



## Impact of aeroponic cultivation and plant growth regulators on the biochemical composition of grapevine leaves

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### ABSTRACT

**Background:** Grapevine (*Vitis vinifera* L.) is a globally important perennial fruit crop, renowned for its economic value and cultural significance. Grape leaves, while often underappreciated, play a vital role in culinary traditions and are valued for their cultural, nutritional, and health benefits. However, the availability of grape leaves is constrained by viticulture practices and chemical treatments that prioritize fruit production over leaf harvesting. This study seeks to compare the biochemical characteristics of grapevine leaves from the cultivar '*Deghin Yerevani*' grown from virus-free, tissue-cultured plants under aeroponic and soil conditions within a greenhouse.

**Objective:** To evaluate the impact of aeroponic versus soil-based cultivation and the application of specific growth regulators on the biochemical composition and nutrient content of '*Deghin Yerevani*' grapevine leaves, with the goal of optimizing conditions for high-quality leaf production.

**Materials and Methods:** This study, conducted from 2021 to 2023 at the Scientific Center of Agrobiotechnology, ANAU, utilized virus-free *in vitro* plants of *Vitis vinifera* L. cv. '*Deghin Yerevani*', sourced from the National Grape Field Collection. Plants were cultured in four treatment groups with varying growth media compositions: PGR-free, with Indole-3-acetic

acid (IAA), with IAA and Gibberellic acid ( $GA_3$ ), and with IAA,  $GA_3$ , and 6-Benzylaminopurine (BAP). Post-culturing, plants were transferred to either an aeroponic system, which used a misting cycle and maintained controlled environmental conditions, or to a greenhouse with a defined soil mix and monitored moisture levels. Both systems operated for 8 weeks, with each treatment replicated three times. Chlorophylls a and b, ascorbic acid, sugars, and macro- and micronutrient contents were analyzed, and statistical significance was determined using standard error and *Student's t-test* ( $p < 0.05$ ).

**Results:** Grapevine leaves from 'Deghin Yerevani' plants grown in aeroponic systems exhibited significantly higher chlorophyll a, chlorophyll b, and total chlorophyll compared to those grown in soil. Growth regulators, particularly IAA,  $GA_3$ , and BAP, enhanced chlorophyll content, sugar levels, and Vitamin C across both cultivation methods. Aeroponic systems showed superior results with increased sugar content (5.7 g/100g) and Vitamin C (20.4 mg/100g) compared to soil-based cultivation. Nutrient analysis revealed higher levels of nitrogen, phosphorus, potassium, calcium, and magnesium in aeroponics. Overall, growth regulators and aeroponic cultivation improved the biochemical and nutrient profiles of grapevine leaves.

**Conclusion:** The study demonstrated that aeroponic cultivation and the application of growth regulators significantly enhanced the biochemical composition of grapevine leaves. Higher levels of chlorophyll, sugar, and Vitamin C, along with improved nutrient content, were observed in aeroponic systems compared to soil-based cultivation. These findings underscore the benefits of aeroponics and growth regulators in optimizing grapevine leaf quality, suggesting potential for improved yield and nutritional value in grapevine production.

**Keywords:** 'Deghin Yerevani', grapevine, *in vitro* propagation, functional foods, Aeroponic Cultivation, Plant Growth Regulators



**Graphical abstract:** grapevine, functional properties, plant growth regulators, Grapevine leaves

## INTRODUCTION

Grapevine (*Vitis vinifera* L.) is a globally significant perennial fruit crop, widely cultivated for its economic value and cultural importance [1-3]. Having been cultivated for thousands of years, grapes are not only consumed fresh but are also the foundation for various products, including wine, juice, and dried fruits [4].

Grape leaves, though often overlooked, hold substantial value in culinary traditions, prized for their cultural, nutritional, and health-related benefits [5-6]. They are especially known for their use in dolmas, a traditional dish where the leaves are stuffed with a mixture of rice, herbs, and sometimes meat, with variations found across many cultures. Additionally, grape leaves are used to wrap foods like fish, cheese, or vegetables before grilling or baking, adding a subtle, earthy flavor to these dishes [7].

Despite their culinary significance, the availability of grape leaves is limited due to viticulture practices and chemical treatments that prioritize fruit production [8]. While freshly harvested leaves are preferred, the removal of young shoots to enhance grape quality reduces their availability. Nevertheless, grape leaves are recognized for their nutritional value and potential health benefits [9].

In regard to nutrition, grape leaves are low in calories but rich in essential nutrients, such as vitamins A, C, and K, as well as fiber, calcium, and iron. They contain biochemical compounds such as polyphenols, flavonoids, and phenolic acids, which are known for their antioxidant, anti-inflammatory, and anti-cancer properties [10-11]. These bioactive compounds play a critical role in combating oxidative stress, supporting bone health, and boosting immune function, thereby contributing to the prevention of chronic diseases [12-14].

The concept of functional foods, which provide health benefits beyond basic nutrition, is largely based on the presence of bioactive compounds. These compounds

are essential in preventing or managing chronic diseases through various physiological mechanisms [15-17]. Recent research has increasingly focused on optimizing the production and concentration of these beneficial compounds through advanced cultivation techniques [18]. The biochemical quality of grapevine plants is influenced by genetic, environmental, and cultivation factors [19-21].

Advances in horticultural practices, such as *in vitro* micropropagation and controlled environment cultivation, have opened new avenues for enhancing the biochemical quality of grape crops. One area of significant interest is the role of plant hormones in this process. Hormonal treatments can greatly influence both the propagation efficiency and the biochemical composition of grapevines [22]. Understanding the impact of different plant growth regulators on the synthesis of bioactive compounds is crucial for improving grapevine productivity and health benefits [23].

Tissue culture and aeroponic systems are advanced cultivation methods that provide controlled environments conducive to promoting plant growth and enhancing leaf quality. Tissue culture offers a sterile, disease-free propagation system that ensures the preservation of consistent genetic traits [24-29]. In contrast, aeroponic systems deliver nutrients directly to plant roots in a mist or air environment, facilitating optimal nutrient uptake and growth [30].

This study aims to compare the biochemical characteristics of grapevine leaves from the cultivar '*Deghin Yerevani*', derived from virus-free, tissue-cultured plants grown in aeroponic versus soil conditions within a greenhouse.

The goal is to evaluate the impact of aeroponic versus soil-based cultivation and the application of specific growth regulators on the biochemical composition and nutrient content of grapevine leaves from the '*Deghin Yerevani*' cultivar, with the goal of optimizing conditions for high-quality leaf production.

## MATERIALS AND METHODS

**Experimental Setup:** The study was conducted from 2021 to 2023 at the Scientific Center of Agrobiotechnology, ANAU. Virus-free *in vitro* plants of the grapevine cultivar *Vitis vinifera* L. 'Deghin Yerevani', a native Armenian seedless variety, were used. These plants were regenerated from the shoot apical meristem and sourced from the National Grape Field Collection (geographic coordinates 40.157419°N, 44.291986°E). The *in vitro* plants were cultured in four distinct groups, each with a different growth medium composition. Group I consisted of MS medium without any plant growth regulators (PGR-free). Group II involved MS medium supplemented with 1.0 mg/L Indole-3-acetic acid (IAA). Group III contained MS medium with 1.0 mg/L IAA and 1.0 mg/L gibberellic acid (GA<sub>3</sub>). Group IV included MS medium supplemented with 1.0 mg/L IAA, GA<sub>3</sub>, and 6-Benzylaminopurine (BAP). Following the *in vitro* culturing phase, plants from each group were transferred to either an aeroponic system or pots in a greenhouse for further growth and analysis.

**Aeroponic System:** The aeroponic system, designed for soil-free cultivation, kept plant roots suspended in a controlled environment, where they received essential nutrients, water, and oxygen through a nutrient-rich mist. This mist, delivered via nozzles or sprayers, allowed for direct root exposure to air, differentiating it from hydroponics, which uses water-based solutions [31]. The system utilized a misting cycle of 5-second bursts every 15 minutes throughout the day. The environment was maintained at a temperature range of 20°C to 25°C with 70% humidity, and with a photoperiod of 16 hours of light followed by 8 hours of darkness, at a light intensity of 50  $\mu\text{mol}/\text{m}^2\cdot\text{s}$ . The nutrient solution was based on that of Buttaro et al. (2012) [32] and Di Lorenzo et al. (2013) [33].

**Greenhouse Environment:** Simultaneously, other plants were transferred to pots in a greenhouse. The greenhouse conditions were controlled with a temperature range of 20°C to 25°C and a relative

humidity of 60% to 70%. It was equipped with both natural and artificial lighting to ensure a photoperiod of 16 hours of light and 8 hours of darkness. Soil moisture was monitored, and watering was performed when the top inch of soil felt dry. A balanced, slow-release fertilizer was applied as needed. The greenhouse pots contained a soil mixture composed of one part perlite, two parts peat, and one part soil.

Both systems ran for 8 weeks, with each treatment (aeroponic and soil) replicated three times. Each group consisted of 10 plants, totaling 40 plants across all groups. To obtain reliable results, 5 to 10 mature, healthy leaves per plant were harvested, ensuring minimal variability. Chlorophylls a and b were determined according to Lichtenthaler (1987) [34], ascorbic acid content via iodine titration (AOAC International, 2000) [35], and sugars were measured using a modified method based on Melgarejo et al. (2000) [36]. Macro- and micronutrient content was assessed according to Jones, Wolf, and Mills (1991) [37].

**Statistical Analysis:** Data from three independent experiments were combined and presented as mean values. Treatment means were compared using the *standard error (SE)* of the mean. Significant differences between means were determined using a *Student's t-test*, with significance set at  $p < 0.05$ .

## RESULTS

This study evaluated the biochemical composition of grapevine leaves from virus-free tissue-cultured 'Deghin Yerevani' plants, comparing those cultivated in aeroponic versus soil conditions. Notable differences were observed in photosynthetic pigments and nutrient content between the two cultivation methods.

Table 1 summarizes the chlorophyll content in grapevine leaves under different treatments and growing conditions. Plants cultivated in the aeroponic system exhibited significantly higher levels of chlorophyll a, chlorophyll b, and total chlorophyll compared to those grown in soil ( $P < 0.05$ ).

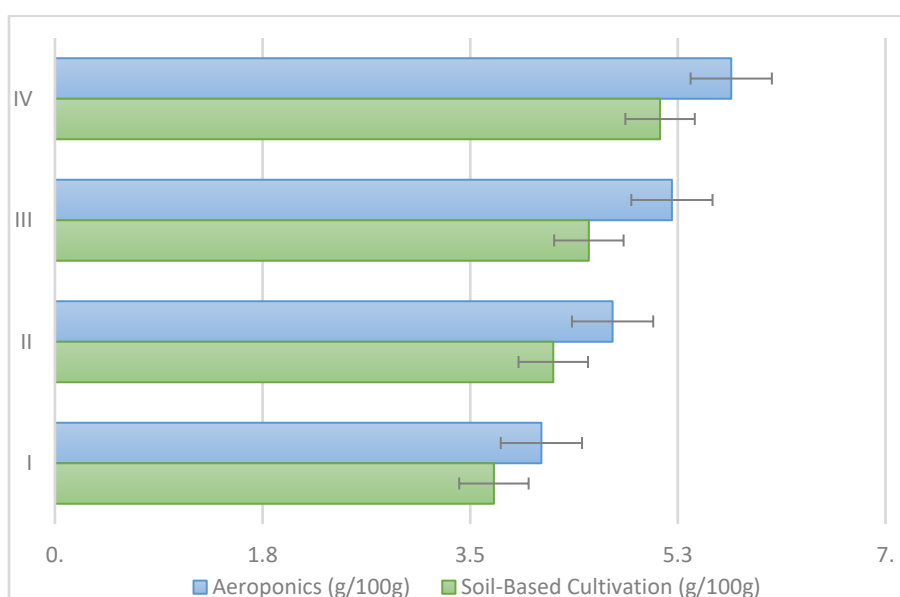
**Table 1.** Comparison of Leaf Chlorophyll a, Chlorophyll b, and Total Chlorophyll in *Vitis vinifera* Under Greenhouse and Aeroponic Conditions.

Treatment Group	Growing Condition	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Chl a/b	Total Chlorophyll (mg/g FW)
Group I (PGR-free) control	Greenhouse	1.35 ±0.009	0.60±0.01	2.24±0.05	1.95
	Aeroponic	1.45±0.005	0.63±0.005	2.30±0.016	2.08
Group II (IAA 1.0 mg/L)	Greenhouse	1.68 ±0.007	0.66±0.02	2.54±0.006	2.34
	Aeroponic	1.75±0.005	0.67±0.006	2.61±0.03	2.42
Group III (IAA 1.0 mg/L +GA 1.0 mg/l)	Greenhouse	1.83±0.01	0.71±0.009	2.58±0.01	2.54
	Aeroponic	1.90 ±0.01	0.72±0.01	2.64±0.03	2.62
Group IV (IAA 1.0 mg/L + GA <sub>3</sub> 1.0 mg/l + BAP 1.0 mg/l)	Greenhouse	2.0 ±0.06	0.74 ±0.006	2.70 ±0.07	2.74
	Aeroponic	2.6 ±0.06	0.83±0.01	3.1± 0.08	3.43

Groups II (IAA 1.0 mg/L) and III (IAA 1.0 mg/L + GA<sub>3</sub>) displayed higher chlorophyll a level compared to the control group. Group IV (IAA 1.0 mg/L + GA<sub>3</sub> + BAP 1.0 mg/L) had the highest chlorophyll a content. Similar to chlorophyll a, Groups II and III showed increased chlorophyll b levels relative to the control. Group IV exhibited the highest chlorophyll b content. The highest ratio (*Chl a/b*) was observed in Group IV, indicating an

enhanced balance between chlorophyll a and b due to the combined treatments. Group IV had the highest total chlorophyll content, with Group III also showing increased levels compared to the control.

**Sugar Content:** The sugar content in grapevine leaves varied with cultivation method and growth regulators [Fig.1].



**Fig. 1.** Sugar Content of *In vitro*-Derived Plantlets leaves Grown in Aeroponic and Soil-Based Conditions.

In terms of sugar content, significant differences were noted between the two cultivation methods and among the growth regulator treatments. In soil-based cultivation, the sugar content increased from 3.7 g/100g in the control group to 5.1 g/100g in Group IV. Aeroponic cultivation led to even higher sugar levels, rising from 4.1

g/100g in the control group to 5.7 g/100g in Group IV. The application of growth regulators consistently enhanced sugar content, with Group IV (IAA 1.0 mg/L + GA<sub>3</sub> + BAP 1.0 mg/L) achieving the highest values in both soil-based and aeroponic systems.

**Table 2.** shows the Vitamin C content in grapevine leaves under various growth conditions and treatments. Vitamin C Content of *In Vitro*-Derived Plantlets Grown in Aeroponic and Soil-Based Conditions

Group	Growth Regulators Applied	Aeroponics, Vitamin C Content, mg/100g	Soil-Based Cultivation, Vitamin C Content, mg/100g
Group I	None	15.24	13.7
Group II	IAA (1.0 mg/L)	17.5	15.0.0
Group III	IAA (1.0 mg/L) + GA <sub>3</sub> (1.0 mg/L)	18.2	16.3
Group IV	IAA (1.0 mg/L) + GA <sub>3</sub> (1.0 mg/L) + BAP (1.0 mg/L)	20.4	18.8

Vitamin C content also varied significantly with cultivation method and growth regulator application. In aeroponic systems, Vitamin C content increased from 15.24 mg/100g in the control group to 20.4 mg/100g in Group IV. Soil-based cultivation showed a similar trend, with Vitamin C content rising from 13.7 mg/100g in the control to 18.8 mg/100g in Group IV. The application of growth regulators, particularly the combination of IAA, GA<sub>3</sub>, and BAP, significantly enhanced Vitamin C levels in grapevine leaves under both cultivation conditions.

The mineral content of fresh grape leaves was evaluated under different cultivation methods, including the application of growth regulators (IAA, GA<sub>3</sub>, and BAP) (table 3).

The mineral content analysis of fresh grape leaves revealed distinct trends across different cultivation methods and treatments. Nitrogen (N) content increased from 7.2±0.03 mg/100g in the soil-based control group to 8.3±0.03 mg/100g with the application of growth

regulators. In aeroponic systems, nitrogen levels were consistently higher, rising from 8.1±0.02 mg/100g in the control group to 9.7±0.04 mg/100g with growth regulators. Phosphorus (P) content also showed a significant increase in both cultivation methods, rising from 73.2±1.7 mg/100g to 87.2±2.6 mg/100g in soil-based cultivation and from 86.1±2.0 mg/100g to 95.2±1.9 mg/100g in aeroponics. Potassium (K) levels exhibited a marked increase, from 235.4±3.4 mg/100g to 255.2±2.16 mg/100g in soil-based cultivation and from 251.2±2.7 mg/100g to 275.4±3.1 mg/100g in aeroponics. Similarly, calcium (Ca) content improved with the application of growth regulators, increasing from 269.8±2.4 mg/100g to 305.1±2.8 mg/100g in soil-based cultivation and from 286.0±3.1 mg/100g to 321.2±3.4 mg/100g in aeroponics. Magnesium (Mg) levels increased slightly, from 80.19±0.70 mg/100g to 81.89±0.60 mg/100g in soil-based cultivation and from 83.9±0.50 mg/100g to 87.99±1.0 mg/100g in aeroponics.

**Table 3.** Mineral Content of Fresh Grape Leaves (mg/100g)

Element	Soil-Based Cultivation Control	Aeroponics Control	Soil-Based Cultivation, IAA (1.0 mg/L) + GA <sub>3</sub> (1.0 mg/L) + BAP (1.0 mg/L)	Aeroponics; IAA (1.0 mg/L) + GA <sub>3</sub> (1.0 mg/L) + BAP (1.0 mg/L)
Nitrogen (N)	7.2±0.03	8.1±0.02	8.3±0.03	9.7±0.04
Phosphorus (P)	73.2±1.7	86.1±2.0	87.2±2.6	95.2±1.9
Potassium (K)	235.4±3.4	251.2±2.7	255.2±2.16	275.4±3.1
Calcium (Ca)	269.8±2.4	286.0±3.1	305.1±2.8	321.2±3.4
Magnesium (Mg)	80.19±0.70	83.9±0.50	81.89±0.60	87.99±1.0
Iron (Fe)	2.0±0.23	2.4±0.10	2.10±0.23	2.60±0.10
Copper (Cu)	0.29±0.01	0.31±0.02	0.31±0.01	0.33±0.01
Zinc (Zn)	0.43±0.02	0.50±0.02	0.51±0.02	0.59±0.02
Manganese (Mn)	2.45±0.01	2.58±0.02	2.61±0.02	2.95±0.01

The iron (Fe) content rose modestly, from 2.0±0.23 mg/100g to 2.1±0.23 mg/100g in soil-based cultivation and from 2.4±0.10 mg/100g to 2.6±0.10 mg/100g in aeroponics. Copper (Cu) content increased slightly from 0.29±0.01 mg/100g to 0.31±0.01 mg/100g in soil-based cultivation and from 0.31±0.02 mg/100g to 0.33±0.01 mg/100g in aeroponics. Zinc (Zn) levels also rose modestly in both methods, from 0.43±0.02 mg/100g to 0.51±0.02 mg/100g in soil-based cultivation and from 0.50±0.02 mg/100g to 0.59±0.02 mg/100g in aeroponics. Finally, manganese (Mn) content increased with growth regulator application, from 2.45±0.01 mg/100g to 2.61±0.02 mg/100g in soil-based cultivation and from 2.58±0.02 mg/100g to 2.95±0.01 mg/100g in aeroponics.

## DISCUSSION

The findings of this study highlight the superior efficacy of aeroponic cultivation systems in enhancing the biochemical properties of grapevine leaves. Aeroponic cultivation consistently resulted in higher levels of chlorophyll a and b, indicating an optimal environment for chlorophyll synthesis [38]. The increased chlorophyll a/b ratio observed in Group IV (IAA at 1.0 mg/L, GA<sub>3</sub>, and BAP) under aeroponics reflects enhanced photosynthetic

efficiency. This supports the notion that controlled environments, such as those provided by aeroponics, are highly effective in optimizing plant physiology, aligning with previous research on the advantages of such systems [39].

Plant Growth Regulators (PGRs) including IAA, GA<sub>3</sub>, and BAP were found to significantly boost chlorophyll content in both cultivation methods. Notably, the combination of these PGRs in Group IV demonstrated the most substantial increase in chlorophyll levels under aeroponics. This finding is consistent with studies suggesting that PGRs enhance chlorophyll production by influencing critical metabolic pathways [40].

Aeroponic systems showed superior performance compared to traditional greenhouse cultivation in terms of chlorophyll content. This can be attributed to the controlled factors such as humidity, nutrient availability, and root oxygenation, which collectively create an environment conducive to optimal nutrient uptake and metabolic activity. The combination of aeroponics with targeted PGRs not only enhances chlorophyll synthesis but also holds potential for improving grapevine growth and yield. Future research should delve into the mechanisms behind these effects and assess their impact

across various grapevine cultivars and environmental conditions.

The impact of growth regulators on sugar content in grapevine leaves was significant across both soil-based and aeroponic systems [41]. The highest sugar content was observed with the application of IAA, GA<sub>3</sub>, and BAP, highlighting the synergistic effects of these hormones on metabolic pathways that lead to increased sugar accumulation [42]. This result aligns with previous studies showing that PGRs influence key physiological processes related to sugar metabolism [43].

Aeroponic cultivation consistently yielded higher sugar content compared to soil-based systems.

This may be due to the more controlled environment in aeroponics, which enhances nutrient uptake and physiological processes more efficiently than traditional soil-based systems. While IAA alone and in combination with GA<sub>3</sub> showed improvements, the most significant enhancement was observed with the combination of IAA, GA<sub>3</sub>, and BAP. This reflects the combined roles of these hormones in regulating growth and sugar metabolism: IAA influences cell elongation and differentiation, GA<sub>3</sub> promotes cell division and elongation, and BAP aids in cell proliferation and shoot formation.

Vitamin C content also increased significantly with the application of growth regulators, particularly in the aeroponic system [44]. The control group under aeroponics had 15.24 mg/100g of Vitamin C, which increased to 20.4 mg/100g in Group IV with the application of IAA, GA<sub>3</sub>, and BAP. This trend was also observed in soil-based cultivation, with the highest Vitamin C content of 18.8 mg/100g in Group IV. Higher Vitamin C levels in aeroponics can be attributed to more efficient nutrient uptake and optimal growth conditions [45]. These findings underscore the role of PGRs in enhancing Vitamin C content, with aeroponics amplifying these effects [46].

The combined use of growth regulators and aeroponics significantly impacted the mineral content of grapevine leaves. Aeroponic systems consistently yielded higher concentrations of all major mineral elements compared to soil-based cultivation, particularly with the application of growth regulators. Enhanced levels of nitrogen, phosphorus, potassium, and calcium observed in aeroponics are attributed to improved nutrient uptake efficiency, as roots are directly exposed to nutrient-rich solutions. Growth regulators further amplified this effect by promoting root development and increasing nutrient absorption.

While magnesium, iron, copper, zinc, and manganese also showed increased concentrations in aeroponics, the differences were less pronounced compared to macronutrients. This suggests that while aeroponics effectively boosts overall mineral content, the impact on micronutrient absorption may vary depending on the specific growth regulators used. The synergistic approach of combining growth regulators with aeroponics optimizes the nutrient profile of grapevine leaves, potentially leading to improved plant growth, higher yields, and enhanced fruit quality.

Further studies are needed to assess the long-term effects of these methods on grapevine growth, yield, and fruit quality.

## CONCLUSION

This study demonstrated that aeroponic cultivation significantly improved the nutritional quality of grapevine leaves, surpassing traditional methods in enhancing chlorophyll, sugar, and Vitamin C content. The addition of plant growth regulators (PGRs) further optimized these benefits, suggesting a combined approach could boost plant growth and yield. The increased mineral content observed in aeroponics, particularly for nitrogen, phosphorus, potassium, and calcium, highlighted its potential to optimize grapevine cultivation. Future



studies should assess the long-term effects of these methods on grapevine growth, yield, and fruit quality.

**Abbreviations:** ANAU: Armenian National Agrarian University, BAP: 6-Benzylaminopurine, Fe: Iron, GA<sub>3</sub>: Gibberellic Acid, IAA: Indole-3-Acetic Acid, IBA: Indole-3-Butyric Acid, Kin: Kinetin, K: Potassium, Mg: Magnesium, Mn: Manganese, MS: Murashige and Skoog, N: Nitrogen, NaClO: Sodium Hypochlorite, P: Phosphorus, PGRs: Plant Growth Regulators, Zn: Zinc.

**Conflicts of interest:** The authors declare that there are no conflicts of interest.

**Author contributions:** GM, AB, YuM, KD drafted the experimental design. GM, NS, KD, AV, YuM performed the experiments, AV, YuM helped in data collection, data analysis and initial draft of manuscript text. All authors read and agreed with the final version of the manuscript.

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