INFLUENCE OF GIBBERELLIC ACID CONCENTRATIONS AND APPLICATION TIME ON PRE AND POST HARVEST PERFORMANCE OF LIMEQUAT (*Citrus floridana*).

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ABSTRACT: *The influence of gibberellic acid concentrations and application time on pre and post-harvest performance of limequat (cv. China Lime) was investigated in District Nowshera, KPK, Pakistan during 2016. The experiments were laid out in Randomized Complete Block Design (RCBD) and Completely Randomized Design (CRD) with two factors repeated three times. Different concentrations of GA_3, i.e. 0, 5, 10 and 15 ppm were used at 0, 7, 14 and 21 days interval after the cell expansion stage. The pre-harvest experiment results indicated that minimum fruit drop (4.49%), maximum number of fruits plant^{-1} (562.22), fruit diameter (3.23 cm), yield plant^{-1} (17.62 kg), juice content (36.90%), titratable acidity (6.00%), ascorbic acid (24.08mg100ml^{-1}), with minimum juice TSS (5.90^{o}Brix) was observed in fruits treated with 15 ppm of GA_3. In application time, minimum fruit drop (5.54%), maximum number of fruits plant^{-1} (540.92), fruit diameter (3.27 cm), yield plant^{-1} (17.39 kg), juice content (36.94%), titratable acidity (5.95%), ascorbic acid (23.89mg100ml^{-1}) with minimum juice TSS (5.99^{o}Brix) was recorded at the onset of cell expansion stage. In postharvest experiment, the GA_3 significantly affected all the quality parameters of limequat fruit. Minimum weight loss (15.15%) with maximum juice content (31.85%), titratable acidity (5.86%), ascorbic acid (19.57 mg100ml^{-1}), and minimum juice TSS (6.68^{o}Brix) was recorded in fruits treated with 15 ppm of GA_3. Similarly in storage durations, the highest TSS (7.90^{o}Brix), weight loss (32.36%) with minimum juice content (22.96%), titratable acidity (5.66%) and ascorbic acid (12.54mg100ml^{-1}) were recorded in fruits stored for 21 days of storage. It is concluded from the current study that GA_3 application at 15 ppm increased the yield and quality of limequat fruit. GA_3 application at the onset of cell expansion stage might be done to reduce the pre and postharvest loses of limequat fruit.*

Key words: GA_3 concentrations, Application Time, Yield, Quality, Limequat.

INTRODUCTION

Limequat (*Citrus floridana.*) fruit is the fundamental crop of the world which belongs to family Rutaceae. Limequat is developed by Walter T. Swingle in 1909 and this was produced by the crossing of key lime and kumquat having flavor, tender, juicy, evergreen and of vigorous nature tree. The limequat were originated in Florida and USA and were spread in Middle East and subtropical region [1].The leading five countries in citrus production are USA, Spain, India, Argentina and Iran [2]. The pre and postharvest losses in citrus is affected by several factors. The pre-harvest factors include temperature, rain, relative humidity and the post-harvest factors include harvesting, handling, and packaging that can cause the post-harvest losses of fruits [3]. The yield and quality of limequat fruit is seriously affected by temperature and other stresses. Gibberellic acid is known to increase the fruit size, yield and quality of limequat fruit. Limequat fruit storability is very short due to high perishability and its shelf life losses are high during storage [4].This research was planned to evaluate the influence of gibberellic acid on pre and post-harvest performance of limequat fruit.

MATERIALS AND METHODS

The research entitled "The influence of gibberellic acid concentrations and application time on pre and post-harvest performance of limequat" was performed at District Nowshera, Khyber Pakhtunkhwa, Pakistan during 2016.

Experimental Design

The experiment was laid out in Randomize Complete Block Design with 2 factors. Treatments were repeated thrice. The trees were sprayed with different concentrations of GA_3 i.e. 0, 5, 10 and 15 ppm after 0, 7, 14 and 21 days of cell expansion stage. Before the application of GA_3 it was important to know the cell expansion stage. For observing the cell expansion stage, after every 7 days the fruit samples were collected from the plants and putted in a beaker or cylinder for observing the cell division process. When the fruits started floating on the surface of water it indicated the end of cell division stage and the fruit entered into cell expansion stage [5].

Parameters to be studied:

Fruit drop (%)

Fruit drop percentage was calculated by using the following formula:

$$\text{Fruit drop \%} = \frac{\text{Total number of dropped fruits}}{\text{Total number of fruits after application}} \text{ x } 100$$

Fruit diameter (cm)

Diameter of fruit was determined randomly with vernier caliper from each treatment.

Yield per plant (kg)

Yield per plant was calculated by weighting the fruit at the time of harvesting and average weight was taken.

Total juice content (%)

Juice from fruit was extracted and weighted. Usual juice weight was measured independently for each treatment. The average juice (%) per fruit was determined by the below formula:

$$Juice\ \% = \frac{Total\ juice\ weight}{Fruit\ weight} \times 100$$

TSS (^{o}Brix)

Total Soluble Solids were measured with the help of digital refractometer by putting 1-2 drops of juice on the prism of refractometer and reading was recorded.

Acidity (%)

Acidity of limequat juice was determined by the method as approved by AOAC (1990) [6].

Ascorbic Acid (mg/100ml)

Ascorbic acid of the fresh and treated fruits was found by dye method as approved by AOAC (1990) for all treatments [6].

Storage Experiment: The experiment was conducted in Completely Randomized Design (CRD) with two factors i.e. factor A was fruit treated with foliar application of different levels of gibberellic acid, i.e. 0, 5, 10, and 15 ppm at the onset of cell expansion stage and factor B was storage durations i.e. 0, 7, 14, and 21 days.

The following parameters were studied during the experiment:

Fruit Weight loss (%)

Average fruit weight loss % was calculated by the following formula:

$$\text{Weight loss \%} = \frac{\text{Fresh weight-Final weight}}{\text{Fresh weight}} \text{ x } 100$$

Total juice content (%), TSS (^{o}Brix), Acidity (%) and Ascorbic Acid (mg100ml^{-1}) were determined according to previous procedures as mentioned in pre harvest experiment.

Data Analysis

Data analysis was done on statistix 8.1 software and the means were compared with the help of LSD test [7].

RESULTS AND DISCUSSION

Fruit drop (%)

The mean data pertaining fruit drop (%) are given in table-1 demonstrated that the GA_3 concentrations and application time significantly affected the fruit drop (%), while their interaction was found non-significant. The mean data regarding GA_3 concentrations show that maximum fruit drop (9.93%) was observed in control, while minimum fruit drop (4.49%) was observed in fruits treated with 15 ppm of GA_3 after cell division stage. In application time, maximum fruit drop (9.38 %) was observed in fruits treated with GA_3 after 21 days of cell expansion, while minimum fruit drop (5.54%) occurred in the fruits, which were treated directly at the onset of cell expansion stage.

GA_3 significantly affected the fruit and flower retention and thus decreased fruit drop. PGRs had an important role in fruits and flowers retention and also enhanced the sink and source association and translocation of photoassimilates thus helping in flower and fruit set and reduced fruit drop [8]. Pre harvest fruit drop was a major problem in horticulture sector and PGRs had ability to delayed rind color, fruit softening and the pre harvest fruit drop [9].

Number of Fruits plant^{-1}

The mean data about number of fruits plant^{-1} are presented in table-1 revealed that GA_3 concentrations and application time significantly affected the number of fruits plant^{-1}, while their interaction was found non-significant. The mean data shows that highest number of fruits plant^{-1} (562.22) was recorded with GA_3 application at 15 ppm, while the lowest number of fruits plant^{-1} (438.06) was recorded in untreated plants. In application time, maximum number of fruits plant^{-1} (540.92) were recorded at the onset of cell expansion stage, while minimum number of fruits plant^{-1} (455.06) were noticed in plants sprayed 21 days after cell expansion stage.

Exogenous application of GA_3 increased the total number of fruits plant^{-1} in mandarins [10]. The foliar application of GA_3 after full blooming stage may enhance the number of fruits plant^{-1} in sweet orange [5]. GA_3 reduced the biosynthesis of ethylene and reduced the fruit drop thus tend to produced more number of fruits [11]. Cell division and cell elongation were stimulated by the foliar application of GA_3 which increased the fruit weight and yield [12].

Fruit Diameter (cm)

The mean data regarding fruit diameter (cm**)** are presented in table-1, revealed that GA_3 concentrations and its application time significantly affected the fruit diameter (cm**)**, while their interaction was found non-significant. Mean data shows that maximum fruit diameter (3.23cm) was observed in GA_3 application at 15 ppm, while minimum fruit diameter (2.99cm) was observed in control treatment. Mean data regarding time of GA_3 application show that maximum fruit diameter (3.27cm) was recorded at the onset of cell expansion stage, while minimum fruit diameter (2.96cm) was noticed in plants sprayed after 21 days of cell expansion stage.

Gibberellic Acid involves in cell division and cell enlargement which increased and improved the fruit size in sweet cherry [13]. Gibberellic Acid at different concentrations increased the fruit diameter in citrus [14]. Gibberellins was involved in the plasticity of the cell wall and hydrolysis of starch into sugars which reduced the water potential of the cell, water entered into the cell and caused cell elongation [15].

Yield plant^{-1}(kg)

Mean data about yield plant^{-1} are existing in table-1 showed that concentrations of GA_3 and its application time significantly influenced yield plant^{-1}, while their interaction was found non-significant. Mean data revealed that maximum yield plant^{-1} (17.62 kg) was observed with GA_3 application at 15 ppm, while minimum yield plant^{-1} (14.11 kg) was observed in control treatment. Mean data of application time demonstrated that maximum yield plant^{-1} (17.39 kg) was recorded at the onset of cell expansion stage, while minimum yield plant^{-1} (14.37 kg) were noticed in plants sprayed 21 days after the cell expansion stage.

Pre harvest foliar application of Gibberellic Acid has significant effect on cell elongation in fruits and yield in citrus species [16]. GA_3 significantly increased the yield in strawberry when it was applied in the form of foliar application [17]. GA_3 develops sink strength in the fruit cells, thus facilitates the movement of water and nutrients [18].

Table 1. Fruit drop, No. of fruits plant^{-1}, fruit diameter, and Yield plant^{-1} as affected by Gibberellic acid concentrations and application time.

GA_3 Conc. (ppm)	Fruit drop (%)	No. of fruits plant^{-1}	Fruit Diameter (cm)	Yield plant^{-1} (kg)
0	9.93 a	438.06 c	2.99 c	14.11 c
5	7.88 b	478.94 b	3.07 bc	15.49 b
10	5.87 c	535.06 a	3.11 b	16.86 a
15	4.49 d	562.22 a	3.23 a	17.62 a
Days of application after cell expansion stage				
0	5.54 c	540.92 a	3.27 a	17.39 a
7	6.05 c	528.17 a	3.13 b	16.52 ab
14	7.21 b	490.14 b	3.04 c	15.80 b
21	9.38 a	455.06 c	2.96 c	14.37 c
LSD	0.80	33.00	0.08	0.87
GA_3* Days of application				
Significance	NS	NS	NS	NS

Fruit Juice content (%)

The mean data regarding fruit juice content (%) are existing in table-1 demonstrated that GA_3 concentrations and its application time significantly affected the fruit juice content (%), while their interaction was found non-significant. Mean data for GA_3 revealed that highest fruit juice content (36.94%) was observed in GA_3 application at 15 ppm, followed by GA_3 at 10 ppm (35.64 %), while minimum fruit juice content (33.57%) was observed in control treatment. In application time, maximum fruit juice content (36.94%) was recorded at the onset of cell expansion stage, followed by (36.00%) 7 days of foliar application after cell expansion stage, while minimum fruit juice content (33.51%) was noticed in plants sprayed after 21 days of cell expansion stage.

The growth regulators had a significant effect on various fruit quality attributes and specially GA_3, which inhibit the ethylene production and maintains the juice content and other quality aspects of the fruit [19, 20]. Since chlorophyll degradation was delayed in GA_3 treated plants which retained the quality attributes of fruits [21]. Plant hormones played a regulating role in the mobilization of metabolites within a plant and developing fruits were extremely active metabolic "sinks" which mobilized metabolites and directed their flow from vegetative structure and hence increased the juice content [4]. GA_3 significantly affected the juice content percentage in mandarins [22].

Total soluble solids (^{o}Brix)

Mean data regarding Total soluble solids (^{o}Brix) are existing in table-2, showed that the concentrations of GA_3, application time and their interaction significantly affected the fruit TSS. Maximum fruit TSS (6.37^{o}Brix) was observed in plants treated with distilled water after 21 days of cell expansion stage. However, minimum fruit TSS (5.63^{o}Brix) was recorded in plants treated with 15 ppm of GA_3 at the onset of cell expansion stage.

Pre harvest GA_3 applications are known to increase a number of postharvest fruit properties in citrus [23]. Increased in TSS might be due to further assimilation and accumulation of photosynthetic activity in the plant [24]. Since chlorophyll degradation was delayed in GA_3 treated plants which retained the quality attributes of fruits [21].

Titratable Acidity (%)

The mean data regarding Titratable Acidity (%) are expressed in table-2, demonstrated that the concentrations of GA_3, application time and their interaction significantly affected titratable acidity (%). Maximum titratable acidity (6.07%) was recorded in plants treated with foliar application of GA_3 at 15 ppm at the onset of cell expansion stage, while the minimum titratable acidity (5.83%) was found in plants sprayed with distilled water after 21 days of cell expansion stage.

Acidity in fruits that received pre-harvest GA_3 applications was higher as compared to those that were untreated [25]. Pre harvest GA_3 applications are known to increase a number of postharvest fruit properties in citrus [23]. Pre and post-harvest application of growth regulators resulted in higher titratable acidity than in control treatments [26].

Ascorbic Acid (mg100ml^{-1})

Mean data about the Ascorbic Acid is existing in table-2, demonstrated that GA_3 concentrations, application time and their interaction significantly affected the Ascorbic acid content (mg100ml^{-1}) of limequat fruit. Maximum Ascorbic Acid content (25.10 mg100ml^{-1}) was observed in plants treated with foliar application of GA_3 at 15 ppm at the onset of cell division stage, while minimum Ascorbic acid content (21.47 mg100ml^{-1}) was recorded in plants treated with distilled water after 21 days of cell division stage.

Exogenous application of PGRs plays an effective role in maintaining ascorbic acid content in fruits [27]. Pre and post-harvest application of growth regulators induced higher ascorbic acid content than in control treatment [26]. Maximum ascorbic acid content was found in GA_3 treated fruits as compared to untreated [28].

Table 2. Juice content, TSS, Acidity and Ascorbic acid content as affected by Gibberellic acid concentrations and application time.

GA_3 Conc. (ppm)	Juice content (%)	TSS(^{0}Brix)	Acidity (%)	Ascorbic acid mg100ml^{-1}
0	33.57 c	6.30 a	5.86 d	21.67 d
5	35.13 b	6.22 b	5.88 c	22.70 c
10	35.64 ab	6.07 c	5.93 b	23.58 b
15	36.90 a	5.90 d	6.00 a	24.08 a
Days of application after cell expansion stage				
0	36.94 a	5.99 c	5.95 a	23.89 a
7	36.00 ab	6.09 bc	5.93 b	23.13 b
14	34.75 bc	6.15 b	5.90 c	22.68 c
21	33.55 c	6.25 a	5.88 d	22.33 d
LSD	1.33	0.074	0.014	0.36
GA3* Days of application				
Significance	NS	*Fig 1	*Fig 2	*Fig 3

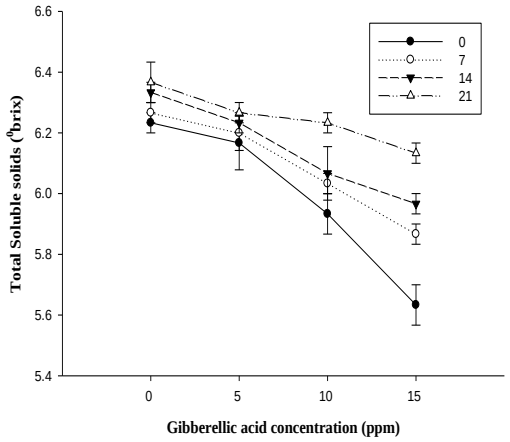
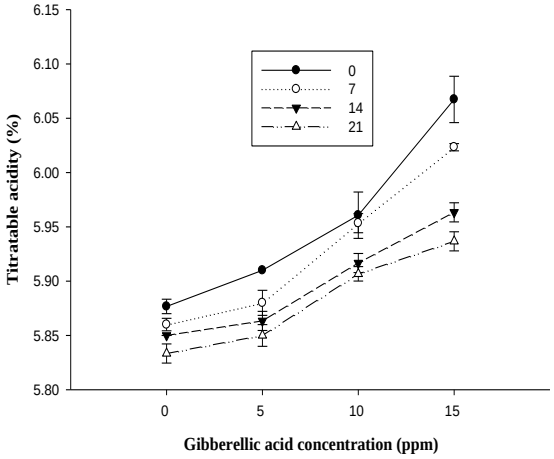


Fig 1



Fig/ 2

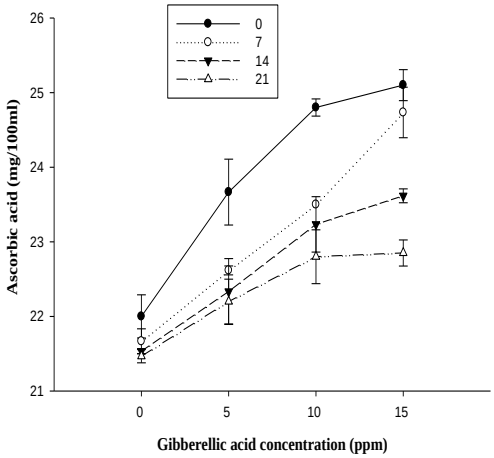


Fig. 3

Mean data about the fruit weight loss (%) are existing in table-3 expressed that GA_3 concentrations and storage durations significantly affected the fruit weight loss (%), while their interaction was found non-significant. Mean data revealed that highest fruit weight loss (19.31%) of limequat was observed in control treatment, followed by weight loss (17.88%) of limequat in fruits treated with foliar application of GA_3 at 5 ppm. While the lowest fruit weight loss (15.15%) of limequat was observed in fruits treated with 15 ppm of GA_3. Similarly, in storage durations, highest fruit weight loss (32.36%) of limequat was recorded in fruits stored for 21 days, while there was no weight loss (0.00%) found in fresh fruits at zero days of storage.

The fruit contains high amount of juice content and have direct relationship with evaporation during storage which leads to increase in weight loss (%). A considerable increased occurred in weight loss during storage because the insoluble

solute converted into soluble form with the passage of time during storage and thus weight loss occurred in fruit [29]. The weight loss of fruit is also influenced by high respiration rate and ethylene production. GA_3 have the ability to reduce the ethylene production due to antagonistic relationship of GA_3 with ethylene and the insoluble solids were converted into soluble contents due to maximum respiration and thus more water released from the surface of fruit and weight loss occurred [30,31].

Fruit Juice content (%)

Mean data regarding fruit juice (%) are existing in table-3 showed that GA_3 concentrations and storage durations significantly affected the fruit juice (%), while their interaction was found non-significant. Mean data revealed that highest fruit juice content (31.85%) was recorded in fruits treated with 15 ppm of GA_3, followed by juice content (30.58%) was observed in fruits treated with 10 ppm of GA_3. However, the least fruit juice content (28.26%) was recorded in control treatment. Data regarding storage durations shows that maximum fruit juice content (36.94%) was observed in fresh fruits at zero days of storage, while minimum fruit juice content (22.96%) was recorded in fruits stored for 21 days.

GA_3 at different concentrations increased the peel thickness and juice contents percentage in grapefruit [32]. GA_3 at different concentrations increased the juice content percentage in citrus [13, 22]. Foliar application of GA_3 increased the juice content percentage in kagzi lime because GA_3 have an antagonistic effect with ethylene. Foliar application of plant growth regulators before harvest absolutely increased percent juice content in many citrus species [33, 34]. The maximum storage duration decreased the juice content in fruits due to increase in respiration rate, which lead to more loss of water from the fruit and decreased the juice content in fruit [35].

Total soluble solids (^{o}Brix)

Mean data regarding fruit TSS (^{o}Brix) are presented in table-3 demonstrated that the concentrations of GA_3, storage durations and their interaction significantly affected the fruit TSS. Maximum fruit TSS (8.03^{o}Brix) was observed in untreated fruits after 21 days of storage, while lowest fruit TSS (5.63^{o}Brix) was recorded with 15 ppm of GA_3 in fresh fruits at zero days of storage.

The increased in TSS might be due to the action of sucrose-phosphate synthesis (SPS) which is mainly triggered by ethylene and repining during storage [36, 37] TSS was significantly affected by foliar application of GA_3 as compared to control [4, 14,38, 39]. Increased in TSS might be due to further assimilation and accumulation of photosynthetic activity in the plant [24]. Since chlorophyll degradation was delayed in GA_3 treated plants which retained the quality attributes of fruits [21]. The decrease in respiration rate during storage leads to lower the ripening process and maintained the fruit TSS and vice versa [40].

Titratable Acidity (%)

Mean data regarding Titratable Acidity (%) are presented in table-3 revealed that GA_3 concentrations, storage durations and their interaction significantly affected the titratable acidity (%). Maximum titratable acidity (6.07%) was observed in fruits treated with 15 ppm of GA_3 at zero days of storage, while lowest titratable acidity (5.60%) was recorded in untreated fruits after 21 days of storage.

The current study indicated that juice acidity was significantly affected by GA_3 application as compared to control treatment. Decreased in acidity occurred during post-harvest storage. Acidity showed a trend of decrease with the storage duration. Reduction of total acidity during market life was earlier in control fruits as compare to treated fruits which showed more use of organic acids and high respiration rate of untreated fruit [41, 42, 43]. Pre harvest treatments postponed the decrease of acidity during storage [44] which led to delay in metabolic changes of organic acids [45] and kept acidity of fruits and this led to improve storability. During the fruit storage, the acids break down into sugars due to the respiration of the fruits which resulted in decline of fruit acidity [46].

Table 3. Weight loss, juice content, TSS, Acidity and ascorbic content as affected by Gibberellic acid concentrations and storage durations.

GA_3 Conc. (ppm)	Weight loss (%)	Juice content (%)	TSS (^{o}Brix)	Acidity (%)	Ascorbic acid mg100ml^{-1}
0	19.31 a	28.26 c	7.23 a	5.71 d	15.89 d
5	17.88 ab	29.58 b	7.04 b	5.74 c	17.80 c
10	16.73 b	30.58 ab	6.86 c	5.77 b	19.08 b
15	15.15 c	31.85 a	6.68 d	5.86 a	19.57 a
Storage Durations					
0	0.00 d	36.94 a	5.99 d	5.95 a	23.89 a
7	14.27 c	32.38 b	6.55 c	5.76 b	19.76 b
14	22.45 b	28.00 c	7.37 b	5.71 c	16.15 c
21	32.36 a	22.96 d	7.90 a	5.66 d	12.54 d
LSD	1.50	1.30	0.13	0.02	0.47
GA3* Days of application					
Significance	NS	NS	*Fig: 4	*Fig: 5	*Fig: 6

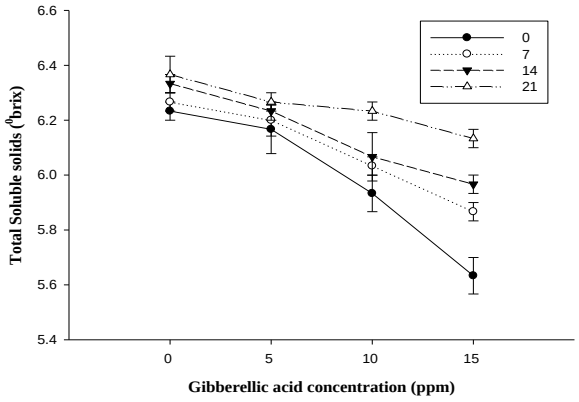


***Fig:4**

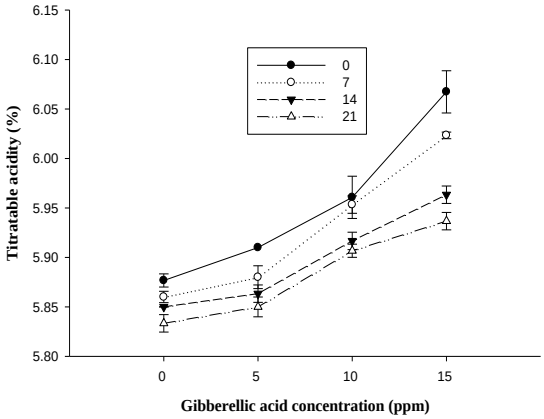


Fig. 5

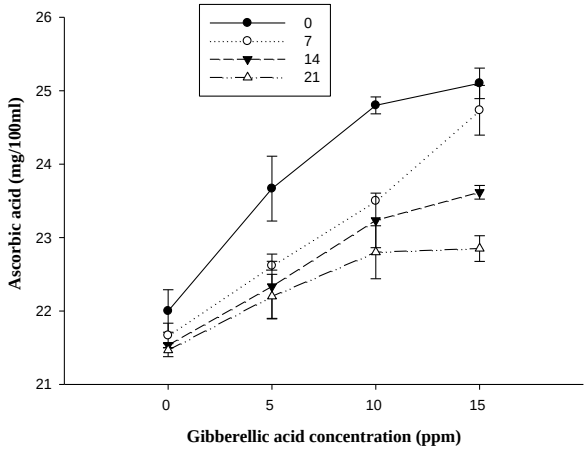


***Fig: 6**

Ascorbic acid (mg100ml^{-1})

Mean data about Ascorbic acid (mg100ml^{-1}) are presented in table-3 expressed that the GA_3 concentrations, storage durations and their interaction significantly affected the ascorbic acid content. Maximum ascorbic acid (25.10mg100ml^{-1}) was observed in fruits treated with 15 ppm of GA_3 in fresh fruits at zero days of storage, while lowest ascorbic acid (9.73mg100ml^{-1}) was recorded in untreated fruits after 21 days of storage.

The influence of GA_3 might be due to decreased or delayed ascorbate oxidase activity [47]. Ascorbic acid is an important nutrient quality factor, which is very sensitive to degradation due to its oxidation compared to other nutrients during storage. Decreased of ascorbic acid during storage could be due to the conversion of dehydroascorbic to diketogulonic acid by oxidation. Higher Ascorbic acid content imparts higher nutritive value to fruits [48]. Decreased in ascorbic acid could be due to enzymatic loss of L-ascorbic acid where it is converted into 2-3-dioxy–L-gluconic acid [49]. The content of Ascorbic acid was recorded higher in the GA_3 treated fruits than in control treatment [28].

CONCLUSIONS

On the basis of results obtained from the experiment the following conclusions were made.

- Foliar application of GA_3 at 15 ppm has shown significant effect on all the pre harvest yield and quality attributes of limequat.
- GA_3 Application directly at the onset cell expansion stage has also shown significant response on pre-harvest performance of limequat.
- For storage, limequat fruits retained most of the quality attributes up to 21 days of storage duration at ambient temperature.
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