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# Enhancing Apricot Growth and Leaf Nutrient Content Through Antioxidant and Bio-Regulator Applications

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***Abstract***: Antioxidants and plant bio-regulators were applied at critical developmental stages, specifically the pink bud and pit hardening stages. The results of the study revealed a significant augmentation in plant growth, along with notable increases in leaf macro- and micronutrient content, attributable to the application of these substances. The gibberellic acid at 50 ppm exhibited the most pronounced effects, with elevated levels observed in plant height, trunk girth, annual shoot growth, leaf area, and the concentrations of nitrogen, potassium, iron, copper, zinc, and manganese within the leaves. Conversely, ascorbic acid at 2000 ppm application demonstrated notable increases in total leaf chlorophyll content, as well as phosphorus, calcium, and magnesium concentrations within the leaf tissue. These findings elucidate the diverse impacts of exogenous substances on apricot physiology, emphasizing their role in fortifying plant vitality and enhancing leaf mineral status. The study contributes to understanding plant physiological dynamics and offers practical insights for optimizing agronomic practices to improve vegetative status as well as the mineral uptake efficiency of apricot across varied agro-ecological contexts.

***Keywords***: Apricot, Antioxidants, Plant bio-regulators, Leaf mineral content, Leaf chlorophyll

Apricot (*Prunus armeniaca* L.), a prominent stone fruit crop within the Rosaceae family and Prunoidae subfamily, thrives in temperate regions worldwide, including the USA, Spain, France, Italy, Turkey, Morocco, Iran, Africa, Australia, and India. Numerous studies have shown that antioxidants can improve photosynthetic efficiency and enhance nutrient uptake in plant by modulating hormonal signalling pathways involved in plant growth and development. Antioxidants are known to have a beneficial role in absorbing the free radicals (hydrogen peroxide, singlet oxygen, hydroxyl radicals, and ozone etc.) produced during plant respiration and photosynthesis (Abd Elhamid et al 2014). In contemporary agricultural practices, there is extensive utilization of naturally occurring and benign substances, notably antioxidants like ascorbic acid and citric acid, aimed at augmenting crop growth and productivity, with the ultimate goal of mitigating health risks. Ascorbic acid is a small and water soluble antioxidants that increases plant growth and photosynthesis attributes in stressful as well as non-stressful environments. It additionally serves a multitude of functions in plant growth (Hajivar et al 2020). Application of exogenous citric acid (CA) has been shown to enhance growth and yield in crop plants under varied conditions by enhancing physiological responses like increased photosynthetic rates, diminished reactive oxygen species levels, and improved osmoregulation, contributing to superior plant performance (Tahjib-Ul-Arif et al 2021).

In addition to these antioxidants, extrinsic application of plant bio-regulators has an advantageous impact on fruit crops' vegetative growth and leaf mineral content. Applying $GA_3$ to the leaves has been demonstrated to improve the mineral contents (N, K, Zn, Mn, and Fe) in the leaves, as well as promote vegetative characteristics including leaf size and chlorophyll content (Zhang et al 2016). PGR benzyladenine (BA), an artificially produced cytokinin molecule that exhibits diverse biological functions in the growth and development of plants, akin to naturally existing cytokinins. Cytokinins are endogenous plant hormones that govern plant growth, encompassing cellular division and leaf senescence (Sardoei et al 2014). The current study aimed to examine the use of naturally occurring and benign compounds, such as ascorbic acid and citric acid, as well as plant growth regulators (PGRs), in agriculture in order to boost crop growth and leaf mineral content.

## MATERIAL AND METHODS

**Location and climate:** The present study was conducted at Dr. Yashwant Singh Parmar University of Horticulture and Forestry, situated in Nauni, Solan (H.P.), India. The experimental site is located at an elevation of 1250 m above mean sea level, namely at 30º 51'N latitude and 76º 11'E longitude. The region in question is situated in the sub-temperate, sub-humid mid-hills agro-climatic zone II of Himachal Pradesh. This zone is distinguished by temperate

summers in May-June and cold winters in December-January. The geographical area under consideration exhibits an annual precipitation range of 110-120 cm, with the predominant occurrence taking place between the months of July to September.

**Planting material and experimental details:** The research was conducted on nine-year-old apricot cultivar New Castle, grafted onto seedling rootstocks. Twenty-seven plants exhibiting uniform vigour and size were selected and planted at a spacing of 5 × 5 m were subjected to various treatments (Table 1). These treatments were meticulously administered at the pink bud stage in February and reiterated at the pit hardening stage in April, over the span of 2019 and 2020. Crop harvesting was conducted during the final week of May.

In order to formulate stock solutions in advance within the laboratory, the preparation process entailed the meticulous weighing. On the day of application, dilutions were conducted directly within the experimental orchard. Bio-regulator ($GA_3$) was first dissolved in a tiny amount of 80 % pure alcohol while being constantly stirred, and then the volume was adjusted to one litre using distilled water. In a similar manner, the compound benzyladenine was initially dissolved in a limited amount of 0.1N sodium hydroxide (NaOH) while stirring continuously. Subsequently, the volume was adjusted to one litre by adding distilled water. The antioxidants were dissolved in distilled water. The final volume of the solution was adjusted to one litre for the stock solution. Subsequent dilutions were prepared according to the experimental specifications. The application of plant bio-regulators and antioxidants was carried out with great attention to detail in the morning hours. This was achieved by utilising a knapsack sprayer equipped with micro fine nozzles, which greatly enhanced the precision of the mist spray. The experiment was conducted in randomized block design with three replications.

**Methodologies:** Plant height measurements were conducted utilizing a graduated flag staff, wherein the distance from the base to the apex of each plant was recorded twice: initially before the onset of growth in January, and subsequently upon growth cessation in December. The increment in plant height was computed and presented as a percentage. Trunk girth was assessed at a standardized height of 15 cm above ground level, initially before the commencement of the experiment and subsequently upon cessation of growth. Measurements were recorded in centimetres (cms) and expressed as a percentage increase in trunk girth.

For annual shoot extension ten shoots from the current season's growth were randomly selected from different locations around the periphery of each plant. Following the cessation of growth, the length of these shoots was measured. To record leaf area, a total of 25 fully expanded and mature leaves were randomly sampled from various locations around the perimeter of each plant during June. Leaf area measurements were conducted utilizing a Leaf Area Meter (LI-COR Model 3100). The average leaf area was computed by dividing the cumulative leaf area measurements by the total number of leaves sampled, and the results were expressed in square centimetres ($cm^2$) per leaf.

For the determination of total chlorophyll content, ten fully expanded and mature leaves were harvested during the initial week of August in the morning hours. The leaves were promptly placed in an icebox to maintain their chlorophyll integrity and transported to the laboratory for analysis, ensuring preservation through refrigeration below 0°C. Upon arrival at the laboratory, the leaves from each sample underwent thorough washing and fine chopping. Subsequently, 10 mg of the finely chopped material was

**Table 1.** Effect of foliar application of antioxidants and plant bio-regulators on increase in growth and chlorophyll content of apricot cv. New Castle

| Treatment | Per cent increase in plant height (%) | Per cent increase in plant girth (%) | Annual shoot extension (cm) | Leaf area ($cm^2$) | Leaf chlorophyll content (mg/100g fresh weight) |
|---|---|---|---|---|---|
| T1-Ascorbic acid (1000 ppm) | 15.89$^{b}$ | 4.41$^{cd}$ | 101.29$^{abc}$ | 42.25$^{b}$ | 2.18$^{abc}$ |
| T2-Ascorbic acid (2000 ppm) | 18.38$^{ab}$ | 5.09$^{bc}$ | 103.62$^{ab}$ | 44.94$^{a}$ | 2.57$^{a}$ |
| 73-Citric acid (1000 ppm) | 14.87$^{bc}$ | 4.7$^{bcd}$ | 94.05$^{de}$ | 43.58$^{ab}$ | 1.98$^{bc}$ |
| T4-Citric acid (2000 ppm) | 17.22$^{b}$ | 5.34$^{b}$ | 97.35$^{cde}$ | 42.79$^{b}$ | 2.48$^{a}$ |
| T5-Benzyladenine (50 ppm) | 15.09$^{bc}$ | 3.95$^{de}$ | 92.78$^{e}$ | 41.58$^{b}$ | 1.88$^{c}$ |
| T6-Benzyladenine (100 ppm) | 16.15$^{b}$ | 4.64$^{bcd}$ | 99.55$^{bcd}$ | 42.44$^{b}$ | 2.16$^{abc}$ |
| T7-Gibberellic acid (25 ppm) | 16.95$^{b}$ | 6.87$^{a}$ | 98.49$^{bcde}$ | 41.95$^{b}$ | 1.93$^{bc}$ |
| T8-Gibberellic acid (50 ppm) | 20.98$^{a}$ | 7.46$^{a}$ | 105.79$^{a}$ | 45.39$^{a}$ | 2.34$^{ab}$ |
| T9-Control (Water spray) | 11.49$^{c}$ | 3.23$^{e}$ | 79.81$^{f}$ | 38.89$^{c}$ | 1.41$^{d}$ |

Figures with same letter in column do not differ significantly

transferred into vials containing 7 ml of dimethyl sulphoxide (DMSO). These vials were then subjected to incubation at a controlled temperature of 65°C for duration of thirty minutes. Following incubation, the resulting extract was transferred into graduated test tubes, and the final volume was adjusted to 10 ml with dimethyl sulphoxide (DMSO), adhering to the methodology outlined by Hiscox and Israelstam (1979). The optical density (OD) of the resultant extract was measured using a Spectronic-20D spectrophotometer at wavelengths of 645 nm and 663 nm, relative to a dimethyl sulphoxide (DMSO) blank to estimate the total chlorophyll content. The results obtained were then expressed as mg of chlorophyll per gram of fresh weight.

For the estimation of leaf mineral content, leaf samples were collected from the midsection of the current season's growth surrounding the tree perimeter, in accordance with the guidelines provided by Kenworthy (1964), during the final week of June to the middle of July. Cleaning, drying, grinding, and storage of the samples were conducted following the procedure outlined by Chapman (1964). The digestion of one-gram leaf samples for the estimation of nitrogen was carried out in concentrated sulphuric acid by adding the digestion mixture (potassium sulphate 480 parts, copper sulphate 20 parts, mercuric oxide 3 parts and selenium powder 1 part) as suggested by Jackson (1967). Total nitrogen content was subsequently estimated using the micro-Kjeldahl method (Jackson 1973). For the estimation of phosphorus, potassium, calcium, magnesium, iron, copper, zinc, and manganese, leaf samples were digested using a diacid mixture containing nitric acid and perchloric acid in a ratio of 4:1 (Jackson 1967). Phosphorus content was determined using the vanadate-molybdate phosphoric yellow color method (Jackson 1973). Potassium content was determined using a flame photometer. Total calcium, magnesium, iron, manganese, zinc, and copper were determined using a Perkin-Elmer Atomic Absorption Spectrophotometer model 400. Macro and micro-nutrient levels were expressed on a dry weight basis as percentage and parts per million (ppm), respectively.

All parameters investigated were recorded over the course of two years (2019-2020) and subjected to statistical analysis. Pooled treatment means were compared in OPSTAT utilizing the Duncan Multiple Range Test (DMRT) at a significance level of 5% (Duncan 1955).

## RESULTS AND DISCUSSION

**Tree growth:** The vegetative traits of apricot underwent significant improvement due to the external application of various antioxidants and plant bio-regulators (Table 1). Gibberellic acid 50 ppm, ascorbic acid 2000 ppm, citric acid 2000 ppm, benzyladenine 100 ppm, and gibberellic acid 25 ppm predominantly led to notable enhancements in plant height during both years and in the aggregated data. The gibberellic acid 25 and 50 ppm, ascorbic acid 2000 ppm, citric acid 2000 ppm, and benzyladenine 100 ppm consistently yielded higher percentage increases in plant girth compared to alternative treatments The annual shoot extension showed higher percentage increases with gibberellic acid at 25 and 50 ppm, ascorbic acid at 2000 ppm and citric acid at 2000 ppm, when compared to other treatments. Among the treatments, gibberellic acid at 50 ppm consistently exhibited the highest percentage increase in plant height, plant girth and annual shoot extension throughout both years and in the aggregated data. The superiority of gibberellic acid in plant growth may be attributed to its role in controlling the cell elongation and cell division (Pires et al 2000) and direct effect on internodal elongation. Gibberellins also play a primary role in stimulating the auxin reaction, which helps in controlling the vegetative growth. The most typical property of gibberellins is promotion of stem growth. These findings are in conformity with Hazarika et al (2016) in papaya cv. Red Lady and Gholap et al (2000) in aonla.

The increase in plant vegetative parameters with the foliar application of antioxidants (ascorbic and citric acid) might be due to its auxin like actions, which ultimately cause cell division and growth (Ortiz-Espin et al 2017). Ascorbic acid is known for its capacity to enhance chlorophyll levels in leaves, hence augmenting photosynthetic efficiency and facilitating energy provision for diverse plant functions, including growth and development. In addition, also plays a role in cell division and enlargement and helps in organizing the growth processes and plant development eventually increasing the plant leaf area (Abd-El-Aziz et al 2006). These findings are in agreement with El-Badawy (2013) in Canino apricot. The application of gibberellic acid at higher concentrations (50 ppm) resulted in a notable increase in leaf area compared to the control and other treatments (Table 1). Gibberellic acid, known for its role in regulating plant growth and development, likely stimulated cell elongation and expansion, contributing to the observed augmentation in leaf area. The increase in leaf area due to gibberellic acid may be attributed to the role of gibberellins in both cell division and elongation (Batlang et al 2006). Gibberellic acid treatments also exerted notable effects on leaf chlorophyll content, particularly at higher concentrations (50 ppm), where significant increases were observed compared to the control and some other treatments. Gibberellic acid, known for its role in promoting cell elongation and expansion, may indirectly influence chlorophyll accumulation by enhancing leaf area and photosynthetic capacity. The present findings are also in

conformity with the results obtained by El-Naby et al (2019) in Canino apricot and Al-Rawi (2016) in peach cv. Peento. The observed increase in leaf area following the application of ascorbic acid, particularly at higher concentrations (2000 ppm), underscores its potential role as a stimulant for promoting leaf expansion and canopy development. This enhancement in leaf area can be attributed to the physiological effects of ascorbic acid, including its involvement in cell division, expansion, and maintenance of cellular integrity, thereby facilitating the proliferation of leaf tissue. Ascorbic acid treatments, particularly at higher concentrations (2000 ppm), elicited a significant increase in leaf chlorophyll content compared to other treatments and the control group. The superiority of ascorbic acid treatment in enhancement of chlorophyll content may be attributed to its role as an antioxidant Ascorbic acid acts as a cofactor for enzymes involved in the mechanisms of photosynthesis and hormone synthesis in plants. Ascorbic acid mitigates oxidative stress-induced senescence in wheat leaves by increasing the activities of catalase and ascorbate peroxidase, and reducing chlorophyll degradation.

Conversely, treatments involving citric acid and benzyladenine exhibited more varied effects on leaf chlorophyll content, with some treatments demonstrating either increases or decreases compared to the control. These contrasting responses may reflect the complex interplay between exogenously applied compounds and endogenous regulatory mechanisms governing chlorophyll metabolism, including its synthesis, degradation, and turnover rates. The augmentation in leaf chlorophyll due to exogenous application of antioxidants and PGR's may also be due to increased plant efficiency in absorbing elements from soil including Mg and Fe (Table 2, 3) leading to increasing their concentration in the leaves as are necessary and important for the formation of chlorophyll molecule (Fayed 2010). Similar findings pertaining to ascorbic acid application have been reported by El-Badawy (2013) in Canino apricot trees, Metep and Hasan (2020) in apricot cv. Zaghenia and Abdel-Salam (2016) in grape cv. Ruby. The control group, subjected to water spray, exhibited relatively

**Table 2.** Effect of foliar application of antioxidants and plant bio-regulators on NPK, calcium and magnesium content in leaf of apricot cv. New Castle

| Treatments | Nitrogen (%) | Phosphorus (%) | Potassium (%) | Calcium (%) | Magnesium (%) |
|---|---|---|---|---|---|
| T1 | $2.36^{e}$ | $0.19^{de}$ | $2.42^{d}$ | $2.64^{ab}$ | $0.7^{bc}$ |
| T2 | $2.75^{a}$ | $0.27^{a}$ | $2.72^{ab}$ | $2.69^{a}$ | $0.74^{a}$ |
| T 3 | $2.41^{de}$ | $0.21^{cd}$ | $2.28^{e}$ | $2.46^{c}$ | $0.64^{e}$ |
| T4 | $2.59^{bc}$ | $0.24^{b}$ | $2.46^{d}$ | $2.52^{c}$ | $0.66^{de}$ |
| T5 | $2.35^{e}$ | $0.21^{cd}$ | $2.59^{c}$ | $2.53^{bc}$ | $0.63^{e}$ |
| T6 | $2.49^{cd}$ | $0.23^{bc}$ | $2.63^{bc}$ | $2.51^{c}$ | $0.69^{bcd}$ |
| T7 | $2.5^{cd}$ | $0.24^{b}$ | $2.64^{bc}$ | $2.49^{c}$ | $0.68^{cd}$ |
| T8 | $2.67^{ab}$ | $0.27^{a}$ | $2.78^{a}$ | $2.56^{bc}$ | $0.72^{ab}$ |
| T9 | $2.13^{f}$ | $0.17^{e}$ | $2.16^{f}$ | $2.22^{d}$ | $0.58^{f}$ |

For treatment details see Table 1
Figures with same letter in column do not differ significantly

**Table 3.** Effect of foliar application of antioxidants and plant bio-regulators on mineral content in leaf of apricot cv. New Castle

| Treatments | Iron (ppm) | Copper (ppm) | Manganese (ppm) | Zinc (ppm) |
|---|---|---|---|---|
| T1 | $157.16^{cd}$ | $6.85^{c}$ | $47.97^{c}$ | $22.53^{d}$ |
| T2 | $158.6^{bc}$ | $7.37^{b}$ | $48.55^{c}$ | $23.53^{c}$ |
| T 3 | $155.54^{cd}$ | $7.06^{bc}$ | $48.78^{c}$ | $21.21^{e}$ |
| T4 | $156.06^{cd}$ | $7.04^{bc}$ | $49.89^{c}$ | $22.24^{d}$ |
| T5 | $157.67^{cd}$ | $8.11^{a}$ | $53.97^{b}$ | $24.2^{bc}$ |
| T6 | $160.46^{abc}$ | $8.25^{a}$ | $55.37^{ab}$ | $24.55^{b}$ |
| T7 | $163.46^{ab}$ | $8.21^{a}$ | $56.25^{ab}$ | $26.23^{a}$ |
| T8 | $165.77^{a}$ | $8.47^{a}$ | $57.42^{a}$ | $26.55^{a}$ |
| T9 | $152.24^{d}$ | $5.91^{d}$ | $44.76^{d}$ | $20.92^{e}$ |

Figures with same letter in column do not differ significantly
For treatment details see Table1

lower increase in plant height, girth, annual shoot extension, leaf area, and chlorophyll content values compared to the treated groups, underscoring the importance of applied treatments in modulating plant vegetative growth, chlorophyll metabolism and overall photosynthetic efficiency.

**Mineral content in leaf:** The leaf nitrogen content exhibited variation among the various treatments, with values ranging from 2.13 to 2.75% (Table 2). The nitrogen content was highest in 2000 ppm ascorbic acid and 50 ppm gibberellic acid, whereas control exhibited the lowest nitrogen concentration. There was variability in the leaf phosphorus percentage among the different treatments, with recorded values ranging from 0.17 to 0.27%. The leaf phosphorus content was maximum in 2000 ppm ascorbic acid and 50 ppm gibberellic acid . The amount of potassium exhibited variation among the different treatments, with recorded values varying between 2.16%to 2.78%. Application of 50 ppm gibberellic acid exhibited the highest concentration of leaf potassium whereas control demonstrated the lowest concentration of potassium. Al-Rawi et al (2016) observed significantly higher leaf nitrogen and potassium content with foliar application of $GA_3$ in peach cv. Peento. Soest (2012) reported that spray of gibberellic acid increased leaf P content in apple cultivar Anna. El-Badawy (2013) also mentioned that foliar application of ascorbic and citric acid gave significantly higher leaf N, P, and K content in "Canino" apricot.

Examination of calcium content in leaf indicated that treatments involving ascorbic acid (1000 ppm and 2000 ppm) demonstrated notable increases in leaf calcium levels compared to other treatments (Table 2). This observation suggested a potential role for ascorbic acid in enhancing calcium uptake in plants. Abd-El-Rhman et al (2017), reported increased leaf Ca content with foliar sprays of ascorbic acid at 2000 ppm in pomegranate. There was notable variation in magnesium uptake across the different treatments. Ascorbic acid 2000 ppm and gibberellic acid 50 ppm consistently showed higher magnesium percentages compared to other treatments suggesting a potential positive effect of these substances on magnesium uptake. The findings are in agreement with Daood and Shahin (2006) in apricot cv. Canino, and Soest (2012) in apple cultivar Anna.

Across the treatments and years, there were discernible variations in leaf iron status (Table 3). Treatments utilizing gibberellic acid at 25 and 50 ppm ($T_8$) consistently resulted in the highest iron percentages, indicating a potential positive influence of gibberellic acid on iron uptake by plants. Treatments with higher concentrations of gibberellic acid ($T_7$ and $T_8$) and benzyladenine ($T_5$ and $T_6$) consistently resulted in higher copper percentages. Treatments containing gibberellic acid ($T_7$ and $T_8$) and benzyladenine ($T_5$ and $T_6$) consistently resulted in higher leaf manganese and zinc compared to other treatments suggesting a potential positive influence of these substances on manganese and zinc uptake by plants. Soest (2012) also observed that spray of gibberellic acid increased leaf Zn and Fe content in apple cultivar Anna. Foliar sprays of 100 ppm $GA_3$ also increased leaf Fe content in peach cv. Peento (Al-Rawi 2016). Mahmoud et al (2015) reported that application of benzyladenine increased leaf Zn, Fe and Mn content over control in "Manzanillo" olives.

## CONCLUSION

The notable influence of antioxidant and bio-regulator interventions on the growth of apricots and the nutritional profile of their leaves was evident. The effectiveness of different treatments, specifically gibberellic acid and high-concentration ascorbic acid, in strengthening plant vigour, increasing chlorophyll content, and improving leaf mineral uptake has been established through rigorous research and analysis. This provides a valuable insight for enhancing apricot cultivation techniques to enhance both crop output and quality.

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