



## Article

## Response of Local Orange Seedlings *Citrus sinensis* L. to Foliar Application of Nano-NPK and Humic Acid and its Reflection on Vegetative Growth and Chemical Characteristics

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### ABSTRACT

A private nursery located in the Al-Dujail District of Salah Al-Din Governorate hosted the experiment during the March 1st – February 28th 2023-2024 growing season. The goal was to determine how foliar application of the nano-fertilizer NPK and humic acid impacted vegetative and chemical growth characteristics of local orange seedlings, *Citrus sinensis* L. Uniform one-year-old seedlings with similar growth were selected as a method to decrease initial variance between experimental units and thus improve accuracy of results. This research was conducted using a two-factor factorial design and Randomized Complete Block Design (RCBD) with three replications for each treatment. The first independent factor was foliar application of the nano-fertilizer NPK 20:20:20 at three concentrations (0, 1, 1.5 g/L), while the second independent factor was foliar application of humic acid at three concentrations (0, 1.5, 2 g/L). A statistical analysis was performed using an analysis of variance table and significant difference comparison between means was done via the LSD test at a 0.05 significance level. The results are summarized below. Most vegetative growth and chemical growth traits of orange seedlings benefited from the use of nano-NPK fertilizer. The most substantial increase in growth occurred in the 1.5 g L<sup>-1</sup> spray application, which produced the maximum average growth rates for all of the vegetative growth traits (i.e., height, total leaf nitrogen, and leaf potassium) measured at 97.56 cm, 2.682 mg/g fresh wt, 3.92%, 1.69%, respectively. The results of this study show that the use of a humic acid spray significantly enhanced the vegetative growth traits and chemical growth traits of orange seedlings. The 2 g L<sup>-1</sup> spray application produced significant increases in plant height, number of branches per seedling, and total chlorophyll, nitrogen, and potassium in leaves by an average of 96.3 cm, 12.29 branches per seedling, 2.677 mg/g fresh wt., 3.56%, and 1.633%, respectively. Most of the vegetative and chemical growth markers measured for the two compounds were significantly affected by their interaction. When nano-NPK at 150 g L<sup>-1</sup> was mixed with humic acid at 2 g L<sup>-1</sup>, the results produced were optimum for all vegetative and chemical growth markers as measured.



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## INTRODUCTION

Oranges *Citrus sinensis* L. belong to the family Rutaceae and are a related type of modified berry called Hesperidium. The pericarp of these fruits becomes mostly leathery, containing many oil glands that give them their distinctive aroma, and they create an outer peel (Obaid & Sharabi, 2017). Citrus fruits have a major place in the fruit market, second only to grapes, with an estimated global production of roughly 152,448,800 tons. The leading citrus fruit is the orange with a global production of 75,413,374 tons, with Brazil being the largest producer with 16,713,543 tons (FAO, 2018). In Iraq, oranges are one of the crops that the central and south-central areas of Iraq grow well due to the environmental conditions. The number of fruit-bearing trees in Iraq is 6,383,881, and the average yield for each tree is 22.4 kg per tree (resulting in 142,998 tons total). The Salah al-Din Governorate produces the most oranges, followed by Baghdad and Diyala Governorates (Central Statistical Organization, 2020). Due to their nutritional, environmental, economic and therapeutic values, citrus species rank high in terms of value when compared to other types of fruit trees. Citrus fruits provide many vitamins (especially vitamin C) and many essential minerals required by the body (Ahmed and Dawood 2020).

Seedling production is recognized as a critical, but also a very fragile, stage during the life cycle of all citrus species. Seedlings are vital to the future productivity of the plant and the quality of the seedlings and amount of vital growth materials (e.g. macro and micronutrients) they receive will directly impact their overall growth (Castle et al., 2016). The intent is to have the seedlings have enough of each macro and micronutrient to have vigorous vegetative and root systems, exactly right amounts of nutrients to allow for proper chemical composition and to adequately prepare the seedlings for moving to their final location (Mattos Jr. et al., 2003; Srivastava & Singh, 2009). For example, the three primary macronutrients that citrus trees require are nitrogen, phosphorus, and potassium. For citrus fruit trees, one of the largest amounts of nitrogen is located within the fruit. Additionally, nitrogen is an important macronutrient for supporting vegetative growth, protein synthesis and chlorophyll production, and nucleic acid formation (Legaz et al., 1995). On the other hand, phosphorus plays an important role in energy transfer, energy utilization, respiration, photosynthesis and root development (Marschner, 2012). Potassium is essential for regulating processes throughout the plant's life cycle like opening and closing stomata, transporting sugars through the plant system and increasing the plant's ability to survive both biotic and abiotic stressors (Alva et al., 2006). Traditional fertilizers are inefficient because of their low percentages of the elements in the fertilizers (i.e. nitrogen = 30%-35%, phosphorus = 18%-20%, potassium = 35%-40%) (Bindraban et al., 2015). One reason why these nutrients are not utilized by the plants is due to the many different ways they can be lost, such as leaching, volatilization, soil fixation, and surface runoff. Loss of nutrients through these processes results in an abundance of wasted dollars (i.e. economic loss) and significant environmental contamination (i.e. contaminants in both groundwater and surface water) (Lassaletta et al., 2014; Sutton et al., 2011). Nanotechnology has undergone tremendous growth in different areas, including agriculture, for around 20 years. It is speculated that nano-fertilizers will serve as a solution to the problem of inefficient conventional fertilizers (DeRosa et al., 2010; Kah et al., 2018).

Nanoferrites are characterised by the small size of their constituent particles (1-100 nm), which give them unique physical and chemical properties, including high overall surface area, high solubility, and ability to easily penetrate the cell wall of plants. Recent research has shown that NPK nano-fertilisers can improve the efficiency of nutrient uptake compared with traditional fertilisers by 80%-90%, thus providing an opportunity to reduce traditional fertiliser application rates by 30%-50% without loss of yield (Subramanian & Tarafdar, 2011; Rameshaiah et al., 2015). Application of nano-fertilisers has shown to greatly enhance growth rates, yields and crop quality of a number of different types of crops including citrus (Davarpanah et al., 2016; Zahedi et al., 2019). Conversely, humic substances, especially humic acid, are important natural organic materials which greatly improve soil fertility and overall health of plants (Canellas & Olivares, 2014). The organic compound humic acid is a large and complex molecule

and is the product of the decomposition of organic matter. It is composed of long carbon chains, and has multiple functional groups, such as carboxylic acid (-COOH), hydroxyl (-OH), phenolic, and carbonyl groups. These characteristics endow humic acid with a high affinity for binding minerals and influencing plant physiology (Stevenson, 1994; Tan, 2014).

There are many benefits to using humic acid in the soil-plant continuum. Humic acid improves the structure of the soil by increasing soil aggregation and porosity, which in turn increases aeration, drainage, and water holding capacity (Chen et al., 2004). Humic acid increases the cation exchange capacity of soils; it is a natural chelator of minerals and thus increases the availability of these minerals for plant uptake and reduces their movement away from the root system by leaching or fixation (Nardi et al., 2002; Cimrin & Yilmaz, 2005). Humic acid is an excellent biostimulant because it mimics plant hormones, stimulates root growth, increases the permeability of cell membranes, activates metabolic enzymes, and increases photosynthetic efficiency (Canellas et al., 2015; Olaetxea et al., 2018). In addition, studies show that humic acid can help plants become more resilient to environmental stressors, such as drought, salinity, and heat, by promoting the synthesis of antioxidants and protective proteins (García et al., 2012; Tahir et al., 2011). Prior studies show that using nano-fertilizers and organic amendments, like humic acid, together may be more effective than using them separately and could potentially create a synergistic effect (Abdel-Aziz et al., 2016; Sharifi et al., 2018). By improving soil quality, enhancing root function, and creating an ideal environment for nutrient uptake, humic acid increases the effectiveness of nano-fertilization. Meanwhile, nano-fertilizers provide nutrients with high efficiency and high availability (Maswada & Abd El-Rahman, 2014).

Although native orange trees have a major economic contribution to this part of Australia. There are several technical and knowledge-based issues impeding the ability to produce an optimal yield. The main issue is with the low efficiency levels of traditional fertiliser programmes used in producing seedlings. In most cases, and under optimal conditions, the efficiency of conventional fertilisers does not exceed 30 - 40% (Bindraban et al., 2015). This project is designed to investigate the effectiveness of applying both nano NPK and humic acid as foliar sprays separately and in combination on the chemical properties of orange trees. This is to assist in developing stronger seedlings for grafting with fewer detrimental environmental and financial effects compared to traditional fertilisers, as there does not appear to be any previous research on this issue.

## **MATERIALS AND METHODS**

### **Study location**

The experiment was done at a private nursery located in the Al-Dujail District, Salah al-Din Governorate, from the beginning of the growing season (March 1, 2023) until the end of the growing season (February 28, 2024). On March 1, 2023, one-year-old *Citrus sinensis* L. seedlings were purchased from a licensed nursery in Baghdad Governorate. The seedlings were selected for uniformity, including height (30-35 cm), stem diameter (4-5 mm), and number of branches (3-4 branches), to minimize differences between experimental units at the start of the experiment and to ensure accuracy of results. The selected seedlings that were grown in polyethylene bags were then transplanted into 10 kg black plastic pots (50 cm diameter × 40 cm height) containing holes in bottom for drainage, which allowed for proper drainage of water and prevented water from accumulating in the pots. The black pots contained commercial growing medium that is a mixture of locally produced loamy soil and peat moss, in equal volumes (3:1 volume). The mixture of any of the components that made up the growth medium was mixed thoroughly to ensure that all physical and chemical characteristics were uniformly distributed throughout the growth media. Maintenance of the plants, including cutting back the plants and controlling for weeds, fertilizing, and watering, will be done constantly throughout the study. Before the experiment was started, random samples of the growth media were taken and sent to the laboratories at Tikrit University's College

of Agriculture in the Horticulture and Soil and Water Resources departments to be analyzed for their chemical and physical characteristics. Table 1 presents the original properties of the growth medium that was used in the study.

**Table 1. Some chemical and physical properties of the soil mixture**

Property	Unit	Value
Ph	-	7.3
EC	dS m <sup>-1</sup>	1.8
Texture	Silty loam	sandy mixture
Sand	gm/kg <sup>-1</sup>	702.6
Silt	gm/kg <sup>-1</sup>	144.2
Clay	gm/kg <sup>-1</sup>	153.2
N	mg/kg <sup>-1</sup>	28.9
P	mg/kg <sup>-1</sup>	45.5
K	mg/kg <sup>-1</sup>	67.8
Organic Matter	%	2.8

**1. Study Factors and Treatments:** The research was executed as a factorial experiment incorporating two factors, utilizing a randomized complete block design (RCBD) with three replications. Each experimental unit comprised three seedlings per treatment, yielding a total of 81 seedlings utilized in the experiment (9 treatments × 3 replicates × 3 seedlings). The two variables being examined are:

A. The initial factor: NPK nano-fertilizer. This factor was indicated by the foliar application of a compound NPK nano-fertilizer at three concentrations:

- N<sub>0</sub>: Control treatment (no application - application of distilled water exclusively) .
- N<sub>1</sub>: Application of NPK nano-fertilizer at a concentration of 1 g/L .
- N<sub>2</sub>: Application of NPK nano-fertilizer at a concentration of 1.5 g/L.

A compound NPK nano-fertilizer with a ratio of (20:20:20), purchased from [Land Crane], was utilized, featuring a particle size of 20-80 nm as per the manufacturer's specifications. The fertilizer comprises nutrients in nanoparticle form, including Nitrogen (20% N as nano-nitrogen), Phosphorus (20% P<sub>2</sub>O<sub>5</sub> as nano-phosphate), Potassium (20% K<sub>2</sub>O as nano-potassium), and nano-micro-elements (Fe, Zn, Mn, Cu) at suitable proportions.

B. The second element is humic acid. The second factor involved foliar application of humic acid at three concentrations: -

- H<sub>0</sub>: Control treatment (no spraying) equals 0 g L<sup>-1</sup>.
- H<sub>1</sub>: Humic acid spray at a concentration of 1.5 g/L.
- H<sub>2</sub>: Humic acid aerosol at a concentration of 2 g L<sup>-1</sup>

1. Foliar sprays were applied in the morning with no direct sunlight, thus reducing evaporation. An 16-liter manual backpack sprayer was used, fitted with a nozzle for spraying. The sprayer has a precise nozzle, which allows for equalising the amount of liquid applied across the upper and lower surfaces of the leaves until they reach the point of run-off. The treatments are administered as three sprays throughout the growing season, with two weeks between each application, using the following schedule: – First spray: April 1, 2023, and one month after transplanting – Second spray: April 15, 2023 – Third spray: April 29, 2023.

2. The seedlings' optimal responsiveness to the different treatments coincides with these times that corresponded to their active period of vegetative growth. Therefore, the seedlings will have received the same level of agricultural treatment throughout the study in order to eliminate any influence of extraneous factors on the results..

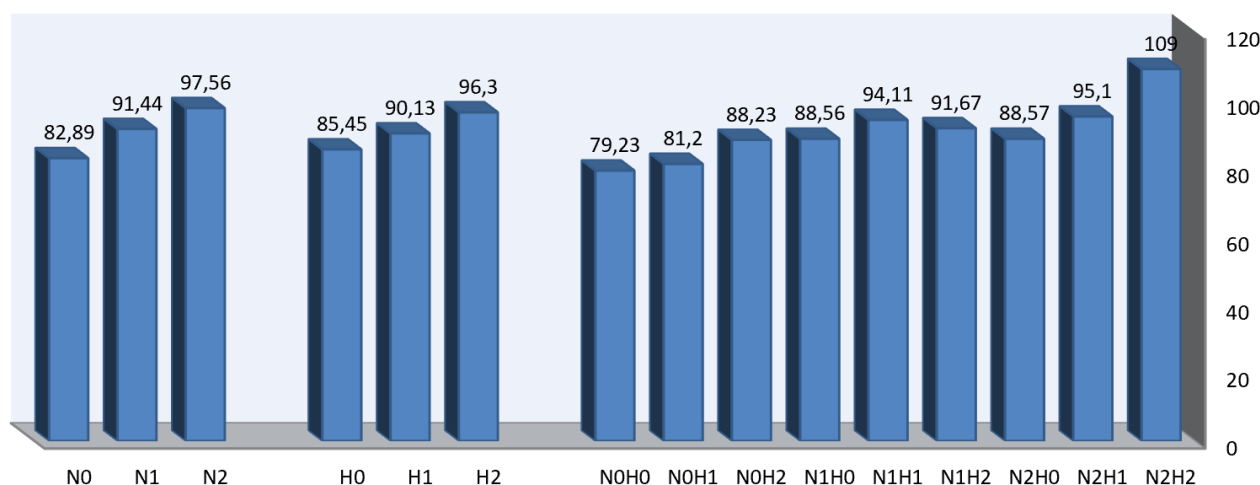
**Examined characteristics:**

1. Seedling height (cm): Measure the height of the plant from the junction of the stem and the growing media to the apical meristem using a ruler.
2. Branch count (seedling branch - 1): Determine the total number of lateral branches for an individual plant by counting how many branches were counted on the main stem of the plant then calculating the average for each treatment.
3. Area of Leaves (cm<sup>2</sup> leaf<sup>-1</sup>): Three mature, fresh leaves were collected from each of the three seedlings. As described by Sadik et al. (2011), the area of each leaf was calculated using the method from Al-Zaidi (2016) and a scanner along with the Image J software loaded on a computer. The average area of each leaf was then calculated for the treatment group and for each rep.
4. The method for determining Total Chlorophyll Content (mg/g - 1g Fresh Weight) was conducted in the laboratories of Science and Technology/ Environment and Water by the Ministry of Science and Technology by using the acetone technique. A 0.02 g of leaves was weighed and soaked in 10 ml of 80% acetone. The leaves were macerated with the acetone, and the chlorophyll was dissolved. The chlorophyll solution was then placed in dark, airtight glass vials. Absorbance of Light at two wavelengths (663 nm and 645 nm) was measured for the sample using Spectrophotometer. The total chlorophyll was then calculated using the following equation (Goodwin, 1976): Total Chlorophyll =  $20.2D(645) + 8.02D(663)$
5. Percentage of nitrogen in leaves: A technique called Micro-Kjeldahl was used to determine the amount of nitrogen contained within the samples. According to Van Dijk et al., (2021) the amount of sample (0.2 grams) is added to a flask along with an appropriate amount of sulfuric acid (5 millilitres) along with an appropriate amount of potassium sulphate and copper sulphate/ as a mixture to assist digestion of the contents through the application of heat. After digestion, the mixture will become a clear, pale blue liquid; the liquid will then be quantitatively transferred into the distillation flask of a Micro-Kjeldahl device containing concentrated (40%) Sodium Hydroxide. A distillation flask will then pass through a cooling unit and flow into a sample collection tube containing a known volume of 20% boric acid solution with added drops of methyl red and bromocresol blue to serve as indicators. After cooling, heat will then be applied to the receiver until enough distillate has been collected, approximately 25 ml or so, is obtained from distilling the sample through the distillation flask and condenser. The liquid from the receiver will then be centrifuged with 0.1 N hydrochloric acid, and a blank (control) solution will be created using the same ingredients, except for the sample, as described above. Nitrogen percent (%) is calculated using the following formula: Nitrogen (%) =  $(\text{Volume of HCl consumed} \times \text{Normality of acid} \times 0.014) / (\text{Weight of sample} \times 100)$ .
6. Leaves Phosphorus Content (%): The phosphorus content in the leaves was determined according to the procedures described in Chapman and Pratt (1961). Briefly, a 0.5 g dried and ground sample was dissolved in 5 ml of H<sub>2</sub>SO<sub>4</sub> and 2 ml of HClO<sub>4</sub>. Ammonium molybdate and ascorbic acid were used in a colorimetric method, and the resulting solution was quantitatively assayed with a spectrophotometer (2005 Bichrom-LibraS22-UK) at 700 nm.
7. Potassium in leaves (%): The potassium concentration in the leaves was assessed utilizing a flame photometer, following the methodology outlined by Estefan et al. (2013).

**RESULTS AND DISCUSSION****Seedling Height (cm):**

According to Figure 1., orange seedling heights are affected by the following variables and how they are related to one another: Nano-NPK fertilizer, humic acid application, and both their application

methods (spraying). The application of Nano-NPK had a positive effect on the height of the seedlings; with 1.5 g L<sup>-1</sup> showing the greatest average height of 97.56 cm compared to control (0 g L<sup>-1</sup>) at 82.89 cm. Likewise, the application of humic acid (2 g L<sup>-1</sup>) also had a significant positive effect on the height of the seedlings with a greatest average seedling height measured at 96.30 cm compared to control (0 g L<sup>-1</sup>) at 85.45 cm. The interaction coefficients for these two applications (Nano-NPK and humic acid) show that orange seedling heights were significantly increased from those resulting from the control group. The highest seedling height measured 109.00 cm after application of Nano-NPK at a concentration of 1.5g/L and Humic acid at 2g/L. The lowest seedling height (79.23 cm) was recorded for seedlings treated with no Nano-NPK (0 g/L) or Humic acid (0 g/L) added..

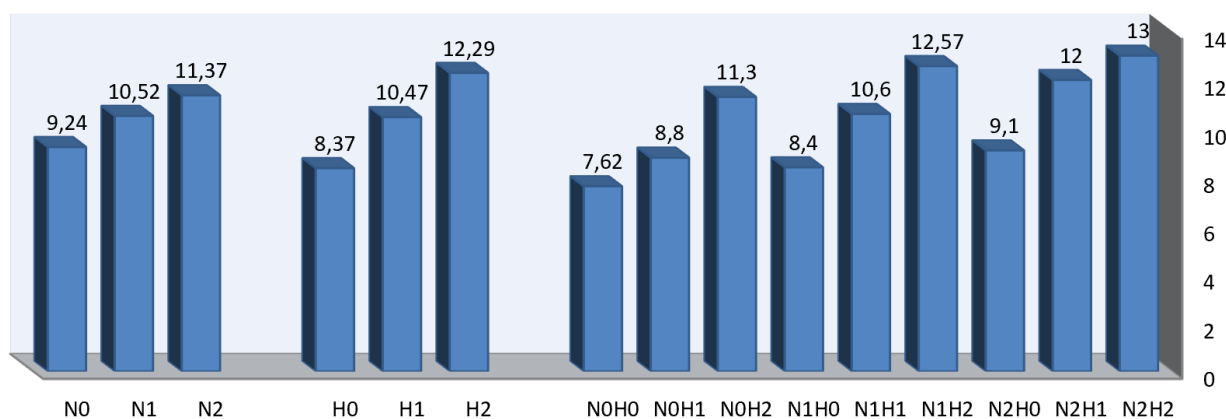


L.S.D (P<0.05)= N= 0.96. H= 0.96. N\*H=1.67

Figure 1. Effect of nano-NPK and humic acid and the interaction between them on the height of local orange seedlings (cm).

### Number of branches (seedling branch<sup>-1</sup>)

Figure 2. demonstrates that there are no significant changes among the treatments including the application of Nano-NPK on orange seedlings. Humic acid spray treatments markedly influenced the branch count in orange seedlings, with treatment (H<sub>2</sub>) at a concentration of 2 g/L yielding the highest average of 12.29 branches per seedling, in contrast to the control treatment (H<sub>0</sub>), which exhibited the lowest average of 8.37 branches per seedling. The statistical analysis reveals no significant differences in the two-way interaction for this attribute.



L.S.D (P<0.05)= N= n.s, H= 1.87, N\*H= n.s

Figure 2. Effect of nano-NPK and humic acid and the interaction between them on the number of local orange seedlings (seedling branch-1).

### Leaf area (cm<sup>2</sup> leaf<sup>-1</sup>):

The impact of study variables and their interactions on the leaf size of orange seedlings was demonstrated by Figure 3. The spraying of seedlings with Nano-NPK proved to significantly improve the leaf size of seedlings with increasing spray concentration, and the 1.5g L<sup>-1</sup> spray treatment was superior in resulting in the largest leaf size of 33.80 cm<sup>2</sup> leaf<sup>-1</sup>, compared to the comparison treatment which resulted in the smallest size of 21.20 cm<sup>2</sup> leaf<sup>-1</sup>. For the spraying of seedlings with humic acid, it also showed a significant increase in the leaf size of orange seedlings when using the 2g L<sup>-1</sup> (H2) spray concentration, having resulted in the largest leaf size of 31.95 cm<sup>2</sup> leaf<sup>-1</sup>, compared to the comparison treatment (H0) that resulted in the smallest leaf size of 24.00 cm<sup>2</sup> leaf<sup>-1</sup>. Regarding the effect of the interaction of Nano-NPK with Humic acid, the results of the same figure show that the leaf area of orange seedlings clearly increases. The maximum leaf area (38.51 cm<sup>2</sup> leaf<sup>-1</sup>) of seedlings treated with Nano-NPK at a concentration of 1.5 g L<sup>-1</sup> plus Humic acid at a concentration of 2.0 g L<sup>-1</sup> is the result of this spray treatment; however, the lowest leaf area (0 cm<sup>2</sup> leaf<sup>-1</sup>) resulted from the combination of Nano-NPK and Humic acid at their respective concentrations of 0 g L<sup>-1</sup>. 18.89 cm<sup>2</sup> leaf<sup>-1</sup>.

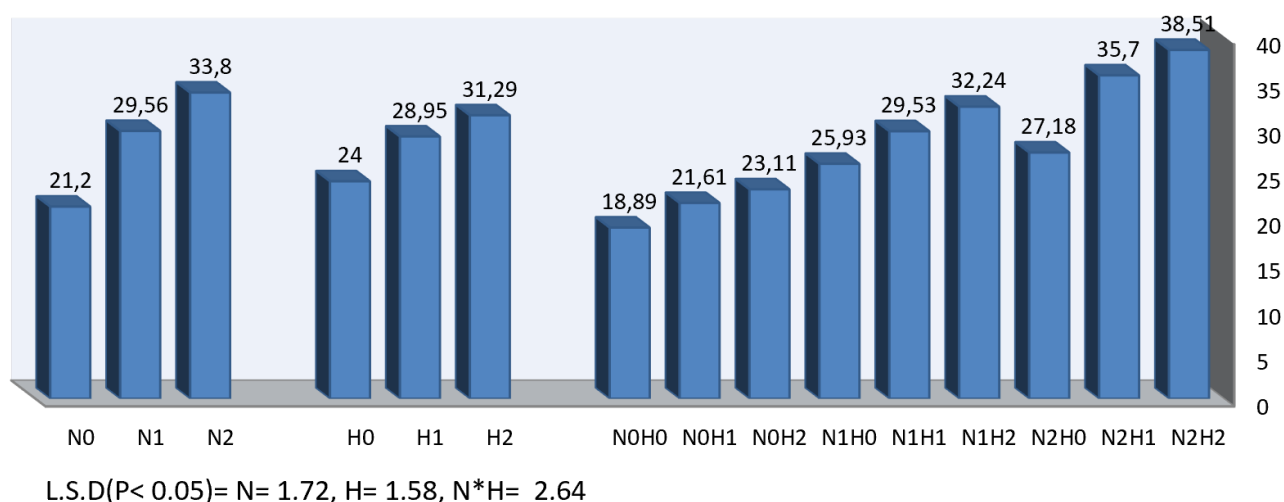
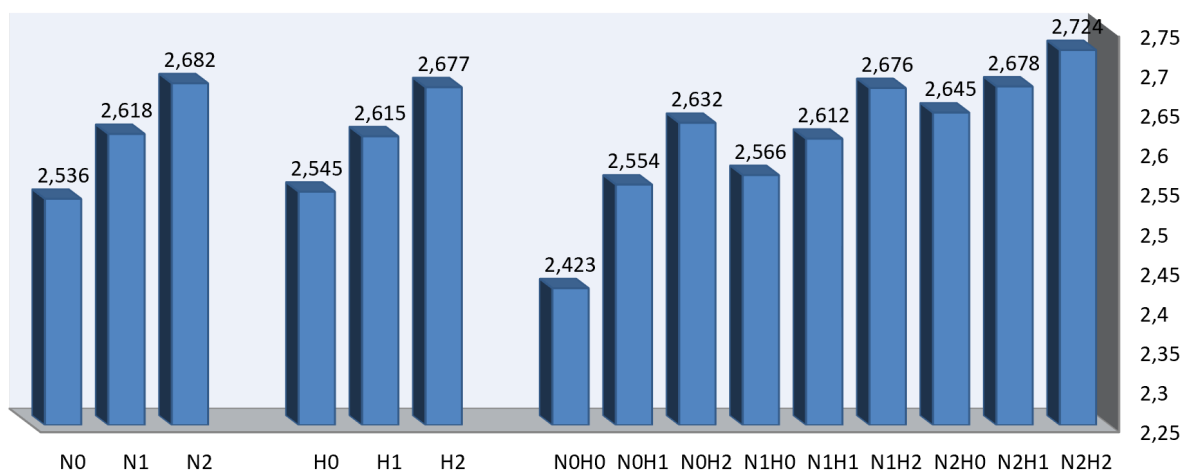


Figure 3. The effect of Nano-NPK and Humic Acid and their interaction on the area of one leaf of local orange seedlings (cm<sup>2</sup> leaf<sup>-1</sup>).

### Total chlorophyll in leaves (mg g<sup>-1</sup> wet weight):

As shown in Figure 4, the results from these trials demonstrated numerous significant differences between treatments. The use of different amounts of Nano-NPK produced significantly different amounts of total chlorophyll in leaves of orange seedlings. Of all treatments, treatment (N2) at a rate of 1.5g L<sup>-1</sup> produced the highest concentration of chlorophyll in the leaves measured at a concentration of 2.682 mg g<sup>-1</sup> wet weight. Conversely, treatment (N0), which received only water, exhibited the lowest concentration of total chlorophyll measured at 2.536 mg/g wet weight. Similarly, the use of Humic acid produced a statistically significant increase in the total chlorophyll concentration of the leaves of indigenous orange seedlings. For treatment (H2) at a rate of 2g L<sup>-1</sup>, there was an increase in total chlorophyll concentration estimated at 2.677mg g<sup>-1</sup>wet weight compared to the treatment (H0) that received only water, which resulted in the lowest concentration of total chlorophyll at 2.545mg g<sup>-1</sup>wet weight. Based on the results of statistical analysis shown in the figure, there is significant variability in chlorophyll concentration due to differences in spraying agent. Of the two treatments analyzed, N2H2 produced the highest level of chlorophyll in leaf tissue (2.724 mg g<sup>-1</sup> wet), while NOH0 produced the lowest level of chlorophyll in leaf tissue (2.423 mg g<sup>-1</sup> wet).

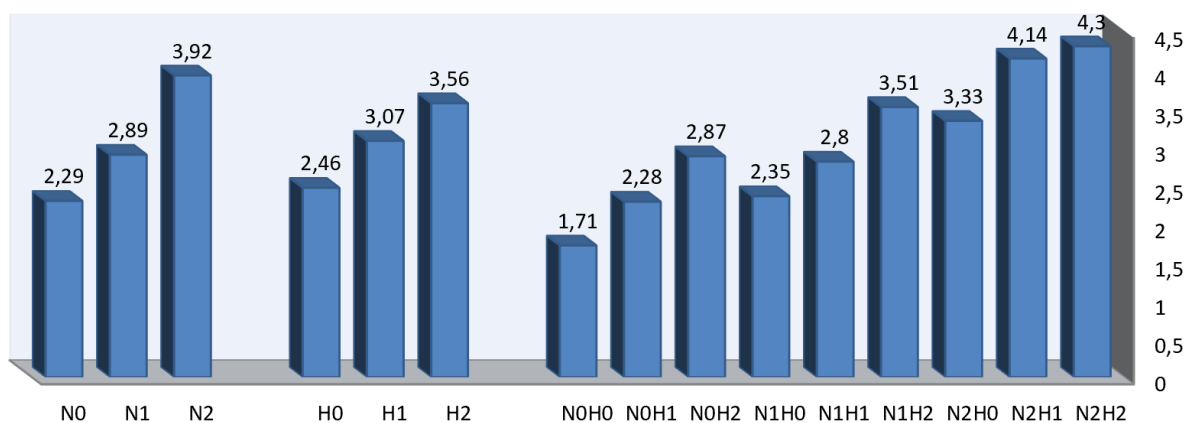


L.S.D. (P<0.05)= N= 0.113, H= 0.113, N\*H= 0.195

**Figure 4.** Effect of nano-NPK and humic acid and the interaction between them on the total chlorophyll content in leaves (mg g<sup>-1</sup> wet weight) of local orange seedlings.

### Nitrogen in leaves (%)

The nitrogen level in orange seedling foliage, as seen in Figure 5, has been significantly affected by the amount of Nano-NPK applied to the local seedling. The highest level of nitrogen in the leaf was achieved with a Nano-NPK application of 1.5 g L<sup>-1</sup> (N2) at 3.92%, while the treatment without Nano-NPK (N0) resulted in the lowest amount of nitrogen at 2.29%. As well, an application of Humic acid to the foliage of local orange seedlings resulted in a higher concentration of nitrogen in the leaf. In this case, the treatment of 2 g L<sup>-1</sup> (H2) resulted in a much greater nitrogen content of 3.56% compared to the control lot (H0), which had a nitrogen percentage of only 2.46%. The results illustrated in the following figure also show that there were statistically significant interactions among the foliar spray treatments, and the N2H2 treatment demonstrated the highest concentration of nitrogen (2.870%). The other treatments and NOH0 treatment contained the least amount of nitrogen in the foliage of the seedlings studied. f 1.71%.



L.S.D (P< 0.05)= N= 0.025, H= 0.025, N\*H= 0.030

**Figure 5.** Effect of nano-NPK and humic acid and their interaction on the percentage of nitrogen in the leaves of local orange seedlings.

### Phosphorus in leaves :(%)

According to Figure 6, there is a distinct connection between the use of Nano-NPK and the nitrogen values of orange seedling leaves shown in figure 5. When comparing the highest (3.92% N) at 1.5g/L of Nano-NPK (N2) with the lower (2.29% N) of no Nano-NPK (N0), referencing the two foil spray treatments, the application of Humic acid to orange seedling foliage caused a significantly greater amount

of nitrogen (3.56% N) than in the control lot (2.46% N) when applied at 2g/L. There are also statistically significant differences between the two foliar spray applications (N2H2 versus N0H0) with N2H2 containing the largest nitrogen (2.87%) and N0H0 containing the fewest nitrogen.

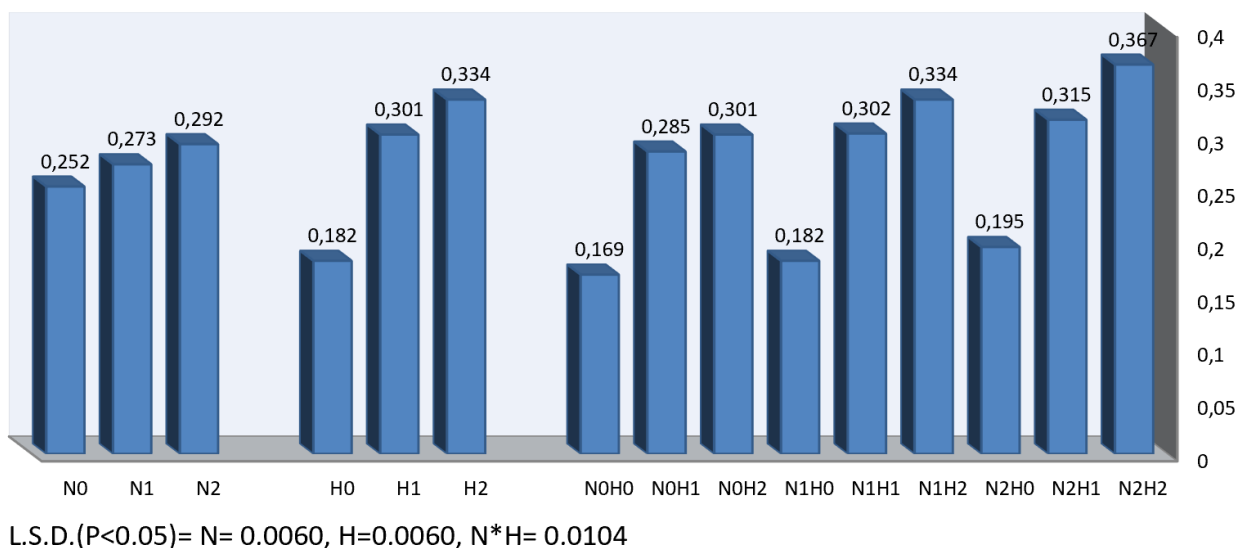


Figure 6. Effect of Nano-NPK and Humic acid, and their interaction, on the percentage of phosphorus in the leaves of local orange seedlings.

**Potassium in the leaves(%):**

As seen in figure 7, there is a dramatic difference in the potassium percentage found in the leaves of orange seedlings treated with the Nano-NPK spray. The N2 treatment of 1.5 g L-1 NPK resulted in the highest potassium percentage in seedlings (1.690%) and the N0 treatment of only H2O resulted in a much lower potassium percentage in seedlings (1.375%). The results show that the application of humic acid also had a significant impact on the potassium percentage in seedlings' leaves. The use of humic acid at 2gL-1 (H2) caused a significant increase in the potassium percentage of seedlings to 1.633%; while the control H0 seedlings that received only H2O showed the lowest value of 1.405%. Both treatments also had interactions that significantly impacted the potassium percentage value that resulted from either of the two spray treatments to seedlings. The maximum potassium concentration was found in treatment N2H2 (1.784%); while the lowest value was found in treatment N0H0 (1.279%).

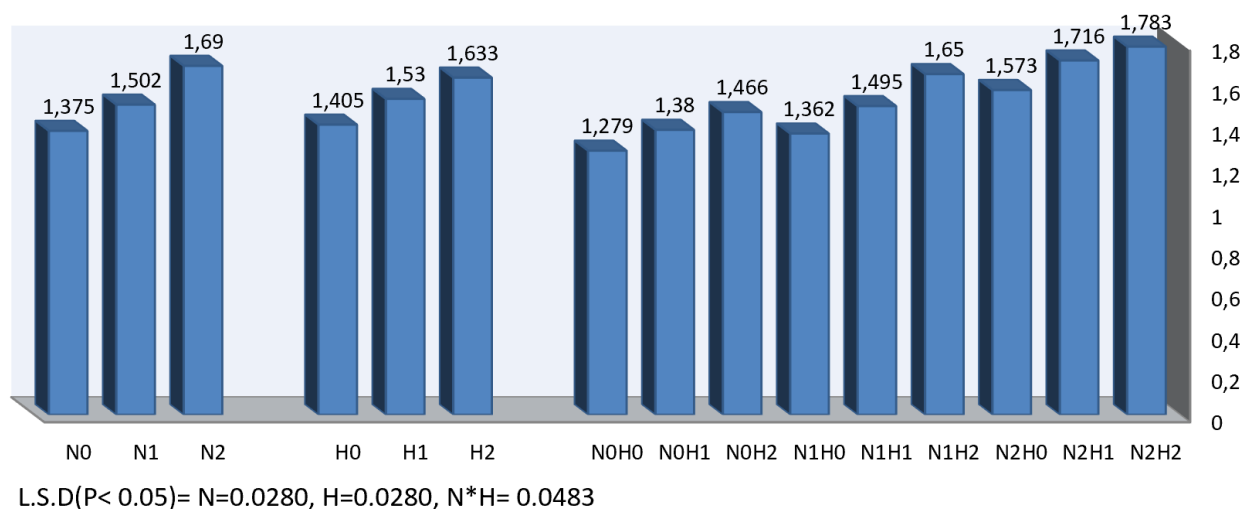


Figure 7. Effect of Nano-NPK and Humic Acid, and their interaction, on the percentage of potassium in the leaves of local orange seedlings.

## DISCUSSION

The use of nano-fertilizers is vital for the growth and development of plants. Figure 1 shows the effectiveness of Nano-NPK fertilizer for increasing the growth of orange seedlings. This effectiveness is due to the characteristics unique to nano-fertilizers. Nano-fertilizers possess a high surface area and a size between 1nm and 100nm, this helps them move through cell membranes and increases the effectiveness of nutrient uptake (particularly when applied as a foliar application)(Liu and Lal, 2015; Elemike et al., 2019). Also, when the macronutrients (N, P, K) are delivered in nanoparticle form, they help activate important physiological processes in the plant. For example, nitrogen helps produce proteins and chlorophyll as well as stimulate the production of plant hormones (e.g., auxins and cytokinins) that promote cell division and elongation (Taiz and Zeiger, 2010). As for phosphorus, it plays an important role in energy transfer process through chemical means. Nitrogen is involved in the formation of adenosine triphosphate (ATP) and nucleic acids, both of which are important for plant vegetative production (Vance et al., 2003). Additionally, potassium works to help regulate osmotic pressure within the cell and help maintain turgor within the cells which are critical for increased cell elongation and greater longitudinal growth (Marschner, 2012).

Use NPK nano-fertilizer at a concentration of 1.5 g/L produced the highest amount of total chlorophyll in the leaves (as shown in Figure 3). This is due to nitrogen being the major part of the chlorophyll molecule as well as contributing to the formation of proteins needed for the function of photosystems in chloroplasts (Taiz & Zeiger, 2010). The addition of magnesium to the nanoparticle structure allows for increased synthesis of chlorophyll because magnesium is the central atom in the chlorophyll molecule's porphyrin ring (Evans, 1989). Figure (4) is an example of this point where the nitrogen content in the leaves was significantly greater after treatment with 1.5 g L<sup>-1</sup> of nano-NPK than at the time of application. According to (Elemike et al., 2019), the nitrogen concentration in leaves increased significantly due to the rapid uptake and transport of nitrogen from the soil to the plant's leaves through the plant's leaves' stomata and epidermis during the nanofertilizer's nano-scale size, as well as the translocation of nitrogen within the plants' vascular tissues by xylem and phloem. Figure 5 shows that applying 1.5 g L<sup>-1</sup> of nano-NPK significantly affected the phosphorus concentration in leaves. The increase is attributed to the ability of nanofertilizers to supply phosphorus in a means by which phosphorus can be readily available and quickly absorbed, thereby improving the efficiency of phosphorus in the plant. Phosphorus is essential for the formation of ATP and ADP, nucleic acids, and the phospholipids in cellular membranes (Vance et al., 2003); The results in Figure 6 indicated that the application of 1.5 g L<sup>-1</sup> of nano-NPK resulted in a significant increase in potassium concentration in the leaves. The beneficial effects of nano-fertilizers on plant growth are due to their ability to transport potassium directly into the plant cells. The importance of potassium in this regard lies in the fact that it is a critical regulating factor for ionic balance and osmotic pressure inside the plant cells, as well as an activator for more than 60 different types of enzymes. Potassium also controls the opening and closing of stomata, which assists in increasing the efficiency of both photosynthesis and vegetative growth of the plant (Marschner, 2012). The results reported here are in agreement with the results published by Dawood (2020) concerning the effect of different fertilization types on growth of pomegranate seedlings. Specifically, they found that seedlings that received nano-fertilizers exhibited increased height and number of branches (compared to the control group).

As shown in Figure 1, humic acid had a major impact on the growth of orange seedlings. When using 2 g/L of humic acid, the average height of seedlings was maximized. This positive effect can be attributed to humic acid's hormone-like properties, stimulating the production of endogenous auxins and activating H<sup>+</sup>-ATPase proton pumps in the plasma membrane. Activation of these pumps leads to increased acidity of the cell wall and improved flexibility of the cells; therefore aiding in cell elongation and longitudinal growth. Furthermore, humic acids improve the absorption and transport of nutrients (e.g., nanoparticles) to the tissues of a plant by activating transport proteins within the membranes of

the cells, therefore improving the nutritional uptake and efficiency of growth of plants (Cieschi et al., 2019). As shown in Figure 2, there was also a significant effect of humic acid on the number of branches produced. This is due to the ability of humic acids to stimulate the development of lateral branches by activating axillary buds and partly disrupting apical dominance. Through the modification of the internal hormones involved in latitude development by increasing the cytokinin to auxin ratio. In addition, humic acids promote the distribution of carbohydrates and nutrients throughout the plant. This helps to meet the essential needs for additional bud development and vegetative branching (Nardi et al. 2016). In figure 3, it can be seen that humic acid, at 2 g/L, resulted in a significant increase in the maximum amount of chlorophyll that could be found in plants' leaves. This is because humic acid helps with the synthesis of chlorophyll through improved absorption of important mineral elements that are required for the process of photosynthesis and by stimulating the activity of several different enzymes that are involved in chlorophyll synthesis, especially  $\delta$ -aminolevulinic acid dehydratase, which is a key enzyme involved in the production of chlorophyll molecules (Tahir et al., 2011). In figure 4, it is clear that humic acid has a substantial positive effect on the nitrogen content found in leaves. Increased absorption of nitrogen through enhanced ion transport mechanisms in cell membranes will likely result from (Nardi et al. 2016). In addition, the activation of the enzyme nitrate reductase, which is responsible for converting nitrate into ammonia, is also an important pathway for the absorption of mineral nitrogen into plants. The effect of humic acid treatment at a concentration of 2 g/L on the leaf phosphorus content is illustrated in Figure 5. The increase in phosphorus was attributed to the chelating ability of humic acids to convert phosphorus that was bound to the soil into soluble phosphorus, which is available for uptake by plants. The humic acids also stimulated the activity of acid phosphatases, which are enzymes that release phosphorus from organic compounds and thus increase the bioavailability of phosphorus to the plant (García et al. 2012). Additionally, as shown in Figure 6, the addition of humic acid at a concentration of 2 g/L significantly enhanced the potassium levels in the leaves. The enhanced uptake of  $K^+$  through  $K^+$  ions channel activation and the facilitated movement of potassium ions and roots from soil into the plant causes an increase in overall plant nutrition. (Canellas et al., 2015).

## CONCLUSIONS

1. Foliar application of nano-NPK nutrients and humic acid markedly enhanced the majority of vegetative and chemical growth attributes of orange seedlings in comparison to the control treatment.
2. The application of nano-NPK nutrition at a concentration of 1.5 g/L and humic acid at 2 g/L produced the most significant enhancements in seedling height, leaf chlorophyll content, and levels of nitrogen, phosphorus, and potassium.
3. The N2H2 interaction treatment yielded superior values for the examined features relative to the other treatments, suggesting a synergistic effect between the two nutrients.

## AUTHORS' CONTRIBUTIONS

**Sabreen Mohammed Lateef:** conceived and designed the study, supervised the experimental work, and revised the manuscript critically for important intellectual content. **Marwa Noman Hussin:** conducted the field experiments, collected the data, and wrote the first draft of the manuscript. **Muntadher Mohammed Al-Mousawi:** performed the statistical analysis and contributed to the interpretation of results. All authors read and approved the final manuscript.

## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflicts of interest.

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