Research Article

Bio-stimulant and Salicylic Acid Application Triggers Antioxidants Activities and Yield Parameters of Bottle Gourd (*Lagenaria siceraria* L.)

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Abstract | Different polyphenolics and biostimulants are used across the globe for enhancing productive and qualitative tarits of horticultural plants. A field trial was carried out in 2018-19 aiming at the evaluation of impacts of new commercial products "Quantis" and "Acevit-C" on growth, yield and antioxidative behaviour of bottle gourd cv. Aakash F_1. Three level of Quantis (6 ml, 8 ml, 10 ml) and Acevit-C (100 ppm, 150 ppm and 200 ppm) were applied as foliar treatment following RCBD design. Results showed that impact of foliar application of both Quantis and Acevit-C on bottle gourd growth and reproductivity were significant. Moreover, application of 100 ppm Acevit-C and 10 ml/L^{-1} Quantis exhibited 6.5 and 11.5 fold increment in yield respectively as compared to control. The use of these plant products at variant concentrations showed an increase of antioxidant activity by upgrading superoxide dismutase activity (64.42 U mg^{-1} protein), peroxidase activity (1.90 μmol min^{-1} g^{-1} protein), catalase activity (37.81 $nmol^{-1}$ g^{-1} protein) and protein (6.28 mg g^{-1} fresh weight) contents. These commercial plant products based on amino acids enriched macro and micronutrients as well as acetyl-salicylic acid combined with ascorbic acid can be used carefully for expecting efficient productivity with high quality produce.

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Introduction

Bottle gourd (*Lagenaria siceraria* L.) from Cucurbitaceae family is one among highly liked vegetables raised in South East Asia (Bisognin, 2002). the vegetable originated from Africa and nowadays cultivated mainly in China, Ukraine, Argentina and Turkey, representing almost 45% of the World production of bottle gourd. Bottle gourd is grown in many old and new places of the world, and they are of great importance as they are enriched with essential edible products and fibres (Palamthodi et al., 2019). In Pakistan, it is cultivated on an area of 5410 ha with a cummulative yield of 56720 tonnes in Pakistan (FVC, 2018).

Bottle gourd yield and quality is dependent upon several factors, including climatic conditions, soil fertility status, cultural and agronomic practices, incidence of pest and diseases, which if unfavourable, limit growth, productivity and quality of bottle gourd (Koike et al., 2007). While modern agriculture tends to adopt new technologies along with genetic improvements and advanced crop management practices to enhance growth and productivity of crops (Silva et al., 2017). In addition, use of low risk pesticides, selective herbicides and a new class of naturally derived compounds often classified as biostimulants or crop protectants are preferably consumed (Michalak and Chojnacka, 2013; Calvo et al., 2014). These later two groups are relatively new class of products which are majorly used for maximizing growth, productivity and quality, particularly under unfavorable growing conditions (Colla et al., 2014; Jardin, 2015). These natural products are comprised of vast range of molecules and compounds that significantly regulate plant's physiological processes upon drench or foliar application (Herrera et al., 2014). These natural products also enhance emergence of seed and biological activity of plants. Their utility at agriculture forms has been increasing to enhance yield and nutritional capacity of food crops because these are enriched of environmental friendly compounds (Schiavon et al., 2010) and thus contribute to sustainable crop production (Jardin, 2015; Radkowski and Radkowska, 2013). The formulations of these natural products are often based on amino acids (AA) (Ertani et al., 2009; Chojnacka et al., 2014; Subbarao et al., 2015; Nardi et al., 2016). Some of the examples of these commercial products are: Isabian (Syngenta Pakistan) Metalosate (Albion Minerals, USA); Agrocean B (Agrimer, France); Tecamin, Tecamin Max and Tecamin Flower (Agritecno Fertilizantes, Spain); Quelant Ca, Quelant Amino-Green (Bioiberica, Spain).

With the recent technological advancement, these naturally derived compounds are augmented with other minerals i.e. N, P, K and Ca etc. Such formulations minimize the nutritional demands of plants by optimizing the utilization of micro and macro-nutrients thus promoting root growth and plant health (Herrera et al., 2014). Such mixtures owing their versatile composition particularly enriched with amino acids and N are well known for their growth-promoting and precursor role in the course of protein formation, catalyst of phytohormones and vital constituents for promoting new growth (Nardi et al., 2016). Moreover, such formulations are well suited for foliar applications since leaf cuticle are highly permeable for amino acids (Arfan et al., 2006). Any other nutrients or minerals attached with AA is also easily absorbed as compared to simple cation diffusion. Apart from amino acid and other minerals formulations, there is also a class of plant growth enhancers based on plant hormones. Acetyl salicylic acid (ASA) and ascorbic acid (Asc. acid) are prominent one among these plant hormones (Gunes et al., 2007). Both compounds are known for their antioxidant capacity and strengthens plant defence against biotic and abiotic stress (Eraslan et al., 2007). Their exogenous application has reported to alter physiological processes by regulating ion uptake, cell division, cell signalling and stomatal conductance (Barth et al., 2006).

The current study was designed to examine the impact of commercially available amino acid (enriched with macro and micronutrients) and plant hormones (ASA and ascorbic acid) on productivity and quality of bottle gourd. Fewer studies are available in Pakistan regarding the influence of amino acid and plant hormones on cucurbits. Therefore, this study was designed and execute to identify the effective concentrations of commercially available "Quantis®" and "Acevit-C" on bottle gourd cv. Aakash F_1. We have hypothesized that these products could serve as a suitable supplement along with the application of traditional fertilizers to improve the productivity and quality of bottle gourd.

Materials and Methods

Plant material

Present experiment was conducted at the vegetable farm, Institute of Horticultural Sciences (31.4303° N, 73.0672° E), University of Agriculture, Faisalabad, Pakistan. Seeds of bottle gourd cv. Aakash F_1 from Kalash Seeds company were raised in seedling trays (32 cells) filled with peat moss and coconut coir. Before transplanting, the field was cultivated and harrowed well to make raised beds (30×10 feet dimension) on finely pulverized soil. However, following 30 days after emergence, these seedlings were transplanted on prepared foundations. For ensuring healthy growth, optimized cultural practices i.e. weeds removal, irrigation, fertigation and pest management were adapted till harvesting. After transplanting, the seedling establishment was observed on a regular basis.

Collection and application of commercial products

A commercial biostimulant from Syngenta Pvt. Ltd. namely "Quantis" was purchased from local market and anti-stress product "Acevit-C" was acquired from

A&K Pharma Private (Ltd), Faisalabad. Quantis is enriched with amino acid (aspartic acid, glutamic acid, proline and alanine), potassium oxide (9%), nitrogen (1%), calcium (1%) and organic carbon (15%). While, Acevit-C consists of 6.7% acetyl-salicylic acid and 20% ascorbic acid. Both of these products were applied in different concentrations viz. control, 6 ml, 8 ml, 10 ml and 100 ppm, 150 ppm and 200 ppm respectively. Three foliar sprays of these compounds were done after seedling establishment (30 days after transplant) with two weeks intervals. Control plants receive distilled water application. Whereas, each plant receive similar number of sprays with 20 ml of solution in each application.

Data collection

Different parameters like leaf area, days to flowering (Gocmen et al., 2017), fruit length, diameter and weight (Fatma et al., 2018), number of fruits per plant, yield per plant and yield per plot (El-Kohly and Hafeez, 1982).

Extraction for antioxidant enzymes assay

Fresh fruit (100 mg) from each treatment were macerated in 4 ml of 50 mM phosphate buffer (pH 7.0), having 1 mM EDTA and 1% PVP (polyvinyl polypirrolidone). The resultant solution was centrifuged for 25 min at 15000 × *g* and supernatant was collected for assay of enzyme activities on immediate basis.

Determination of antioxidant enzymes activity

Superoxide dismutase (SOD) enzyme was assayed by measuring 50% blocking of photochemical reduction of nitro blue tetrazolium (NBT). Aliquots of supernatant (100 µl) were mixed with 500 µl of phosphate buffer, 200 µl of methionine, 100 µl of riboflavin with 800 µl of distilled water and placed under UV light for 15 minutes followed by absorbance determination at 560 nm by using a micro plate reader. SOD activity was expressed on the basis of protein contents as U mg^{-1} protein (Stagner and Popovic, 2009).

Catalase (CAT) and Peroxidase (POD) assay were determined using protocol of Chance and Maehly (1955). Enzyme extract (100 µl) was mixed in 500 µl solution containing 300 mM of phosphate buffer with 100 µM of 5.9 mM H_2O_2. Changes in reaction solution were observed by absorbance at 240 nm and expressed in nmol min^{-1} g^{-1} protein. Whereas, for POD activity assay, a solution containing 500 µl of 50 mM phosphate buffer (5.0 pH) with 200 µl of 40 mM H_2O_2, 500 µl of 20mM guaiacol and 0.1 ml of enzyme extract. Reaction of the solution was observed as changes recorded at an interval of 20 seconds at wavelength of 470 nm. While results were expressed in µmol min^{-1} g^{-1} protein.

Determination of total protein contents

Total protein contents of treated and control plants were determined by following the protocol of Bradford (1976). Twenty micro liter of sample from each treatment was poured in micro-centrifuge tubes. Following this, 1000 µl of Bradford reagent was added and samples were incubated for fifteen minutes at 37°C and absorbance was taken at 595 nm.

Experimental layout and statistical analysis

Current research trials was led following Randomized Complete Block Design (RCBD), having seven treatments with triplicate replications. Statistical analysis were performed utilizing Statistix 8.1 software while ANOVA technique was adopted to determine the overall significance and difference in treatment means was done by using the LSD test at 5% probability level (Steel et al., 1997).

Results and Discussion

Foliar application of Quantis and Acevit-C have led to significant increment in vegetative growth of bottle gourd. About 2-2.5 folds increment in leaf area was assessed in treated plants in comparison with control plant (Figure 1A). However, non-significant differences were observed regarding earliness to flower induction. Treated plants and control ones both show a typical range of 25 to 28 days for flower induction in bottle gourd (Figure 1B).

A significant interaction between Acevit-C and Quantis applications was found for fruit length. It was observed that the use of Quantis at 10 ml concentration yielded maximum fruit length (55.68 mm) followed by 150 ppm Acevit-C recording (46.89 mm) fruit length (Figure 1C). Similar outcomes were observed regarding fruit diameter, where Quantis and Acevit-C application have resulted in ameliorated fruit diameter (Figure 1D).

Foliar application of phytohormones, and minerals enriched biostimulant had a significant impact on

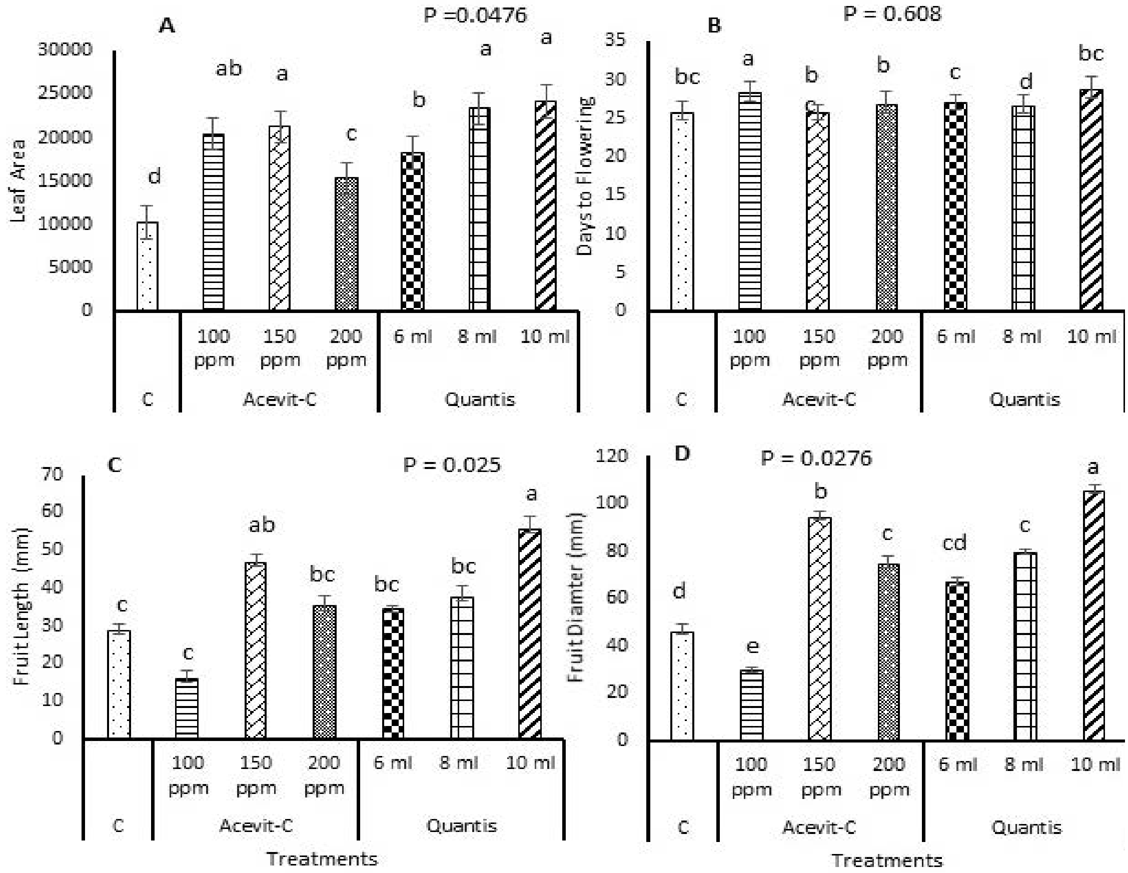

Figure 1: *Impact of Activit-C and Quantis application on the vegetative and reproductive behaviour of bottle gourd. Means sharing different letters are significantly different from each other. The error bars indicates the standard error of means.*

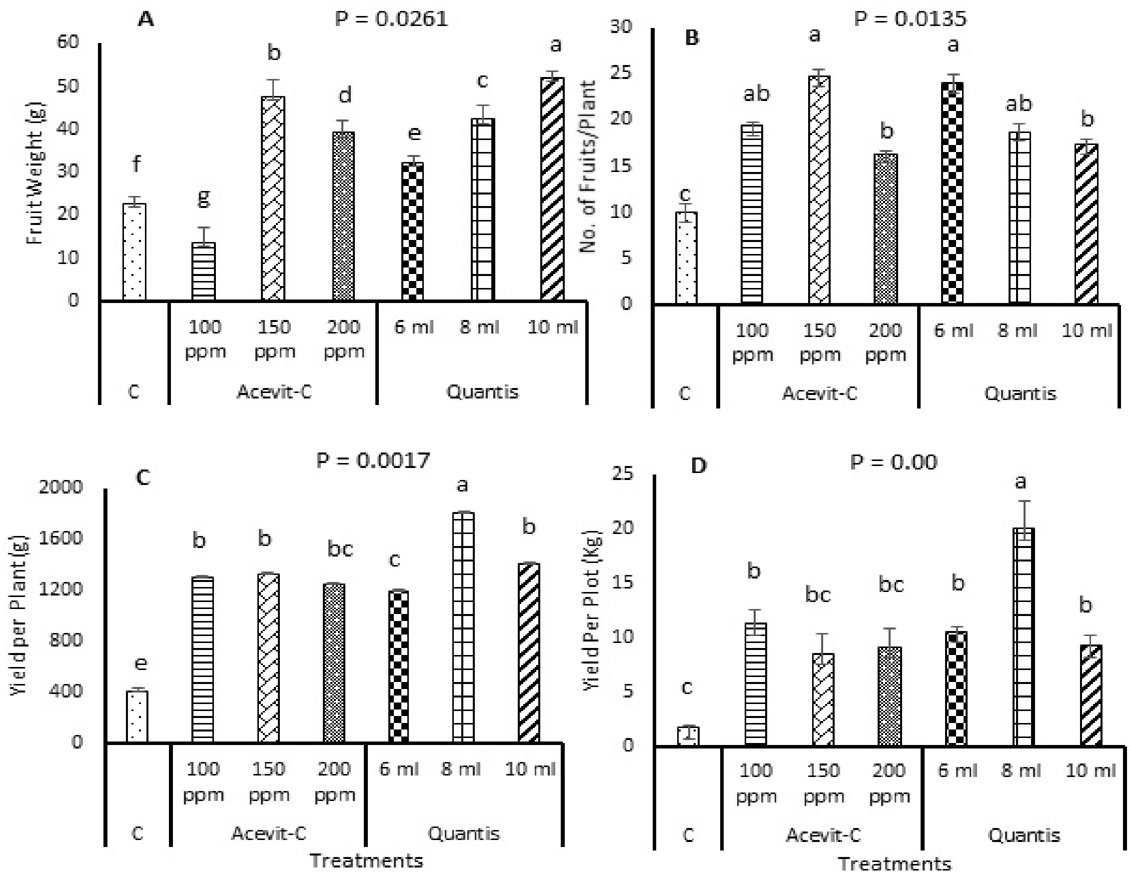

Figure 2: *Impact of Activit-C and Quantis application on the reproductive behaviour of bottle gourd. Means sharing different letters are significantly different from each other. The error bars indicates the standard error of means.*

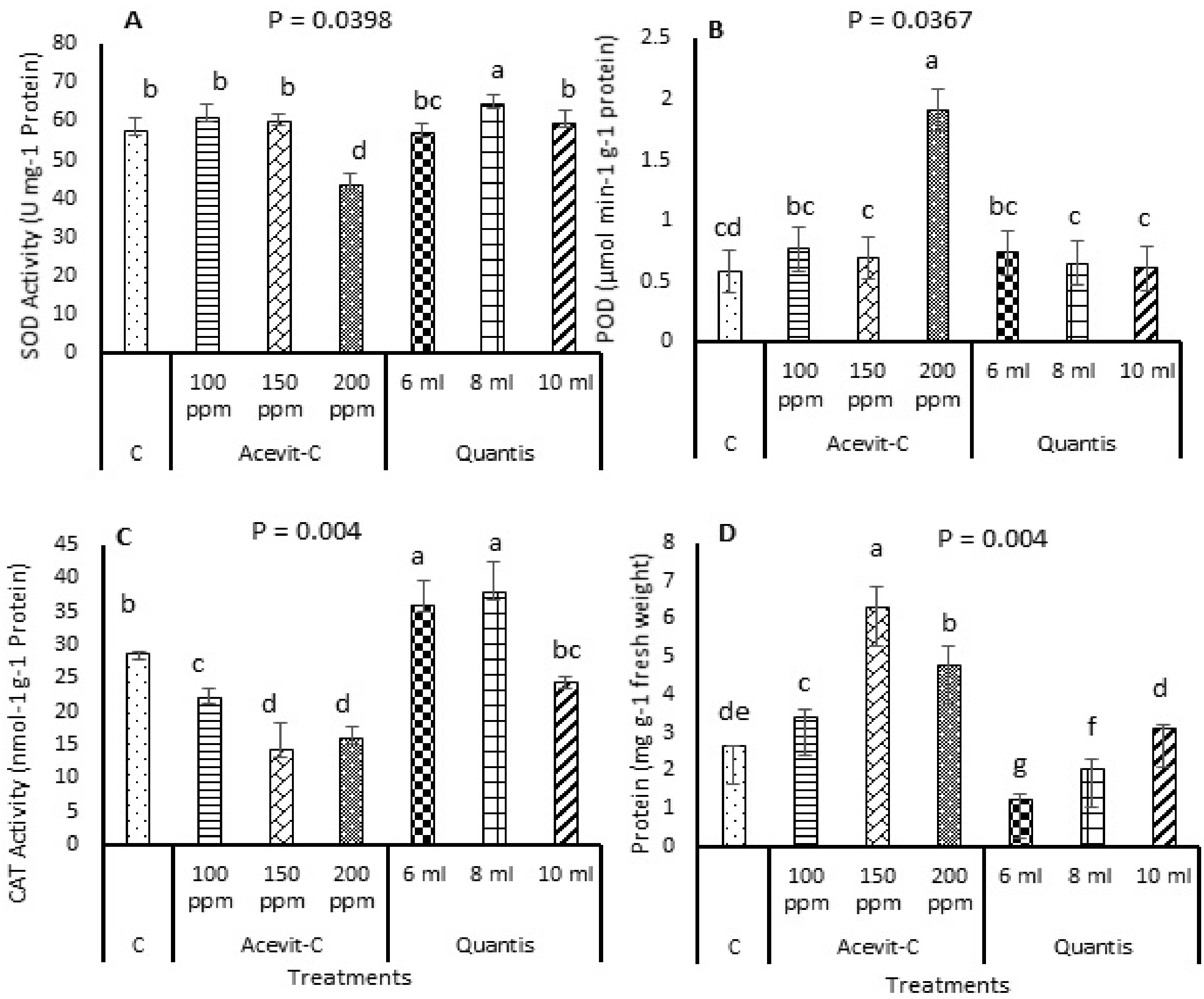

Figure 3: *Impact of Activit-C and Quantis application on the antioxidant potential of bottle gourd. Means sharing different letters are significantly different from each other. The error bars indicates the standard error of means.*

fruit weight as compared to control plants (Figure 2A). Maximum fruit weight (52.97 g) was observed in plants receiving 10 ml Quantis followed by 150 ppm Acevit-C (47.85 g) respectively. Number of fruits per plant were also remarkably affected by foliar application of these commercial products. The number of fruits varies from a range of 16 to 24.5 fruits per plant among treated plants, where the highest number of fruits (24.5) were recorded by the application of Acevit-C (150 ppm) and least number of fruits (16.33) by 200 ppm Acevit-C. Moreover, control plants only resulted in 10 fruits per plant (Figure 2B).

Highly significant results were observed for yield per plant and per plot (Figure 2C and 2D). The Acevit-C and Quantis application remarkably enhanced 4-4.5 folds yield per plant as compared to control plants. While, the yield per plot increased to 15 folds (Figure 2D). Foliar application of Acevit-C and Quantis had significant, profound effects on SOD activity of bottle gourd cv. Aakash F_1 (Figure 3A). Interestingly 200 ppm Acevit-C treated plants have resulted in lower SOD contents (43.41 U mg^{-1} Protein) among all treatments. Moreover, outcomes also concluded that Quantis treated bottle gourd plants have reported overall higher SOD contents (56.07, 64.42 and 59.62 U mg^{-1} Protein).

POD activity was significantly influenced by foliar treatment of these commercial products. However, it was observed that the plants treated with 200 ppm Acevit-C had maximum POD activity (1.90 µmol min^{-1} g^{-1} protein). At the same time, all other treatments have resulted in average POD activity in the range of (0.58 to 0.76 µmol min^{-1} g^{-1} protein) (Figure 3B).

Similar reseults were found against the CAT contents of plants treated with minerals enriched amino acid based commercial product. The application of these products has reported higher CAT levels, particularly bottle gourd plants treated with 6 and 8 ml Quantis exhibited CAT activity of 35.97 and 37.06 nmol-1 g-1 protein, respectively (Figure 3C). Exogenously applied biostimulants had a significant impact on the total soluble protein contents. Maximum total soluble protein (6.23 mg g^{-1} fresh weight) was recorded in 150 ppm Acevit-C treated plants followed by the 200 ppm Acevit-C treated plants (4.77 mg g^{-1} fresh weight) (Figure 3D). However, results regarding Quantis treated plants depicted lower protein contents in the range of 1.24 to 3.1 mg g^{-1} (fresh weight), respectively (Figure 3D).

Pearson's correlation was carried out to estimate the effect of treatments on relation among quantitative traits (Table 1). Results reveal that fruit yield had highly significant positive linkage with fruit diameter (0.88), fruit length (0.95) and fruit weight (0.87) while it had significantly negative relationship with peroxidase activity (-0.26). However, fruit weight had significant positive association with fruit diameter (0.86) and similar context with that of fruit length (0.86). Whereas fruit weight exhibited a positive relation with number of fruits per plant (0.50) and negative association with peroxidase activity (-0.17). Fruit diameter and length were highly correlated with each other (0.93). Results regarding relation between vegetative and reproductive phase of bottle gourd crop revealed remarkable association among these parameters. Leaf area had highly significant positive association between fruit diameter (0.84), fruit length (0.82), number of fruits (0.83) and to some lesser extent with fruit weight (0.71**). However, enzymatic activity has negative correlation with most of these reproductive parameters (Table 1). POD has highly negative association with fruit diameter (-0.32), fruit length (-0.33), fruit weight (-0.17) and number of fruits (-0.34).

Table 1: *Correlations among different quantitative traits of bottle gourd under influence of biostimulants and Salicylic acid application.*

	CAT	Days to flower	Fruit diameter	Fruit length	Fruit weight	Leaf width	Leaf area	No. of fruits/ plant	POD	Protein	SOD	Yield per plant
Days to flower	0.0461 0.9218											
Fruit diameter	0.0481 0.9184	0.3944 0.3813										
Fruit length	0.1851 0.6911	0.4003 0.3736	0.951** 0.001									
Fruit weight	0.1139 0.8078	0.1783 0.7021	0.900* 0.0057	0.913* 0.004								
Leaf width	0.0181 0.9693	0.4883 0.2663	0.8363 0.019	0.883 0.0084	0.903** 0.0053							
Leaf area	0.0583 0.9013	0.5386 0.2122	0.591 0.0069	0.903** 0.0052	0.845** 0.0166	0.934** 0.002						
No of fruits/ plant	0.0685 0.8841	0.381 0.3991	0.9569 0.0007	0.9789 0.0001	0.9479 0.0012	0.9352 0.002	0.9197 0.0034					
POD	0.4707 0.2864	0.0562 0.9047	-0.0628 0.8937	-0.0709 0.8799	0.1056 0.8217	-0.0147 0.9751	-0.2933 0.5232	-0.033 0.944				
Protein	0.9333 0.0021	0.2791 0.5444	0.1863 0.6892	-0.0377 0.936	0.3055 0.5052	0.1085 0.8168	0.0423 0.9282	0.0827 0.8601	0.3749 0.4073			
SoD	0.4603 0.2986	0.1207 0.7966	0.3783 0.4028	0.3715 0.4119	0.2006 0.6663	0.2978 0.5165	0.5326 0.2184	0.3661 0.4193	0.917** 0.0036	0.3052 0.5056		
Yield per plant	0.0957 0.8383	0.3817 0.3981	0.902** 0.0054	0.959** 0.0006	0.943** 0.0014	0.953** 0.0009	0.887** 0.0077	0.986** 0.000	0.0541 0.9082	0.0423 0.9282	0.2793 0.5441	
Yield per plot	0.2958 0.5195	0.2282 0.6226	0.882** 0.0086	0.957** 0.0007	0.886** 0.0079	0.835** 0.0194	0.809** 0.0275	0.954** 0.0008	0.0561 0.9049	0.0988 0.8332	0.3962 0.3789	0.953** 0.0009

*Upper values indicated Pearson's correlation coefficient; Lower values indicated level of significance at 5% probability. *: Significant (P<0.05); **: Highly significant (P<0.01).*

The use of hormonal and biostimulants compounds in horticultural plants enhances vegetative growth, reproductive behaviour and quality of final produce (Bulgari et al., 2019). Among different commercial biostimulants, formulations having amino acids in addition with major (N, P, K and Ca) and trace elements (Zn and B) are preferred due to their multiple roles i.e. provision of various nutrients, signal transduction, protein formation and promoting new growth structures (Khan et al., 2019). However, it was assessed that foliar application of Quantis resulted in enhanced leaf area that could be ascribed to presence of N who could have augmented protein synthesis involved in cell division and differentiation (El-Shiaty et al., 2004) along with an ample supply of organic carbon (C) thus affecting C: N ratio. This C:N partitioning have significant impacts on plant productivity as in majority about more than 70% of plant biomass is allocated to above ground parts i.e. leaves, stems and fruit etc. Moreover, as N contents increases, major proportion of plant biomass is allocated to leaves. However, provision of C and N have not only incremented of leaf area but also affected fruit quality attributes i.e. length and diameter. Augmentation of fruit length and diameter could also be ascribed to role of amino acid that have enhanced K levels (Bahari et al., 2013) which is known for quality enhancement in fruits (Woldemariam, 2018).

Regarding the impact of Acevit-C on fruit length and width, similar results were obtained, but up to a lesser extent, as in the case of Quantis. ASA and ascorbic acid are known to regulate defence mechanism particularly modulating biochemical, molecular and physiological processes, i.e. nutrient absorption and transport, photosynthetic ability and membrane permeability under stress conditions (Bassem, 2015). However, increment in fruit quality parameters could be attributed to cell division, growth promoting and signal transduction capacity of ascorbic acid (Gallie, 2013). Similar results were reported in soybean plants treated with different salicylic acid applications that resulted in incremented levels of photosynthetic pigments, upgraded antioxidant capacity and phenolic contents (Fahad et al., 2015).

The role of natural products (derived from organic compounds or plant hormones) for yield enhancement is always focused as these are known to pose significant impacts on productivity and quality of produce (Tasgin et al., 2003; Ullah et al., 2019). Similar results were observed regarding number of fruits per plant and fruit weight where Quantis and Acevit-C treated plants have reported higher fruit weight along with retention of a maximum number of fruits per plant. This increment in productivity could be related to various factors under the influence of amino acids or plant hormones (i.e. SA and AA). Number of fruits per plant could be due to increased pollen tube ovule penetration and delayed ovule senescence that led to retention of enhanced fruit set (Arabloo et al., 2017). While increment of fruit weight could be attributed to upgraded sugar levels under the influence of salicylic and ascorbic acid application that substantially leads to enhancement of osmotic gradient and reduction in water loss, thus strengthening vines and ultimately producing more fruits having better weight (Paradikovic et al., 2013). In contrast, the exogenous application of amino acids has reduced protein breakdown, thus conserves the plant energy, which helps in enhancing productivity. Similar outcomes were observed in the case of sweet pepper (Champa, 2015) berries (Colla et al., 2017) and tomato (Rajjou et al., 2006).

Antioxidant enzyme activities was also higher in bottle gourd fruits when treated with Quantis and Acevit-C. This coincides with literature data: increment of antioxidant activities could be ascribed to the protective role of SA and AA, that regulates nitrate reductase, thus prevents enzyme damage (Hamilton and Heckathorn, 2001). Moreover**,** SA is known to enhance endogenous proline contents, also acts like osmolyte, thus protects complex II electron transport (Makela et al., 2000), strengthens cell membrane its three-dimensional structure (Carneiro et al., 2019) along with antioxidant enzymes, i.e. SOD, POD and Rubisco.

Correlation findings have exhibited a highly significant positive relation between vegetative characters i.e. leaf width and leaf area on fruit length, diameter and weight of bottle gourd. This whole scenario suggests the most suitable application time for biostimulants or any other amendments for the sake of improving the yield productivity. While positive association between leaf area and enzymatic activity (SOD and CAT) also expressed the possible improvement in plant immunity against biotic and abiotic stresses when treatment are applied on leaf stages. This could be ascribed to a well known fact that improved vegetative growth is an index of plant health

(Yadav et al., 2007). Moreover, indirect selection for yield enhancement could also be made on the basis of this quantitative traits correlation. There has been a lot of previous research studies that also exhibited similar positive impacts of different quantitative traits on productivity of bottle gourd (Husna et al., 2011; Janaranjani and Kanthaswamy, 2015).

Conclusions and Recommendations

The foliar application of Quanits and Acevit-C significantly influenced the growth, yield, and quality attributes of bottle gourd cultivar. In general, the higher rate of Quantis (8 ml/L^{-1}) had a valuable impact on the vegetative and reproductive growth along with yield. Whereas, in comparison Acevit-C was not as efficacious for enhancing growth and yield attributes other than increased POD activity. Further investigations are recommended on other cucurbits under variant environmental and soil conditions to explore the effective concentrations of these commercially available biostimulants.

Author's Contributions

MA conceptualization and KJ methodology. MA, MZH and RIK formal analysis. KJ, TR, ZM data curation. KJ, MA, RIK, TR and MZH writing original draft preparation. MZH and ZM writing review and editing. MA supervision.

Conflict of interest

The authors have declared no conflict of interest.

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