244 *Egypt. J. Chem.* **Vol. 68**, No. 5 pp. 559 - 615 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Effect of Foliar Spraying with Spirulina platensis Extract and the Phyco-synthesized Scenedesmus obliquus-Zn/Fe Nanoparticles on Growth, Leaf Mineral Content and Soil Microbial Activity of Nemaguard Peach Seedlings

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Abstract

A nursery experiment was conducted during the period from 2-7-2022 to 14-9-2023 to study the effect of foliar spray with *Spirulina* extract at concentrations 5 and 10 mL/L or nano-micronutrients (Iron and Zinc) at concentrations 2 and 4 mL/L and their interactions on vegetative growth parameters and nutritional status of Nemaguard peach seedlings, beside the control treatment (spray with distilled water only). Results showed that the binary interaction between *Spirulina* fertilization and nano-microelements, the combination SE1 + (NF2+NZ2) treatment gave the highest significant increase in seedling height and diameter, total chlorophyll leaf content, fresh and dry weight of leaves, potassium, magnesium, and zinc. Meanwhile, the combination SE2 + (NF2+NZ2) treatment significantly increased the number of branches and leaves/seedlings and leaf area, as well as the leaf content of total carbohydrates, nitrogen, phosphorus, and iron. Nano-Fe/Zn and *Spirulina* extract also increased the activity of microbes in the rhizosphere of seedlings compared to the control treatment. it could be concluded that applying *Spirulina* extract and Nano-micronutrients (iron and zinc) on Nemaguard rootstock seedlings is an effective way to improve vegetative growth and leaf mineral content and promote seedling growth, and shorten the time needed to reach the appropriate size to be grafted onto.

keywords: Spirulina ; Nanoelements; Iron; Zn; Nemaguard; vegetative growth; mineral contents.

1. Introduction

Nemguard peaches, Prunus persical X Prunus davidiana Carrere, are favored for almonds and are often used as rootstock for peach cultivars. Compared to other rootstocks in use, it has demonstrated resistance to nematodes known to cause knotting in roots, grows vigorously, is highly disease-resistant, and is more resilient to the gall crown [1-4]. Additionally, under Nubaria circumstances, the Nemaguard rootstock significantly improved nutrient absorption, resulting in the highest level of leaf mineral content and a notable increase in vegetative vigor of the Ne Plus Ultra and Nonpareil almond cultivars [5].

Because iron uses its entry into the porphyrin complexes that make up chlorophyll to aid in the production and activation of the chlorophyll pigment, it is regarded as one of these elements that is most vital. It is also a component of the cytochrome that powers the plant's respiration mechanism. It also contributes to the synthesis of chloroplasts and plant proteins [6]. Furthermore, iron is necessary for the synthesis of numerous enzymes, including cytochrome oxidase, peroxidase, and catalase, which initiate a number of essential plant processes, particularly oxidative reactions, because these reactions involve the transfer of electrons [7]. Zinc is a nutritional element that increases motility in the vegetative group of seedlings. This increases absorption and transmission speed within the plant and prevents zinc from depositing on the surfaces of calcareous soil colloids. Additionally, it is crucial for the synthesis and activation of chlorophyll, the synthesis of proteins and carbohydrates, and the overall efficacy of these processes. In addition to their significant significance in boosting plant resistance to various diseases, enzymes and oxygen play a crucial role in the synthesis of essential plant hormones, including auxins [8].

Since their faster and higher translocation to different areas of plants, nano-fertilizers (NFs) are widely utilized in fruit crop

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Received date 13 August 2024; revised date 20 September 2024; accepted date 25 September 2024
DOI: 10.21608/ejchem.2024.311951.10188

nutrition as soil-based and spray-based applications that give nutrients with high efficiency and little waste [9, 10]. Once NFs have penetrated the cuticle tissue of leaves or roots, they proceed through apoplastic, symplastic, lipophilic, and hydrophilic pathways. These pathways affect the effectiveness and ultimate destination of the nanoparticles and their properties, reactivity, delivery, and translocation within plant tissues. This can lead to different reactions of different plant parts to the same nanoparticle [11, 12].

NFs are significantly smaller than conventional materials and can penetrate leaf tissues directly through stomata. They also have a higher surface area-to-weight ratio, various forms, and higher penetrability, which may have a greater impact on growth and developmental processes. NFs' impact on plants and other plant processes can be influenced by their concentration and timing of consumption [13]. NPs are very soluble in plant tissues because of their small size, and excessive NF concentrations may be detrimental to plant growth and development. They are often used at low concentrations (mg/L) to avoid these side effects. Nutrient utilization efficiency is increased and nutrient doses are decreased when using nano-fertilizers because they control the release of nutrients from fertilizers, increasing the amount of nutrients available to plants. Accordingly, we also reduce the use of a large amount of inorganic fertilizers (50%) in the conventional method to achieve a higher yield and lower damage applied in lower concentrations to reach the nutrients required for the plants. We also save about 10% of the cost of conventional fertilizers [12]. Numerous studies have discovered that applying nano-fertilizers topically to fruit trees improves both root and vegetative growth on fig trees [14], Aggizi olive seedlings [15], Keitte mango trees [16], Pomelo seedlings [17] and olive trees [18] without having a deleterious effect on soil microbial activity.

According to Verkleij [19], one of the organic sources utilized as a complement to fertilizer in agricultural production is *spirulina* extract (SE). According to Spinelli et al. [20], SEs are complex compounds that do not require fertilizer and are used to stimulate plant growth at low concentrations. They are composed of macro and micro elements as well as several groups of plant stimulants, including cytokinins, auxins and auxin-like compounds, gibberellins, abscisic acid, vitamins, amino acids, antibiotics, polysaccharides, micro- and macronutrients, and amino and organic acids. In comparison to using commercial, traditional fertilizers, SE was shown to have a positive effect on crop plant growth and yield by increasing the activities of plant enzymes such as nitrate reductase, phosphatase, and chelate reductase when applied sufficiently [21, 22]. Many studies and research studies have demonstrated the beneficial effects of *spirulina* extract on fundamental chemical content features as well as numerous vegetative development attributes [23, 24, 25]. The purpose of this study was to assess the effects of nano-iron, zinc, and *spirulina* extracts on the vegetative growth and nutrient content of Nemaguard rootstock seedlings in order to obtain strong-growing seedlings for grafting on them. This was due to the significance of nano-produced micronutrients and *spirulina* extracts in new directions of agricultural production.

2. Materials and Methods

2.1. Microalgae and cultivation conditions

Two types of microalgae were used in this study, *i.e.,* the blue-green microalga *Spirulina platensis* and the green microalga *Scenedesmus obliquus.* The dried powder of *S. platensis* was provided by the Biofertilizer Production Unit, National Research Centre, Cairo, Egypt. On the other hand, *S. obliquus* had been previously isolated and characterized as *S. obliquus* NRCIbr1 with accession number KY621475 [26]. *S. obliquus* was cultivated on Bold Basal Medium [27] in a flat-panel photobioreactor under continuous illumination with white, fluorescent lights. The cultivation process was stopped after three weeks of cultivation, and the biomass was harvested by centrifugation for 10 min at 6000 rpm. The cell-free supernatant was used for Zn- and Fe-NPs biosynthesis.

2.2. Extraction of microalga metabolites

S. platensis was extracted aqueously in accordance with Darwesh et al. [28], with a few minor adjustments made as follows: Separately, 5 g of ground-up, dried microalga were combined with 50 mL of distilled water and incubated for 30 minutes at 50 °C in a water bath sonicator. Following a centrifugation process (at 5000 rpm for 10 min), the cells and their solid residues were separated from the aqueous extract. They were then re-placed in 50 ml of distilled water for a subsequent sonication/extraction cycle, which was carried out three times at elevated temperatures (60, 70, and 80 °C, respectively). Following the four extraction cycles, the aqueous fractions were collected, filtered, and the volume was adjusted to 200 ml.

2.3. Phyco-synthesis of zinc and iron nanoparticles (Zn- and Fe-NPs)

Zn and Fe nanoparticles were individually biosynthesised in a reducing environment provided by the aqueous extract of *S. obliquus* (SE). With a few little modifications, Zn-NPs biosynthesis was carried out using the procedure outlined by Abdel-Gawad et al. [29]. In short, a dropwise addition of SE was made to 0.5 M aqueous zinc acetate, with constant stirring until the resulting solution took on a pale white hue. The mixture was stirred, and then 0.01 M NaOH solution was added dropwise to get the mixture's pH to 12. The resulting residue was recovered by centrifugation (4000 rpm for 5 min.), and contaminants were removed with ethanol and distilled water until the pH was neutral. In preparation for more research, the sediments were gathered, vacuum-dried, and pulverized in a mortar. Fe-NPs were biosynthesized using the technique outlined by Darwesh et al. [30]. In summary, 0.1M of $FeCl_3 \cdot 6H_2O$ was gradually added to a 1:1, v/v *S. obliquus* microalgal extract, and the mixture was constantly stirred until the precipitates of Fe-NPs formed and the color of the solution turned black.

Centrifugation (5000 rpm for 5 min.) was used to collect the resultant precipitate, and the NPs precipitates were then twice re-suspended in DW for washing before being washed in 100% ethanol to get rid of contaminants and water residues. For additional research, the precipitate was gathered, vacuum-dried, and crushed in a mortar.

2.4. Transmission electron microscopy characterization of biosynthesized Zn- and Fe-NPs

Using high-resolution transmission electron microscopy (HRTEM) (JEOL 2100 Japan®), the morphological characterization of the phyco-synthesized Zn-NPs and Fe-NPs was carried out to determine the size and form of the produced nanostructures. The samples were made by drop-coating carbon-coated copper grid with nanoparticle solutions, and then loading the grid into a specimen holder once it had dried. The size and shape of the water-suspended nanostructures were noted when they were observed using HRTEM.

2.5. Chemical characterization of ZnO-NPs and Fe-NPs using FTIR

Fourier transform infrared spectroscopy (FTIR) was used in a diffuse reflectance mode to record the potential biomolecule guild for capping, reduction, and effective stabilization of the phyco-synthesized ZnO-NPs and Fe-NPs. In a mortar and pestle, one milligram of each sample was combined with 2.5 milligrams of dry potassium bromide. The resulting powder was loaded onto an FTIR device at 26 ± 1 °C and placed in a micro-cup with an internal diameter of 2 mm. The materials were scanned with an FTIR spectrometer (Agilent System Cary 630 FTIR Model®) in the 400:4000 cm1 infrared ranges. To determine whether functional groups were present in the sample, the resulting spectrum data were compared with the reference chart.

2.6. Agriculture experiment design

In the years 2022–2023, two-year-old Nemaguard peach seedlings were cultivated in 5-kg plastic culture pots at the National Research Centre in Egypt. The study aimed to determine the impact of foliar spraying with Spirulina extract, nano-iron and nano-zinc on the growth and nutritional status of the Nemaguard seedlings. Three levels of micronutrients (Fe and Zn) produced by nanotechnology (0, 2, and 4 mL/L) and three levels of *Spirulina* extract (0, 5, and 10 mL/L) were sprayed on the seedlings; these levels received the codes SE0, SE1, and SE2, respectively. Nine treatments were used in the trial, and a randomized complete block design (RCBD) with five replications was used. The physical and chemical examination of the soil is displayed in Table 1. In each growing season, the seedlings were sprayed three times, with a gap of two weeks between applications. The dates of the first spraying were July 2, the second was July 16, and the third was July 30. After two months following the final spraying of both seasons, the experimental parameters were recorded.

2.7. Soil's total microbial enzyme activities

Using the methodology described by Patle et al. [31] with slight modifications, the total microbial enzyme activities of soils were determined by measuring the rate of hydrolysis of the colorless fluorescein diacetate (FDA) into fluorescein (fluorescent yellow-green). In brief, 50-mL sealed centrifuge tubes were filled with two grams of rhizosphere soil samples (three times each). The reaction was initiated and horizontally incubated for 20 minutes at 30 °C after adding 15 mL of potassium phosphate buffer (60 mM, pH 7.6) and 0.2 mL of 0.1% FDA (in acetone). The reaction was halted after color development and incubation by adding 15 mL of chloroform/methanol (2:1) and vortex for one minute. The tubes were centrifuged for 10 minutes at 5000 rpm in order to settle the turbidity and soil and separate the color-containing methanol-water layer from the chloroform layer. The colored fluorescein generated in the chloroform layer was measured using spectrophotometry at 490 nm and compared to fluorescein standards. FDA hydrolysis levels, expressed as µg of released fluorescein g-1 soil, were one way to express the total quantity of soil microbial activity.

Table 1: Some of the soil's physical and chemical characteristics before treatment

Character	Value	Character	Value
Sand (g/100g)	66.8	Na^{+} (ppm)	16
Silt (g/100g)	14	N (%)	0.15
Clay (g/100g)	19.2	P (%)	0.17
Texture	Sandy loam	K (%)	0.17
Organic Matter (%)	1.02	Mg (%)	0.51
EC dSm^{-1}	0.24	Fe (ppm)	25
pH	7.81	Mn (ppm)	10
HCO_3^- (meq/100g soil)	1.24	Cu (ppm)	0.5
Cl^- (ppm)	53.19	Zn (ppm)	8.89

2.7.1. Seedling height (cm): A tape measure was used to record the average height of five seedlings per treatment, extending from the base to the main tip.
2.7.2. **Seedling diameter (mm):** The Vernier calipe method was used to measure the average diameter of five seedlings for each treatment at a height of 10 cm above the ground.
2.7.3. **Number of branches/seedlings:** The average number of branches of five seedlings for each treatment was counted.
2.7.4. **Number of leaves/seedlings:** The average number of leaves per five seedlings per treatment was calculated.
2.7.5. **Leaf area (cm^2):** Using a Cl-202 portable laser leaf area meter, the average area of twenty-five leaves per treatment was measured.
2.7.6. **Total chlorophylls (SPAD unit):** The average total chlorophyll content of twenty-five leaves for each treatment was determined using the chlorophyll measuring device model SPAD-502.
2.7.7. **Fresh and dry weight of leaves (g):** In each treatment, an average of twenty-five leaves was weighed to determine their fresh weight. After being cleaned with distilled water and dried in an oven at 70 °C until a constant weight was achieved, the leaves were again weighed to determine their dry weight.
2.7.8. Determination of leaf carbohydrate and mineral contents:
Total carbohydrate content of leaves (mg/g dry weight) was determined [32]. The leaf samples were crushed in a porcelain mortar to prevent contamination with any minerals. 0.5 g of the ground dried material of each sample was digested with H_2O_2 and H_2SO_4. Then, appropriate aliquots were obtained to determine the minerals. Nitrogen (N %) was determined using Micro-Kjeldahl [33]. According to Chapman and Pratt [34], the Phosphorus (P %) was determined calorimetrically. While, the Potassium (K %) measured using flame photometer [35]. Also, using atomic absorption, the Iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) measured according to Jackson [36]. The concentrations of Fe, Mn, Cu and Zn were expressed as ppm on a dry weight basis.

3. Statistical analysis

The data from both seasons were statistically analyzed using the analysis of variance (ANOVA) method [37]. The experimental design used was a random block design with five replicates. Using the least significant differences test (LSD) at a 5% probability level, a means comparison of the treatments was carried out.

4. Results

4.1. Biosynthesis of Fe/Zn-nanoparticles as foliar fertilizers

Two microelements (Zn and Fe) were phyco-synthesized in nanoform using the extract of *S. obliquus* microalga. The size, shape and particle morphology of FeNPs and ZnNPs were examined using HRTEM. The results illustrated in **Fig. 1** showed that both micro-elements's nano forms were spherical in shape. The size of FeNPs and ZnNPs was less than 10 nm.

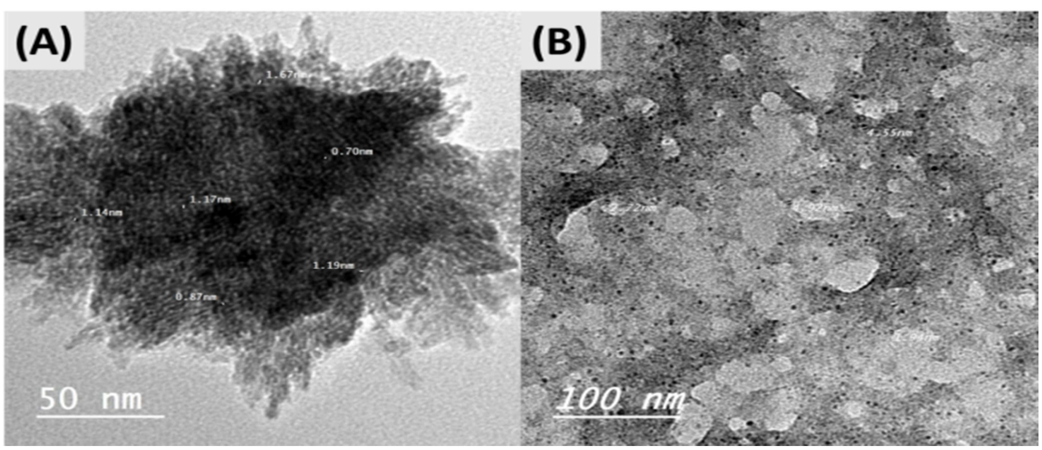

Figure 1: Characterizations of phyco-biosynthesized Fe-NPs (A) and Zn-NPs (B) using TEM microscopy

For chemical composition and functional groups with a biological nature, they were determined by the FTIR technique. The chemical compositions of the phyco-synthesized iron nanoparticles characterized by FTIR spectroscopy at room temperature exhibited well-defined peaks at 580 and 3370 cm^{-1} for Fe-O and Fe-OH, respectively (**Fig. 2A**). The presence of iron oxide (FeO) in the produced nanoparticles is confirmed by the development of a well-defined peak at 580 cm^{-1}. The presence of a nitrate group (caused by the nitrate reductase enzyme) is the reason for the development of a tiny peak at 1000. Furthermore, the O-H stretching modes and surface hydroxyl bending vibration are responsible for the peak located at 1600 cm^{-1}. Zinc oxide (ZnO) stretching vibrations are the vibration mode associated with the absorption peak located at 575.9 cm^{-1}. Both the primary alcohol's C-O bond and the primary amine's C-N bond are thought to be responsible for the peak at 1005.3 cm^{-1}. Both alkyls and aromatic nitro compounds are thought to be responsible for the peak at 1550 cm^{-1}. Hydroxyl compounds' stretching vibration was identified as the cause of the peaks at 3005 cm^{-1} (Fig. 2B).

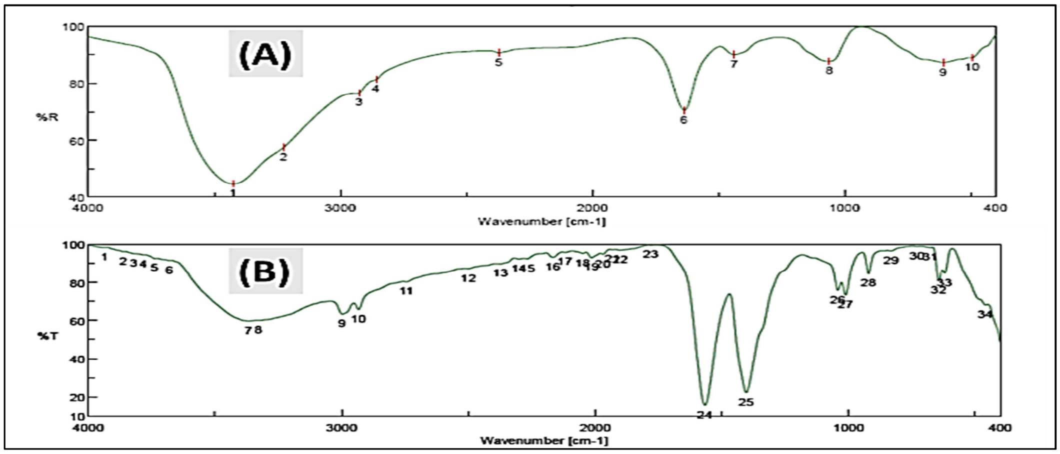

Figure 2: Characterizations of phyco-biosynthesized Fe-NPs (A) and Zn-NPs (B) using FTIR

4.2. Seedling height (cm)

Table 2 presents statistically significant variations between the spraying treatments of *Spirulina*; SE1 performed better and gave the best mean (120.11 and 123.67 cm); in contrast to the SE2 treatment, which provided the lowest mean (81.00 and 88.67 cm) during the first and second seasons, respectively, there was a significant difference in the superior treatment's rate, amounting to 45.25 and 39.47%). The findings demonstrated that, while using the nano-micronutrient (Fe and Zn) fertilizers, there were significant differences in the treatment means. In the first and second seasons, respectively, the addition of NF2+NZ2 fertilizer greatly outperformed (126.56 and 126.83 cm) by 86.97 and 48.04% relative to the comparator treatment. Regarding the binary interaction, there was a notable impact of micro-nano-fertilizers and *spirulina* fertilizers. The SE0 + (NF0 + NZ0) treatment recorded the lowest values of seedling height (51.33 and 69.50 cm) during the first and second seasons, respectively. While the SE1 + (NF2 + NZ2) treatment recorded higher values of seedling height (179.67 and 169 cm) during the first and second seasons, respectively

4.3. Diameter of seedling (mm)

The spraying of *Spirulina* treatments varies significantly, as Table 3 demonstrates. Among the control groups, SE0 produced the lowest mean (0.56 and 0.47 mm) and a non-significant difference from the SE2 addition level (0.59 and 0.52 mm) in the first and second seasons, respectively. In contrast, SE1 outperformed and gave the best mean (0.73 and 0.67 mm) in the 2022 and 2023 seasons, with a significant difference from the SE2 treatment. Additionally, the results demonstrated that, while using the nano-micronutrient (Fe and Zn) fertilizers during the two research seasons, there were significant differences in the means of the treatments. With a non-significant difference from NF1+NZ1, the fertilizer NF2+NZ2 considerably outperformed (0.68 and 0.62 mm), increasing by 21.43 and 37.78% in comparison to the NF0+NZ0 treatment, which recorded the lowest values (0.56 and 0.45 mm) during the first and second seasons, respectively. There was a notable impact from the binary interaction between micro-nano-fertilizers and *Spirulina* fertilizers. The control group of SE0 + (NF0+NZ0) recorded the lowest values of seedling diameter (0.47 and 0.40 mm), while the SE1 + (NF2+NZ2) treatment the greatest values of seeding diameter (0.83 and 0.80 mm), during the first and second seasons, respectively,

Table 2: Effect of foliar application with *Spirulina* and nano-fertilizes on height of Nemaguard seedlings

	1st season				**2nd season**			
Treatment	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**
SE_0	51.33g	95.67c	96.00c	**81.00C**	69.50g	98.00d	98.50d	**88.67C**
SE1	76.33e	104.33b	179.67a	**120.11A**	90.00f	112.00c	169.00a	**123.67A**
SE2	81.00d	73.33f	104.00b	**86.11B**	97.50d	95.00e	113.00b	**101.83B**
Mean	**69.55C**	**91.11B**	**126.56A**		**85.67C**	**101.67B**	**126.83A**	

a,b,c,d and e means at the same column with different superscript are significantly (P<0.05) different.

Table 3: Effect of foliar application with *Spirulina* and nano-fertilizes on diameter of Nemaguard seedlings

	1st season				2nd season			
Treatment	**NF0+NZ0**	**NF1+NZ1**	**NF2+NZ2**	**Mean**	**NF0+NZ0**	**NF1+NZ1**	**NF2+NZ2**	**Mean**
SE0	**0.47d**	**0.60c**	**0.60c**	**0.56B**	**0.40d**	**0.50c**	**0.50c**	**0.47B**
SE1	**0.60c**	**0.77b**	**0.83a**	**0.73A**	**0.45c**	**0.75ab**	**0.80a**	**0.67A**
SE2	**0.60c**	**0.57cd**	**0.60c**	**0.59B**	**0.50c**	**0.50c**	**0.55b**	**0.52B**
Mean	**0.56AB**	**0.65A**	**0.68A**		**0.45AB**	**0.58A**	**0.62A**	

a,b,c,d and e means at the same column with different superscript are significantly (P<0.05) different.

4.4. Number of branches/seedlings

Table 4 shows significant differences between the *Spirulina* fertilizer treatments. In comparison to the SE0 treatment, which had the lowest mean (7.67 and 7.39 branches and seedlings), respectively, the SE2 treatment resulted in a higher increase in branches and seedlings as a mean (13.70 and 14.17) during the first and second seasons by about 78.62 and 91.75% than the control treatment. There were significant differences between the means of the treatments when adding the nano-micronutrient fertilizers (Fe and Zn). During the first and second seasons, the NF2+NZ2 fertilizer addition significantly outperformed the NF1+NZ1 treatment (10.22 and 9.5 branches/seedling, respectively), with an increase of 53.62 and 29.93% compared with the control group during the first and second seasons, respectively. (11.66 and 11.33 branches/seedlings, respectively). The SE2 + (NF2+NZ2) treatment outperformed and gave the highest values for this trait (16.33 and 16.50 branches/seedlings during the first and second seasons, respectively) in the binary interaction between fertilizing *Spirulina* and nano-Fe and Zn fertilizer. In contrast, the control group SE0 + (NF0 + NZ0) recorded the lowest values (3 and 4.67 branches/seedlings during the first and second seasons, respectively).

4.5. Number of leaves/seedlings

The averages of spraying with different Spirulina treatments are very different, as Table 5 illustrates. The highest value for this trait in transaction SE2 was 121.45 and 110.83 leaves/seedlings during the first and second seasons, respectively, as the number of leaves/seedlings and the level of addition increased. In contrast, the control group SE0 recorded the lowest average for this trait, which was 60.11 and 61 leaves/seedlings, respectively, during the first and second seasons. When nanotechnology-derived micronutrient fertilizer (Fe and Zn) was sprayed, the number of leaves significantly increased. In comparison to the addition of the NF1+NZ1 treatment, which gave an average of (88.89 and 93.39 leaves/seedling during the first and second seasons, respectively), the seedlings fertilized with NF2 + NZ2 had the highest average number of leaves, with (119.11 and 112.22 leaves/seedling during the first and second seasons, respectively).

The control group recorded the lowest average number of leaves/seedlings, with (57.55 and 46.5 leaves/seedlings) during the first and second seasons, respectively. Concerning this attribute, the combined interaction between *spirulina* and nano fertilizer spraying treatments had a significant impact. Comparing the results of the first and second seasons, the SE2 + (NF2 + NZ2) treatment had the highest values for this trait (179.67 and 169.00 leaves/seedling, respectively) than the control group SE0 + (NF0 + NZ0), which had the lowest values (37.33 and 34.00 leaves/seedling, respectively).

Table 4: Effect of foliar application with *Spirulina* and nano-fertilizes on No. branches of Nemaguard seedlings

	1st season				2nd season			
Treatment	**NF0+NZ0**	**NF1+NZ1**	**NF2+NZ2**	**Mean**	**NF0+NZ0**	**NF1+NZ1**	**NF2+NZ2**	**Mean**
SE0	**3.00i**	**9.67de**	**10.33d**	**7.67B**	**4.67g**	**7.00f**	**10.50cd**	**7.39C**
SE1	**8.67d-f**	**7.33 d-h**	**8.33 d-g**	**8.11B**	**10de**	**7.00f**	**7.00f**	**8.00B**
SE2	**11.09c**	**13.67b**	**16.33a**	**13.70A**	**11.50c**	**14.50b**	**16.50a**	**14.17A**
Mean	**7.59C**	**10.22B**	**11.66A**		**8.72C**	**9.50B**	**11.33A**	

a,b,c,d and e means at the same column with different superscript are significantly (P<0.05) different.

Table 5: Effect of foliar application with *Spirulina* and nano-fertilizes on No. leaves of Nemaguard seedlings

SE_0	37.33i	66.67g	76.33e	**60.11C**	34.00i	72.00f	77.00e	**61.00C**
SE1	66.33h	84.33d	101.33c	**84.00B**	53.50g	96.67c	90.67d	**80.28B**
SE2	69.00f	115.67b	179.67a	**121.45A**	52.00h	111.50b	169.00a	**110.83A**
Mean	**57.55C**	**88.89B**	**119.11A**		**46.50C**	**93.39B**	**112.22A**	

a,b,c,d and e means at the same column with different superscript are significantly (P<0.05) different.

4.6. Leaf area (cm2)

Table 6 demonstrates that there were slight variations in the Spirulina treatment averages throughout the study's two seasons. In comparison to the control group SE0, which recorded the lowest average (2.32 and 2.34 cm2), respectively, the treatment SE2 had a higher leaf area of Nemaguard seedlings (2.59 and 2.63 cm2, respectively). The treatments with nanotechnology-made micronutrient fertilizers had no significant effect. Additionally, the NF2+NZ2 treatment had the greatest mean leaf area (2.87 and 2.73 cm2) in each of the two seasons, while the NF0+NZ0 control group had the lowest mean leaf area (2.17 and 2.15 cm2) in both two seasons, respectively. Concerning the combined effect between *Spirulina* and micro-nutrient nano fertilizer treatments, it was found that the treatment SE2 + (NF2+NZ2) significantly outperformed it, yielding the highest leaf area values of 2.97 and 2.92 cm2 in 2022 and 2023, respectively. In contrast, the control group SE0 + (NF0 + NZ0) recorded the lowest leaf area values (1.91 and 1.96 cm2) in the two study seasons.

Table 6: Effect of foliar application with *Spirulina* and nano-fertilizes on leaf area of Nemaguard seedlings

	1st season				**2nd season**			
Treatment	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**
SE_0	1.91f	2.32cd	2.72b	**2.32B**	1.96fg-i	2.43f	2.62a-c	**2.34B**
SE1	2.28c-e	2.46c	2.91ab	**2.55A**	2.09f-h	2.58a-e	2.64ab	**2.44B**
SE2	2.33cd	2.47c	2.97a	**2.59A**	2.39fg	2.59a-d	2.92a	**2.63A**
Mean	**2.17B**	**2.42B**	**2.87A**		**2.15B**	**2.53AB**	**2.73A**	

a,b,c,d and e means at the same column with different superscript are significantly (P<0.05) different.

4.7. Total leaf chlorophyll (SPAD unit)

There are notable variations in the total leaf chlorophyll of Nemaguard seedlings during both seasons and the mean of the *Spirulina* treatment, as shown in Table 7. Compared with the control group SE0, which had the lowest mean of 22.00 and 20.70 during both seasons, respectively, treatment SE1 had the best value for the total leaf chlorophyll, which was 28.51 and 28.62 throughout both seasons.

Furthermore, the results indicated that the addition of nano-fertilizers significantly increased this characteristic; the treatment NF2+NZ2 recorded the highest mean of (26.66 and 28.12 during both seasons, respectively) and differed significantly from treatment NF1+NZ1, which recorded (25.70 and 26.65 during both seasons, respectively); the lowest mean was recorded for this trait (20.95 and 19.07) during both seasons, respectively, compared to the control group NF0+NZ0. The total leaf chlorophyll content was significantly impacted by the combined effect of spraying with *Spirulina* and micronutrients (Fe and Zn) nano-fertilizers. The treatment SE1 + (NF2+NZ2) performed best, with the highest values of 32.47 and 34 during both seasons, respectively. The lowest value was observed for the control group SE0 + (NF0+NZ0) at 16.43 and 16.05 in two seasons.

Table 7: Effect of foliar application with *Spirulina* and nano-fertilizes on total leaf chlorophyll of Nemaguard seedlings

	1st season				**2nd season**			
Treatment	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**
SE_0	16.43f	24.67bc	24.90b	**22.00BC**	16.05h	22.80c-e	23.25cd	**20.70C**
SE1	22b-e	31.07ab	32.47a	**28.51A**	18.90g	32.95ab	34.00a	**28.62A**
SE2	24.43bc	21.37de	22.60b-d	**22.80B**	22.25c-f	24.20c	27.10b	**24.52B**
Mean	**20.95C**	**25.70B**	**26.66A**		**19.07C**	**26.65B**	**28.12A**	

a,b,c,d and e means at the same column with different superscript are significantly (P<0.05) different.

4.8. Leaves fresh weight (g)
Table 9 shows statistically significant variations in the mean values of fertilizer treatments while using *Spirulina* for spraying. In both seasons, treatment SE1 caused a higher leaf dry weight (3.22 and 2.77 g, respectively) compared to the control group SE0, which recorded the lowest average leaf fresh weight (2.29 and 2.49 g, respectively). On the other hand, the leaf dry weight had no significant difference in treatment SE1 compared to treatment SE2 (3.10 and 2.76 g, respectively). The weight of the leaf was significantly impacted by the spraying of nanotechnology-manufactured micronutrient fertilizer; in both seasons, the treatment NF2+NZ2 had the highest average dry weight of the leaf (3.58 and 2.88 g, respectively), in comparison to the control group, NF0+NZ0 (2.18 and 2.53 g, respectively). Dry leaf weight was significantly affected by the combination of nano-fertilizers and *Spirulina* spraying in both research seasons. The combination treatment of SE1 + (NF2+NZ2) and SE2 + (NF2+NZ2) had the greatest value of the dry weight of leaves (3.91 g) but did not differ significantly in the first season. In the second season, the combination treatment of SE1 + (NF2 + NZ2) had the greatest value of the dry weight of leaves (3.44 g). In contrast, during the first and second seasons, the control group SE0 + (NF0 + NZ0) had the lowest values for the dry weight of leaves (1.49 and 2.30 g, respectively).

Table 8: Effect of foliar application with Spirulina and nano-fertilizes on leaves fresh weight of Nemaguard seedlings

	1st season				2nd season			
Treatment	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**
SE_0	3.05g	4.23f	5.03c	**4.10C**	4.12g	4.21fg	4.37de	**4.23BC**
SE1	4.43e	6.14b	7.27a	**5.95A**	4.22fg	5.24b	5.71a	**5.06A**
SE2	4.62d	4.33ef	6.13b	**5.03B**	4.67c	4.24def	4.41d	**4.44B**
Mean	**4.03C**	**4.90B**	**6.14A**		**4.34B**	**4.56AB**	**4.83A**	

a,b,c,d and e means at the same column with different superscript are significantly (P<0.05) different.

4.9. Leaves dry weight (g)
Table 9 shows statistically significant variations in the mean values of fertilizer treatments while using Spirulina for spraying. In both seasons, treatment SE1 caused a higher leaf dry weight (3.22 and 2.77 g, respectively) compared to the control group SE0, which recorded the lowest average leaf fresh weight (2.29 and 2.49 g, respectively). On the other hand, the leaf dry weight had no significant difference in treatment SE1 compared to treatment SE2 (3.10 and 2.76 g, respectively). The weight of the leaf was significantly impacted by the spraying of nanotechnology-manufactured micronutrient fertilizer; in both seasons, the treatment NF2+NZ2 had the highest average dry weight of the leaf (3.58 and 2.88 g, respectively), in comparison to the control group, NF0+NZ0 (2.18 and 2.53 g, respectively). Dry leaf weight was significantly affected by the combination of nano-fertilizers and Spirulina spraying in both research seasons. The combination treatment of SE1 + (NF2+NZ2) and SE2 + (NF2+NZ2) had the greatest value of the dry weight of leaves (3.91 g) but did not differ significantly in the first season. In the second season, the combination treatment of SE1 + (NF2 + NZ2) had the greatest value of the dry weight of leaves (3.44 g). In contrast, during the first and second seasons, the control group SE0 + (NF0 + NZ0) had the lowest values for the dry weight of leaves (1.49 and 2.30 g, respectively).

Table 9: Effect of foliar application with Spirulina and nano-fertilizes on leaves dry weight of Nemaguard seedlings

	1st season				2nd season			
Treatment	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**
SE_0	1.49h	2.44f	2.95c	**2.29B**	2.30e	2.46d	2.72c	**2.49B**
SE1	2.25fg	3.50b	3.91a	**3.22A**	2.58cd	2.27c	3.44a	**2.77A**
SE2	2.80d	2.59e	3.90a	**3.10A**	2.70c	3.09b	2.48d	**2.76A**
Mean	**2.18C**	**2.84B**	**3.58A**		**2.53C**	**2.61B**	**2.88A**	

a,b,c,d and e means at the same column with different superscript are significantly (P<0.05) different.

4.10. Total carbohydrate content of leaves (mg/ g dry weight)
Table 10 shows significant differences between the averages of the *Spirulina* treatments compared to the control group. Treatment SE2 increased the leaf's total carbohydrate content by 3.56 and 3.39%, respectively, in both seasons, which was significantly higher than the leaf's total carbohydrate content of the SE1 treatment, which recorded an average of 3.49 and 3.23%, respectively. Moreover, the lowest content of the leaf's total carbohydrate was recorded for the control group in both

seasons by 3.22 and 2.88%, respectively. Additionally, the results demonstrated that spraying iron and zinc nano-fertilizers significantly increased the content of total carbohydrate in the leaves. In comparison to the control group NF0+NZ0, which obtained the lowest mean for this attribute (3.15 and 2.84%) during both seasons, the treatment NF2+NZ2 recorded the greatest mean of 3.57 and 3.41% during the two study seasons, respectively.

Throughout the 2022 and 2023 seasons, there was a notable impact on the overall carbohydrate content of leaves because of the combination of spraying *Spirulina* and nano-fertilizers. Treatments of SE2 + (NF1+NZ1) and SE2 + (NF2+NZ2) had the highest significant values for the carbohydrate content in the leaves of Nemaguard seedlings (3.67 and 3.65%, respectively), therefore there was no discernible difference between them throughout the first season. In the second season, SE2++ (NF2+NZ2) had the greatest content of the carbohydrate by 3.78%. During the first and second seasons, respectively, the control group SE0 + (NF0 + NZ0) had the lowest content leaf total carbohydrate by 2.82 and 2.45%, respectively.

Table 10: Effect of foliar application with Spirulina and nano-fertilizes on leaves total carbohydrate content of Nemaguard seedlings

	1st season				2nd season			
Treatment	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**
SE_0	2.82f	3.40cd	3.44c	**3.22C**	2.45f	3.07de	3.12d	**2.88BC**
SE1	3.28e	3.59b	3.60b	**3.49B**	3.03e	3.32cd	3.34c	**3.23B**
SE2	3.35de	3.65a	3.67a	**3.56A**	3.05de	3.35b	3.78a	**3.39A**
Mean	**3.15B**	**3.55A**	**3.57A**		**2.84C**	**3.25B**	**3.41A**	

a,b,c,d and e means at the same column with different superscript are significantly (P<0.05) different.

4.11. Mineral content of leaves

4.11.1. Macro elements

4.11.1.1. Nitrogen (%)

The findings presented in Table 11 demonstrate that spraying micronutrient fertilizers made with nanotechnology and *Spirulina* during both seasons had a substantial impact on the nitrogen content of Nemaguard seedlings. When it came to the impact of spraying *Spirulina*, it was observed that the SE2 treatment outperformed the SE1 treatment in terms of the nitrogen content of the leaves (0.99 and 1.17% during the 2022 and 2023 seasons, respectively). The SE0 control group recorded the lowest nitrogen content (0.72 and 0.75% during the 2022 and 2023 seasons, respectively). Regarding the impact of using the iron and zinc nano-fertilizers via spraying, it is evident that the percentage of nitrogen in the leaves increased significantly. When comparing the leaf nitrogen content of the two seasons, the treatment NF2+NZ2 recorded the greatest mean of 1.02 and 1.19%, respectively, while the control group NF0+NZ0 attained the lowest mean (0.64 and 0.66% across both seasons, respectively).

Concerning interaction, during the 2022 and 2023 seasons, there was a notable difference in the nitrogen content of leaves sprayed with a combination of *Spirulina* and nanofurans. The SE2 + (NF1+NZ1) therapy came in second to last, achieving (1.04 and 1.29%) in the 2022 and 2023 seasons, respectively, while the treatment outperformed SE2 + (NF2+NZ2) and gave the greatest average (1.22 and 1.48%) during the times. In contrast, during the seasons of 2022 and 2023, the lowest average of 0.54 and 0.56%, respectively, was seen for the control group SE0 + (NF0 + NZ0).

4.11.1.2. Phosphorus (%)

The phosphorus content of the leaves was slightly affected by treatment with either individual spraying with *Spirulina*, Nano-iron and Nano-zinc or by overlapping treatment between them during the two seasons.

4.11.1.3. Potassium (%)

Table (13), which displays the effects of both single and combination treatments throughout the research seasons, demonstrates a considerable change in the potassium content of seedling leaves. Treatment SE1 fared better than treatment SE2, which recorded an average of 2.26 and 2.16%, respectively, for the potassium content of the leaf during the two seasons. Treatment SE0 recorded the lowest means (2.13 and 1.98%) for both seasons. These significant differences between the averages of the *Spirulina* treatments were observed. The treatment with SE1 + (NF2+NZ2) recorded the highest value of leaf potassium content in the interaction between spraying *Spirulina* and nano-fertilizers, followed by SE1 + (NF1+NZ1), with no discernible differences between them over both seasons. In the first and second seasons, the control group SE0 + (NF0 + NZ0) had the lowest values of leaf potassium content, 1.76 and 1.60%, respectively.

Table 11: Effect of foliar application with *Spirulina* and Nano-fertilizes on leaves nitrogen content of Nemaguard seedlings

	1st season				2nd season			
Treatment	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**
SE_0	0.54i	0.79f	0.83e	**0.72C**	0.56i	0.83f	0.86e	**0.75C**
SE1	0.65h	0.97d	1.01c	**0.88B**	0.68h	1.06d	1.22c	**0.99B**
SE2	0.72g	1.04b	1.22a	**0.99A**	0.74g	1.29b	1.48a	**1.17A**
Mean	**0.64C**	**0.93B**	**1.02A**		**0.66C**	**1.06B**	**1.19A**	

a,b,c,d and e means at the same column with different superscript are significantly ($P<0.05$) different.

4.11.1.4. Phosphorus (%)

The phosphorus content of the leaves was slightly affected by treatment with either individual spraying with *Spirulina*, Nano-iron and Nano-zinc or by overlapping treatment between them during the two seasons.

4.11.1.5. Potassium (%)

Table (13), which displays the effects of both single and combination treatments throughout the research seasons, demonstrates a considerable change in the potassium content of seedling leaves. Treatment SE1 fared better than treatment SE2, which recorded an average of 2.26 and 2.16%, respectively, for the potassium content of the leaf during the two seasons. Treatment SE0 recorded the lowest mean (2.13 and 1.98%) for both seasons. These significant differences between the averages of the *Spirulina* treatments were observed. The treatment with SE1 + (NF2+NZ2) recorded the highest value of leaf potassium content in the interaction between spraying *Spirulina* and nano-fertilizers, followed by SE1 + (NF1+NZ1), with no discernible differences between them over both seasons.

Table 12: Effect of foliar application with *Spirulina* and nano-fertilizes on leaves phosphorus content of Nemaguard seedlings

	1st season				2nd season			
Treatment	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**
SE_0	0.427g	0.450d-f	0.473bc	**0.45AB**	0.380ef	0.423bcd	0.443bc	**0.42AB**
SE1	0.437e-g	0.480a-c	0.487ab	**0.47AB**	0.407de	0.453b	0.457ab	**0.44AB**
SE2	0.453cd	0.487ab	0.497a	**0.48A**	0.423bcd	0.457ab	0.473a	**0.45A**
Mean	**0.44B**	**0.47AB**	**0.49A**		**0.40B**	**0.44AB**	**0.46A**	

a,b,c,d and e means at the same column with different superscript are significantly ($P<0.05$) different.

Table 13: Effect of foliar application with *Spirulina* and nano-fertilizes on leaves potassium content of Nemaguard seedlings

	1st season				2nd season			
Treatment	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**
SE_0	1.76g	2.15e	2.44d	**2.13C**	1.60efg	2.01de	2.33c	**1.98C**
SE1	1.93f	2.95a	2.98a	**2.62A**	1.98d-f	2.85a	2.89a	**2.57A**
SE2	1.94f	2.65c	2.19E	**2.26B**	1.86ef	2.47b	2.14cd	**2.16B**
Mean	**1.88C**	**2.58A**	**2.54B**		**1.81B**	**2.44A**	**2.45A**	

a,b,c,d and e means at the same column with different superscript are significantly ($P<0.05$) different.

In the first and second seasons, the control group SE0 + (NF0 + NZ0) had the lowest values of leaf potassium content, 1.76 and 1.60%, respectively.

4.11.2. Micro elements

4.11.2.1. Iron (ppm)

The results presented made it abundantly evident that there were significant differences between the *Spirulina* fertilizer treatments (Table 14) that were sprayed and the iron content of Nemaguard seedlings. Specifically, SE2 performed better than SE1 treatment, which gave the lowest mean (69.69 and 69.74 ppm) during the first and second seasons, respectively, and was significantly different from SE1 treatment (76.32 and 76.90 ppm). Additionally, the superior treatment's rate increased to 12.71 and 13.35% during the first and second seasons, respectively, compared to the control group SE0, which produced the lowest mean (69.69 and 69.74 ppm) during the first and second seasons, respectively.

Also, the results showed significant differences between the means of the treatments when adding nano Fe and nano Zn as fertilizers. Throughout both seasons, the addition of the NF2+NZ2 fertilizer significantly outperformed (78.65 and 79.14 ppm, respectively), with a significant difference from the NF1+NZ1 treatment (77.99 and 78.25 ppm during the first and the second seasons, respectively), with an increase of (15.77 and 15.87%) compared with the control group.

Regarding the combination of using Nano-Fe and Nano-Zn fertilizer and fertilizing *Spirulina*, the SE2 + (NF2+NZ2) treatment had the highest iron content values in the leaves (83.85 and 84.30 ppm), while SE2 + (NF1+NZ1) (82.88 and 83.43 ppm) was the next best treatment during the first and second seasons, respectively. The control group SE0 + (NF0 + NZ0) had the lowest iron content values in their leaves, measuring 65.94 and 66.05 ppm in both seasons, respectively.

Table 14: Effect of foliar application with *Spirulina* and Nano-fertilizes on leaves iron content of Nemaguard seedlings

	1st season				2nd season			
Treatment	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**
SE_0	65.94f	71.23d	71.90d	**69.69C**	66.05g	70.78e	72.39d	**69.74C**
SE1	68.90e	79.86c	80.19c	**76.32B**	69.41f	80.55c	80.74c	**76.90B**
SE2	68.93e	82.88b	83.85a	**78.55A**	69.43f	83.43b	84.30a	**79.05A**
Mean	**67.92C**	**77.99B**	**78.65A**		**68.30C**	**78.25B**	**79.14A**	

a,b,c,d and e means at the same column with different superscript are significantly (P<0.05) different.

4.11.2.2. Manganese (ppm), Cupper (ppm) and Zinc (ppm)

As shown in Tables (15, 16 and 17), the content of manganese, copper, and zinc in the leaves of Nemaguard seedlings was significantly affected by treatments with both *Spirulina* and Nano- (iron and zinc), singly or mixed, during the two seasons of the study. The results showed significant differences between the averages of treatments with *Spirulina*, the SE_1 treatment was superior in the content of Mn, Cu and Zn in the leaves of Nemaguard seedlings (67.03, 16.95, and 34.52 ppm), respectively during the first season, and (65.08, 16.22, and 33.39 ppm), respectively during the second season compared to the control treatment SE_0, which showed the lowest average leaf content of Mn, Cu and Zn (57.12, 14.25, and 29.76 ppm), respectively during the first season, and (55.58, 13.84, and 28.18 ppm), respectively during the second season.

As for spraying with iron and zinc manufactured using nanotechnology, it was noted that the treatment with NF_2+NZ_2 was superior in achieving the highest average leaf content of these elements (67.60, 16.77, and 35.02 ppm), respectively during the first season and (65.68, 16.09, and 33.94 ppm), respectively during the second season. While the comparison NF_0+NZ_0 recorded the lowest average leaf content of manganese, copper, and zinc (50.99, 12.80 and 26.11 ppm), respectively during the first season, and (49.31, 12.02, and 24.60 ppm), respectively during the second season. Regarding the interaction between spraying with *Spirulina* and Nano fertilizers, the SE_1+ (NF_2+NZ_2) treatment recorded the highest rate of these elements in the leaves of Nemaguard seedlings (75.23, 19.86, and 39.00 ppm) respectively during the first season, and (73.25, 18.49, and 38.72 ppm), respectively during the second season. At the same time, the control group SE_0+ (NF_0+NZ_0) recorded its lowest rate (48.12, 12.43, and 25.99 ppm), respectively during the first season, and (46.65, 11.74, and 24.12 ppm), respectively during the second season.

Table 15: Effect of foliar application with *Spirulina* and nano-fertilizes on leaves manganese content of Nemaguard seedlings

	1st season				2nd season			
Treatment	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**
SE_0	48.12h	57.97f	65.23c	**57.12C**	46.65i	56.38f	63.72c	**55.58C**
SE1	52.19g	73.67b	75.23a	**67.03A**	50.24h	71.76b	73.25a	**65.08A**
SE2	52.67g	63.92d	62.34e	**59.64B**	51.03g	61.64d	60.08e	**57.58B**
Mean	**50.99C**	**65.19B**	**67.60A**		**49.31C**	**63.26B**	**65.68A**	

a,b,c,d and e means at the same column with different superscript are significantly (P<0.05) different.

Table 16: Effect of foliar application with *Spirulina* and nano-fertilizes on leaves cupper content of Nemaguard seedlings

	1st season				2nd season			
Treatment	**NF0+NZ0**	**NF1+NZ1**	**NF2+NZ2**	**Mean**	**NF0+NZ0**	**NF1+NZ1**	**NF2+NZ2**	**Mean**
SE0	12.43h	15.00e	15.33d	**14.25C**	11.74g	14.56e	15.21d	**13.84C**
SE1	12.76g	18.23b	19.86a	**16.95A**	12.10f	18.08b	18.49a	**16.22A**
SE2	13.22f	16.79c	15.12de	**15.04B**	12.21f	15.62c	14.58e	**14.14B**
Mean	**12.80C**	**16.67B**	**16.77A**		**12.02B**	**16.09A**	**16.09A**	

a,b,c,d and e means at the same column with different superscript are significantly (P<0.05) different.

Table 17: Effect of foliar application with *Spirulina* and nano-fertilizes on leaves zinc content of Nemaguard seedlings

	1st season				2nd season			
Treatment	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**	**NF_0+NZ_0**	**NF_1+NZ_1**	**NF_2+NZ_2**	**Mean**
SE_0	25.99e	30.34d	32.96c	**29.76C**	24.12h	28.72f	31.70e	**28.18C**
SE1	26.00e	38.56a	39.00a	**34.52A**	24.14h	37.30b	38.72a	**33.39A**
SE2	26.34e	35.01b	33.11c	**31.49B**	25.53g	34.79c	31.39e	**30.57B**
Mean	**26.11B**	**34.64A**	**35.02A**		**24.60B**	**33.60AB**	**33.94A**	

a,b,c,d and e means at the same column with different superscript are significantly (P<0.05) different.

4.11.3. Influence of *Spirulina* and Fe/Zn-Nano foliar applications on microbial enzyme activity of Nemaguard seedlings rhizosphere

In the current study, the effect of using *Spirulina* extract and/or phyco-biosynthesised Fe/Zn-NPs as foliar fertilizers for Nemaguard seedlings on the activity of microorganisms in the soil was investigated. Two levels of nano-fertilization as well as two levels of *Spirulina* extract were applied as foliar applications. The effect of fertilizers can be attributed to the direct use of nutrients that reach the soil by microbes. In addition, plant nutrition affects the microbial activity of the soil indirectly by stimulating root exudates that contain microbial growth stimulants.

According to the results illustrated in Figure 3,, individual applications of nano-Fe/Zn and *Spirulina* extract resulted in higher microbial activity in the seedling rhizosphere compared to the control treatment. However, significant enhanced soil microbial activities were recorded in the rhizosphere of plants treated with Fe/Zn-NPs and *Spirulina* extract (5 or 10 mL/L). These results demonstrate the positive effect of *Spirulina* extract individually or in combination with Fe/Zn-NPs as foliar fertilizer(s) for plants.

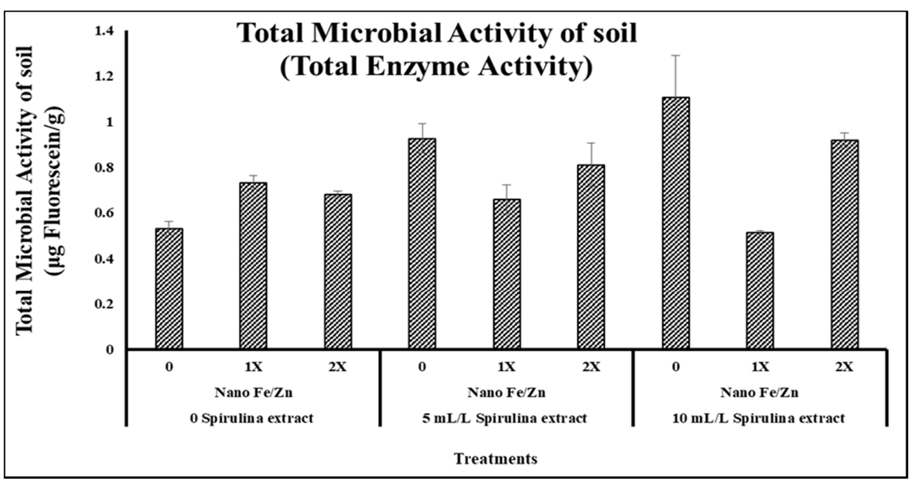

Figure 3: Influence of different levels of Spirulina and Fe/Zn-Nano foliar applications on microbial enzyme activity of Nemaguard seedlings rhizosphere. 1X nano Fe/Zn: 2 mL/L , 2X nano Fe/Zn: 4 mL/L

5. Discussion

Many attempts have been made recently to produce new fertilizers in improved forms in an attempt to lessen the issues brought on by old fertilizers, such as air, water, and soil pollution. At the moment, nanotechnology is crucial to the improvement of fertilizers [38]. This is because nanotechnology allows for the synthesis of materials in novel forms with particles that have dimensions of one millionth of a millimeter and are characterized by superior and generally useful properties in a wide range of fields, including chemical, physical, biological, mechanical, magnetic, optical, and electrical ones. By utilizing the special qualities of nanoparticles, nano-fertilizers help to improve nutrient utilization efficiency [39]. In addition to extending the fertilizer's duration of action, nano-fertilizers release nutrients in a manner that is ordered and in accordance with crop requirements. This results in decreased environmental pollution and nutrient loss over an extended period of time, as well as improved and developed nutrient use efficiency [40, 41]. Furthermore, *Spirulina* is a well-known source of organic matter, nutritional fertilizers, complex polysaccharides, amino acids, and vitamins, all of which are crucial for plant metabolism and productivity. It has recently been used as a fertilizer additive in horticulture and agriculture, as foliar sprays containing *Spirulina* extract improve plant nutrient absorption and growth [42,43].

The investigation's findings showed that, when compared to the untreated control seedlings, the administration of *Spirulina* extract and nano-iron and zinc, either separately or in combination, increased the analyzed growth metrics and nutritional status of Nemaguard seedlings. Our research's findings were in line with those of Al-Abedy and Al-Abbasi [25] and Kareem et al. [42], who discovered that, in comparison to untreated control plants, foliar spraying with nano-micronutrients or *Spirulina* extract alone and their interactions increased all the plant growth parameters studied, including plant height, stem diameter, number of branches per plant, number of leaves per plant, leaf area, total chlorophyll content in leaves, carbohydrates, leaf dry matter, and percentages of N, P, K, and Cu. This is because of the beneficial effects of nano nutrients on soil properties, enhanced plant photosynthesis products, increased efficiency and absorption of water and nutrients in the soil, easier transportation of carbohydrates from the source to the downstream, and increased plant fullness [44-47]. The effect of nano-iron as a catalyst in the formation of chlorophyll pigment may be responsible for the increase in leaf content of elements, carbohydrates, and chlorophyll by accelerating the condensation of glutamate to aminolevulinic acid and the conversion of the Mg-Protoporphyrin 1x methyl ester into the Protochlorophyllid complex, which is a crucial step in the production of chlorophyll. Additionally, iron is necessary for the creation of several enzymes, including cytochrome oxidase, peroxidase, and catalase, which activate a variety of biological processes within the plant and promote growth [7].

In addition to this moral superiority, it might also be caused because seedlings' roots grow faster when nano chelated iron is added. This means that the plant absorbs more nitrogen and iron to meet its needs, including those of its numerous metabolic processes [48]. The leaves show a rise in the buildup of these components. The function of chelated zinc nano-fertilizer in dramatically boosting seedling growth can be attributed to zinc's involvement in the synthesis of tryptophan, an amino acid that serves as the building block for the natural hormone indole acetic acid (IAA), which is essential for plant cell expansion and elongation. Zinc also plays a role in the synthesis and activation of chlorophyll, the synthesis of carbohydrates and proteins, and the efficiency of enzymes [49, 50]. This study's findings are consistent with those of Yaseen and Kadim [51] and Al-Abedy and Ghalib [52], who noted that the small size of nano fertilizers gives them special properties. Due to its size and vast surface area, it increases the amount of surface area available for absorption. This, in turn, increases the efficiency with which nutrients are absorbed and promotes the growth of seedlings, which in turn increases the demand for nutrients and their concentration inside the plant. The study's findings were consistent with those of Dawood et al. [53] in Pistachio and Ayad et al. [17] in Pomelo seedlings, who discovered that foliar spraying with iron nano-fertilizer increased the height and chlorophyll and carbohydrate content of the seedlings. Furthermore, Hasan et al. [54] found that adding zinc nano fertilizer increased the dry weight and chlorophyll content of pears' leaves.

These results concurred with those of Rezaei and Abbasi [55] as well as Mamyandi et al. [56]. The results of Farnia and Omidi [57] and Al-Abedy and Al-Abbasi [25] confirmed that the foliar spray containing nano-micronutrients increases plant growth and all activities related to metabolism, including photosynthesis, CHO synthesis, and the activities of involved plant enzymes. These enzymes are important in the synthesis of amino acids, carbohydrates, and energy compounds. Since nano-formulations easily pass through leaf stomata through gas exchange, foliar spraying them increased the availability of nutrients [58]. The larger surface area and high absorption of nano-fertilizer contribute to its unique properties, which also help to increase the processes of photosynthesis, metabolic enzyme activation, sugar biosynthesis and translocation, protein assimilation, water absorption, and nutrient transport. These processes help to improve the physiological activities of the plant and raise the amount of carbohydrates in the leaves, which in turn increases plant height. The findings are consistent with those found by Mohasedat et al. [59] regarding apples, Sabir et al. [60] regarding blueberries, Abdelaziz et al. [16] in the case of mangos, and Roshdy and Refaai [61] in the case of date palm, made the same observations.

Spirulina extract contains essential amino acids and organic acids that are involved in vital activities, which increase absorption and positively affect the growth of seedlings. As a result, spraying Nemaguard seedlings with it significantly increased the characteristics of vegetative growth. It also had a clear effect on seedling height, number of branches/seedlings, number of leaves/seedlings, leaf area, total chlorophyll percentage, and the percentage of fresh and dry weight of the leaves. Regarding apples, these findings align with the findings of Thanaa et al. [24], Hassan et al. [62], and Mosa et al. [43]. Furthermore, the increase in the percentage of dry matter in leaves could be attributed to the *Spirulina* extract, which contains macro- and microelements as well as plant hormones that are essential for growth, including gibberellins, auxin, auxin-like substances, cytokinin, and vitamins [63]. This increased leaf area raised the products of photosynthesis and increased the dry weight of the leaves. Furthermore, most of the essential organic product activities that boost the buildup of carbohydrates in leaves are also enhanced by *Spirulina* [64, 65]. El-Bassiouny et al. [66] attribute this to the beneficial effects of *Spirulina* fertilizer on the physical and chemical properties of the soil, as well as the increased activity of microorganisms present in the soil, which enhanced better nutrient absorption by plant root cells and increased photosynthesis. Consequently, there was a rise in vegetative growth, as evidenced by an increase in shoots, an increase in the average number of leaves per seedling, and an increase in the fresh weight of leaves. Together with the *Spirulina* extract's content of macro- and microelements, phytohormones, and vitamins, the observed improvement in leaf elemental content may be the result of iron and zinc nanoparticles' size, unique surface area, and reactivity, which all influence their solubility, diffusion, and accessibility in plants.

The findings of Al-Janabi et al. [67] suggested that nanomaterials may be used to create novel, well-balanced crop nutrition fertilizers, which is in line with the findings of Mosanna and Khalilvand [68]. In agricultural soils, rhizosphere bacteria facilitate nutrient uptake, produce compounds that stimulate plant growth, bioremediate toxic chemicals, and prevent disease. One crucial indicator of soil quality is total bacterial enzyme activity. It displays the microbial population's activity, which provides an oblique indicator of the fertility and nutrition of the soil. According to this process, the colorless fluorescein diacetate can be cleaved into fluorescein (with a detectable fluorescent color) by the enzymes generated by microbial populations in soil, such as lipases, esterase, and proteases [69-71, 31].

6. Conclusion

The study found that Spirulina extract, nano-iron, and zinc significantly improved seedling height, diameter, leaf area, number of branches, leaf weight, chlorophyll content, and leaf percentages of macronutrients and micronutrients in Nemaguard rootstock seedlings, outperforming the control group. The study found that foliar application of Spirulina extracts and iron and zinc nano-fertilizers effectively promoted Nemaguard rootstock seedling growth, increasing microbial activity in the rhizosphere. The combination of Spirulina extract and Fe/Zn- nanoparticles was also beneficial.

7. Conflict of interest

The authors declare that there was no conflict of interest in carrying out this work.

8. Acknowledgements

The authors would like to express their gratitude to the National Research Centre (NRC), Egypt, for supporting and offering generous funding for the project "Technology of Nemaguard rootstock production vegetatively with the technique of tissue culture, saving hard currency spent on importing the seeds".

9. Funding

This work was supported and funded by the National Research Centre through the project titled Technology of Nemaguard Rootstock Production Vegetatively with the Technique of Tissue Culture, saving hard currency spent on importing the seeds. Project ID: 130502133, during 2022/2024.

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