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# Effect of the Exogenous Application of Different Concentrations of Indole-3-Acetic Acid as a Growth Regulator on Onion (*Allium cepa* L.) Cultivation

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**Abstract:** Indole-3-acetic acid (IAA) is a key plant hormone that plays crucial roles in plant growth and development. This study investigated the effects of exogenous application of IAA as a growth regulator in onion cultivation (*Allium cepa* L.). Various IAA concentrations were evaluated to determine their effects on onion growth and chemical parameters. Several agronomic properties and chemical parameters, including total fresh weight, plant height, chlorophyll content, nitrates, total phenols, and antioxidant capacity (DPPH), were analyzed. The results revealed that the exogenous application of different concentrations of IAA had a significant impact on onion growth and quality. Specifically, it was found that certain concentrations of IAA fostered a significant increase in fresh bulb weight and a notable elevation in the levels of phenolic compounds. However, the onion's response to IAA was concentration dependent. In conclusion, the present study offers evidence that the exogenous application of IAA as a growth regulator can enhance onion growth and quality. These findings hold relevance for the advancement of sustainable agricultural practices and can be directed towards crop enhancement.

**Keywords:** auxins; phytohormones; phenolic compounds; nitrate; chlorophylls



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## 1. Introduction

Onion (*Allium cepa* L.) is a widely produced crop throughout the world due to its nutritional importance and low-cost production [1]. It is a bulb vegetable belonging to the Amaryllidaceae family and is used in multiple culinary preparations owing to its distinctive flavor and organoleptic properties [2,3]. In addition, onions are recognized for their medicinal properties and benefits to human health. Despite its importance, onion cultivation currently faces various challenges that affect its productivity and quality. The main challenges are the impact of diseases caused by pathogens, such as fungi and bacteria [4,5], as well as the presence of insect pests that affect plant health and bulb quality [6,7]. Moreover, abiotic stress, such as adverse weather conditions and water availability, can negatively influence the growth and development of the onion crop [8,9]. The quality of crops are fundamental aspects to guarantee food safety [10]. Producers constantly face the challenge of optimizing production and obtaining high-quality crops under variable conditions, according to market demand [11].

In this context, the use of growth regulators has become a promising alternative to enhance plants' resistance to various stresses [12]. They can have applications in agriculture, where they promote increased plant growth and development, enhancing resistance to both abiotic and biotic stress [13], as well as increase the quality of agricultural products. Among the most widely used growth regulators, indole-3-acetic acid (IAA) is a key phytohormone in the regulation of plant growth and development [14,15]. The use of IAA as a growth regulator in various crops has shown positive effects in terms of germination [16], grounding [17], blossoming [18], fruit set [19], and accumulation of secondary metabolites, such as antioxidants [20,21]. In addition, the use of growth regulators can offer advantages in terms of agricultural sustainability, as they can reduce the excessive use of fertilizers and promote good agricultural practices [22]. Likewise, its selective and dosed application can optimize the use of resources, such as water and nutrients, contributing to production efficiency in cultivation [23]. Other studies have reported that the effect of IAA improves onion bulb quality [24], increases grain yield and quality in quinoa [25], and enhances rooting and yield in rice [26]. It is a promising tool for enhancing the development and performance of various crops [27].

This study aimed to investigate the impact of exogenously applied IAA at varying concentrations on onion (*Allium cepa* L.) crops as growth regulators to enhance their productivity. *Allium cepa* L. was chosen due to its global economic and agricultural significance. This study aimed to assess diverse IAA concentrations on growth, photosynthesis, phenolic compounds, and antioxidants in *Allium cepa* L. The primary objective was to identify optimal IAA levels for maximizing these attributes without harming crop health.

## 2. Materials and Methods

### *2.1. Experimental Design*

The experiment was carried out in the experimental field of Universidad Autònoma de Barcelona, (Cerdanyola del Valles, Barcelona, Spain). The plants were grown in pots and brought to a greenhouse on 23 September 2022.

#### 2.1.1. Substrate for Cultivation

The seedlings of onion (*Allium cepa* L.) were of the Hamaemi variety. It is among the most commonly cultivated varieties in the region due to its tender quality, which is valued by the local market, and its suitability to the soil and climate conditions. These seedlings were obtained from a commercial nursery "Planters Faura" (Castellbisbal, Barcelona, Spain) at one month old. They were transferred into pots filled with a mixture of peat, sand, and sterilized vermiculite (1:1:1, *v*/*v*/*v*) [28].

#### 2.1.2. Preparation of Indole-3-Acetic Acid Solution

A 5000 ppm stock solution of IAA was prepared from the commercial product Sigma-Aldrich (product reference 1.00353 of CAS 87-51-4) with a concentration $\geq$ 99%, which was then diluted to different concentrations according to the proposed experimental design, as explained later. The solution was preserved in a dark container and stored in a cold room at 4 °C.

The experimental design was a completely random design (CRD) with several treatments and four replications. Six treatments were implemented: without application of IAA-IA0 (control treatment), and 50, 100, 150, 200, and 250 ppm of IAA [29], named IA50, IA100, IA150, IA200, and IA250, respectively. All treatments were replicated four times for a total of twenty-four pots (six concentrations of IAA (D) for four replicates).

IAA solution was sprayed onto the onion leaves. The application commenced during transplanting through root immersion, followed by spraying with 2 mL of the IAA solution per plant on days 20, 45, 60, 70, and 80, starting from 23 September 2022.

### *2.2. Agronomic Characteristics*

Crop evapotranspiration (ETc) (mm/day) was determined using the FAO Penman–Monteith equation (Equation (1)):

$$\text{ETc} = \text{ETo} \times \text{Kc} \quad (1)$$

where evapotranspiration (ETo) (mm/day) with limited data and the onion crop coefficient (Kc) was calculated, applying scheduled drip irrigation throughout the vegetative period.

Irrigation management was initiated from crop establishment until one day before harvest. Irrigation was performed using a per plant drip irrigation system. The total amount of irrigation water was 5.16 L per plant. Crop management was carried out in accordance with the guidelines of good agricultural practices for onion cultivation. Accordingly, NPK fertilizers equivalent to 140, 60, and 200 kg $ha^{-1}$ were applied to all treatments, in accordance with the practical guide of the rational fertilization of crops in Spain and *Real Decreto* 47/2022 on water protection against diffuse pollution produced by nitrates from agricultural sources [30,31].

During the phenological cycle of the onion crop, chemical protection with cupric fungicide was carried out for preventive control in all treatments. Onions were harvested on 9 March 2023 (all treatments were harvested on the same day). The fresh biomass (root, bulb, and aerial biomass) and roots were immediately washed with distilled water and weighed on the same day. The collected bulbs with aerial parts were stored in a cold room at 4 °C with a ventilation system for subsequent analysis of chemical parameters and dry material.

### 2.3. Greenhouse Climatic Variables

Figure 1 shows the climatic variables of the external experimental site during the onion crop growth period. The maximum temperatures oscillated around 32.5 °C, registered in September, and the average minimum temperature oscillated in the range of 6.8 °C, registered in January.

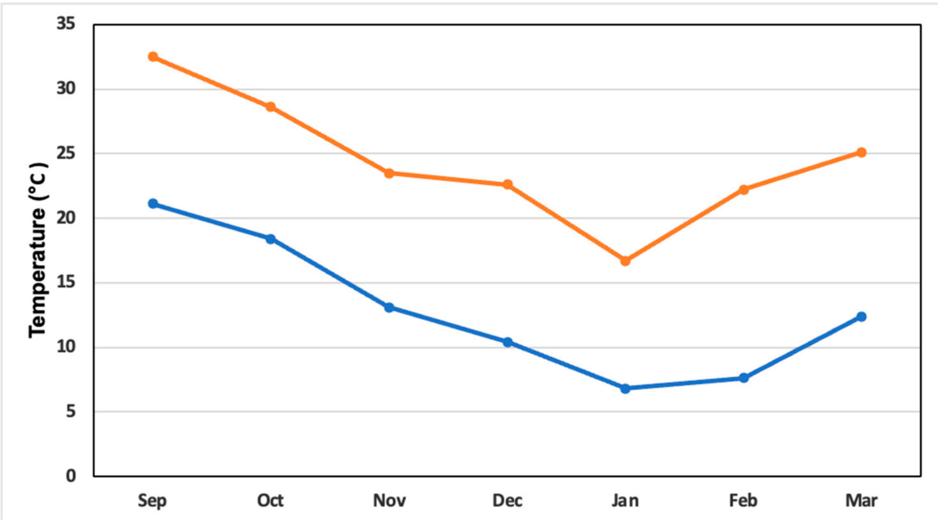


**Figure 1.** Maximum monthly temperature (°C, orange line) and average monthly temperature (blue line) during the growing season of the onion crop (September 2022–March 2023).

### 2.4. Determination of Growing Parameters

At harvest, onion plants were extracted from all pots, and the total fresh biomass of the plant (FW), root fresh weight (FR), fresh weight of the leaves (FF), and bulb fresh weight (FB) were measured from all repetitions. The dry weights of the roots, leaves, and bulb were determined separately from three representative samples from each replicate and dried in an oven at 105 °C until a constant weight was obtained to determine the percentage of dry matter (DM). Plant height and central bulb diameter were also measured.

### 2.5. Chlorophyll, Carotenoids, and Nitrates

Chlorophyll a and b and carotenoid contents were tested in triplicate for each plant from the fresh leaf samples. Extraction was carried out with 96% ethanol, according to the method of Lichtenhaler and Wellburn [32], and the absorbance of the solution was measured using a spectrophotometer (HACH LANGE DR 3900) at 649, 665, and 470 nm. The total chlorophyll content was calculated as the sum of chlorophyll a and b.

Nitrates were tested in all the plants using the Cataldo method for the extraction [33], and DR test kits for nitrates of 22–155 ppm were used. According to the manufacturer's methodology, 100 μL of the extracted sample was passed through a 0.22 μm filter and measured using a spectrophotometer (HACH LANGE DR 3900, Hach Lange, Düsseldorf, Germany). The concentration was calculated using the fresh weight (FW).

### 2.6. Total Phenolic Content and Antioxidant Activity (DPPH)

The total phenolic content (TP) was determined through employing the Folin–Ciocalteu method [34], using gallic acid as the standard equivalent. The absorbance of the blue-colored reaction solution was read at 750 nm using a spectrophotometer (HACH LANGE DR 3900). Extracts were obtained from onion bulbs, and from each repetition, they were measured in triplicate. The results were expressed in milligrams of gallic acid (AGE) equivalent per gram of dry matter (DM) (mg AGE $g^{-1}$ of dry weight).

A slightly modified DPPH (2,2-diphenyl-1-picrylhydrazil) free radical scavenging assay was used to measure the antioxidant activity of the methanol extracts of onion crops [35]. An 80% methanol solution of DPPH was prepared and poured into flasks covered with metal paper, and 100 μL of the extract was used in triplicate for each repetition. Following the preparation of the solution, 2900 μL of the DPPH solution was added, shaken for 1 min, and left for 30 min at room temperature, and the absorbance of each sample was read at 517 nm using a spectrophotometer (HACH LANGE DR 3900). The results were expressed in micromoles of Trolox (TE) equivalent per gram DM of the bulb (μmol TE g $dm^{-3}$), with the antioxidant activity calculated using Equation (2):

$$\text{Antioxidant activity} = \text{Ab} \times \text{V} \times \text{D}/\text{Wm} \times \text{Dm} \quad (2)$$

where Ab represents the extract concentration; V denotes the extract volume; D represents the dilution used; Wm denotes the sample weight; and Dm designates the dry weight (Dm).

### 2.7. Statistical Analysis

The average of the triplicate data was determined and then subjected to an analysis of variance (ANOVA) using the MINITAB 19 software package, InfoStat 2020.

#### 2.7.1. InfoStat 2020

For data analysis, the statistical software InfoStat version 2020 was employed. This program offers a wide range of statistical tools and analysis capabilities that enable precise exploration and modeling of the data. Descriptive analyses, significance tests, analysis of variance, and principal component analysis were carried out using the functionalities provided by InfoStat.

#### 2.7.2. Minitab 2019

Furthermore, Minitab version 19 was used to corroborate the results obtained with Infostat and to perform additional robustness analyses. Minitab is widely recognized for its versatility in statistical analysis and its ability to generate effective graphical visualizations. Through Minitab, regression, cluster, and principal component analyses were conducted, contributing to a deeper understanding of the results.

These tools were selected based on their recognition by the scientific community and their capability to handle complex datasets, allowing for a comprehensive assessment of the treatment effects in this study.

In addition, the differences between the means were evaluated using a post hoc LSD test ($p \leq 0.05$). A principal component analysis (PCA) was also performed on the agronomic properties and chemical parameters with the effect of the IAA concentrations. Hierarchical cluster analysis (HCA) was performed based on the agronomic properties, chemical variables, and IAA concentrations. All data are presented as mean ± standard deviation ($n = 4$).

## 3. Results

### *3.1. Effect of the Concentrations of IAA on the Onion Growth Agronomic Properties Parameters*

For the data analysis, the agronomic property parameters were determined, corresponding to the total fresh weight per plant, fresh weight of the root per plant, bulb per plant, plant height, diameter, and height of the bulb. This evaluation was performed on the day of harvest, except for the fresh weight of the bulb, which was kept in a cold room until the evaluation of the chemical parameters.

The total fresh weight (FW) did not exhibit statistically significant differences among the IAA concentration treatments (Table 1), and a closer analysis using the LSD $\leq 0.05$ post hoc test revealed significant differences in their mean values. Specifically, the IA50 concentration demonstrated a statistically higher effectiveness in increasing the total fresh weight compared to the control, making it the most effective treatment for promoting overall growth. Interestingly, the IA200 dose appeared to be less effective in this regard. Notably, treatments with IA100, IA150, and IA250 also exhibited effectiveness, albeit without statistically significant variations compared with the IA0 treatment. The fresh weight of the root (FR) did not present statistically significant differences between the treatments, according to the LSD post hoc test ($p \leq 0.05$) (Table 1). In all the treatments evaluated in relation to the fresh weight of the root, they did not present statistically significant differences among themselves. These results may be indicative that the exogenous application of IAA in the concentrations used did not significantly influence the development and growth of the root system of the onion plants.

**Table 1.** Effect of IAA concentrations through exogenous application on the total fresh biomass of the plant (FW), root fresh weight (FR), bulb fresh weight (FB), and leaf fresh weight (FF) in the pots test of *Allium cepa* L.

| Treatment | FW (g) | FR (g) | FF (g) | FB (g) |
|---|---|---|---|---|
| IA50 | 92.00 ± 23.30 a | 13.63 ± 2.78 a | 36.47 ± 7.58 a | 3.68 ± 0.35 a |
| IA100 | 63.88 ± 6.05 ab | 9.500 ± 0.70 a | 26.25 ± 6.97 a | 3.32 ± 0.14 ab |
| IA150 | 79.40 ± 32.00 ab | 13.25 ± 5.78 a | 31.87 ± 15.14 a | 3.47 ± 0.37 ab |
| IA200 | 53.00 ± 16.75 b | 9.38 ± 2.02 a | 27.21 ± 13.15 a | 2.79 ± 0.13 c |
| IA250 | 62.50 ± 30.90 ab | 12.00 ± 6.45 a | 25.23 ± 13.50 a | 3.12 ± 0.51 bc |
| Control IA0 | 57.50 ± 26.80 ab | 7.75 ± 3.07 a | 24.13 ± 11.35 a | 3.09 ± 0.41 bc |
| Significance concentration | ns | ns | ns | * |

The results in Table 1 represent the mean ± standard deviation of the mean. The letters (a, ab, bc, b, and c) in each column indicate significant differences based on the LSD post hoc test ($p \leq 0.05$). ns: not significant; *: significant ($p \leq 0.05$). FB data did not meet the normality test and were transformed using the natural logarithm of LN (X).

Based on the fresh weight (FF) results, no significant differences were found between the treatments in relation to this parameter. The results according to the post hoc LSD test ($p \leq 0.05$) indicate that the exogenous application of different IAA concentrations did not have a statistically significant effect on the FF of the onion plants.

Based on the results for the bulb weight (FB), it is evident that there are statistically significant differences (according to the LSD post hoc test ($p \leq 0.05$)) between the treatment with IA50, which presented the highest FB per plant, and the rest of the treatments along with the control. These results suggest that the application of IA50 can have a beneficial effect on the growth and development of onion crops by increasing the FB. In contrast, a negative effect was also evident at high IAA concentrations.

In relation to the plant height (PH), there was no significant difference in the PH between the treatments with different concentrations of IAA (Table 2). However, in the post hoc LSD test ($p \leq 0.05$), it was observed that the concentration of IA150 turned out to have a higher PH compared to the control. However, the IA200 concentration resulted in a significantly lower PH compared to the IA150 concentration. In the same way, the bulb height (BH) and central bulb diameter (CDB) did not present significant differences.

**Table 2.** Effect of exogenous applications of IAA on the plant height (PH), bulb height (BH), and central bulb diameter (CDB) in an *Allium cepa* L. pot trial.

| Treatment | PH (cm) | BH (cm) | CDB (cm) |
|---|---|---|---|
| IA50 | 56.00 ± 5.23 ab | 5.00 ± 0.89 a | 4.02 ± 0.62 a |
| IA100 | 56.50 ± 4.51 ab | 4.62 ± 0.98 a | 3.22 ± 0.26 a |
| IA150 | 58.25 ± 5.56 a | 4.80 ± 0.78 a | 3.85 ± 0.99 a |
| IA200 | 51.00 ± 4.90 b | 5.25 ± 0.50 a | 3.20 ± 1.23 a |
| IA250 | 54.50 ± 3.87 ab | 4.67 ± 0.57 a | 3.05 ± 0.85 a |
| Control | | | |
| IA0 | 52.25 ± 2.06 ab | 4.42 ± 0.83 a | 3.07 ± 0.35 a |
| Significance concentration | ns | ns | ns |

The results represent mean ± standard deviation of the mean. The letters (a, ab, b) in each column indicate significant differences based on the LSD post hoc test ($p \leq 0.05$). ns: not significant.

Regarding the dry weight (RDW; Table 3), there was no significant difference in the effects of the IAA concentrations. However, it was observed that there is a significant difference according to the LSD post hoc test ($p \leq 0.05$). The results showed that the IA200 and IA250 treatments had significantly higher root dry weights compared to the control treatment. In contrast, the control treatment, IA0, presented a significantly lower root dry weight than the other treatments. The IA50, IA100, and IA150 treatments did not show significant differences between them. Regarding the bulb dry weight (BDW) and leaf dry weight (FDW) relationship, no statistical significance was found in the effects of the IAA concentrations on the BDW and FDW, as revealed from the LSD post hoc test ($p \leq 0.05$), indicating that all the treatments did not present a significant difference in relation to the mean. These results suggest that the different concentrations of IAA did not have a significant effect on the BDW and FDW.

**Table 3.** Effect of IAA concentrations through exogenous application on the root dry weight (RDW), bulb dry weight (BDW), and leaf dry weight (FDW) in the *Allium cepa* L. pots trial.

| Treatment | RDW (g) | BDW (g) | FDW (g) |
|---|---|---|---|
| IAA | | | |
| IA50 | 0.45 ± 0.16 ab | 0.53 ± 0.35 a | 0.793 ± 0.40 a |
| IA100 | 0.45 ± 0.18 ab | 0.50 ± 0.09 a | 0.631 ± 0.21 a |
| IA150 | 0.43 ± 0.15 ab | 0.79 ± 0.60 a | 0.749 ± 0.38 a |
| IA200 | 0.55 ± 0.12 a | 0.44 ± 0.46 a | 0.4419 ± 0.19 a |
| IA250 | 0.53 ± 0.16 a | 0.49 ± 0.29 a | 0.6107 ± 0.14 a |
| Control | | | |
| IA0 | 0.31 ± 0.06 b | 0.51 ± 0.35 a | 0.458 ± 0.31 a |
| Significance concentration | ns | ns | ns |

The results represent the mean ± standard deviation of the mean. The letters (a, ab, b) of each column indicate significant differences according to the LSD post hoc test ($p \leq 0.05$). ns: not significant ($p \leq 0.05$). The BDW was transformed using the square root of (X).

### *3.2. Effect of the Concentrations of IAA on Chlorophyll, Carotenoids, and Nitrates*

Chlorophyll a and b, along with carotenoids and total pigments did not present statistical significance between treatments; likewise, according to the post hoc LSD test ($p \leq 0.05$), no significant differences were found between the medians of the treatments.

The amount of nitrates presented no statistical significance; however, the post hoc LSD test ($p \leq 0.05$) demonstrated that the concentration of IA100 had a significantly higher mean compared to the IA0 control (as shown in Table 4).

**Table 4.** Effect of IAA concentrations through exogenous application on the photosynthetic indicators chlorophyll a, chlorophyll b, carotenoids, total pigments, and nitrates in the *Allium cepa* L. pots trial.

| Treatment | Chlorophyll a (mg fw$^{-1}$) | Chlorophyll b (mg fw$^{-1}$) | Carotenoids (mg fw$^{-1}$) | Total Pigments (mg fw$^{-1}$) | Nitrates (mg kg fw$^{-1}$) |
|---|---|---|---|---|---|
| IA50 | 0.44 ± 0.23 a | 0.41 ± 0.26 a | 0.20 ± 0.11 a | 0.44 ± 0.35 a | 1424 ± 349 b |
| IA100 | 0.37 ± 0.05 a | 0.45 ± 0.05 a | 0.19 ± 0.04 a | 0.58 ± 0.06 a | 3734 ± 1526 a |
| IA150 | 0.20 ± 0.11 a | 0.29 ± 0.14 a | 0.12 ± 0.10 a | 0.41 ± 0.18 a | 2422 ± 1104 ab |
| IA200 | 0.41 ± 0.11 a | 0.35 ± 0.08 a | 0.15 ± 0.03 a | 0.55 ± 0.08 a | 2278 ± 732 ab |
| IA250 | 0.26 ± 0.11 a | 0.24 ± 0.16 a | 0.12 ± 0.11 a | 0.36 ± 0.18 a | 2104 ± 1239 b |
| Control IA0 | 0.25 ± 0.02 a | 0.35 ± 0.11 a | 0.12 ± 0.04 a | 0.4473 ± 0.07 a | 2072 ± 742 b |
| Significance concentration | ns | ns | ns | ns | ns |

The results represent the mean ± standard deviation of the mean. The letters (a, ab, b) of each column indicate significant differences according to the LSD post hoc test ($p \leq 0.05$). ns: not significant ($p \leq 0.05$). The CHA, CHB, and TP data were transformed using the square root (X).

### 3.3. Effect of the Concentrations of IAA on Phenolic Content and Antioxidant Activity (DPPH)

Regarding total phenols (TPs), the results revealed a statistically significant difference (according to the post hoc LSD test ($p \leq 0.05$)); the IA200 treatment exhibited a higher TP than the IA0 control treatment, followed by the IA50 treatment (Table 5). Conversely, IA100 treatment resulted in an intermediate amount of TP. With regard to the antioxidant capacity (DPPH), the results indicate that there was no statistically significant difference among the treatments in terms of their antioxidant capacity measured through DPPH. However, the post hoc LSD test ($p \leq 0.05$) revealed that IA200 exhibited the highest antioxidant capacity compared to the control, while the IA150 treatment demonstrated the lowest antioxidant capacity.

**Table 5.** Effect of IAA concentrations through exogenous application on the total phenols (TPs) and antioxidant capacity (DPPH) in the *Allium cepa* L. pot assay.

| Treatments | TPs (mg AGE g dm$^{-3}$) | DPPH (µmol TROLOX g dm$^{-3}$) |
|---|---|---|
| IA50 | 0.69 ± 0.53 c | 7.57 ± 0.82 ab |
| IA100 | 1.60 ± 1.19 bc | 6.00 ± 0.54 bc |
| IA150 | 2.69 ± 0.72 abc | 5.42 ± 1.85 c |
| IA200 | 3.98 ± 1.53 a | 7.94 ± 2.00 a |
| IA250 | 2.97 ± 0.78 ab | 6.66 ± 1.09 abc |
| Control IA0 | 0.63 ± 0.89 c | 7.49 ± 0.72 ab |
| Significance concentration | * | ns |

The results represent mean ± standard deviation of the mean, letters (a, ab, bc, abc, and c) of each column indicate significant differences according to the LSD post hoc test ($p \leq 0.05$). ns: not significant; *: significant ($p \leq 0.05$). TPs and DPPH data were transformed using the natural logarithm of LN (X).

### 3.4. Classification Analysis

In this section, chemical and agronomic properties were studied separately by means of a principal component analysis and afterwards in a combined manner using the hierarchical cluster analysis.

#### 3.4.1. Principal Component Analysis (PCA) for Chemical Parameters

In the first interaction, when principal component analysis (PCA) was performed on the evaluated variables, a two-component model was obtained considering 66.3%

of the total variance. The principal component 1 (PCA1) explained 53.8% of the data variability, while component 2 (PCA2) explained 19.3%, according to Figure 2a. The variables chlorophyll a, b, carotenoids, total pigments, and DPPH were in the positive quadrant of PCA1, indicating that they are strongly related. In addition, as shown in Figure 2b, the IA200 and IA100 concentrations were located close to these points, which may suggest that these concentrations are related to higher levels of chlorophyll, total pigments, and carotenoids. In the same main component analysis (PCA1), the TPs, nitrates, and antioxidant capacity were measured through DPPH. These factors were slightly close to each other, indicating a relationship between them. The concentrations of IA100 and IA250 were also close to these points, suggesting that these concentrations could influence the levels of TPs, nitrates, and antioxidant capacity. For PCA2, the chemical parameters were separated into two groups of variables. In the positive quadrant, TPs, DPPH, and nitrates corresponded to the concentrations of IA100 and IA200. In contrast, in the negative quadrant, chlorophyll a, chlorophyll b, total pigments, and carotenoids were present. This separation in PCA2 suggests an inverse relationship or contrast between the metabolites and characteristics associated with the total phenols, DPPH, nitrates, and those associated with chlorophylls, carotenoids, and total pigments. In other words, higher concentrations of IA100 and IA200 appear to have a positive effect on total phenols, DPPH, and nitrates, while they have a negative effect on chlorophylls, carotenoids and nitrates.

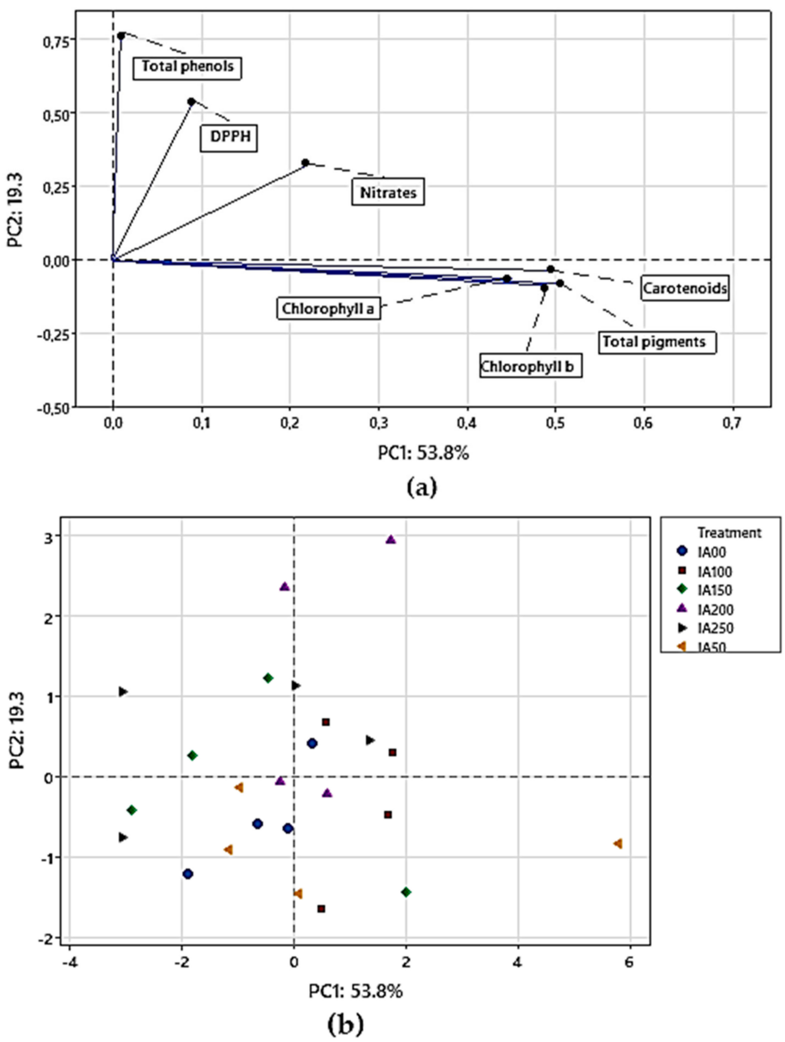


**Figure 2.** (**a**) Principal component analysis (PCA) of the relationship between the chemical parameters; (**b**) IAA concentrations and their relationship with the chemical parameters.

3.4.2. Principal Component Analysis (PCA) for Agronomic Properties

The principal component analysis (PCA) was performed on the evaluated agronomic properties, obtaining a two-component model considering 65.3% of the total variance. The principal component analysis 1 (PCA1) explained 51.2% of the data variability, while component 2 (PCA2) explained 14.1%, according to Figure 3a. The agronomic properties (fresh waste weight, root fresh weight, fresh weight leaves, bulb fresh weight, plant height, bulb height, bulb center diameter, roots dry weight, bulb dry weight, and leaves dry weight) were all found in the positive quadrant of PCA1, indicating that these parameters are strongly related. In addition, the IA50 and IA150 concentrations were located close to these points, suggesting that the values of these agronomic properties are related to higher levels.

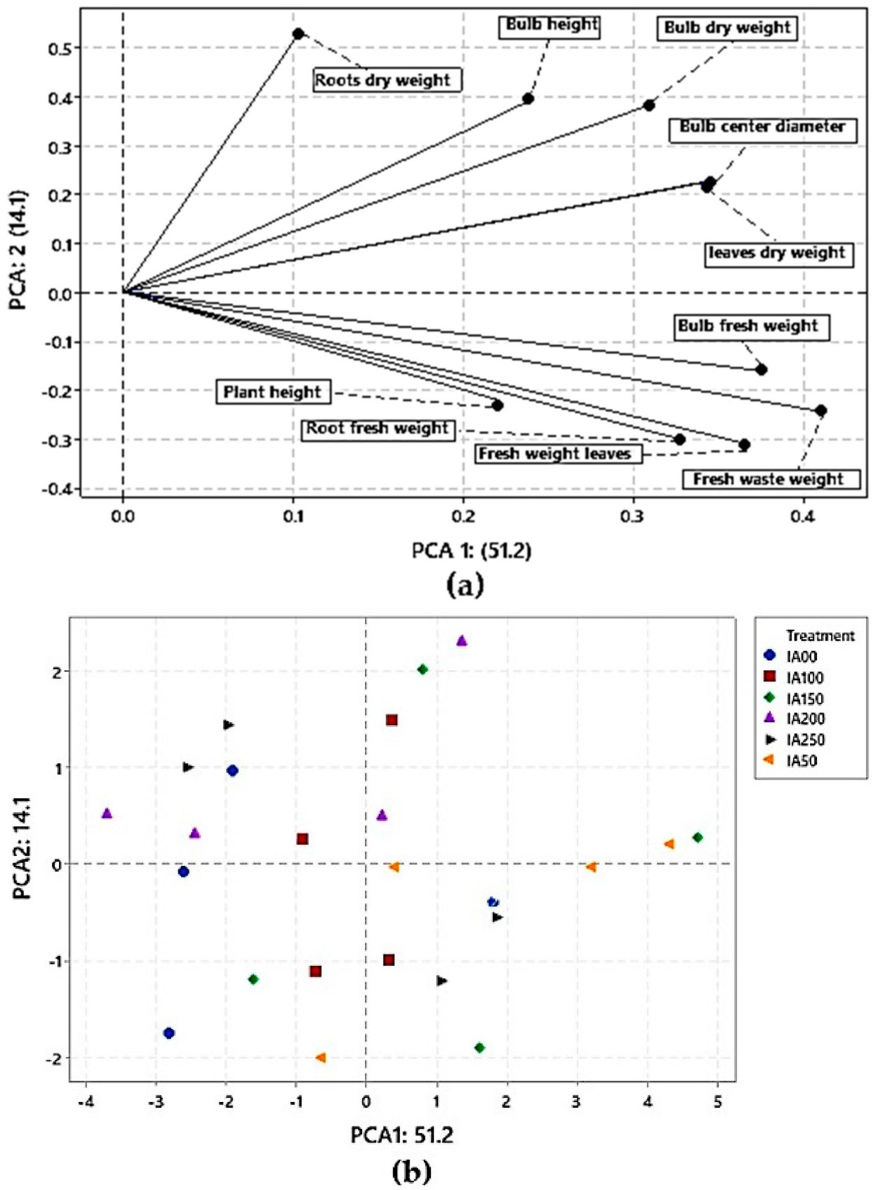


**Figure 3.** (**a**) Principal component analysis (PCA) of the relationship between the agronomic properties; (**b**) IAA concentrations and their relationship with the agronomic properties.

On the other hand, most of the variables of the agronomic properties were found in the same PCA1, which indicates that these variables are related to each other and have a lower contribution to the variability of the data. Also, the concentrations of IA150 and

IA50 (Figure 3b) were located close to these points, suggesting that these concentrations are related to the root dry weight, bulb height, bulb dry weight, bulb center diameter, and leaves fresh weight. The concentrations of IA0, IA100, IA200, and IA250 were distributed on the negative side of the positive quadrant of PCA1.

Regarding PCA2, the variables root dry weight, bulb height, bulb dry weight, bulb center diameter, and leaves dry weight were in the positive quadrant; in the negative quadrant, the variables bulb fresh weight, fresh waste weight, fresh weight leaves, root fresh weight, and plant height were found.

PCA2 explained 14.1% of the total variability of the data, which suggests that there is a moderate correlation between the variables in this component.

#### 3.4.3. Combined Agronomic Properties and Chemical Analysis

The hierarchical cluster analysis (HCA) analysis was carried out on the entire dataset, which corresponds to a tree diagram where the objects are grouped in rows by their similarities based on the agronomic properties and the IAA concentrations according to Figure 4a, which suggests that there are two groups of concentrations. They differ on the basis of their agronomic properties. The first group is made up of concentrations IA50 and IA150, as they have similar growth patterns in terms of their agronomic properties, and the second group is made up of concentrations IA200, IA250, IA100, and IA0. The cluster analysis based on the agronomic properties and the different concentrations of IAA also reveals the existence of two distinct groups. In the first group, made up of the IA50 and IA150 concentrations, a similarity was observed in the growth and development of the onion in terms of the fresh weight of the bulb, fresh weight of the leaves, fresh weight of the root, height of the plant, among other agronomic properties. These results suggest that these IAA concentrations may have a similar effect on onion vegetative development. The second group is made up of concentrations IA200, IA250, IA100, and IA0. It is important to highlight that this group differs from the first group in terms of the agronomic properties evaluated, which suggests that these IAA concentrations may have a different impact on onion vegetative development. These findings support the importance of selecting the appropriate concentration of IAA to influence the vegetative development of the onion crop.

In the cluster analysis presented in Figure 4b, which is based on the chemical parameters and the IAA concentrations, it is suggested that there are two groups that differ on the basis of their chemical parameters. The first group is made up of concentrations IA50, IA100, and IA200, as they have similar levels in their chemical parameters, and the second group is made up of concentrations IA250, IA150, and IA00.

The cluster analysis based on the agronomic properties and chemical parameters, according to Figure 5, reveals the formation of two distinct groups depending on the IAA concentrations evaluated. In the first group, which is solely composed of the treatments with concentrations of IA50 and IA150, a similarity was observed in the agronomic properties and chemical parameters analyzed. This suggests that these IAA concentrations may have a similar effect on onion development under both an agronomic property level and chemical level. On the other hand, the second group was made up of the treatments with concentrations of IA0, IA100, IA200, and IA250. These concentrations revealed significant differences in both their agronomic properties and chemical parameters, indicating a divergent response in onion growth and development compared to the first group. These results suggest that the IAA concentrations used in this study may have a different impact on the agronomic properties and chemical aspects of the onion crop.

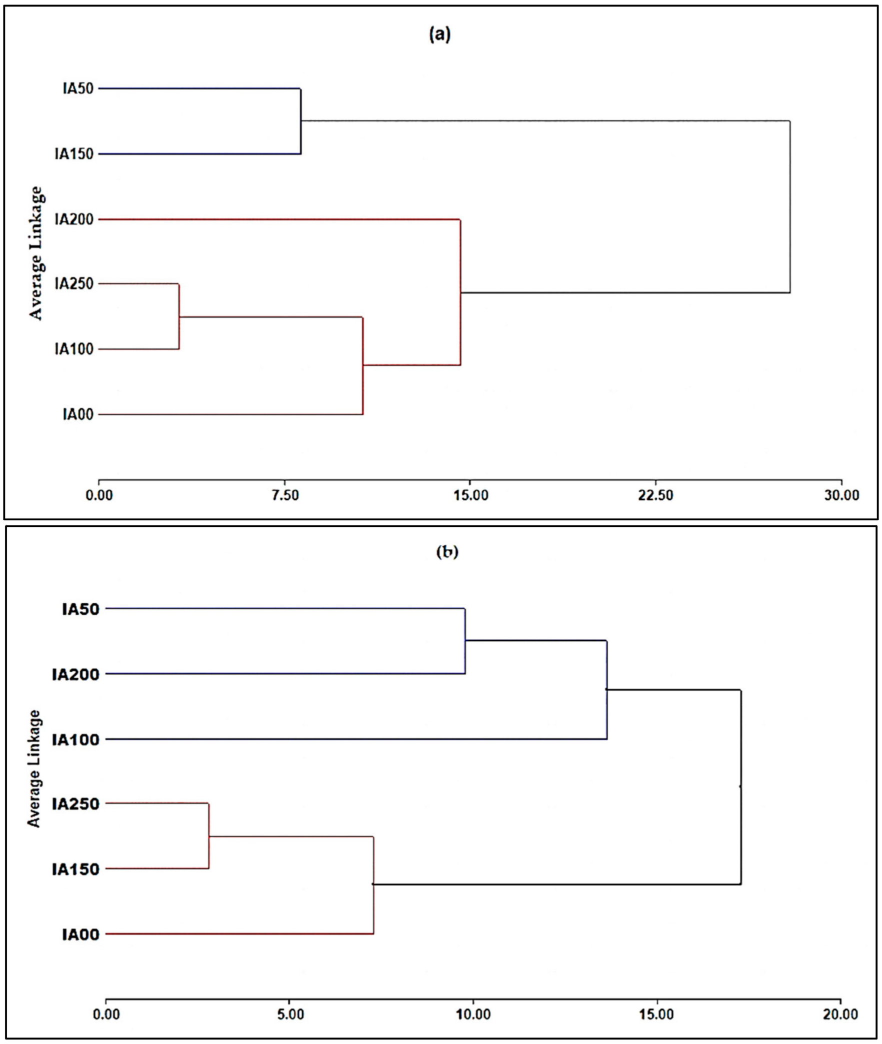


**Figure 4.** (**a**) Cluster dendrogram based on the IAA effect on the agronomic properties of the onion crop. (**b**) Cluster dendrogram based on the effect on the chemical parameters of the onion crop.

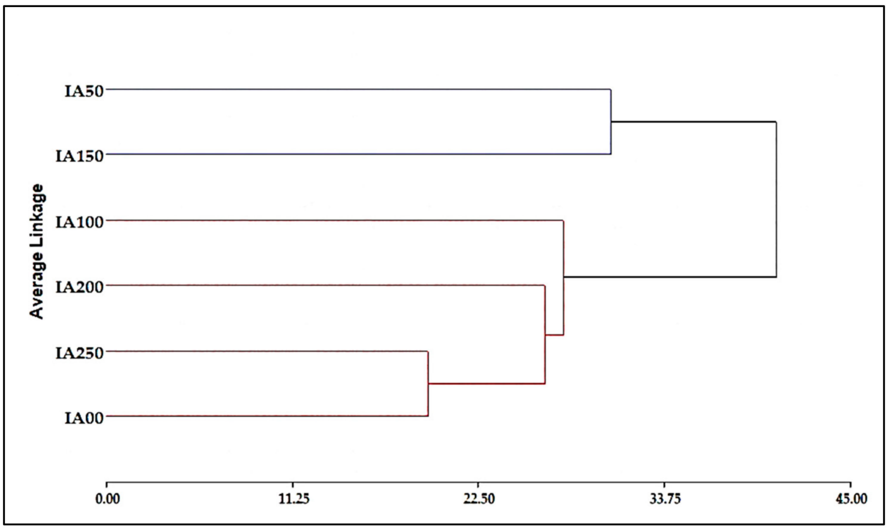


**Figure 5.** Cluster dendrogram of the agronomic properties and chemical parameters in the application of IAA in onion cultivation.

Figure 6 provides a detailed comparison of the various treatments applied to onion cultivation, highlighting their distinct characteristics. Firstly, the IA50 treatment stands out, showcasing a significant increase in the bulb size compared to the IA0 control and IA100 and IA150 treatments. This prominent difference in the bulb size in the IA50 treatment clearly indicates its positive effect on onion growth. In the second category, the IA100 and IA150 treatments exhibited comparable bulb sizes, being slightly smaller than the IA50 treatment. As we move to the third treatment group, constituting IA250 and IA0, a reduction in bulb size was evident compared to the previously mentioned treatments. Lastly, in the fourth group, the IA250 treatment displayed a bulb size similar to that observed in the third group, solidifying the relationship between the applied IAA concentration and the bulb size. This figure provides a visual insight into how different IAA concentrations impact onion bulb development, which can be crucial in understanding the treatment effects on the overall onion crop yield.


**Figure 6.** Comparison of plant growth between the control (IA0) and different concentrations of IAA (IA50, IA100, IA150, IA200, and IA250).

## 4. Discussion

In this study, the effect of the exogenous application of six concentrations of IAA (IA0, IA50, IA100, IA150, IA200, and IA250) in onions grown under greenhouse conditions were evaluated.

In terms of the total fresh weight, which represents the total fresh biomass of the crop (root, bulb, and leaves), this result can have interesting economic benefits, considering that more biomass is produced. The results from this study are consistent with previous studies that have demonstrated the beneficial effects of IAA on plant growth and development. Several studies have reported that IAA application can promote root growth, increase biomass accumulation, and improve nutrient uptake in different crops [36–38]. Regarding the optimal concentration of IAA, the results of this study support the trend observed in previous research that suggests that low or moderate concentrations of IAA are more effective than higher concentrations. For instance, a study conducted in maize cultivation found that the concentrations of 50 and 100 ppm of IAA had a positive effect on the yield compared to the application of 200 ppm of IAA [39]. Furthermore, our results from this study indicate that a concentration of 200 ppm of IAA may not be effective and could even have negative effects on onion cultivation. These findings are supported by previous studies that have reported inhibitory or toxic effects of IAA at high concentrations [40].

The results obtained in this study agree with previous investigations that have demonstrated the significant effect of IAA on the FB. Several studies have reported that the application of IAA can promote onion bulb development and increase its weight in different horticultural crops [39,41]. These results indicate that the IA50 concentration had a significant positive effect on the fresh weight of the onion bulb, being greater in comparison with the other evaluated treatments and the control. These findings are consistent with previous research that has found that low concentrations of IAA in *Guizotia abyssinica* [42] can enhance growth and development in different crops [43,44]. Regarding the root dry weight (RDW), the IA200 and IA250 treatments presented better results compared to the other treatments and the control. However, it is important to note that other studies have shown that specific IAA concentrations can influence the root dry weight in this crop [36]. These findings suggest that the response of the root dry weight to IAA may be variable depending on the experimental conditions and the specific characteristics of each crop. Regarding the other agronomic properties evaluated in this research (including the FR, FF, BH, CDB, BDW, and FDW), they did not show significant differences between the treatments and differences in the LSD post hoc test ($p \leq 0.05$). These findings are consistent with previous research that has shown that IAA concentrations do not have a significant effect on certain agronomic properties in crops [45]. However, it is important to note that a number of studies in the scientific literature have reported some positive effects of IAA on the leaf and root fresh weight in other crops, suggesting that these effects may be species-specific [46]. Studies have shown that IAA can stimulate root growth and the development of the aerial part of plants, which is reflected in an increase in the fresh weight of the distinct parts of the plant [47].

No statistical significance between treatments was observed when analyzing the results obtained for chlorophyll a, chlorophyll b, carotenoids, and total pigments. Several previous studies have also evaluated the effect of IAA on these photosynthetic pigments in different crops. Some reported results are similar to those obtained in this study, since days after the application of IAA, the effect of these pigments begins to decrease, not having a significant impact on the levels of chlorophyll and carotenoids. For example, a study conducted by the authors of [48] on *Cinnamomum camphora* plants found no significant differences in the chlorophyll and carotenoid contents 60 days after IAA application. Another study carried out by the authors of [49] in *Lolium perenne* L. plants showed no significant differences related to the treatment in the proportion of chlorophyll at 60 days of evaluation. These findings may suggest that the effect of the IAA concentrations used in our study at the end of the harvest may not have a direct effect on the degradation of the photosynthetic pigments in the onion crop. However, it is important to highlight that the pigment content can be influenced by several factors, such as the interaction with the environmental conditions, the genotype of the plant, and the stage of growth. Therefore, the lack of a significant effect of IAA in our results may be due to these variations and the specific sensitivity of onion to this growth regulator.

Regarding the effect of the exogenous application of IAA on the accumulation of nitrates in the onion crop, no significant differences were found. The presence of a higher quantity of nitrates in the IA100 treatment may suggest that this concentration promoted the accumulation of nitrates in the onion crop. However, it has also been reported that IAA application can improve root architecture and nitrogen metabolism in apple trees [50]. Some studies have also indicated that the presence of IAA improved the content of nitrates in wheat sprouts [51]. However, it is important to consider several explanations for these results. The response to IAA may be specific to each plant species. Different plants may have different regulatory mechanisms for nitrate accumulation and may respond differently to IAA [37]. It is possible that the onion crop is not as sensitive to IAA in terms of nitrate accumulation, which would explain the lack of significant differences observed in the present study. Furthermore, the accumulation of nitrates in plants is a complex process that is influenced by multiple factors [52,53], such as the availability of nitrates in the soil [54], the activity of enzymes related to their metabolism [55], and the response of other phytohormones [56]. These factors can interact in a complex way with IAA and nitrate signaling [57].

Our results agree with previous studies that have investigated the IAA effect on total phenol levels in onion crops. Several studies have shown that the exogenous application of IAA can positively influence the accumulation of total phenols in plants [58]. Supporting these findings, several studies have reported the role of IAA in the production of total phenols in different plant species, observing an increase in the levels of total phenols in response to the application of IAA, which is consistent with our results [59]. Furthermore, it has been reported that IAA can induce phenol synthesis in plants by acting as a regulator of secondary metabolism [60]. Furthermore, the presence of total phenols in plants is of particular interest due to their antioxidant properties and health benefits [61]. These bioactive compounds have been associated with antimicrobial, anti-inflammatory, and anticancer activities, among others [62]. Therefore, the increase in the levels of total phenols in the onion crop in response to the concentrations of IAA used in our study could have positive implications from the nutritional and health points of view. However, it is important to highlight that the effect of the exogenous application of IAA may vary according to the plant species and the specific environmental conditions. Therefore, further investigations are required to better understand the underlying mechanisms involved in the regulation of total phenols in response to IAA.

The results obtained in relation to the antioxidant capacity measured using DPPH indicate that there was no statistically significant difference between the treatments in general. These results are confirmed by other studies, such as the effect of IAA on the contents and metabolites in *Hibiscus sabdariffa* plants, showing no considerable difference between concentrations with IAA [63]. For example, an increase in the total phenols and antioxidant capacity measured using DPPH could indicate a response to plant defense mechanisms under stress conditions [64], or as a form of protection against oxidative damage [65]. In our case, it is suggested that the concentration of IAA may have an impact on the antioxidant capacity of the onion crop. Treatment with IA200 showed a higher level of antioxidant activity, which could indicate its potential to protect plant cells against oxidative stress [66]. This antioxidant capacity may be due to the presence of phenolic compounds in IAA, which are known for their antioxidant activity [59]. Importantly, antioxidant capacity measured using DPPH is only one of many ways to assess the antioxidant potential of a compound or extract [67]. Other experiments, such as free radical scavenging capacity or lipid peroxidation inhibitory activity, can provide a more comprehensive view of the antioxidant profile [68]. In the scientific literature, studies on the antioxidant activity of phenolic compounds have been reported with the presence of different biostimulants or plant extracts [69]. These studies have demonstrated the ability of phenols to neutralize free radicals and protect plants against oxidative stress [70,71]. This can be beneficial in formulating biostimulants that contain phenols that can stimulate plants against external limiting factors [72]. The results indicate that the IA200 treatment had the highest antioxidant capacity, as measured using DPPH, closely followed by the IA250 treatment. These findings support the idea that IAA may contribute to the antioxidant activity in onion cultivation, although more research is necessary to fully understand the mechanisms of action and effects of antioxidant capacity in relation to the concentration of IAA and other components that are present. These findings support the importance of selecting the proper IAA concentrations to influence the chemical composition of the onion crop and potentially improve its nutritional quality and antioxidant properties. These findings are consistent with previous studies that have shown that concentrations of plant hormones, such as IAA, can modulate both agronomic properties and chemical parameters in plants [73]. The exogenous application of IAA can influence the morphology and other chemical parameters of the plants [74,75]. The cluster analysis showed the formation of two distinct groups depending on the concentrations of IAA and its impact on the agronomic properties and chemical parameters of the onion [41]. These findings underscore the significance of carefully selecting the appropriate concentration of IAA to influence onion growth, development, and its chemical content. Within the framework of agricultural practices striving for sustainability and safety, it is of paramount importance to emphasize the need for comprehensive and exhaustive research into the long-term effects of growth regulators. While our study provides valuable insights into the immediate impacts of exogenously applied IAA on onion cultivation, it is essential to acknowledge that potential consequences, particularly regarding biosecurity, warrant further investigation.

## 5. Conclusions

This study assessed the impact of exogenous IAA application as a growth regulator on onion cultivation. Varied IAA concentrations significantly affected multiple agronomic properties and chemical parameters. Notable differences were observed in the bulb and leaf fresh weights, plant height, root dry weight, and total phenol content. Cluster analysis identified distinct groups based on the IAA concentrations. Two clusters emerged for agronomic properties, notably with the IA50 and IA150 concentrations. For chemical parameters, the IA50, IA100, and IA200 concentrations shared similarities. IA50 proved to be most effective for the bulb fresh weight, while IA200 displayed elevated total phenol levels, closely followed by IA150 and IA250. Although no significant variations occurred regarding the levels of nitrates due to the IAA concentrations, IA100 exhibited a slightly higher median. Our conclusions suggest that IAA concentrations influence onion growth, development, and chemical composition. Yet, these responses can vary by parameter, highlighting the need for tailored IAA application strategies.

These findings provide a basis for optimizing IAA application strategies as a growth regulator in onion crops, seeking concentrations that maximize the desired parameters without causing negative effects on the crop and environment. Further research is needed to better understand the underlying mechanisms and the dose–response relationship of IAA in different varieties of the onion crop, with the aim of harnessing its potential as a growth regulator in agriculture.

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