

## Application of nano-nitrogen fertilizers to enhance nitrogen efficiency for lettuce growth under different irrigation regimes

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Climate change has worsened the existing scenario by increasing temperature, severity of extreme droughts, elevating evapotranspiration and severe water shortage. Furthermore, excessive and unwisely application of fertilizers ultimately produce degraded agricultural land. All these consequences reduced the yield and quality of agricultural commodities to feed the increasing population of the world. Innovative products of trending technology in the field of agriculture, nanotechnology, contribute a significant boost for food production. The shortage of fresh water can be managed by adopting different efficient irrigation methods, also promote the quality and quantity of agricultural crops. By keeping in view, the above all, an experiment was conducted to evaluate the effect of nano nitrogen (nN) under different water regimes and assessed the growth attribute and other essential nutrient uptake by lettuce plant in different combination of bulk and nano nitrogen forms with surface and drip irrigation method. In this experiment, for the comparison of different irrigation methods, two control treatments were chosen, such as 100% bulk size nitrogen (bN) in surface irrigation and 100% bN application by drip irrigation. While nN was applied with bN in different combinations through drip irrigation and foliar application. Among all the combinations of nitrogen (N) fertilizer, application of 75% nN through drip irrigation and 25% of nN in foliar application significantly affect the growth and biochemical parameters such as plant biomass, leaf area, absolute growth rate, net assimilation rate,  $\beta$ -carotene, crude protein and yield. Similarly, N uptake, N use efficiency and apparent N recovery were increased by this combination as compared to lower N rates. The results indicated that the combined application of nN as a soil and foliar treatment was more efficient than that of soil application of bN. Furthermore; it could minimize the required N fertilization rate to reduce environmental pollution without any yield loss.

**Keywords:** Climate change, severe water shortage, drip irrigation, growth parameters, surface irrigation, nanoparticles, N-use efficiency.

### INTRODUCTION

The population of the world is increasing rapidly and facing the risk of hunger and food insecurity (Molotoks *et al.*, 2020). All these challenges are the outcome of climate change, global warming, unbalanced use of resources, and the decrease in arable agricultural land, which ultimately limit the production of food and enhance environmental hazards (Upadhyay, 2020; Molotoks *et al.*, 2020). Furthermore, it has been estimated that the population is going to reach 9.8 billion by the year 2050, indicating that 70% more food production is needed for the people than in 2005 (Kopittke *et al.*, 2019). To face this increase, the use of agrichemicals has

dramatically increased for high crop production (Ramírez-Rodríguez *et al.*, 2020). The application of mineral fertilizers is very important for ensuring food security, which about a 30-50% increase in crop yield reported (Steward and Roberts, 2012). Among the commercial fertilizers, nitrogen (N) is the most crucial nutrient for crop production globally, but its use efficiency (NUE) is still less than 50% (Mejias *et al.*, 2021). Nitrogenous fertilizers were counted as the biggest contributor to crop production, but about 40-70% of nitrogen is lost into the environment and is not utilizable by crops, which causes economic losses as well as very serious environmental pollution (Trenkel, 2010; Solanki *et al.*, 2015). Emission of nitrous oxide in the air and leaching of nitrate in

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the water cause contamination in the environment (Ramírez-Rodríguez *et al.*, 2020).

Agricultural production is enhanced by adopting sustainable agricultural practices that ultimately mitigate the risk of shortage of food (Adegbeye *et al.*, 2020; Sharma *et al.*, 2020; Rajput *et al.*, 2021). Now days, 6 billion people are continuously feed by sustainable agricultural practices without harming the environment. Nanotechnology, play a significant role in the continuous production of food crops in sustainable agriculture. Nano fertilizers may have the ability to enhance sustainable agriculture and increase the productivity of crops, mainly through boosting the nutrient use efficiency of these crops (Zulfiqar *et al.*, 2019). For example, nano-fertilizers were produced as a potential solution to reduce fertilizer loss in the environment and increase fertilizer use efficiency for crop production, hence decreasing the recommended dose of traditional fertilizers (Subramanian *et al.*, 2015). The nano-formulations of N-fertilizer may minimize N-losses by leaching, emissions, and soil microbial immobilization (Baruah and Dutta, 2009). Moreover, there are limited water resources in Egypt; several farmers are still using the surface irrigation method, which has low water use efficiency as well as less nutrient use efficiency. Therefore, alternative irrigation methods were applied in Egypt many years ago to overcome the water scarcity, like drip irrigation, which has a high-water use efficiency and nutrient use efficiency as well. Several bulk and nano size of fertilizers, particularly N-fertilizers, have been applied through drip irrigation by the fertigation technique. This technique promoted the yield stability of tomatoes by increasing N-use efficiency (Hu *et al.*, 2021), increased the yield of vegetables by reducing the emission of N<sub>2</sub>O and N-leaching under solar greenhouse production (Zhao *et al.*, 2021) and increased crops and water productivity and N-use efficiency (Li *et al.*, 2021). Now a days, drip fertigation has become a common practice for vegetable crops in field like lettuce and major crops (Li, 2017). This technique may also be considered a proper candidate to apply nano-fertilizers.

Nitrogen in nano-form has slow-release properties (Kottegoda *et al.*, 2011) and enhance nitrogen use efficiency (NUE) when applied through drip irrigation and foliar application. The NUE is improved because of the high specific surface area of nano fertilizers; they earned their properties as easier absorption by the plant, which improved the efficiency and the economic benefits with some extra benefits of improving soil properties and the ability of water and fertilizer conservation (Shalaby *et al.*, 2016; Usman *et al.*, 2020). High crop yield and quality were reported with the application of nano-fertilizers (Tantawy *et al.*, 2015; Prifti and Maci, 2017; Abd El-Azeim *et al.*, 2020). A few studies have been published on nano-N, which discuss the synthesis of nano crystalline N-doped-TiO<sub>2</sub> (Chaturvedi and Singh, 2021).

Hereafter, the main purposes of this study was to assess the growth and biochemical attributes in lettuce plant by (1) surface and drip irrigation methods; (2) forms of nitrogen fertilizer such as bulk and nano-N fertilizers (3), different portions of bulk and nano fertilizers in combinations, and (4) full application of fertilizers as soil application vs partial adding as foliar application in nano-form. Furthermore, the impact of these treatments on lettuce growth, yield, quality, and N use efficiency parameters was also investigated.

## MATERIALS AND METHODS

**Material collection and characterization:** The experiment was conducted at the experimental farm of the Faculty of Agriculture, Kafrelsheikh University, which is located in the northern part of Egypt and situated between 31° 37' N latitude and 20° 30' E longitude during the summer seasons of 2017 and 2018. Soil samples (0 to 30 cm depth) were collected from the field for pre-sowing analysis of soil during both years. Random soil samples were taken for chemical analysis (in supplementary file Table 1) as described by (Cottenie *et al.*, 1982). Chemical analysis such as saturated soil pH and electrical conductivity of saturated soil extract were determined by preparing the soil-saturated paste after air-drying, grinding and sieving through a 2 mm mesh size sieve. Soluble cations such as Ca+Mg were determined by the titration method, while Na and K were determined by flame photometer and anions like total carbonates were determined by titration method. Other mineral essential nutrients such as nitrogen (total and available) and phosphorus were analyzed by Kjeldahl and Olsen method. The organic matter status of the soil was determined by the Wakley-black method in laboratory. The nano nitrogen fertilizer was purchased from the Bio-Nano Technology Company, Egypt. The morphological characteristics of the nano-nitrogen fertilizer was imaged by Transmission Electron Microscopy (TEM) micrograph. The TEM images indices the presence of a droplet type Nano formulation structure, where the hydrophilic portions of the droplets were stained black, while the hydrophobic components were unstained and had a size range between 36 nm of nitrogen Nanoparticles in the foliar fertilizer (12% N) and 32.13 nm (20.4% N) of nitrogen Nanoparticles in the soil added fertilizers (liquid). Iceberg lettuce seeds from Aviram cv. (Hazera Co.) were planted during the summer season in trays and followed-up by the normal practices until the age of 25 days.

**Climatic conditions of experimental site and agricultural practices:** The temperature ranged between 25 and 30°C as maximum and 13-22°C as minimum, while RH was 77-81% as maximum and 41-48% as minimum, during the experiment periods from April to May. On April 1<sup>st</sup>, the seedlings were transplanted in rows with 7.84 m length and 0.70 m wide with 30 cm between plants. There is a raised bed as a border

between plots 100 cm to prevent any seepage. The harvest time was on 30<sup>th</sup> of May.

Two irrigation systems were applied in this experiment as follows:

1. Surface irrigation system (control), the fertilizers were added to the soil which traditionally followed in the experiment site. 2. Drip irrigation system was applied for all treatments except the control and the fertilizers were added by fertigation system.

Two nitrogen fertilizer sources were used as follows:

1. Ammonium nitrate (33% N) was added to the soil in the control plots or with drip irrigation system in the other related treatments. 2. Nitrogen nanoparticles from two sources were added either through drip irrigation system (20.4% N with particle size of 32.13 nm) by different portions, or as foliar application (12% N with particle size of 36 nm) at the concentration of 3 g L<sup>-1</sup> in the related treatments which 25% from the recommended N dose was sprayed. The studied treatments could be arranged as given in Table 1.

The treatments were arranged in a randomized complete block design with 7 treatments in three replicates. Each experimental unit included 7 rows with total area of 38.5 m<sup>2</sup>. The recommended dose of nitrogen for growing lettuce (210 kg N/ha) was considered as a base for calculations the needed dose of N in each treatment.

**Growth attributes:** Ten plants from each plot were randomly taken to evaluate the vegetative growth characteristics i.e., plant leaf area (cm<sup>2</sup>) was measured using a portable leaf area meter LI-3100 (LI-COR, Lincoln, Nebraska, USA), fresh and dry weights for head, leaves, stem and main root (g) were measured by using electrical weighing balance, total yield (ton/ha). Absolute Growth Rate (AGR) is defined as increase of head dry matter per unit of time (g/ day) (Gul *et al.*, 2013).  $AGR = (W_2 - W_1) / (T_2 - T_1)$ , where W1 and W2 are dry weights of heads (g) at Time T1 and T2 (45 - 60 days after transplanting).

Net assimilation rate (NAR): is defined as the increase of head dry matter per unit leaf area per unit of time (g/ m<sup>2</sup>/ day) (Gul *et al.*, 2013).  $NAR = [(W_2 - W_1) / (T_2 - T_1)] \times [(Ln L_2 - Ln L_1) / (L_2 - L_1)]$ , where, W1 and W2 are dry weight of head at time T1 and T2, L1 and L2 are leaf area at T1 and T2 (45 - 60 days after transplanting).

$$\text{Increase in yield\%} = \frac{\text{Treatment yield} - \text{Control yield}}{\text{Control yield}} \times 100 \quad (i)$$

Moisture content of leaves % =

$$\frac{\text{Fersh wieght (g)} - \text{Dry wieght(g)}}{\text{Fersh wieght (g)}} \times 100 \quad (ii)$$

The fresh leaves were used to determine nitrate content according to the described method by (Sah, 1994). Total chlorophyll content was recorded in the outer fourth leaf by using chlorophyll Meter (SPAD-502, Minolta Co, ltd., Japan). Ascorbic acid (mg/100g fresh weights) and carotene contents were determined according to the described methods in (A.O.A.C, 2000). As for the total nitrogen, it was determined at harvesting time in the digestion product, using the Micro-Kjeldahl method (A.O.A.C, 2000). Crude protein % =  $N\% \times 6.25$  (Salo-vaananen and Koivistoinen, 1996).

Nitrogen use efficiency and its related parameters:

Nitrogen uptake (N uptake) = kg N in dry yield per ha (Karam *et al.*, 2002).

Apparent nutrient recovery efficiency (Baligar *et al.*, 2001)

$$ANR (\%) = \frac{\text{N uptake, kg}}{\text{Quantity of N applied + N in soil befor seedling, kg}} \times 100 \quad (iii)$$

Nitrogen use efficiency (kg kg<sup>-1</sup>) (Karam *et al.*, 2002).

$$(NUE) = \frac{\text{Dry yield}}{\text{Quantity of N applied + N in soil befor transplanting}} \quad (iv)$$

Physiological efficiency (kg kg<sup>-1</sup>) (Baligar *et al.*, 2001)

$$(PE) = \frac{\text{Dry yield}}{\text{N up take}} \quad (v)$$

**Statistical analyses:** Analysis of variance (ANOVA) was done using Statistical Assistance (Assistat 7.7 beta) program. The significant differences between means were determined by Duncan's multiple range tests.

## RESULTS

**Vegetative growth:** In the field of agriculture, nanotechnology play a significant role in the crop production and protection by inventing most valuable products in the form of nano-fertilizers. Nano-fertilizers not only enhance the growth and yield of crops, also reduce the environmental pollution by minimizing the extra use of fertilizers in agriculture. Furthermore, smart way of irrigation application to agricultural crops like drip irrigation contribute in water management and increase fertilizer use efficiency. In the current study, nanoparticles of nitrogen (nN) and bulk form of nitrogen (bN) were applied in different combinations through different irrigation methods like surface irrigation

**Table 1. Applied treatments during experiment.**

No.	Bulk size of nitrogen fertilizer (bN)	+ Nanoparticle size of fertilizer (nN)	Total units%
1-	100% bN with surface irrigation (control)	+ 0%	100
2-	100% bN with drip irrigation	+ 0%	100
3-	50% bN with drip irrigation	+ 25% nN with drip irrigation	75
4-	25% bN with drip irrigation	+ 50% nN with drip irrigation	75
5-	25% bN with drip irrigation	+ 25% nN with drip irrigation	50
6-	0% bN with drip irrigation	+ 75% nN with drip irrigation + 25% nN as foliar	100
7-	75% bN with drip irrigation	+ 25% nN as foliar	100

and drip irrigation. The growth attributes of lettuce plant were examined by sampling on 45 DAT and 60 DAT in both seasons (2017 and 2018). Data given in Table 2 indicates that dry weights of plant parts (head, root, stem and leaves) were significantly affected by the application of nN in different combinations with bN through drip irrigation. The traditional soil application of bN fertilizer with surface irrigation (control) produced the lowest weight of plant parts followed by the application of bN through drip irrigation system. The

nN application at the rate of 75% (out of recommended dose of N 210 kg ha<sup>-1</sup>) through drip irrigation and the remaining dose of N which was 25% by foliar application significantly increased the plant weight, plant head weight, main root weight, leaves weight in both seasons 2017 and 2018. While in 2017, 25% bN and 50% nN through drip irrigation significantly enhanced the main stem weight as shown in the Table 2. Other combination of bN and nN also increased the growth

**Table 2. Effect of different combinations of bulk and nano size of nitrogen fertilizers on dry matter partitioning (g) of lettuce plant at 60 DAS after transplanting**

Treatments*	Plant	Head	Main root	Stem	Leaves
<b>2017</b>					
Control 100% bNS	8.1 g	6.3 g	1.8 d	1.2 f	5.1 g
Control 100% bNs	46.2 c	44.2 c	2.0 c	2.2 e	42.1 c
50% bNs +25% nNs	34.2 e	32.4 e	1.8 d	3.1 b	29.3 e
25% bNs +50% nNs	44.0 d	41.2 d	2.8 a	3.4 a	37.8 d
25% bNs +25% nNs	33.0 f	31.1 f	2.0 c	2.7 c	28.4 f
0% bNs+100% nN(75% nNs+25% nNf)	103.4 a	100.6 a	2.8 a	2.7 c	97.9 a
75% bNs +25% nNf	79.9 b	77.7 b	2.3 b	2.4 d	75.2 b
<b>2018</b>					
Control 100% mNS	12.7 g	1.8 d	0.93 e	1.2 e	10.7 g
Control 100% mNs	44.7 d	43.6 d	1.1 d	1.9 d	41.7 d
50% mNs +25% nNs	34.6 e	33.1 e	1.6 c	1.9 d	31.2 e
25% mNs +50% nNs	60.6 c	59.1 c	1.9 b	2.0 c	57.1 c
25% mNs +25% nNs	32.7 f	31.2 f	1.5 c	2.0 c	29.3 f
0% mNs+100% nN(75% nNs+25% nNf)	116.0 a	113.9 a	2.1 a	2.7 a	111.2 a
75% mNs +25% nNf	84.9 b	82.8 b	2.1 a	2.2 b	80.5 b

Means in the same column (for each year) followed by different letters are significantly different according to Duncan's test at the  $P \leq 0.05$  probability level. \* bNS = bulk size of N fertilizer as soil surface application; bNs = bulk size of N fertilizer as drip application; nNs = nano N fertilizer as fertigation by drip and nNf= nano N as foliar application.

**Table 3. Effect of different combinations of bulk and nano nitrogen fertilizers on leaves number, plant leaf area, absolute growth rate (AGR) and net assimilation rate (NAR) of lettuce**

Treatments*	Leaves number/ plant		Leaf area /plant (m <sup>2</sup> )		AGR (g/day)	NAR (g/m <sup>2</sup> /day)
	45 DAT	60 DAT	45 DAT	60 DAT	45-60 DAT	45-60 DAT
<b>2017</b>						
Control 100% bNS	21.8 a	30.0 d	0.30 a	0.32 e	0.31 g	0.58 d
Control 100% bNs	23.3 a	43.0 c	0.11 a	0.80 c	2.55 c	3.14 b
50% bNs +25% nNs	20.9 a	41.3 c	0.08 a	0.56 d	1.60 f	2.84 c
25% bNs +50% nNs	23.3 a	41.1 c	0.13 a	0.75 c	2.12 d	2.64 c
25% bNs +25% nNs	18.4 a	40.5 c	0.06 a	0.55 d	1.66 e	3.20 b
0% bNs+100% nN(75% nNs+25% nNf)	25.9 a	62.1 a	0.17 a	1.80 a	6.11 a	3.82 a
75% bNs +25% nNf	23.5 a	55.6 b	0.15 a	1.20 b	4.72 b	3.97 a
<b>2018</b>						
Control 100% bNS	19.1 d	27.0 b	0.05 f	0.33 g	0.66 g	1.92 e
Control 100% bNs	22.7 b	41.1 ab	0.06 e	0.80 c	2.59 d	0.09 f
50% bNs +25% nNs	20.2 c	41.3 ab	0.06 e	0.60 f	1.92 e	3.43 c
25% bNs +50% nNs	22.3 b	40.7 ab	0.12 c	0.78 d	3.48 c	4.25 b
25% bNs +25% nNs	19.0 d	39.9 ab	0.08 d	0.61 e	1.69 f	2.82 d
0% bNs+100% nN(75% nNs+25% nNf)	24.3 a	53.9 a	0.16 a	1.60 a	7.10 a	4.94 a
75% bNs +25% nNf	24.0 a	50.3 a	0.15 b	1.30 b	5.10 b	4.17 b

Means in the same column (for each year) followed by different letters are significantly different according to Duncan's test at the  $P \leq 0.05$  probability level. \* bNS = bulk size of N fertilizer as soil surface application; bNs = bulk size of N fertilizer as drip application; nNs = nano N fertilizer as fertigation by drip and nNf= nano N as foliar application.

attributes through drip irrigation method relative to control treatments.

Other growth parameters i.e., leaves number per plant, plant leaf area, absolute growth rate (AGR) and net assimilation rate (NAR) were significantly affected by the application of nN through drip irrigation by combining with bN form of nitrogen as shown in Table 3. The number of leaves per plant, leaf area, AGR and NAR was increase by the fertigation of nN at the rate 75% through irrigation and combined with remaining 25% dose of N by foliar application at 45 DAT and 60 DAT in both lattice growing seasons (2017 and 2018) by comparing with control treatments. However, number of leaves per plant at 45 DAT in 2017 growing season non-significantly affect by the applied treatments.

**Yield attributes of plant:** All studied treatments (with drip irrigation) increased fresh and dry yields compared to the control (surface irrigation), (In supplementary file Fig. 2). It was noticed that dry yield followed the same trend as of the fresh one. Marked increase was obtained from application of full nitrogen demand in nano form as 25% foliar plus 75% fertigation. It recorded 246.1 and 139.3% higher than the control (In supplementary file Fig. 3). The treatment of 25% nano N as foliar plus 75% mineral N as fertigation came in the second rank and recorded 206.1 and 106.7% in 2017 and 2018, respectively). However, reducing N dose up to 50% (25% nano + 25% mineral) led to intermediate values (109.9 and 25.8%, in 2017 and 2018, respectively) but still higher than the control.

**Biochemical parameters:** Biochemical attributes such as leaf  $\text{NO}_3$  contents, leaf moisture contents, ascorbic acid,  $\beta$ -

carotene and crude protein were significantly affected by the application of nano-nitrogen through drip irrigation in different combinations of recommended dose of N as shown in the Table 4. The leaves  $\text{NO}_3$  contents was increased by 75% dose of N in bulk form through fertigation and 25% dose of N in foliar application. The surface irrigation method and 100% dose of N application in bulk form increased the leaves moisture contents in both (2017 and 2018) seasons of cultivation as shown in Table 4. In the case of ascorbic acid, the low N application doses (50% in nano form and 25% in bulk form) applied through drip irrigation method increased the ascorbic acid contents in 2017. While in 2018, the contents of ascorbic acid 50% dose of N in both form (25% bN and 25% nN) in drip irrigation system.  $\beta$ -carotene and crude protein were increased by the application 75% dose of N in nano form through drip irrigation and 25% dose of N in nano form was applied through foliar as shown in the Table 4.

**Physiological parameters and nutrient acquisition:** The physiological parameters such as chlorophyll contents in the leaves of lettuce plant were significantly affected by the application of nano nitrogen through drip irrigation at 45 DAT and 60 DAT in both seasons of lettuce sowing (2017 and 2018) as shown in the Table 5 and 6. The maximum chlorophyll contents were measured by the application 50% dose of N in the nano form and 25% in bulk through drip irrigation. The application N fertilizer in different combinations also significantly affected essential nutrient concentration in lettuce plant, both bulk and nano form in surface and drip irrigation methods in both seasons and at 45 DAT and 60 DAT as shown in the Table 5 and 6. The

**Table 4. Effect of different combinations of bulk and nano nitrogen fertilizers on quality properties of lettuce leaves**

Treatments*	leaf $\text{NO}_3$ content (ppm)	Moisture content of leaves (%)	Ascorbic acid (mg/100g fw)	$\beta$ -carotene (mg/100g fw)	Crude protein (%)
<b>2017</b>					
Control 100% bNS	1299 d	94.9 a	1.76 d	-	15.8 e
Control 100% bNs	1399 c	84.3 c	1.52 e	-	18.1 c
50% bNs +25% nNs	1274 f	88.2 b	2.67 c	-	16.1 e
25% bNs +50% nNs	1277 e	87.2 b	3.99 a	-	16.9 d
25% bNs +25% nNs	750 g	88.3 b	3.67 b	-	10.7 f
0% bNs+100% nN(75% nNs+25% nNf)	1420 b	79.7 d	1.66 de	-	22.2 b
75% bNs +25% nNf	1449 a	76.8 e	1.55 e	-	22.9 a
<b>2018</b>					
Control 100% bNS	1300 d	93.8 a	1.71 de	1217 e	15.7 d
Control 100% bNs	1392 c	84.2 cd	1.45 f	1474 c	15.8 c
50% bNs +25% nNs	1267 e	87.5 b	1.73 c	1370 d	14.0 c
25% bNs +50% nNs	1264 f	82.1 d	3.69 b	1373 d	12.9 f
25% bNs +25% nNs	753 g	86.8 bc	3.89 a	1173 f	11.1 g
0% bNs+100% nN(75% nNs+25% nNf)	1416 b	78.9 e	1.77 d	1512 b	22.1 b
75% bNs +25% nNf	1453 a	74.8 f	1.69 e	1579 a	23.7 a

Means in the same column (for each year) followed by different letters are significantly different according to Duncan's test at the  $P \leq 0.05$  probability level. \* bNS = bulk size of N fertilizer as soil surface application; bNs = bulk size of N fertilizer as drip application; nNs = nano N fertilizer as fertigation by drip and nNf= nano N as foliar application.

**Table 5. Effect of different combinations of bulk and nano nitrogen fertilizers on chlorophyll, N, P, K contents of lettuce leaf at 45 days after translating**

Treatments*	Chlorophyll (SPAD)	N (%)	P (%)	K (%)
<b>2017</b>				
Control 100% bNS	30.2 c	4.82 e	0.44 d	4.77 c
Control 100% bNs	38.0 ab	5.61 b	0.55 b	5.19 b
50% bNs +25% nNs	38.0 ab	5.16 d	0.43 e	4.59 d
25% bNs +50% nNs	40.3 a	5.43 c	0.47 c	4.67 cd
25% bNs +25% nNs	37.2 b	4.23 f	0.35 f	3.70 e
0% bNs+100% nN(75% nNs+25% nNf)	38.0 ab	5.66 b	0.55 b	5.52 a
75% bNs +25% nNf	39.7 ab	5.87 a	0.58 a	5.09 b
<b>2018</b>				
Control 100% bNS	35.2 b	5.24 d	0.46 d	4.65 d
Control 100% bNs	43.0 a	5.57 c	0.57 b	5.10 b
50% bNs +25% nNs	43.0 a	5.09 e	0.43 e	4.63 d
25% bNs +50% nNs	45.3 a	5.32 d	0.49 c	4.89 c
25% bNs +25% nNs	42.2 a	4.14 f	0.37 f	3.31 e
0% bNs+100% nN(75% nNs+25% nNf)	43.0 a	5.86 b	0.57 b	5.20 a
75% bNs +25% nNf	44.6 a	5.15 a	0.58 a	5.33 a

Means in the same column (for each year) followed by different letters are significantly different according to Duncan's test at the  $P \leq 0.05$  probability level. \* bNS = bulk size of N fertilizer as soil surface application; bNs = bulk size of N fertilizer as drip application; nNs = nano N fertilizer as fertigation by drip and nNf= nano N as foliar application.

**Table 6. Effect of different combinations of bulk and nano nitrogen fertilizers on chlorophyll, N, P, K contents of lettuce leaf at 60 days after translating.**

Treatments*	Chlorophyll (SPAD)	N (%)	P (%)	K (%)
<b>2017</b>				
Control 100% bNS	23.1 c	2.53 e	0.39 d	4.00 b
Control 100% bNs	27.0 c	2.89 c	0.51 b	4.50 a
50% bNs +25% nNs	33.0 b	2.58 e	0.38 e	3.70 c
25% bNs +50% nNs	33.0 b	2.71 d	0.43 c	4.10 b
25% bNs +25% nNs	34.2 ab	1.71 f	0.31 f	3.10 d
0% bNs+100% nN(75% nNs+25% nNf)	32.3 b	3.56 b	0.51 b	4.40 a
75% bNs +25% nNf	38.7 a	3.67 a	0.53 a	4.40 a
<b>2018</b>				
Control 100% bNS	30.1 c	2.35 d	0.41 c	4.15 c
Control 100% bNs	31.7 b	2.52 c	0.52 a	4.45 b
50% bNs +25% nNs	31.0 bc	2.24 e	0.37 d	3.84 d
25% bNs +50% nNs	32.5 b	2.07 f	0.45 b	4.05 c
25% bNs +25% nNs	31.0 bc	1.78 g	0.34 e	3.13 e
0% bNs+100% nN(75% nNs+25% nNf)	34.0 a	3.52 b	0.52 a	4.69 a
75% bNs +25% nNf	34.1 a	3.79 a	0.52 a	4.79 a

Means in the same column (for each year) followed by different letters are significantly different according to Duncan's test at the  $P \leq 0.05$  probability level. \* bNS = bulk size of N fertilizer as soil surface application; bNs = bulk size of N fertilizer as drip application; nNs = nano N fertilizer as fertigation by drip and nNf= nano N as foliar application.

maximum contents of N, P and K were detected by the application of N at the rate of 75% in drip irrigation as bulk form and 25% as nano form through foliar application in both seasons. While the maximum K contents in 2017 at 45 DAT were detected by the application of nano form on N in 75% through drip irrigation and 25% nN in foliar application.

**Nitrogen use efficiency and its related parameters:** Although, the plant took up more N when the drip-fertigated

N was increased up to the full recommended dose, the surface irrigation with full N dose gave the lowest N uptake (In supplementary file Table 2). The highest N uptake was obtained when the full dose was added in the form of nano as 75% soil application plus 25% foliar application. Moreover, the combined treatments of mineral and nano sources were better than the mineral source only. Generally, the percentage of N-uptake from the total added amount expressed as

apparent nutrient recovery efficiency (ANR) followed the same trend as that of the absolute N uptake (Table 6). Every added unit of N produced higher yield (expressed by nitrogen use efficiency, NUE) in the case of spraying 25% of the recommended dose in nano form with favorable effect when associated with applying the left dose (75%) in nano form also through drip irrigation (Table 6). When the Full dose of N was added as only mineral form either with surface or drip irrigation, the use efficiency of N was extremely reduced which the applied units of N produced the lowest yield with surface irrigation followed by the drip irrigation treatment. The uptake unit of N produced higher yield (expressed as physiological efficiency) in the case of reduced N dose (25% nano + 25% mineral), (Table 6). However, the uptake unit of N in the case of spraying 25% of the full dose as nano seemed to produce lower yield.

**Chemical characters of the soil:** The comparison between soil characters before transplanting and after harvesting indicates that there was no effect of the treatments on soil pH

(In supplementary file Table 3). All treatments decreased soil EC after harvest except the treatments of 75% N as soil application + 25% N as foliar application. The lowest EC was obtained from the full dose of N with surface irrigation without observed differences with the treatments that contain 75% from the full N dose. Generally, total N of the soil decreased after lettuce harvest. The treatment of the full-recommended dose of N as mineral with 25% foliar application left more N in the soil after harvest comparing with the other treatments. However, the reduced doses (50% and 75% from the recommended dose) left lower N comparing with the full dose. In the case of available N, the full dose as soil application of either surface or drip irrigation reduced the available N comparing with its content before transplanting. However, the full dose in nano form (75% as fertigation + 25% as foliar application) left the highest available N in the soil after harvest (In supplementary file Table 3).

**Supplementary Table 1. Soil analysis of the experimental site before lettuce growing**

Soil depth	Organic matter (g kg <sup>-1</sup> )	Total carbonate (g kg <sup>-1</sup> )	Total N (%)	Available N (mg kg <sup>-1</sup> )	Soluble cations (cmol (+) kg <sup>-1</sup> )					pH	EC (dS m <sup>-1</sup> )
					P	Mg <sup>++</sup>	Na <sup>+</sup>	Ca <sup>++</sup>	K <sup>+</sup>		
Season 2017											
0 -30 cm	10.3	5.6	3.9	30.8	1.04	0.40	10.2	0.8	0.76	7.59	1.59
Season 2018											
0 -30 cm	12.8	7.0	3.9	30.9	1.10	0.34	10.1	0.8	0.78	7.50	1.61

**Supplementary Table 2. Effect of different combinations of mineral and nano nitrogen fertilizers on N uptake, apparent nutrient recovery efficiency (ANR%), N use efficiency (NUE), physiological efficiency (PE) of lettuce plant**

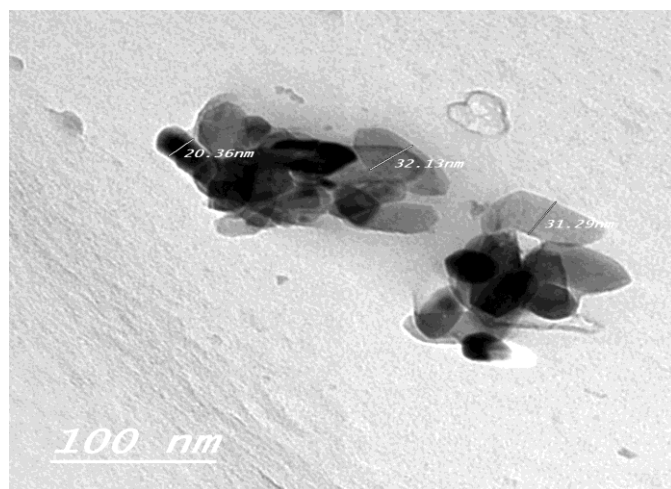
Treatments*	N uptake (Kg/ha)	ANR (%)	NUE (Kg <sub>yield</sub> /kg N)	PE (Kg <sub>yield</sub> /kg N uptake)
2017				
Control 100% bNS	7.5 g	3.6 f	1.4 f	39.6 b
Control 100% bNs	60.0 c	28.6 d	9.9 e	34.6 d
50% bNs +25% nNs	39.3 e	25.0 de	9.7 e	38.8 bc
25% bNs +50% nNs	52.5 d	33.3 c	12.3 d	36.9 c
25% bNs +25% nNs	25.1 f	23.9 e	13.9 c	58.3 a
0% bNs+100% nN(75% nNs+25% nNf)	168.2 a	80.1 a	22.5 a	28.1 e
75% bNs +25% nNf	133.8 b	63.7 b	17.4 b	27.3 e
2018				
Control 100% bNS	13.0 e	6.2 e	2.6 e	42.6 c
Control 100% bNs	51.7 c	24.6 d	9.8 d	39.7 d
50% bNs +25% nNs	34.8 d	22.1 d	9.9 d	44.8 c
25% bNs +50% nNs	57.4 c	36.5 c	17.7 b	48.4 b
25% bNs +25% nNs	26.1 d	24.6 d	14.0 c	56.4 a
0% bNs+100% nN(75% nNs+25% nNf)	188.7 a	89.8 a	25.5 a	28.4 e
75% bNs +25% nNf	147.6 b	70.3 b	18.5 b	26.4 e

Means in the same column (for each year) followed by different letters are significantly different according to Duncan's test at the  $P \leq 0.05$  probability level. \* bNS = bulk size of N fertilizer as soil surface application; bNs = bulk size of N fertilizer as drip application; nNs = nano N fertilizer as fertigation by drip and nNf= nano N as foliar application.

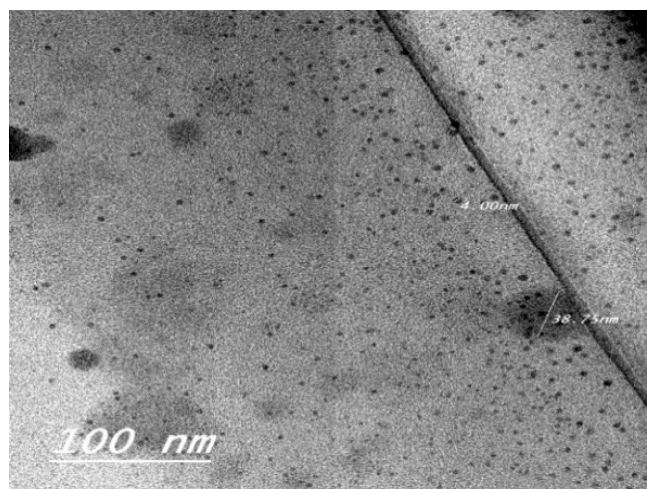
**Supplementary Table 3. Effect of different combinations of mineral and nano nitrogen fertilizers on chemical characteristics of the soil after lettuce harvest comparing with before transplanting**

Treatments*	pH	EC (dS m <sup>-1</sup> )	Total N (%)	Available N (mg/kg soil)
<b>2017</b>				
			Before transplanting	
	7.6	1.6	3.90	30.8
			After harvest	
Control 100% bNS	7.6	0.9	0.70	22.4
Control 100%bNs	7.5	1.5	0.75	16.8
50% bNs +25% nNs	7.5	0.9	0.56	36.4
25% bNs +50% nNs	7.6	0.9	0.56	36.4
25% bNs +25% nNs	7.4	1.5	0.64	36.4
0% bNs+100% nN(75% nNs+25% nNf)	7.5	1.9	0.84	42.0
75% bNs +25% nNf	7.5	1.8	0.66	36.2
<b>2018</b>				
			Before transplanting	
	7.5	1.6	3.91	31.4
			After harvest	
Control 100% bNS	7.4	0.9	0.74	21.8
Control 100%bNs	7.4	1.5	0.64	15.6
50% bNs +25% nNs	7.5	0.9	0.54	35.6
25% bNs +50% nNs	7.5	0.9	0.56	37.4
25% bNs +25% nNs	7.4	1.5	0.64	36.6
0% bNs+100% nN(75% nNs+25% nNf)	7.5	1.9	0.79	40.6
75% bNs +25% nNf	7.5	1.9	0.76	39.6

\* bNS = bulk size of N fertilizer as soil surface application; bNs = bulk size of N fertilizer as drip application; nNs = nano N fertilizer as fertigation by drip and nNf= nano N as foliar application.



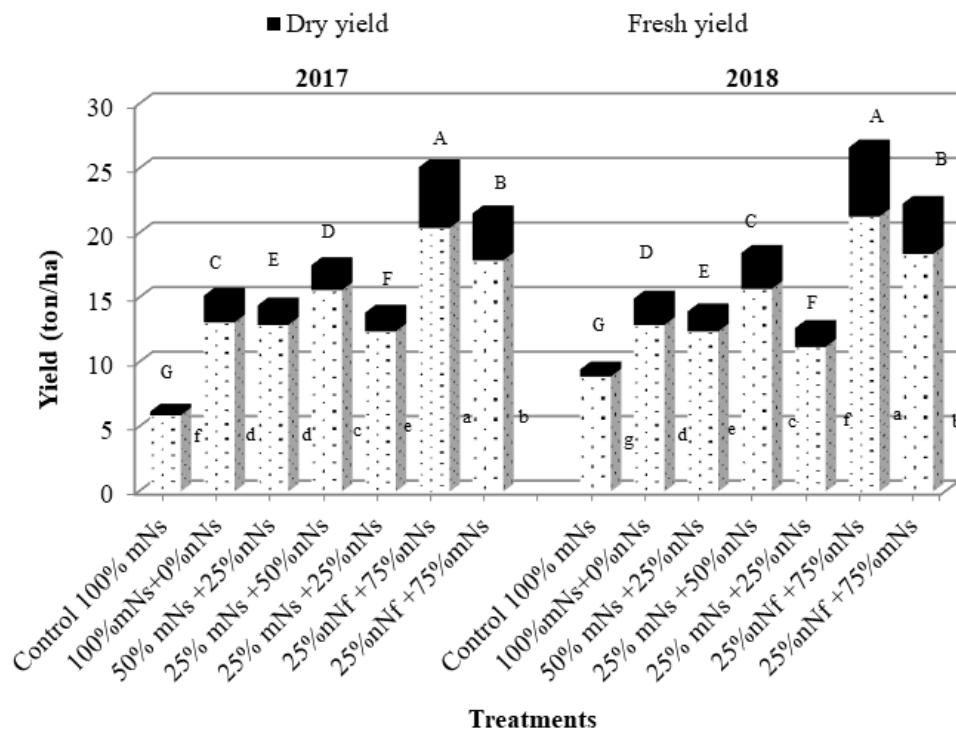
**Nitrogen nanoparticles of the soil added fertilizer (20.4% N, 32.13 nm)**



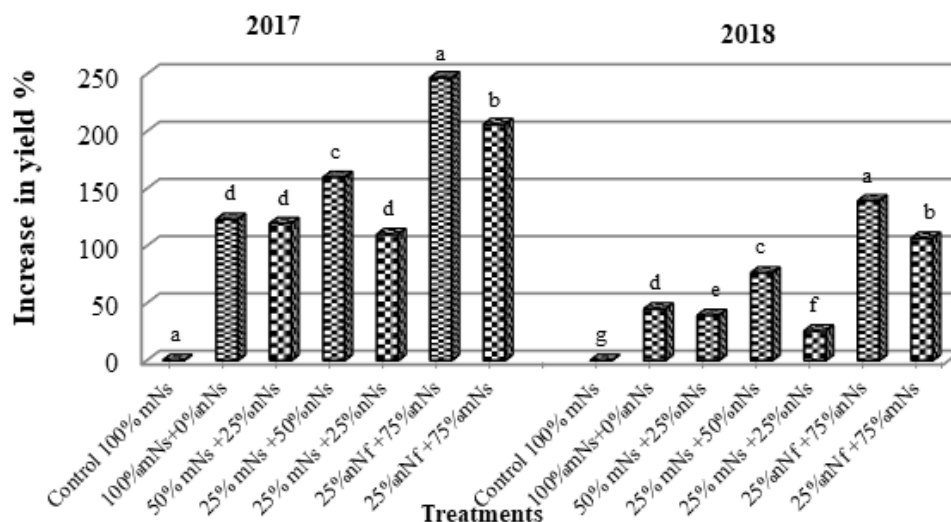
**Nitrogen nanoparticles of foliar application fertilizer (12% N, 36 nm)**

**Supplementary Figure 1. Transmission electron microscopy (TEM) micrograph of morphology of the applied Nano fertilizers in the experiment**





Supplementary Figure 2. Effect of different combinations of mineral and nano nitrogen fertilizers on total fresh yield and dry yield (ton /ha) of lettuce. Means in the same column (for each year) followed by different letters are significantly different according to Duncan's test at the  $P \leq 0.05$  probability level. \* mNS = bulk size of N fertilizer as soil surface application; mNs = bulk size of N fertilizer as drip application; nNs = nano N fertilizer as fertigation by drip and nNf= nano N as foliar application.



Supplementary Figure 3. Effect of different combinations of mineral and nano nitrogen fertilizers on increase in yield % of lettuce. Means in the same column (for each year) followed by different letters are significantly different according to Duncan's test at the  $P \leq 0.05$  probability level. \* mNS = bulk size of N fertilizer as soil surface application; mNs = bulk size of N fertilizer as drip application; nNs = nano N fertilizer as fertigation by drip and nNf= nano N as foliar application.

## DISCUSSION

Traditional way of agricultural crops cultivation contributing the major losses of food production and increase the expenses on agricultural inputs such as good quality irrigation water and fertilizers. Excessively and unwisely, use of these inputs not only decrease the optimum production of crops to feed the increasing population of world also affect the economics of nation. Recently, trending technology called as nanotechnology in the field of agriculture, improved the fertilizer use efficiency and crop produced by inventing nano fertilizers. Furthermore, to increase the water use efficiency drip irrigation method is favorable for regions having low available water resources like Egypt. By keeping in view, the main purpose of this study was to compare 1-The traditional growing method of lettuce includes surface irrigation and soil application of mineral fertilizers vs drip irrigation with fertigation system. 2-Bulk vs nano N fertilizers. 3-Combinations of different portions of mineral and nano fertilizers. 4- Adding full N fertilizer as a soil application vs adding part of the N demand as a foliar application in nano form. Furthermore, the impact of these treatments on lettuce growth, yield, quality and N use efficiency parameters was investigated. Growth attributes in plants were increased by the application of nitrogen in nano form because nitrogen use efficiency is increased by fertigation in drip irrigation (Eleiwa *et al.*, 2012). Similar results were observed in this study; the application of nano nitrogen with drip irrigation significantly enhanced the growth of lettuce plant as shown in the Table 2 and 3. The increase in growth attributes just because of nitrogen as it improved the physiology of plants leaves by increasing chlorophyll contents (Elhindi *et al.*, 2016). Chlorophyll contents enhanced the rate of photosynthesis, increased the accumulation of glucose and improved fresh and dry weight of plants consequently (Elhindi *et al.*, 2016; Elemike *et al.*, 2019; He *et al.*, 2022). In another experiment, it was reported that nitrogen is necessary for the synthesis of vitamins, energy production compounds like ATP, enzymes and synthesis of amino acids such as Tryptophan; responsible for the cell elongation (Galili *et al.*, 2016; He *et al.*, 2022). All these also improved the health and working of plants that contribute the accumulation of high biomass. Our results also similar with Ali and Al-Juthery (2017), when nano fertilizers were applied through foliar application and also with drip irrigation (Al-juthery, 2018). The results also consistent with Moosapoor *et al.* (2013) who examined that foliar application of nano fertilizers play a significant role in photosynthesis, increased leaf area and finally increased yield.

The obtained results were showed that nano N fertilizer had a favorable effect on lettuce growth comparing to the traditional ammonium nitrate. The smaller particles size (In supplementary file Fig. 1) and the high specific surface areas of the nano fertilizers could be the reason due to their higher dissolution rate and extent in water/soil solution than the

related bulk solids from traditional fertilizers (Liu and Lal, 2015). Furthermore, to enhance the fertilizer use efficiency, essential nutrients were applied in nano form and improved the plant growth as shown in Table 2. Fertilizer use efficiency significantly enhanced by the application of nutrients in nano form because nano fertilizers once enters the plant, bind with carrier proteins like aquaporin, ion channels and endocytosis (Schwab *et al.*, 2015). This behavior of nano fertilizers lead to the formation of new openings that penetrate the cell wall and stimulate the absorption of water and other essential nutrients that encourage the growth of plant (Abyaneh and Maryam, 2014). It was reported that non fertilizers were more effective and efficient than the traditional fertilizers due to their positive effects on the growth, quality nutrition of crops and as well as reduced the stress in plants (Morales-Diaz *et al.*, 2017, Singh, 2017, Ali and Al -juthery, 2017). Bloom (2015) reported that the application nitrogen in nano form increased the growth, quality of yield by enhancing the protein contents, absorption of other essential nutrients (Hemerly, 2016). Similar results were obtained in the current study as shown in the table 2 and 4 and consistent with Haleema *et al.* (2018) the nano form of nutrients make a complex with numerous specific and nonspecific membrane transporter proteins or chemicals in root exudates then directly transported to the plants (Shukla *et al.* 2016). The high N-use efficiency of nano fertilizer (In supplementary file Table 2) is supported by the results of (Subramanian and Tarafdar, 2011) on  $^{15}\text{N}$ , whom indicated that N-use efficiency from nano fertilizer reached 82% compared to 42% from conventional fertilizer (urea) which suggested that nano fertilizers may be regulate nutrient release that commensurate with plant requirement. On lettuce, Wang *et al.* (2017) explained the important function of nitrogen absorption in nanoparticles form as a result of some important nitrogen metabolism-related unigenes (3 genes of glutamine dumper family, glutamine synthetase gene 7 nitrate transporter genes and 3 ammonium transporter genes) which are involved in amino acids metabolism and transport as upregulated in roots by stimulation of nanoparticles.

When 25% of N demand was applied as foliar, N-use efficiency was increased. In this research, we decreased 25% from N demand as it added as foliar application in nano form to give the maximum efficiency from N fertilization (In supplementary file Table 2). In this concern, Gagne *et al.* (2019) reported that foliar nitrogen application is a practical and sustainable way and it could reduce nitrogen loss to the environment. It also noted that Nano particles can move in all types of tissues including both stomatal and cuticular pathways and the washing did not remove significant amount (Larue *et al.*, 2014).

The best results were obtained from the combination between soil and foliar applications. These results may be due to the double benefits of absorption and translocation through xylem and phloem. In this concern, Wang *et al.* (2016) reported that

nano particles could enter the plant through the roots and leaves according their size. In our results the particle size ranged between 32-36 nm, hence leaf and root pathway are possible to enter the plant and play their role inside the cell series of barriers for uptake and transport of nanoparticles in plant (Wang *et al.*, 2016).

**Conclusions:** Drip irrigation was recommended for lettuce high growth, yield and quality comparing with surface irrigation. The application of nitrogen in nano form not only increase the fertilizer use efficiency at lower dose than recommended, also reduce the extra losses and environmental contamination. The best results were obtained when the plants were fertilized by 75% of N demand as fertigation plus 25% as foliar application in nano form. Nitrogen fertilizer in nano form (75% fertigation + 25% foliar application) increased N uptake, apparent recovery efficiency% and N use efficiency. Foliar application of nano N significantly minimized the nitrate contents in leaves, while increased  $\beta$ -carotene and crude protein contents by enhancing the growth and fertilizer use efficiency.

**Conflict of Interest:** The authors declared that there is no conflict of interest

**Authors Contribution:** MASE and MBE planed, conducted the experiment and collected the data, MYE collected data, analyzed, HER and MU Interpreted the data, and wrote first draft, MU and MZR Edited, submitted and revised the paper draft.

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