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RESPONSE OF "MANZANILLO" OLIVE SEEDLINGS TO UNCONVENTIONAL NITROGEN APPLICATIONS AND GROWTH STIMULI

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ABSTRACT

This study was undertaken to evaluate the response of "Manzanillo" olive (*Olea europea* L.) seedlings to unconventional nitrogen applications and growth stimuli treatments on growth parameters and plant chemical compositions. A pot experiment was carried out during two consecutive seasons of 2018 and 2019: from March to October of each season at the Experimental Farm of Plant Production Dept. Fac. Environ. Agric. Sci., Arish Univ. Egypt. An approximate one-year-old uniform "Manzanillo" olive seedlings were transferred into black polyethylene bags (30 cm diameter and 40 cm depth) filled with 4.0 kg growth media *q.e.* mixture of sandy soil and peat moss at mixture ratio of 4:1 by volume. Olive seedlings were sprayed with different sources of nitrogen (0.0 mg N L^{-1} as control, 500 mg nano-N L^{-1} and 1000 mg urea L^{-1}) followed with growth stimuli additions (humic acid, amino acid and humic acid+amino acids) at different intervals. The obtained results showed that three times application of nano-N foliar fertilizer in combination with humic+amino acids improved plant growth characteristics such as root system, vegetative growth, pigments and leaf nutrients content. Moreover, using N fertilizer in the form of nanoparticles reduced the required amount of N to plant comparing with traditional form of N reflected on saving the environment from contamination and the loss of access addition of traditional N fertilizer by leaching or ammonification. In general, the foliar application of N fertilizer at different forms was the easiest way to fertilize olive plant and most suitable for plant absorption process.

INTRODUCTION

Olive trees had high economic values in Egypt and many countries in Mediterranean's sea regions since they use it for pickling, oil extraction or for both purposes. Olive (*Olea europaea* L.) is an evergreen tree belonging to the Oleaceae family that includes 30 genera **(Azawi and Salih Majeed, 2019)**. Olive tree spreads a lot in the Mediterranean basin and is considered one of the most important and oldest major crops in this region **(Hagag *et al.*, 2013 and 2015; Shahin *et al.*, 2015; Osama, 2015)**. Olive is successfully cultivated in the irrigated semi-arid areas in Egypt. In 2019, the harvested area in Egypt reached approximately 899,42 ha and the production was about one-million ton **(FAO, 2019)**. "Manzanillo" is the most important commercial group in the world as it is an early ripening variety **(Hussein and Abd-Elallc, 2018)**.

Although olive's nutrient requirements are lower than that for many other fruit trees, shortage in these requirements costs the tree major physiological disorders **(Dimassi *et al.*, 1999; Popovic *et al.*, 1999)**. Nitrogen is one of the essential nutrients needed by plants, mainly for chlorophyll buildup and associated with high

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photosynthetic activity **(Jasrotia *et al.*, 1999; Bouranis *et al.*, 2001)**. However, nitrogen uptake and metabolism are a key factor for olive roots to change the pH of their surrounding solution, which facilitates nutrients uptake by increasing their availability to the plant **(Cesco *et al.*, 1999)**.

Nano-fertilizers are responsible for providing one or more types of nutrients for developing plants, accelerating their growth, and increasing productivity. However, the response of plants to Nano-fertilizers varies according to the plant type, growth stages, and the nature of the nanomaterials themselves **(Liu and Lal, 2015; Chhipa, 2017).**

Growth stimuli have a major impact on plants, so they improve the physiological processes of crops, making them more efficient and effective, improving flowers and nodes to increase productivity. Amino acids contribute to the regulation of metabolic processes to give plants the ability to withstand environmental conditions and also increase the content of plant tissues of proteins in addition to their effect in increasing number of leaves and their area **(Azawi and SalihMajeed, 2019).** Humic acid is the main component of humus, are known to stimulate plant growth and help in cell respiration, photosynthesis, water and nutrient absorption, enzyme activities, protein synthesis, tolerance to dehydration, seed germination, and overall plant performance (**Hagag *et al.*, 2013; Shahin *et al.*, 2015**). One of the techniques that provides the tree's nutrient needs and is considered environmentally friendly is the foliar spray technology (**Ali *et al.*, 2019**). Therefore, the main objective of the current study is to evaluate the response of using different nitrogen sources (0.0 mg N L^{-1} as control, 500 mg nano-N L^{-1} and 1000 mg urea L^{-1}) in combination with different treatment of growth stimuli (humic acid, amino acid and humic acid+amino acids) applied at different time intervals on Manzanillo seedlings growth and chemical properties.

MATERIALS AND METHODS

This study was started on March 2018 and lasted until October 2019 at the Experimental Farm of Plant Production Dept. Fac. Environ. Agric. Sci., Arish Uni. Egypt. Forty-five "Manzanillo" olive one-year-old uniform seedlings were transferred into black polyethylene bags (30 cm diameter and 40 cm depth). Bags were filled with 4 kg growth media *q.e.* mixture of sandy soil and peat moss (mixture ratio of 4:1 by volume). Growth media was treated with a mixture of Rizolex-T 50 WP (Wettable Powder), fungicide (contains 20% Tolclophos-methyl and 30% Thiram) and fermented organic matter (Compost).

These seedlings were subjected to three different nitrogen fertilizer sources: (i) control as tap water, (ii) urea (1000 mgL^{-1}) and (iii) N nanoparticles (nano-N, 500 mgL^{-1}). The proposed fertilizers were sprayed to the plant three times, at mid-March, mid-April, and mid-May. Moreover, three different growth stimuli treatments (humic acid, amino acid and humic acid + amino acids) were sprayed five days later to spraying them with different nitrogen fertilizer sources. Seedlings were regularly irrigated with tap water to maintain water field capacity.

Preparation of Different Sources of N Forms

Urea-N fertilizer

Briefly, 1000 mg urea-N L^{-1} was prepared by dissolving 2.14 g urea-N (46%, 99% purity) in 250 mL water and diluted to final volume of 1 liter for further use.

Nano-N fertilizer

Nano-N fertilizer was prepared based on chitosan nanoparticles**.** Chitosan (Cs) nanoparticles were prepared by 0.9 g low molecular weight chitosan were dissolved in 250 ml distilled water contains 2.5 ml acetic acid for 20 min, then 50 ml sodium tripolyphosphate solution (TPP) contains 0.208g was dropped into the chitosan beaker at room temperature, after that chitosan solution was magnetically stirred for 45 min in order to obtain chitosan nanoparticles. These chitosan nanoparticles could be stored in distilled water at 2-8°C. It will be stable at the prepared size nearly 40-60 nm for two months. The prepared chitosan nanoparticles solution is of concentration 3mg/ml- 3000 ppm **(Tang *et al.*, 2007)**. After preparing chitosan nanoparticles, a sample was taken to take a microscopic image of it using a high-contrast Transmission electron microscope (TEM) model JEOL-1200 EX, Japan and Fourier transmission infrared (FTIR) spectroscopy (BRUKER ALPHA 11) **(Mahmoud *et al.*, 2018; Hussain *et al.*, 2016)**. Chitosan nanoparticles loaded with pure urea (46%) as a source of nitrogen. Briefly, 200 mL of Cs nanoparticles with a concentrate of 3000 $mgL^{-1}$and size volume of 40-60 nm were added in a conical flask with 100 mL of distilled water. A proportion of 0.33 urea-N (46%) were then added and stirred for continuous 6 hours using magnetic stirrer at room temperature. A stock solution of chitosan nanoparticles loaded with urea at final concentration of 3000 mg N L^{-1} was obtained and stored 5°C until the time of application. The preparation of working solution prepared from the stock solution was freshly prepared before every spraying time for olive seedlings **(Mohamad *et al.*, 2013)**. After preparing N nanoparticles, a sample was characterized using and a high-contrast transmission electron microscope (TEM) model JEOL-1200 EX, Japan and Fourier transmission infrared (FTIR) spectroscopy (BRUKER ALPHA 11) **(Hussain *et al.*, 2016; Mahmoud *et al.*, 2018).**

Amino acids mixture (1000ppm N) to confirm the quality of manufactured nano-N forms.

Growth Stimuli Treatments

Aminolom Complex was used as it contains micro and some elements and free amino acids and was composed of nitrogen 5.3%, iron 2.6%, manganese 2.6%, boron 0.9%, zinc 1.3% and sulfur 3.6% and free amino acids 10.6% are vegetable sources. As it contains 14 types of amino acid, (1.5 ml) was dissolved in 1 liter of water was used.

Humic Acid at 1000ppm N

Two mL of concentrated humic acid (HUMATEX) was dissolved in 1000 mL water to reach a concentration of 1000 mgL^{-1}. The chemical properties of humic acid used in the current study was obtained from the supplier as it contains 5.4% K and 32% Na. Moreover, 1.5 mL of concentrated amino acid (Aminolom Complex) was dissolved, as well, in 1000 mL water to reach a 1000 mgL^{-1}. The chemical properties of amino acid were provided from the supplier as it contains N 5.3%, Fe 2.6%, Mn 2.6%, B 0.9%, Zn 1.3% and S 3.6% with 14 types of amino acids.

Vegetative Growth Measurements

At the end of the growing season (mid-September) of both season, fresh weight of leaf and root were recorded by weighting on electrical balance, leaves dry weight (g) were measured. Briefly, all the leaves were individually separated from the grown seedlings and allocated on perforated paper bags in the electric oven at a temperature of 70°C for three days then their weights were measured using digital sensitive scale. Moisture content (M.C%) was calculated using this equation; (M.C% $=W_w - W_d / W_w \times 100$ where Ww and Wd were assigned to fresh weight and dry weight respectively. Seedling height (cm), shoot length (cm) and stem diameter (cm) were measured at a height of 5 cm from the mixture surface by Vernier Caliper **(Javed *et al.*, 2017).** Number of shoots, number of leaves and leaf area (LA cm^2) were measured. Briefly, leaf area was calculated using the fresh weighted method by taking ten leaves randomly from each seedling and the fresh weight of them was recorded with their corresponding disk known area for each leaf. The area of the leaf is calculated by compensation in equation according to **(Ackley, 1964)**.

Chemical Analyses

Random leaves were taken from each treatment and oven dried at 60ºC and dried samples were subjected for multi-element analyses. The plant content of N was measured using Nessler reagent and both P and K were measured using spectrophotometer (Model JENWAY 6300) and flame photometer, respectively according to the method described by **Bremner and Mulvaney (1982) and Mahmoud *et al*. (2018)**, respectively. Iron, Zn and Mn concentrations (mgL^{-1}) were determined using atomic absorption spectroscopy according to the method described by **AOAC (2000)**.

Biochemical Analyses

Chlorophyll a and b and carotenoids contents were estimated by taking samples from the third pair of leaves from the bottom of the branch. This was done by soaking 100 mg of fresh leaves after washing them thoroughly with water in 5 ml of N, N-Dimethylformamide (DMF) **(Azad *et al.*, 2019)** in dark-colored glassware and storing at a temperature of 4°C for 72 hours. The supernatants were then measured at wavelengths of 480, 647, 664 nm using the spectrophotometry. After that, compensation was given in the following equations accordin g to **(Moran, 1982; Vicas *et al.*, 2010; Purcarea and Cachita, 2008 and 2010):**

Chlorophyll a (mg/g sp) = (11.65 a664 – 2.69 a647) • V/sp

Chlorophyll b (mg/g sp) = (20.81 a647 – 4.53 a664) • V/sp

Carotenoids (mg/g sp) = (1000 A480 – 1.28 chloroph.a – 56.7 chloroph.b)/ 245 • V/sp

Statistical Analysis

Data were statistically analyzed with a complete randomized design (CRD) by using Co-STAT software, V.6.13 (CoHort software, Berkeley, CA 94701) on 6 treatments (3 N-fertilizers x 3 growth stimuli) and five replicates. Mean values of treatments were differentiated by using least significant range (Duncan's multiple range tests) at 0.05 level probability **(Duncan 1955).**

RESULTS

Charctrization Chitosan-TPP (Cs-TPP) / Urea (Cs-TPP-U) Nanoparticles

During the synthesis of chitosan nanoparticles (Cs-NPs), it was observed that the chitosan solution in sodium tripolyphosphate solution (TPP) changed from a clear to an opalescent suspension. This transformation is evidence of the formation of chitosan nanoparticles with TPP. In the present study synthesized Cs-TPP NPs (Photo-1) was immediately used to loaded by urea (u) in order to avoid change in its nanosize. However, **Fan *et al.* (2012)** reported that chitosan-TPP nanoparticles (Cs-TPP NP) prepared in acetic acid at their optimized conditions exhibited no significant changes in particle size and particle distribution after 20 days of storage at room temperature (10−20°C). The authors ascribed the stability of the particles to the following features of the nanoparticle suspensions. Low ionic strength, appropriate pH (5.4), small size, high cross-linking density, and high surface potential of the particles. On the contrary, **Lopez-Leo ́n *et al.* (2005)** found that Cs-TPP particles incubated in a nonbuffered, and low-ionic-strength solution were unstable in suspension over time. The authors suggested that Cs−TPP nanoparticles should be stored as freeze-dried samples. According to these opposing findings in the literature the manufactured Cs with urea was fresh prepared (as working solution) and applied to the plant (Photo-2 and Fig. 1).

Photos 1 and 2 show a transmission electron microscopy (TEM) image of the chitosan nanoparticles (Cs-TPP) with and without urea. The nanoparticles showed a spherical shape with a homogeneous size distribution. The mean diameter of the chitosan nanoparticles (in the dry state) was of approximately 45 ± 1.5 nm, which is higher in suspension due to the nanoparticles' swelling ability. It was found a clear hallow surrounding the Cs-TPP in photo (2) as evidence of urea loaded to Cs-TPP.

Fig. 1 shows the FTIR spectra of pure chitosan (Cs-TPP) and Cs-TPP-U (loaded with urea) nanoparticles. The FTIR spectrum obtained that the chitosan had characteristic peaks of; 3435 cm^{-1} corresponding to the stretching of the NH_2 and OH groups; 1649 cm^{-1} corresponding to the C=O group of amide I; 1083–1020cm^{-1} due to the stretching of C–O and 620 cm^{-1}, due to vibrations of the pyranosidic rings **(Tonhi and Plepis, 2002).** It is observed that the band at 1649 cm^{-1}, characteristic of pure chitosan, stretched more in the spectrum of the loaded urea nanoparticles and two new bands appeared at 1638 cm^{-1} (group –COO–) and 1545 cm^{-1} (group–NH_3^+), indicating the interaction between Cs and TPP. The main changes observed in the spectrum of the nanoparticles loaded with urea (Cs-TPP-U), when compared to the spectrum of Cs-TPP, occurred at a region between 2000–1000 cm^{-1} region. A strong peak was observed at 1500 cm^{-1} due to deformation of ammonium ions (–NH_4^+), **(Sterne *et al*., 1982).** This finding confirms the formation of interaction between –NH_2^+ groups of urea and –COO– groups of Cs-TPP nanoparticles to form the nitrogen fertilizer nanoparticles on chitosan materials.

Effect of Spraying Nitrogen Fertilizer and Growth Stimuli Treatments

Results in Tables 1, 2, 3 and 4 indicate that fertilization of olive seedlings of urea (1000 mgL^{-1}) significantly increased seedling height (129.2a cm) and Carotenoids (0.540a mg/100g F.W) in first Season. Leaf area (2.58a cm^2) and number of shoots/seedling (39.11 a) in second season comparing with control. Whereas in most cases, fertilization with N-nano (500 mgL^{-1}) is more effective in both seasons, and gave the highest value of No. of roots/seedling (290.7 and 300.3), shoot length (87.61cm and 89.29cm), moisture content (55.87% and 54.67%), chlorophyll a (1.23 and 1.24 mg/100g F.W), chlorophyll b (0.750 and 0.755 mg/ 100g F.W), N (2.82% and 2.80%), phosphorus (0.211% and 0.233%) and potassium (1.24% and 1.26%). Also, seedling height (145.3a cm) and Carotenoids (0.540a mg/100g F.W) in second season. Leaf area (2.50a cm^2) and No. of shoots/seedling (43.77a) in first season. while the least values in both seasons were recorded by control treatment.

Regarding the specific effect of growth stimuli treatment (Tables 1, 2, 3 and 4) results clearly showed that in 2018 and 2019 seasons, the olive seedling treated with either humic acid or humic acid and amino acid gained significantly greater shoot length (85.00 and 83.19 cm), leaf area (2.53 and 2.54 cm^2), No. of shoots/seedling (90.89 and 43.88), chlorophyll a (1.22 and1.3 mg/100g F.W), chlorophyll b (0.700 and 0.692 mg/100g F.W), N(2.42 and 2.41%), Phosphorus (0.196 and 0.223%) and Potassium (1.27 and 1.29 %). Also, moisture content (55.32%) in first season.

Meanwhile, amino acid treatment gave the highest seedling height (134.0 cm), No. of roots/seedling (279.88 and 288.33) in both seasons and moisture content (54.65%) in second season. Obviously, humic acid treatment gave the highest value of seedling height (123.3 cm) in first season and Carotenoids (0.546 and 0.540 mg/100g F.W) in both seasons. While, when olive seedlings fertilized with humic acid and humic acid and amino acid treatments gave the highest value of shoot length, as compared with the amino acid.

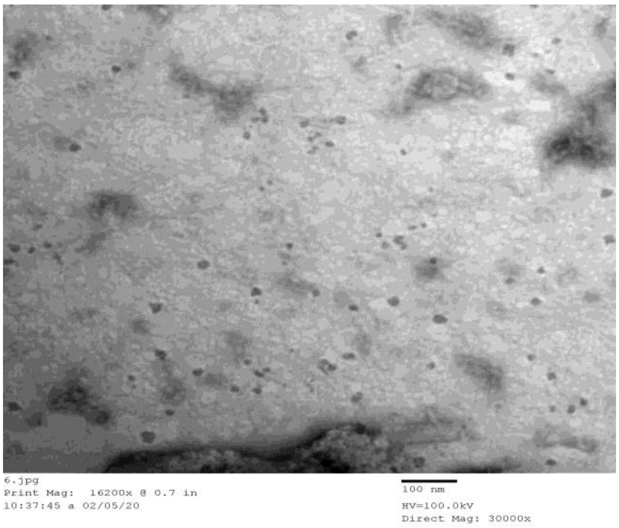

Photo 1. TEM microphotographs of Raw Cs-TPP nanoparticles

Photo 2. EM microphotographs of Cs-TPP nanoparticles loaded with Urea

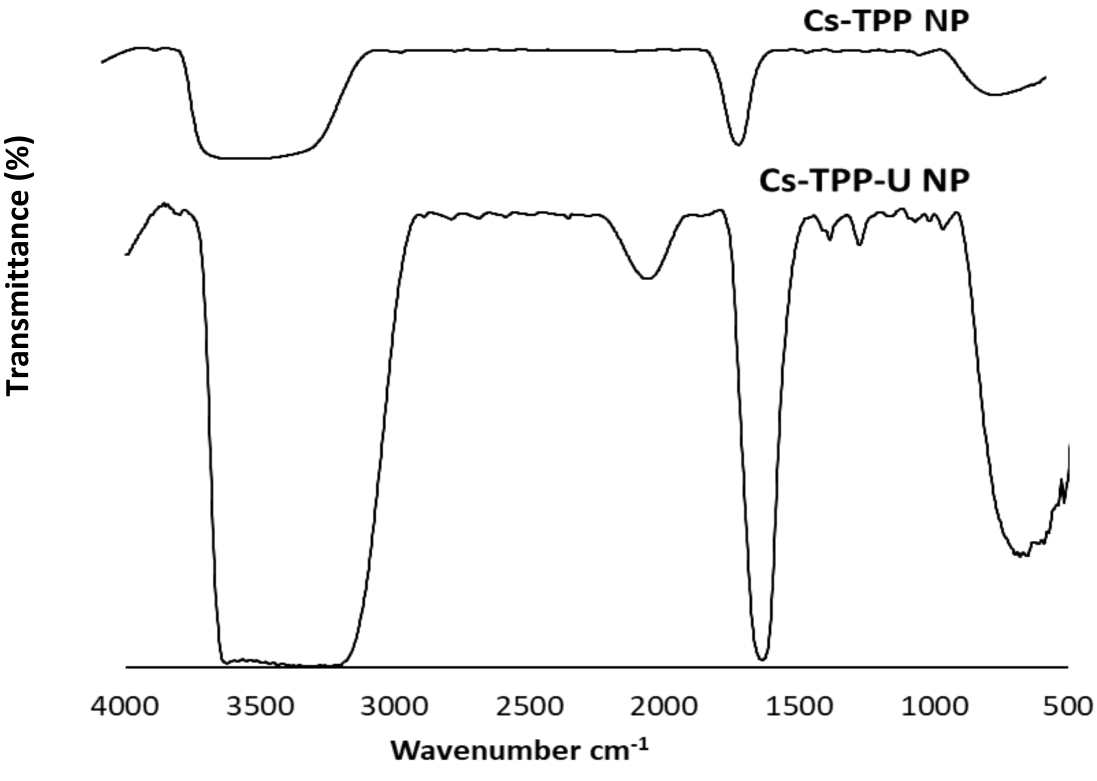

Fig. 1. Representative FT-IR transmittance spectra of raw Cs-TPP NP and Cs–TPP-U NP

Table 1. Effect of spraying nitrogen fertilizer and growth stimuli treatments on seedling height (cm), No. of roots/seedling and shoot length (cm) of "Manzanillo" olive seedlings during 2018 and 2019 seasons

Treatments	Seedling height (cm)				No. of roots/seedling				Shoot length (cm)			
	Amino acids	Humic acid	Humic & Amino acids	Mean	Amino acids	Humic acid	Humic & Amino acids	Mean	Amino acids	Humic acid	Humic & Amino acids	Mean
2018 Season												
Control	123.0 bc	132.0ab	114.8 cd	123.3 b	250.33 i	260.66 g	252.33h	254.4b	80.66 c	59.36 e	79.20 c	73.07 b
Urea (1000 ppm)	123.1 bc	124.5 b	140.0 a	129.2 a	282.66 d	293.33 b	282.33e	286.1a	65.80 d	92.46 b	94.56ab	84.27 a
Nano-N (500 ppm)	122.8 bc	113.5 d	113.3 d	116.5 c	306.66 a	273.00 f	292.33c	290.7a	81.36 c	100.23 a	81.23 c	87.61 a
Mean	122.97 a	123.3 a	122.7 a		279.88 a	275.66 a	275.66a		75.94 b	84.02 a	85.00 a	
2019 Season												
Control	116.0 fg	112.5 g	119.8 ef	116.1 c	272.33 e	261.00 f	253.00g	262.1c	80.26 d	74.00 e	76.53 e	76.93c
Urea (1000 ppm)	121.0 e	132.6 d	142.16 c	131.9 b	288.33 c	291.33 bc	280.33d	286.7b	84.33 c	73.56 e	86.43bc	81.44b
Nano-N (500 ppm)	165.00 a	149.3 b	121.6 e	145.3 a	304.33 a	301.66 a	295.00b	300.3a	81.43 d	99.83 a	86.60bc	89.29a
Mean	134.0 a	131.5 a	127.9 b		288.33 a	284.66 b	276.11c		82.01a	82.46a	83.19a	

* Means followed by the same letter(s) within each column are not significantly different at the 0.05 level, according to Duncan's multiple range test.

Table 2. Effect of spraying nitrogen fertilizer and growth stimuli treatments on leaf area, number of shoots/seedling and moisture content (%) of "Manzanillo" olive seedlings during 2018 and 2019 seasons

Treatments	leaf area (cm^2)				No. of shoots/seedling				Moisture content (%)			
	Amino acids	Humic acid	Humic & Amino acids	Mean	Amino acids	Humic acid	Humic & Amino acids	Mean	Amino acids	Humic acid	Humic & Amino acids	Mean
2018 Season												
Control	2.21 d	2.30 cd	2.60 b	2.37 b	35.33 b	36.66 b	33.66 b	35.22 b	54.26 e	50.20 i	53.64 g	52.70 b
Urea (1000 ppm)	2.59 b	2.30 cd	2.22 b	2.37 b	36.66 b	35.00 a	35.33 b	35.66 b	56.56 c	53.70 f	54.56 d	54.94 a
Nano-N (500 ppm)	2.40 c	2.31 cd	2.78 a	2.50 a	51.00 a	46.00 a	34.32 b	43.77 a	53.03 h	56.83 b	57.76 a	55.87 a
Mean	2.40 b	2.30 c	2.53 a		41.00 a	39.22 a	34.44 b		54.62 a	53.58 b	55.32 a	
2019 Season												
Control	2.22 e	2.30 d	2.59 b	2.37 b	33.00 c	42.66 b	36.00 c	37.22 a	57.90ab	52.26 de	52.36de	54.51 a
Urea (1000 ppm)	2.63 b	2.32 d	2.78 a	2.58 a	33.66 c	41.00 b	42.66 b	39.11 a	54.40cd	55.90 bc	49.46 e	53.25 a
Nano-N (500 ppm)	2.42 c	2.30 d	2.25 de	2.32 c	32.66 c	48.00 a	34.66 c	38.44 a	51.66de	54.26 cd	58.98a	54.67a
Mean	2.41 b	2.31 c	2.54 a		33.11 c	43.88 a	37.77 b		54.65 a	54.14 ab	53.60 b	

* Means followed by the same letter(s) within each column are not significantly different at the 0.05 level, according to Duncan's multiple range test.

Table 3. Effect of spraying nitrogen fertilizer and growth stimuli treatments on chlorophyll a (mg/100g F.W), chlorophyll b (mg/100g F.W) and carotenoids (mg/100g F.W) of "Manzanillo" olive seedlings during 2018 and 2019 seasons

Treatments	Chlorophyll a (mg/100g F.W)				Chlorophyll b (mg/100g F.W)				Carotenoids (mg/100g F.W)			
	Amino acids	Humic acid	Humic & Amino acids	Mean	Amino acids	Humic acid	Humic & Amino acids	Mean	Amino acids	Humic acid	Humic & Amino acids	Mean
2018 Season												
Control	1.05 h	1.18 de	1.16 e	1.13 c	0.615 f	0.665 d	0.645 e	0.642 c	0.513 h	0.526 f	0.493 i	0.511 b
Urea (1000 ppm)	1.27 b	1.19 d	1.14 f	1.20 b	0.656 de	0.640 e	0.673 d	0.656 b	0.536 c	0.553 b	0.530 e	0.540a
Nano-N (500 ppm)	1.25 c	1.10 g	1.35 a	1.23 a	0.716 c	0.754 b	0.781 a	0.750 a	0.533 d	0.558 a	0.522 g	0.538ab
Mean	1.19 b	1.16 c	1.22 a		0.662 c	0.686 b	0.700 a		0.527 b	0.546a	0.515 c	
2019 Season												
Control	1.04 f	1.18 c	1.16 d	1.13 c	0.617 f	0.664 d	0.649de	0.643 b	0.510cd	0.538abc	0.497 d	0.515 b
Urea (1000 ppm)	1.26 b	1.18 c	1.15 d	1.20 b	0.661 d	0.636 ef	0.635 ef	0.644 b	0.543ab	0.52bcd	0.520bc	0.530ab
Nano-N (500 ppm)	1.25 b	1.10 e	1.37 a	1.24 a	0.720 c	0.753 b	0.794 a	0.755 a	0.53abc	0.561 a	0.52bcd	0.540 a
Mean	1.18 b	1.15 c	1.23 a		0.666 b	0.684 a	0.692 a		0.528ab	0.540 a	0.512 b	

* Means followed by the same letter(s) within each column are not significantly different at the 0.05 level, according to Duncan's multiple range test.

Table 4. Effect of spraying nitrogen fertilizer and growth stimuli treatments on nitrogen (%), phosphorus (%) and potassium (%) of "Manzanillo" olive seedlings during 2018 and 2019 seasons

Treatments	Nitrogen (%)				Phosphorus (%)				Potassium (%)			
	Amino acids	Humic acid	Humic& Amino acids	Mean	Amino acids	Humic acid	Humic & Amino acids	Mean	Amino acids	Humic acid	Humic & Amino acids	Mean
2018 Season												
Control	1.82 gh	1.74 h	1.94 f	1.83 c	0.165 f	0.173 ef	0.184de	0.174 c	0.89 e	0.98 e	1.14 cd	1.00 b
Urea (1000 ppm)	1.86 fg	2.07 e	2.22 d	2.05 b	0.194 cd	0.208 ab	0.185de	0.196 b	1.12 cd	1.29 b	1.26 b	1.22 a
Nano-N (500 ppm)	2.89 b	2.46 c	3.11 a	2.82 a	0.211 ab	0.201 bc	0.221 a	0.211 a	1.10 d	1.22 bc	1.41 a	1.24 a
Mean	2.19 b	2.09 c	2.42 a		0.190 a	0.194 a	0.196 a		1.04 c	1.16 b	1.27 a	
2019 Season												
Control	1.86 fg	1.74 g	1.95 ef	1.85 c	0.165 c	0.172 c	0.177 c	0.171 c	0.89 e	1.08 d	1.17 cd	1.05 c
Urea (1000 ppm)	1.86 fg	2.06 e	2.24 d	2.05 b	0.196 b	0.208 b	0.203 b	0.202 b	1.14 cd	1.13 cd	1.29 b	1.19 b
Nano-N (500 ppm)	2.87 b	2.51 c	3.03 a	2.80 a	0.211 b	0.200 b	0.289 a	0.233 a	1.13 cd	1.24 bc	1.42 a	1.26 a
Mean	2.20 b	2.10 c	2.41 a		0.191 b	0.193 b	0.223 a		1.05 c	1.15 b	1.29 a	

* Means followed by the same letter(s) within each column are not significantly different at the 0.05 level, according to Duncan's multiple range test.

Concerning, the interaction effect between varietal differences of nitrogen fertilizer and growth stimuli treatments, Table 1 reveals that the highest value of olive seedling height in the first seasons (140.0 cm) came from spray olive seedlings with urea (1000 mgL^{-1}) × humic acid& amino acid treatments (1000 mgL^{-1}), while in second season Nano-N (500 ppm) × Amino acids give the highest value (165.00 cm). But N-nano (500 mgL^{-1}) x amino acid treatment (1000 mgL^{-1}) gave the highest No. of roots/seedling (306.66 and 304.33) in 1^{st} and 2^{nd} seasons, shoot length give highest value (100.23 and 99.83 cm) with using Nano-N (500 ppm) × Humic acid in both seasons.

On the other hand, results in Tables 2, 3 and 4 proved that the highest value of leaf area (2.78 cm^2) came from spray with Nano-N (500 ppm) × Humic & Amino acids in first season, while in second season Urea (1000 ppm) × Humic & Amino acids give the highest value (2.78 cm^2). On the other hand, Nano-N (500 ppm) × Humic acid gave the highest No. of shoots/seedling (51.00 and 48.00) in 1^{st} and 2^{nd} seasons. As for, the interaction effect between nitrogen nanoparticles treatment (500 mgL^{-1}) × humic acid & amino acid treatments (1000 mgL^{-1}) shows a significant difference in both seasons for moisture content (57.76% and 58.98%), chlorophyll a (1.35 and 1.37 mg/100g F.W), chlorophyll b (0.781 and 0.794 mg/ 100g F.W), Carotenoids (0.558 and 0.561 mg/100g F.W), N (3.11% and 3.03%), phosphorus (0.221% and 0.289%) and potassium (1.41% and 1.42%). While the least values in both seasons was record by control treatment with spray amino acid or humic acid or humic acid & amino acid treatment in both seasons. The other treatments came in between with significant difference among them in this sphere.

DISCUSSION

The current results showed that the interaction effect between nitrogen nanoparticles treatment (500 mgL^{-1}) and humic acid and amino acid treatments (1000 mgL^{-1}) gave the highest vegetative growth parameters, organic and chemical compositions and improving the root system.

Nano fertilizers are important in increasing the efficiency of nutrients, having a higher yield, better quality, and safer environment. It reduces soil contamination as well as potential adverse effects when conventional mineral fertilizers are applied. These results are obtained by **Hayyawi *et al*. (2020)**. The authors reported that nano fertilizers (NF) are more efficient and effective than conventional fertilizers because of their positive effects on the quality of food crops, reduce stresses that occur to the plant, small, applied quantities and costs, their fast of absorption by plant cells and penetration of cells and the fats of transport and representation within plant tissue**.** However, nitrogen consider to be essential element for plant growth and development, The N gave the highest shoot and root dry weight, this probably due to nitrogen concentration which increased dry matter accumulation in roots and decreased shoot: root ratio **(Hegazy *et al*., 2007)**. Leaf nitrogen content with the mineral nitrogen only and mineral nitrogen source 100% + humic acid (monthly doses from March to November each 20 mL/plant) treatments showed the highest significant values compared with all other treatments. Nitrogen fertilization increased dry matter accumulation in roots and decreased shoot\root ratio. As to the nonconventional sources of organic matter suitable for soil amendments, different humic acid- derived materials have improved soil characteristics and plant growth **(Hagag *et al*. 2011).**

The positive effect of humic acid may be play very important role in stimulating plant growth by acting on mechanisms involved in cell respiration, photosynthesis, protein synthesis, water and nutrient uptake and enzyme activities **(Chen *et al.*, 2004; Ali *et al*., 2007)**. Humic acid is complex substances derived from organic mattercomplex substances derived from organic matterenhance nutrient uptake, drought tolerance, drought tolerance under field conditions **(Hagag *et al*. 2011)**.

Sladky and Tichy (1959) found that humic acid improved chlorophyll content of tomato leaves by 63% in plants grown in humic acid containing solution and by 15% in those grown in folic acid containing solution. Research shows that humic acid stimulated photosynthesizing activity in plants through increasing the activity of rubisco enzyme **(Delfine *et al*., 2005)**. Also, humic acid improves the uptake of nutrients and the yield of plants *via* forming stable complexes with nutrients, especially micronutrients like Fe and Zn **(Varanini and Pinton, 1995; Hagag *et al.*, 2016)**. **Danyaei *et al.* (2017)** found that the treatment with humic acid improved the uptake of Fe, Zn, Cu and Mn and that higher uptake of Fe and Mn enhanced chlorophyll content. Also, humic acid effect as a chelating and considered as induce for nutrient element act to increase capable capacity and increase availability of nutrient elements and then easier absorbed by plants and increase its concentration in plant tissue and building root system with highly efficiency for absorption of macro and micro nutrient elements which help to increase the quality of synthesized substances in leave to build plant tissues **(Al-Niemi, 1999; Fernandez-Escobar *et al.*, 1999)**. The results are in harmony with those obtained by **EL-Shazly and Ghieth (2019).**

On the other side, the previous results could be explained according to **Ghanta and Mitra (1993)** reported that improving growth characters in response to the foliar application of micronutrients, may be due to their positive action on increasing cell division in the meristematic tissues and accelerating carbohydrates and proteins formation **(Yousef *et al*., 2011; Ali *et al.*, 2019)**. Also, its might be due to that the application of humic acid increased the concentration of micronutrients in plants **(Atiyeh *et al.*, 2002 and Abdel-Mawgoud *et al.*, 2007).** As well as providing nutrient base that increase the activity of the microorganisms (**Tisdale *et al*., 1997**). Further than, improving the physical and chemical characteristics of the soil, all these have a positive impact on the plant growth and yield **(Hagag *et al.* 2014).** The ameliorative effect of amino acids might be linked to the observable increase in photosynthetic pigments and fact plant growth through their influence As well as, amino total as a source of amino acids may play an important role in plant metabolism and protein assimilation which is necessary for cell formation and consequently increase in fresh and dry matter **(Sh Sadak *et al.*, 2015)**. Also, amino acids contain 5% of components is enzymes of which very important to the process of photosynthesis as well as the processing of plant nitrogen directly, particularly if foliar spraying on the leaves **(Ali *et al*., 2019)**.

Conclusion

Finally, vegetative growth parameters, mineral content and total pigments analysis indicated that spray nitrogen nanoparticles at a concentration of 500 mgL^{-1} in combination with humic + amino acids three times annually gave promising results in terms of enhancing overall seedling positive characteristics.

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الملخص العربي

استجابة شتلات الزيتون المنزانيللو لمعاملات النيتروجين غير التقليدية ومحفزات النمو

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أجريت هذه الدراسة خلال موسمين متتاليين 2018-2019 حيث يبدأ كل موسم في شهر مارس وينتهي في شهر أكتوبر بمزرعة قسم الإنتاج النباتي، كلية العلوم الزراعية البيئية، جامعة العريش، مصر. وهدفت التجربة الى دراسة استجابة شتلات الزيتون المنزانيليو إلى معاملات من النيتروجين غير التقليدية ومحفزات النمو على قياسات النمو، والمحتوى من العناصر الكيميائية والعضوية. وتمت زراعة شتلات المنزانيليو عمر سنة فى أكياس بلاستيكية سوداء بقطر 30سم وارتفاع 40 سم تم ملؤها بـ 4كجم بيئة رمل: بيتموس بنسبة 4: 1 على التوالي. تضمنت هذه التجربة 3 معاملات لمصادر نيتروجينة (معاملة المقارنة، 1000ملجم/لتر يوريا، 500 ملجم/لتر نانو نيتروجين) وتم رشهم ثلاث مرات/العام، بالإضافة إلى رش 3 معاملات محفزات النمو (حمض الهيوميك، الأحماض الأمينية، مخلوط الأحماض الامينية وحمض الهيوميك). أظهرت النتائج أن رش النانو نيتروجين بتركيز 500 ملجم/لتر مع مخلوط الأحماض الأمينية وحمض الهيوميك ثلاث مرات/العام أعطى أعلى القيم لقياسات النمو الخضري والمحتوى من العناصر الكيميائية والعضوية مع تحسين المجموع الجذري.

الكلمات الاسترشادية: الزيتون، المنزانيللو، النانو نيتروجين، حمض الهيوميك، الأحماض الأمينية.

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