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Photosynthetic efficiency and chlorophyll fluorescence responses of *Viola ignobilis* Rupr. subjected to different biostimulants and two light intensities

Roshanak Ansari[1], Khodayar Hemmati[*,1], Sara Khorasaninejad[1] and Nahid Niari Khamsi[2]

1. Horticultural Sciences Department, Plant Production Faculty, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
2. Department of Agronomy and Plant Breeding, Faculty of Engineering, Islamic Azad University, Kermanshah branch, Kermanshah, Iran

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***Corresponding author:**
Horticultural Sciences Department, Plant Production Faculty, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Email: kh_hemmati@gau.ac.ir

ABSTRACT

Purpose: *Viola ignobilis* Rupr. is one of the important medicinal species which is in danger of extinction due to the over harvesting from the main habitats. Nowadays, domestication of medicinal plants in accordance with sustainable agricultural methods is a new important challenge. In this research, we used biostimulants and optimization of light intensity as eco-friendly approaches to improve yield and photosynthetic efficiency of *V. ignobilis* Rupr. **Research Method:** The experiment was set up in a split plot arranged in a randomized complete block design with 3 replications. The main factor was two light levels (50% and 100% of full sunlight) and as sub factors plants were treated with animal derived protein hydrolysate (A.PH), vegetal derived protein hydrolysate (V.PH), seaweed extract (SE), the combination of A.PH + SE and V.PH + SE and also, water served as a control. **Findings:** Light intensities and biostimulant application significantly impacted the morphological parameters including fresh and dry weight of roots and shoots compared to control plants. Furthermore, the photosynthetic pigments did not differ significantly in two light intensities, but, biostimulant application considerably increased the photosynthetic pigments concentration. The obtained results indicated that the highest value of assimilation rate, transpiration rate and stomatal conductance, and also chlorophyll fluorescence parameters including the highest values of qP, Fv/Fm and (ΦPSII) were connected to plants treated with A.PH + SE biostimulants under full irradiance. **Research limitations:** No limitations were found. **Originality/Value:** Optimizing light condition and combined use of PHs + SE biostimulants due to synergistic effects can improve crop yield and photosynthetic efficiency in violet, when no other sources of fertilizers are available.

INTRODUCTION

Sweet violet (*Viola ignobilis* Rupr.) is a member of the *Violaceae* family is facing extinction because of over-harvesting. *Viola* is the largest genus in this family with approximately 664 species which is used for medicinal and ornamental purposes (Marcussen et al., 2022).

During the last decades, excessive application of chemicals in conventional agriculture has been contaminated environment due to heavy metals, and harmful residues (Alengebawy et al., 2021). Therefore, sustainable agricultural practices such as nature-based solutions can be helpful to enhance production and reduce harmful impacts on the ecosystem (Artmann & Sartison, 2018). It follows that must be attended to the technical aspects of plants growth and development such as temperature, nutrition, humidity, and light condition (Hamidah et al., 2018). In this context, there are novel cultivation methods, that able ameliorate destructive impacts of conventional farming. One of these methods contains the use of naturally derived biostimulants. Du Jardin (2015) describes a plant biostimulant as an organic and inorganic compounds or microorganism that when apply to plants, enhances nutrition efficiency, biotic and abiotic stresses tolerance and crop quality parameters. Biological stimulants reduce the need to use chemical fertilizers by influence on root growth and architecture and consequently increasing the nutrients acquisition (Sun et al., 2024).

Protein hydrolysates (PHs) are natural bio-stimulants consisting of oligopeptides, polypeptides, and free amino acids, which can be produced through chemical and/or enzymatic hydrolysis of obtained organic materials from wastes of plants or animals origin (Carillo et al., 2019). The researchers found that plants easily absorb low molecular size peptides and amino acids, which can significantly influence plant growth and physiology by acting on photosynthesis and mechanisms involved in abiotic stress resistance (Schiavon et al., 2008). These products have ability to improve nutritional uptake, nutrient-use efficiency and boosting yield and quality of treated crops (Polo & Mata, 2018).

Seaweed extracts (SE) and their derivative products are another group of biostimulants. One of the common species is *Ascophyllum nodosum* which is comprised of polysaccharides, primarily alginate, laminaran, polyphenols, betaines, amino acids, and vitamins (Ertani et al., 2018). It has been reported that SE contains essential micro and macro nutrients, phyto-hormones including auxin, ABA and cytokinines and other crucial ingredients, which may have influence on biochemical reaction in plant cells (Baltazar et al., 2021).

Light modulation may also be considered as an effective strategy which different aspects of it consisting quality, intensity and duration strongly influence on plant growth and development (Vitale et al., 2021). Light intensity deeply impact on plant morphogenesis, anatomy, cellular biochemistry, photosynthesis, and secondary metabolite production (Badmus et al., 2022; Tang et al., 2022), thus, optimizing the light condition could be improved yield and physiological responses in medicinal herbs.

Up to now, no research has been published on the subject of the optimal protocol of light requirements of violet (*Viola ignobilis* Rupr.) and also applying the biostimulants on growth and physiology of this important medicinal herb. Moreover, there are a few published studies that describe the interaction effects of light intensity and plant biostimulants. The purpose of the current study was to evaluate the probable effects of the biostimulants application and two light conditions on the growth, photosynthetic pigments, leaf gas exchange, and chlorophyll fluorescence parameters in *Viola ignobilis* Rupr.

MATERIALS AND METHODS

Experimental conditions

The seedlings of *Viola ignobilis* Rupr. were collected at the 4-leaf stage from the valley in Kaleybar County, Eastern Azerbaijan province (38° 51' 59.99" N., 47° 01' 60.00" E., 1144 m a.s.l) and confirmed by the Guilan Agriculture and Natural Resources Research Center. This study was carried out in Roudesar, a city in Guilan province in northern Iran (37° 08' 15.40" N, 50° 17' 16.80" E, 0 m a.s.l) from December 2021 to April 2022.

Treatments and experimental design

The experimental design was a split plot arrangement based on randomized complete blocks with three replicates. The main factor was two light levels included: 100% full sunlight and 50% full sunlight and sub-factors made of three bio-stimulants treatments: animal protein hydolysate (A.PH), vegetal protein hydrolysate (V.PH), seaweed extract (SE) and combination of A.PH + SE and V.PH + SE compared with foliar application of water as controlled treatment. Each treatment consisted of 4 pots with 3 replications. Amounting to a total of 12 experimental unit plots, each plot consisting of 48 plants in each treatment (576 plants in total). The four-leaf stage seedlings were transplanted in December 2021 to 3 L pots (density = 4 plants per pot). The final substrate was prepared from equal proportions of forest soil and leaf mold; pH: 7.35; electrical conductivity: 1.08 $dS \cdot m^{-1}$; organic matter (%): 10; total N (%): 3.1; available P ($mg.kg^{-1}$): 10; exchangeable K ($mg.kg^{-1}$): 145.2.

The protein hydrolysate treatments were started 3 weeks after cultivation (on January 15) and were applied weekly 12 times until the flowering stage on 15 April. The A.PH was obtained from enzymatic hydrolysis of fish under alkaline condition containing 75% free amino-acids. The V.PH was used in this experiment obtained through enzymatic hydrolysis of soybean seeds, containing 48% amino acids and soluble peptides. Foliar spray the above-quoted biostimulants were applied on the leaves of violet at the concentration of 0.2 $g L^{-1}$, in a solution with distilled water (Cristiano et al., 2018). Moreover, the third biostimulant used in this research was Acadian seaweed (Acadian Plant Health, Canada) extract which is made from the brown seaweed, *Ascophyllum nodosum* is contained amino acid 4.4%, mannitol 4%, alginic acid 10%, and other organic compounds 55%. The elemental composition of Acadian as follows: N 1.5%, K 17%, P 0.2%, sulphur 1%, Mg 0.3%, Ca 0.4%, Fe 150 ppm. The SE was applied directly to the soil (500 mL per pot) every two weeks from 3 weeks after cultivation at the concentration of 2 $g L^{-1}$. The relative dose of the SE was based on manufacturer recommendations. No fertilizer has been applied and cultivation practices were performed following standard methods.

Shade treatments

Shade treatments were imposed using green shading nets 50% above the wooden frames and fixed at a height of 3 m above the ground to provide a 50% reduction in light. Green agro shade nets with a standard size of 3 m width and 50 m length with 50% shade were used. This was made with high-density polyethylene plastics. Plants were randomly divided into two groups which were subjected to two different light intensities. The mean daily variation in full sunlight from January to April measured by using a HT620 Digital Lux Meter (Habotest, China). To control light condition, light intensity was measured three times a day at 10 am, 12 noon, and 2 pm, and at the end of each month.

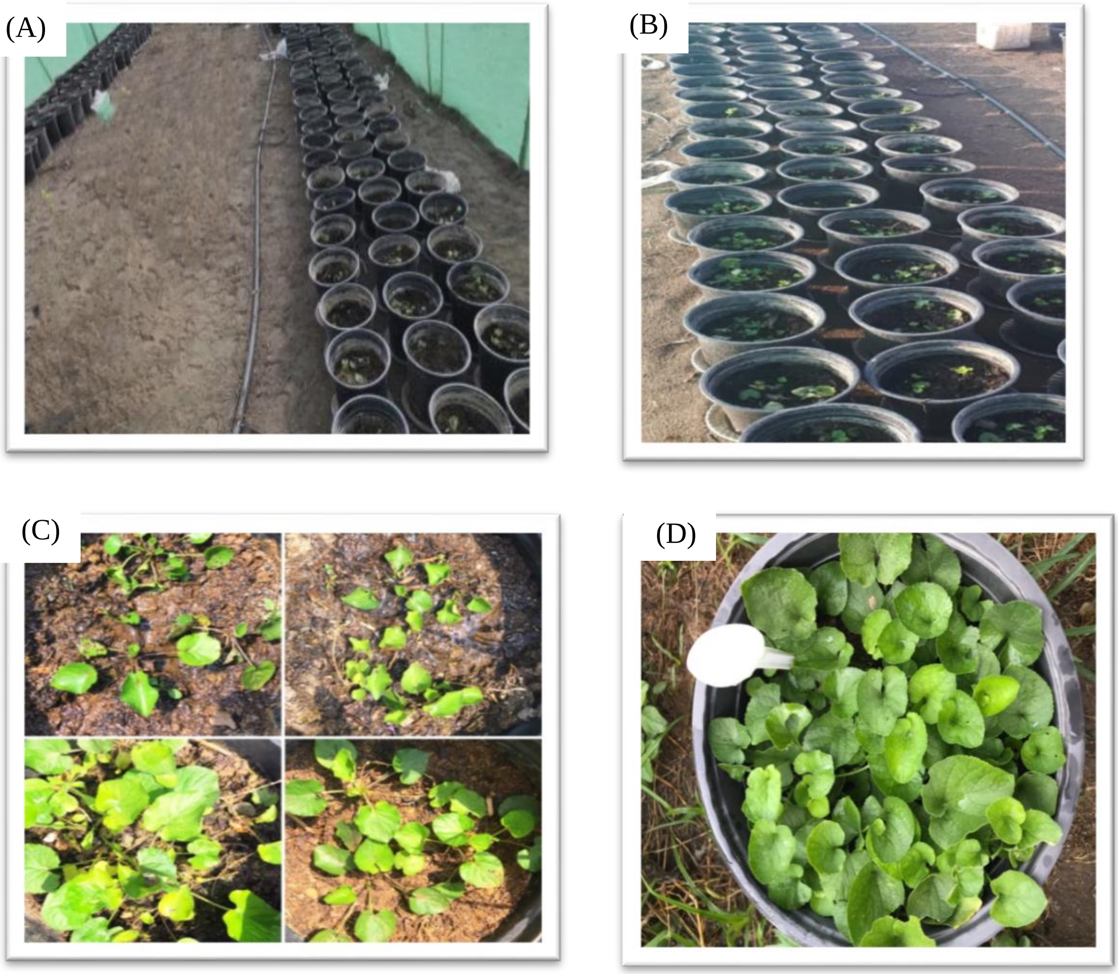

Fig. 1. (A): Shade treatment (50% natural light), (B): 100% light intensity, (C): Different stages of violet growth, (D): Plant growth after 8 weeks.

Morphological parameters

At the end of the flowering stage (121 days after cultivation), six plants were harvested and their morphological parameters were measured. Plants were removed from pots and the growing medium was gently washed from the roots to measure the fresh weight of the aerial part and the root, then, plant samples were dried in an oven at 70°C for 72 h to reach a constant weight for measuring dry weight.

Photosynthetic pigments

The evaluation of total carotenoids and chlorophylls was carried out according to the method reported by Porra (2002). To preparing methanolic extracts, 0.5 g of fresh leaf tissue extracted by grinding leaves in 80% acetone, the samples were kept in the dark at room temperature for 24 hours, and then the absorbance values of the solutions were measured by spectrophotometer at 663.2, 645.4 and 470 nm. The amount of chlorophyll and carotenoid was calculated based on the following formulas (1, 2) and the results were expressed in milligrams/gram FW.

$$(Chl\ a) = (12/25\ A\ 663)-(2/79\ A645),\ (Chl\ b) = (21/21\ A645)-(5/1\ A663),$$
$$(Chl\ T) = (Chl\ a) + (Chl\ b) \qquad (1)$$

$$Carotenoid = (1000A470)-(1/8\ Chl\ a)-(85/02\ Chl\ b)/198 \qquad (2)$$

Gas Exchange parameters

Photosynthetic parameters were investigated using a portable gas exchange fluorescence system (GFS-3000, Heinz Walz Effeltrich, Germany) to measure gas exchange parameters including assimilation rate (A µmol m^{-2} s^{-1}), transpiration rate (E mmol m^{-2} s^{-1}), and stomatal conductance (G H_2O mmol m^{-2} s^{-1}). The measurements were carried out on a fully expanded leaf from 10:00 am to 2:00 pm (Xu et al., 2020). The cuvette temperature, photosynthetic active radiation and CO_2 concentration were maintained at 28.4° C, 700 µmol m^{-2} s^{-1}, and 578.48 ppm respectively. At each conducted time point, five plants were randomly selected from each replication and analyzed for the mentioned parameters.

Chlorophyll Fluorescence

At the end of flowering stage, measurement of Chlorophyll Fluorescence parameters was carried out on a last fully expanded leaf with a fluorometer GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany). F_o (initial minimal fluorescence) and F_m (maximal fluorescence) were determined after a 30 min dark- adaptation period and the maximum quantum efficiency of PSII was calculated as $F_v/F_m = (F_m\ \ F_o)/F_m$ (Fig. 2A). The plants to be measured were placed in a dark room. Leaves were light-adapted for approximately 20 min before measurements of other parameters were calculated including, qP, F_v/F_m and ΦPSII (Genty et al., 1989). The Chlorophyll fluorescence data presented are means from at least 5 leaves per replication (Fig. 2B).

(A) (B)

Fig. 2. (A): fluorometer GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany), (B): measurement of Chlorophyll Fluorescence parameters of *Viola ignobilis* Rupr.

Table 1. Chlorophyll fluorescence parameters used in current study.

Parameter	Formula	Description
F_v/F_m	$(F_m - F_0)/F_m$	Maximum quantum yield of PSII photochemistry measured in the dark-adapted state
qP	$(F'_m - F_s)/(F'_m - F'_0)$	Photochemical quenching of PSII
Y(PSII)	$(F'_m - F_s)/F'_m$	Effective quantum yield of photochemical energy conversion in PSII

Ref: Shin et al. (2021).

Statistical analysis

Data Analysis was performed using the ANOVA procedure in SAS version 9.2 (SAS Ins., Cary, NC, USA). Differences between treatment means were separated by the least significant difference (LSD) at the 95% confidence level ($p < 0.05$). All graphs were drawn using Excel software.

RESULTS AND DISCUSSION

Morphological parameters

The experimental results of the collected data revealed that the simple effect of light intensity showed significant influence ($p < 0.01$) on shoot fresh and dry weight, but root biomass appeared to be unaffected by light intensity (Table 2). Also, application of biostimulants improved significantly all studied morphological traits compared to untreated plants in *Viola ignobilis* Rupr ($p < 0.01$). As can be seen from the Table 2, the interaction of light intensity and biostimulants application didn't reveal any significant effect on the morphological parameters.

Table 2. Variance analysis of the effect of light intensity (A), biostimulants (B) and their interaction (A × B) on morphological traits, and photosynthetic pigments of *Viola ignobilis* Rupr.

		Mean Squares							
S.o.V	df	Shoot FW	Root FW	Shoot DW	Root DW	Chla	Chl b	TChl	Car
R	2	1.6955^{ns}	32.668**	0.9466^{ns}	3.4721**	0.01798^{ns}	0.0018^{ns}	0.0114^{ns}	0.01074**
A	1	37.108**	15.8404^{ns}	0.9801**	2.6028^{ns}	0.07933^{ns}	0.0004^{ns}	0.0756^{ns}	0.01361^{ns}
R × A	2	10.662^{ns}	19.5784*	1.40605^{ns}	1.7230^{ns}	0.00911^{ns}	0.0010^{ns}	0.0870^{ns}	0.00067^{ns}
B	5	338.80**	276.125**	40.8545**	27.990**	0.66426**	0.016**	0.893**	0.11627**
A × B	5	1.5572^{ns}	0.18567^{ns}	0.01027^{ns}	0.0974^{ns}	0.00231^{ns}	0.0002^{ns}	0.0022^{ns}	0.00028^{ns}
Error	20	88.590	95.939333	18.331844	11.39904	1.7225222	0.05034	1.18033	0.035688
Total	35	1852.239	1597.8301	228.34162	164.8307	5.1889638	0.14195	5.93307	0.6549555
CV (%)		7.69	8.21	18.45	10.41	13.14	7.61	8.4	5.87

S.o.V: Source of variation, df: Degree of freedom, CV: Coefficient of variation. Chla: chlorophyll a, Chlb: chlorophyll b, TChl: Total Chlorophyll, Car: Carotenoid. Asterisks (*) represent the level of significance for each factors (A, B) and their interaction (A × B): NS: non-significant; * $p < 0.05$; ** $p < 0.01$.

The fresh and dry weight of violet shoot increased by 6.5% and 7% respectively in 100% light intensity compared with shaded plants. Furthermore, the biostimulant application strongly improved the fresh and dry weight compared to biostimulant-untreated plants. The obtained results exhibited that the highest shoot fresh weight (38.98g), and shoot dry weight (8.52g) were observed in A.PH + SE treatment, without any significant differences with V.PH + SE treatment, also, the untreated plants showed the lowest shoot fresh weight (18 g) and shoot dry weight (2.37g) respectively (Table 3).

As Table 3 shows, the highest root fresh weight (32.57 g) is connected to A.PH + SE, although, had no significant difference with V.PH + SE treatment and the minimum root fresh weight (13.86 g) was belonged to untreated plants. Moreover, the higher root dry weight (8.91 g) was related to A.PH + SE, but no significant differences were found between A.PH +SE and V.PH + SE. The minimum root dry weight (3.21 g) was recorded in untreated plants.

The findings of this experiment indicate that, the fresh and dry weight of shoot and root were increased in full light intensity, although, in terms of root biomass, no statically significant difference was found between two light conditions. In accordance with the present results, previous studies have demonstrated that the plant yield decreased under lower irradiance. Hirano et al. (2019) reported that the total plant mass in *Datura inoxia* and *D. stramonium* decreased under lower light intensity. Szymborska-Sandhu et al. (2020) recorded the highest number of shoots and biomass of *Melittis melissophyllum* L. in full sunlight.

Today, the use of biostimulants in sustainable agriculture is a profitable strategy for improving crop yield and quality (Rouphael & Colla, 2020), hence, many researchers has been focused on effectiveness of new products in order to improving crop production. In this experiment, the use of plant biostimulant enhanced strongly all of evaluated morphological parameters, not only in high light intensity, but also in shade condition compared to untreated plants. Regarding to obtained results, in this work, PHs revealed more strongly effects on morphological parameters of *Viola ignobilis* Rupr. Than SE. Several authors found similar results in agreement with our findings in respect to positive influences of PHs on morphological parameters in various plants. For instance, Carillo et al. (2019) found that the fresh yield of greenhouse spinach was significantly increased in PH-treated plants compared to control. They explained that the great amount of amino acids and small peptides in PH exhibited hormone-like activities on plant which were responsible for increase nutrient acquisition and improve growth. Jolayemi et al. (2023) proved that the protein-based biostimulants increased all agronomic and physiological parameters of sugar beet. In current work, the roots treated with biostimulants considerably improved in comparison with control. Several authors demonstrated that root formation regulated by hormone-like activities of PHs. In a study conducted by Kim et al. (2019) it was shown that PHs extremely increased root growth and development in basil, tomato and chrysanthemum. The current study revealed that the combination of SE and PHs exhibited additive and synergistic effects on enhance growth and physiological traits of *Viola ignobilis* Rupr.

Table 3. Means comparison for morphological traits of *Viola ignobilis* Rupr. in response to two light intensities and biostimulants application.

Treatments	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Light Intensity				
L1	32.43a	27.30a	5.35a	7.51a
L2	30.45b	25.98a	5.00b	6.97a
Biostimulants				
A.PH	31.90b	28.46b	4.31b	8.14ab
V.PH	31b	27.51bc	4.19b	7.81b
SE	30.32b	25.65c	3.37bc	6.53c
A.PH + SE	38.98a	32.57a	8.52a	8.91a
V.PH + SE	38.15a	31.79a	8.34a	8.71a
H_2O	18c	13.86d	2.37c	3.21d

L1 (100% light intensity), L2 (50% light intensity), A.PH (animal protein hydrolisate), V.PH (vegetal protein hydrolysate), seaweed extract (SE). Plants treated with H_2O served as a control. Different letters within each column indicate significant differences according to the least significant difference (LSD) ($p < 0.05$). Asterisks (*) represent the level of significance for factors (Light, Biostimulant) and their interaction (Light × Biostimulant). NS: non-significant; *: $p < 0.05$; **: $p < 0.01$.

Chlorophyll pigments and carotenoid

As shown in Table 2, the ANOVA analysis demonstrated that the light intensity and the interaction between light intensity and biostimulants had not significant effects on chlorophyll concentration. Furthermore, biostimulant application showed significant impact on all evaluated photosynthetic pigments ($p < 0.01$). As shown in Table 4, the highest concentration of chlorophyll a (2.49 $mg.g^{-1}$ FW) was achieved in A.PH + SE application, although, there was not considerable difference between all biostimulants treatments. Also, the lowest content of chlorophyll a (1.58 $mg.g^{-1}$ FW) was related to control plants. In terms of chlorophyll b, the higher concentration (0.71 $mg.g^{-1}$ FW) was related to treated plants with A.PH + SE, without any significant difference between all biostimulants except for, SE treatment. Moreover, the lowest (0.57 $mg.g^{-1}$ FW) occurred in untreated plants. The maximum amount of total chlorophyll (3.21 $mg.g^{-1}$ FW) was observed in V.PH + SE application, without any significant difference with A.PH + SE. Also, the lowest value (2.15 $mg.g^{-1}$ FW) was belonged to untreated plants (Table 4). Looking at Table 4, it is apparent that the highest carotenoid content (0.81 $mg.g^{-1}$ FW) obtained in plants treated with A.PH + SE treatment, without any significant difference with other treatment except for SE, while, untreated plants showed that, the lowest amount of carotenoid content (0.48 mg g^{-1} FW).

Chlorophyll content is a critical indicator that shows the adaptability of plants to environmental conditions (Liu et al., 2007), indeed plants grown under low light intensity increase pigment density in order to optimize light absorption efficiency (Khoshbakht et al., 2018). In accordance with the present results, previous studies have demonstrated that the highest concentration of chlorophyll a and chlorophyll b were obtained in low light irradiance. For instance, Duan et al. (2018) reported that shading significantly increased the contents of chlorophyll a, chlorophyll b and chlorophyll a+b in *Lespedeza Buergeri* seedlings. Furthermore, He et al. (2019) indicated that the contents of chlorophyll a, chlorophyll b and total chlorophyll of *Castanopsis kawakamii* seedlings were higher in low light intensity in non-gap environment.

In current study application of biostimulants had a positive influence on the chlorophyll contents of *Viola ignobilis* Rupr compared to control plants. However, combination of PHs and SE enhanced chlorophyll content more than when SE and PHs individually were used. These results are in agreement with Caruso et al. (2020) findings which showed both the protein hydrolysates and *Trichoderma* treatments alone or in combination, were led to increase in chlorophyll content in perennial wall rocket compared to the untreated plants. Munaro and et al. (2024) confirmed that chitosan nanoparticles and microalgae-based protein hydrolysate enhanced chlorophyll and carotenoid in tomato.

In this investigation, the concentration of carotenoid did not differ significantly between two light condition, although, it was higher in full sunlight. In addition to light intensity effects, the carotenoid content can be influenced by biostimulants. Similar to our observation, the positive correlations between carotenoids content and biostimulants application have been reported in several researches. In a research carried out by Rachidi et al. (2020) carotenoid content significantly enhanced in tomato treated by microalgae polysaccharides as a biostimulant. Also Aktsoglou et al. (2021) reported that the PHs is responsible for increasing the content of total carotenoids in spearmint plants.

Gas exchange parameters

Based on the results of analysis of variance (Table 5), the simple effect of light intensity and biostimulant application on the gas exchange parameters was significant ($p < 0.01$), but the interaction of these two factors didn't show any significant influence.

The value of assimilation rate of CO_2 in 100% light conditions was 10.95% higher compared to plants that grew in 50% light intensity (Fig. 3A). The obtained results showed that the highest value of assimilation rate (8.41 µmol m^{-2} s^{-1}) was observed in A.PH + SE treatment and the lowest value (3.36 µmol m^{-2} s^{-1}) related to untreated plant (Fig. 3B).

As it can be seen from Fig. 3C, the transpiration rate of plants under 100% light intensity was 13% higher than plants in 50% light condition. Based on the mean comparison results, the highest transpiration rate (3.75 mmol m^{-2} s^{-1}) was obtained in V.PH + SE treatment, without any significant difference with A.PH + SE application. The lowest rate of transpiration (2.46 mmol m^{-2} s^{-1}) was recorded in control plants (Fig. 3D).

According to the results (Fig. 3E), the stomatal conductance of violet increased by 16.95% at 100% light intensity compared to shade condition. The highest value of stomatal conductance (35.75 mmol m^{-2} s^{-1}) was observed in A.PH + SE treatment without any significant difference with V.PH + SE, furthermore the lowest value (24.82 mmol m^{-2} s^{-1}) was recorded in untreated plants (Fig. 3F).

Table 4. Means comparison for morphological traits of *Viola ignobilis* Rupr. in response to two light intensities and biostimulants application.

Treatments	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
Light Intensity				
L1	2.27a	0.65a	2.93a	0.73a
L2	2.18a	0.66a	2.84a	0.69a
Biostimulants				
A.PH	2.32a	0.65ab	2.97ab	0.77a
V.PH	2.38a	0.68ab	3a	0.77a
SE	2.18a	0.63b	2.8b	0.73b
A.PH + SE	2.41a	0.70a	3.11a	0.81a
V.PH + SE	2.49a	0.71a	3.21a	0.79a
H_2O	1.58b	0.57c	2.15c	0.48c

L1 (100% light intensity), L2 (50% light intensity), A.PH (animal protein hydrolisate), V.PH (vegetal protein hydrolysate), seaweed extract (SE). Plants treated with H_2O served as a control. Different letters within each column indicate significant differences according to the least significant difference (LSD) ($p < 0.05$). Asterisks (*) represent the level of significance for factors (Light, Biostimulant) and their interaction (Light × Biostimulant). NS: non-significant; *: $p < 0.05$; **: $p < 0.01$.

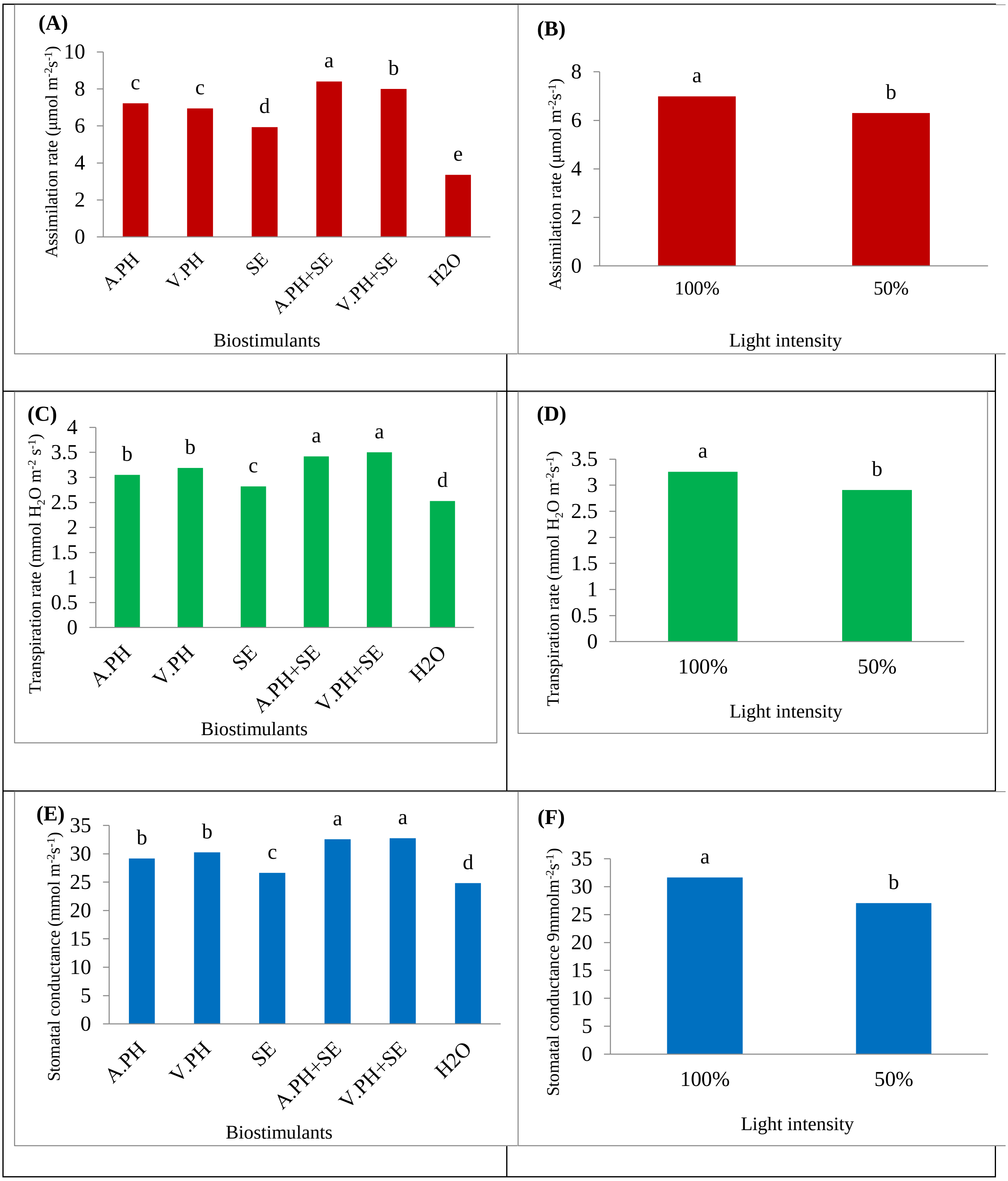

Fig. 3. Simple effect of the biostimulants on assimilation rate (A), transpiration rate (C) and stomatal conductance (E). Simple effect of light intensity on assimilation rate (B), transpiration rate (D), and the stomatal conductance (F). Different letters on bars indicate significant differences at ($p < 0.05$).

Table 5. Variance analysis of the effect of light intensity (A), bio-stimulants (B) and their interaction (A × B) on gas exchange parameters and chlorophyll fluorescence traits of *Viola ignobilis* Rupr.

Mean Squares							
S.o.V	df	Assimilation rate (A)	Transpiration rate (E)	Stomatal conductance (GH_2O)	qP	F_v/F_m	Yield (ΦPSII)
R	2	0.01757^{ns}	0.02235278ns	1.5606750ns	0.00026178ns	0.00059643ns	0.00019242ns
A	1	4.340277^{**}	1.11654444^{**}	189.2458778^{**}	0.16321600^{**}	0.04956560^{**}	0.04096576^{**}
R × A	2	0.010352^{ns}	0.00713611^{ns}	1.1311694^{ns}	0.00164233^{*}	0.00031228^{ns}	0.00089224^{ns}
B	5	20.04544^{**}	0.80371778^{**}	60.6758267^{**}	0.00914978^{**}	0.00589189^{**}	0.00700321^{**}
A × B	5	0.081864^{ns}	0.04514444^{ns}	0.2625711^{ns}	0.00055653^{ns}	0.00006401^{ns}	0.00021226^{ns}
Error	20	0.0601706	0.01039778	1.6061022	0.00046039	0.00068067	0.00085941
Total	35						
CV (%)		3.687751	3.299390	4.315018	2.515109	3.423534	3.953115

S.o.V: Source of variation, df: Degree of freedom, CV: Coefficient of variationAsterisks (*) represent the level of significance for each factors (A, B) and their interaction (A × B): NS: non-significant; * $p < 0.05$; ** $p < 0.01$.

The respiratory behavior of plants is different among species, in this experiment, all gas exchange parameters of *Viola ignobilis* Rupr. were higher in full sunlight. Importance of stomatal conductance correlated to water and CO_2 exchange between leaves and the atmosphere, which caused an increase in photosynthesis. Overall, plants grown under high light intensity are distinguished by the greatest stomatal conductance than plants grown at low light condition (Warren et al., 2007).

According to finding of Idris et al. (2019) the assimilation rate in some species of Malaysian plants in high light intensity was higher than shaded. Moreover, Proietti et al. (2023) demonstrated that an assimilation rate was three times higher in spinach leaves exposed in high light intensity compared to those at low light intensity. Therefore, these findings indicated that the increasing CO_2 gain improved photosynthetic efficiency and growth in plants.

In addition to light as a main factor in photosynthesis process, the findings indicate that there are a positive relationship between biostimulants application and photosynthetic behavior of plants. Colla (2015) declared that PHs promote the photosynthetic rate and energy supply for metabolic process due to the raise of N assimilation and amino acid biosynthesis in plant cells. Cristiano et al. (2018) indicated that the animal PH use in snapdragon had a positive effect on the photosynthetic parameters related to the leaf gas exchange. As a result of this experiment, the leaf net photosynthesis (+52%), transpiration rate (+55%), and stomatal conductance (+0.8%) significantly increased compared to control plants.

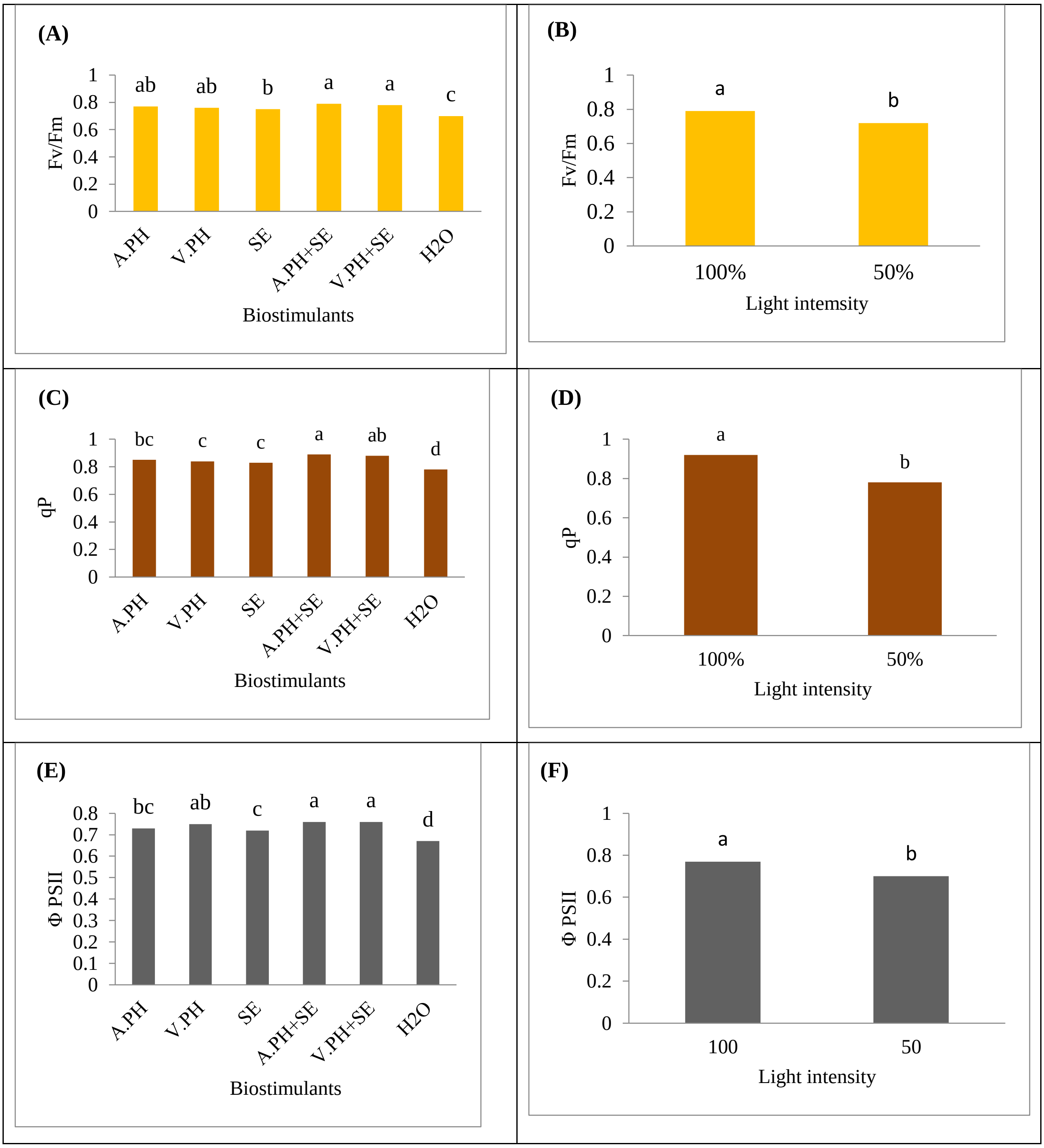

Fig. 4. Simple effect of the biostimulants on the value of F_v/F_m (A), the qP value (C), and the ΦPSII (E). Simple effect of light intensity on the value of F_v/F_m (B), the qP value (D), and the ΦPSII (F). Different letters on bars indicate significant differences at ($p < 0.05$).

Chlorophyll Fluorescence parameters

As shown in Table 5, the simple effect of light intensity and biostimulant application on the Chlorophyll Fluorescence parameters was significant ($p < 0.01$), although did not significantly respond to their interaction.

The value of F_v/F_m increased by 11.26% when the plant was grown in 100% light intensity rather than shaded plants (Fig. 4A). Furthermore, treated plants with biostimulants exhibited significant effect on F_v/F_m value ($p < 0.01$). The highest value of F_v/F_m (0.838) was detected in plants treated with A.PH + SE, on the other hand the lowest value (0.689) was found in untreated plants. The interaction between light intensity and biostimulant didn't show any significant effect on F_v/F_m value (Fig. 4B).

The qP value increased by 17.94 % at 100% light intensity compared to the plants grown at 50% light intensity (Fig. 4C). The highest qP value (0.89) and lowest (0.78) were recorded respectively in the application of A.PH + SE treatment, and untreated plants (Fig. 4D).

The ΦPSII (quantum yield of photosynthetic electron transport) was 10% greater in plants grown at 100% light intensity (Fig. 4E). The highest value (0.76) of ΦPSII was recorded in plants treated with A.PH+SE and V.PH+SE treatments and minimum value (0.63) was related to control plants (Fig. 4F).

In recent years, chlorophyll fluorescence measurements have been known as useful and non-invasive tools to measures the quantum yield of photosystem II under different light conditions. In plants, PSII is an important component of photosynthesis. Maximum photochemical efficiency of PSII is determined by F_v/F_m ratio. Environmental condition like plant stresses influence on PSII and lead to remarkable decrease in the F_v/F_m ratio (Niari khamsi & Najaphy, 2012). Maximum photochemical efficiency of PSII (F_v / F_m), has been widely used for such researches in various species under different situation (Genty et al., 1989). In this study the higher F_v/F_m ratio was observed for treated *viola ignobilis* Rupr. were grown under full sunlight. All kinds of biostimulants caused to remarkable increase efficiency of photosystem II compared with untreated plant in both evaluated light intensities.

These results indicated that optimum light intensity improves the efficiency of PSII by increasing the energy transport from PSII to PSI. The qP value shows the amount of energy consume by photochemical reactions to the energy absorbed by antenna pigments in PSII and is correlated to CO_2 assimilation. High qP value is advantageous for electron transport and PSII yield (Guo et al., 2006).

Furthermore, the application of biostimulants separately and in combination with together enhanced qP value compared with untreated plants in both light intensities. Di mola et al. (2021) revealed that the application of PH effectively mitigated the impacts of salinity with regard to maintenance of higher F_v/F_m, and ΦPSII at salinity level, as a result of this, improve the photosynthetic productivity. A study set out by Asadi et al. (2022) to assess the effects of *Arbuscular Mycorrhizal* fungus and SE foliar application on growth and physiological traits of *Lactuca sativa* L. in this experiment the combination of AMF and SE enhanced photochemical efficiency of PSII (F_v/F_m). Overall, these results in terms of the maximum values of F_v/F_m, qP and ΦPSII indicated that full sunlight is necessary for normal growth of *Viola ignobilis* Rupr.

CONCLUSION

In current study, a sustainable approach was used in order to sustain medicinal plants productivity without the use of chemical fertilizer. The finding indicated that the plants were grown in full sunlight revealed maximum photosynthetic efficiency and yield. Moreover, the application of all kind of biostimulant, separately and in combination with each other resulted in an improvement in morphological and photosynthetic traits in both of light conditions. Overall, combined PHs and SE provided additive and synergistic effects on growth and development of *Viola ignobilis* Rupr. Future studies on the current topic are therefore recommended on other valuable medicinal plants.

Conflict of interest
The authors declare that there is no conflict of interest.

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