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# Influence of Plant Growth Regulators, Urea and Micronutrient on Growth, Yield and Quality of Rangpur Lime (*Citrus limonia* Osbeck)

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***Abstract*:** The present study was conducted at Central Agricultural University. Pasighat, on influence of the pre-harvest application of NAA and $GA_3$ combined with 1% urea and 0.5% $ZnSO_4$ on vegetative growth, fruit drop, yield and fruit quality of *Citrus limonia* Osbeck, during 2020-2021. The experiment consisted of ten treatments with NAA, $GA_3$, $ZnSO_4$ and Urea. The trees sprayed with NAA @ 30 ppm + $GA_3$ @ 30 ppm + 0.5% $ZnSO_4$ + 1% urea recorded the maximum plant height, minimum days for flowering, fruit drop percentage, acidity, higher ascorbic acid, total, reducing and non-reducing sugars and fruit juice content. The treatment combination of NAA @ 10 ppm + $GA_3$ @ 10 ppm + 0.5% $ZnSO_4$ + 1% urea performed better with respect to stem girth (38.98 cm). The current study revealed that NAA @ 30 ppm + $GA_3$ @ 30 ppm + 0.5% $ZnSO_4$ + 1% urea is the best treatment for enhancing plant growth, reproductive and biochemical parameters of fruit.

***Keywords*:** PGRs, Urea, $ZnSO_4$, Fruit quality, Rangpur lime

Citrus is a globally important commercial fruit crop. It is India's third most important fruit crop, behind mango and banana. Citrus fruits are regarded as an excellent diet and the most popular fruit in the country. With consideration to generating quality planting material many rootstocks used for different citrus species out of which 'Rangpur lime' is also one of the widely used rootstock in India. It is a salt and drought tolerant, cold hardy and resistant to tristeza, xyloprosis and exocortis. It is also commonly used rootstock, notably in Brazil, due to its tolerance to the tristeza virus and its adaptation to water deficiency. With scion variations, Rangpur lime also has excellent agronomic qualities that enable high production and good fruit quality (Silva et al 2019). Among the citrus fruits grown in India, Rangpur lime is not only a crucial rootstock for Nagpur mandarin, sweet range, Coorg mandarin and pummelo, but also a popular rootstock for "Khasi Mandarin," which is grown from seeds. Because of its tolerance to salt and drought, early fruit set, lengthy fruit retention on the tree, and resistance to the tristeza virus, the cultivar is used as a rootstock in many other states (Dilip et al 2017).

Among PGRs, auxins have a direct effect on abscission by promoting a delay in abscission, resulting in an increase in fruit yield and quality. For the decrease of fruit physiological drop, 2-4 dichlorophenoxy acetic acid, NAA and Gibberellic acid have been tried (Fahad et al 2014). The use of PGRs can re-enforce hormone balance or delay precocious fall and losses prior to harvest. Auxins inhibit fruit drop by keeping cells at the abscission zone and preventing the formation of hydrolytic enzymes such as cellulase and polygalacturonase, which destroy the cell wall. The activity of auxin in strengthening the cells in the abscission zone (Basu et al 2013), which is located at the peduncle, may be the cause of the decreased fruit drop percentage. The use of gibberellic acid to prevent fruit drop at various phases throughout fruit growth and development has become a widely used practice in the citrus (Hikal et al 2013). The major application of plant growth regulators enhances internal fruit quality, influence flowering, fruit set and fruit drop as well as to influence fruit quality traits in different citrus species (Harsimrat et al 2015). Exogenous applications of these growth regulators have been tested on a variety of citrus species either alone or in combination either at full bloom or at the preharvest stage(Nawaz et al 2008). Bhatt et al (2016) observed that on *Citrus limon* the least fruit cracking was found under NAA @ 50 ppm, the lowest fruit drop was under $GA_3$ (20 ppm), the maximum fruit number and seed weight was under $GA_3$ (10 ppm). Choudhary et al (2013) in Nagpur mandarin observed that the application of $GA_3$ increased the number of vegetative shoots, which significantly increase plant spread and crown volume. Considering this in point, the present experiment was undertaken to study the effect of pre-harvest spray of PGRs, urea and micronutrient on reducing fruit drop, increasing yield and quality of lime cv. Rangpur lime.

## MATERIAL AND METHODS

The present investigation was carried out in the year 2020 at Central Agricultural University, Pasighat, Arunachal

Pradesh, India. Nine years old lime trees cv. Rangpur Lime planted at a spacing of 6 m × 6 m was used as experimental materials. The experiment was laid out in RBD consisting of ten treatments and four replications. The treatments applied were: $T_1$ (Control), $T_2$ (NAA @ 10 ppm + 0.5% $ZnSO_4$ + 1% urea), $T_3$ ($GA_3$ @ 10 ppm + 0.5% $ZnSO_4$ + 1% Urea), $T_4$ (NAA @ 20 ppm + 0.5% $ZnSO_4$ + 1% urea), $T_5$ ($GA_3$ @ 20 ppm + 0.5% $ZnSO_4$ + 1% Urea), $T_6$ (NAA @ 30 ppm + 0.5% $ZnSO_4$ + 1% urea), $T_7$ ($GA_3$ @ 30 ppm + 0.5% $ZnSO_4$ + 1% Urea), $T_8$ (NAA @ 10 ppm + $GA_3$ @ 10 ppm + 0.5% $ZnSO_4$ + 1% urea), $T_9$ (NAA @ 20 ppm + $GA_3$ @ 20 ppm + 0.5% $ZnSO_4$ + 1% urea) and $T_{10}$ (NAA @ 30 ppm + $GA_3$ @ 30 ppm + 0.5% $ZnSO_4$ + 1% urea). Regular manures, fertilizer application, and plant protection measures were undertaken in the field. Two PGRs *viz*., NAA and $GA_3$ @ 10, 20 and 30 ppm were applied as a foliar spray to the plants along with 1% urea and 0.5% $ZnSO_4$. The water-insoluble PGRs were initially dissolved in a small quantity of ethanol. All spray solutions were prepared separately and made up the volume of the required quantity with tap water. PGRs *viz*., NAA and $GA_3$ were applied on the same day but separate foliar spray and 0.5% $ZnSO_4$ and 1% Urea were applied 10 days prior to their application with a knap-sack sprayer without any adhesive chemical to the foliar spray. First foliar spray of NAA and $GA_3$ @ 10, 20 and 30 ppm and $ZnSO_4$ (0.5%) along with urea (1%) was applied during April, June and October. The fruits were harvested when they are fully matured and start to develop attractive color. Plant growth and reproductive observations like plant height (cm), stem girth (cm), tree canopy (m), days to flowering (50% flowering), and fruit quality attributes like TSS (%), titratable acidity (%), total sugar (%), reducing sugar (%) and vitamin C (mg/100 g) were estimated as per standard procedures of AOAC (2002) using five fruits randomly selected from each treatment. The data on the mean values of all characters were statistically evaluated using the OPSTAT programme.

## RESULTS AND DISCUSSION

**Tree growth:** The various treatment combinations had a substantial impact on plant height (Table 1). The treatment $T_{10}$ had the highest increase in plant height (346.25 cm) being followed by $T_7$ (322.50cm), $T_8$ (318.00cm) and $T_5$ (310.50cm) while the control ($T_1$) had the lowest rise in plant height (269.00 cm). The gibberellins ($GA_3$), function as a growth promoter and accelerate the cell elongation in part by activating the intercalary meristematic area of developing shoots, also lengthen internodal distances between branches as reported by Awati M and Kiran K C (2018) in acid lime. Prasad et al (2015) mentioned that plant growth regulator (2,4-D) treated in conjunction with urea in 'Kinnow Mandarin', were more successful in promoting vegetative development than PGRs applied alone. Urea's ability to stimulate plant growth may be ascribed to nitrogen, a crucial elemental component of chlorophyll, the substance that allows plants to absorb solar energy and use it to convert atmospheric carbon dioxide into carbohydrates through photosynthesis. Energy for plant development and growth is provided by the carbohydrates so synthesized. The growth of plants is increased when urea is applied to foliar. Kacha et al (2012) also mentioned that 200 ppm of NAA spray, resulted in the greatest height (177.3cm) of the phalsa bush.The maximum tree canopy N-S (4.67m) and E-W (4.43m) was in trees sprayed with NAA @ 30 ppm + $GA_3$ @ 30 ppm + 1% urea + 0.5% $ZnSO_4$, whereas minimum spread was in $T_4$ from N-S

**Table 1.** Effect of pre-harvest spray of PGRs, urea and micronutrient on plant growth parameters and flowering period of rangpur lime

| Treatments | Plant height (cm) | Stem girth (cm) | Tree canopy (m) | | Flowering period (Days) |
|---|---|---|---|---|---|
| | | | N-S | E-W | |
| $T_1$ | 269.00 | 30.78 | 3.97 | 3.62 | 14.38 |
| $T_2$ | 299.50 | 34.23 | 4.25 | 3.97 | 15.25 |
| $T_3$ | 293.50 | 33.28 | 4.27 | 3.65 | 13.81 |
| $T_4$ | 287.25 | 38.25 | 3.83 | 3.95 | 14.63 |
| $T_5$ | 310.50 | 35.65 | 4.28 | 4.17 | 13.00 |
| $T_6$ | 289.25 | 35.78 | 4.22 | 4.11 | 14.50 |
| $T_7$ | 322.50 | 34.38 | 4.29 | 3.93 | 13.00 |
| $T_8$ | 318.00 | 37.13 | 3.98 | 4.06 | 13.50 |
| $T_9$ | 253.25 | 38.03 | 4.39 | 3.96 | 12.50 |
| $T_{10}$ | 346.25 | 38.98 | 4.67 | 4.43 | 11.63 |
| C.D @ 1% | 29.82 | 4.53 | 0.30 | 0.29 | 0.87 |

(3.83 m) direction and in control trees from E-W (3.62 m) direction (Table 1). This expansion of the tree canopy may be caused by the efficient conversion of food components that have been stored for the beginning of more side branches in the trees. After spraying $GA_3$, the growth metrics such as plant height, mean spread, and canopy volume increased greater in all treatments (apart from control). It has been primarily attributed to the effect of $GA_3$, which encourages cell elongation, increases cell size and also stimulates cell multiplication. The findings of the current study agreed with those of Baskaran et al (2009) in acid lime. The treatments had a significant influence on the stem girth of the tree. Treatment $T_{10}$ (38.98 cm) greatly increased the stem girth than control (30.78 cm), however treatments $T_9$, $T_8$ and $T_4$ were at par with $T_{10}$.The number of days to reach flowering was significantly affected by the quantity of growth regulators and chemicals used (Table 1). These chemicals are also used to control tree production by either limiting vegetative development or promoting blooming. The minimum flowering period was observed in $T_{10}$ (11.63 days) and maximum was recorded in $T_2$(15.25 days). Applying $GA_3$ and $ZnSO_4$ resulted in a rise in the number of fruits per tree, which may have been caused by the treatment's initial tendency to increased flowering. The $GA_3$ treatment's effect on the promotion and diversion of metabolites and flower-inducing compounds may be the cause of the lateness in flowering in the control group by changing the destiny of reproductive buds to vegetative buds and inhibiting flower development. The cultivar, genetic make-up, habitat, and cultural traditions are only a few of the variables that influence the occurrence of blooming in plants (Harsimrat et al 2015).

**Influence on yield and yield attributing traits:** There was a significant relationship between the various treatments and yield attributing traits (Table 2). Due to higher blossoming in March, there were significantly more fruits/trees. Treatment $T_{10}$ was determined to be the most effective, producing 814.25 fruits per tree, subsequently followed by $T_9$ (767.50), whereas control trees had the fewest fruits (437.25). $T_{10}$ had the largest fruit output per tree due to its increased blooming inclination (129.71 Kg). The pool data for this feature showed the greatest value in treatment $T_{10}$ (129.71 kg), which is at par with the results of treatment $T_9$ (120.85 kg), and the lowest yield was recorded in control trees (72.53 kg). The lowest fruit drop percentage were observed in treatment $T_{10}$ (33.47%), which is equivalent to $T_8$, $T_7$ and $T_9$ and the highest was noticed in the control trees (56.48%).$GA_3$ enhanced the number of fruits per tree and the total yield both independently and in combination with auxin. Citrus fruit quality and yield are improved by auxins, among PGRs that directly affects abscission by delaying it (Harsimrat et al 2015). Multiple abscission zones arise within the same inflorescence because the abscission of fruits is typically accompanied by structural changes (Bermejo et al 2018). Because the cells become free in the abscission zone and fruits abscised. The corresponding enzymes breakdown pectin, hemicelluloses, and cellulose as mechanical tension separates and softens the cell wall (Zhang et al 2022). In plants, NAA significantly boost the production of cellulose fibre and spraying NAA in various fruit crops at varying concentrations prevents fruit drop in the majority of fruit trees. Plant growth regulators have a significant impact on early reproductive processes in citrus, showing that hormones play a crucial role in the regulatory systems that regulate fruit set and the abscission of ovaries and fruitlets. Ghosh et al (2012) observed that the NAA applied at 15ppm was the most efficient in reducing fruit drops at various months after fruit

**Table 2.** Effect of pre-harvest spray of PGRs, urea and micronutrient on fruit drop, yield and physical parameters of rangpur lime

| Treatments | Fruit drop (%) | No. of fruits/tree | Yield (Kg/tree) | Fruit length (cm) | Fruit width (cm) | Fruit weight (g) | No. of seeds/fruit |
|---|---|---|---|---|---|---|---|
| $T_1$ | 56.48 | 437.25 | 72.53 | 5.43 | 5.40 | 119.50 | 17.68 |
| $T_2$ | 47.25 | 473.75 | 80.53 | 5.45 | 5.93 | 122.85 | 16.45 |
| $T_3$ | 43.26 | 511.00 | 75.98 | 5.70 | 5.98 | 124.53 | 15.50 |
| $T_4$ | 41.23 | 529.50 | 87.63 | 6.13 | 6.29 | 126.23 | 13.75 |
| $T_5$ | 40.26 | 630.25 | 88.96 | 6.38 | 5.99 | 135.00 | 11.50 |
| $T_6$ | 35.69 | 680.75 | 103.24 | 6.27 | 6.05 | 147.92 | 12.00 |
| $T_7$ | 35.10 | 735.75 | 113.04 | 6.50 | 6.12 | 153.58 | 11.00 |
| $T_8$ | 34.70 | 728.75 | 115.34 | 6.72 | 6.31 | 158.25 | 12.13 |
| $T_9$ | 35.15 | 767.50 | 120.85 | 7.20 | 6.67 | 166.25 | 8.75 |
| $T_{10}$ | 33.47 | 814.25 | 129.71 | 7.48 | 7.08 | 171.38 | 10.75 |
| C.D. @ 1% | 4.39 | 59.54 | 11.874 | 0.48 | 0.77 | 6.28 | 1.82 |

set, resulting in a twofold increase in fruit output over the control and better fruit size in sweet orange. Kachave and Bhosle (2009) observed that in Kagzi lime concluded that the most effective treatment to enhance total soluble solids, acidity, ascorbic acid and reducing sugar of fruit is NAA 200 ppm + micronutrients mixture @ 1% spray. When combined with zinc, NAA enhanced the yield per tree more than the control. Due to its role in auxin synthesis, zinc may have contributed to the greater increase in fruit production.

**Fruit physio-biochemical attributes:** The influence of different treatments on fruit length was significant with maximum increase in treatment $T_{10}$ (7.48 cm) followed by $T_9$ (7.20 cm) and minimum was in $T_1$ (5.43 cm) (Table 2). Similarly, the treatments had a substantial impact on fruit width. Among all the treatment studied, $T_{10}$ showed highest fruit width (7.08 cm) whereas $T_1$ depicted the least (5.40 cm). As a consequence of a strong impact on fruit length and width from PGR's, urea and micronutrient foliar spray, the maximum fresh weight of the fruit was in $T_{10}$ (171.38 g) followed by $T_9$ and the minimum was in $T_1$ (119.50 g). The increase in fruit weight might be the result of zinc spray which is related to tryptophan, a critical amino acid for fruit growth and development. The contribution of auxin to fruit growth, is based on a higher cell expansion, may account for the improvement in the fruit's overall physical characteristics (fruit length, width, and weight). The increased cell vacuolization that results in larger vesicles, locules, and ultimate fruits is most likely the cause of this enlargement. Hesami and Abdi (2010) observed that NAA @ 100 ppm enhanced bunch weight and improved physical attributes (fruit weight, height, diameter, and size) in date palm. Treatment ($T_9$) with NAA @ 20 ppm + $GA_3$ @ 20 ppm + 0.5% $ZnSO_4$ + 1 % urea had few average numbers (8.75) of seeds per fruit . The control group had the most seeds per fruit (17.68 seeds), nevertheless spraying $GA_3$ can possibly lower the number of seeds in citrus cultivars that produce them, however, this depends on the cultivar (Hung et al 2023). Ullah et al (2012), also indicated that a 200 mg/L $GA_3$ spray treatment one month after anthesis decreased the number of seeds in Kinnow mandarin.

The treatments significantly increased TSS, acidity, total sugar, reducing sugar, and ascorbic acid (Table 3). The total soluble solid content was highest in $T_{10}$ (8.55 °Brix) while the control depicted the least (7.30 °Brix). The combination of PGR's and micronutrient may respond to the more accumulation of total soluble solids by enriching the nutrient content in the juice sacs. Significantly, the lowest acidity percentage (0.60 %) was with NAA @ 30 ppm + $GA_3$ @ 30 ppm + 0.5% $ZnSO_4$ + 1% urea, whereas the highest levels (1.30 %) was in control. The acidity of acid lime is mainly due to the presence of citric acid, which is one of the most widely distributed acids in fruits. Sawale et al (2021) concluded that foliar applications of urea, zinc sulphate, and borax in various amounts had an immense effect on reducing acidity. Singh et al (2018) findings in sweet orange cv. Mosambi are also in support of our study. There was substantial difference in vitamin C content between the fruit that had been treated with NAA and $GA_3$ and untreated. The greatest concentration of vitamin C was in t $T_{10}$ (38.52 mg/100g). The control had the lowest vitamin C content (20.87 mg/100 ml). Fruits with increased vitamin C concentration are more nutrient-dense. The fruits that weren't treated, however, showed a more pronounced decline. Enzymatic loss of L-ascorbic acid, which is transformed into 2-3-dioxy-L-gluconic acid, may be the cause of a drop in ascorbic acid in non-treated fruits (Dosedel et al 2021). Treatment $T_{10}$ recorded the highest total

**Table 3.** Effect of pre-harvest spray of PGRs, urea and micronutrient on biochemical parameters of lime cv. Rangpur lime

| Treatments | TSS (°Brix) | Acidity (%) | Vitamin C (mg/100g) | Total sugars (%) | Reducing sugars (%) | Non-reducing (%) | Fruit juice (ml) |
|---|---|---|---|---|---|---|---|
| $T_1$ | 7.30 | 1.30 | 20.87 | 2.41 | 0.28 | 1.92 | 78.50 |
| $T_2$ | 7.98 | 0.75 | 22.16 | 2.62 | 0.36 | 2.04 | 86.00 |
| $T_3$ | 7.95 | 0.62 | 23.22 | 2.82 | 0.33 | 2.24 | 85.13 |
| $T_4$ | 7.55 | 0.71 | 24.64 | 3.07 | 0.38 | 2.42 | 90.50 |
| $T_5$ | 7.55 | 0.72 | 25.98 | 2.89 | 0.49 | 2.12 | 89.23 |
| $T_6$ | 8.33 | 0.62 | 27.42 | 2.72 | 0.58 | 1.93 | 88.75 |
| $T_7$ | 7.40 | 0.66 | 26.57 | 3.06 | 0.55 | 2.26 | 89.5 |
| $T_8$ | 8.15 | 0.72 | 29.67 | 3.22 | 0.64 | 2.33 | 92.50 |
| $T_9$ | 8.30 | 0.61 | 30.33 | 3.16 | 0.66 | 2.25 | 94.25 |
| $T_{10}$ | 8.55 | 0.60 | 38.52 | 3.86 | 1.11 | 2.48 | 113.25 |
| C.D @ 1% | 0.59 | 0.223 | 9.44 | 0.76 | 0.37 | NS* | 11.36 |

sugar content (3.86 %). Same treatment combination also showed the significant variation with greatest levels of reducing sugar (1.11 %) whereas with non-significant differences in non-reducing sugar (2.48 %). On 'Kinnow mandarin', foliar applications of Zn up to 0.6% enhanced tree growth, productivity, and mineral nutrients with greater fruit quality compared to control was also reported by Razzaq et al (2013). Zinc has been shown to play a specific role in the hydrolysis of complex polysaccharides into simple sugars, the synthesis of metabolites, and the quick translocation of minerals and photosynthetic products from other parts of the plant to developing fruits, which increases the weight, volume and size of the fruit (Parmar et al 2014). The treated fruits had considerably more fruit juice than untreated fruits (Table 3). Eventually, juice content recovered was higher in $T_{10}$ (113.25 ml) treated with NAA, $GA_3$ @ 30 ppm than in control (78.50 ml), which might be due to enhancement of cell division and cell elongation, likely as a result of the $GA_3$ treated fruits retained firmness and the characteristics of the non-treated fruit as overripe fruit. The increased proportion of juice may also be explained by the fact that hormones control how metabolites are mobilized within a plant, and is well known that growing fruits are particularly active metabolic "sinks" that mobilize metabolites and guide their movement from vegetative structure (Rokayaet al 2016).

## AUTHORS CONTRIBUTION

S. Bhanu Varsha – conception, data collection, analysis and interpretation of results, manuscript preparation, P.K. Nimbolkar – conception, design and manuscript preparation, L. Wangchu – Conception and design , Siddhartha Singh - data analysis and interpretation of results

## CONCLUSIONS

Rangpur lime' requires constant nutrient management for balancing the vegetative growth reproductive growth as well for quality fruit production. Citrus growers are encouraged to apply the foliar application of NAA @ 30 ppm + $GA_3$ @ 30 ppm + 0.5% $ZnSO_4$ + 1% Urea, as it appears to be a promising treatment that improved the growth morphology, yield attributing traits and fruit quality. The foliar application of NAA @ 20 ppm + $GA_3$ @ 20 ppm + 0.5% $ZnSO_4$ + 1% Urea would be recommended with less number of seeds in processing industries of rangpur lime for beverage preparations.

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