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Pre Harvest Foliar Application of Methyl Jasmonate on Fruit Quality and Quality Enzymes and Phenolic Compounds Changes During Storage of Grapefruit.

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ABSTRACT

Grapefruit is an important fruit crop in the world as well as in Pakistan. The signalling molecule like methyl jasmonate (MeJA) are endogenous plant growth substances which can play a key role in plant growth and development and responses to environmental stresses. Phytochemicals in grapefruit are considered very important for human body due to their medicinal properties. The effects of methyl jasmonate (MeJA) applied before fifteen days of harvesting to estimate the fruit firmness, fruit color, total soluble solid (TSS), weight loss, total phenolic (TP) and separate phenolic compounds , total antioxidant activity (TAA) of "grape fruit were analyzed at initial stage of harvesting and after storage. Foliar spray of methyl jasmonate (MeJA) significantly decreased fruit weight loss after 90 days storage. Quality related parameters were maintained after 90 days storage. The higher doze of MeJA @ 4mM significantly maintained the total phenolics and antioxidant after 90 days of storage. Enzymes peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX), and superoxide dismutase (SOD), Polycultrase (Poly) were found higher at 90 days storage these enzymes play an important role to developed a defense mechanisms in stored fruit those were delay fruit repining and improve shelf life of grapefruit during storage. MeJA applications has significantly increased different individual phenolic compounds especially chlorogenic acid contents after 90 days storage. These phytonutrient have significant role for antioxdative stress anti-viral, anti-cancer, anti-inflammatory activities of human body. The application of MeJA has potential to produce the different enzymes linkage which increased the fruit internal change and produce the resistance for disease resistance and improve the quality of fruits.

Keywords: Grapefruit, Phytochemicals, Methyl Jasominate, postharvest, foliar application

1. INTRODUCTION

Postharvest quality of grape fruit is greatly affected by several pre-and postharvest factors. Grape fruit face critical period after harvest and being highly perishable fruits are subjected to rapid deterioration[1]. Generally, the magnitude of postharvest losses in fresh fruits and vegetables is extraordinarily high resulting in proportionately higher economic losses than that of preharvest losses[2]. After harvesting the postharvest quality of any produce cannot to be improved however, accurate measurements can be minimized these bumper quality losses. The storage life cycle of fruit adopted much physiological process. MeJA are one of the simplest of non-traditional plant hormones with diverse roles and functions, including a potential role in plant defense as part of a complex signalling pathway[3]. Postharvest quality of grape fruit is greatly affected by several pre-and postharvest factors. Grapefruit face critical period after harvest and being highly perishable fruits are subjected to rapid deterioration[1]. Generally, the magnitude of postharvest losses in fresh fruits and vegetables is extraordinarily high resulting in proportionately higher economic losses than that of pre harvest losses[2]. After harvesting the postharvest quality of any produce cannot to be improved however, accurate measurements can be minimized these bumper quality losses. The storage life cycle of fruit adopted much physiological process. MeJA are one of the simplest of non-traditional plant hormones with diverse roles and functions, including a potential role in plant defense as part of a complex signaling pathway[3]. Plant growth and development are controlled in part, by endogenous growth substances which are affected by biotic and abiotic signals and events. A particular class of growth regulators, collectively called 'jasmonates' are involved in plant responses to such events and elicit unique responses Jasmonates are plant bio and abiotic stress hormones that play a prominent role in signaling path way of against plant defense[2]. MeJA are one of the simplest of non-traditional plant hormones with diverse roles and functions, including a potential role in plant defence as part of a complex signaling pathway, disease control[2]. MeJA increased the production of different resistance enzymes[4] that resistance against the free radical production in cell. Many observations suggest that MeJA could link to oxidative stress[5] MeJA have vast system of production of different enzymes which could protect against different stress during storage Ali *et al.*[6] Jasmonates: hormonal regulators or stress factors in leaf senescence. Grape fruit is one of the most important members of citrus species (*Citrus paradisi Macf*.) Mostly pigmented grapefruit varieties were planted in the world[4]. Grapefruit (*Citrus paradisi Macf*.) is most prominent fruit crops grown in world for local consumption and export. Judging by palatability and external appearance, the quality of grapefruits grown in Pakistan has been commended as superior to fruits grown in other leading citrus producing areas[7]. Pakistan has great potential to produce an export the high quality of grapefruits, harvesting methods and postharvest handling practices are still problem of many producers and distributors in whole world. Faulty harvesting and rough handling at the orchard directly affect fruit quality and nutritive values. The growers of grapefruit in world don't consider the cultural operation and quality related parameters which were

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directly effects on their shelf life. Modern era the consumption of plant growth regulators has been attention to grower for the improvement of the quality and shelf life of fruits. The application of pre and post harvest spray of modern growth regulators in fruit is a novel approach for extending the internal fruit quality and shelf life. Preharvest application of these growth regulators more effective after storage and maintained the shelf life of fruits. Different modern growth regulators available in world such as polyamines[8] methyl jasomante), chlorine dioxide[9] nitric oxide Singh et al.[10], oxalic acid[11] salicylic acid, 1-methylcyclopropene[9].These growth regulators were applied before and after harvest improves the quality and shelf life of grapefruit. The bioactive compounds mostly effect by several factors. These compounds rarely lower due to variety, growing periods environmental changes, plant nutritional level, production pattern, harvest maturity and cultural practices (training system, pruning, shading)[12],Shin et al.[13]. MeJA significantly improves these bioactive compounds and developed a vast enzymatic system which maintained fruit quality during storage[13], Rudell *et al.*[14], Fan *et al*.[15-16]. Growth regulators significantly improve the physiological process related to quality during storage. Different scientist were reported that growth regulators showed significantly effects quality ,resistance, improvement different postharvest diseases control and the reduction of these bioactive compounds during storage. (Kondo *et al*.[17-18]. Pre-storage MeJA application is important for improvement and maintained these bioactive compounds after storage. MeJA have a mechanism for maintained these bioactive compounds due to their fast gene linkage creation and formation of quick trigger system these genes important role to improvement these compound during storage[16] but there no enough studies to explain these mechanism and maintained these compounds during storage. Phenolic compounds, like any other antioxidant, protect cells from ROS, either by scavenging or by neutralizing free radicals[14]. The aim of this study to investigate the effects of MeJA on phytochemicals and fruit quality changes during and after storage maximum quantities of these phytochemicals present in grape fruit juice after storage.

2. EXPERIMENTAL SITE

Our experiment was conducted in Orange Research Station Sargodha in Pakistan during the mid-seasons. Fifteen uniform 12-year-old grapefruit trees grafted onto sour orange rootstock, 6 m *6 m spacing were used. Each tree was represented as a replicate. The trees were grown using similar cultural practices of irrigation, fertilization, pest management and weeding. The orchard soil texture was sand loamy, pH was 8.2, electrical conductivity (EC) was l.0 dS^m-1 and $CaCO_3$ content was 22.0%.

2.1 *Foliar application of Methyl jasomiante before harvesting of grapefruit*

Me JA was applied @ of 3 and 4 mM at 15 days interval before harvesting (115 days after full bloom). Triton X-100 was used as surfactant to enhance the mobility and efficiency of chemical. Distilled water was used as control treatment. The application was carried out in early morning. The volume of solution was measured and applied by calibration of[19]. Each treatment contained 1500 mL solution was applied in each selected tree. The calibration also considered according to height and row of tress.

2.2 *Harvesting of fruits and cold storage treatments of grapefruit*

125 fruits were randomly harvested from each tree side after harvest the fruit were measured the TSS and fruit firmness to insured the maturity standard. The reaming 20 fruit were used to determine the quality parameters and other 5 were used to determine the individual bioactive compounds at early harvesting time. All other fruit were placed in plastic trays and transferred to cold storage and stored at 8 °C temperature and relative humidity with (90 ± 5%). Storage period of these fruit were analyzed after 30, 60 and 90 days to find out the fruit change in quality and bioactive compounds and quality related enzymes.

2.3 *Color Characteristics, Weight Loss Changes during Cold Storage*

Fifty fruits from each treatment were stored in cardboard boxes in single rows. Weight loss and color changes were noted day after storage of 30, 60 and 90.

2.4 *Quality Related Properties of fruit*

2.4.1 Physiological Fruit weight loss

Ten fruits (n=10) were randomly selected from each treatment unit. These fruits were weighted as fresh and at 30 days internal during the storage period and weight was calculated using the following formula (Takur, 1996).

$$\text{PWL (\%)} = \frac{\text{Original Fruit weight- final fruit weight after storage}}{\text{Average fruit weight}} \times 100$$

2.4.2 *Fruit firmness*
Fruit firmness was measured with the help of penetrometer and expressed pressure necessary to force a plunger of specified size into the pulp of the fruit then average reading was calculated in the fruit.

2.5 *Chemical Characteristic of grapefruit*
Total soluble solids of juice were recorded by using digital hand refractometer (Atago, RX 5000 and Japan).

2.6 *Bioactive Compounds of grapefruit*
Five fruit each tree was washed after storage interval than samples were kept in -20 °C temperature for bioactive compound analyzed.

2.7 *Total Phenolic compounds*
Total phenolic contents (TPC) were calculated by using Folin-Ciocalteu reagent method as reported by Ainsworth and[22]. The FC-reagent (10 mL) was dissolved in distilled water to make the solution 100 mL. In each sample (100 mL), FC-reagent (200 μL) was added and vortex thoroughly. The 700 mM Na_2CO_3 (800 μL) was added into each sample and incubated at room temperature for 2 h. Sample (200 μL) was transferred to a clear 96-well plate and absorbance of each well was measured at 765 nm. Amount of TPC was calculated using a calibration curve for Gallic acid. The results were expressed as Gallic acid equivalent.

2.8 *Total Antioxidant Activity of grapefruit by two different standard method of ABTS+ and FRAP*
Grapefruit juice after day storage 30, 60 and 90 the total antioxidant activity was measured by two standards methods the $ABTS^+$ and FRAP reported by [20] ABTS+ radical scavenging activity of 2 mM and 2.45 mM of $K_2S_2O_8$ solutions were prepared by using 0.1 M of PO_4^{-3} buffer solution with pH =7.4 After the preparation of $ABTS^+$ and $K_2S_2O_8$ solutions they were mixed with ratio of 1:2.The ABTS+ $K_2S_2O_8$ was incubated for 6 hour. Then, the absorbance value for this mixture was read at 734 nm then 20 mL sample were taken of mixture into tubes, 1mL of of $ABTS^+$- $K_2S_2O_8$ solution and buffer solution were added to marked up volume of 4mL then values were read at 734nm. The result was expressed as mmol (TE)/g fw.

2.9 *Individual Phenolic Compounds*
Individual bioactive compounds were measured by HPLC with detector of 280 nm. The mobile phase contained of acetonitrile, water and formic acid. The mobile phase flow rate was set at 1 mL/min and the injection volume was 20 mL.

2.10 *Fruit quality related enzymes extraction*
Enzymes (POD), SOD was determined according to Lu *et al.*[21] with slight modifications. Briefly, 10 ml of fresh juice sample were extracted with 30 mL phosphate buffer 50 mM, pH = 6.8. After Days centrifugation at 15,000 × g for 30 min at 4°C, the supernatant was used as enzymatic extract for peroxidase quantification in a reaction mixture containing: 50 mM phosphate buffer pH = 7.0,12 mM H_2O_2, 7 mM guaiacol and 100 L of enzymatic extract in a total volume of 3 mL. For catalase (CAT) and ascorbate peroxidase (APX) activities, the protocol described by[23].

2.11 *Statistical Analysis*
Collected data were statistically analyzed using computer software MSTAT-C. Analysis of variance was used to test the significance of variance. While difference among treatment means were compared using LSD test (P=0.05) Steel *et al*[22]. Standard errors (SE) were computed by MS-Excel and data were presented graphically using the same program.

3. RESULTS AND DISCUSSION
3.1 *Effects of MeJA on Fruit Color changes during storage*
Fruits treated with Me JA showed maximum fruit colour as compared to fruit of untreated (Table-1).

Table: 1 Effects of Methyl Jasomiante treatment on colour of fruit during storage of grapefruit

Treatments	0 days	30 days	60 days	90 days
Control	3.3 b	3.6a	3.7a	3.8a
3mM, MeJA	3.6b	3.8b	4.0a	4.4a
4mM, MeJA	3.8b	3.9b	4.2a	4.3a LSD =1.21

Color of fruit (C*, L*, h° values) increased during storage. Fruit treated with Me JA @ 4 mM showed higher values of L*. The greatest changes in hue angle were showed that reddening in fruit color by application of MeJA. Higher doses of MeJA showed significantly improves the fruit color during storage[23] Krishna *et al*[24]. Me JA have capacity to increase the ethylene production rate in fruit during development stage and enhanced ripening[24]. Fruit color is one of the important characteristics for quality of grapefruit. The fruit color is mostly contributed for quality these colour related changes due to different compounds such as anthcyanins and total carotenoids accumulation[10]. MeJA stimulates ethylene and improved the repining and breakdown of chrophyll and development for accumulation of carotenoids[24].

3.2 *Total Soluble Solids changes during storage*

Fruit were applied Me JA @ 4mM showed higher TSS as compared to fruits of control. Maximum TSS was recorded after 90 days storage (14.3 °brix) (Table-2).

Table: 2 Effects of Methyl Jasomiante treatment on TSS (°Brix) of fruit during storage of grapefruit

Treatments	0 days	30 days	60 days	90 days
Control	12.1 c	13.7 b	14.0 a	11 .6d
3mM, MeJA	12.1 d	13.8 c	14.5a	13.9bc
4mM, MeJA	12.3 d	13.6c	14.8a	14.3b LSD =1.21

Total soluble solid were increased during storage period 30, 60 90 days and lower TSS were recorded in untreated fruit (11.6 °brix) at end of storage. TSS of grapefruit is directly related to aroma and taste. Total soluble solids are a key factor to judge the fruit quality. For determination of fruit quality, SSC, titratable acidity, firmness, size and color are main criterion of consumer for selection of any fruit Hoehn et al.[25] It is understood that soluble solids concentration is increased during storage period as a result of insoluble starch conversion into soluble solids. This change in soluble solids concentration may be correlated with hydrolytic regulation of starch concentrations during postharvest storage which ultimately results in starch conversion (breakdown) to sugars (key fruit ripening indicator)[26]. Our results indicate that higher concentrations of Me JA @ 4 mM increased the levels of TSS in fruits as compared to the fruits of control.

3.3 *Fruit firmness changes during storage of grapefruit*

Fruit firmness showed more in early storage (0, 30 days) then decreased (60, 90 days) of storage (Table-3) Lower fruit firmness was (50.31Nm^2) and higher fruit firmness were showed in control (60.4 Nm^2)) respectively.

Table: 3 Effects of Methyl Jasomiante treatment on fruit firmness (Nm^2) of fruit during storage of grapefruit

Treatments	0 days	30 days	60 days	90 days
Control	52.84 c	51.83d	55.75 b	60.41a
3mM, MeJA	55.23a	49.6b	43.65c	37.51d
4mM, MeJA	53.40a	39.95b	38.5.26c	34.31d LSD =0.12

There is correlation between total soluble solid and fruit firmness during storage in our study. Fruit firmness and TSS play important role in fruit quality and improvement shelf life of grape fruit. Grower is linked market for longer times due to improvement quality by application of Me JA.

Rudell et al[14] reported that fruit firmness of lemon was improved by pre harvest spray of different growth regulators before and after cold storage. Different scientists were reported that fruit firmness and quality related changes due to improper harvesting time and maturity level of fruit for storage[28]. Fruit become more softer during storage due to break down of pectin and cell wall layer which is major caused for lower shelf life of fruits in storage[20-28] reported that decreased fruit firmness in "Fuji" apples by increased the application of Me JA. In present study Me JA application showed lower fruit firmness as compared to untreated fruit. Gonzalez- Aguilar et al.[29] conduct experiment on different fruit and prove that fruit firmness decreased significantly during storage. Me JA application reduced the fruit firmness due to different enzymes creation which caused lower fruit firmness. Fan *et al.*[30] reported that fruit firmness was lower due to polygalacturonase biosynthesis was inhibited by Me JA application.

3.4 *Physiological fruit weight loss during storage*

Fruit those were treated with Me JA showed no significantly change in fruit weight loss after 30 days. Application of Me JA treated fruit showed lower fruit weight loss as compared to fruit those were untreated. Fruit weight loss in control more after day storage of 60, 90 days than the fruit those were treated. Lowest fruit weight loss was noted in fruit those were treated with Me JA @ 3 and 4 mM (Table-7). Fruit weight losses are known as most significant physiological disorder during postharvest life. Low fruit losses during storage in treated fruit also singled the superiority of MeJA by reducing the respiration, transpiration and metabolic activities of fruit and these three activities are directly related with fruit weight loss during storage.

Table: 7 Effects of Methyl Jasomiante treatment on fruit weight loss of fruit during storage of grapefruit

Treatments	0 days	30 days	60 days	90 days
Control	0 d	10.1a	9.1b	12 12c
3mM, MeJA	0d	2.53c	3.17b	4.32a
4mM, MeJA	0d	2.84b	2.43b	3.16a LSD =0.12

Treatment of MeJA cause the hindrance in respiration by generating free radicals[31] by closing stomata Manthe *et al*.[10-32] and slowing down respiration which may have ultimately reduced the weight loss of fruit. The finding of also shown that strawberry fruits showed less fruit weight loss than control when salicylic acid was supplied accomplished with nutrients.

3.5 *Effects of MeJA on Total Phenolic compounds changes during storage*

Fruit those were treated with Me JA showed significantly maintained TPC during storage (Table 4) Maximum TPC were found in fruit that treated with Me JA @ 4 mM 0.70 mg GAE/100 g after 30 days storage and minimum was noted in fruit those were untreated. Me JA application significantly maintained phenolic compounds during storage.

Table: 4 Effects of Methyl Jasomiante treatment on total phenolic compounds (mgGAE/100g) of fruit during storage of grapefruit

Treatments	0 days	30 days	60 days	90 days
Control	0.61a	0.47 b	0.46 c	0.39 d
3mM, MeJA	0.72a	0.72.1 a	0.71b	0.70.1c
4mM, MeJA	0.70a	0.70a	0.69.5a	0.69 b LSD = 0.12

While untreated were showed more reduction (0.39 mg GAE/100 g). Phenolic being secondary plant metabolites are synthesized by all plants. These compounds are responsible for the flavor and taste of products Jeong *et al.* [33] Robert *et al.* [34] stated that phenolic is involved in several functions such as nutrient absorption in plants, protein synthesis, enzymatic activities and photosynthesis. Many phenolic compounds act as antioxidants, but in higher quantity they become browning substrates. PPO and reactive oxygen species involves reduction of these phenolic compound during storage Robarts *et al.*[34]. Fruits treated with MeJA @ 4mM showed minimum reduction in phenolic compound during the storage (maintained) as compared to the fruits those were without treatments (control). The loss of phenolic compounds during storage can be associated with several enzymatic and non-enzymatic reactions, ethylene production being superior[35] Similar findings have also been described by Huang *et al.*[36] who reported that that MeJA treated 'Cara cara' navel oranges showed increased total phenolic content, higher concentration of MeJA having more profound effect in this respect.

3.6 *Fruits during storage of Total Antioxidant Activity (ABTS+ and FRAP)*

TAA were maintained in all treatment but in control treatment it's decreased (Table-5).

Table: 5 Effects of Methyl Jasomiante treatment on Total antioxidant activity of fruit during storage of grapefruit

Treatments	0 days	30 days	60 days	90 days
Control	19.50a	18.5b	17.3c	14.1d
3mM, MeJA	20. 2d	24.09b	25.34a	23.1c
4mM, MeJA	21.91c	25.73a	25.1a	24.2b LSD =0.14

Maximum TAA was recorded by treatment of Me JA @ 4mM (25.1 mmol TE/g fw)) after 60 days of storage. ABTS test were showed lowest values in control treatment (14.1) the highest values was obtained from 4mM of MeJA treatment and lowest values at 0 days (25.73). Any other test FRAP also proved same results if Me JA @ 4mM showed maximum FRAP values (12.43 mmol TE/g fw) in Table-6.

Table: 6 Effects of Methyl Jasomiante treatment on FRAP of fruit during storage of grapefruit

Treatments	0 days	30 days	60 days	90 days
Control	10.83bc	11.13a	10.04b	9.65b
3mM, MeJA	10.40c	11.53b	12. 00a	11.37b
4mM, MeJA	10.2c	11.22b	12. 43a	12.16a LSD =0.14

Fruit of untreated showed lowers values (9.65 mmol TE/g fw). They exist generally as flavonols in fruit peel[33]. There is also evident that exogenously applied MeJA with suitable dose enhanced the efficiency of antioxidant system in plants Hayat *et al.*[37] Antioxidants are compounds capable of quenching ROS without undergoing conversion, themselves, to destructive radicals[38]To ascertain dietary importance of fruits and vegetables it is also important to estimate their antioxidant activity. Higher concentration of MeJA maintained TAC during storage which might be due to counteracted balancing between increased free radicals and increased FRSA. TAA which might be due to imbalance electronics structure and more leakage of cell membrane. Results are supported by the previous findings of various scientists[37]. The other reason for lower reduction in TAA contents changes might be due to decreased respiration rate and prevents fruit senescence during storage. It seems that these compounds prevent enzymatic activities which have a role in anthocyanin synthesis path way that slowing down ripening process. It is well documented that Me JA affects to trigger many physiological events including colour in fruit[16].

3.7 *Individual phenolic compounds changes during treatment application and storage*

The results were showed in Table 8.

Table: 8 Effects of Methyl Jasomiante treatment on individual phenolic compounds of grape fruit after storage

Individual Phenolic	Treatments	0 days	30 days	60 days	90 days
Chlorogenic acid	Control	3.68 a-A	4.62 a-B	6.19. 5 a-C	7.4 7 b-D
	3mM, MeJA	6.23 b-C	10.26 a-C	11.32 b-BC	13.30 a-AB
	4mM, MeJA	7.15 c-B	11.96 c-B	12.27 c-B	14.84 b-A
Ferulic acid	Control	2.23 a-A	2.12 a-A	1.94 a-A	1.42 b-A
	3mM, MeJA	1.80 b-D	2.34 b-C	2.72 b-C	10.45 a-B
	4mM, MeJA	2.34 b-B	2.559 b-B	2.99 b-B	3.33 c-B
Epicatechin	Control	0.8 b-A	0.06 a-A	0.04 a-A	0.03 a-A
	3mM, MeJA	0.14 b-A	0.0 8 a-A	0.09 a-A	0.07 a-A
	4mM, MeJA	1.38 a-A	0.39 a-B	0.41 a-B	0.40 a-B
p-Coumaric acid	Control	2.56 a-A	2.53 a-A	2.40 a-AB	2.39 a-AB
	3mM, MeJA	2.48 b-A	2.70 ab-A	2.79 a-A	2.56 a-A
	4mM, MeJA	2.80b-B	2.89 b-B	2.97 a-B	2.95 a-B
Caffeic acid	Control	3.19 a-A	2.21 a-AB	2.89a-AB	2.90 b-AB
	3mM, MeJA	4.55 a-B	3.59 a-B	3.24 a-B	4.63 a-A
	4mM, MeJA	4.88a-A	3.89 a-A	3.97 a-A	5.07b-A
Rutin	Control	2.38 a-D	3.03 a-C	4.21 b-B	5.03 a-A
	3mM, MeJA	4.79 a-E	5.17 b-D	6.55 b-C	7.24 a-B
	4mM, MeJA	4.98 a-D	5.60 a-C	7.90 a-B	8.40 a-B
Naringenin	Control	2.56 a-A	2.38 a-A	2.13 ab-AB	1.78 b-BC
	3mM, MeJA	1.43 b-C	2.33 a-A	1.98 b-AB	1.86 ab-BC
	4mM, MeJA	1.44 b-B	2.52 a-A	2.47a-A	2.28 a-A
Quercetin	Control	2.28 a-C	2.67 a-BC	3.59 a-A	2.99 b-B
	3mM, MeJA	2.19 a-D	2.63 a-BC	2.76 b-AB	3.16ab-A
	4mM, MeJA	2.36 a-B	2.58 a-B	3.36 a-A	3.46 a-A
Kaempferol	Control	3.31 b-A	3.90 a-B	2.81 b-C	2.44 b-C
	3mM, MeJA	4.25 b-A	3.90 a-B	3.78b-C	3.60 b-CD
	4mM, MeJA	5.07 a-A	4.99 a-B	4.80 a-BC	3.90 a-C

Me JA application significantly increased chlorogenic acid during storage. While it may decreased in untreated fruit. The ferulic acid, P -coumaric acid and catechin increased by MeJA application. The values of kaempferol, rutin, caffeic acid, epicatechin and naringenin were maintained during storage but untreated reduced these compounds after storage. Day after storage 30, 60 and 90 showed that the values of ferrulic acid, chlorogenic acid and epicatechin were different from each other's. Our results were showed that there is a relation between antioxidant activity and total phenolic compounds. These result further reported Rupasinghe *et al.*[52] in Plum. TPC significantly increased by MeJA treatments the chlorogenic acid were increased but decreased in untreated fruit. Ozoglu et al.[53] conduct experiment on "Rio red" variety of grape fruit showed significantly maintained the phenolic compounds during storage. There were alots of factors which effects the changes of phenolic compound such as variety, root stock, nutrient uptakes and cultural practices maturity storage methods[39-40] Scalzo *et al.* [41]. Phenolics and antioxidant significantly improves by growth regulators application (before and after storage)[16]. Kondo et al.[17] reported that jasmonates application before harvesting showed significant improves the antioxidants and phenolic compounds, therefore Me JA different concentration applied different growth stage also needs for study on the fruits. Grape fruit juice contained the abundant amount of phenolic compounds, flavonoids and antioxidant activity which is role in antioxdative stress anti-viral, anti-cancer, anti-inflammatory activities and an observed inhibition of human platelet aggregation of cell. Wang et al.[18] reported that the antioxidant activity in grape fruit was more as compared to apple. Foliar application of MeJA before harvesting showed significantly increased phenolic, flavonoids content in fruit[42]. Different scientist were reported in different crops such as strawberries, Plum showed application of MeJA significantly improves the total phenolic compounds[42], Diaz-Mula *et al.*[43].Total phenolic improves by application of different doses of Me JA on different fruit such as red plums, apricot and lemon Karakaya *et al.* [44], Proteggente *et al.*[45]. Khan and Singh, [30] reported in his experiment that phytochemicals of fruit were improves due to Me JA spray. Amakura *et al.*[47], Kim *et al.*[40] reported that quercetin and chlorogenic acid were more in grapefruit. Usenik *et al.* [49] reported that rutin and chlorogenic acid contents of cherry increased by application of Me JA. Lombardi *et al.* [48] explained that ferulic acid, neo-chlorogenic acid, chlorogenic acid kaempferol and p-coumaric acid are rich source of these phenolic compounds in grape fruit. Slimestad *et al.* [49] conduct experiment on grape fruit that contained maximum amount of rutin content in his study.

3.8 *Quality related enzymes of grapefruit*

3.8.1 *Ascorbate peroxidase (APX)*

Fruit treated MeJA @ 4 mM showed maximum peroxidase activities after 90 days storage as compared to fruit of untreated. Untreated fruit reduces the peroxidase activities after 90 days storage.

3.8.2 *Catalase (CAT)*

The result were presented in figure-1 (b) showed maximum catalase activities was recorded in 90 days stored fruit and lower values was recorded in untreated fruit.

3.8.3 Polycultrase (Poly)

The result showed in figure-1 (c) maximum poly was observed after 90 days storage. The values of Poly were 0.05 µg/L, while lower values and continuous reduction was observed in untreated fruit. The trend show in storage was that the gurally increased the enzymatic activities

3.8.4 *Peroxidase (POD)*

The result showed in figure-1 (D) the maximum values was recorded in 90 days stored fruit, while lower values was noted in untreated fruit.

3.8.5 *Superoxide dismutase (SOD)*

The result showed in figure-1 (D) the maximum values was recorded in 90 days stored fruit, while lower values was noted in untreated fruit. Enzymes have ability to active during postharvest due to catalyze the fruits cells. Inside the fruit the ezymes involved in different reaction , SOD and CAT are the quality related enzymes which enhanced the quality and increased the sequence based protein and regulator the cell membrane which improve the taste and other quality related parameters. Polygalacturonase (Poly; APX) hydrolyzes the glycosidic bonds of deesterified polygalacturonides, producing sugars and organic acids. Increased the constitutes level of these enzymes that cause fruit texture degradation and result in losses of galacturonic acid which lead to more softing of fruit hence caused the pathogen attack more[50]. MeJA have capacity to modification these enzymes and improves the metabolites which promote disease resistance genes that altering the internal concentrations of ROS like superoxide anion ($O2^-$) and hydrogen peroxide. These enzymes have direct involves the antimicrobial activity, developed a mechanism of singling pathway to active the enzymatic links with membrane and cellular components which improves and maintained the quality during storage[51]. The enzyme of POD which cause the major oxidation of phenolic and resulting the fruit spoiling). POD can oxidize phenols to quinones, then condense tannins to brown polymers in the presence of H_2O_2, which may then contribute to enzymatic browning POD activity was inhibited by the MEJA application of these enzyme type has not been assigned a precise location within a metabolic pathway and that its biological function is not clear yet[47].

4. CONCLUSIONS

Grapefruit juice is rich source of different phytochemical which play a vital role for health improvement. In this study it proves that before harvesting the application of MeJA significantly maintained these essential compounds during and after storage. The best doze for application of MeJA @ 4mM. MeJA application on different fruits for their developmental stages needs attention to scientists for better fruit quality. Per harvest application of MeJA is significantly improves the defense mechanism in grapefruit during storage. Application of MeJA have sigficant effects on quality related enzymes and block the PPO activities which caused the major fruit browning during storage.

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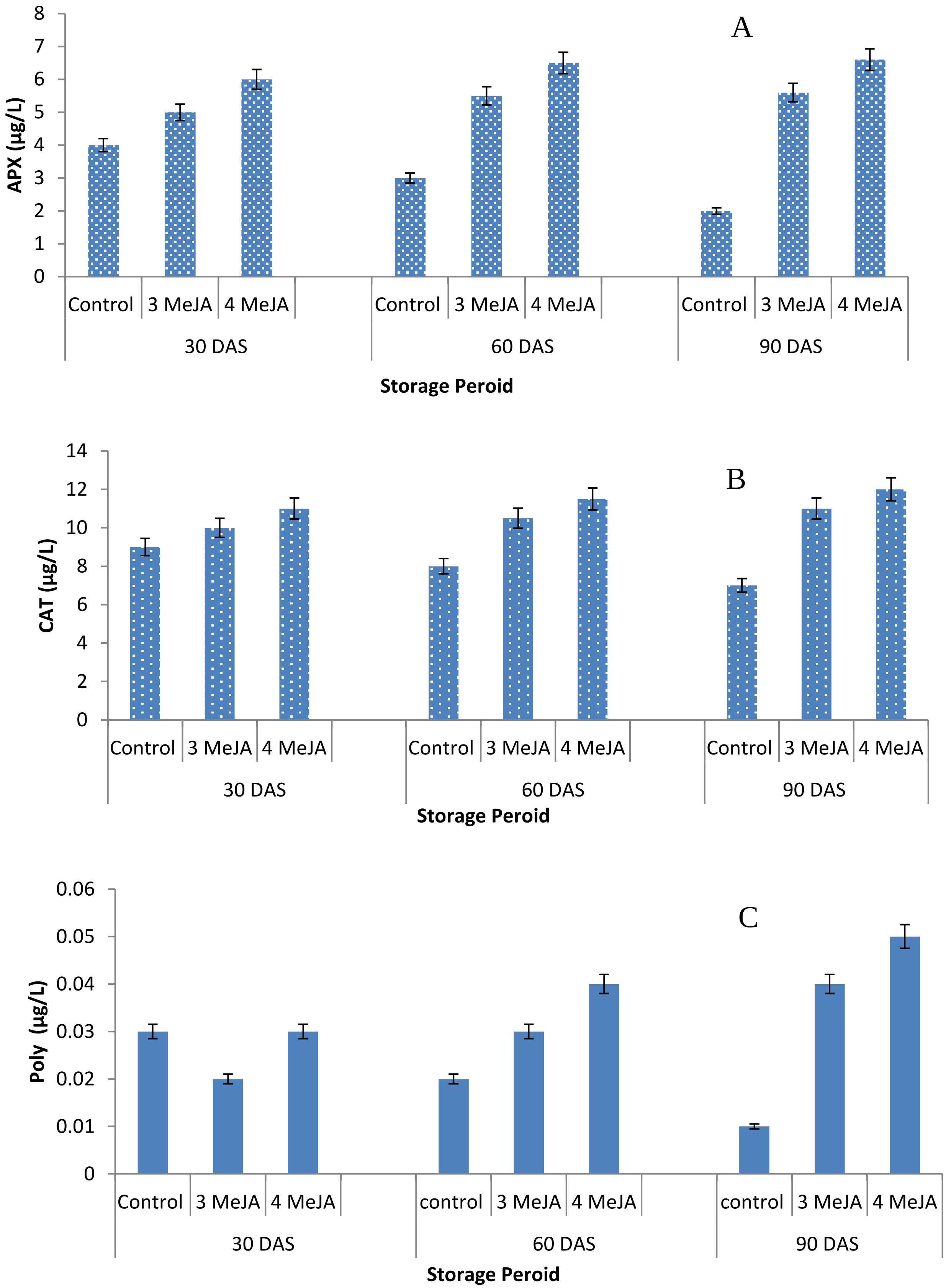
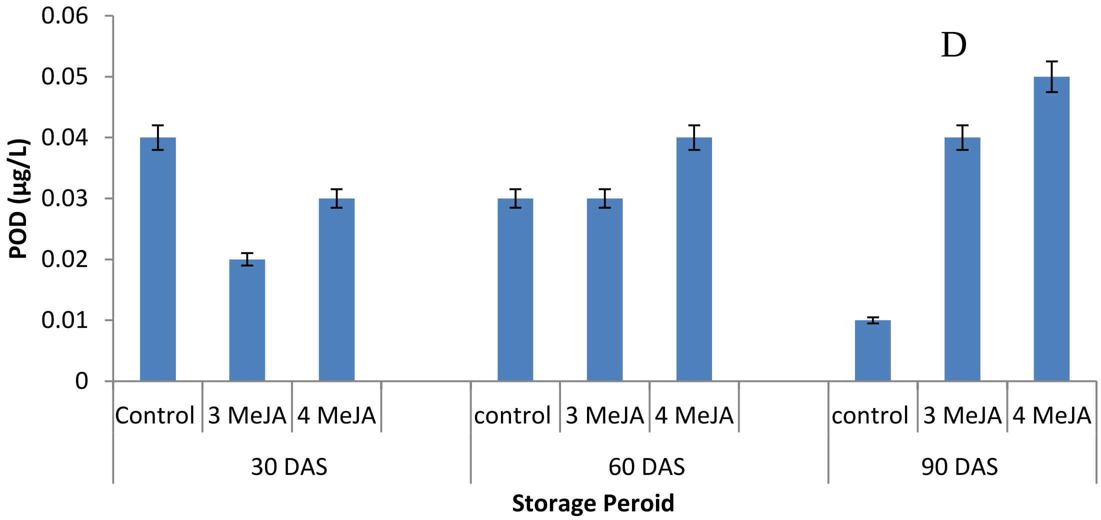
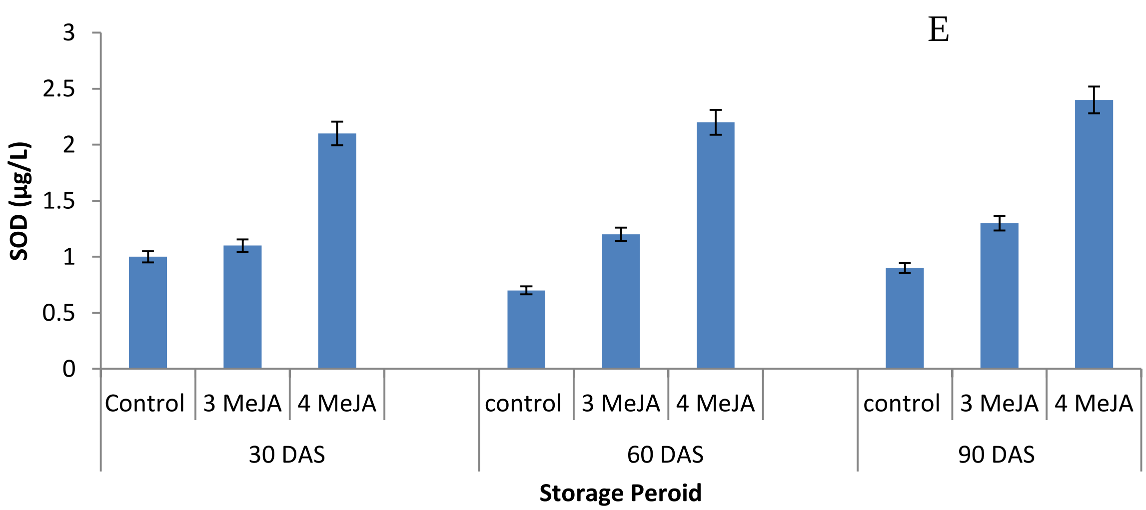


The difference between mean values shown on the same column but lower letter is non-significant (*P* < 0.05). Ten fruit for each replication.
LSD= 0.14





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