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Original Article

Effect of foliar application of bio-stimulants on growth, yield and nutritional quality of broccoli

Efeito da aplicação foliar de bioestimulantes no crescimento, produtividade e qualidade nutricional de brócolis

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Abstract

Broccoli is one of important cole crop grown all over the world due to its unique nutritional profile consumed fresh as well as processed. It contains a wide range of nutrients, vitamins, minerals and specific anti-cancer compounds such as glucosinolates. Broccoli cultivation in Pakistan is increasing rapidly, however, till now there is no standardized cropping technology for broccoli cultivation under local climate. Considering research gap (lack of suitable varieties, poor growth, and unavailability of optimized crop technology), trial was conducted at Vegetable research area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad to evaluate the impact of bio-stimulants on different broccoli cultivars under local climatic conditions. The set of experimental treatments was laid out in Randomized Complete Block Design (RCBD) with three replications. Pre-harvest application of Isabion and Seaweed extract significantly enhanced the plant height (11%), dry weight (4%), leaf area (7%), and yield plant^{-1} (5%). Moreover, Isabion and seaweed extract application led to the increase in antioxidant enzymes i.e., superoxide dismutase (18%), peroxidase (38%) and catalase (12%). In crux, the foliar application of bio-stimulants (Isabion and seaweed extract) on broccoli enhanced the growth, yield, and contents of antioxidant enzymes.

Keywords: amino acid, antioxidant, broccoli, cultivars, seaweed extract.

Resumo

O brócolis é uma das importantes culturas de repolho cultivadas em todo o mundo devido ao seu perfil nutricional único consumido fresco e processado. Contém uma ampla gama de nutrientes, vitaminas, minerais e compostos anticancerígenos específicos, como glucosinolatos. O cultivo de brócolis no Paquistão está aumentando rapidamente, no entanto, até agora não há tecnologia de cultivo padronizada para o seu cultivo sob o clima local. Considerando a lacuna de pesquisa, como a falta de variedades adequadas, o baixo crescimento e a indisponibilidade de tecnologia de cultivo otimizada, o teste foi realizado na área de pesquisa de vegetais, no Instituto de Ciências Hortícolas na Universidade de Agricultura, em Faisalabad, Paquistão, para avaliar o impacto de bioestimulantes em diferentes cultivares de brócolis nas condições climáticas locais. O conjunto de tratamentos experimentais foi disposto em Randomized Complete Block Design (RCBD) com três repetições. A aplicação pré-colheita de Isabion e extrato de algas marinhas aumentou significativamente a altura da planta (11%), peso seco (4%), área foliar (7%) e rendimento planta (5%). Além disso, a aplicação de Isabion e extrato de algas marinhas levou ao aumento das enzimas antioxidantes, ou seja, superóxido dismutase (18%), peroxidase (38%) e catalase (12%). No crux, a aplicação foliar de bioestimulantes (Isabion e extrato de algas marinhas) melhorou o crescimento, rendimento e conteúdo de enzimas antioxidantes do vegetal

Palavras-chave: aminoácido, antioxidante, brócolis, cultivares, extrato de algas marinhas.

1. Introduction

Broccoli (*Brassica olerica* L.) belongs to cruciferous family, a plant of genus Brassica, with 18 chromosomes (Dixon, 2007). It is famous for its good taste, flavor and maximum nutritional and curative or palliative value. It contains 89.1% water, 2500 IU of vitamin A and 113 mg of vitamin C/100g (Dhaliwal, 2012). High contents of active phytochemicals (glucosinolates and sulphoraphane) in broccoli proved it as vegetable having anti-cancer attributes (Guo et al., 2001; Zhao et al., 2007). In the recent decade, the consumption and interest of broccoli increased due to its nutritive value (Martínez-Sánchez et al., 2008). The busy lifestyle abridged the time devoted to cook

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food, so the use of slightly processed leafy vegetables marketed as ready-to-eat salads has been augmented (Martínez-Sánchez et al., 2012). In the world market, about 40% of this vegetable is sold as fresh, while the remaining 60% is sold as frozen food (Stoessel et al., 2012). However, lack of promising varieties and production technology, retarded crop growth, poor plant population, hampered yield and low quality are major bottlenecks in successful production of broccoli.

Bio stimulants are substances applied to agricultural and horticultural crops to enhance yield, mend quality, augment plant tolerance to biotic or a-biotic stress, and increase nutritional efficacy (Du Jardin, 2015). The use of bio stimulants in agriculture has known as suitable alternative of synthetic fertilizer to increase the yield and dietary value of food crops. Bio stimulants are known as good healthy compounds that promote healthy impacts on plants (Haider et al., 2012). Majorly, these bio stimulants reduce the synthetic fertilizer usage, thus positively influencing vegetative and reproductive growth of plant (Nardi et al., 2009). The application of bio stimulants in vegetables induces earliness; enhances fruit set and fruit size (Yakhin et al., 2017). Bio stimulants affects plant processes by influencing physiological (Vaccaro et al., 2009) and biochemical mechanism (Ertani et al., 2013). Bio stimulants enhance nitrogen assimilation through onset and transcription of N metabolism (Ertani et al., 2009). Furthermore, initiation of the metabolic systems associated with the production of phenyl propanoids in plants treated with bio stimulants thus entails that how these molecules react to modulate the stress situations (Schiavon et al., 2010).

Among bio stimulants, seaweed and amino acid extract have been used throughout the world for promoting plant growth and yield (Herrera et al., 2014). Seaweed based bio stimulants improve drought tolerance water use efficiency, and nutrient utilization in plants (Arioli et al., 2015). Seaweeds constitute many important compounds like amino acid, proteins, lipids, phenol, fibre, polysaccharides and pigments which are valuable for health benefits and agriculture utilization (Mišurcová et al., 2011; Pérez and Rojas, 2016). Seaweed extracts application in agricultural practices improve germination of seed, flowering, leaf quality and functioning, root development and architecture, improves defense mechanism against pest and pathogens (Zhang et al., 2002), fulfills deficiency of nutrients (Arioli et al., 2015). Amino acids are obtained by enzymatic protein hydrolysis from agro based industrial by-products, both from animal waste e.g. epithelial tissues, collagen, and plants e.g. crop residues (Du Jardin, 2015). These bio-stimulants contain macro and microelements as well as fats (Parrado et al., 2008). Protein hydrolysates act as stimulus for nitrogen and carbon metabolism (Schiavon et al., 2008). Amino acids not only augment plant defense mechanism but also the ability to tolerate stresses e.g. salinity, temperature and oxidative stress (Ertani et al., 2013).

In Pakistan, broccoli was introduced as salad crop however it could not make a significant mark in its cultivation (Zubair et al., 2017). At present, there is a little or information on the effect of foliar application of seaweed extract and amino acids on growth, yield and quality of broccoli. In this regard, the effects of pre-harvest foliar spraying of seaweed extracts and amino acids on five promising broccoli varieties were investigated under local climate conditions. Therefore, the aim of this study was to evaluate the crop growth, yield and activity of antioxidant enzymes of promising broccoli varieties under foliar application of bio-stimulants.

2. Material and Methods

2.1. Experimental material

Seeds of the five cultivars vis., Marathon, Barostar, Waltham-29, Green Pia and Premium were acquired from the local market of Faisalabad. Two commercial bio-stimulants "Wokozim" (active ingredient seaweed extract) and "Isabion" (a source of amino acid) were purchased from "Jaffar Brothers limited" and "Syngenta Pakistan", respectively. Three types of fertilizers viz. Urea, Diammonium phosphate (DAP) and Muriate of Potash (MOP) acquired from Institute of Horticultural Sciences, University of Agriculture Faisalabad.

2.2. Experimental site and land preparation

This field-based research trial was conducted at Vegetable Research Area, Institute of Horticultural Sciences, University of Agriculture Faisalabad, during 2018-19. The experimental site was ploughed with the help of cultivator, followed by rotavator application to finely pulverize the soil. After ploughing and planking a tractor driven ridger was used to prepare ridges having dimensions (36" × 36"). Recommended dose of fertilizer (by Agriculture Department of Punjab) was broadcasted at experimental site uniformly. Urea, DAP and MOP were broadcasted at the rate of 50 kg, 80 kg and 15 kg acre^{-1} respectively to meet the nitrogen, phosphorous and potash requirement.

2.3. Design and treatments

The experiment was conducted in randomized complete block design (RCBD) having three replications. Five promising varieties i.e., Baroster = V_1, Green Pia = V_2, Marathon = V_3, Premium = V_4, Waltham-29 =V_5, and nine treatments of bio-stimulants viz., T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1} were used in the experiment.

2.4. Field management

The 30 days old broccoli seedlings of five different cultivars were transplanted on top of ridge while maintaining 1.5 feet plant to plant distance. After transplanting, seedlings were observed on regular basis and dead or injured seedlings were replaced with healthy ones from the nursery. Ten treatments of bio-stimulants were evaluated for their efficacy to stimulate growth, yield and nutritional quality. In the control treatment of bio-stimulant plants were not treated with biostimulants.

While other nine treatments of bio-stimulants were applied through foliar application on five broccoli cultivars using hand held sprayer. Application of foliar sprays was repeated at fortnightly starting from 30 days after transplanting. The constant numbers of sprays per plant were applied with 20 ml solution per plant.

2.5. Observations and measurements

2.5.1. Growth and development traits

Plant height of randomly selected three plants was measured from the bottom horizontal to the top end of the main pole with a measuring tape. Numbers of leaves $plant^{-1}$ of each treatment were counted manually. Leaves were collected from each treatment of each replicate to measure leaf area using a digital leaf area meter in cm^2. Broccoli plants were uprooted and excised into roots and shoots with the help of sterilized scissor and then their shoot fresh weight was measured with the help of electronic digital weighing balance (3000g/0.1g Electronic Balance, made in China) (Arshadullah and Zaidi, 2007). Stem diameter of randomly selected three plants from each treatment was recorded at final harvest by the use of digital Vernier clipper at stem base. Previously, shoots taken from each individual plant were placed in separate paper bag and kept in air dry oven for 72 hours at 70 °C then shoot dry weight was measured using electronic digital weighing balance. The fresh and dry weight was expressed in grams (g). Broccoli heads from each harvested plant were kept separately, followed by weighing on electronic digital weighing balance. The shoots arising from leaf axils after harvesting of main curd develop into secondary curds. The numbers of these secondary curds were recorded. The secondary curds from three randomly selected plants of each treatment were harvested and weighed on electronic digital weighing balance and their means were calculated. Broccoli heads from each harvested plant were kept separately, followed by weighing on electronic weighing balance to get the broccoli yield and expressed in grams.

2.5.2. Biochemical traits

At commercial maturity stage fresh sample of broccoli were harvested from each treatment. Ice box was used to transport samples from field to lab. To measure total soluble solids (TSS) juice of fresh samples was acquired by using juice extractor (Philips HR 1860, Amsterdam, Netherlands). One drop of extracted juice from each sample was placed on absolutely dry refractometer (N-50E handheld refractometer, Atago Co. Ltd., Tokyo, Japan) prism and readings were recorded and expressed as °Brix as explained by Esturk et al. (2014). Total soluble sugars of fruit juice were determined by following method of Yemm and Willis (1954). Juice of one gram fresh sample was extracted in 3 ml distilled water and centrifuged for 15 minutes at room temperature.

Then, anther solution (0.2 g in 100 mL of H_2SO_4), was added in 1 mL of extract, cooled for 5 minutes (in ice) and mixed thoroughly. Each sample was observed at 620 nm and the sugar concentration was expressed in % according to glucose calibration curve as followed by Cocetta et al. (2015). Superoxide dismutase (SOD) activity was assayed after following the protocol of Štajner and Popović (2009) by measuring 50% blocking of photochemical reduction of NBT. Aliquots of plant extracts (100 µL) were mixed with 500 µL of phosphate buffer (pH 5), 200 µL of methionine, 100 µL of riboflavin and 800 µL of distilled water and placed under UV light for 15 minutes, followed by analysis at 560 nm absorbance by using UV-Vis spectrophotometer (U2020, IREMCO). One unit of SOD activity was expressed on the basis of protein contents as U mg^{-1} protein. The catalase activity was detected by mixing 100 µL H_2O_2 (5.9 mM concentration) in 100 uL of plant extract as explained by Bartosz (1995). UV-Vis spectrophotometer (U2020, IREMCO) was used to take the reading at absorbance of 240nm. Peroxidase activity was determined by following method explained by Chance and Maehly (1955). 100 uL of plant extract was taken and mixed with reaction mixture that involves 800 µL of phosphate buffer (50 mM, pH 5), 100 µL of H_2O_2 (40 mM) and 100 µL of 20 mM guaiacol. Readings were taken at absorbance of 470 nm by using UV-Vis spectrophotometer (U2020, IREMCO).

2.6. Statistical analysis

This research trial was laid out following factorial arrangement under RCBD with three replications having three plants each (Steel et al., 1997). The data was subjected to Tuckey's test for estimation of significance between treatments. Values of $p \leq 0.05$ were taken as significant. Sigma plot 12.3 was used for developing graphs.

3. Results

The application of bio stimulants in terms of Isabion and seaweed extract depicted significant impact on plant height and number of leaves of broccoli (Figure 1 and 2). The highest plant height (48 cm) was observed in T3 (6 ml L^{-1} Isabion application) that is also statistically at par with T9 (combination of 6 ml L^{-1} Isabion + 6 ml L^{-1} seaweed extract). Contrarily, the lowest plant height was observed in control treatment (T0) that was 35 cm. Amongst cultivars; Barostar gave the highest plant height (46 cm) while the lowest plant height (34 cm) was recorded for Waltham-29. Similarly, foliar application of 6 ml L^{-1} Isabion resulted in maximum number of leaves (19) that was statistically at par when combination of 6 ml L^{-1} Isabion and 6 ml L^{-1} seaweed extract (T9) was applied (Figure 2). In case of cultivars; Green pia produced maximum number of leaves (17) that was statistically at par with Baroster while, Premium gave the minimum maximum number of leaves (14). Application of 6 ml L^{-1} Isabion alone or when applied in combination with seaweed extract (T8 and & T9) increased the stem diameter to maximum value (24 mm). While, reduced dose of Isabion (2 & 4 ml L^{-1}) or seaweed extract (2 & 4 ml L^{-1}) did not increased the stem diameter significantly (Figure 3). In case of cultivars; on an average maximum stem diameter (22 mm) was recorded for Marathon and Green Pia followed by Premium (20 mm) cultivar. Whereas, the lowest stem diameter (19 mm) was recorded for cultivar Barostar.

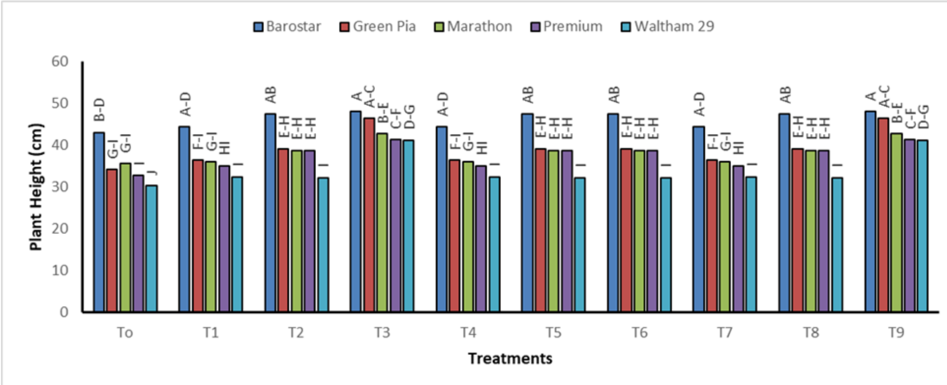

Figure 1. Impact of bio-stimulants foliar application on plant height (cm) of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}; Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

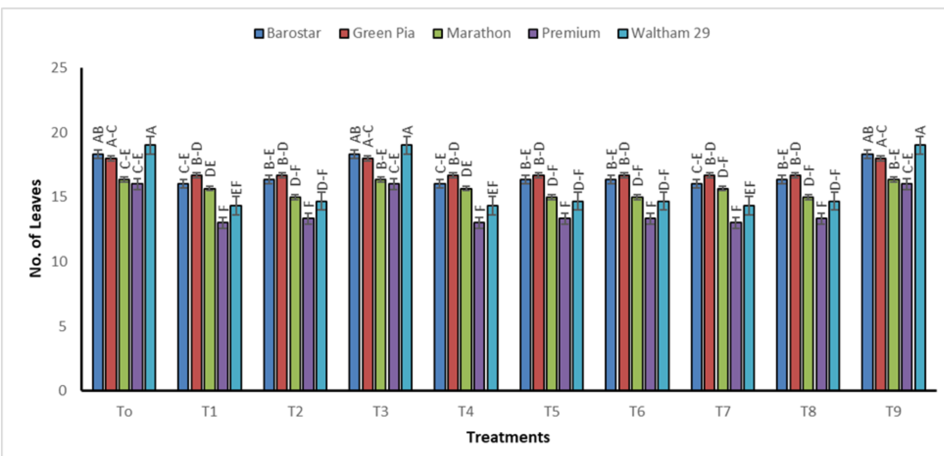

Figure 2. Impact of bio-stimulants foliar application on numbers of leaves of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}; Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

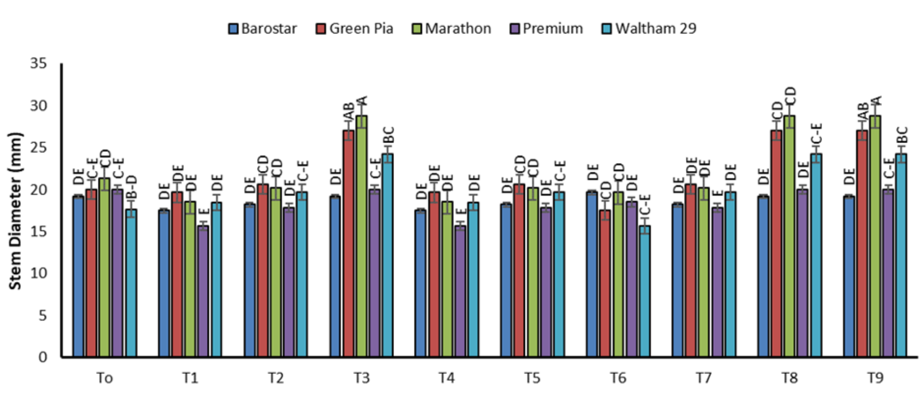

Figure 3. Impact of bio-stimulants foliar application on stem diameter (mm) of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}; Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

On an average, the maximum value for leaf area (509 cm^2) was recorded when 4 ml Isabion (T2) and 4 ml L^{-1} Seaweed extract (T5) alone or in combination (T8) applied exogenously. Whereas, the control treatment gave the minimum leaf area (461 cm^2) as presented in Figure 4. In case of cultivars, maximum leaf area was recorded for Marathon (607 cm^2) followed by Barostar (559 cm^2), Waltham-29 (465 cm^2), Green Pia (431 cm^2), and Premium (394 cm^2).

Date presented in Figure 5 and Figure 6 showed that bio-stimulant application in the form of Isabion (T3) or Seaweed extract (T5) alone or in combination gave maximum shoot fresh weight (193 g) and dry weight (27 g) as compared to control treatment (182 g fresh weight and 25 g dry weight). Regarding cultivars: the highest shoot fresh weight (213 g) dry weight (29 g) was attained in Waltham-29 followed by cultivar Premium. Whereas, lowest fresh and dry weight was recorded for cultivar Green pia (Figure 5 and 6). It was observed that application of bio-stimulant did not influence the number of secondary curds (Figure 7). However, Waltham-29 cultivar produced significantly higher number of secondary curds (9) followed by Marathon (6) and Green Pia (5). While minimum number of secondary curds (4) were recorded in Barostar and Premium cultivars. It was observed that exogenous application of bio-stimulant increased the broccoli yield significantly as represented in Figure 8. Among the bio-stimulant treatments, 4 ml Isabion or 4 ml L^{-1} Seaweed extract either in alone or in combination exhibited the highest yield potential (569 g) as compared to control treatment (529 g). In case of cultivars, the highest yield (751 g) was observed in broccoli cultivar Waltham-29 followed by Marathon (645 g), and Green Pia (509 g). While minimum broccoli yields (437 g) was recorded for Baroster cultivar (Figure 8).

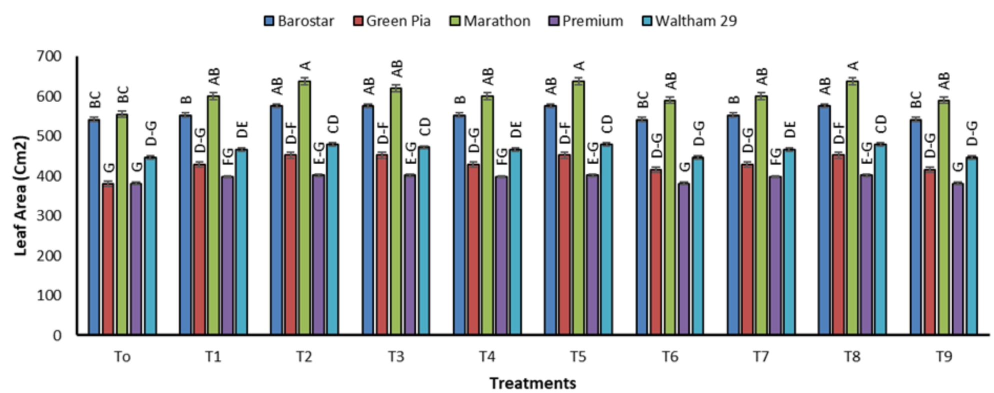

Figure 4. Impact of bio-stimulants foliar application on leaf area (cm^2) of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}; Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

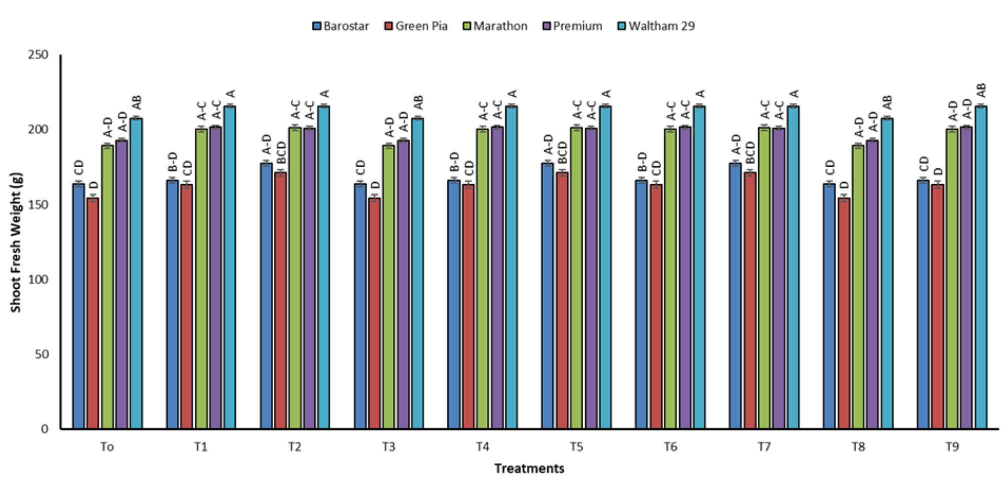

Figure 5. Impact of bio-stimulants foliar application on shoot fresh weight (g) of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}; Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

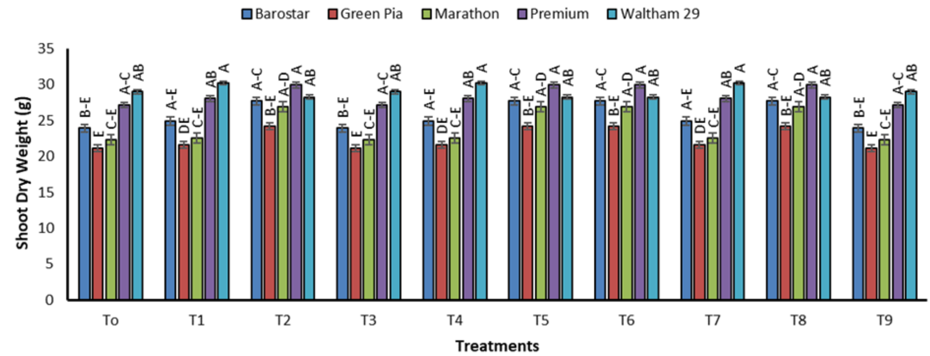

Figure 6. Impact of bio-stimulants foliar application on shoot dry weight (g) of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}. Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

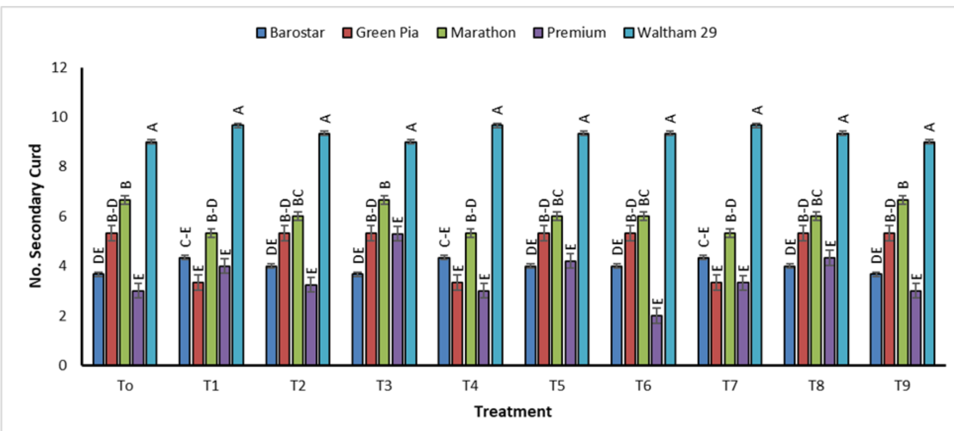

Figure 7. Impact of bio-stimulants foliar application on number of secondary curds of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}; Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

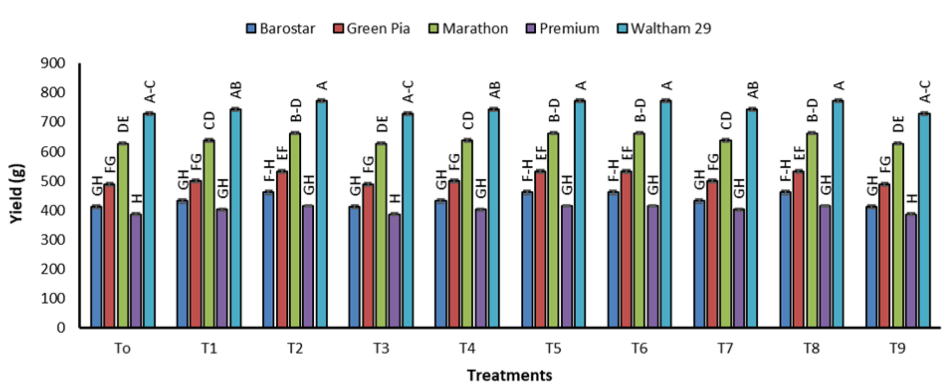

Figure 8. Impact of bio-stimulants foliar application on yield potential (g) of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}; Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

Data presented in Figure 9 sowed that total soluble solids (TSS) were not influence by bio-stimulant application or across the cultivar. However, maximum TSS (7 °Brix) was recorded when 4 ml L^{-1} Seaweed extract was exogenously applied on cultivar Barostar. While, least TSS (4.7 °Brix) value was recorded when Seaweed extract was applied on Green Pia cultivar. Among bio-stimulant treatments, 4 ml Seaweed extract and 4 ml L^{-1} Isabion application exhibited the increased TSS contents (Figure 9). Statistical analysis revealed that total soluble sugars did not influence significantly across the cultivars or bio-stimulant treatments. However, on an average the highest total sugar (86%) was recorded in non-treated Barostar plants while, minimum total sugar contents (63%) was observed in cultivar Marathon when it was treated with 6 ml L^{-1} Isabion (Figure 10). Data revealed that application of 2 ml L^{-1} Seaweed extract increased the SOD activity and gave the maximum value of SOD activity (868 U mg^{-1} Protein). Increasing rate of application of bio-stimulant decreased the SOD activity and least SOD activity was recorded when 4 ml L^{-1} Isabion in combination with 4 ml Seaweed extract was applied (Figure 11). In case of cultivars, the highest SOD activity (742 U mg^{-1}) was noted in Green Pia cultivar. Whereas, the lowest SOD activity (710 U mg^{-1}) was noted in Barostar cultivar. The highest protein contents values (0.27) were recorded under the exogenous application of bio-stimulant in treatment T5 (2 ml L^{-1} Seaweed extract), T7 (2 ml L^{-1} Seaweed extract in combination with 2 ml L^{-1} Isabion) and T8 (4 ml L^{-1} Seaweed extract in combination with 4 ml L^{-1} Isabion). Regarding cultivars maximum protein contents were noted for Barostar cultivar (0.27), while minimum protein contents (0.24) were recorded in Premium cultivar (Figure 12). Data regarding CAT activity depicts that the highest CAT activity (4.71 nmol min^{-1} g^{-1} proteins and 4.46 nmol min^{-1} g^{-1} proteins) were recorded when 2 ml L^{-1} and 4 ml L^{-1} Isabion was applied on Waltham-29 and Green pia cultivars, respectively (Figure 13). While, minimum CAT activity was recorded in control treatment as compared to treatments where bio-stimulant were exogenous applied. Data regarding POD activity revealed that application of 6 ml L^{-1} Isabion (T3) and application of 6 ml L^{-1} Isabion in combination with 6 ml L^{-1} Seaweed extract (T9) enhanced the POD activity to maximum 38 µmol min^{-1} g^{-1} protein and 30 µmol min^{-1} g^{-1}, respectively (Figure 14). However, minimum POD activity was recoded for premium cultivar (26 µmol min^{-1} g^{-1}) when reduced dose of Isabion (2 ml L^{-1} Isabion) was used (23.7 µmol min^{-1} g^{-1}).

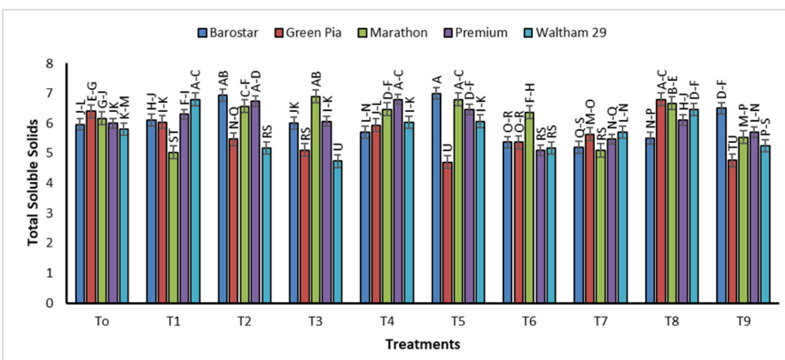

Figure 9. Impact of bio-stimulants foliar application on total soluble solids (cm) of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}; Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

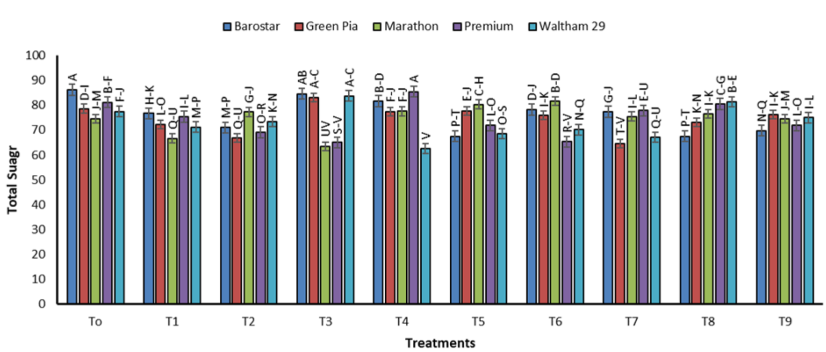

Figure 10. Impact of bio-stimulants foliar application on total soluble sugars of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}; Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

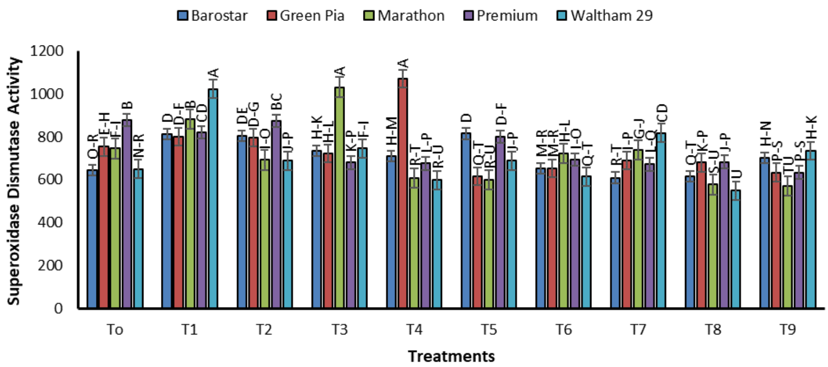

Figure 11. Impact of bio-stimulants foliar application on superoxide dismutase of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}. Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

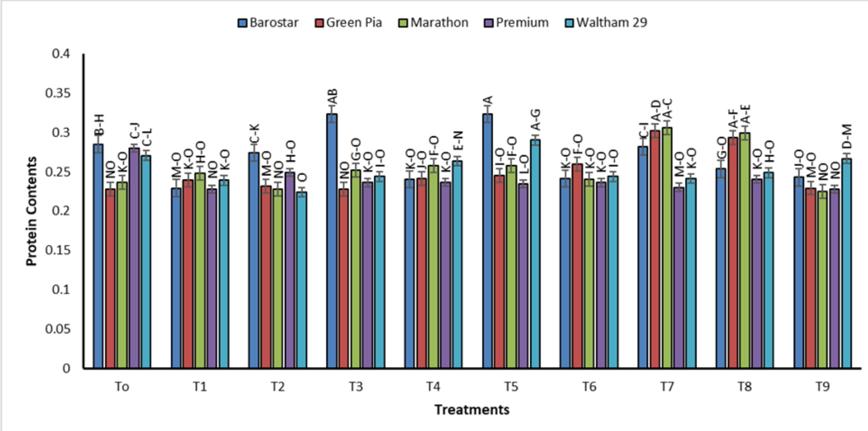

Figure 12. Impact of bio-stimulants foliar application on protein contents of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}; Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

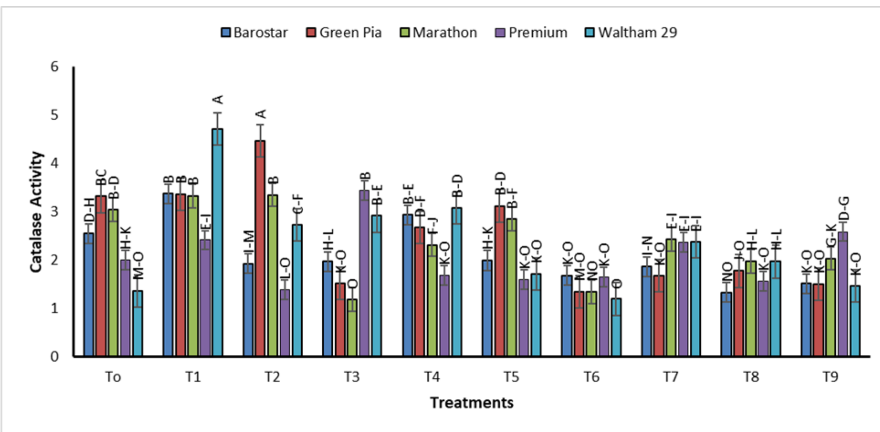

Figure 13. Impact of bio-stimulants foliar application on catalase activity of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}; Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

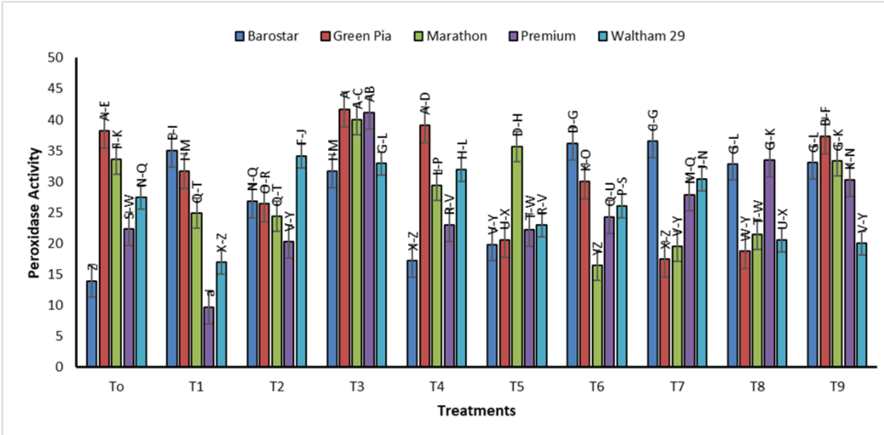

Figure 14. Impact of bio-stimulants foliar application on peroxidase activity of broccoli cultivars. T_o = control; T_1 = Izabion 2 ml L^{-1}; T_2 = Izabion 4 ml L^{-1}; T_3 = Izabion 6 ml L^{-1}; T_4 = Seaweed Extract 2 ml L^{-1}; T_5 = Seaweed Extract 4 ml L^{-1}; T_6 = Seaweed Extract 6 ml L^{-1}; T_7 = Izabion 2 ml L^{-1}+ Seaweed Extract 2 ml L^{-1}; T_8 = Izabion ml L^{-1}+ Seaweed Extract ml L^{-1}; T_9 = Izabion 6 ml L^{-1}+ Seaweed Extract 6 ml L^{-1}; Values sharing different lettering for a parameter are different significantly ($p \leq 0.05$).

4. Discussion

The growth, yield and quality of broccoli were considerably improved by application of bio-stimulants (Figure 1-14). Exogenous application of bio-stimulant limits the negative effect of environmental stress on crop especially leafy vegetables. The research study of Toscano et al. (2021) concluded that application of natural bio-stimulants acquired from plants resulted in improving crop yield and quality. Similarly, study of Kałużewicz et al. (2018) concluded that the nutritional quality of vegetables increased significantly when treated with bio stimulants. The growth and development of broccoli depends on cell growth and cell cycle progression (Sablowski and Dornelas, 2014). Exogenous application of bio-stimulant in the form of Seaweed extract and Isabion contain compounds such as hormones and plant growth regulators, which are known to promote cell growth, basal metabolism, and gene expression regulating nutrient uptake (Du Jardin, 2015). Hence, for enhanced growth rate under normal growing conditions, an adequate supply of free amino acid (Isabion) is essential (Zheng et al., 2016). Application of bio-stimulant augmented the root and shoot growth that could be attributed to availability of phosphorus and potassium in seaweed extract. Phosphorus and potassium in seaweed extract facilitated the root growth that enhanced the root shoot growth ratio along with positive effects on enhancing photosynthesis and meristematic growth in treated plants (Herrera et al., 2014). Beside these macronutrients seaweed extract also contains natural growth hormones (auxins and cytokinins).

These growth hormones resulted in enhanced growth of plant via stimulating enlargement and division of cells, which accordingly increase plant height (Marhoon and Abbas, 2015). Results of this study are also supported by the study of Awad et al. (2007) which concluded that foliar application of Isabion enhanced plant growth, yield and other attributes in garlic. It also explained that enhanced plant growth, yield and other attributes in garlic could be attributed to influence of amino acid directly or indirectly on physiological activities of the plants. Similarly, El-Abagy et al. (2014) also investigated the effect of Isabion on growth and productivity of onion and found that plant height responded positively to different application rates and sources of Isabion. Similar trends were shown in case of seaweed extract foliar application on potato and found that seaweed extract foliar application increased plant growth significantly in comparison to control treatment (Haider et al., 2012). Research study of Khan et al. (2012) explained that application of seaweed extract and amino acid mixtures resulted in increased leaf size (41.5%), number of bunches cane^{-1}, chlorophyll content (18.15 mg g^{-1}), (6.66%), rachis length (13.5%), and berry weight (14.78%). Increment in leaf size could be attributed to increase in level of endogenous hormonal that was attained due to the hormonal action of seaweed extract application. Similarly, the increase in leaf size could be attributed to the increased physiological response caused by the organic matter in the seaweed extract. Sugar act as an effective signal molecule and a direct substrate of intermediate metabolism. Hence the availability of sugar is a powerful driving force for plant growth and development (Zheng et al., 2016). In addition, the increase in leaf area is due to the iron and manganese content in the seaweed extract, which strengthens the endogenous hormone levels of the treated plants, thereby enhancing the photosynthesis process, resulting in an increase in chlorophyll production, and a positive impact on growth parameters. Foliar application of seaweed extract significantly increased the size of watermelon leaves because of the presence of auxins, gibberellins, cytokinins, ethylene precursors and betaine, which may enhance the growth response of plants (Abdel-Mawgoud et al., 2010). The results confirmed by the study of Khan et al. (2012) that applying seaweed during flowering and fruit setting can increase the leaf area of grapes by 1.7 times. Increased chlorophyll contents due to exogenous application of seaweed extract could be attributed to increase in the respiration and photosynthesis rates and higher level of amino acid in treated plants (Awad et al., 2007).

Enhanced plant growth and yield due to seaweeds extract application could be attributed to increased plant nutrient absorption, improved nutrient status through several mechanisms including asymbiotic nitrogen fixation, dissolved nutrients, iron-absorbing, and production of volatile organic compounds (Calvo et al., 2014). Exogenous application of Isabion plays a signal transduction role in regulating plant roots to obtain nitrogen, reducing nitrate and ammonium influx and transport carrier transcription (Fan et al., 2006; Miller et al., 2008). Increased flowering in plants treated with different levels of bio-stimulants was noted as compared to control that could be attributed to enhanced cell division (Calvo et al., 2014). Cytokinins present in bio-stimulants (seaweed extract) interferes with the cellular mechanism, helps in reduction of chlorophyll degradation, enhances chlorophyll production and photosynthesis processes that led to positive effects on growth parameters (Khan et al., 2012).

The increase in the diameter of the treated broccoli head may be due to the activity of the plant growth regulator present in the bio stimulants. Since the diameter of the fruit is the result of the activity of endogenous growth regulators, such as increased activity of auxin, it stimulates cell division and expansion, and cytokinin is related to cell division and thickening of other plant organs (Kitano et al., 2008). The increase in curd weight might be attributed to the increase in leaf chlorophyll content, improved the photosynthesis rate and overall health of the broccoli plant. Our results are confirmed by the study of Norrie and Keathley (2006) who explained that exogenous application of seaweed extract increased the berry weight by 26.5%. Seaweed extract is known to be yield booster as it enhances activity of nitrate reductase which convert nitrate to nitrite. As it is an important element for plants to produce proteins, nucleic acids and chlorophyll (Thiviya et al., 2022). A biostimulants contain micro, macro nutrients along with promoting hormones like cytokinins, auxins and betaines, which increase chlorophyll production that leads to increase vegetative growth and productivity (Shehata et al., 2011).

Results clearly showed that total sugars contents vary significantly among treated plants. However, control treatment exhibited higher total sugars as compared to other bio stimulants treated plants. The increase in fruit sugar content could be due to the conversion of certain components of carbohydrates such as starch into sugars by the action of enzymes during ripening (Karemera and Habimana, 2014). Moreover, increase in total soluble sugars might be due to the increase in the hydrolysis of starch and polysaccharides into soluble sugars during plant growth. Similarly, higher respiration rate of the curd converts stored sugars or starches into energy, to ensure early ripening. On the other hand, preharvest-treated amino acids and seaweed maintained the rapid growth of total sugars during plant growth, which may be related to slow plant respiration, high levels of antioxidant enzymes, and defense mechanisms against hyper maturity. In Addition, the increasing tendency of reducing sugars in fruits and vegetables during ripening might be due to the conversion of fruit starch to sugars (Wills et al. 1989).

Exogenous application of bio-stimulants augmented the anti-oxidative behavior in the form of increased SOD, POD and CAT activities. Research study of Vasconcelos et al. (2009) concluded that influence of bio-stimulant on anti-oxidative behavior is mediated by its composition. Accumulation as well as synthesis of antioxidant enzymes might be connected to higher nutrients contents and enzymatic activity that is involved in phytochemical homeostasis (Ertani et al., 2014). Bio-stimulant application in the form of amino acid on *Capsicum chinensis* L. not only enhanced the antioxidant activity but also ascorbate and capsaicin concentration. Study of Kulkarni et al. (2019) concluded that application of bio-stimulant governs a positive effect on increasing phenylalanine ammonia lyase, an enzyme involved in synthesis phenolic acids. Similarly, increased antioxidant activity was observed in garlic leaves under the application of humic acid (Ekin, 2019). Vasantharaja et al. (2019) stated a positive bio-stimulant effect related to the on antioxidant activity and phenols contents of *Vigna unguiculata*. Furthermore, bio-stimulants primed maize plants protected against oxidative stresses by augmenting the the activity of superoxide dismutases (Trevisan et al., 2019) a key enzymes associated to the antioxidant defence by catalysing the enzymatic dismutation of superoxide to H_2O_2 (Wang et al., 2016).

5. Conclusion

The results exhibited that different concentrations of amino acid and seaweed extract have enhanced growth, yield and nutritional quality of different selected broccoli cultivars. The results also exhibited that combined application of 4 ml L^{-1} seaweed extracts + 4 ml L^{-1} amino acid yielded the highest production, and quality and upregulated the antioxidative behaviour of selected broccoli cultivars. Moreover, among cultivars Walthom-29 and Marathon outperformed in the local condition.

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