

## Potential impact of iron oxide conjugated nano-fertilizer on growth, flowering and isozyme expression in *Gardenia jasminoides*

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### Abstract

Nano-fertilizers protect the soil from the excessive addition of traditional fertilizers, enhancing the efficiency of the elements and diminishing the number of additive fertilizers. The effect of Fe<sub>2</sub>O<sub>3</sub>NPs-Boron (Fe<sub>2</sub>O<sub>3</sub>NPs-B), and Fe<sub>2</sub>O<sub>3</sub> NP-Humic Acid (Fe<sub>2</sub>O<sub>3</sub>NPs-HA) at 100, 150 and 250 ppm, Fe<sub>2</sub>O<sub>3</sub>, and control (without any iron fertilizers) on the vegetative growth, flowering, photosynthetic pigments, nutrient element content and isozymes activity (peroxidase, superoxide dismutase and polyphenol oxide) of *Gardenia jasminoides* plants was investigated. Gamma-rays at 25 kGy were conducted for the promising synthesis of Fe<sub>2</sub>O<sub>3</sub>NPs-B, and Fe<sub>2</sub>O<sub>3</sub>NPs-HA. The experiment was carried out under greenhouse conditions during two successive seasons. The results stated that Fe<sub>2</sub>O<sub>3</sub>NPs-B and Fe<sub>2</sub>O<sub>3</sub>NPs-HA at the highest concentration (250 ppm) had a significant positive effect in all vegetative characteristics, photosynthetic pigments, nutrient element content and isozymes activity. Fe<sub>2</sub>O<sub>3</sub>NPs-HA showed the optimal result in all morphological and biochemical characteristics. The highest activity of enzymes appeared in the treated plants with Fe<sub>2</sub>O<sub>3</sub> NPs-B followed by Fe<sub>2</sub>O<sub>3</sub> NPs-HA at 250 ppm. The advantage nano-fertilizer usage may be summarized as saving the soil from the unreasonable accumulation of classic fertilizers, improving the use efficiency of parts and reducing the number of different fertilizers as a consequence of their increased surface area and their nano-size.

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## Introduction

Gardenia or cap jasmine (*Gardenia jasminoides*) is a tropical shrub ranging in height from 4 to 8 feet, with white flowers that have a distinctive fragrant smell and bright dark green leaves (Al-Maathedi *et al.*, 2017). This plant belongs to *Rubiaceae* family and grows naturally in tropical and subtropical regions of Africa, South Asia and Australia (Davidson, 1989; Kobayashi and Kaufman, 2006). The main purpose of growing gardenia plants is to spread the fragrant smell in the place and enjoy the bright color of the leaves. Gardenia may grow as potted flowering plants, hedges plant, groups in gardens, as specimens. This plant can to spread their fragrance indoors and out by planting them near patios, doors, or windows (Mousa *et al.*, 2015; Mok *et al.*, 2020). It is normal for old leaves to turn yellow and fall off, especially during the spring and fall seasons, In the case of yellowing of the new leaves, this indicates a lack of micro-nutrients, especially iron (Mousa *et al.*, 2015).

Environmental concerns increase with the use of mineral fertilizers, so it is necessary to consider suitable alternatives (Attia *et al.*, 2016; Farrag *et al.*, 2017). Staying away from traditional fertilizers and pesticides is considered a supreme goal for most modern research in order to avoid their destructive impact on the environment, human health, and the soil (Hashem *et al.*, 2023; Khattab *et al.*, 2022). Nano fertilizers are nutrient fertilizers with nanoscale formulations that are applied to plants and allow slow but efficient uptake of active ingredients (Hashem *et al.*, 2021; Abdelaziz *et al.*, 2022).

In horticultural crops such as ornamental, medicinal, and aromatic plants, Nano fertilizers have successfully increased yield and nutrient content, improved physiological and biochemical characteristics, and global food security (Albalawi *et al.*, 2022; Elbasuney *et al.*, 2022). Additionally the synthesized nano-fertilizers are designed from standard fertilizers via physical, chemical, or biological methods that guide to greatest outcomes than conventional fertilizers about expansion speeds and nutrient values (Lal, 2008; Abdelaziz *et al.*, 2023).

The advantage of using Nano fertilizers could be outlined as saving the soil from the extreme expansion of conventional fertilizers, improving the benefits efficiency from the consumed elements, and reducing the number and quantity of other standard fertilizers as a consequence of their increased surface area and the noted nanosized (Khan *et al.*, 2019). Nano fertilizers maintain an influential function in plant nutrition via their capacity to be slow-release fertilizers that supply plants with a great quantity of some minerals (Naderi and Abedi, 2012). The form of recently recycled soils (sandy soil) bind the agriculturalists to operate slow or steady release fertilizers which include more increased reactivity, more detailed surface area, and better density of reactive area which improve the performance of this place on particle texture (Ruffini Castiglione and Cremonini, 2009). The impact of NPs on crops relies on their chemical components, dimensions, surface coating, response among details compounds and plants and eventually the persistent dose which influences the physiological function and directed to an influential impact on tested crops (Khodakovskaya *et al.*, 2012; Seleiman *et al.*, 2020).

Iron (Fe) considered the major micronutrients in many crops; it retains a critical part in the natural processes of numerous enzymes that experience in many important functions like protein structure, and photosynthesis (Elbasuney *et al.*, 2022; El-Batal *et al.*, 2023). Heme protein contains cytochrome an important enzymes (oxidase, catalases, peroxidases), and leghemoglobin, which recreate an crucial function in controlling and scavenging ROS and improving nitrogen content in legume root nodules through a symbiotic response among crops and plant growth promoting rhizobacteria (Osorio and Habte, 2014; Khalil *et al.*, 2015) and respiration function which finally improving the grade of plant product (Briat *et al.*, 2015).

Humic acid (HA) is a biological polymer including phenolic and carboxyl functions for the relations function (Ampong *et al.*, 2022; Abdelaziz *et al.*, 2023). The way of action of HA on plant development can diverge into direct and indirect impacts because it improves cell membrane permeability (Clapp *et al.*, 2006), improved water retention, and cation interaction capability and enhanced ATP and some amino acid formalization, leading to the most promising development and crop expansion (MacCarthy *et al.*, 1990).

Piccolo *et al.* (1992) reported that it can be utilized as a growth regulator to promote growth of plant, organize hormone levels, and enhance ability to tolerant. Positive effects of HA on philodendron plant growth parameters were demonstrated, including plant height, leaf area, leaf number, stem diameter, root length, fresh and dry weights of leaves, stems, and roots, and leaf mass compared with untreated photosynthetic pigment plants as shown by (El-Shawa *et al.*, 2022).

Boron (B) element is one of the micronutrient has a vital different functions; the primary function of B is in plant cell wall structural integrity (Cakmak *et al.*, 2023; Elkhodary *et al.*, 2023). It plays an essential role in protein formation, nitrogen metabolism and cell division, cell membrane integrity, cell wall formation, nucleic acid and antioxidant system formation (Johnson *et al.*, 2005; Koshiha *et al.*, 2009). It also plays an important role in carbohydrate transport, cell differentiation and development, nitrogen metabolism, active salt uptake, hormone metabolism, water balance and photosynthesis in plants (Gauch and Dugger, 1953; Das *et al.*, 2022). The aim of this study was to study the effect of iron oxide nanocomposites at various levels in different forms ( $\text{Fe}_2\text{O}_3$  NPs-B, and  $\text{Fe}_2\text{O}_3$  NPs-HA) to compare with Fe-EDTA, and control on the vegetative, flowering growth and isozymes activity of *Gardenia jasminoides* seedlings.

## Materials and Methods

### *Chemicals and reagents*

Analytical grade chemicals such as ferrous sulphate heptahydrate, humic acid, and boric acid (Sigma Aldrich, UK), were used to produce different nanocomposites.

### *Gamma radiation*

Gamma irradiation as an eco-friendly method (El-Batal *et al.*, 2020; Fathy and Mahfouz, 2021; Sivaselvam *et al.*, 2021) were proceed in the NCRRT, Cairo, Egypt. The applied radiation origin was  $^{60}\text{Co}$ -Gamma chamber 4000-A-India, and the tested solutions were irradiated; behind liquefying the starting materials, a radiation dose rate was decided to be 1.04 kGy/hour.

### *Preparation of iron oxide nanocomposites ( $\text{Fe}_2\text{O}_3$ NP-B and $\text{Fe}_2\text{O}_3$ NP-HA)*

$\text{Fe}_2\text{O}_3$  NP-B and  $\text{Fe}_2\text{O}_3$  NP-HA were produced in the existence of gamma rays which serve as a distinct reducing agent. Gamma rays generated removal of metal ions due to the free reducing electrons ( $e^-_{\text{aq}}$ ).

For the synthesis of  $\text{Fe}_2\text{O}_3$  NP-HA, about 4.2 gm of ferrous sulphate heptahydrate was dissolved in 100 ml distilled water and kept for about 30 minutes in stirring. After that the solution of humic acid was prepared after dissolving about 0.5 gm of boric acid in 50 ml distilled water. Finally, the solution of ferrous sulphate, and humic acid were mixed to give final volume of 150 ml. Before the gamma-irradiation, the prepared sample were checked for their pH and adjusted to be neutral (pH=7) after the adjustment with sodium hydroxide. Then, solution was gamma-irradiated with fixed dose at 35.0 kGy.

*Characterization of iron oxide nanocomposites (Fe<sub>2</sub>O<sub>3</sub> NP-B and Fe<sub>2</sub>O<sub>3</sub> NP-HA)*

Surface morphology, and outer appearance of iron oxide nanocomposites was analyzed by SEM, JEOL JSM-5600 LV, Japan. The elemental accounting of the formed iron was performed employing EDAX detector (JEOL JSM-5600 LV, Japan). The crystalline design of the synthesized nanocomposites was tested by the XRD analysis (Shimadzu XRD-6000, Japan). Dynamic light scattering test (DLS-PSS-NICOMP 380-ZLS particles sized system St. Barbara, California, USA) was performed to specify the intermediate particle size diffusion of the produced iron oxide nanocomposites. A high-resolution transmission electron microscope (HR-TEM, JEM2100, Jeol, Japan) was applied as an excellent analysis for examining the constitution, and the intermediate particle size of the designed iron oxide nanocomposites.

*Experiment design*

The experiment was carried out under glass greenhouse conditions of Horticulture Research Institute (HRI), Agricultural Research Center, Giza, Egypt during two successive seasons of 2020/2021 and 2021/2022, while the chemical analysis implemented in Ornamental Plants and Woody Trees Dept. and Faculty of Science, Al-Azhar University, and Fe<sub>2</sub>O<sub>3</sub>NP fertilizer in different forms were prepared in NCRRT, EAEA, Cairo, Egypt to find out the best form and concentration of iron fertilizer suitable for the production of *Gardenia jasminoides* plants with vigorous growth and abundant flower production.

*Preparation of Gardenia jasminoides seedlings*

Seedlings of *Gardenia jasminoides* 12-15 cm height and contained 5-7 leaves/plant were obtained from Horticulture Research Center, Agricultural Research Center. The seedlings were cultivated on the 1<sup>st</sup> March in plastic pots 30 cm (one plant/pot,) filled with 9 kg soil mixture (peat moss+ sand 2:1v/v). The physical and chemical analysis were shown in Tables 1 and 2, which was determined according to Jackson (1958). The soil drench application of the treatment was started a month after cultivation and continued monthly during the growing season.

**Table 1.** Physicochemical characteristics of the sand soil

| Soil Sample | Coarse sand              | Fine sand |          | Silt                          |                 | Clay                          |                 |                 |                |
|-------------|--------------------------|-----------|----------|-------------------------------|-----------------|-------------------------------|-----------------|-----------------|----------------|
|             | 68%                      | 20.7%     |          | 5.2%                          |                 | 6.1%                          |                 |                 |                |
| Sand        | EC (dS.m <sup>-1</sup> ) | pH        | Fe (ppm) | Anion (meq.l <sup>-1</sup> )  |                 | Cation (meq.l <sup>-1</sup> ) |                 |                 |                |
|             | 1.74                     | 6.3       | 11       | HCO <sub>3</sub> <sup>-</sup> | Cl <sup>-</sup> | Ca <sup>+</sup>               | Mg <sup>+</sup> | Na <sup>+</sup> | K <sup>+</sup> |
|             |                          |           |          | 1.96                          | 21              | 3.9                           | 3.2             | 17              | 5.1            |

**Table 2.** Chemical analysis of peatmoss

| Soil sample | Sp% | pH        | Ash%      | O. M.%  | N%   | P%   | K%   | Fe (ppm) |
|-------------|-----|-----------|-----------|---------|------|------|------|----------|
| Peat moss   | 35% | 3.6 – 3.9 | 1.5 – 2.3 | 93 – 97 | 2.55 | 0.06 | 2.37 | 15       |

*Preparation of iron oxide nanocomposites treatments*

The tested treatments were prepared as the following:

1. Control treatment without iron application.
2. Fe<sub>2</sub>O<sub>3</sub> (100 ppm).
3. Fe<sub>2</sub>O<sub>3</sub>NPs-B (100 ppm).
4. Fe<sub>2</sub>O<sub>3</sub>NPs-B (150 ppm).
5. Fe<sub>2</sub>O<sub>3</sub>NPs-B (250 ppm).
6. Fe<sub>2</sub>O<sub>3</sub>NPs-HA (100 ppm).
7. Fe<sub>2</sub>O<sub>3</sub>NPs-HA (150 ppm).

8. Fe<sub>2</sub>O<sub>3</sub>NPs-HA (250 ppm).

Additionally, the treated plants were harvested at the end of April in the next year for each season.

*Data recorded*

Vegetative growth

Plant height (cm) stem diameter (cm), number of leaves/plants, root length (cm) number of branches/plants, leaf area (cm<sup>2</sup>) fresh and dry weight of leaves, stem and root (g/ plant) were investigated.

Flowering parameters

Number of flowers/plants, flower diameter (cm), flowers fresh and dry weights (g/ plant) were investigated.

*Chemical analysis*

Photosynthetic pigments

Photosynthetic pigments (mg g<sup>-1</sup> F.W.) were determined in methanol (80%) according to Boger (Böger, 1964). chlorophyll a, chlorophyll b, and carotenoids were extracted, and the green color was assessed spectrophotometrically at 665, 649, and 470 nm.

Nutrient elements content

The leaves obtained from each treatment were dried in the oven at 60 °C for 3 days. 0.5 g of the dried leaves was crushed and digested using H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> according to Cottenie (1980). The digested solution was used to determine the following mineral contents: Nitrogen contents (%) was determined by the modified method according to Mu and Plummer (2001), phosphorus content (%) was determined as given by Jackson (1958), potassium (%) was determined according to Piper (2019), and iron content (ppm; the available iron) was determined by Atomic Absorption Spectrophotometer (AAS-4141) according to Lindsay and Norvell (1978).

Native-PAGE profiling of isozymes

Electrophoresis was performed to identify isozymes (POD, PPO, and SOD) differences between treatments. Peroxidase isozymes (POD, E.C. 1.11.1.7) in leaves sample were assessed by the procedure defined by Barcelo *et al.* (1987). Polyphenol oxidase isozymes (PPO, E.C. 1.10.3.1) in leaves sample were estimated as described by the published articles (Bradford, 1976; Thipyapong *et al.*, 1995). Finally, superoxide dismutase isozymes (SOD, EC 1.15.1.1) in fresh leaves were carried out as described by Winterbourn *et al.* (1975).

*Experimental layout and statistical analysis*

The experiment was laid out in completely randomized design (CRD). The treatments were applied with three replicates for each treatment during two seasons. The recorded Data statistically analysed using the Least Significant Difference(LSD) test at the 5% level as described by Little and Hills (1978). All the statistical analyses were performed by using CoStat (CoHort software, Monterey, CA, USA) V6.4 (2005), and standard deviation ( $\pm$  SD) was calculated.

## Results

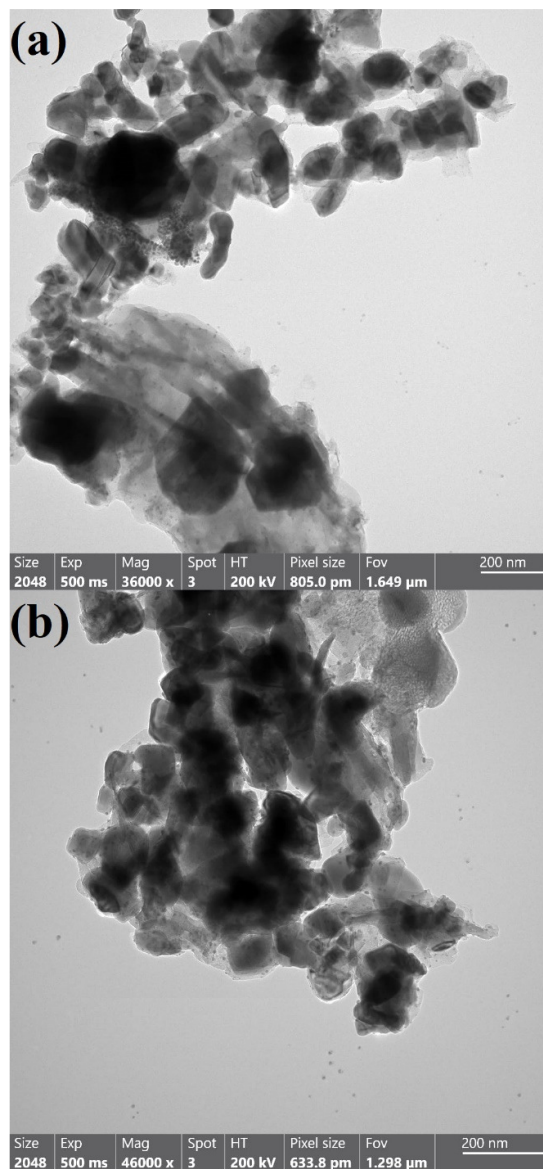
*Characterization of iron oxide nanocomposites (Fe<sub>2</sub>O<sub>3</sub> NP-B and Fe<sub>2</sub>O<sub>3</sub> NP-HA)*

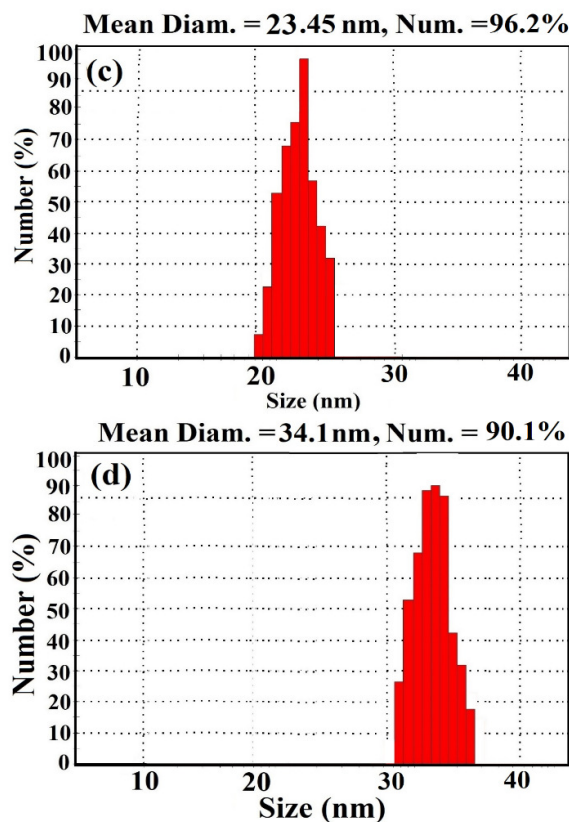
The synthesized iron oxide nanocomposites were produced using gamma-rays synthesis technique. The synthesized iron oxide nanocomposites demonstrated deep brown color, and the formed nanocomposite did

not aggregate with duration. The stabilization means was related to electrostatic attraction with the coated B, and HA.

HRTEM micrographs demonstrated mono-dispersed iron oxide nanocomposites which have a rounded shape of 12.25 nm average particle size for Fe<sub>2</sub>O<sub>3</sub>NPs-B, and 15.80 nm average particle size for Fe<sub>2</sub>O<sub>3</sub>NPs-HA (Figure 1 (a) for Fe<sub>2</sub>O<sub>3</sub>NPs-B, and Figure 1 (b) for Fe<sub>2</sub>O<sub>3</sub>NPs-HA). HRTEM images demonstrated high-rate mono-dispersed nanoparticles with consistent particle size which a coated faint layer (B, or HA). Additionally, particle size diffusion was estimated by DLS, and the outcome showed that the intermediate Fe<sub>2</sub>O<sub>3</sub> NPs-B particle size allotment was calculated to be 23.45 nm by 100 % as shown in Figure 1 (c), and 34.10 nm by 100% for Fe<sub>2</sub>O<sub>3</sub> NPs-B as shown in Figure 1 (d).

In our result we noted the particle size dispersal calculated from the DLS study was more than the calculated from HRTEM imaging (average particle size). The explanations are described as the DLS process assessed the hydrodynamic radius founded close to Fe<sub>2</sub>O<sub>3</sub> NPs-B, or Fe<sub>2</sub>O<sub>3</sub> NPs-HA and surrounded by the water molecules which helps in the immense sizes of the produced Fe<sub>2</sub>O<sub>3</sub> NPs (Baraka *et al.*, 2017).

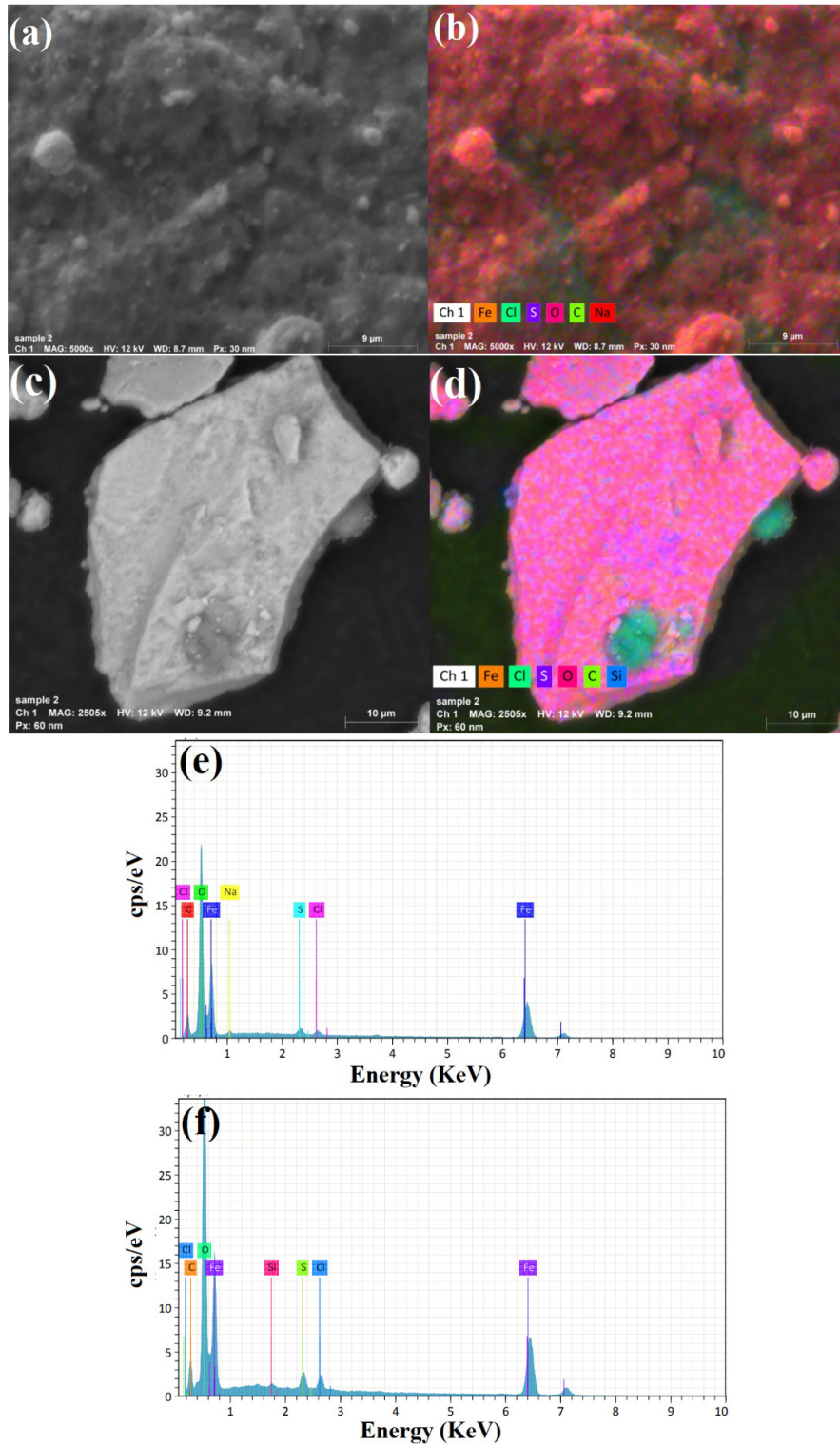




**Figure 1.** Shape, average particle size, and particle size distribution of the synthesized iron oxide nanocomposite; where (a) HRTEM of  $\text{Fe}_2\text{O}_3$  NPs-B, (b) HRTEM of  $\text{Fe}_2\text{O}_3$  NPs-HA, (c) DLS of  $\text{Fe}_2\text{O}_3$  NPs-B, and (d) DLS of  $\text{Fe}_2\text{O}_3$  NPs-HA.

The external morphology, purity, and the elemental composition of the prepared iron oxide nanocomposites were studied, as shown in Figure 2. SEM analysis of the synthesized  $\text{Fe}_2\text{O}_3$  NPs-B (Figure 2 a) showed that the prepared  $\text{Fe}_2\text{O}_3$  NPs was stabilized with the boron layer and after a magnification, and mapping as shown in Figure 2 b,  $\text{Fe}_2\text{O}_3$  NPs included a semi-spherical construction, with a consistent distribution as a bright particle at the surface of boron. The same situation was noted in case of  $\text{Fe}_2\text{O}_3$  NPs-HA, where SEM imaging illustrated the distribution of  $\text{Fe}_2\text{O}_3$  NPs with HA layer, and noted bright particles were seen on the surface (Figure 2 c, d).

EDX study showed the increased purity of the designed nanocomposites, as shown by the existence of atoms expected to per part of it (Fe, B, and O atoms) in the synthesized  $\text{Fe}_2\text{O}_3$  NPs-B and the lack of other atoms that may seem as an impurity. Carbon atom (C) was resembled to the holder which operated for the SEM imaging technique, the present Na atom was from the pH fixation, and S was present as traces because of the ferrous sulfate precursors as shown in Figure 2 e. The identical concern was reported in the chance of  $\text{Fe}_2\text{O}_3$  NPs-HA, where the existence of atoms expected to individually part of it (Fe and O atoms) from the synthesized  $\text{Fe}_2\text{O}_3$  NPs and S, C, and O atoms from the HA layers. Furthermore, carbon atom (C) resembled to the holder employed for the SEM imaging technique. Finally, the presence of Si atom was due to some contaminants in the imaging method (Figure 2 f).



**Figure 2.** Surface morphology, and elemental analysis of the synthesized iron oxide nanocomposite; where (a, b) SEM of Fe<sub>2</sub>O<sub>3</sub> NPs-B, (c, d) SEM of Fe<sub>2</sub>O<sub>3</sub> NPs-HA, (e) EDX of Fe<sub>2</sub>O<sub>3</sub> NPs-B, and (f) EDX of Fe<sub>2</sub>O<sub>3</sub> NPs-HA.



*Vegetative growth traits*

Iron fertilization showed positive results in the vegetative characteristics of *Gardenia jasminoides* plants compared to untreated plants, but fertilization with compounds of Fe<sub>2</sub>O<sub>3</sub>NPs-B and Fe<sub>2</sub>O<sub>3</sub>NPs-HA at different concentrations had a significant positive effect compared to both plants treated with chelated iron and untreated plants. The data tabulated in Tables 3 and 4 showed that the highest values obtained for each of the traits: plant height, stem diameter, number of leaves, number of branches, root length, leaf area, fresh and dry weights of leaves, stems and roots were obtained from treatment with Fe<sub>2</sub>O<sub>3</sub>NPs-HA at a concentration of 250 ppm in both seasons followed by treatment with Fe<sub>2</sub>O<sub>3</sub>NPs-B for the traits plant height, stem diameter, root length and fresh and dry weight of the roots; while the rest of the vegetative characteristics increased with treatment the Fe<sub>2</sub>O<sub>3</sub>NPs-HA at a concentration of 150 ppm in both seasons.

**Table 3.** Effect of iron oxide nanocomposited fertilization with different forms on plant height, stem diameter, no. of leaves, no. of branches, root length and leaf area of *Gardenia jasmionides* plant during two seasons

| Treatments (ppm)                          | Plant height (cm) | Stem diameter (cm) | No. of leaves/plant | No. of branches/plant | Root length (cm) | Leaf area (cm <sup>2</sup> ) |
|---|-------------------|--------------------|---------------------|-----------------------|------------------|------------------------------|
| <b>1<sup>st</sup> season</b>              |                   |                    |                     |                       |                  |                              |
| Control                                   | 40.87± 1.55       | 0.48± 0.02         | 50.00± 2.65         | 6.33± 0.58            | 20.00± 1.73      | 12.00± 0.63                  |
| Fe <sub>2</sub> O <sub>3</sub> 100        | 43.60± 1.84       | 0.52± 0.02         | 55.33± 2.88         | 6.67± 0.58            | 21.80± 1.06      | 12.73± 0.87                  |
| Fe <sub>2</sub> O <sub>3</sub> NPs B100   | 46.67± 1.93       | 0.55± 0.03         | 68.67± 3.22         | 7.00± 1.73            | 25.67± 0.58      | 13.24± 0.58                  |
| Fe <sub>2</sub> O <sub>3</sub> NPs B150   | 50.70± 2.29       | 0.60± 0.03         | 97.67± 2.08         | 9.67± 1.53            | 28.00± 1.00      | 15.46± 0.60                  |
| Fe <sub>2</sub> O <sub>3</sub> NPs B250   | 54.70± 2.36       | 0.71± 0.03         | 119.33± 2.88        | 11.33± 1.15           | 37.67± 1.53      | 19.25± 0.83                  |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA100  | 47.00± 1.76       | 0.54± 0.02         | 79.00± 2.65         | 8.67± 1.53            | 23.67± 1.15      | 17.25± 0.81                  |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA 150 | 52.30± 1.31       | 0.63± 0.03         | 133.67± 2.52        | 14.67± 2.52           | 33.10± 1.65      | 20.23± 0.47                  |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA 250 | 58.67± 1.42       | 0.80± 0.03         | 220.00± 2.65        | 17.00± 1.73           | 48.33± 1.53      | 20.75± 0.95                  |
| LSD 5%                                    | <b>3.19</b>       | <b>0.04</b>        | <b>4.69</b>         | <b>2.67</b>           | <b>2.30</b>      | <b>1.27</b>                  |
| <b>2<sup>nd</sup> season</b>              |                   |                    |                     |                       |                  |                              |
| Control                                   | 37.67± 1.39       | 0.44± 0.01         | 48.33± 2.88         | 6.00± 1.00            | 21.67± 1.53      | 9.37± 0.65                   |
| Fe <sub>2</sub> O <sub>3</sub> 100        | 40.67± 1.95       | 0.52± 0.03         | 59.67± 3.05         | 6.67± 1.15            | 23.33± 0.58      | 10.63± 0.68                  |
| Fe <sub>2</sub> O <sub>3</sub> NPs B100   | 42.00± 1.51       | 0.52± 0.03         | 75.33± 2.08         | 8.00± 1.00            | 24.00± 1.00      | 12.12± 0.71                  |
| Fe <sub>2</sub> O <sub>3</sub> NPs B150   | 47.33± 1.89       | 0.59± 0.04         | 111.33± 3.22        | 10.67± 0.58           | 32.50± 0.50      | 18.06± 0.70                  |
| Fe <sub>2</sub> O <sub>3</sub> NPs B250   | 56.87± 1.37       | 0.75± 0.03         | 127.00± 2.65        | 11.67± 1.53           | 44.67± 1.53      | 20.85± 0.69                  |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA100  | 42.67± 1.49       | 0.55± 0.03         | 85.67± 2.52         | 9.33± 1.15            | 26.67± 1.15      | 13.93± 0.64                  |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA 150 | 51.70± 1.56       | 0.62± 0.02         | 136.00± 2.65        | 13.67± 1.53           | 40.00± 1.73      | 26.22± 0.75                  |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA 250 | 61.33± 1.67       | 0.82± 0.03         | 215.67± 3.05        | 16.67± 0.58           | 53.87± 1.63      | 35.46± 0.99                  |
| LSD 5%                                    | <b>2.79</b>       | <b>0.05</b>        | <b>4.82</b>         | <b>1.94</b>           | <b>2.23</b>      | <b>1.27</b>                  |

**Table 4.** Effect of iron oxide nanocomposites fertilization with different forms on fresh and dry weight of leaves, stems and roots of *Gardenia jasmionides* plant during two seasons

| Treatments(ppm)                           | Leaves F.W.<br>(g) | Stems F.W.<br>(g) | Roots F.W.<br>(g) | Leaves D.W.<br>(g) | Stems D.W.<br>(g) | Roots D.W.<br>(g) |
|---|--------------------|-------------------|-------------------|--------------------|-------------------|-------------------|
| <b>1<sup>st</sup> season</b>              |                    |                   |                   |                    |                   |                   |
| Control                                   | 10.02 ± 0.86       | 11.74 ± 1.04      | 27.44 ± 1.10      | 3.24 ± 0.46        | 5.17 ± 0.62       | 9.97 ± 0.63       |
| Fe <sub>2</sub> O <sub>3</sub> 100        | 10.85 ± 1.10       | 13.99 ± 1.10      | 30.17 ± 1.03      | 3.52 ± 0.44        | 6.37 ± 0.59       | 11.02 ± 0.68      |
| Fe <sub>2</sub> O <sub>3</sub> NPs B100   | 11.07 ± 0.78       | 15.37 ± 0.94      | 37.52 ± 1.25      | 3.61 ± 0.45        | 7.03 ± 0.57       | 13.90 ± 0.82      |
| Fe <sub>2</sub> O <sub>3</sub> NPs B150   | 18.26 ± 0.80       | 22.07 ± 0.78      | 40.03 ± 1.87      | 6.04 ± 0.62        | 10.24 ± 0.63      | 14.89 ± 0.86      |
| Fe <sub>2</sub> O <sub>3</sub> NPs B250   | 21.96 ± 1.11       | 24.58 ± 1.16      | 46.65 ± 1.01      | 7.30 ± 0.43        | 11.46 ± 0.71      | 17.53 ± 0.64      |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA100  | 14.75 ± 1.27       | 19.66 ± 0.87      | 32.37 ± 1.24      | 4.85 ± 0.44        | 9.07 ± 0.78       | 11.88 ± 0.76      |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA 150 | 25.11 ± 0.76       | 28.90 ± 0.91      | 42.29 ± 1.59      | 8.44 ± 0.56        | 13.62 ± 0.86      | 15.82 ± 0.66      |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA 250 | 37.76 ± 1.14       | 34.23 ± 1.07      | 54.12 ± 1.64      | 12.76 ± 0.84       | 16.25 ± 0.72      | 20.44 ± 0.66      |
| LSD 5%                                    | <b>1.72</b>        | <b>1.71</b>       | <b>2.38</b>       | <b>0.95</b>        | <b>1.20</b>       | <b>1.24</b>       |
| <b>2<sup>nd</sup> season</b>              |                    |                   |                   |                    |                   |                   |
| Control                                   | 9.37 ± 0.67        | 12.06 ± 0.66      | 28.07 ± 1.40      | 3.01 ± 0.73        | 5.45 ± 0.89       | 10.18 ± 0.57      |
| Fe <sub>2</sub> O <sub>3</sub> 100        | 10.63 ± 0.69       | 13.17 ± 0.81      | 34.98 ± 0.94      | 3.44 ± 0.54        | 6.00 ± 0.57       | 12.76 ± 0.64      |
| Fe <sub>2</sub> O <sub>3</sub> NPs B100   | 12.12 ± 0.81       | 15.80 ± 0.83      | 36.27 ± 1.06      | 3.94 ± 0.72        | 7.24 ± 0.63       | 13.29 ± 0.50      |
| Fe <sub>2</sub> O <sub>3</sub> NPs B150   | 18.06 ± 0.78       | 21.75 ± 0.64      | 47.36 ± 1.34      | 5.95 ± 0.66        | 10.14 ± 0.62      | 17.61 ± 0.60      |
| Fe <sub>2</sub> O <sub>3</sub> NPs B250   | 20.85 ± 0.93       | 25.65 ± 0.91      | 54.81 ± 1.40      | 6.91 ± 0.63        | 12.06 ± 0.55      | 20.59 ± 0.71      |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA100  | 13.93 ± 0.85       | 18.96 ± 0.81      | 40.45 ± 1.49      | 4.56 ± 0.49        | 8.75 ± 0.68       | 14.93 ± 0.80      |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA 150 | 26.22 ± 0.84       | 30.49 ± 0.64      | 50.93 ± 1.80      | 8.81 ± 0.54        | 14.42 ± 0.72      | 19.07 ± 0.78      |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA 250 | 35.46 ± 1.17       | 35.00 ± 0.68      | 57.68 ± 1.77      | 11.99 ± 0.82       | 16.64 ± 0.66      | 21.80 ± 0.83      |
| LSD 5%                                    | <b>1.48</b>        | <b>1.30</b>       | <b>2.47</b>       | <b>1.12</b>        | <b>1.16</b>       | <b>1.19</b>       |

*Flowering traits*

The flowering characteristics of the *G. jasmionides* plants were affected by a positive and significant effect, as the results presented in the Table 5 which showed that the number of flowers, flower diameter n, the fresh and dry weight of the flowers increased when treated with Fe<sub>2</sub>O<sub>3</sub>NPs-HA at a concentration of 250 ppm, followed by treatment with Fe<sub>2</sub>O<sub>3</sub>NPs-B at a concentration of 250 ppm when compared to plants treated with Fe<sub>2</sub>O<sub>3</sub>NPs and untreated.

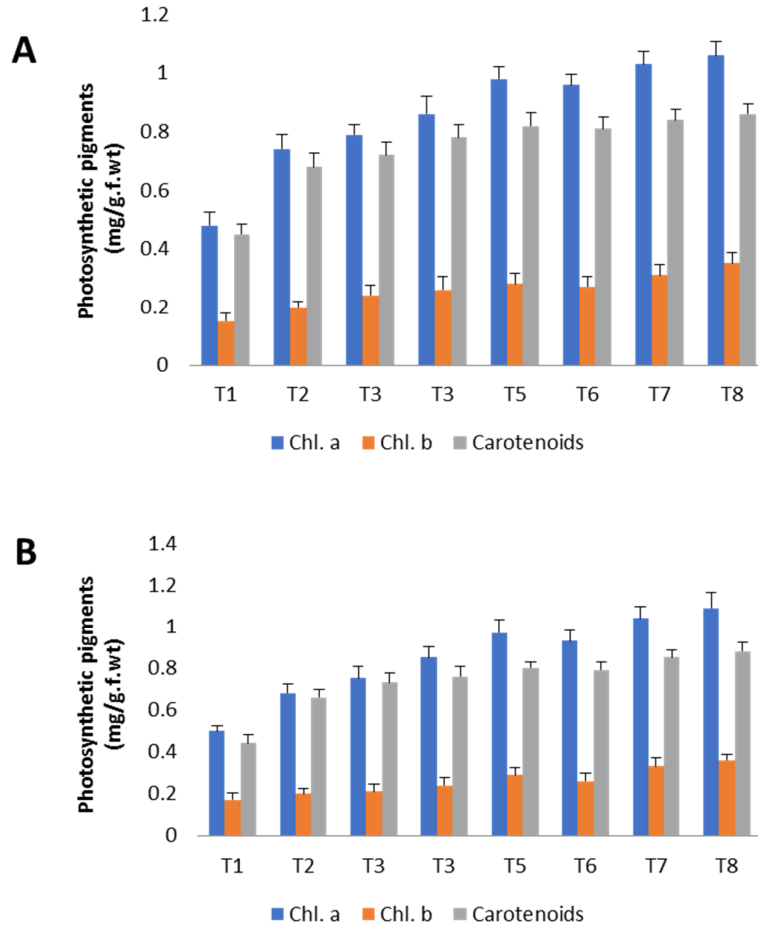
**Table 5.** Effect of iron oxide nanocomposites fertilization with different forms on the flowering traits of *Gardenia jasminoides* plant during two seasons

| Treatments(ppm)                           | No. of flowers | Flower diameter | Flowers F.W. | Flowers D.W. |
|---|----------------|-----------------|--------------|--------------|
| <b>1<sup>st</sup> season</b>              |                |                 |              |              |
| Control                                   | 2.00 ± 0.00    | 2.50 ± 0.08     | 1.63 ± 0.10  | 0.28 ± 0.03  |
| Fe <sub>2</sub> O <sub>3</sub> 100        | 2.00 ± 1.00    | 2.72 ± 0.07     | 1.83 ± 0.09  | 0.32 ± 0.02  |
| Fe <sub>2</sub> O <sub>3</sub> NPs B100   | 2.33 ± 0.58    | 3.25 ± 0.11     | 2.16 ± 0.05  | 0.40 ± 0.03  |
| Fe <sub>2</sub> O <sub>3</sub> NPs B150   | 3.67 ± 0.58    | 4.57 ± 0.09     | 2.35 ± 0.07  | 0.44 ± 0.02  |
| Fe <sub>2</sub> O <sub>3</sub> NPs B250   | 4.64 ± 1.52    | 5.46 ± 0.07     | 3.46 ± 0.11  | 0.68 ± 0.03  |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA100  | 2.00 ± 1.00    | 3.83 ± 0.09     | 1.97 ± 0.06  | 0.36 ± 0.02  |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA 150 | 4.00 ± 1.00    | 4.91 ± 0.10     | 3.17 ± 0.08  | 0.61 ± 0.02  |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA 250 | 7.33 ± 1.15    | 6.08 ± 0.07     | 3.96 ± 0.07  | 0.79 ± 0.04  |
| LSD 5%                                    | <b>1.66</b>    | <b>0.14</b>     | <b>0.14</b>  | <b>0.04</b>  |
| <b>2<sup>nd</sup> season</b>              |                |                 |              |              |
| Control                                   | 1.67 ± 0.58    | 2.24 ± 0.06     | 1.52 ± 0.06  | 0.27 ± 0.03  |
| Fe <sub>2</sub> O <sub>3</sub> 100        | 2.33 ± 0.58    | 2.89 ± 0.08     | 2.08 ± 0.08  | 0.37 ± 0.02  |
| Fe <sub>2</sub> O <sub>3</sub> NPs B100   | 3.00 ± 0.00    | 3.58 ± 0.05     | 2.27 ± 0.06  | 0.41 ± 0.02  |
| Fe <sub>2</sub> O <sub>3</sub> NPs B150   | 3.67 ± 1.15    | 4.44 ± 0.06     | 2.50 ± 0.07  | 0.46 ± 0.02  |
| Fe <sub>2</sub> O <sub>3</sub> NPs B250   | 5.67 ± 0.58    | 5.83 ± 0.06     | 3.88 ± 0.08  | 0.75 ± 0.03  |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA100  | 2.67 ± 0.58    | 3.95 ± 0.07     | 2.52 ± 0.08  | 0.47 ± 0.03  |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA 150 | 5.00 ± 1.00    | 5.27 ± 0.07     | 3.64 ± 0.10  | 0.69 ± 0.03  |
| Fe <sub>2</sub> O <sub>3</sub> NPs HA 250 | 8.67 ± 1.53    | 6.93 ± 0.09     | 4.37 ± 0.09  | 0.85 ± 0.03  |
| LSD 5%                                    | <b>1.50</b>    | <b>0.12</b>     | <b>0.13</b>  | <b>0.04</b>  |

*Chemical composition*Photosynthetic pigments

Our study revealed that the fertilization with iron oxide nanocomposites in the different forms and different concentrations increased the content of chlorophyll a, b and carotenoids in the fresh leaves of *G. jasminoides* plants as compared to control plants.

The data included in the Figure 3 showed that the highest content of chlorophyll a, b and carotenoids (1.06±0.05, 0.35±0.04 and 0.86±0.04 mg.g<sup>-1</sup> F.W, respectively, in the 1<sup>st</sup> season) and (1.09±0.08, 0.36±0.03 and 0.88±0.04 mg.g<sup>-1</sup> F.W, respectively, in the second season) were obtained from plants treated with Fe<sub>2</sub>O<sub>3</sub>NP-HA at a concentration of 250 ppm as compared to untreated plants.

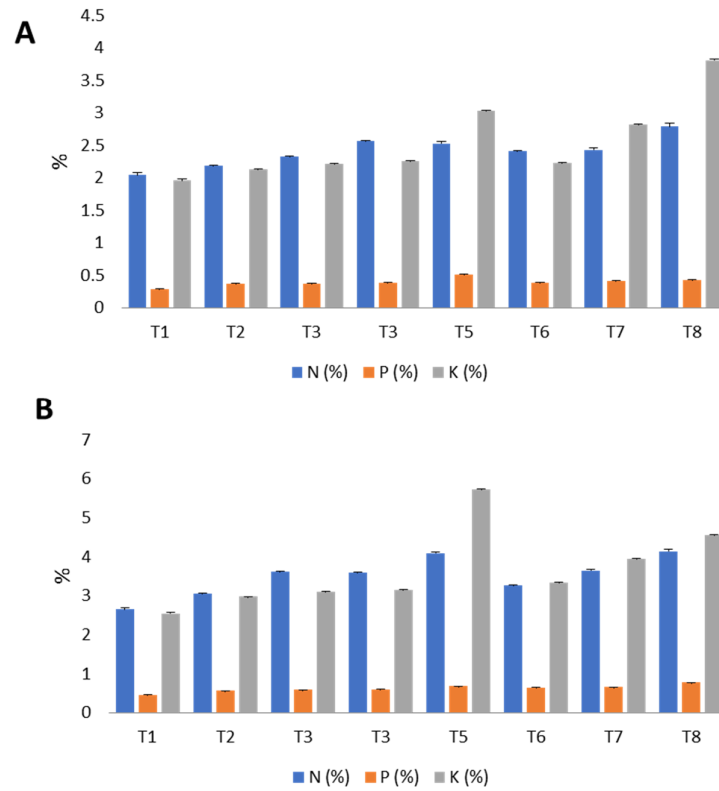


**Figure 3.** Effect of iron oxide nanocomposites fertilization with different forms on photosynthetic pigments (A) 1st season and (B) 2nd season of *Gardenia jasminoides* plant during two seasons. where, T1: control, T2: Fe<sub>2</sub>O<sub>3</sub> 100, T3: Fe<sub>2</sub>O<sub>3</sub>NPs B 100ppm, T4: Fe<sub>2</sub>O<sub>3</sub>NPs-B 150 ppm, T5: Fe<sub>2</sub>O<sub>3</sub> NPs-B 250 ppm, T6: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 100 ppm, T7: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 150 ppm, and T8: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 250 ppm.

#### Nutrient elements content

Our study showed that the plants were fertilized with Fe<sub>2</sub>O<sub>3</sub>NP in the different forms and various concentrations increased the content of nutrient elements content (N, P and K%) in the leaves of *G. jasminoides* plants as compared to control plants. The data included in the Figure 4 showed that the highest content of N and K (2.79±0.05, and 3.81±0.02%, respectively, in the 1<sup>st</sup> season) were obtained from plants treated with Fe<sub>2</sub>O<sub>3</sub>NPs-HA at a concentration of 250 ppm as compared to all treatment's plants.

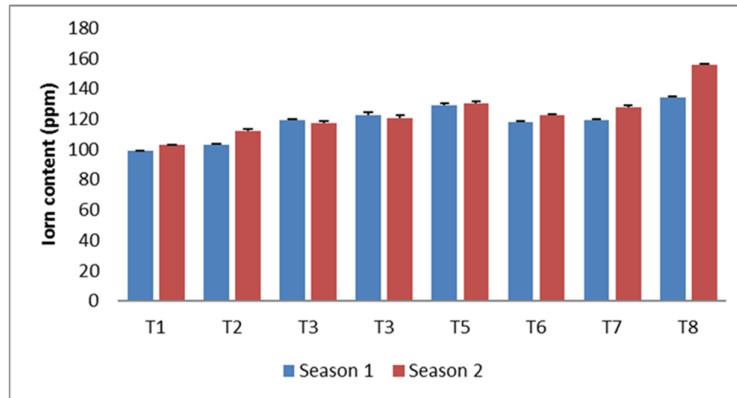
While the highest content of P% (0.51±0.01%) in the 1<sup>st</sup> season was obtained from plants treated with Fe<sub>2</sub>O<sub>3</sub> NPs-B at a concentration of 250 ppm as compared to all treatments. On the other hand, it was observed that plants treated with Fe<sub>2</sub>O<sub>3</sub>NPs-HA at a concentration of 250 ppm recorded the highest content of N and P (4.14± 0.05 and 0.77±0.01%, respectively, in the 2<sup>nd</sup> season), while plants treated with Fe<sub>2</sub>O<sub>3</sub>NPs-B at a concentration of 250 ppm recorded the highest content of K (5.72±0.01 % in the 2<sup>nd</sup> season).



**Figure 4.** Effect of iron oxide nanocomposites fertilization with different forms on nutrient element content (N, P and K%) (A) 1st season and (B) 2nd season of *Gardenia jasminoides* plant during two seasons. where, T1: control, T2: Fe<sub>2</sub>O<sub>3</sub> 100, T3: Fe<sub>2</sub>O<sub>3</sub>NPs B 100ppm, T4: Fe<sub>2</sub>O<sub>3</sub>NPs-B 150 ppm, T5: Fe<sub>2</sub>O<sub>3</sub> NPs-B 250 ppm, T6: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 100 ppm, T7: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 150 ppm, and T8: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 250 ppm.

Iron content (ppm)

The content of iron in dry leaves of *G. jasminoides* plants was positively affected by Fe<sub>2</sub>O<sub>3</sub>NPs treatment. The results presented in Figure 5 showed that the highest value content of iron was recorded in plants treated with Fe<sub>2</sub>O<sub>3</sub> NPs-HA at a concentration of 250 ppm giving (134.33±0.58 and 155.71±0.56 ppm, respectively, in first and second seasons) followed by treatment with Fe<sub>2</sub>O<sub>3</sub> NPs-B at a concentration of 250 ppm (129.33±1.2 and 130.64 ±1.34 ppm, respectively, in first and second seasons) while the lowest content of iron was found in untreated plants that recorded giving values (99.26 ± 0.15 and 102.85±0.19 ppm) in first and second seasons, respectively.

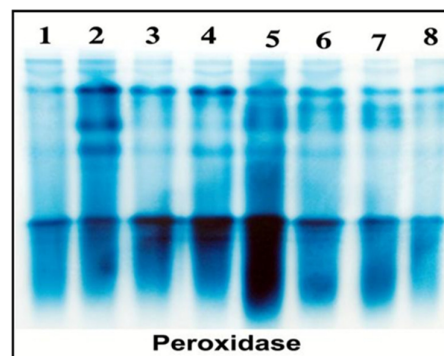


**Figure 5.** Effect of iron oxide nanocomposites fertilization with different forms on iron content (ppm) of *Gardenia jasminoides* plant during two seasons where, T1: control, T2: Fe<sub>2</sub>O<sub>3</sub> 100, T3: Fe<sub>2</sub>O<sub>3</sub>NPs B 100ppm, T4: Fe<sub>2</sub>O<sub>3</sub>NPs-B 150 ppm, T5: Fe<sub>2</sub>O<sub>3</sub>NPs-B 250 ppm, T6: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 100 ppm, T7: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 150 ppm, and T8: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 250 ppm.

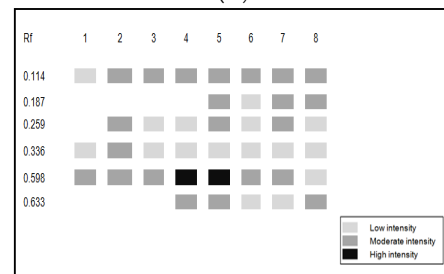
*Native-PAGE profiling of isozymes*

Peroxidase (POD)

Peroxidase electrophoretic patterns are shown in Figure 6A and B and Table 6. Six bands with different intensities were found among the profiles of all treatments. Three bands were present in all treatments (monomorphic bands) at *R<sub>f</sub>* (retention factor) 0.114, 0.336 and 0.598 but in different intensity. The other three bands which have *R<sub>f</sub>* 0.178, 0.259, and 0.633 were polymorphic.



(A)



(B)

**Figure 6.** Effect of iron oxide nanocomposites fertilization with different forms on peroxidase isozyme activity where, (A) banding pattern, and (B) Ideogram analysis of *G. jasminoides* plant where, 1: control, 2: Fe<sub>2</sub>O<sub>3</sub> 100: Fe<sub>2</sub>O<sub>3</sub> NPs-B 100 ppm, 4: Fe<sub>2</sub>O<sub>3</sub> NPs-B 150 ppm, 5: Fe<sub>2</sub>O<sub>3</sub>NPs-B 250 ppm, 6: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 100 ppm, 7: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 150 ppm and 8: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 250 ppm).

Band at  $R_f$  (0.187) disappeared in control treated plant,  $Fe_2O_3$  100 and  $Fe_2O_3$ NPs-B at 100 and 150 ppm but appeared in  $Fe_2O_3$ NPs-B at 250 ppm and  $Fe_2O_3$ NPs-HA at 100, 150 and 250 ppm. The band which has  $R_f$ 0.259 became very intensified with all treatments but not appeared with control treated plant. Isozyme at  $R_f$ (0.633) disappeared in control treated plant,  $Fe_2O_3$  100 and  $Fe_2O_3$ NPs-B at 100 ppm while appeared in all other treatments. The peroxidase (POD) activity was highest by application of  $Fe_2O_3$ NPs-B followed by  $Fe_2O_3$ NPs-HA,  $Fe_2O_3$  NPs and control plant, respectively as showed in Table 6.

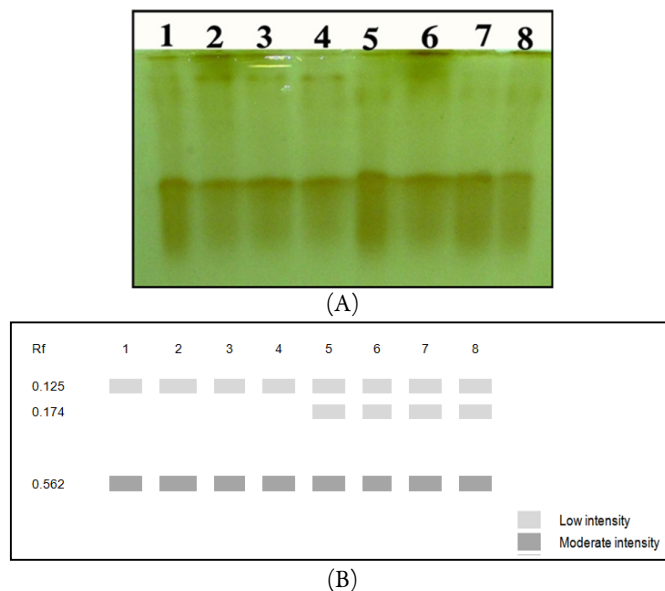
**Table 6.** Isomers of peroxidase enzymes (+/-) and their Retention factor (Rf) in response of Effect of iron oxide nanocomposites fertilization with different forms on peroxidase isozyme activity of *G. jasmionides* plant (where, 1: control, 2:  $Fe_2O_3$  100, 3:  $Fe_2O_3$ NPs-B 100 ppm, 4:  $Fe_2O_3$  NPs-B 150 ppm, 5:  $Fe_2O_3$  NPs-B 250 ppm, 6:  $Fe_2O_3$  NPs-HA 100 ppm, 7:  $Fe_2O_3$ NPs-HA 150 ppm and 8:  $Fe_2O_3$  NPs-HA 250 ppm)

| Retention factor (Rf) | 1  | 2  | 3  | 4   | 5   | 6  | 7  | 8  |
|-----------------------|----|----|----|-----|-----|----|----|----|
| 0.114                 | +  | ++ | ++ | ++  | ++  | ++ | ++ | ++ |
| 0.187                 | —  | —  | —  | —   | ++  | +  | ++ | ++ |
| 0.259                 | —  | ++ | +  | +   | ++  | +  | ++ | +  |
| 0.336                 | +  | ++ | +  | +   | +   | +  | +  | +  |
| 0.598                 | ++ | ++ | ++ | +++ | +++ | ++ | ++ | +  |
| 0.633                 | —  | —  | —  | ++  | ++  | +  | +  | +  |

- absent  
 + low intensity band  
 ++ moderate intensity band  
 +++ high intensity

Polyphenol oxidase (PPO)

Expression of the Polyphenol oxidase isozyme was detected in Figure 7A and B. The results showed that three bands with various intensities were shown in Table 7 two bands showed in all treatments (monomorphic bands) at  $R_f$  0.125 and 0.562. The other band at  $R_f$  (0.174) appeared with  $Fe_2O_3$ NPs-B at 250 ppm and  $Fe_2O_3$ NPs-HA at 100, 150 and 250 ppm. In addition, no clear differences of PPO enzymatic activity were shown between control and plants treated with  $Fe_2O_3$  100 and  $Fe_2O_3$ NPs-Bat 100 and 150 ppm while PPO activities increased with  $Fe_2O_3$ NPs-B at 250 ppm and  $Fe_2O_3$ NPs-HA at 100, 150 and 250 ppm.



**Figure 7.** Effect of iron oxide nanocomposite fertilization with different forms on polyphenol oxidase isozyme activity where (A) banding pattern, and (B) Ideogram analysis of *G. jasmionides* plant (where, 1: control, 2: Fe<sub>2</sub>O<sub>3</sub> 100, 3: Fe<sub>2</sub>O<sub>3</sub> NPs-B 100 ppm, 4: Fe<sub>2</sub>O<sub>3</sub> NPs-B 150 ppm, 5: Fe<sub>2</sub>O<sub>3</sub> NPs-B 250 ppm, 6: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 100 ppm, 7: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 150 ppm, and 8: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 250 ppm)

**Table 7.** Isomers of polyphenol oxidase enzymes (+/-) and their Retention factor (Rf) in response of effect of iron oxide nanocomposites fertilization with different forms on peroxidase isozyme activity of *G. jasmionides* plant (where, 1: control, 2: Fe<sub>2</sub>O<sub>3</sub> 100, 3: Fe<sub>2</sub>O<sub>3</sub> NPs-B 100 ppm, 4: Fe<sub>2</sub>O<sub>3</sub> NPs-B 150 ppm, 5: Fe<sub>2</sub>O<sub>3</sub> NPs-B 250 ppm, 6: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 100 ppm, 7: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 150 ppm, and 8: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 250 ppm)

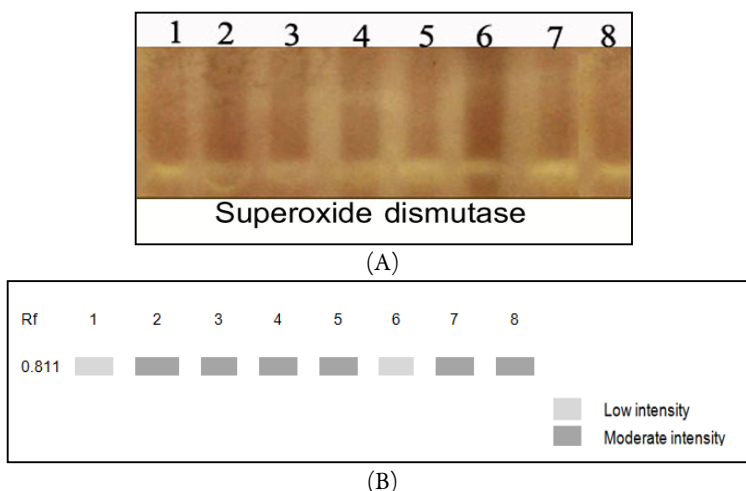
| Retention factor (Rf) | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
|-----------------------|----|----|----|----|----|----|----|----|
| 0.125                 | +  | +  | +  | +  | +  | +  | +  | +  |
| 0.174                 | -  | -  | -  | -  | +  | +  | +  | +  |
| 0.562                 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |

- absent  
 + low intensity band  
 ++ moderate intensity band  
 +++ high intensity

Superoxide dismutase (SOD)

Table 8 and Figure 8A and B revealed that one band monomorphic at Rf (0.811) with different intensities. The highest SOD activity was recorded with at control, Fe<sub>2</sub>O<sub>3</sub>NPs-HA at (100 and 250 ppm), while was lowest value at control, Fe<sub>2</sub>O<sub>3</sub>100, Fe<sub>2</sub>O<sub>3</sub>NPs-B at 100, 150 and 250 ppm and Fe<sub>2</sub>O<sub>3</sub>NPs-HA at 150 ppm.





**Figure 8.** Effect of iron oxide nanocomposites fertilization with different forms on superoxide dismutase isoenzyme activity; where (A) banding pattern, and (B) Ideogram analysis of *G. jasmionides* plant (where, 1: control, 2: Fe<sub>2</sub>O<sub>3</sub> 100, 3: Fe<sub>2</sub>O<sub>3</sub> NPs-B 100 ppm, 4: Fe<sub>2</sub>O<sub>3</sub> NPs-B 150 ppm, 5: Fe<sub>2</sub>O<sub>3</sub> NPs-B 250 ppm, 6: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 100 ppm, 7: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 150 ppm, and 8: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 250 ppm)

**Table 8.** Isomers of superoxide dismutase enzymes (+/-) and their Retention factor (Rf) in response of effect of iron oxide nanocomposites fertilization with different forms on peroxidase isozyme activity of *G. jasmionides* plant (where, 1: control, 2: Fe<sub>2</sub>O<sub>3</sub> 100, 3: Fe<sub>2</sub>O<sub>3</sub> NPs-B 100 ppm, 4: Fe<sub>2</sub>O<sub>3</sub> NPs-B 150 ppm, 5: Fe<sub>2</sub>O<sub>3</sub> NPs-B 250 ppm, 6: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 100 ppm, 7: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 150 ppm, and 8: Fe<sub>2</sub>O<sub>3</sub> NPs-HA 250 ppm)

| Retention factor (Rf) | 1 | 2  | 3  | 4  | 5  | 6 | 7  | 8 |
|-----------------------|---|----|----|----|----|---|----|---|
| 0.811                 | + | ++ | ++ | ++ | ++ | + | ++ | + |

+ low intensity band  
 ++ moderate intensity band  
 +++ high intensity

## Discussion

Gardenia plants are considered needy to iron element, so the efficiency of the form used for iron fertilization is evident on the morphological characteristics of this plant species, therefore; the previous results showed that the fertilization with Fe<sub>2</sub>O<sub>3</sub>NPs-HA at a concentration of 250 ppm had an effective effect on the vegetative and flowering characteristics and the photosynthetic pigment content of leaves, followed by the fertilization with Fe<sub>2</sub>O<sub>3</sub>NPs-Bat a concentration of 250 ppm.

Several studies have reported that iron NPs has a critical role in metabolic processes such as respiration, DNA synthesis, it is essential for the maintenance of chloroplast structure and function (Ghafari and Razmjoo, 2013). Rout and Sahoo (2015) stated that the iron fertilization causes an increase in morphological parameters as a result to its role in cytochrome formation and ferredoxin compounds that necessary for the photosynthesis process which pushes towards increasing growth rate.

El-Shawa *et al.* (2022) reported that the iron NPs treatments gave the highest values of chlorophyll and carotenoids content in leaves of *Philodendron bipinnatifidum* plants, this increase may be a result of stimulating the activity of some specific enzymes which play an important role in chlorophyll synthesis (Elfeky *et al.*, 2013), such as NADPH proto-chlorophyllide oxidoreductase (POR), which is the main enzyme of chlorophyll synthesis in flowering plants (Zalat *et al.*, 2021).

Additionally, iron functions in the synthesis of a specified type of RNA that regulates chlorophyll synthesis (Apel *et al.*, 1980; Ma *et al.*, 2012). These results are in the same line on *Rosa hybrida* plant (Ibrahim, 2019) on *Hibiscus sabdariffa* plant, (Alalaf *et al.*, 2020) on pomelo seedlings and Abdulazeez *et al.* (2020) on two cultivars of *Fressia × hybrida* plants.

Many of studies cleared the constructive effect of boron on plant growth (Emara and Am, 2017), found that nano-boron treatment increased the values of plant height, chlorophyll a, b and carotenoids. Al-Rubaye and Khudair (Al-Rubaye and Khudair 2020; Abdelaziz *et al.*, 2021) stated that the boron treatment increased plant height, number of leaves, root length, shoot and roots fresh and dry weights, number of flowers and flowers dry weight of gazania plant. The positive functions of boron on ornamental plants were attributed to its essential roles in translocation of sugars as well as enhancing the formation of meristems, cell division and root development. The beneficial effect of boron on preventing the abortion of flowers, the conversion of starch to soluble sugars (Blevins and Lukaszewski, 1998; Al-Qubaie, 2013).

The study showed that the addition of HA in the soil medium resulted in the production of the highest values in vegetative growth, regrowth and content of photosynthetic pigments in plant leaves. HA caused some useful changes in physical and chemical properties of the soil, such as water retention capacity, airing, pH and ion transportation (Lodhi *et al.*, 2013; Yu *et al.*, 2018). Vickers (2017) reported that HA substances are similar to hormones in terms of promoting plant growth. HA could enhance plant growth by increasing the permeability of cell membrane, facilitate transport of essential elements within the roots and support respiration (Cacco and Dell'Agnola, 1984; Masciandaro *et al.*, 2002).

HA also positively affects the nutrient absorption of plants and is particularly important for the transport and the availability of micronutrients (Sharif *et al.*, 2002; El-Sayed *et al.*, 2023). Various studies manifested that the treatment with HA led to an improvement in the growth characteristics of the plant as the same in *Passiflora edulis* (Cavalcante *et al.*, 2013), *Achillea mille folium* (Bayat *et al.*, 2021), and garden cress (Yildirim *et al.*, 2021).

Our resulted indicated that, the activities of POD, PPO and SOD isozymes increased with increasing iron oxide nanocomposites rates compared with Fe<sub>2</sub>O<sub>3</sub>NPs application and control plant (without iron fertilizers) hence, indicates the positive impact of iron oxide nanocomposites on the isozymes theses agreement with (Adrees *et al.*, 2020; Hashem *et al.*, 2023) which stated that the NPs treatment linearly improved these enzyme activities when compared with the control either normal conditions and there was a positive correlation between enzyme activities and the plant biomass, chlorophyll contents (El-Sayed *et al.*, 2022; Hashem *et al.*, 2022).

The obtained results indicated that the seedlings treated with Fe<sub>2</sub>O<sub>3</sub>NPs-HA had a lower rate of increase in the enzymatic activity of POD, SOD and PPO than the seedlings treated with Fe<sub>2</sub>O<sub>3</sub>NPs-B. This resulted agreement with Allison (2006) which stated that HA are very important for enzyme functions because they compose a large proportion of soil organic matter and may help to stabilize enzymatic activities.

Recent evidence suggests that plant Glutathione peroxidases family (GPxs) can be implicated in plant growth and development, and peroxidases has important roles for control cell growth either by restriction or promotion of cell elongation; they have a role in auxin catabolism, destruction of flavonoids, biosynthesis of ethylene and secondary metabolites (Cosio and Dunand, 2009; Csiszár *et al.*, 2012; Bela *et al.*, 2015).

Our results for PPO activity were agreement with El-Shawa *et al.* (2022) which found a higher PPO activity compared to the control when treated with HA at 2 ml/L in Philodendron plant.

Our results revealed that iron oxide nanocomposites are promising for improving the growth and flowering of *Gardenia jasminoides* which agreement with heavily studies that stated that NPs enhanced the plant growth and production when compared with the control (Attia *et al.*, 2023; El-Batal *et al.*, 2023). The synthesized Fe<sub>2</sub>O<sub>3</sub>NPs-HA enhanced the mobilization of the nutrient element (iron) and the special structure of Fe<sub>2</sub>O<sub>3</sub>NPs-HA could protect and prolong soil enzyme activity (Mousa *et al.*, 2021), soil organic matter, soil

enzyme it may enhanced the plant growth and flowering characteristics of plant which agree with Xiang *et al.* (2019).

### Conclusions

We may conclude that Fe<sub>2</sub>O<sub>3</sub> NPs-B and Fe<sub>2</sub>O<sub>3</sub> NPs-HA showed a significant improvement in the desirable characteristics of *G. jasminoides*, whether vegetative or flowering, based on the improvement in the chemical content of plant. HRTEM micrographs demonstrated mono-dispersed iron oxide nanocomposites which owns a sphere-shaped of 12.25 nm ordinary particle size for Fe<sub>2</sub>O<sub>3</sub>NPs-B, and 15.80 nm average particle size for Fe<sub>2</sub>O<sub>3</sub>NPs-HA. HRTEM micrographs confirmed high quality mono-dispersed particles with uniform particle size which a coated faint layers (B, or HA). Additionally, particle size scattering was premeditated by DLS, and the consequence showed that the average Fe<sub>2</sub>O<sub>3</sub> NPs-B particle size spreading was originated to be 23.45 nm by 100%, and 34.10 nm by 100% for Fe<sub>2</sub>O<sub>3</sub> NPs-B. The best results were obtained from plants treated with Fe<sub>2</sub>O<sub>3</sub> NPs-HA at 250 ppm for all vegetative, flowering parameters, photosynthetic pigments, nitrogen, and iron content followed by Fe<sub>2</sub>O<sub>3</sub> NPs-B at concentration in both seasons. The plants treated with Fe<sub>2</sub>O<sub>3</sub>NPs-HA showed an enhancement in the potassium content in the first season and phosphorus content in the second season. However, the highest activity of enzymes (POD, PPO, and SOD) appeared in the treated plants with Fe<sub>2</sub>O<sub>3</sub>NPs-B at 250 ppm, followed by Fe<sub>2</sub>O<sub>3</sub>NPs-HA at 250 ppm.

### Authors' Contributions

Conceptualization, S. M. E., A. S. M., M.S.A., G.S.E., and A. I. E. Methodology, S. M. E., A. S. M. M.S.A.G.S.E., and A. I. E. Software, S. M. E., A. S., M. M.S.A., G.S.E., and A. I. E.; Formal analysis, S. M. E., A. S. M., M.S.A., G.S.E.; and A. I. E.; Investigation, S. M. E., A. S. M. M.S.A.G.S.E., and A. I. E.; Resources, S. M. E., A. S. M. M.S.A.G.S.E., and A. I. E.; Data Curation, S. M. E., A. S. M., M.S.A.G.S.E., and A. I. E.; Writing original draft preparation, S. M. E., A. S. M. M.S.A.,G.S.E., and A. I. E.; Writing Review and Editing, S. M. E., A. S. M. M.S.A.,G.S.E.,Y.A.A., M.K.O and A. I. E.; and M. S. A.; Supervision, S. M. E., A. S. M., M.S.A.G.S.E., H.A.A.; and A. I. E. All authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable.

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### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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