Pak. J. Bot., 57(2): DOI: http://dx.doi.org/10.30848/PJB2025-2(28)

THE POTENTIAL ROLE OF GIBBERELLIC ACID IN REGULATING PHOTOSYNTHETIC PIGMENTS, FRUIT QUALITY AND ANTIOXIDANT ENZYMES IN SWEET LIME *CITRUS LIMETTA* RISSO

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Abstract

Sweet lime is one of the well-known citrus species, but due to its poor yield and quality farmers hesitate from its cultivation. Gibberellic acid is the key plant growth regulator which regulates various physiological activities in plants. In horticulture crops, Gibberellic acid is particularly applied to improve flowering, fruit set and fruit quality. Therefore in the present work the influence of Gibberellic acid concentrations (Control, 40, 50 and 60 ppm) and time of application (5, 10 and 15 days after bud break, DABB) on photosynthetic pigments, fruit quality and antioxidant enzymes of sweet lime was evaluated during the year 2020. Results of the current study demonstrated that Gibberellic acid at 50 ppm produced high values of Chlorophyll (*Ch*) *a*, *Ch b*, *Ch a+b*, carotenoid content, fruit juice content, vitamin C, superoxide dismutase (SOD) and peroxidase dismutase (POD) activity, while the highest fruit firmness and total soluble solids (TSS) were recorded with 40 ppm GA_3. Whereas, the application of GA_3 on 15 DABB significantly increased Chlorophyll *a*, *Ch b*, *Ch a+b*, carotenoid content, fruit juice content, vitamin C, POD and SOD activity. Maximum TSS, and total sugars were produced on 10 DABB application that was statistically at par with 15 DABB application. Hence it is recommended that GA_3 must be applied at 50 ppm on 15 DABB for enhanced photosynthetic pigments, fruit quality and antioxidant enzymes.

Key words: Sweet lime; Gibberellic acid; Time of application; Photosynthetic pigments; Fruit quality; Antioxidant enzymes.

Introduction

Sweet lime (*Citrus limetta* Risso) locally known as Mettah is famous for its excellent nutritional and medicinal properties throughout the globe. In Indian sub-continent sweet lime is commonly known as Mosambi. It is indigenous to Asia and well grown in India, China, southern Japan, Vietnam, Malaysia, Indonesia and Thailand (Khan *et al*., 2016). Sweet lime is one of the earliest fruit that come in the market among the sweet group of citrus fruits. Sweet lime has great demand during Aug-Sept due to its refreshing juice. Along with its importance in juice industry sweet lime is known for its anti-fever and other therapeutic properties (Kumar *et al*., 2020).

Gibberellins (GAs) are group of plant hormones that regulates different processes like, stem elongation, cell division, germination, dormancy, flowering, sex expression, enzymes activation and senescence of leaves and fruits (Mazher *et al*., 2014). In citriculture, GA_3 is commonly used for flower reduction, fruit setting, improving fruit quality and increase of storage life by controlling ripening (Agusti & Almela, 1991). Photosynthetic pigments particularly chlorophyll are very much necessary for optimum photosynthesis. Islam *et al*., (2021) stated that GA_3 positively increased leaf *Ch a*, *Ch*, *b Ch a+b* and carotenoid content in mungbean. Gibberellic acid improves chlorophyll formation by effecting number and size of chloroplast (Arteca, 1996) Gibberellic acid has the ability to enhance cell enlargement (Davis, 2004) which improve size of the fruit particularly in citrus (Eman *et al*., 2007) and litchi (Stern & Gazit, 2000). Gibberellic acid is also known to effect fruit quality in terms of, color, TSS, sugar content, fruit firmness, acidity, vitamins and storage life.

During the growth period plants face numerous biotic and abiotic stresses which greatly affects photosynthesis rate thus affecting yield and quality of crops. In response of unfavorable conditions plants produce immense amount of reactive oxygen species (ROS) which causes damages to lipid membrane, protein, DNA and photosynthetic pigments (Damanik *et al*., 2019). Super oxide dismutase (SOD). Peroxidase (POD) and catalase (CAT) are key antioxidant enzymes that plays key role in elimination of free radicals (Zhang *et al*., 2014). Various workers reported that GA_3 played key role in the regulation of these antioxidant enzymes (Tepe, 2021; Alharby *et al*., 2021). Importance of GA_3 in physiological and biochemical parameters in different citrus species has been reported by various scientists however there is no proper recommendation of GA_3 in term of concentration and timing for sweet lime (Garmendia *et al*., 2019; Thanaa *et al*., 2012), hence the current research was conducted to study the influence of GA_3 concentrations and time of application on photosynthetic pigments, fruit quality and antioxidant enzymes. This study may provide useful information to maximize word wide productivity of sweet lime.

Material and Methods

An experiment "Potential role of Gibberellic acid in the regulation of photosynthetic pigments, fruit quality and antioxidant enzymes of sweet lime" was conducted during the year 2020 in private orchard in Rustam, Mardan, Pakistan. The experiment consisted of two factors, factor A was time of application of Gibberalic acid i.e., 5, 10 and 15 days after bud break (DABB), while factor B was Gibberellic acid concentrations i.e., 0, 40, 50 and 60 ppm. Design used for the experiment was Randomized Complete Block Design (RCBD) with split plot arrangement having

three replications. Trees of uniform size and age (20 years old) were selected for the experiment. The cultivar used in the experiment was Palestine line which was grafted on sour orange rootstock. Fruits were physico-chemical analyzed at Biochemistry laboratory of Agriculture Research Institute Tarnab Peshawar. How did you compare your results without a control? It seems only time of application and concentration ratio were compared as there was no control.

Preparation and application of GA_3 solution: Required concentration of GA_3 was first dissolved with ethanol drops and then the required solution was made with the addition of distilled water. For example for 40 ppm solution 40 mg of GA_3 was dissolved in one liter of distilled water. The prepared solutions were than sprayed on different application times with the help of hand sprayer (Fig. 1). Each tree was fully bathed with the solution during early morning. The solution was sprayed only once on a tree.

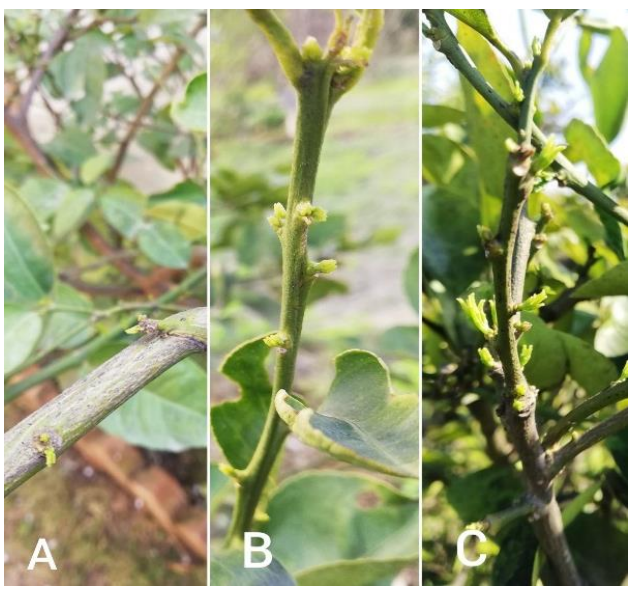

Fig. 1. Growth stages at the time of Gibberellic acid application, A= 5 DABB, B= 10 DABB, C= 15 DABB.

Determination of photosynthetic pigments: Photosynthetic pigments were measured with the help of spectrophotometer as described briefly by Lichtenthaler, (1987). 0.2 g of leaf was pulverized with 10ml of 80% acetone and the solution was filled using wattman filter paper the solution was filtered. The solution was increased upto 15ml with the addition of 80% acetone, then the solution was kept in refrigerator for 48 hours. After 48 hours 3ml from the solution was poured in cuvette and its absorbance was measured in wavelengths of 663 nm (chlorophyll a), 645.8 nm (chlorophyll b) and 470 nm for carotenoids. Concentration of the pigments was calculated with the formula given below.

$$\text{Ch-a} = 12.25A_{663.2} - 279A_{646.8}$$
$$\text{Ch-b} = 21.5A_{646.8} - 5.1A_{663.2}$$
$$\text{C x+c} = (1000A_{470} - 1.82C_a - 85.02C_b)/198$$

A = Absorbance, Ch-a = Chlorophyll a, Ch-b = Chlorophyll b, C x+c = Carotenoids

Determination of fruit quality parameters: Fruit juice was determined with the formula:

$$\text{Average juice weight} = \frac{(\text{Average juice weight} \times 100)}{\text{Average fruit weight}}$$

Penetrometer (FTFT011, Italian Equipped with 4 mm probe) was used for the measurement of fruit firmness, for this purpose a little portion of peel was removed from selected fruits and then the tip of the penetrometer was inserted in the flesh of the fruit and the reading was noted and average fruit firmness was recorded. TSS was calculated with Digital refractometer (Atgo Master-α), sample of juice extracted from fruit was placed on dry prism and then reading was taken. For determination of vitamin C method described by Ruck, (1969) was used. Total sugars were estimated by titration method according to the method described by Hortwitz (1960).

Determination of antioxidant enzymes: The SOD activity was determined by measuring its capacity of inhibit the photo reduction of nitro blue tetrazolium (NBT) according to the procedure explained by Stajner & Popvic (2009). Similarly Peroxidase activity (POD) was determined according to the procedure given by Liu *et al*., (2009). POD reaction solution was consisted of 50mM phosphate buffer (pH was 5), 40mm of H_2O_2, 20MM guaiacum and 0.1 ml enzyme extract. The absorption was measured at 470 by spectrophotometer.

Statistical analysis

For statistical analysis of the collected data statistix-8.1 software as described by Steel *et al*., (1997) was used. Recorded data were subjected to Analysis of variance (ANOVA) for variations between treatments and their interactions. The LSD test was applied when the data were found significant i.e., $p \leq 0.05$.

Results

Influence of GA_3 concentrations and its time of application on photosynthetic pigments: There were significant variations in photosynthetic pigments in response of GA_3 concentrations and its time of application (Table 1). In this study GA_3 application at 50 ppm on 15 DABB resulted in the highest Ch *a*, *b* (Fig. 2). Whereas, the highest carotenoid content was recorded in trees treated with 40 ppm GA_3 on 15 DABB (Fig. 2).

Influence of GA_3 concentrations and its time of application on fruit quality: There were significant differences in fruit quality parameters in response of GA_3 concentrations and its time of application. Fruits with maximum juice content were produced with 50 ppm GA_3 application on 15 DABB (Fig 3). Fruit firmness was the maximum with 40 ppm GA_3 application on 5 DABB (Fig. 3). The highest vitamin C was obtained with 50 ppm GA_3 application on 15 DABB (Fig. 3). Whereas, the highest TSS and total sugars were recorded on 40 ppm GA_3 and 10 DABB application time, in application timing 10 DABB application was statistically similar with 15 DABB application timing (Table 2).

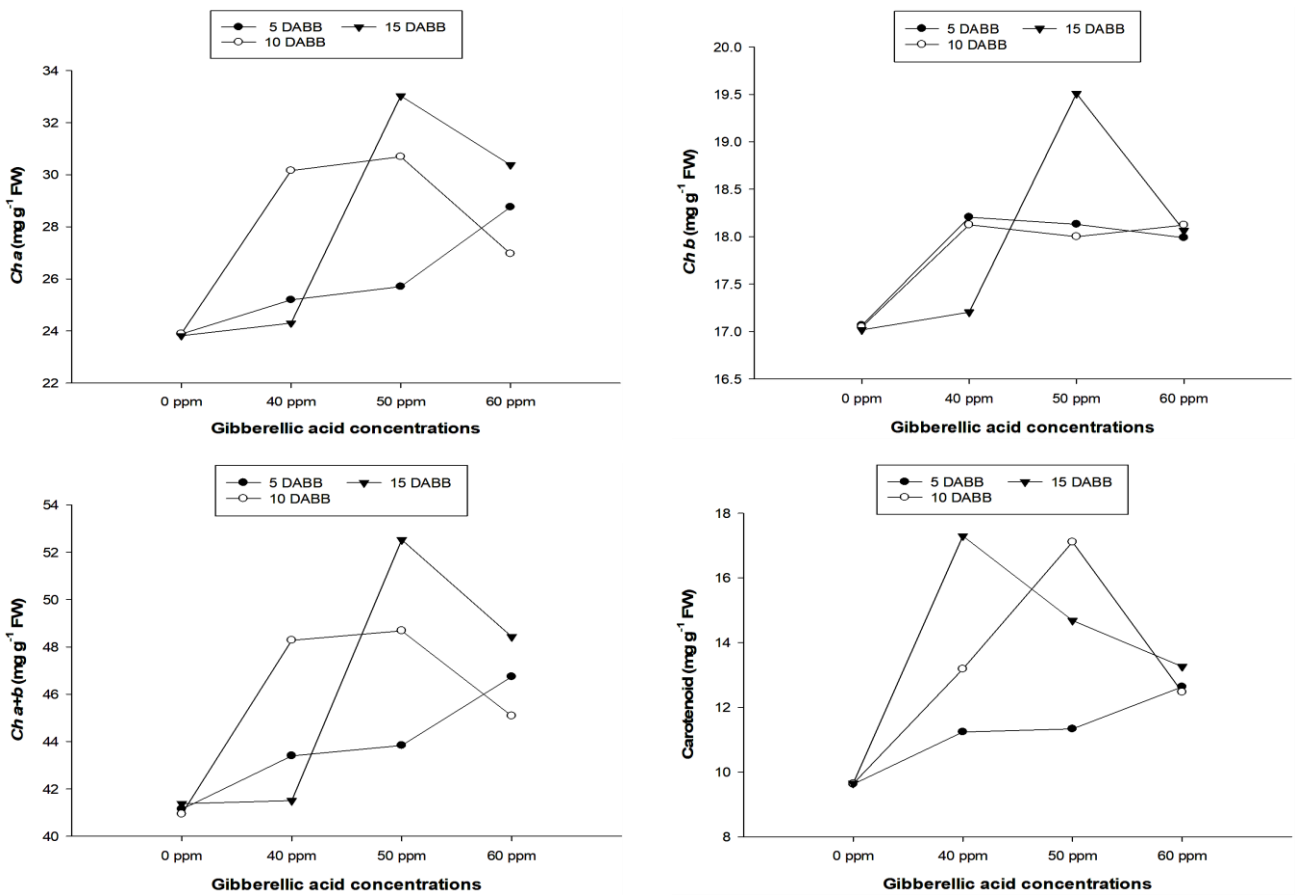

Fig. 2. Chlorophyll *a*, *b*, *a*+*b* and carotenoid (mg g^{-1} FW) as affected by the interaction of GA_3 concentrations and time of application.

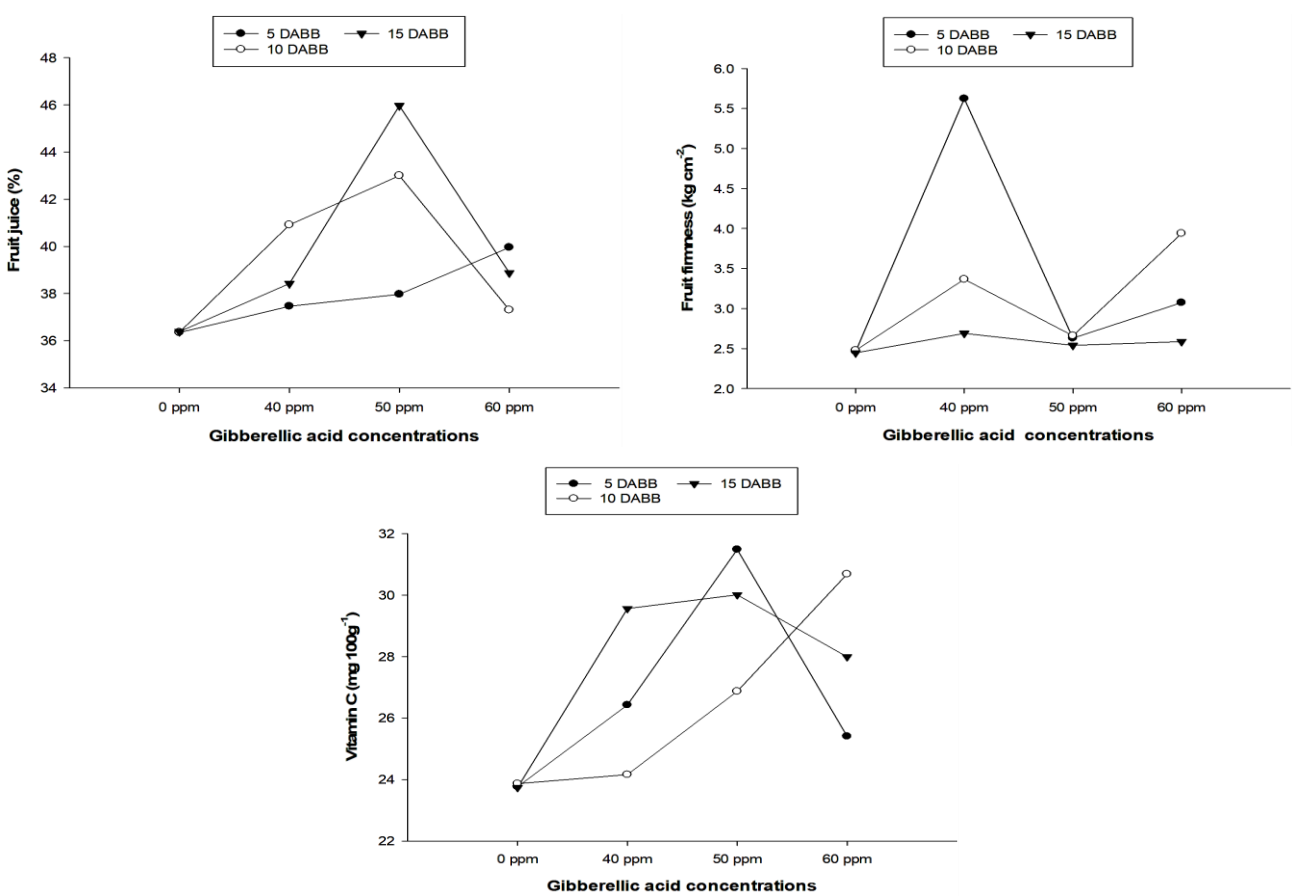

Fig. 3. Fruit juice (%), fruit firmness (kg cm^2) and vitamin C (mg $100g^{-1}$) as affected by the interaction of GA_3 concentrations and time of application.

Table 1. Influence of GA_3 concentrations and its time of application on photosynthetic pigments.

Gibberellic acid concentrations (GA_3) ppm	*Ch a* (mg g^{-1} FW)	*Ch b* (mg g^{-1} FW)	*Ch a+b* (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)
Control	23.86 c	17.04 c	41.15 c	9.63 c
40	26.55 bc	17.84 b	44.39 b	13.90 ab
50	29.80 a	18.54 a	48.34 a	14.37 a
60	28.69 ab	18.05 ab	46.75 ab	12.79 b
LSD ($p \leq 0.05$)	2.780	0.584	2.791	1.496
Time of application (TA)				
5 DABB	25.88	17.84	43.77	11.20 b
10 DABB	27.92	17.82	45.74	13.10 ab
15 DABB	27.88	17.94	45.96	13.72 a
LSD ($p \leq 0.05$)	NS	NS	NS	1.937
GA_3 x TA	*	*	*	**

*, ** = Significant at $p \leq 0.05$ and $p \leq 0.01$ respectively
DABB stands for days after bud break

Table 2. Influence of GA_3 concentrations and its time of application on fruit quality parameters.

Gibberellic acid concentrations (GA_3) ppm	Fruit juice (%)	Fruit firmness (kg cm^{-2})	Vitamin C (mg $100g^{-1}$)	TSS (^{0}Brix)	Total sugars (%)
Control	36.36 c	2.46 b	23.79 c	8.10 ab	1.09 a
40	38.93 b	3.89 a	26.17 b	8.58 a	1.15 a
50	42.31 a	2.61 b	29.45 a	7.87 b	0.95 b
60	38.71 b	3.20 ab	28.03 ab	7.94 b	0.91 b
LSD ($p \leq 0.05$)	2.165	0.757	2.544	0.521	0.115
Time of application (TA)					
5 DABB	37.93 b	3.44 a	26.77 b	7.77 b	0.94 b
10 DABB	39.39 a	3.11 ab	26.71 b	8.35 a	1.12 a
15 DABB	39.91 a	2.56 b	27.82 a	8.25 a	1.01 ab
LSD ($p \leq 0.05$)	1.284	0.534	1.056	0.340	0.126
GA_3 x TA	*	*	*	NS	NS

NS = Non-significant and * = Significant at $p \leq 0.05$
DABB stands for days after bud break

Influence of GA_3 concentrations and its time of application on antioxidant enzymes: There were significant variations in SOD and POD with GA_3 concentrations and time of application. GA_3 at 50 ppm on 15 DABB resulted in increased SOD (Fig. 4). Similarly the highest POD activity was recorded with the same concentration and application timing (Fig. 5).

Discussion

In the current study GA_3 application and its time of application significantly affected photosynthetic pigments, fruit quality and antioxidant enzymes of sweet lime. Gibberellic acid affects different physiological and morphological processes by effecting DNA, RNA and cell division and elongation and biosynthetic pigments (Ihl *et al*., 1994). In current study Photosynthetic pigments were increased with GA_3 application. Similar trend of results were reported by Mazher *et al*., (2014) and Sardoei & Shahdadneghad, (2014). Increased content of photosynthetic pigment (both chlorophyll and carotenoid) was due to increased number and size of chloroplast, enhanced plastid activity (Arteca, 1996), Rubisco and photosynthetic CO_2 assimilation rate by GA_3 (Taiz & Zeiger, 2006).

Juice content was also increased with GA_3 concentrations. Davies *et al*., (1997) reported that with GA_3 application 10% more juice content was recorded. This increase is considered as a great economic benefits for the grower because the value of processed fruits increases with juice percentage and Brix (Braddock, 1999). The enhancement of Juice content might be related with the positive effect of GA_3 in increasing cell expansion which consequently increases the capacity of vesicle to accumulate more juice (Agusti *et al*., 2002). GA_3 concentrations had a significant effect on fruit firmness. Gibberellic acid has been consistently used in fruit orchards because of its role in increasing fruit size and firmness (Kappel & Macdonald, 2002; Clayton *et al*., 2006). GA_3 application increased firmness due to inhibition of polygalacuronases and Pectin methylesterases (PMEs) cell wall hydrolytic activity (Souzaa *et al*., 2019; Andrews & Li, 1995). These enzymes are responsible for fruit softening, GA_3 maintain fruit firmness by regulating these enzymes.

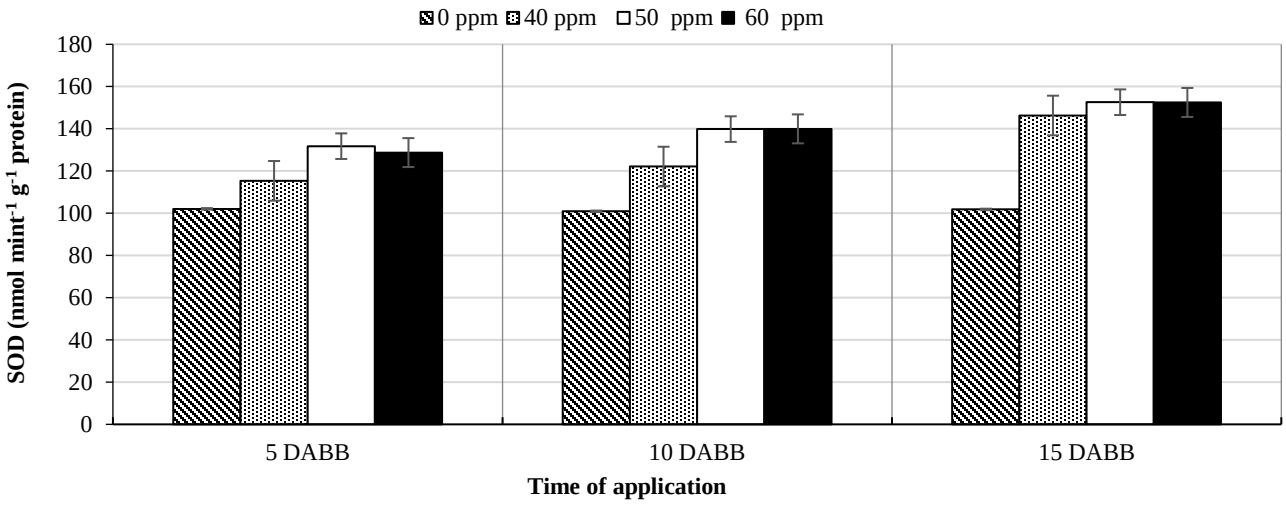

Fig. 4. Superoxide Dismutase (nmol mint^{-1} g^{-1} protein) as affected by the interaction of GA_3 concentrations and time of application.

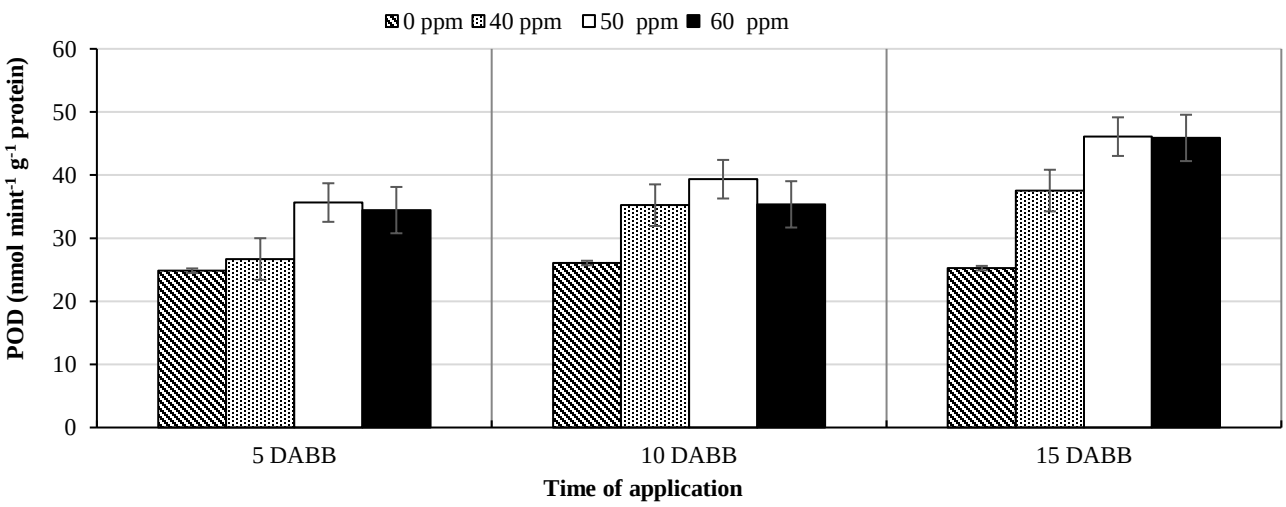

Fig. 5. Peroxidase Dismutase (nmol mint^{-1} g^{-1} protein) as affected by the interaction of GA_3 concentrations and time of application.

Vitamin C is also increased by GA_3 concentrations, similar results has been published by Hifny *et al*., (2017). To maintain ascorbic content plants need sufficient amount of nitrogen (Miceli & Miceli, 2014; Dizda & Pitura, 2008), thus gibberellic acid may have increased nitrogen content in plants which is used by plant for different biochemical processes (Miceli *et al*., 2019). Total soluble solids and total sugars were enhanced by GA_3 at lower concentrations. In citrus fruits with bigger size, high in juice content and high in sugar/acid ratio and sugars content are considered best in term of quality. Gibberellic acid is known for storage of sugars and starch and translocation of sugars to the developing sinks which ultimately improve growth and development (Iqbal *et al*., 2011). The increase in total sugars may be related to increased carbohydrates production and improved photosynthetic pigments. Kumar *et al*., (2011) stated that increase in sugars was related with the conversion of complex sugars into simple sugars or conversion of acids into sugars. Increased in TSS could be due to the improved ability of fruits to store more carbohydrates through faster translocation of carbohydrates induced by gibberellic acid (Iqbal *et al*., 2011).

SOD and POD were significantly increased by GA_3 concentrations. SOD, POD and CAT are important enzymes in fruit which play vital role in oxidative defense mechanism (Tareen *et al*., 2012). Gibberellic acid is known to improve plant defense system by eliminating excess ROS and by increasing antioxidant enzymes, however their response is different under varied conditions such as different plant species, duration of application and method of exposure (Alharby *et al*., 2021). The current results were in agreement with Didi *et al*., (2022) who reported that increase in POD and SOD with GA_3 was because of increase in protective enzymes and removal of ROS. Similarly Shahzad *et al*., (2021) demonstrated that GA_3 significantly increased POD and SOD enzymes under stressed and non-stressed conditions.

In the current study 15 DABB application was found to be the most appropriate timing to influence majority of parameters. Fifteen days after bud application was close to flowering compared to others. It has been reported that plant growth regulators when applied on active stage perform best. Plant growth regulators causes different effects with different time of application, for more desirable results PGRs must be applied in a narrow time

period usually within a few days (Roper, 2005). Time of application, concentration and species all have effect on the influence of PGRs on plant growth and development (Sing *et al*., 2015). Hence it is cleared from the above findings that time of application is very much important to get more beneficial results.

Conclusion

Gibberellic acid has a potential role in enhancing photosynthetic pigments, fruit quality and antioxidant enzymes. In the current study application of 50 ppm GA_3 on 15 DABB proved beneficial for the photosynthetic pigments, fruit quality and antioxidant enzymes. At higher level (60 ppm) decline was noted in majority of parameters. It is therefore recommended that GA_3 must be applied at 50 ppm on 15 DABB for more desirable results.

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(Received for publication 06 June 2023)