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**Shareef et al. nano-fertilizer of date palm****Foliar nano-fertilization enhances fruit growth, maturity and biochemical responses of date palm****Hussein J. Shareef<sup>1\*</sup>, Rashid A. Al-Yahyai<sup>2</sup>, Alaa El-Din K. Omar<sup>3,4</sup>, Wan Arfiani Barus<sup>5</sup>**<sup>1</sup>Dept. of Date Palm Research Center, University of Basrah, Basrah, Iraq<sup>2</sup>Dept. of Plant Sciences, Sultan Qaboos University, Muscat, Oman.<sup>3</sup>Institute of Research and Consulting, King Faisal University, Hofuf, Kingdom of Saudi Arabia,<sup>4</sup> Horticulture Dept., Faculty of Agriculture, Kafresheikh University, Kafr El-Sheikh 33516, Egypt<sup>5</sup>Dept. of Agrotechnology, Universitas Muhammadiyah Sumatera Utara, Medan, Indonesia\*Corresponding author ([husseinshareef@live.com](mailto:husseinshareef@live.com))**Abstract**

The experiment was conducted in the Abi Al-Khaseeb orchard, Basrah, Iraq during the 2019 season, on date palm (Hillawi cv.). The effect of foliar nano-fertilizer on the response of the growth and fruit ripening rate was amid. Adding nano-fertilizer to the annual date palm fertilization program improved growth and increased production. A comparison of foliar NPK (1, 2 g L<sup>-1</sup>) as nano-fertilizer and traditional fertilizer, and combined, was applied. The results revealed that the treatment of foliar traditional and nano-fertilizers together increased the weight of fruit and bunches, water content, indoleacetic acid, and gibberellic acid relative to other treatments. Nano-fertilizers (1g L<sup>-1</sup>) led to an increase in fruit ripening rate, dry mass, and total soluble solids, activity of the enzymes peroxidase, and superoxide dismutase, and abscisic acid content. The leaflet protein expression shows that the appearance of protein bands 1 to 5 and 6 was up-regulated by control and traditional fertilizer. Whereas the protein bands 6 and 7 were down-controlled under nano-fertilizer. Hierarchical cluster analysis of proteins in the leaf in response to traditional and nano-fertilizer showed two distinct clusters. The use of nano-fertilizer individually leads to the acceleration of fruit ripening. while the production fruit that is increased using foliar nano-fertilizer with traditional fertilizer.

**Keywords:** Ascorbic acid, Cluster analysis, Fruit ripening, Indoleacetic acid, Protein pattern

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### Introduction

Fertilizers play a significant role in plant growth and improving production. Using environmentally friendly nano-fertilization instead of traditional fertilizer is a major impact on crop nutrition (Rameshaiah et al. 2015). Environmentally friendly fertilizers are characterized by reducing nutrient loss, whether in the soil or directly on the plant, controlling the way it is used directly on the plant or in the soil (Chen et al. 2018).

Nano-fertilization could even be utilized to control the delivery of the fertilizers to such an extent that the nutrients are taken up by the plant, and not lost to non-targeted soil, water, or microorganisms (Kopittke et al. 2019). The large surface area to volume of the nanomaterials helps in rapid response and hence high plant growth effectiveness (Zahedi et al. 2019). The effect of nanostructures can be attributed to easier absorption and increased nutrient absorption through the leaves or roots (Amira et al. 2015). Foliar spray application of macronutrients and micronutrients has been considered a successful strategy to build salt resilience in plants and has been proposed to enhance the antagonistic impact of salt stress (Zouari et al. 2016). Foliar application may likewise offer an answer for root limitation brought about by salt stress (Amira et al. 2015). Foliar nutrient application has become common in date palm nutrient management (Shareef 2020).

Date palm (*Phoenix dactylifera* L.) is important in reducing the damage of climate change because of its high ability to convert carbon dioxide into oxygen and carbohydrates (Shareef 2019). Date palm growth is impacted by environmental stresses, such as drought, salinity, and heat, which unfavorably influence the development and profitability of the plants (Jasim et al. 2016). Plant hormones such as auxin (IAA), gibberellic acid (GA3), and abscisic acid (ABA) are sensitive to the influence of environmental factors on physiological changes in plants (Fahad et al. 2015).

Nowadays, the use of fertilizers containing NPK is crucial for the improvement of harvest yield and fruit quality supplying vital nutrients for plant development (Shareef 2011a). A nano-built composite, which comprises N, P, K, and micronutrients, when applied to grain crops, improved the uptake and utilization of supplemental nutrients (Abdel-Aziz et al. 2018). A nano-fertilizer may be useful in date palm production.

Several studies have recently been done on the effect of foliar nano-fertilizer on date palm production (Roshdy and Refaai 2016; Amiri et al. 2016; Altemimy et al. 2019; Jubeir and Ahmed 2019a,b; Jubeir and Ahmed 2019). Also, Roshdy and Refaai (2016) found that soil-applied nano-fertilizer improved the growth and production of date palm compared to the use of traditional fertilizer. Therefore, it is imperative to understand physiological and biochemical mechanisms that result from the application of nano-fertilizer on date palm fruit compared to traditional fertilizers. Knowing the effects of

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nano-fertilizers on fruit growth and developmental and phytochemical activities in the date palm fruit is essential to decide how to adopt the technology. This experiment was aimed to assess the effect of foliar nano-fertilizers and traditional (NPK) as a foliar spray on the growth, yield, and metabolic activities of dates.

### Materials and Methods

#### Description of the experimental site

Date palm, cultivar 'Hillawi,' was used in this study. The palms were grown in an orchard at Abi Al-Khaseeb, Basrah, Iraq (30°28'40.2"N 47°52'07.1"E), during the growing season of 2019. Thirty palms 15 years of age were selected and pollinated with cv. Ghanami Akhdar. Date palm clusters were thinned at one bunch per eight leaves rate (8 leaves / 1bunch ratio). The palms were divided into five blocks, each block had six palms, and the treatments were distributed equally among them. Palms were sprayed with fertilizers on the 1<sup>st</sup> of April and repeated a month later. All horticultural operations (such as tree management and irrigation) were carried out following the regular traditional practices for date palm in the region. The soil of the experiment was a clay loam (Sandy 10%, Silt 30%, Clay 60%, Organic matter 1.28 %, K 1.15 g Kg<sup>-1</sup>, P 0.56 g Kg<sup>-1</sup>, N 3.89 g Kg<sup>-1</sup>, CaCO<sub>3</sub> 397.4 g Kg<sup>-1</sup>, Mg 503 mg Kg<sup>-1</sup>, CEC 16.62 cm Kg<sup>-1</sup>, SO<sub>4</sub> 3.38 mg Kg<sup>-1</sup>), and surface irrigation was used during the study. The average soil electric conductivity (EC) was (6 dS m<sup>-1</sup>), and irrigation water EC was (3 dS m<sup>-1</sup>).

#### Experimental setup and sampling

Super micro plus nano-fertilizer (Nano spuer) (Sepeher Parmis, Agriculture & Livestock /Agriculture /Agricultural Pesticides and fertilizers, Iran) with the following chemical composition: (Mn 0.7%, Mg 6%, Ca 6%, Fe 4.5%, Zn 8%, K 3%, P 3%, N 5%, Mo 0.1%, B 0.65%, and Cu 0.65%) was used as source of nano-fertilizer in this study. Whereas NPK (Vista Leaf, Jordan) (20-20-20-1% MgO) with the following chemical analysis (N 20%, P<sub>2</sub>O<sub>5</sub> 20%, k<sub>2</sub>O 20%, MgO 1.0%, Fe 700 ppm, Zn 450 ppm, Mn 350 ppm, Cu 50 ppm, B 200 ppm) was used as the traditional supplemental fertilizer. The following six treatments were applied on the 1<sup>st</sup> of April and May: (a) Control, sprayed with distilled water, (b) Nano spuer 1 g, sprayed Super micro plus at 1 g L<sup>-1</sup>, (c) Nano super 2 g, sprayed Super micro plus at 2 g L<sup>-1</sup>, (d) NPK 1 g, sprayed NPK at 1 g L<sup>-1</sup>, (e) NPK 2 g, sprayed NPK at 2 g L<sup>-1</sup>, (f) Nano super 1 g +NPK 1 g, sprayed Super micro plus at 1 g L<sup>-1</sup> + sprayed NPK at 1 g L<sup>-1</sup>. All the treatments contained the surfactant Tween 20 at 0.1%. All fruit samples were taken at the *Rutab* stage (At this stage the tissues of the fruit begin to soften, become moist, and begin gradually starting from the tip of the fruit and continues towards its base. The fruits are characterized by good flavor and high sweetness, and they are completely suitable for eating, and it is considered the stage of complete ripeness) during August 2019 to determine the fruit quality parameters.

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### Data collection

#### Yield (bunch weight/palm) and physical fruit characteristics

Bunches were harvested at mid-August (*Rutab* stage), and bunch weight was recorded in kg. A fifty fruit sample from each replicate was taken to determine the average fruit weight (g). The ripening percentage was calculated when the fruits entered the *Rutab* phase for the period from 1 to 15 August. Five strands from each bunch taken to calculate the rate of ripening.

#### Fruit chemical characteristics

Fifty fruit samples to determine the moisture content and dry mater according to (Association of Official Analytical Chemists (AOAC) 2005).The total soluble solids percentage (TSS) was determined using a hand refractometer, according to (AOAC 2005). Ascorbic acid (AsA) content was determined by employing a slightly changed methodology of Luwe et al. (1993) by homogenizing the samples in ice-cold trichloroacetic acid (TCA, 1% w/v) instead KCl solution.

Assay of antioxidant enzymes. Fruit tissues were homogenized with three volumes (w/v) of an ice-cold extraction buffer (50mM Tris-HCl, pH 7.8, 1mM EDTA, 1mM MgCl<sub>2</sub> and 1.5% (w/w) polyvinylpyrrolidone). The homogenate was centrifuged at 15000 xg for 20 min at 4 °C. The supernatant was used as the crude extract for the assay of enzyme activities. The activity of SOD was determined according to Beauchamp and Fridovich (1971) by following the photoreduction of nitro blue tetrazolium (NBT). The reaction mixture contained: 50mM phosphate buffer (pH 7.8), 0.1mM EDTA, 13 mM methionine, 75 IM NBT, 2l M riboflavin and 100 lL of the supernatant. Riboflavin was added as the last component, and the reaction was initiated by placing the tubes under fluorescent lamps. The reaction was terminated after 10 min by removing the reaction tubes from the light source. Nonilluminated and illuminated reactions without supernatant served as calibration standards. The absorbance of the solution was measured spectrophotometrically (EMCLAB/4000U model) at 560 nm. The activity of POD was assayed by the method of Upadhyaya et al. (1985). The reaction mixture contained 2.5 ml of 50mM potassium phosphate buffer (pH 6.1), 1ml of 1% hydrogen peroxide, 1 mL of 1% guaiacol, and 10–20µl of enzyme extract. Absorbance was read at 420 nm.

#### Hormones Analysis

Five grams of a fresh fruit tissue sample, which was homogenized in 70 % methanol was stirred overnight at 4 °C. The extract was filtered through Whatman filter paper (No. 1) and evaporated under vacuum. The pH of the aqueous phase was adjusted to 8.5, using 0.1 M phosphate buffer. Later the aqueous phase was partitioned using methanol twice. A rotary evaporator removed the methanol phase. The aqueous phase pH was adjusted to

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2.5, using 1 N hydrochloric acid (HCl). The concentrate was injected into a reversed-phase HPLC, from Shimadzu Company (LC10AVP) using C18 column in an isocratic elution mode utilizing a portable stage comprising of acetone: water (26:74) with 30 mM phosphoric acid as per Tang et al. (2011). The pH was kept up at 4, utilizing 1 N sodium hydroxide. The temperature was kept at 25 °C. The flux rate was 0.8 ml min<sup>-1</sup>, and the elution of the phytohormones was observed at 208 and 265 nm for indoleacetic acid, gibberellic acid (GA3), and abscisic acid, respectively.

### Extraction of Proteins and Gel Electrophoresis

Proteins were extracted by homogenizing the 333 mg of solidified dried leaf segments in 1 ml of extraction buffer [0.2 M, tris-hydroxymethyl aminomethane (Tris) + 0.001 M ethylenediaminetetraacetic acid + (Na<sub>2</sub> + EDTA) + 12 % glycerol + 0.01 M dithiothreitol (DTT) + 0.05 mM phenylmethylsulfonyl fluoride (PMSF)] utilizing a mortar and pestle. At that point, the samples were centrifuged at 15,000 xg for 15 min. Supernatant buffer consisted of 0.125 M Tris HCl (pH 6.8) + 4 % SDS + 20 %, glycerol + 10 % β-mercaptoethanol + 0.01 % bromophenol blue. Protein samples were denaturated by bubbling in a water bath at 90 °C for 3 min. Protein electrophoresis was performed in an irregular SDS polyacrylamide gel (Invitrogen, Carlsbad, CA model), Laemmli (1970). Protein groups were investigated by ImageJ programming (v1.50i, <https://imagej.nih.gov/ij/>).

### Data analysis

Treatments in the field were arranged in a completely randomized design with five replications. The collected quantitative data were subjected to analysis of variance (ANOVA) as well as descriptive statistics using Duncan's multiple range test at the  $P \leq 0.05$  level. Cluster analysis was done using IBM SPSS (Ver. 23.0).

## Results

### *Fruit weight, bunch weight, and fruit ripening rate in response to foliar traditional and nano-fertilizer*

The applications of traditional and nano-fertilizers affected the increase in fruit weight, bunch weight, and ripening rate of fruits in the *Rutab* stage (Table 1). The combination of traditional and nano-fertilizers together increased the weight of fruit and bunches relative to other treatments (Table 1). On the contrary, the treatment of nano-fertilizers at 1 g L<sup>-1</sup> led to an increase in the percentage of ripe fruits related to other treatments. Also, the application of nano-Fertilizers, whether 1 or 2 g L<sup>-1</sup>, did not increase the fruit or bunch weight. These treatments differed substantially in the ratio of ripeness of fruits. Moreover, the individual traditional fertilizer treatments did not differ in their effect on fruit weight, bunch weight, and percentage of ripe fruits.

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### *Water content, dry matter, and TSS in response to foliar traditional and nano-fertilizer*

The water content of fruit increased with the use of traditional fertilizers, while the dry weight increased with the use of nano-fertilizers (Table 2). Treatment of traditional and nano-fertilizers increased the percentage of the water content of fruits relative to other treatments. Whereas, the treatment of nano-fertilizers alone at 1 g L<sup>-1</sup> increased dry weight and total soluble solids compared to other treatments (Table 2).

### *Ascorbic acid, the activity of POD, and SOD in response to foliar traditional and nano-fertilizer*

Traditional and nano-fertilizers improved ascorbic acid content, as well as the peroxidase and superoxide dismutase activity (Fig. 1). Nano fertilizer 1 g L<sup>-1</sup> increased ascorbic acid in fruits relative to other treatments. The concentrations used for traditional and nano-fertilizers differed substantially with each other in affecting the fruit content of ascorbic acid (Fig. 1 a). The activity of enzymes increased by using nano fertilizers, especially the concentration of 1 g L<sup>-1</sup> (Fig.1 b-c). In contrast, traditional fertilizers increased the activity of enzymes compared to the control treatment, which did not differ from the treatment of traditional and nano fertilizers accompanying.

### *IAA content, GA3, and ABA in response to foliar traditional and nano-fertilizer*

Plant hormones substances such as indoleacetic acid (IAA), gibberellic acid (GA3), and abscisic acid (ABA) changed during the ripening stage of Hillawi date palm fruit (Fig. 2). The combination treatment of traditional and nano-fertilizers together increased IAA and GA3 compared to other treatments (Fig. 2 a-b). Only a 1 g L<sup>-1</sup> nano-fertilizer treatment increased ABA content compared to other treatments. While traditional fertilizer accompanying the nano fertilizer reduced the ABA content as compared to the control treatment (Fig. 2 c).

### *Protein pattern and cluster analysis in response to foliar traditional and Nano-fertilizer*

The protein pattern of the leaflets in one-dimensional polyacrylamide gel electrophoresis (SDS-PAGE) and examination of groups by the ImageJ program demonstrated some striking contrasts (Fig. 3). Protein expression shows that the appearance of protein bands 1 to 5 and 6 (molecular mass were 132.072, 69.333, 40.642, 34.389, 28.232, 14.646 kD) was up-regulated by control and traditional fertilizer. In contrast, the expression of protein bands 6 and 7 (molecular mass were 17.091, 13.980 kD, respectively) was down-controlled under nano- fertilizer treatments.

Hierarchical cluster analysis of leaf protein patterns showed two distinct clusters (Fig. 4). The first group included nano-fertilizer treatments (Nano super 2 g, Nano super

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1 g+NPK 1 g, Nano super 1 g). The second group included control and traditional fertilizer treatments (NPK 1 g, NPK 2 g, control).

### Discussion

Using foliar-applied nano-fertilizers appears to reduce the need for large amounts of traditional NPK in date palm. There have many advantages in using nano-fertilizers in agriculture, including faster uptake by plants, improved growth, and better yields. The increase in weight of fruit and weight of bunch increases the productivity of date palm by using nano-fertilizer. Nano-fertilizers facilitates the delivery of nutrients in the plant (Shang et al. 2019). Nano-fertilizer was used in this experiment (Super-micro plus) contains many macros and microelements to compensate for the deficiency in the absorption from the soil of these elements such as Mn, Mg, Ca, Fe, Zn, Mo, and B. Date palms suffer from salinity and contamination with heavy elements such as cadmium, lead, and mercury. While having a lack of microelements such as Mn, Mg, Fe, Zn, Mo, and B in the south of Iraq (Altemimy et al. 2019).

The increase in weight of the fruit due to nano-fertilizers application confirms the importance of nanoparticles as unique in behavior and characteristics, such as the small size, delivery of nutrients, and highly active area, which increased the rapidity of fruit chemical reactions, and these results are consistent with Roshdy and Refaai (2016). Also, micronutrients have a decisive role in the various biological processes (Dimkpa and Bindraban 2016) that reflected reduce the days to maturity of the fruit. Nano-fertilizer promotes and improves growth traits. Nutrients also play a significant role, as nitrogen affects the growth and development of plants through the regulation of the action of plant hormones such as auxins and gibberellins (Fig. 2). Potassium has a positive effect on the process of dividing and expanding cells by stimulating the expansion of the cell wall necessary for the operation of division (Salem et al. 2017). Potassium encourages many enzymes responsible for synthetics the plant's structure (Sanchez-Calderon et al. 2013). All of these factors (Nano-micronutrients, Nano-macronutrients) will push the plant towards increasing rates of metabolism and expanding the synthesis and accumulation of dry matter, which leads to improved growth rates.

The fruits of date palm were affected by the treatment with nano-fertilizers, and this is consistent with many studies on date palm (Roshdy and Refaai 2016; Amiri et al. 2016; Altemimy et al. 2019; Jubeir and Ahmed 2019a, b; Jubeir and Ahmed 2019). Ripening rate reduced due to the slow growth of fruit, continued accumulation of metabolites, high levels of auxins and gibberellins, and low activity of enzymes such as POD and SOD (Fig. 2, 1), and increased the water content (Table 2). The high water content of the date palm fruit delays the fruit reaching the final ripening stage (Jubeir and Ahmed 2019b). Also, delaying the ripening of the fruits may be because of high levels of growth regulators such as IAA and GA3 in the fruit (Cheruth et al. 2015).



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In contrast, the low concentration of nano-fertilizer led to an acceleration of ripening due to the high activity of enzymes and the high level of ascorbic acid as well as the high concentration of total soluble solids and dry matter (Fig. 1). The increase in dry matter and total soluble solids improved the quality of the fruits and accelerate ripening (Kassem and Marzouk 2010). Ascorbic acid stimulated enzymes involved in ripening, such as invertase and cellulase, and improving the quality of fruits (Shareef 2016). The increase in total soluble solids can be attributed to hydrolysis of starch in the fruit and transformation into reduced sugars and accumulation of nutrients (Shareef 2011b)

The main reason for preventing the plant from absorbing nutrients in the local soil solution is the high salinity of the soil and water (Shareef 2020). While the presence of foliar spray of nano-fertilizer enabled the plant to deliver the necessary elements (Altemimy et al. 2019). Therefore, availability elements in the leaves contribute rapid transfer photoassimilates to the fruit, which leads to an increase in the weight of the fruits. Nano-fertilizer delivers the nutrients, not adsorbed from the soil, which leads the necessary elements to perform some vital processes. We believe that these nutrients were mostly micronutrients, which helped to accelerate growth and increase metabolic activity and encouraged early maturity. Nano-fertilizer alone or associated with traditional fertilizer increased the weight of fruit, and increasingly linked to a positive effect of nutrients absorbed by the leaves on growth and yield. The process involves increasing the production of photoassimilates (source) and increasing their transportation and storage in fruits (sink).

The complete mechanism of the effect of nanomaterials on plants is yet not precise. Recent studies indicated that nano-fertilizers are causing changes and modifications in proteins through gene expression and building of new proteins during the development of grains (Elemike et al. 2019). Our results showed the formation of new proteins that were not previously present as a result of the use of nano-fertilizer (Fig. 3), which is due to the occurrence of oxidative stress (Peralta-Videa et al. 2014). The dynamics of nano-fertilizers rapidly penetrate cell membranes to be available in the cytosol. While the biochemical effect provides the necessary elements such as potassium and magnesium, to build new specific proteins and increase secondary metabolites, to reduce the occurrence of oxidative stress (Naser et al. 2016).

## Conclusions

Nano-fertilizers targeted the plant growth program by regulating the phytohormones such as IAA, GA3, and ABA. Also, increasing antioxidants such as vitamin C with increased POD and SOD activity accelerate plant growth. The use of foliar-applied nano-fertilizers with lower concentrations can lead to a faster growth process by increasing the metabolic activity in fruits, which accelerates the fruit ripening process. Early ripening of fruit is desirable for farmers to fetch the highest prices for dates.

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**Shareef et al. nano-fertilizer of date palm**Table 1. Change of fruit weight, bunch weight, and fruit ripening rate in response to the application of foliar traditional and nano-fertilizer<sup>a</sup>.

Treatments	Fruit weight (g)	Bunch weight (Kg)	Fruit ripening (%)
Control	8.18±0.05 <i>d</i>	4.87±0.05 <i>e</i>	57.164±0.87 <i>d</i>
Nano spuer 1 g	9.12±0.14 <i>b</i>	6.4 ±0.07 <i>b</i>	76.214±1.21 <i>a</i>
Nano super 2 g	9.15±0.21 <i>b</i>	6.44±0.08 <i>b</i>	66.758±1.39 <i>b</i>
NPK 1 g	8.32±0.02 <i>cd</i>	5.47±0.12 <i>d</i>	62.114±1.23 <i>c</i>
NPK 2 g	8.36±0.01 <i>c</i>	5.63±0.04 <i>c</i>	63.042±0.92 <i>c</i>
Nano super 1 g +NPK 1g	9.67±0.08 <i>a</i>	7.28±0.13 <i>a</i>	66.306±0.74 <i>b</i>

<sup>a</sup>Means of 5 replications±SE. Means with different letters are different at  $p \leq 0.05$  using Duncan's multiple range test.

**Shareef et al. nano-fertilizer of date palm**Table 2. Change of water content, dry matter, and total soluble solids in response to the application of foliar traditional and nano-fertilizer<sup>a</sup>.

Treatments	Water content (%)	Dry matter (%)	Total soluble solids (%)
Control	60.37±0.22 <i>b</i>	39.63±0.22 <i>d</i>	41.75±0.33 <i>c</i>
Nano spuer 1 g	54.10±0.52 <i>e</i>	45.89±0.52 <i>a</i>	44.67±0.41 <i>a</i>
Nano super 2 g	57.31±1.26 <i>d</i>	42.69±1.26 <i>b</i>	42.89±0.40 <i>b</i>
NPK 1 g	59.03±0.53 <i>c</i>	40.96±0.53 <i>c</i>	41.98±0.03 <i>c</i>
NPK 2 g	60.30±0.79 <i>b</i>	39.69±0.79 <i>d</i>	36.73±0.98 <i>e</i>
Nano super 1 g +NPK 1 g	64.08±0.44 <i>a</i>	35.91±0.44 <i>e</i>	38.48±0.73 <i>d</i>

<sup>a</sup>Means of 5 replications±SE. Means with different letters are different at  $p \leq 0.05$  using Duncan's multiple range test.

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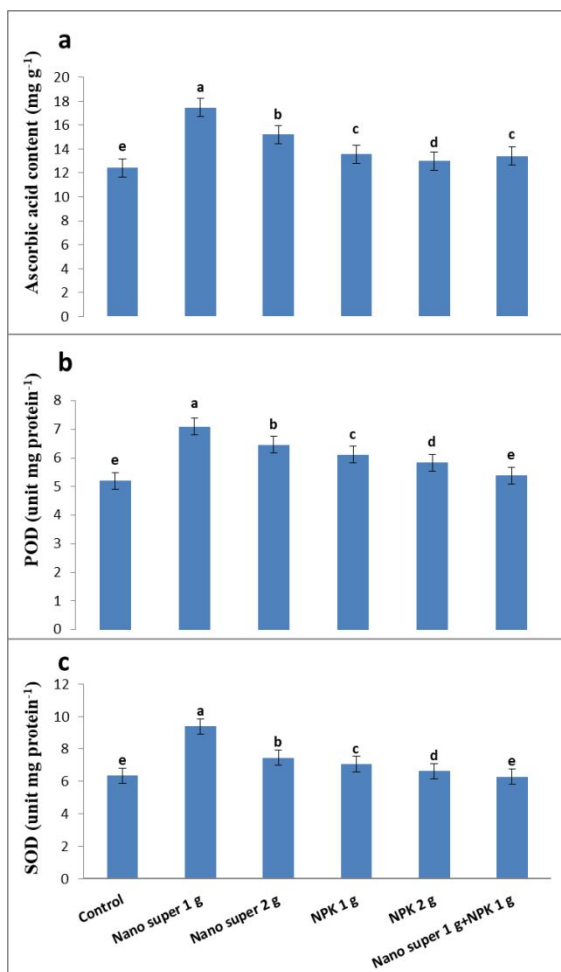


Fig. 1. Change of ascorbic acid content (a), the activity of peroxidase (b), and superoxide dismutase (c) in response to foliar traditional and nano-fertilizer applications. The means of five replicates  $\pm$ SE. Bars with different letters are different at  $p \leq 0.05$  using Duncan's multiple range test.



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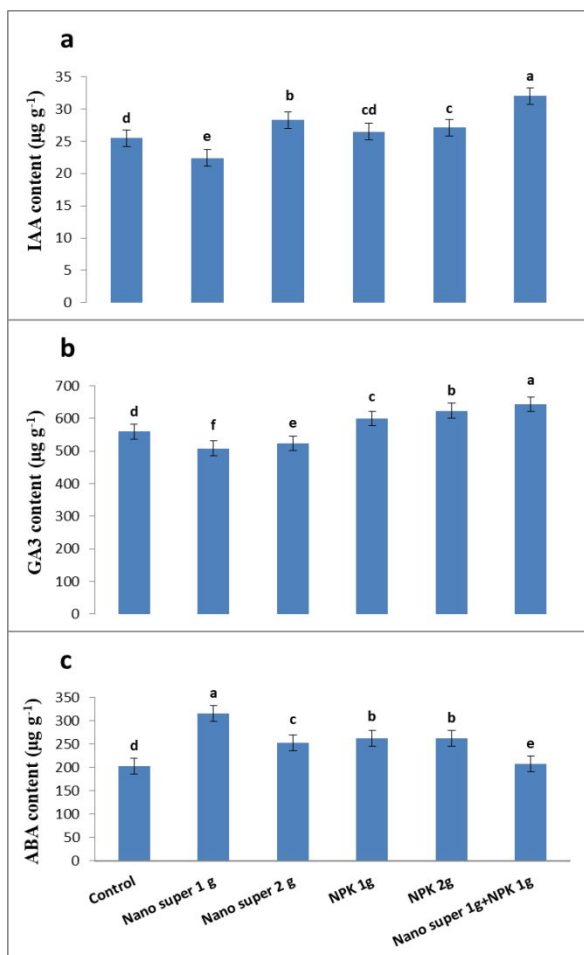


Fig. 2. Changes of IAA content (a), GA3 (b), and ABA(c) in response to foliar traditional and nano-fertilizer applications. The means of five replicates  $\pm$ SE. Bars with different letters are different at  $p \leq 0.05$  using Duncan's multiple range test.

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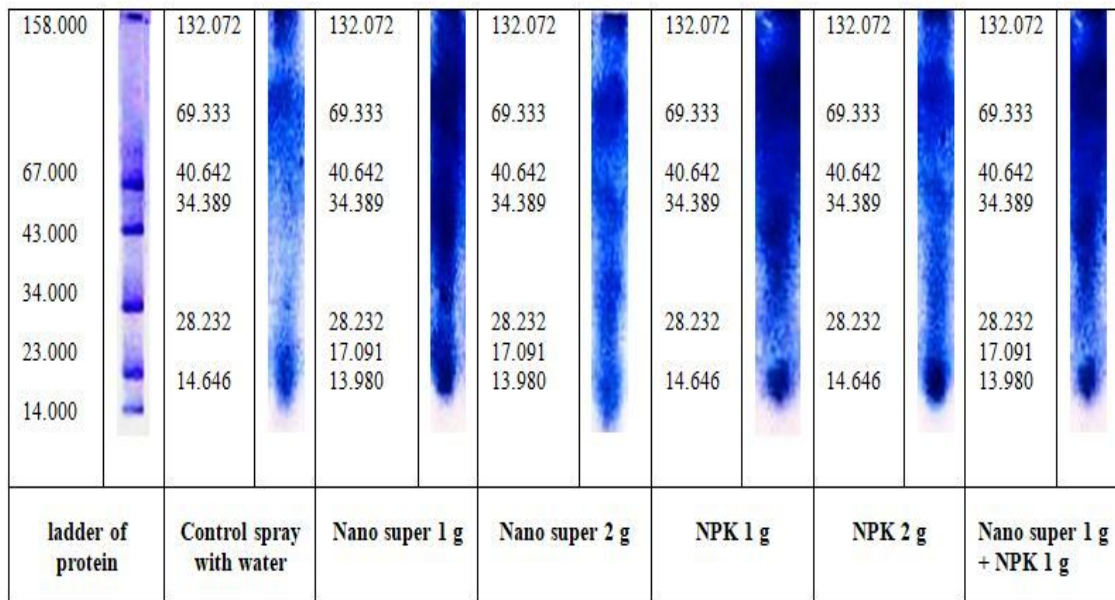


Fig. 3. Analysis of protein patterns by one-D SDS-PAGE extracted from the leaf, showing protein pattern changes in response to application of foliar traditional and nano-fertilizer.

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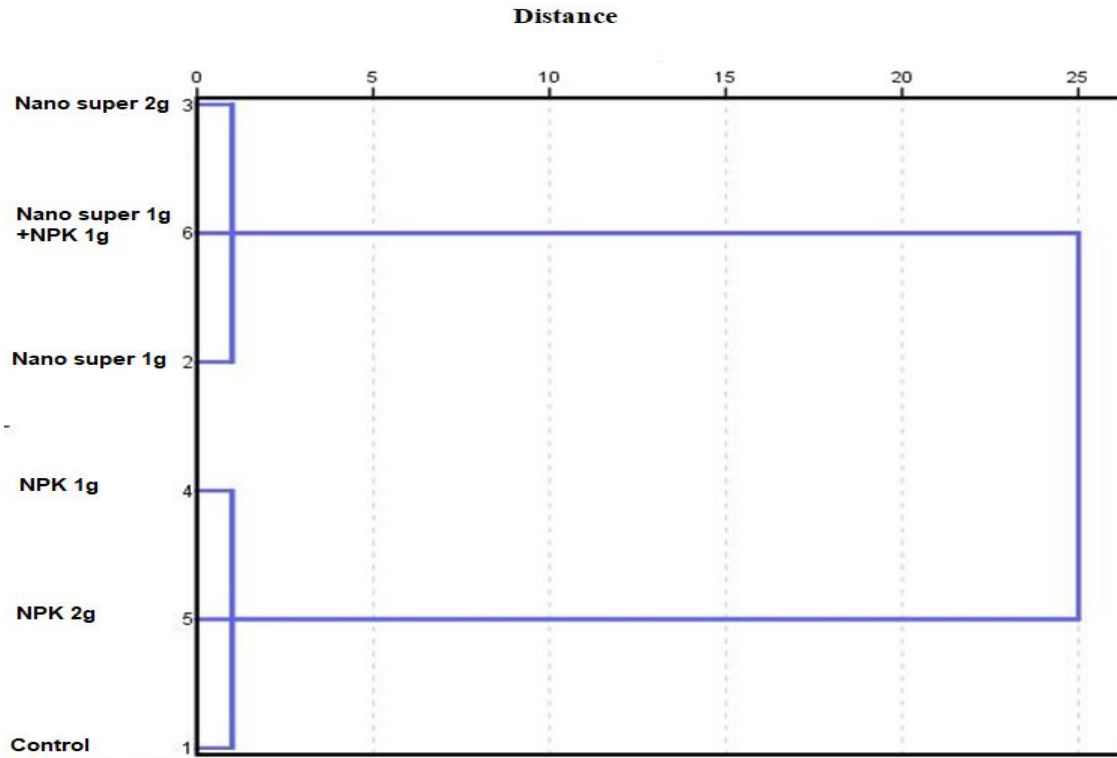


Fig. 4. Dendrogram of hierarchical clustering of leaf proteins pattern of date palm in response to foliar traditional and nano-fertilizer applications.