew has been controlled through the use of genetic resistance and pesticides application (Lebeda & Mieslerová, 2011). However, the first one is impaired because most lettuce cultivars are highly susceptible to this disease (Lebeda et al., 2012). Then, the successful management of powdery mildew often requires the spraying of specific fungicides (e.g., triazoles, strobilurins) or preventive application of broad-spectrum ones (e.g., sulphur) on plants (Lebeda & Mieslerová, 2011).

The growing concern regarding the harmful effects of pesticides in the environment as well as in human health together with an increase in production costs and food demand have intensified the search for alternative management methods. In this scenario, biostimulants arise as a low cost and environmentally friendly strategy (Du Jardin, 2015).

Biostimulants are substances or microorganisms applied to plants to improve nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content. For these purposes, they have been largely used in order to promote growth, increase yield as well as obtain products with higher quality and appearance. The biostimulants can be composed of a single bioactive ingredient, a mixture combining them and often added to fertilizers or crop protection products. Seaweed extracts and amino acids are important constituents frequently employed in formulations of current commercial plant biostimulants (Du Jardin, 2015).

Brown seaweeds, particularly *Ascophyllum nodosum* and *Ecklonia maxima* are the main species present in commercial biostimulants. However, other algae species have been recently used to formulate several fertilizers, but scientific results supporting their efficiency for agricultural purposes are still scarce. The red seaweed *Kappaphycus alverezii* has gained attention for improving the growth and yield of many crops such as maize (Kumar et al., 2019) and wheat (Patel et al., 2018). In general, its benefits are associated with improvement in photosynthetic efficiency, regulation of enzymes related to metabolism nitrogen, modulation of phytohormones, and tolerance to biotic and abiotic stresses. Moreover, this seaweed is rich in algal polysaccharides called carrageenans which are able to induce plant defense responses against diseases, such as, tobacco mosaic virus (TMV), *Botrytis cinerea* and *Pectobacterium carotovorum* in tobacco plants (Vera et al., 2012).

Biostimulants containing amino acids also have been reported to increase yield of several crops such as soybean (Kocira, 2019) and maize (Hassan et al., 2020). Similarly, the foliar spraying of amino acids in combination with an algal extract (*Ascophyllum nodosum*) can enhance the vegetative growth of fenugreek plants (Tarraf et al., 2015). The positive effects of amino acids on yield have been explained by regulation of enzymes related to N reduction and assimilation and phytohormones biosynthesis. This, in turn, improves root development, increasing uptake, translocation and retention of macro- and micro-elements (Du Jardin, 2015).

A foliar fertilizer composed of algal extract (i.e., *K. alverezii*) enriched with amino acids from animal origin (AEAAs; Biostimul®) is commercially available in Brazil. According to the manufacturer, this product is registered as organic fertilizer and has been used in a broad spectrum of crop plants, such as, soybean, wheat, maize, rice, potato, bean, sugarcane, citrus, apple and coffee in order to improve growth and yield and induce abiotic resistance. Thus, considering the potential of this foliar fertilizer and the absence of studies on lettuce, this work was carried out to evaluate the effects of AEAAs on yield and content of leaf pigments in lettuce as well as on the severity of powdery mildew under hydroponic conditions.

Materials and methods

Biological material

Lactuca sativa L. cv. Rubinela (Feltrin Ltd., Brazil) was used in all experiments. This cultivar is a curly

lettuce, characterized by purple leaves, late cycle (30—45 days after transplanting) and resistance to downy mildew.

An aggressive strain of *Golovinomyces orontii* was obtained from infected lettuce and maintained by inoculating new plants every week.

Growing conditions

Seed germination was initiated on a moistened spongy phenolic foam plate (Greenup Ltd., Brazil) in the dark at 25 °C for 24 h. Thereafter, the plate with seeds was transferred to a growing chamber (28 ± 2 °C, 11 h of photoperiod), and seedlings cultivated using the Nutrient Film Technique (NFT). A hydroponic nutrient solution (0.3 $dS.m^{-1}$ electrical conductivity) composed of calcium nitrate (4.5 mM), potassium nitrate (4.9 mM), monoammonium phosphate (1.3 mM), magnesium sulphate (3.3 mM), copper sulphate (0.006 mM), zinc sulphate (0.012 mM) manganese sulphate (0.04 mM), boric acid (0.13 mM), sodium molybdate (1.9 mM) and iron chelate EDDHA (Ethylenediamine Di-Hydroxyphenylacetic) (0.08 mM) was used.

Seven-day-old seedlings were transferred to a greenhouse and further grown in an NFT system using a 14-cm plant spacing and a hydroponic solution with electrical conductivity of 1.1 $dS.m^{-1}$. After 21 days, plants were then transferred to another NFT system with 20-cm plant-spacing and 1.75 $dS.m^{-1}$ electrical conductivity.

The experiments were carried out at 73% (± 12%) relative humidity and 32 °C (± 10 °C).

Treatments

The commercial algal extract enriched with amino acids (AEAAs; Biostimul®) was obtained from Dominisolo (Ltd. Londrina, Brazil) and is composed of 12% (w/w) of *K. alvarezii* extract (50% of k-carrageenan) and 28.4% of amino acids from animal origin (glycine (6.8), proline (3.9), hydroxyproline (3.2), glutamic acid (2.8), alanine (2.5), arginine (2.1), acid aspartic (1.8), lysine (1.0), serine (0.9), leucine (0.8), valine (0.6), threonine (0.5), phenylalanine (0.6), isoleucine (0.5), tyrosine (0.2), and histidine (0.2)), 59.57% of water and 0.03% of benzoisothiazolinone. AEAAs were kept at room temperature until use.

Seven-day-old plants were weekly sprayed with treatments until reaching 50 days (a total of 7 applications). Treatments were supplemented with the surfactant Silwet L-77Ag (Momentive, USA) at 0.01%. AEAAs-based product at doses of 0.1, 0.2, 0.4, 0.8 and 1.6 $mL.L^{-1}$ were dissolved in distilled water and, subsequently, applied to plants using an electric sprayer gun (Powner Ltd., China). An increasing volume of 2, 5, 10, 15, 25 and 30 mL was delivered on each plant at 7, 14, 28, 35, 42 and 49 days after sowing (DAS), respectively. During treatment, each plant was surrounded with a bottomless plastic box to prevent spray drift. Plants sprayed with water served as control.

Inoculation

Twenty-four-day-old plants (4 fully expanded leaves) were inoculated. For this, lettuce plants highly infected with powdery mildew were placed in front of two portable electric fans, for 24 h in the greenhouse, in order to simulate the natural infection.

Determination of SPAD index

The SPAD (Soil Plant Analyses Development) meter is a rapid and non-destructive approach to measure chlorophyll content. For this, the index values were recorded using a hand-held chlorophyll meter (SPAD-502, Minolta corporation, Ltd., Osaka, Japan). Two measurements were made on the 6th leaf (on upper marginal area, from each foliar opposite side), at 38, 41, 44, 47 and 50 days after sowing.

Quantification of leaf pigments

The contents of chlorophyll *a* and *b*, total chlorophyll and carotenoids were determined according to Hiscox and Israelstam (1979) at 50 days after sowing. Briefly, four 11-mm discs were excised from the 7th leaf (on upper marginal area, two from each foliar opposite side) using a cork borer and immediately transferred to a plastic vial containing 7 mL of pure DMSO (Neon Ltd., Brazil) and incubated at 65 °C in a water bath for 30 min. Vials were topped up to 10 mL with DMSO at room temperature, and a 3 mL-aliquot transferred to polystyrene microplates for absorbance measurements using a spectrophotometer (Molecular Devices, Ltd. USA). Absorbances

were measured at 663, 645 and 480 nm, and pure DMSO served as blank control. Pigments content was expressed as $mg.g^{-1}$ FW.

The content of anthocyanins was determined as described by Baslam et al. (2012), with some modifications. For that, two leaf discs were collected from 6th leaf, at 50 days after sowing. Each disc was homogenized in 1 mL of acidified methanol (2.27 mL of 37% HCl + 97.73 mL of methanol) and kept at 4 °C in dark for 18 h. To this mixture 665 µL of distilled water and 1.6 mL of chloroform were sequentially added. Thereafter, samples were centrifuged at 3500 rpm for 15 min. The absorbance of the supernatant was measured spectrophotometrically at 530 and 657 nm and pigments content was expressed as $mg.g^{-1}$ FW.

Disease severity evaluation

The disease severity was assessed by means of a diagrammatic scale (Camara et al., 2018) with 8 severity levels ranging from 0.37 to 74%. The 2nd, 3rd and 4th leaves (emitted before inoculation) and 5th, 6th and 7th leaves (emitted after inoculation) were individually evaluated from the appearance of first powdery mildew colonies and, subsequently, every 4 days until 50 days after sowing.

Determination of sporulation rate and diameter of colonies

At 50 days after sowing (26 DAI), four leaf discs were excised randomly from 7th infected leaf. One powdery mildew colony was chosen randomly for each leaf disc. Diameter of colonies were measured using a binocular stereoscopic microscope equipped with a ruled scale.

The sporulation rate was determined according to Stadnik et al. (2003), as follows: after counting the number of colonies on the infected leaf discs used for colony diameter assessment, the discs were placed floating with the adaxial surface faced up on 50 mL plastic vials containing 2 mL of sterile distilled water and 0.01% Tween®. After an incubation time of 12 h under fluorescent light (180 µmol. m^{-2}. s^{-1}) at 24 °C (± 2 °C), the discs were turned over, vials centrifuged at 3500 rpm for 2 min and then shaken at 3800 rpm for 15 s to release conidia in water. Conidial concentration of suspension was determined using a Neubauer's counting chamber. The sporulation rate was expressed in conidia per colony.

Determination of plant growth and yield variables

At 50 days of sowing, plants were carefully harvested, their leaves counted and leaf fresh mass (LFM, g) and root fresh mass (RFM, g) were determined. Root volume (RV, cm^3) was calculated by the water-displacement method, according to Pang et al., (2011). Root length (RL, cm), plant height (PH, cm) and canopy diameter (CD, cm) were measured with a steel tape ruler. Root length was taken from the root insertion point to the root apex. Plant height was measured from the base of the stem to the top of the plant. In order to measure the canopy diameter, the tape was got around the canopy. Finally, roots and leaves were dried in a forced air oven at 65 °C for 72 h, and thereafter, leaf dry mass (LDM, g) and root dry mass (RDM, g) were determined.

Experimental design and statistical analysis

Two independent experiments were carried out to investigate separately the effect of treatments on inoculated and non-inoculated lettuce plants. Each experiment was arranged as a completely randomized design within two sets of 48 plants, where four plants (replicates) per treatment were destined to growth variables and yield determinations, and four others to SPAD index and leaf pigments quantifications. Additionally, powdery mildew severity was assessed on all individuals in the set of inoculated plants. Considering that there was no difference between the values of severity disease on leaves of 2nd, 3rd, 4th as well as the 5th, 6th and 7th ones, the values were averaged to a single severity value for each group of leaves. The same was done for pigments contents for each group of discs.

After verification of homogeneity of the variances through the Cochran test, data were subjected to analysis of variance (ANOVA). Means were compared by Student's t test or Regression analysis, both at 5% of significance level, using the software SISVAR (v.5.6). All experiments were repeated twice with similar results.

Results

Considering all product doses and evaluation times, SPAD average values varied from 19 to 31 for non-inoculated plants and from 19 to 26 for inoculated ones (Fig. 1).

On non-inoculated plants (Fig. 1A), the older the 6th leaf, the higher the SPAD index. At all evaluation times, the application of AEAAs increased SPAD index in relation to control plants in a dose dependent manner until a saturation point at 0.4 mL.L^{-1}, where a 19% increase was observed. No relationship between leaf age and SPAD index was found in infected leaves (data not shown). For plants inoculated with powdery mildew fungus (Fig. 1B), the AEAAs increased the SPAD index at 38 and 41 DAS, in a dose dependent manner until a saturation point at 0.4 mL.L^{-1}. In this dose, a 24% increase in SPAD index was observed. However, from this time on, no increase was detected.

Leaf pigments

The content of chlorophyll *a* at 50 DAS varied from 0.4 to 0.9 mg.g^{-1} in non-inoculated plants and remained around 0.7 mg.g^{-1} in inoculated ones (Fig. 2A). In non-inoculated plants, the spraying of the foliar fertilizer at all doses increased the content of chlorophyll *a* in relation to control, until a saturation point at 0.4 mL.L^{-1}. This dose caused the highest increase of chlorophyll *a* (158%). On the other hand, no change in its content was recorded in inoculated plants.

The content of chlorophyll *b* at 50 DAS varied from 0.1 to 0.4 mg.g^{-1} in non-inoculated plants and remained around 0.2 mg.g^{-1} in inoculated ones (Fig. 2B). For non-inoculated lettuces, the spraying of the AEAAs increased significantly the chlorophyll *b* content in a dose dependent manner until a saturation point at 0.4 mL.L^{-1}. In this situation, a 208% increase in chlorophyll *b* content was observed. On the other hand, no changes in the content of chlorophyll *b* were recorded in inoculated lettuces.

The total chlorophyll values varied from 0.5 to 1.4 mg.g^{-1} in non-inoculated plants and remained around 0.9 mg.g^{-1} in inoculated ones (Fig. 2C). For non-inoculated lettuces, the application of AEAAs increased the total chlorophyll in a dose dependent manner until a saturation point at 0.4 mL.L^{-1} where a 176% increase was observed. On the other hand, no changes in the content of total chlorophyll were recorded in inoculated plants.

Carotenoids at 50 DAS varied from 0.05 to 0.3 mg.g^{-1} in non-inoculated lettuces, whereas in inoculated ones all values remained around 0.2 mg.g^{-1} (Fig. 2D). The application of the foliar fertilizer increased significantly the carotenoids content in non-inoculated plants in a dose dependent manner until a saturation point at 0.4 mL.L^{-1} where a 775% increase was observed. On the other hand, no changes in the content of carotenoids were recorded in inoculated plants.

Anthocyanins at 50 DAS varied from 0.6 to 0.9 mg.g^{-1} in non-inoculated lettuces and from 0.3 to 0.6 mg.g^{-1} in inoculated ones (Fig. 3). The spraying of the AEAAs increased significantly the anthocyanins content in a dose dependent manner until a saturation point at 0.8 mL.L^{-1} where a 58% increase was observed. On the other hand, in inoculated plants, all

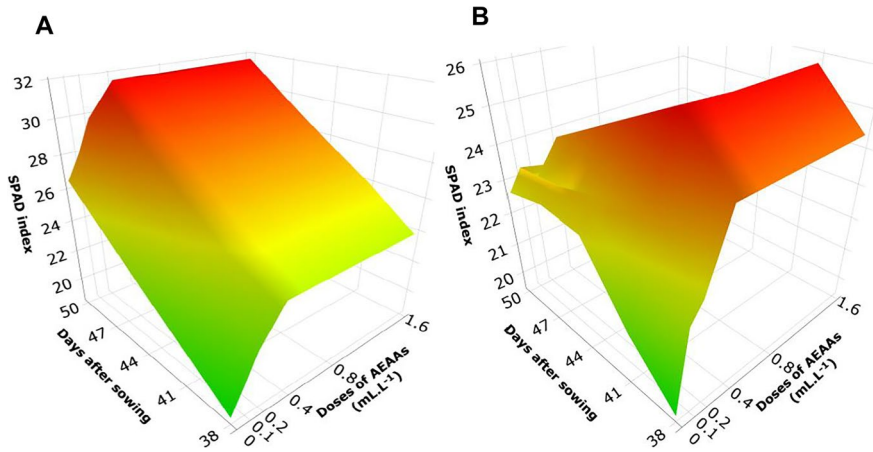

Fig. 1 SPAD index of lettuce (*Lactuca sativa* L., cv. Rubinela) non-inoculated (A) and inoculated (B) with *Golovinomyces orontii* and weekly sprayed with different doses of a commercial algal extract enriched with amino acids (AEAAs/Biostimul®). SPAD index was measured on the 6th leaf. Plants were inoculated at 24 days after sowing

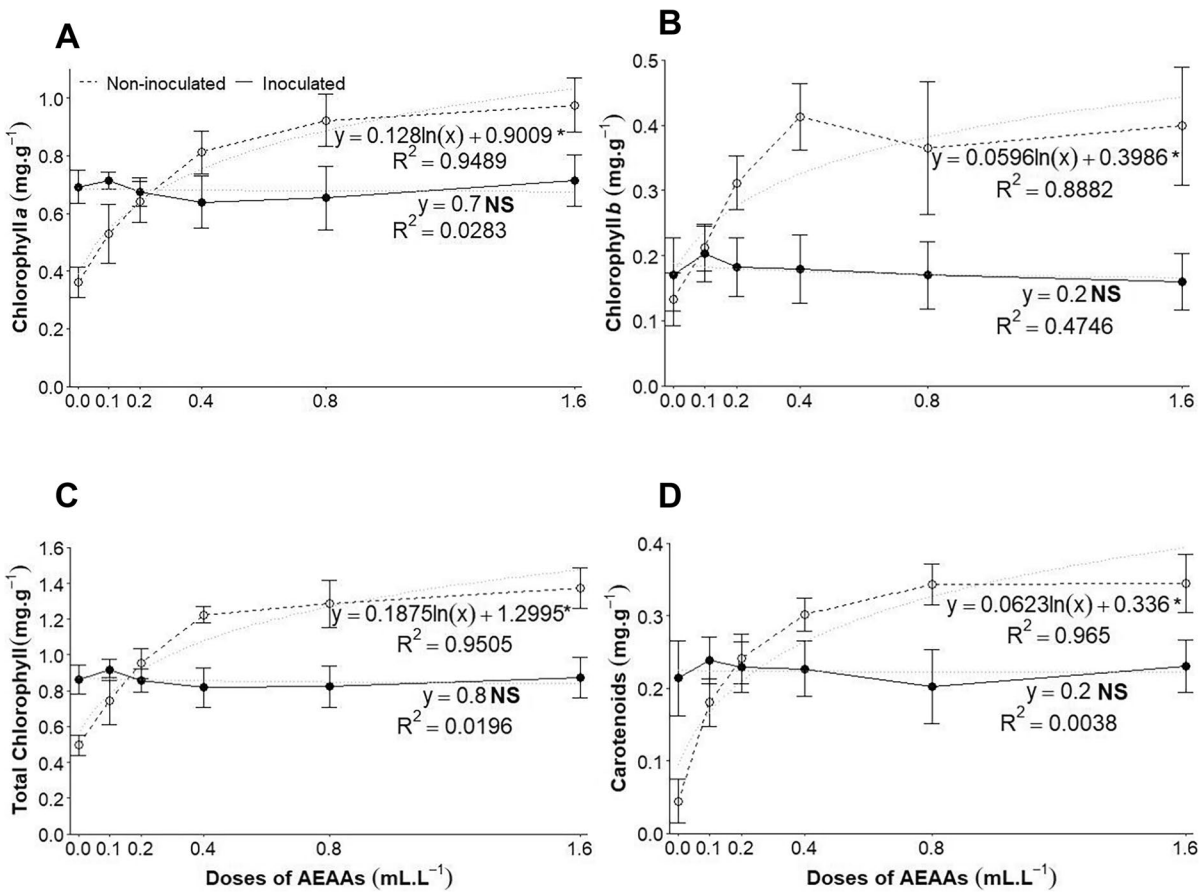

Fig. 2 Content of chlorophylls *a* (A), *b* (B), total (C) and carotenoids (D) of lettuce (*Lactuca sativa* L., cv. Rubinela) weekly sprayed with different doses of a commercial algal extract enriched with amino acids (AEAAs/ Biostimul®) and inoculated with *Golovinomyces orontii* at 24 days after sowing (DAS). Photosynthetic pigments were quantified on the 7th leaf at 50 DAS. * Indicates significant difference and NS indicates non-significant difference by regression analysis ($p \leq 0.05$). Bars represent the standard deviation of the mean

tested doses of AEAAs decreased the anthocyanins content. This reduction was more pronounced (45%) from 0.4 to 1.6 mL.L^{-1}.

Disease severity

First powdery mildew colonies were observed on 2nd, 3rd and 4th leaves at 10 days after inoculation (DAI) with powdery mildew (Fig. 4A).

Disease severity was higher on older leaves (fully developed before inoculation and treated preventively) than on younger leaves (emitted and treated after inoculation), varying from 0 to 95% and from 0 to 35%, respectively. The AEAAs increased powdery mildew severity (Fig. 5) in a dose-dependent manner. However, the increase was more pronounced on older leaves (up to 96% at 26 DAI) than on younger ones (up to 40% at 26 DAI) (Fig. 4B).

At 26 DAI, the sporulation rate of fungus varied from 30 to 43 conidia per colony (Fig. 6A). AEAAs increased significantly the sporulation rate at all doses. However, the most significant increase was by 40% at 0.8 mL.L^{-1}. After that, the increase was at a lesser extent. Moreover, the disease severity was correlated with the sporulation rate (r = 0,68).

The colony diameter of the powdery mildew fungus varied from 2 to 3 mm, at 26 days after inoculation (Fig. 6B). The spraying of the foliar fertilizer increased the colony diameter, in a dose dependent manner until a saturation point at 0.4 mL.L^{-1}, where

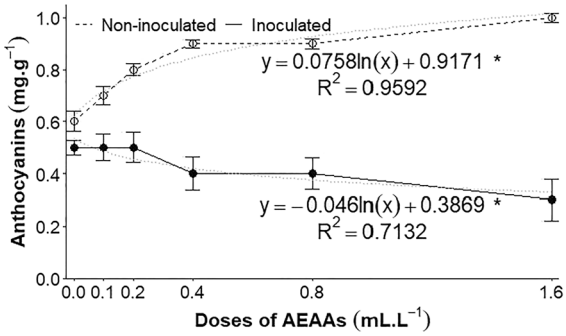

Fig. 3 Content of anthocyanins of lettuce (*Lactuca sativa* L., cv. Rubinela) weekly sprayed with different doses of a commercial algal extract enriched with amino acids (AEAAs/Biostimul®) and inoculated with *Golovinomyces orontii* at 24 days after sowing (DAS). Anthocyanin content was quantified on the 6th leaf at 50 DAS. * Indicates significant difference by regression analysis ($p \leq 0.05$). Bars represent the standard deviation of the mean

a 50% increase was observed in relation to control. Moreover, disease severity was correlated with colony diameter (r = 0.43).

Plant growth and yield

At 50 days after sowing, number of leaves per plant ranged between 17 and 18. The leaf and root fresh weight ranged from 153.7 to 161.6 g and from 27.2 to 28.7 g, respectively. Root volume and root length varied from 125 to 135 cm^3 and from 22.9 to 24 cm, respectively. Plant height and canopy diameter varied from 19.6 to 20.2 cm and from 32.6 and 33.6 cm, respectively. Finally, leaf and root dry weight ranged from 6.7 to 7.1 g and from 1.8 to 1.9 g, respectively. Plant growth and yield were not affected by both spraying of AEAAs and powdery mildew (Tables 1 and 2).

Discussion

Although a large number of commercial products have claimed to affect plant metabolism, scientific evidences are still scarce, and to our best knowledge, non-existent for lettuce. Our study revealed that an algal extract enriched with amino acids can stimulate the biosynthesis and/or avoid degradation of photosynthetic pigments and anthocyanins in lettuce, without leading to both better yield or higher levels of resistance to powdery mildew.

The application of AEAAs increased the SPAD index in non-inoculated plants in a dose dependent manner. Increase in SPAD index has been explained by up-regulation of enzymes related to nitrogen assimilation and reduction of chlorophyll photodamage due to increasing antioxidant activity, as demonstrated by Hassan et al. (2020) and González et al. (2014), respectively. Thus, it is possible to argue

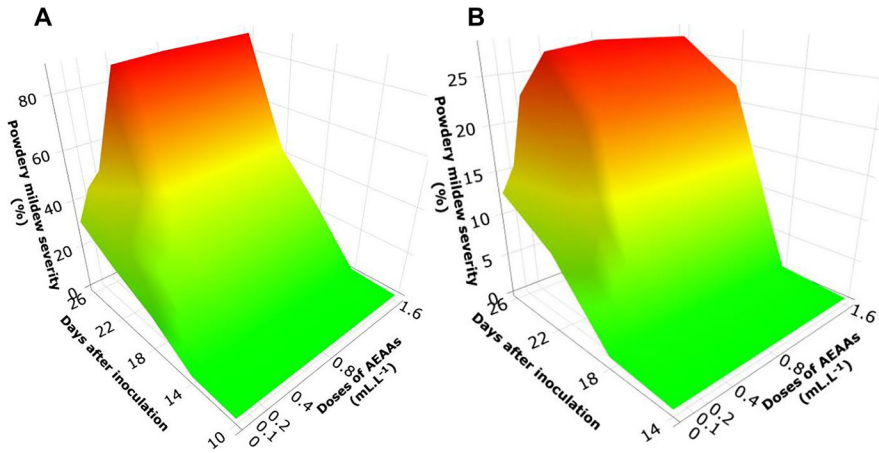

Fig. 4 Powdery mildew severity of lettuce (*Lactuca sativa* L., cv. Rubinela), weekly sprayed with different doses of a commercial algal extract enriched with amino acids (AEAAs/Biostimul®) before inoculation with *Golovinomyces orontii* (A) and after inoculation (B). The severity on leaves previous treated was evaluated at the 2nd, 3rd and 4th leaves (emitted before inoculation) from 34 to 50 days after sowing (DAS). The severity on leaves treated after inoculation was evaluated at the 5th, 6th and 7th leaves (emitted after inoculation) from 42 to 50 DAS. Plants were inoculated at 24 DAS

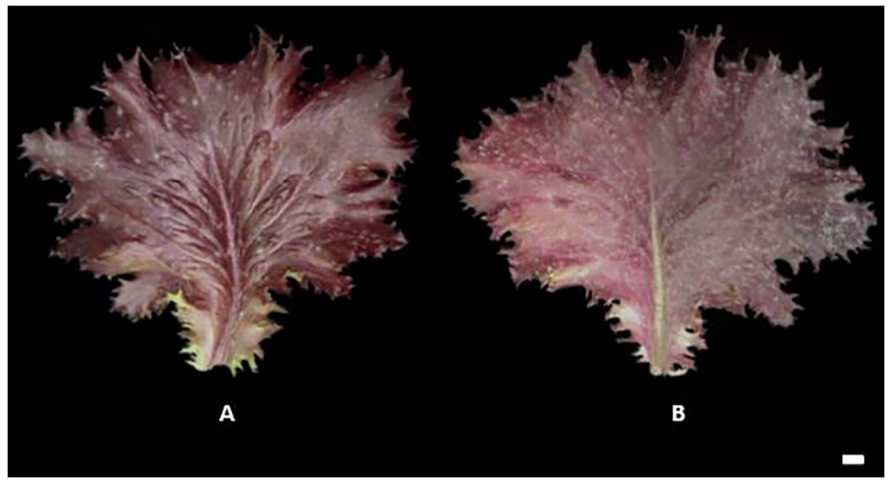

Fig. 5 Powdery mildew symptoms on the 4th leaf of lettuce (*Lactuca sativa* L., cv. Rubinela) at 26 days after inoculation with *Golovinomyces orontii.* Non-treated (A) and treated plants with algal extract enriched with amino acids (AEAAs, Biostimul®) (B) at 1.6 mL.L-1. Bar = 0.5 cm

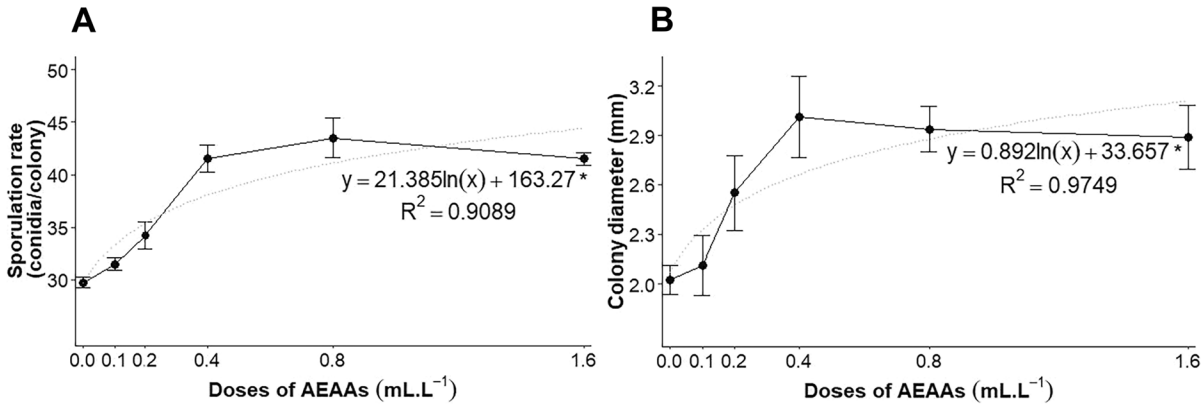

Fig. 6 Sporulation rate (A) and diameter (B) of colonies of *Golovinomyces orontii* on lettuce (*Lactuca sativa* L., cv. Rubinela) weekly sprayed with different doses of a commercial algal extract enriched with amino acids (AEAAs/ Biostimul®). Sporulation rate and diameter were performed on the 7th leaf at 50 days after sowing (DAS). Plants were inoculated at 24 DAS. *Indicates significant difference by regression analysis ($p \leq 0.05$). Bars represent the standard deviation of the mean

that one or more of the extract multicomponents act by one or both of these mechanisms. Interestingly, the spraying of *Kappaphycus alvarezii* extract was reported to increase the SPAD index in rice (Layek et al., 2018). On the other hand, amino acids were not found to affect it in lettuce (Lucini et al., 2015). Pigments quantification by destructive analysis at 50 DAS also corroborated with the SPAD index results, where AEAAs increased the content of chlorophyll and carotenoids in non-inoculated plants in a dose dependent manner as well. Such effect was reported for other plant species including tomato (Cerdán et al., 2013) and wheat (Patel et al., 2018). However, mechanisms how so diverse kind of compound sources would be able to enhance both photosynthetic pigments still remain fully uncovered. Increase in the content of chlorophyll and carotenoids by *K. alvarezii* extract has been associated to the presence of betaines in the seaweed which may be attributed to the reduction in chlorophyll degradation (Layek et al., 2018). Therefore, identifying individual compound(s) in the AEAAs responsible for causing these positive changes will be an exciting topic for future research.

AEAAs increased the SPAD index in inoculated plants only until 41 DAS. Two hypotheses may be raised to explain this result. On one hand, light reflection by leaves covered with powdery mildew colonies is known to be different when compared to uninfected ones (Tartachnyk et al., 2006). Coincidently, the disease onset occurred at 41 DAS. On the other hand,

Table 1 Analysis of variance (ANOVA) in order to determine the significance of differences of growth and yield among inoculated and non-inoculated lettuce (*Lactuca sativa* L., cv. Rubinela), at 50 days after sowing and 26 days after inoculation

Growth and yield variables	F_c	P
NL	0.970	0.327
LFM (g)	3.227	0.076
RFM (g)	1.141	0.288
RV (cm^3)	0.375	0.542
RL (cm)	1.715	0.194
PH (cm)	0.139	0.710
CD (cm)	0.123	0.727
LDM (g)	0.685	0.410
RDM (g)	0.559	0.457

NL Number of leaves; *LFM* Leaf fresh mass; *RFM* Root fresh mass; *RV* Root volume; *RL* Root length; *PH* Plant height; *CD* Canopy diameter; *LDM* Leaf dry mass; *RDM* Root dry mass. F Calculated F-test value; P Probability of F calculated > F expected

nitrogen uptake by the fungus can also reduce the biosynthesis of chlorophylls (Tartachnyk et al., 2006) and, consequently, the SPAD index. In addition, pigments quantification by destructive analysis at 50 DAS showed AEAAs failed to increase the content of chlorophyll and carotenoids in inoculated plants. This effect might be associated to the colonization of host tissues by the pathogen. As a matter of fact, the fungus develops a complex system of infection structures within the leaf tissue leading to damage in photosynthetic apparatus and chlorophyll breakdown. Indeed, a dramatic reduction in chlorophyll content was observed during wheat-powdery mildew interaction at 9 days after inoculation (Kuckenberg et al., 2009). Interestingly, the SPAD index decreased but the content of chlorophylls remained stable in infected leaves. This discrepancy might be related to the evaluation mode of each method. While SPAD is highly affected by changing in light leaf reflection caused by powdery mildew, the absorbance of chlorophylls is determined directly after their extraction from leaves with DMSO.

This is the first study showing that a product containing amino acids and/or *K. alvarezii* extract can increase the anthocyanin content in plants. Similar effects were observed with the application of products containing *Ascophyllum nodosum* in grapevines likely due to modulating of growth regulators. Abscisic acid, for instance, is ultimately involved in anthocyanin biosynthesis (Frioni et al., 2018) and its spraying is able to increase the anthocyanin content in the skin of grapefruit (Peppi et al., 2007). Thus, AEAAs could be further investigated to improve nutraceutical quality of some crops including fruits and vegetable. Curiously, an opposite effect was seen in inoculated

Table 2 Number of leaves (NL), leaf fresh mass (LFM), root fresh mass (RFM), root volume (RV), root length (RL), plant height (PH), canopy diameter (CD), leaf dry mass (LDM) and root dry mass (RDM) of lettuce (*Lactuca sativa* L., cv. Rubinela), at 50 days after sowing (DAS) and weekly sprayed with different doses of a commercial algal extract enriched with amino acids (AEAAs/ Biostimul®)

Growth and yield variables	Doses of AEAAs ($mL.L^{-1}$)						
	0	0.1	0.2	0.4	0.8	1.6	
NL	17.0 ± 1.0	18.0 ± 1.0	17.0 ± 1.0	18.0 ± 1.0	17.0 ± 1.0	18.0 ± 1.0	NS
LFM (g)	158.7 ± 9.1	161.6 ± 9.9	155.1 ± 12.9	159.0 ± 13.5	153.7 ± 8.4	155.8 ± 14.3	NS
RFM (g)	28.1 ± 1.7	28.7 ± 1.6	27.4 ± 2.2	28.1 ± 2.3	27.2 ± 1.5	27.6 ± 2.4	NS
RV (cm^3)	130.0 ± 14.0	135.0 ± 14.0	130.0 ± 14.0	130.0 ± 14.0	125.0 ± 14.0	130.0 ± 15.0	NS
RL (cm)	23.5 ± 1.4	24.0 ± 1.5	23.1 ± 1.8	23.7 ± 1.9	22.9 ± 1.3	23.3 ± 2.1	NS
PH (cm)	19.8 ± 0.7	19.7 ± 0.7	19.6 ± 0.7	19.8 ± 0.7	19.6 ± 0.7	20.2 ± 0.5	NS
CD (cm)	33.4 ± 2.0	33.6 ± 2.0	32.6 ± 2.8	33.1 ± 2.8	32.5 ± 2.2	32.8 ± 3.2	NS
LDM (g)	7.1 ± 0.5	7.1 ± 0.5	6.8 ± 0.7	6.8 ± 0.7	6.7 ± 0.4	6.8 ± 0.7	NS
RDM (g)	1.8 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.8 ± 0.2	1.8 ± 0.1	1.8 ± 0.2	NS

The values correspond to the average of plants inoculated and non-inoculated with *Golovinomyces orontii*

NS indicates no significant difference among product doses by regression analysis ($p \leq 0.05$)

The treatments started at 7 DAS

plants. Anthocyanin content was lower in grape berries infected with powdery mildew (Calonnec et al., 2004). This is because light has a pivotal role in the anthocyanin biosynthesis (de Pascual-Teresa & Sanchez-Ballesta, 2008), and the epiphytic mycelial growth of powdery mildew on the leaf surface might decrease its passage and consequently the anthocyanin content.

AEAAs increased powdery mildew severity and diameter as well as sporulation rate of colonies of the pathogen. To date, there is no similar study with lettuce showing that biostimulants can increase the powdery mildew severity. *G. orontii* is a biotrophic fungus, i.e., it exclusively takes its nutrients from living host cells, and therefore it is highly affected by the host nutritional status. As consequence, it is expected that providing nutrients including amino acids and sugars could enhance plant susceptibility to this fungus. Indeed, it has been reported that the application of nitrogen compounds can increase the powdery mildew severity in wheat (Olesen et al., 2003) and the sporulation rate of the pathogen in tomato (Hoffland et al., 2000). Moreover, AEAAs could improve photosynthesis and increase the soluble sugars in plant cells, as demonstrated in wheat with foliar application of *K. alvarezii* extract (Patel et al., 2018), which could, in turn, facilitate the growth of powdery mildew fungus.

Despite increasing SPAD index and photosynthetic pigments, AEAAs did not affect growth and yield of lettuce. This apparent contradiction may be explained by the excellent nutritional condition of lettuce plants. Indeed, the hydroponic solution used throughout the experiments provide optimal amounts of all nutrients required for fast-growing lettuce plants. However, some studies have suggested that many biostimulants can provide the most significant effects on yield when plants are subjected to abiotic stresses such as high salinity (Lucini et al., 2015), drought (Patel et al., 2018, Kumar et al., 2019) and nutrient deficiency (Cerdán et al., 2013). Hence, it would be interesting to further investigate whether and how AEAAs treatments could affect lettuce plants growing under non-optimal nutritional conditions.

Interestingly, even high severity levels of powdery mildew did not affect neither plant growth nor yield. Although cv Rubinela is susceptible to powdery mildew, this genotype seems therefore to exhibit a high level of tolerance to disease. Recent screening studies demonstrated that most *L. sativa* cultivars are susceptible to *G. cichoracearum* (Lebeda & Mieslerová, 2011). Even though the visual appearance of powdery usually outweighs the damage potential, highly infected lettuce plants have their market value reduced. Then, the planting of lettuce cultivars with some disease tolerance may be important.

Although the AEAAs did not show any positive effect on plant growth and yield, this study revealed an important practical information, *i.e.* some biostimulant products can increase the severity of biotrophic fungus like the powdery mildew on susceptible cultivars of lettuce. Thus, the application of such product has limitations for use under disease-conducive growing conditions.

Conclusion

AEAAs demonstrated a biostimulating effect on plant physiology characterized by the increase of photosynthetic pigments and anthocyanins, but no positive effect on plant growth and yield. On the other hand, as an undesirable side effect, AEAAs increased the severity of lettuce powdery mildew.

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Authors' contributions SR performed all experiments and wrote the first draft of the manuscript. MBF contributed to data analysis and manuscript writing. MJS and JLBO supervised this study. All authors contributed to the article and approved the submitted version.

Data availability All data and material referred in the manuscript are available upon reasonable request.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Authors grant all consents to publish the manuscript.

Competing interests/conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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