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Hydroxyapatite Nanoparticles as Effective Phosphorus Nano-fertilizer on Italian Parsley Plants

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ABSTRACT

Nanomaterials have been developed for various applications due to their unique features, and anew interest is now growing in their use in the agricultural sector as nano-fertilizers. Among the developed nanomaterials, hydroxyapatite nanoparticles (Nano-HAP) possess a relevant potential to be used as a source of nano-phosphorus fertilizer. Here, we evaluated the effect of these nanoparticles on growth traits, herb and essential oil yields, and histological changes of Italian parsley. The following fertilizer treatments were applied: control, NPK normal solution, Nano-HAP (0.25, 0.50, 1.0 g/L). The results showed that the foliar application of Nano-HAP tended to be comparable (and even superior) to that of conventional NPK fertilizer. Italian parsley responded positively to Nano-HAP treatments at 0.5 g/L with higher fresh (~1.5 to 3-fold increase) and dry herb yields and higher essential oil yields (10.64−20.97 L/ha) compared with the control and NPK treatments. Conversely, Nano-HAP at a high concentration of 1.0 g/L adversely influenced the growth and yield characteristics of the Italian parsley. In terms of anatomical traits, the Nano-HAP foliar application showed an improvement in the lamina and petiole measurements. Nano-HAP at 0.5 g/L produced the greatest values for the thickness of lamina, phloem tissue, xylem tissue, main vein of leaf lamina and oil gland diameter, as well as for the thickness of phloem and xylem tissues, and pith diameter of leaf petiole. Overall, our findings indicate that Nano-HAP at 0.5 g/L seems to be the most appropriate treatment for Italian parsley production.

KEYWORDS: Anatomical Traits, Essential Oil Yield, Herb Yield, Hydroxyapatite Nanoparticles and Italian Parsley

1. INTRODUCTION

One of the most promising nanotechnology applications in the agricultural sector is represented by nano-fertilizers, which contribute to improving plant growth and achieving sustainability in the global food production. The term nano-fertilizer refers to a material at the scale of nanometer (dimension of 1 to 100 nm), which allows a more efficient penetration into the plant, and delivery of macro and micronutrients in a controlled manner (Mejias et al., 2021). Nano-fertilizers characterized by targeted delivery of nutrients, controlled nutrient release, high mobility, as well as triggered cellular internalization have a great potential to boost plant productivity and overcome the nutrient deficiencies (Chugh et al., 2021). This consequently improves nutrient use efficiency, reduces fertilizer applications and costs, and it minimizes nutrient losses, thereby avoiding the extensive use of regular fertilizers, and reducing the deleterious impacts that they cause on the soil and ecosystem (Mandal, 2021; Zulfiqar et al., 2019). Also, nano-based materials are currently being explored for sustainable modern agriculture through various aspects as growth regulators, genetic transfer, pesticides, and antimicrobial agents (Farooq et al., 2022 a). Furthermore, one reasonable reason to apply nano-based fertilizers is to provide healthy food production which is free or limited from toxic heavy metals (such as arsenic) uptake by plants (Farooq et al., 2022b; Ulhassan et al., 2022).

Hydroxyapatite (HAP, Ca_{10} (PO_4) 6 (OH_2) is one of several crystal calcium phosphate forms that naturally occur in bones, teeth, and tendons, ensuring their stability, hardness, and functioning (Ferraz et al., 2004). The main advantage of using engineered HAPNPs compared to other nano materials is their substantial biocompatibility and biodegradability. For applications in agriculture, the HAPNP surface chemistry aids the efficiency of soil phosphorus allocation and plant uptake when the nanoparticles are added to the soil (Szameitat et al., 2021). Hydroxyapatite is insoluble in water, thus its involvement in chemical reactions with the soil, such as precipitation and adsorption on colloids, is reduced. HAPNPs enhance soil mobility and thereby they could improve phosphorus uptake by roots. Due to their higher dissolution rate and the faster release of soluble ions, they have been proposed as an alternative type of phosphorus fertilizers. During foliar application, nanoparticles (NPs) are deposited directly inside plants through nano sized pores of plasmodesmata, which usually carry ions between cells, or the stomata in leaves encouraging NPs uptake and transport into the plant leaf system. This leads to a higher nutrient use efficiency and consequent reduction in the use of P and waste of fertilizer (Mittal et al., 2020).

Phosphorus (P) is one of the macro-nutrients needed for plant growth and productivity. Only 10%−20% of the popular conventional P fertilizers are absorbed by plants from soil (Kopittke et al., 2019), while the remaining 80%−90% are rapidly converted to low-availability or fixed forms to insoluble inorganic compounds, or are precipitated with Fe / Al and Ca minerals (Folle et al., 1995). Consequently, excessive P applications are usually required, which may lead to the soil being saturated with P and to reduced sorption ability, as well as to increased P losses (Mozaffari & Sims, 1994). Moreover, the application of conventional P fertilizers superficially, leads to the accumulation of this element in the topsoil, and to the consequent reduction in its uptake by plants and increased loss through leaching or runoff (Ulén, 2006). Despite using scarcely soluble sources of P, such as rock phosphate and apatite to decrease phosphorus losses, the availability of this element to plants is reduced (Fellet et al., 2021). Thus, Nano-HAP could represent a more efficient and environmentally friendly innovation to replace conventional fertilizers.

Parsley (*Petroselinum crispum* L.) is a biannual herb, belongs to the Apiaceae family, widely grown in tropical and subtropical regions throughout diverse parts of the world. The three main parsley types cultivated worldwide are *Petroselinum crispum* var. neapolitanum (Italian-leafed), *Petroselinum crispum* var. crispum (curly-leafed), and *Petroselinum crispum* ssp. tuberosum (turnip-rooted or Hamburg type) (Petropoulos et al., 2004). Parsley is a culinary vegetable grown for its edible and pungent leaves; it is internationally exported and is consumed in fresh, dried, frozen, and preserved forms. Plain leaf parsley is employed to supplement flavor to fresh dishes, sauces, and soups, while curly-leafed parsley is ordinarily utilized for garnishing dishes or as a dried ingredient in herb mixtures (Zhang et al., 2006). Diverse parsley plant parts (leaves, stems, and roots) are precious sources of bioactive ingredients that have therapeutic properties. These active therapeutic natural compounds include furanocoumarins (Kovač-Besović et al., 2008), flavonoids (El-Sayed et al., 2018), polyphenols (Ancuceanu et al., 2018) and essential oils (Petropoulos et al., 2004). In addition, parsley has great nutraceutical values due to presence of e.g., vitamins, minerals, and fatty acids (Agyare et al., 2017; Aishwaya, 2018;

Dobričević et al., 2019).With respect to essential oil, the main components are 1,3,8-p-menthatriene and β-phellandrene, is presented for flat parsley, while curly-leafed parsley contains myrcene, 1,3,8-p-menthatriene, and myristicin (Liberal et al., 2020). Although its essential oil composition, it is of lower value than fresh and dried products which are more economically important and the main use. Due to presence these various therapeutic natural agents in parsley, it has shown many medicinal properties such as antioxidant, anti-tumor, anti-diabetic, antibacterial, antifungal, analgesic, diuretic,anti-inflammatory, immunosuppressant effects, spasmolytic, carminative, hypotensive, hepatoprotective,anticoagulant,gastroprotective ,neuroprotective, and nourishing agent (Agyare et al., 2017; Aishwaya, 2018; Farzaei et al., 2013; Fernandes et al., 2020).

Our aim in this investigation was to focus on the effects of using Nano-HAP (by foliar spray) as a source of P fertilizer on the growth, herb and total essential oil yields compared to the use of NPK normal solution (as conventional chemical fertilizer) for Italian parsley plants. To show the anatomical changes with and without Nano-HAP, we therefore studied the anatomy parameters following different treatments. This research is in the line with the growing interest for suitability of nano-fertilizers application includes Nano-HAP as a novel strategy to provide enhanced efficiency of plant production and minimize adverse environmental impacts with excessive use of conventional fertilizers. In our recent study we proved that Nano-HAP is efficient nano-fertilizer when employed through foliar application for *Rosmarinus officinalis* (Elsayed et al., 2022).

2. MATERIALS AND METHODS

2.1. Hydroxyapatite nanoparticales (Nano-HAP) synthesis.

Nano-HAP were synthesized via the methods of wet chemical precipitation modified from (Puvvada et al., 2010; Paz et al., 2012; Rodríguez-Lugo et al., 2018) according to our previous study (Elsayed et al., 2022). Calcium chloride dihydrate ($CaCl_2$ $2H_2O$) and ammonium dihydrogen phosphate (NH_4 H_2PO_4) were used as the starting precursors for calcium and phosphorus keeping their ratio at 1.67. Typically, solutions were separately prepared by dissolving calcium chloride (Fisher Scientific Co. UK) and ammonium dihydrogen phosphate (EL Nasr Pharmaceutical Chemicals Co. Egypt) in double distilled water under vigorous stirring at room temperature for thirteen minutes (RT) until obtaining the full dissolution. Subsequently, the solution of ethanolamine was dropped gradually into the calcium chloride solution and the total was then warmed, while stirring. The solution of phosphate was then slowly dropped into the calcium solution under continuous stirring, and the pH was adjusted 10 with adding a precipitating agent like NH_4OH solution. The action mixture was left to stir at 40°C, followed by an additional stirring phase of few hours at RT, and it was then aged for 24 hours at RT. Finally, the resulting product was filtered and washed several times with double distilled water and was placed in an oven at 70°C until dry. The resulting material was ground to obtain the Nano-HAP.

2.2. Site description and soil properties

The experiment was conducted in the open field during two successive seasons (2020–2021 and 2021–2022) at the Experimental Station of the Faculty of Agriculture, Cairo University, Giza (Egypt), located at 30° 05′ N, 31° 22′ E. Maximum and minimum temperatures during the growing season presented in Table (1) .The soil type is classified as a sandy clay loam and the physical and chemical analyses of the experimental soil are presented in Table (2).

2.3. Experimental design, plant materials, and treatment details

Italian parsley seeds (*Petroselinum crispum* L. var. *neapolitanum*) was planted directly in the field in mid-December in both seasons. The *Petroselinum crispum* L. the seeds were imported by Enza Zaden (Assem Doss Company, Egypt). Five fertilizer treatments including; control (distilled water only), NPK, NK+ 0.25g Nano-HAP/L, NK+ 0.50g Nano-HAP/L, NK+ 1.00g Nano-HAP/L.

Table 1. Maximum and minimum temperatures during the growing season.

Giza – Egypt				
Date	Maximum Temp. °C	Minimum Temp. °C	Relative humidity %	Soil temperature °C
Nov	25.6	19	58.4	20.3
Dec	24.1	18.5	62.1	19
Jan	22.5	13.5	59.1	14.1
Feb	25.4	14.3	57.4	15.7
Mar	28.2	15.7	53.2	17.4
Apr	30.4	16.5	46.2	20.9

Table 2. Physical and chemical analysis of the experimental soil.

Year	Sand	Silt	Clay	Texture
2020–2021	57.2	19.6	23.2	**SCL**
2021–2022	56.8	19.9	23.3	**SCL**

	pH	**E.C.**	**Millie equivalent/Liter**							
		(dSm^{-1})	**Cations**				**Anions**			
Year	**(2.5:1)**	**(5:1)**	**Ca^{++}**	**Mg^{++}**	**Na^{+}**	**K^{+}**	**CO_3^{--}**	**HCO_3^{-}**	**Cl^{--}**	**SO_4^{--}**
2020–2021	7.42	0.38	1.3	0.7	1.6	0.3	Nil	1.1	2.5	0.3
2021–2022	7.64	0.39	1.1	0.8	1.8	0.4	Nil	1.1	2.2	0.4

Nano-HAP Was assigned randomly in a randomized complete block design within the plots. The treatment was replicated three times. Each plot consisted of five rows, placed 50 cm apart, and the distance between plants in each row was 20 cm, for a total of 100,000 plants per ha. The central three rows in each plot were the experimental units, while the other two served as border rows. Irrigation, plant protection, and weed control were carried out as required. The fertilizer treatments used in the field experiment were: control (distilled water only), NPK, different doses of hydroxyapatite nanoparticles (0.25, 0.50 and 1.00 g/L). Based on soil testing and parsley nutrient requirements, nitrogen (N), (P) phosphorus and potassium (K) were applied as ammonium nitrate (33.5% N), calcium superphosphate (15.5% P2O5), and potassium sulfate (48.5% K2O) at the rates of 250, 120, and 125 kg/hectare (ha), respectively. The plants were sprinkled straightway with Nano-HAP using an Italian hand sprayer (Gamma 10), after strong shaking. Spraying was performed earlier in the morning until the leaves were fully water logged, four times during each season (every 15 days two times before the first harvest and anther two times before the second harvest) starting two months after the seeds were planted. Parsley plants were hand-harvested twice in early March and early April at a marketable foliage stage. During each harvest, plants were collected at 5 cm above ground level, and plant height, fresh and dry herb yield, as well as essential oil yield, were determined. The oil content of the foliage (a sample of 100 g from each treatment) was determined by a three hours hydro-distillation, using a Clevenger-type apparatus based on the method recommended in British pharmacopoeia. The total essential oil yield/ha was calculated per treatment based on the fresh herb yield, oil content, and harvested area. Samples were dried at 60°C for 72 hours for dry weight determination.

2.4. Essential oil extraction

To obtain the essential oil, fresh herb samples (100 g of each sample was placed in a 1 L distilling flask with adequate quantity of water) were subjected to hydro-distillation using a Clevenger apparatus with a distillation time of 3 hours until no further increase in the essential oil was noticed. The vapor is cooled by cold water in the condenser and the oil trap connected to the flask. The oil was collected after completing the distillation process and cooling the apparatus. The essential oil volume was recorded and calculated as (V/W) then dried over anhydrous sodium sulfate, and placed in dark glass vials at 4°C until GC analysis.

2.5. Essential oil components

Quantitative and qualitative data of the essential oil samples obtained from the second harvest of the second season were determined by Gas Chromatography (GC) analysis according to the method represented by Mihajilov-Krstev et al., 2009. Shimadzu capillary Gas Chromatograph 2010 plus was used for GC analysis, coupled with Flame Ionization Detector 2010 Plus detector (Shimadzu) fitted with 30 m_0.25 mm i.d. Stabilwax column and film thickness of 0.25 mm was used, with the helium as carrier gas at the flow rate of 1.0 mL/min with 1:10 split ratio. The temperatures of injector and detector were 210˚C and 250˚C, respectively. Temperature programming was applied at 4°C/ min heating rate starting from 40˚C for 1 min (initial temperature) to 150°C for 6 min then to 210˚C for 1 min (final temperature). Each essential oil sample was diluted (1:10, v/v) with GC-grade *n*-hexane then 0.2 μL was always injected into the GC system for analysis. Identification of oil components was performed mostly using GC standards. The automatic integrator was used to compute each peak area. The qualitative analysis was based on the percent area of each peak of the sample compounds. From the obtained chromatogram and analysis report, the concentration of each component as a percent of the total was calculated. The total area of the peaks represented the whole sample were obtained by summing the peak areas. The relative peak area for each component of the oil was calculated as the ratio between their peak areas and the total peak area, multiplied by 100.

2.6. Anatomical studies

The specimens collected during the second growing season of 2021−2022 included leaflet lamina and petiole of the parsley cultivar under study, namely, Italian-leafed (*Petroselinum crispum* var. *neapolitanum*). Samples were prepared according to the method of Nassar and EL-sahhar (1998) at the Laboratory of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza. Permanent sections were analyzed microscopically and photomicrographed.

2.7. Statistical analysis

The obtained data of each season in the randomized complete block design were subjected to the analysis of variance, which was conducted in MSTAT-C V.2.1 (Freed et al., 1989) separately for each harvest, based on the procedures reported by (Gomez & Gomez, 1984). Differences among means were compared for each trait using the Duncan multiple range test at a probability level of 5% (Duncan, 1955).

3. RESULTS AND DISCUSSION

3.1. Nano particles of hydroxyapatite

The Nano-HAP was less than 100 nm, with a calculated average size of ~ 24 nm, and they exhibited both a rod- and sphere-like morphology. In addition, they were aggregated in clusters, which is a common behavior for these nanoparticles (Elsayed et al., 2022).

3.2. Growth and yield characteristics

It is evident from Table (3) and Fig. (1) that Italian-leafed parsley plants responded significantly to all fertilizer treatments compared with the control in both harvests across both seasons. The addition of NPK, or any of the three Nano-HAP treatments, resulted in taller plants with larger fresh and dry herb yields (tons ha^{-1}) compared to the control. Maximum plant heights were gained from plants sprayed with Nano-HAP at 0.50 g/L, except for the first harvest in the second season, where NPK produced the tallest plants with no significant differences detected between Nano-HAP at 0.25 g/L and 0.50 g/L.

The improved plant height was associated with marked increases in the fresh herb yield. Of the four fertilizer treatments that significantly improved parsley biomass (NPK and all the Nano-HAP treatments), Nano-HAP at 0.50 g/L was significantly more efficient in enhancing the fresh herb yield than the others, increasing it by around 1.5 to 3-fold in both seasons, compared to the control. The maximum values were achieved by Nano-HAP at 0.50 g/L (ranging from 17.20 to 21.29 tons/ha^{-1}) followed by the application at 0.25 g/L (ranging from10.28 to 15.30 tons/ha^{-1}), or

Table 3. Growth and yield characteristics of Italian-leafed parsley treated with foliar application of Nano-HAP and NPK fertilizers in the first and second seasons.

Season 1						
	1st harvest			2nd harvest		
Treat.	**Plant height**	**Fresh herb (tons/ha)**	**Dry herb (tons/ha)**	**Plant height (cm)**	**Fresh herb (tons/ha)**	**Dry herb (tons/ha)**
Control	14.0 b	8.61 d	3.00 d	25.0 c	13.00 b	4.26 b
NPK	15.3 b	14.68 b	4.93bc	25.7 bc	18.41 a	6.11 a
0.25	23.0 a	15.30 b	5.31 b	29.0 b	10.28 c	3.59bc
0.50	23.3 a	21.29 a	6.68 a	39.3 a	17.20 a	6.20 a
1.00	13.0 b	12.40 c	4.12 c	23.3 c	7.72 d	2.61 c
Season 2						
	1st harvest			2nd harvest		
Control	11.0 c	7.33 d	2.58 c	24.0 c	10.97 c	3.74 c
NPK	23.0 a	11.26 c	4.29 b	29.3 b	15.03 b	5.18 b
0.25	21.3 a	12.83 bc	4.38 b	33.7 b	10.66 c	3.46 c
0.50	21.0 a	20.32 a	6.74 a	39.7 a	20.92 a	6.69 a
1.00	15.7 b	13.81 b	5.01 b	32.0 b	10.13 c	3.29 c

Nano-HAP at doses of 0.25, 0.50 and 1.00 g/L.
*Values with the same letters in each column indicate no significant difference between treatments at the 5% level of probability.

Fig.1. Effect of foliar spray with NPK conventional solution and different concentrations of Nano- hydroxyapatite (0.25, 0.5 and 1.0 g/L) on Italian parsley plants.

NPK treatment (ranging from 11.26 to 18.40 tons/ha^{-1}). No significant differences were noticed between the last two treatments in the first harvest of both seasons. By increasing the concentration of Nano-HAP from 0.50 g/L to 1 g/L, the fresh herb yield was significantly reduced. This may be due to the higher concentration affecting the internalization of Nano-HAP into the leaves, although the yield was still above or equal to that in the control, except for the second harvest of the first season where it was lower than in the control.

The pattern observed for the fresh yield was also detected for the dry herb yield (tons ha^{-1}), with significant differences between fertilizer treatments and control. The dry herb yield was highest in plants sprayed with Nano-HAP at 0.50 g/L (ranging from 6.20 to 6.74 tons/ha^{-1}) resulting in more than twice the yield observed in the control treatment. The positive effects of Nano-HAP on plant growth can be ascribed to their ability to penetrate cells, and their small particle size, in addition to the large surface area that regulates release kinetics to specific sites, making them a smart delivery approach for improving P use efficiency and plant uptake, either through absorption by roots or as foliar fertilizer. Studies have shown that the sustained and slow release of Nano-HAP appears to be a readily available and more effective and environmentally friendly P source than that derived from conventional fertilizers (Borm et al., 2006; Bindraban et al., 2015;Montalvo et al., 2015; Fellet et al., 2021; Szameitat et al., 2021). In addition, using apatite nanoparticles as an alternative P source can potentially enhance the agronomical yield and reduce the risks of water eutrophication. Also, the dissolution of Nano-HAP results in the release of orthophosphate ions which are the same ions released by common phosphate salts, and the sole P form used in plant metabolism (Szameitat et al., 2021). Another possible reason can be calcium nourishment, in addition to P nutrition and enhanced gibberellin hormone in plants (Maghsoodi et al., 2020).

A better plant growth was observed with nano-fertilizers compared to the use of traditional NPK. On one hand, this could be related to the fact that P is an essential component of the ATP molecule, which is responsible for storing and transferring energy. This is needed for the vital metabolic processes that occur in plants and that control photosynthesis, respiration, protein synthesis, glycolysis, nutrient translocation and transport through the plant's cells. In addition, these processes have the potential to transfer genetic characteristics from one generation to the next. On the other hand, calcium participates in enzymatic and hormonal processes, and in the metabolic process related to another nutrient uptake, as well as promoting proper plant cell elongation and strengthening of cell walls (Farhan et al., 2021).

Similarly, to what observed the present study, some reports have highlighted the beneficial effects of Nano-HAP on the growth and development of different plant species. (Lin et al., 2021) investigated the influence of Nano-HAP on *Camellia oleifera* seedlings and showed that Nano-HAP with the size of 200nm significantly improved the height, stem diameter, and biomass, followed by those measuring20nm and 80μm. This improvement is due to the increased content of available P in the rhizosphere soil, leaf N, and P concentration, particularly in unfavorable climatic conditions. Another report on the exposure of dragon's head *Lallemantia iberica* plants to nano iron-containing NPK fertilizer showed that it improved plant production compared with the use of traditional fertilizers(Mohammad et al., 2020). In addition, baobab (*Adansonia digitata*) plants sprayed with Nano-HAP showed a significant increase in various growth attributes (plant height, stem diameter, number of leaves per plant, leaf area, root length, total dry weight) compared to those exposed to conventional P fertilizers (Soliman et al., 2016). The same effects on plant growth and phosphorus concentration were observed for apatite nanoparticles applied on lettuce plants, possibly attributed to their small size and high surface to volume ratio (Taşkın et al., 2018).

The obtained results agree with our recent finding shows that Nano-HAP improve growth and biomass production of rosemary plants when treated through foliar application compared to NPK (Elsayed et al., 2022). The impact on crop production and yield efficiency due to the foliar spray of Nano-HAP can be related to easier penetration and translocation in plant leaves (El-Saadony et al., 2021).

3.3. Essential oil yield

Oil content (% dry mass basis) of Italian parsley ranged from 0.06 to 0.23 in the first harvest and from 0.16 to 0.37 in the second harvest. As shown in Fig. (2) Nano-HAP and NPK fertilizers significantly affected the essential oil yield (L/h^{-1}) of Italian parsley plants during both harvests in both seasons. The maximum values were produced from plants treated with Nano-HAP at 0.50 g/L, amounting to 10.64 to 11.43 L/ha in the first harvest and 20.97 L/ha in the second harvest of the second season, except for the second harvest of the first season where the NPK treatment displayed the greatest oil yield (22.09 L/h^{-1}).

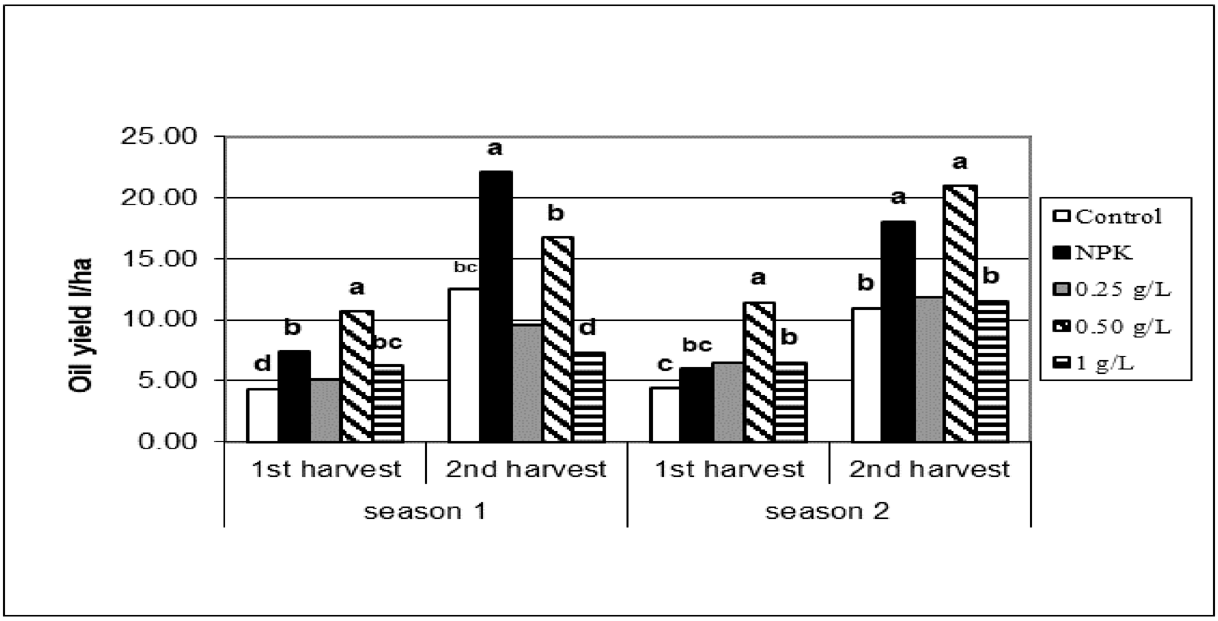

Fig.2. Effect of foliar spray with NPK conventional solution & different concentrations of Nano-HAP (0.25, 0.50 and 1.00 g/L ; three doses of Nano-HAP) on essential oil yield of Italian parsley in two seasons.

The higher oil yield obtained with Nano-HAP at 0.50 g/L or NPK treatments could be due to the greater fresh herb yield compared to essential oil content %. This result coincides with the findings of (Mikhak et al., 2017) in that study, it was shown that the application of both nCp/Nano-HAP particles and conventional P fertilizer could promote chamomile growth. Both Cp-$NH4^{+}$ and Nano-HAP exhibited the highest mean values for plant height, branches number, flowers number, P content, and fresh and dry weights of shoots and flowers, which were reflected in the oil and chamazulene yields. In addition, (Alhasan, 2020) stated that different rates of NPK nano-fertilizer enhanced basil yield of essential oil compared to the control. Moreover, nano-fertilizers and nano-zeolites had superior effects on sage essential oil yield, although the nano-zeolites and nano-NPK proved to be more efficient in the enhancement of essential oil productivity than single nano-phosphorus did. This may be attributed to their effects on the available elements, vitamins, gibberellins, cytokines, hormone-like substances, amino acids, and sugars, which lead to an increase in the biochemical processes occurring in the plant and to a consequent increase in volatile oil content (Mahmoud & Swaefy, 2020).

3.4. Essential oil composition

The aromatic profiles of Italian parsley plants are detailed in Table (4). GC analysis (Gas Chromatography) of the essential oil constituents led to the identification of 15 compounds accounting for 98.8 % of the volatile constituents. Italian parsley was characterized by the predominance of 1,3,8-p-Menthatriene (32.5%), Myristicin (24.7%) and Sabinene (16.5%). Considerable amounts of α-pinene, β-pinene, β-Myrcene, α-Phellandrene, α-terpinolene, p-cymene, 5-Isopropenyl-2-methylcyclopent-1-enecarboxaldehyde, β-Elemene and Germacrene D. (Craft & Setzer, 2017). In addition to Table (4) showed the effect of nano-fertilizers on the essential oil constituents of Italian parsley plants which turned out to be the highest value of essential oil constituents for the plants were treated by Nano-HAP0.5 g/L for Italian parsley plants (Elsayed et al., 2022).

3.5. Leaf anatomy

The anatomical measurements of lamina and petiole of the Italian parsley plant cultivar sprayed with different treatments are presented in Table (5). Microphotographs that depict these treatments are shown in Figures (3 & 4).

Table 4. Essential oil constituents (%) of Italian parsley plants in the second season.

	Ret. Time	Kovats index	Formula	Control	NPK	Nano-HAP		
						0.25	0.5	1.0
α-pinene	6.4	939	C10H16	2.1	2.5	2.3	2.8	2.6
β-pinene	8.7	980	C10H16	1.1	1.4	1.2	1.6	1.3
β-Myrcene	10.6	991	C10H16	4.7	4.9	5.7	5.6	5.5
α-Phellandrene	11.3	1005	C10H16	2.2	2.5	2.5	3.3	3.0
Sabinene	11.7	976	C10H16	14.3	15.6	15.4	16.5	15.9
α-terpinolene	12.9	1088	C10H16	0.9	1.1	1.0	1.1	0.8
p-cymene	14.3	1026	C10H14	1.5	1.8	2.6	2.7	2.5
1,3,8-p-Menthatriene	18.7	1110	C10H14	31.6	32.4	32.1	32.5	32.0
5-Isopropenyl-2-methylcyclopent-1-enecarboxaldehyde	20.0	1271	C10H14O	2.9	2.8	3.1	3.5	3.3
β-Elemene	20.1	1375	C15H24	1.2	1.1	1.4	1.6	1.4
Germacrene D	25.1	1480	C15H24	0.7	1.1	1.2	1.0	1.1
2-Caren-4-ol	25.3	1178	C10H16O	0.8	0.5	1.1	0.8	0.9
trans-Caryophyllene	28.4	1428	C15H24	0.5	0.6	0.5	0.6	0.4
γ-Elemene	30.8	1430	C15H24	0.4	0.6	0.4	0.5	0.5
Myristicin	49.2	1520	C11H12O3	23.5	24.0	23.9	24.7	24.5

Table 5. Anatomical measurements (µm) of leaflet lamina and petiole of Italian parsley affected by foliar spray with NPK normal solution & three concentrations of Nano-HAP (0.25, 0.5 and 1.0 g/l).

Characters	Treatments				
	Control	NPK	Nano- HAP		
			0.25	0.5	1.0
a- Lamina					
Upper epidermis	19.5 d	25.07 a	20.5 c	23.4 b	13.9 e
Lamina thickness	252.9 d	330.9 b	289.3 c	339.2 a	251.6 e
Palisade T. thickness	97.93 c	131.23 a	85.17 d	107.2 b	107.6 b
Spongy T. thickness	133.4 d	174.6 c	187.5 ab	189.9 a	184.03 b
Vascular bundle thickness	243.7 a	177.5 d	218.3 b	217.7 b	180.6 c
Xylem thickness	130.6 c	126.0 d	134.6 b	141.7 a	112.2 e
Phloem thickness	68.9 a	53.9 d	47.0 e	66.7 b	64.4 c
Gland diameter	64.8 e	70.6 d	89.6 b	93.1 a	76.2 c
Main vein thickness	707.8 b	583.4 e	643.1 d	718.4 a	684.5 c
Lower epidermis	13.7 b	10.5 c	13.6 b	16.7 a	12.0 c
b. Petiole					
Epidermis thickness	15.9 e	29.4 a	17.0 d	22.9 b	21.1 c
Collenchyma thickness	104.1 e	114.1 d	125.8 c	217.8 a	134.8 b
Parenchyma thickness	206.7 e	235.4 c	217.2 d	373.5 a	288.0 b
Xylem thickness	243.3 d	262.2 c	321.9 a	292.7 b	218.3 e
Phloem thickness	133.2 a	71.6 d	56.6 e	115.2 b	95.8 c
Pith diameter	1223.7 d	1554.6 b	1699.2 a	1557.6 b	1536.3 c
Cross section diameter	2151.3 e	2453.5 c	2587.5 b	2595.5 a	2432.9 d

Nano-HAP applied with three concentrations; 0.25, 0,50 and 1.00 g/L

*Values with the same letters in each row indicate no significant difference between treatments at the 5% level of probability.

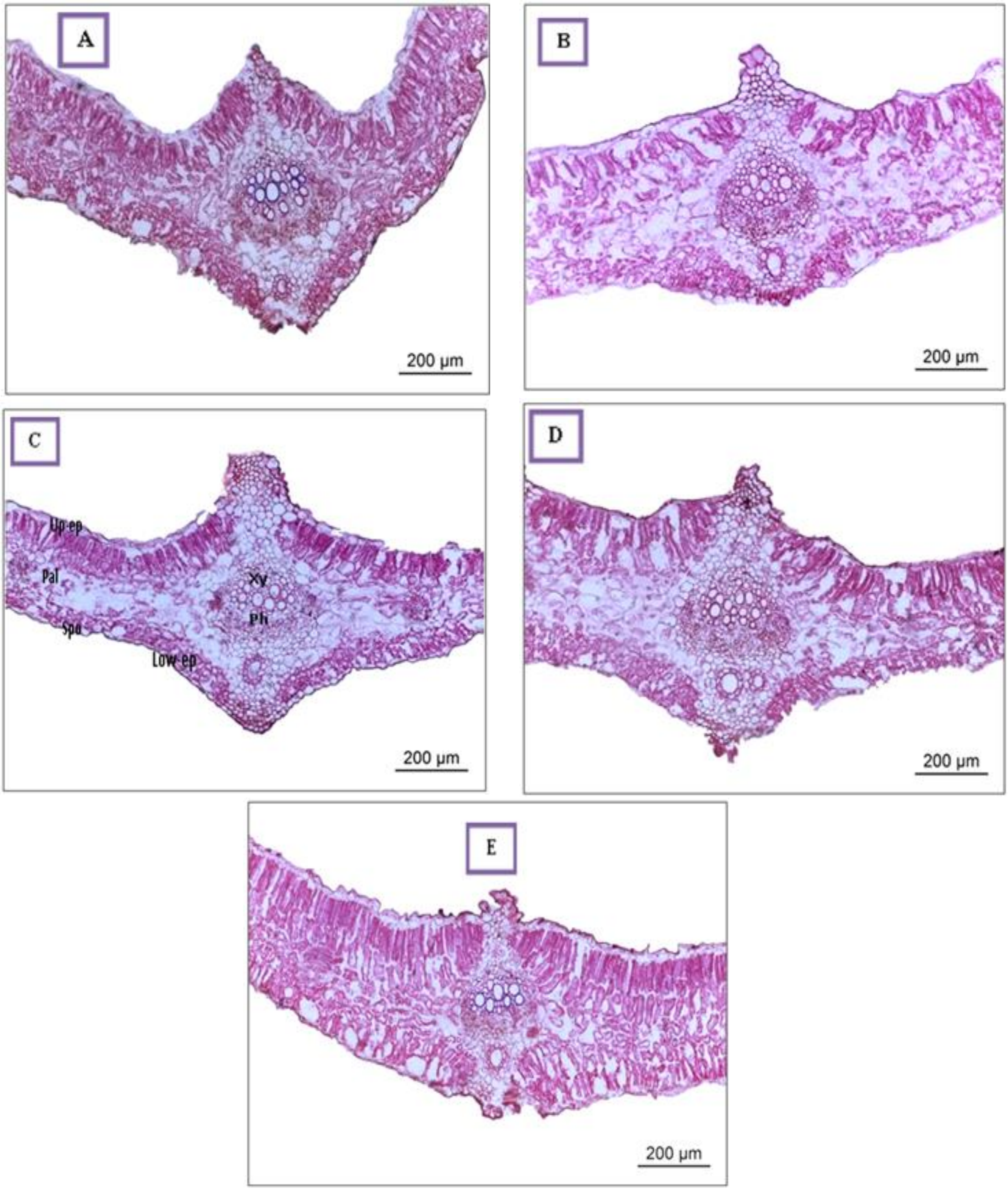

Fig.3. Cross sections of the leaflet lamina of Italian leafed parsley. A: untreated plant (control). B, C and D: plants sprayed with different concentrations of Nano-HAP 0.25, 0.5 and 1.0 g/l, E: plant sprayed with NPK normal solution.

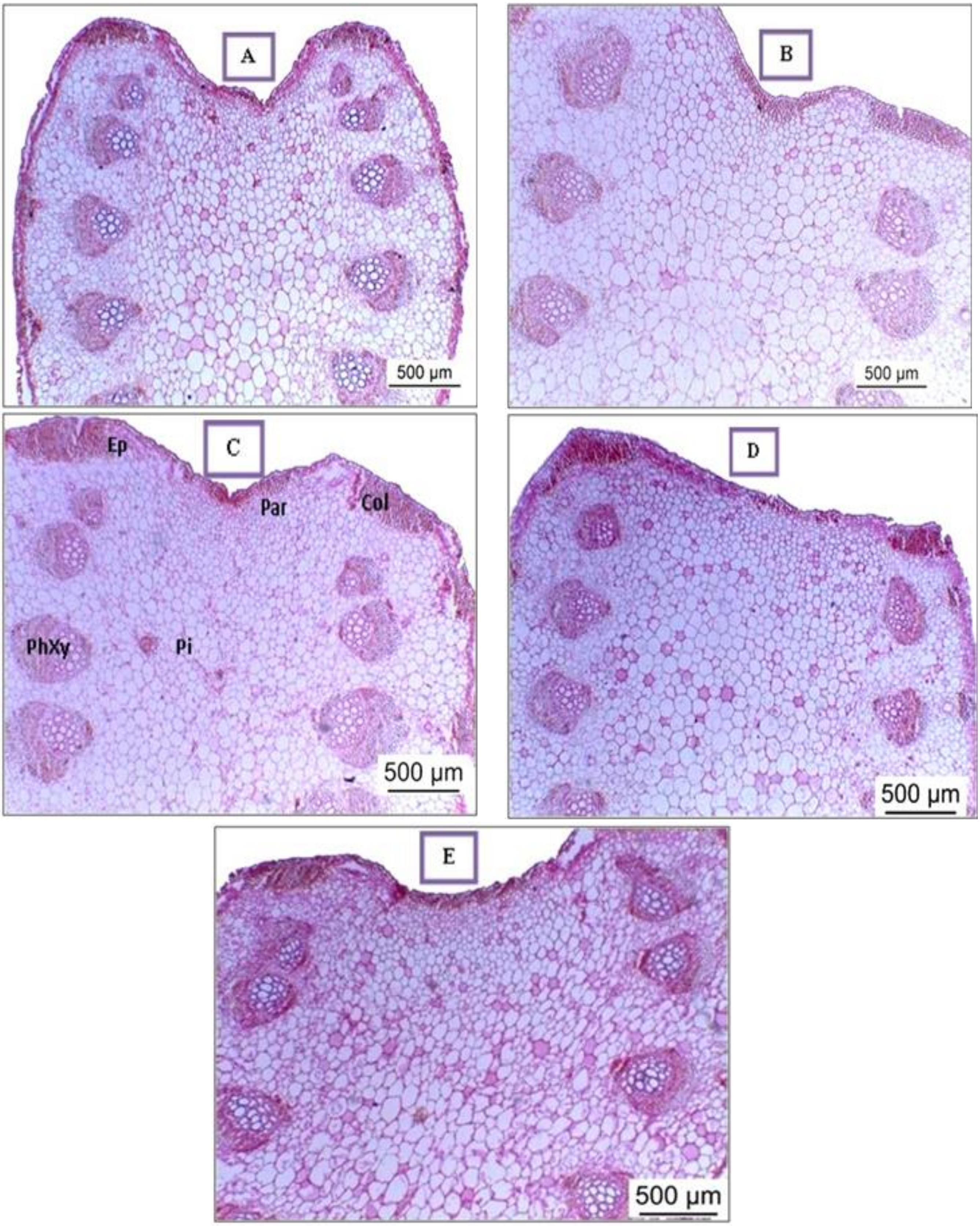

Fig.4. Cross sections through the petiole of Italian leafed parsley. A: untreated plant (control). B, C and D: plants sprayed with different concentrations of Nano-HAP 0.25, 0.5 and 1.0 g/l, E: plant sprayed with NPK normal solution.

a. Lamina

Mature leaf of the Italian parsley cultivar is pinnately combined with numerous leaflets (five to seven). Under a light microscope, the lamina through each leaflet consists of two epidermal layers (upper and lower) with a mesophyll in between (Figure, 3). The mesophyll differentiates into palisade and spongy cells. The palisade tissue consists of two layers of cells and the spongy tissue is composed of four to five layers of chlorenchymatous loosely arranged cells with intercellular space.

It is obvious from Table (5) that foliar spraying with Nano-HAP led to an increase in lamina thickness at all concentrations for the Italian cultivar; the highest thickness values were achieved by Nano-HAP at 0.5 g/l which was 339.2 µm compared to 252.9µm for the control plants.

It is noteworthy that Nano-HAP treatments had a clear effect on the mesophyll area (palisade and spongy tissues) of Italian-leafed parsley, which was greater than the effect on the vascular bundle in the main vein, and this may be associated with nanoparticle uptake and translocation within tissues. (Grillo et al., 2021) mentioned that particle uptake by leaves can occur in different ways. Nanoparticles can internalize the leaves either through cuticular or stomatal pathways, via symplastic or apoplastic transport. The stomata openings in most of the leaves display apertures in the range of micrometers, and these are spontaneous routes for particle uptake and transportation through the leaf mesophyll.

The oil gland is located below the vascular bundle of the midrib, which achieved the highest diameter when the leaves were treated with Nano-HAP at a concentration of 0.5 g/l with values of93.1µm for Italian cultivar compared with 64.8 µm recorded in the control. Increases in the diameter of the secretory glands may be the reason for the increase in the essential oil content of parsley plants. However, Metcalfe and Chalk (Rowson, 1950) mentioned that secretory canals contain a mixture of oils, resin, and mucilage. They are present in the pith, pericycle, and cortex of the petiole and extend into the lamina next to the vascular bundles. These canals probably occur in all species of the Apiaceae family and their arrangement, which is associated with the vascular bundles of the veins, is assumed to be of specific diagnostic value.

b. Leaf petiole

As shown in the cross-sections in Figure (4) the adaxial petioles of Italian-leafed parsley leaves presented a deeply or slightly concave outline and became relatively flat at 1.0 g/L Nano-HAP. The internal structure of the petiole consists of four main parts: the epidermis, hypodermis (collenchyma cells), ground tissue (parenchyma cells), and vascular bundles. In all treatments, the vascular bundles were arranged in a crescent shape that ranged between nine and11 bundles.

In Italian-leafed parsley, it is clear that the use of NPK normal solution led to the highest epidermis thickness (29.4 µm) compared to the other treatments, while the Nano-HAP treatment at 0.5 g/L showed the best results for collenchyma thickness, parenchyma thickness, and cross-section diameter (217.8, 373.5, and 2595.5 µm, respectively). Moreover, Nano-HAP at 0.25 g/L produced the highest values for both xylem thickness and pith diameter (321.9 and 1699.2 µm, respectively). At the same time, the highest phloem thickness (133.2 µm) was recorded in the control compared to all treatments.

In general, the results obtained from the Nano-HAP treatments as foliar applications showed improved leaf anatomical measurements (lamina and petiole) for Italian cultivar compared with those obtained from the NPK normal solution application. These improvements may be due to the beneficial effects of nanoparticles, which have a high reactivity as they emerge from a greater density of reactive areas and more specific surface area (Soliman et al., 2016). Once nanoparticles internalize the cell, they translocate via apoplast or symplast pathways, traveling through the plasmodesmata of one cell to another's and finally reaching the cytoplasm. In the cytoplasm, they begin to distribute among different cytoplasmic organelles and participate in various metabolic pathways of cells (Mittal et al., 2020). The xylem and phloem also play crucial roles in the transport of NPs, while the vacuole and cell wall serve as the main nanoparticle accumulation sites (Hong et al., 2021). It is evinced that nanosized particles (43 nm) are deeply inserted in the bulk of the leaf's interior compartment, which suggests the effectiveness of nano-fertilizers in enhancing nutrient uptake (Eichert et al., 2008).

4. CONCLUISON

The prepared Nano-HAP treatments showed great significance throughout the planned studies on the industrial crop production of Italian parsley cultivar, used mainly for export due to their benefits in food and health as well. Indicating the suitability of Nano-HAP as phosphorus fertilizer for enhanced plant productivity of Italian parsley cultivar and was comparable to the traditional application of NPK fertilizer. The main finding was related to the growth, yield and essential oil yield– the main characteristics considered for parsley production and use. Nano-HAP treatment at 0.50 g/L was significantly more efficient that enhanced fresh herb yield with a maximum fresh yield achieved was more than 20 tons/ha^{-1}and dry yield more than 6.5 tons/ha^{-1}. Also, essential oil yield reached more than 20 L/ha for Italian parsley. Similarly, this treatment enhanced thickness of many anatomical leaf and petiole characteristics (such as upper epidermis, lamina, palisade T., Spongy T., vascular bundle, xylem, phloem, gland, epidermis, and collenchyma) as result of foliar spray of Nano-HAP at 0.5 g/L compared with other treatments. Taken all together, it is recommended to supply parsley plants with Nano-HAP at a concentration of 0.5g/L, which, the results of the present study support the use of nano-fertilizer materials in the cultivation of medicinal and aromatic plants with potential for industrial production.

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الملخص العربي

جزيئات الهيدروكسي اباتايت النانومترية كسماد نانومتري فوسفوري فعال على نباتات البقدونس الايطالي

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تم تطوير المواد النانونية لتطبيقات مختلفة نظرا لميزاتها الفريدة ، ويتزايد الإهتمام الآن بإستخدامها فى القطاع الزراعى كأسمدة نانونية. ومن بين المواد النانوية المطورة، تمتلك جزيئات الهيدروكسي اباتيت النانوية (Nano–HAP) إمكانية مناسبة لإستخدامها كمصدر للأسمدة الفوسفورية النانوية. ومن هنا، قمنا بتقييم تأثير هذه الجزيئات النانونية على صفات النمو، وعلى محصول كلا من العشب والزيت العطرى، والتغيرات التشريحية لنباتات البقدونس الإيطالى. تم تطبيق معاملات الأسمدة التالية: الكنترول، المحلول التقليدى لسماد (NPK)، وتركيزات مختلفة لسماد الهيدروكسي اباتيت النانونية ؛ ٠,٢٥ ، ٠,٥ و ١,٠ جم/ لتر. أظهرت النتائج أن التطبيق الورقى لجزيئات الهيدروكسي اباتيت النانونية تميل أن تكون قابلة للمقارنة (بل وهى متفوقة) مع سماد (NPK) التقليدى. استجاب البقدونس الإيطالى بشكل إيجابى للمعاملة بجزيئات الهيدروكسي اباتيت النانونية عند تركيز ٠,٥ جم / لتر حيث أعطى أعلى إنتاجية من العشب الطازج (حوالى ١,٥ إلى ٣ أضعاف) والجاف وإنتاجية أعلى لمحصول الزيت العطرى (١٠,٦٤ – ٢٠,٩٧ لتر/هكتار) مقارنة بالكنترول والمعاملة بسماد (NPK) التقليدى. وعلى العكس من ذلك، أن المعاملة بتركيز عالى من الجزيئات النانونية قدره ١,٠ جم/لتر أثر سلباً على صفات النمو والمحصول للبقدونس الإيطالى.

ومن حيث الصفات التشريحية ، أظهر التطبيق الورقى بجزيئات الهيدروكسي اباتيت النانونية تحسنا فى قياسات نصل الورقة والعنق مقارناً بسماد (NPK) التقليدى. حيث أنتجت المعاملة بسماد النانو عند تركيز ٠,٥ جم/لتر أكبر قيم لسمك نصل الورقة،وكلا من أنسجة اللحاء والخشب و سمك العرق الوسطى وقطر الغدة الزيتية، وكذلك لسمك أنسجة اللحاء والخشب وقطر النخاع لعنق الورقة. بشكل عام، تشير النتائج التى توصلنا إليها أن جزيئات الهيدروكسي اباتيت النانونية عند تركيز ٠,٥ جم/لتر تبدو أنها أفضل معاملة لإنتاج البقدونس الإيطالى.

الكلمات المفتاحية: الصفات التشريحية، محصول الزيت العطرى، محصول العشب، جزيئات الهيدروكسي اباتيت النانونية و البقدونس الإيطالى.