Influence of plant growth regulators and potassium nitrate on the qualitative parameters of acid lime (Citrus aurantifolia Swingle) during hasta Bahar in Northern dry zone of Karnataka

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Abstract
Qualitative parameters of acid lime (Citrus aurantifolia Swingle) were found to be influenced with the different concentrations of plant growth regulators and chemical during hasta bahar. GA$_3$ @ 50 ppm in June - Cycocel @ 1000 ppm in September - KNO$_3$ @ 2% in October resulted in maximum titrable acidity (7.19), total soluble solids (7.25), total sugar (1.81), reducing sugars (0.87), non-reducing sugars (0.94) and minimum number of seeds (8.06). However, the maximum juice percent (49.31) and least rind thickness were found in GA$_3$ @ 50 ppm in June - Cycocel @ 2000 ppm in September - KNO$_3$ @ 2% in October. Similarly, the maximum ascorbic acid (30.46) and least weight of seeds per fruit (0.62) were found in GA$_3$ @ 50 ppm in June - Cycocel @ 1500 ppm in September - KNO$_3$ @ 2% in October and GA$_3$ @ 50 ppm in June respectively.

Keywords: Acid lime, hasta bahar, plant growth regulators, chemicals

Introduction
Acid lime is botanically known as Citrus aurantifolia Swingle belongs to the family, Rutaceae and has chromosome number (2n=18). It is considered third most commercially important citrus fruit just next to mandarin and sweet orange among citrus species with an annual production of 2.84 million tonnes and productivity of 9.90 MT/ha (Anon, 2017) [1]. The major growing states includes Andhra Pradesh, Telangana, Karnataka, Odisha, Madhya Pradesh, Maharashtra and minorly in Assam, Bihar, Chhattisgarh, Manipur, Jharkhand, Tamil Nadu, Tripura, and Mizoram. Acid limes are usually used as fresh fruit or in preparation of pickles and beverages. It is a good source of vitamin C and has a good anti-oxidant property. It helps in neutralizing the radical particles in human guts and act as a coolant during summer. It is well-liked and appreciated for its appearance, pleasing flavor and better fruit qualities.

In south Indian conditions, the flowering in acid lime occurs in three seasons with varying percentages. As reported by Pawar et al. (2016) [25], the flowering percentage of ambe, mrig and hasta bahar occurs as 47%, 36% and 17% respectively. However, the high demand of acid lime during summer months has widened up the scope for manipulation of flowering and harvesting of fruits during April to May. Despite of several conventional methods for flower manipulation and crop regulation, which does not hold true influence under different conditions in acid lime. This has led to the advent of plant growth regulators and chemical to regulate flowering in acid lime with maximum production and better qualities of fruits. Better quality fruits with high juice content, ascorbic acid, titrable acidity with less seeds per fruit and thinner rind are always marketed with high prices which helps in fetching increased remunerative to the growers. However, citrus canker and leaf miner are the major constraints in the production and quality of acid lime. Therefore, the present study was conducted to investigate the effect of different concentration of PGRs and chemical on biochemical parameters of acid lime during hasta bahar as well as pest and disease incidence.

Materials and Methods
Experiment was conducted on five years old acid lime (Citrus aurantifolia Swingle) trees with the spacing of 6m × 6m. The experimental site was geographically located at an altitude of 629
m from mean sea level and latitude 16.97° N and longitude 75.75° E. The experiment was laid out in split plot design with two varieties of acid lime as main plot treatment and different combination of plant growth regulators and chemical as sub plot treatment with three replications. The experimental details are shown in the table below as follows:

Table 1: Shows main plot treatments

| Main plot treatments | Phule Sharbati | Sai Sharbati |
|----------------------|----------------|--------------|
| S1                   | GA3 @ 50 ppm in June | KNO3 @ 2% in October |
| S2                   | Cycocel @ 1000 ppm in September | Cycocel @ 1500 ppm in September |
| S3                   | GA3 @ 2000 ppm in September | GA3 @ 50 ppm in July - Cycocel @ 1000 ppm in Sept - KNO3 @ 2% in October |

Accordingly, the different concentration of growth regulators and chemicals were prepared by dissolving them in ethanol and applied as foliar spray with knapsack sprayer until the trees were completely wet. The titrable acidity of the juice was determined according to method described in A.O.A.C. (1975) [1]. The total soluble solid were estimated with digital hand refractometer. Ascorbic acid was estimated by titration against standard dye (2, 6-dichlorophenyl). Similarly, the reducing and total sugars were estimated using Di-nitro salicylic (reagent) method and Anthrone (reagent) method respectively. The data collected were subjected to Fisher ’F’ test. The degree of probability employed in deciding the critical difference was 5 percent (P= 0.05).

Results and Discussion

The experimental results obtained from the present investigation are summarized as follows:

Effect of plant growth regulators and chemical on qualitative parameters of acid lime

Juice (%)

The juice percentage was significantly influenced by the application of plant growth regulators and chemicals. However, there were no significant differences found in main plot and significant differences in sub plot and interactions. The maximum juice percent (49.31%) was obtained in S3 (GA3 @ 50 ppm in June - Cycocel @ 1000 ppm in September - KNO3 @ 2% in October) which was statistically significant over the other treatment and was on par with S4 (47.46%) and S7 (45.66%). These results are in similarity with the findings of Mukunda et al., (2014) [24], Pawar et al. (2016) [25] and Ranganna et al. (2017) [28] respectively.

Similar findings which increased juice percentage with gibberellins were reported by Kumar et al. (1975) [21] in sweet lime, Mazumdar and Bhatt (1976) [22], Khalid et al. (2012) [18] in Kinnow mandarin, Sandhu (2013) [10] in lemon, Baghdady et al. (2014) [9] in Valencia orange, Rokaya et al. (2016) [29] in mandarin and with cytocel were reported by Brahmacari et al. (1995) [7] in guava. Similar results were obtained with potassium nitrate were reported by Debaje et al. (2011) [11] and Khayyat et al. (2012) [10] in pomegranate.

Titrable acidity (%)

Titrable acidity had been significantly influenced by the application of plant growth regulators and chemicals in main plot, sub plot and interactions. In main plot, the maximum titratable acidity was found in M2 (6.80%) and followed by M1 (6.39%). As regard to sub plot, the maximum titrable acidity (7.19%) was observed in S3 (GA3 @ 50 ppm in June - Cycocel @ 1000 ppm in September - KNO3 @ 2% in October) and was found to be on par with S7 (21.32%). However, the minimum titrable acidity (5.46%) was observed in S2 i.e. KNO3 @ 2% in October.

Similar results were obtained by Rattanpal et al. (2008) where in maximum acidity was observed in KNO3 5% + 2, 4-D @ 20 ppm when sprayed 60 days after full bloom and Debbarma and Hazarika (2016) [13] in which GA3 100 ppm - Cycocel 1000ppm - KNO3 1 per cent resulted in higher titrable acidity (8.27%) but contradicted with the findings of Debaje et al. (2011) [11] which reported reduced acidity and peel percentage with KNO3 (2 per cent) and GA3 (100 ppm).

Table 2: Effect of plant growth regulators and chemical on juice, titratable acidity, TSS and ascorbic acid in different cultivars of acid lime

| Treatments | Juice (%) | Titratable acidity (%) | TSS (*Brix) | Ascorbic acid (mg/100ml juice) |
|------------|-----------|------------------------|-------------|-------------------------------|
|            | M1 | M2 | Mean | M1 | M2 | Mean | M1 | M2 | Mean | M1 | M2 | Mean |
| S1         | 42.69 | 43.76 | 43.22 | 6.00 | 5.96 | 5.98 | 6.66 | 6.73 | 6.70 | 29.30 | 28.16 | 28.73 |
| S2         | 44.07 | 43.04 | 43.55 | 5.33 | 5.60 | 5.46 | 6.33 | 7.20 | 6.76 | 26.83 | 26.50 | 26.66 |
| S3         | 44.80 | 42.20 | 43.50 | 6.90 | 6.93 | 6.91 | 6.46 | 7.66 | 7.06 | 28.83 | 28.00 | 28.41 |
| S4         | 39.63 | 37.39 | 38.51 | 6.81 | 6.98 | 6.90 | 6.80 | 7.03 | 6.91 | 27.50 | 27.83 | 27.66 |
| S5         | 39.84 | 45.66 | 42.75 | 6.66 | 6.96 | 6.81 | 6.50 | 7.13 | 6.81 | 27.13 | 25.70 | 26.41 |
| S6         | 48.72 | 46.19 | 47.46 | 6.81 | 7.56 | 7.19 | 7.13 | 7.36 | 7.25 | 28.50 | 31.65 | 30.07 |
| S7         | 41.72 | 49.59 | 46.66 | 6.60 | 7.61 | 7.10 | 6.46 | 6.60 | 6.53 | 31.23 | 29.70 | 30.46 |
| S8         | 53.14 | 45.48 | 49.31 | 6.46 | 7.63 | 7.05 | 6.46 | 7.03 | 6.75 | 28.43 | 29.36 | 28.90 |
| S9         | 45.09 | 41.80 | 43.45 | 5.93 | 5.93 | 5.93 | 6.20 | 6.40 | 6.30 | 25.26 | 25.83 | 25.55 |
| Mean       | 44.41 | 43.90 | - | 6.39 | 6.80 | - | 6.55 | 7.01 | - | 28.11 | 28.08 | - |
| S.Em± | C.D. @ 5% | S.Em± | C.D. @ 5% | S.Em± | C.D. @ 5% | S.Em± | C.D. @ 5% |
| M          | 0.90 | NS | 0.04 | 0.29 | 0.90 | 0.58 | 0.18 | 0.53 | 0.81 | 2.34 |
| S          | 1.70 | 4.39 | 0.13 | 0.37 | 0.27 | 0.75 | 1.09 | 3.31 |
| MxS        | 2.44 | 6.92 | 0.20 | 0.53 | 0.27 | 0.75 | 1.09 | 3.31 |
### Sub plot treatments

| Sub plot treatments | Main plot treatments |
|---------------------|----------------------|
| $S_1$               | GA$_3$ @ 50 ppm in June |
| $S_2$               | KNO$_3$ @ 2% in October |
| $S_3$               | Cycocel @ 1000 ppm in September |
| $S_4$               | Cycocel @ 1500 ppm in September |
| $S_5$               | Cycocel @ 2000 ppm in September |
| $S_6$               | GA$_3$ @ 50 ppm in June + Cycocel @ 1000 ppm in Sept + KNO$_3$ @ 2% in October |
| $S_7$               | GA$_3$ @ 50 ppm in June + Cycocel @ 1500 ppm in Sept + KNO$_3$ @ 2% in October |
| $S_8$               | GA$_3$ @ 50 ppm in June + Cycocel @ 2000 ppm in Sept + KNO$_3$ @ 2% in October |
| $S_9$               | Control |

### Total soluble solids (°Brix)

The results indicated the significant differences for total soluble solids in main plot, sub plot and interaction effect. Treatment $M_2$ (7.01 °Brix) recorded highest total soluble solids followed by $M_1$ (6.55 °Brix) in main plot. In sub plot, treatment $S_8$ with GA$_3$ @ 50 ppm in June - Cycocel @ 1000 ppm in September - KNO$_3$ @ 2% in October (7.25 °Brix) recorded the highest total soluble solids.

The results are in conformity with the findings of Mukunda et al. (2014)\[24\] with application of GA$_3$ @ 50 ppm during June - CCC @ 1000 ppm during September - KNO$_3$ @ 2% during October. Similar results were also reported by Debaje et al. (2011)\[11\], Jagtap et al. (2013)\[16\] in acid lime, Tuan and Ruey (2013)\[13\] in apple and Debbarma and Hazarika (2016)\[12\] in acid lime.

### Ascorbic acid (mg/100 ml juice)

There was no statistical significant difference found in main plot treatments. However, the maximum ascorbic acid (30.46 mg/100 ml juice) was found in $S_9$ (GA$_3$ @ 50 ppm in June - Cycocel @ 1500 ppm in September - KNO$_3$ @ 2% in October) and was found to be on par with $S_6$ (30.07 mg/100 ml juice) i.e. GA$_3$ @ 50 ppm in June - Cycocel @ 1000 ppm in September - KNO$_3$ @ 2% in October, $S_8$ (28.90 mg/100 ml juice) and $S_4$ (28.73 mg/100 ml juice). Increase in ascorbic acid may be due to the role of various plant growth regulators and chemicals in degradation of organic acids. Further, GA$_3$ might take part in sugar and starch hydrolysis and cycocel in diverting carbohydrates into the developing fruits. It is believed that potassium might have involved in active sugar metabolism and translocation. These findings were in agreement with Debbarma and Hazarika (2016)\[12\] and Ranganna et al. (2017)\[28\] in acid lime respectively.

Similar results were reported by Kumar et al. (1975)\[21\] in sweet lime, Jonsan et al. (1998)\[17\], Kumar et al. (2012)\[20\] in strawberry, Jagtap et al. (2013)\[19\] and Sandhu (2013)\[30\] in lemon, Meena et al. (2017)\[23\] in phalsa which increase in ascorbic acid content with GA$_3$ and Hari Om et al. (1975)\[15\] in apple cv. Red Delicious, Thukral et al. (1993)\[13\] in lemon cv. Pant Lemon-1 with cycocel application. Lastly, similar results were observed by Rattanpal et al. (2008) and Debaje et al. (2011)\[11\] with potassium nitrate.

### Total sugars (%)

The different concentration of plant growth regulators and chemical had showed significant differences in sub plot and their interaction but not in main plot. The highest total sugar (1.81%) was recorded in $S_8$ (GA$_3$ @ 50 ppm in June - Cycocel @ 1000 ppm in September - KNO$_3$ @ 2% in October) and was significantly superior than other treatments followed by $S_7$ (1.77%) and $S_8$ (1.71%) respectively. The lowest total sugars was recorded in control i.e. $S_9$ (0.91%). It is observable that the different plant growth regulators and chemical concentrations influenced the sugar content of the treated fruits. This may be due to the promotive role of growth regulators in consistent diversion of carbohydrates into the developing fruit and hydrolysis of starch into simple sugars.

Similar findings regarding increase the total sugar have been reported by Mazumdar and Bhatt (1976)\[22\] in sweet orange, Bhujbal et al. (2013)\[6\] in sapota with gibberellins, Hari Om et al. (1975)\[15\] in apple cv. Red Delicious with CCC, Daberao et al. (2016)\[10\] in sapota with 2% KNO$_3$ + 50 ppm GA$_3$.

### Reducing sugars (%)

The different concentration of plant growth regulators and chemical showed significant differences for reducing sugars in sub plot treatments.

Treatment $S_6$ (GA$_3$ @ 50 ppm in June - Cycocel @ 1000 ppm in September - KNO$_3$ @ 2% in October) recorded the highest reducing sugars (0.87%) and was followed by $S_7$ (0.85%) and $S_8$ (0.81%) respectively. The lowest reducing sugar (0.55%) was recorded in $S_2$ (KNO$_3$ @ 2% in October) and was on par with $S_9$ i.e. control. The raised concentration of reducing sugar in the treated fruits may be attributed to the prolonged attachment of fruits in the tree caused by gibberellins might have facilitated the accumulation of more carbohydrates and degradation of complex starch into simpler sugar form by alpha-amylase regulated by the gibberellins.

The results were in close proximity with the findings of Pawar et al. (2016)\[26\] wherein GA$_3$ @ 50 ppm in June + Cycocel @ 1500 ppm in September + KNO$_3$ @ 2% in October recorded the maximum reducing sugar. Similar finding were reported by Mazumdar and Bhatt (1976)\[22\] in sweet orange.
Non-reducing sugars (%)
The data showed in Table 3 had significantly influenced non reducing sugar content of acid lime with different concentrations of plant growth regulators and chemical in sub plot only.

The highest non reducing sugars (0.94%) was recorded in S₄ (GA₃ @ 50 ppm in June - Cycocel @ 1000 ppm in September - KNO₃ @ 2% in October) which was followed by S₃ (0.91%) and S₅ (0.89%) respectively. The lowest non reducing sugar was recorded in S₆ (0.34%) i.e. control. The results were in close proximity with the findings of Pawar et al. (2016) [25] wherein (GA₃ @ 50 ppm in June + Cycocel @ 1500 ppm in September + KNO₃ @ 2% in October) recorded the maximum non-reducing sugar.

Similar finding were reported by Mazumdar and Bhatt (1976) [26] in sweet orange, Hari Om et al. (1975) [27] in apple cv. Red Delicious and Bhattacharyya and Bhatt (2017) [28] in Pant lemon-I.

Number of seeds per fruit (g)
There was no statistical significant differences found in main plot and interactions however significant differences was found in sub plot for number of seeds per fruit (g) (Table 4).

However, in sub plot, the minimum number of seeds (8.06 g) was recorded in S₆ (GA₃ @ 50 ppm in June - Cycocel @ 1000 ppm in September - KNO₃ @ 2% in October) but was on par with S₃ (9.00 g), S₅ (9.20 g), S₄ (9.60 g), S₂ (10.03 g) and S₁ (10.30 g) respectively. The reduction in seed number of the fruits was seen only in the treatment containing gibberellins. This indicated that gibberellins were involved in the physiology of seed development thereby reducing the seed number. However, gibberellins may also be involved in stimulation of parthenocarpic fruit development phenomena.

Similar results were reported by Debaje et al. (2011) [29] with GA₃ 100 ppm. Garasriya et al. (2013) [30] with GA₃ 50 ppm in guava. Jagtap et al. (2013) [31] Bhatt et al. (2016) [32] in lemon.

Randhawa et al. (1964) [33] also reported decreased seed number in guava and grapefruit with GA₃ @ 100 ppm.

Weight of seeds per fruit (g)
There were statistical significant differences found in main plot, sub plot and their interactions as data showed in Table 4. The least weight of seeds per fruit (0.62 g) was observed in S₁ (GA₃ @ 50 ppm in June) and was found to be on par with S₂ (0.63 g) i.e. (GA₃ @ 50 ppm in June - Cycocel @ 1000 ppm in September - KNO₃ @ 2% in October) followed by S₇ (0.65 g) i.e. (GA₃ @ 50 ppm in June - Cycocel @ 1500 ppm in September - KNO₃ @ 2% in October) and S₅ (0.63 g) in Pant lemon-I. However, the highest weight of seeds was observed in S₆ (0.88 g) i.e. control. It is clear that the treatment which contained gibberellins showed relatively lower seed weight as compared to the ones which do not contained gibberellins. It indicated that gibberellins may have played some physiological intervention in fruit development with rudimentary seeds.

These results were in similarity with the findings of Chaudhari et al. (1992) who reported reduced seed weight with GA₃ @ 40 ppm.

Rind thickness (mm)
The data presented in Table 4 showed that the application of different concentration of growth regulators and chemical had significantly influenced the rind thickness of the acid lime. As regard to main plot, the least rind thickness (1.47 mm) was observed in M₂ (Sai Sharbati) and followed by M₁ (1.67 mm) i.e. Phule Sharbati. In sub plot, S₁ (GA₃ @ 50 ppm in June - Cycocel @ 2000 ppm in September - KNO₃ @ 2% in October) recorded the least rind thickness (1.45 mm) and found to be significantly different than other treatments but on par with S₆ (1.46 mm) i.e. GA₃ @ 50 ppm in June Cycocel @ 1000 ppm in September - KNO₃ @ 2% in October followed by...
by $S_2$ (1.46 mm), $S_9$ (1.51 mm), $S_5$ (1.55 mm), $S_7$ (1.56 mm) and $S_1$ (1.60 mm) respectively. The obtained results were in close conformity with the findings of Debaje et al. (2011) \cite{11} where peel percentage were reduced with KNO$_3$ @ 2 per cent and GA$_3$ 100 ppm and Sharma and Randhawa (1966) \cite{31} reported decrease in rind thickness with GA$_3$ @ 50 ppm. However, the results found to be contradictory with the findings of Daberao et al. (2016) \cite{10} who reported maximum peel weight with two percent KNO$_3$ + 50 ppm GA$_3$, Chundawat and Randhawa (1972) \cite{9} in grapefruit var. Saharanpur Special and Dinar et al. (1977) \cite{13} in Marsh grapefruit with GA$_3$.

Table 4: Effect of plant growth regulators and chemical on number of seeds, weight of seeds per fruit and rind thickness in different cultivars of acid lime

| Treatments   | Number of seeds per fruit | Weight of seeds per fruit (g) | Rind thickness (mm) |
|--------------|---------------------------|-------------------------------|---------------------|
|              | $M_1$ | $M_2$ | Mean   | $M_1$ | $M_2$ | Mean   | $M_1$ | $M_2$ | Mean   |
| $S_1$        | 7.87  | 10.53 | 9.20   | 0.62  | 0.63  | 0.62   | 1.65  | 1.50  | 1.60   |
| $S_2$        | 11.00 | 9.06  | 10.03  | 0.72  | 0.78  | 0.75   | 1.48  | 1.39  | 1.46   |
| $S_3$        | 10.13 | 10.46 | 10.30  | 0.69  | 0.78  | 0.73   | 2.22  | 1.57  | 1.87   |
| $S_4$        | 11.60 | 9.80  | 10.70  | 0.71  | 0.77  | 0.74   | 1.94  | 1.51  | 1.70   |
| $S_5$        | 11.73 | 10.00 | 10.86  | 0.70  | 0.78  | 0.74   | 1.69  | 1.43  | 1.55   |
| $S_6$        | 8.80  | 7.33  | 8.06   | 0.62  | 0.64  | 0.63   | 1.44  | 1.43  | 1.46   |
| $S_7$        | 10.33 | 8.86  | 9.00   | 0.62  | 0.67  | 0.65   | 1.65  | 1.49  | 1.56   |
| $S_8$        | 10.33 | 8.87  | 9.60   | 0.78  | 0.70  | 0.74   | 1.49  | 1.42  | 1.45   |
| $S_9$        | 11.46 | 11.13 | 11.30  | 0.87  | 0.89  | 0.88   | 1.53  | 0.97  | 1.51   |
| Mean         | 10.22 | 9.57  | 9.97   | 0.70  | 0.74  | 0.72   | 1.67  | 1.47  | 1.54   |

Sub plot treatments

- $S_1$: GA$_3$ @ 50 ppm in June
- $S_2$: KNO$_3$ @ 2% in October
- $S_3$: Cycocel @ 1000 ppm in September
- $S_4$: Cycocel @ 1500 ppm in September
- $S_5$: Cycocel @ 2000 ppm in September
- $S_6$: GA$_3$ @ 50 ppm in June + Cycocel @ 1000 ppm in Sept + KNO$_3$ @ 2% in October
- $S_7$: GA$_3$ @ 50 ppm in June + Cycocel @ 1500 ppm in Sept + KNO$_3$ @ 2% in October
- $S_8$: GA$_3$ @ 50 ppm in June + Cycocel @ 2000 ppm in Sept + KNO$_3$ @ 2% in October
- $S_9$: Control

Main plot treatments:

- $M_1$: Phule Sharbati
- $M_2$: Sai Sharbati

Yield per tree (kg/tree)

The data regarding yield per tree (kg/tree) showed non-significant difference in main plot as depicted in Figure 1. However, there were statistical significant differences found among the sub plot treatments. The maximum yield per tree was observed in $S_6$ (14.93) and significantly superior over all other treatments and on par with $S_7$ (12.82). However, the minimum yield per tree was observed in $S_9$ (7.11).

![Fig 1: Shows yield tree (kg/tree)](image)
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