Effect of nano chelated nitrogen and urea fertilizers on wheat plant under drought stress condition

Naimeh ASTANEH1, Foroud BAZRAFSHAN1, Mahdi ZARE1*, Bahram AMIRI1, Abdollah BAHRINI2

1 Department of Agriculture, Firoozabad Branch, Islamic Azad University, Firoozabad, Iran.
2 Department of Agriculture, Ramhormoz Branch, Islamic Azad University, Ramhormoz, Iran.
*E-mail: mahdi.zare2009@gmail.com

ABSTRACT: This study was performed to evaluation of nano chelated nitrogen and urea fertilizers on wheat plant under drought stress condition. Experiments were carried out in two locations in Fars province, Iran. Experimental design was performed as split-split plot in RCBD design. The first factor included irrigation treatments (normal and withholding irrigation at flowering stage), sub factor was nitrogen treatment (0, 37, 74 and 110 kg.ha\(^{-1}\)) in the form of Urea fertilizer, and sub-sub factor was nitrogen (0, 14, 27 and 41 kg.ha\(^{-1}\)) in the form of nano chelated nitrogen fertilizer. Studied traits included stem height, spike weight, spike length, plant height, tilled number, stem diameter, anthocyanin, flavonoid, proline, soluble carbohydrates, chlorophyll a and b, carotenoid, 1000-seed weight, biological yield, seed yield and harvest index. According to analysis of variances, stress, nitrogen (urea) and nano chelated nitrogen had significant effects on all studied traits. According to mean comparisons, drought stress led to 51% reduction in seed yield compared to normal irrigation. Application of 37, 74 and 110 kg.ha\(^{-1}\) urea led to 9, 19 and 27% increase in seed yield compared to control, respectively. Application of 14, 27 and 41 kg.ha\(^{-1}\) nano fertilizer led to 31, 44 and 98% increase in seed yield compared to control, respectively. In interaction between stress, urea and nano fertilizer on seed yield, it was found that at normal and stress condition the highest mean of seed yield (7591 and 4091 kg.ha\(^{-1}\)) was obtained by 110 kg.ha\(^{-1}\) urea and 41 kg.ha\(^{-1}\) nano fertilizer. In conclusion replacing urea by nano chelated nitrogen can increase the yield even in the drought stress and decrease amount of needed fertilizer.

Keywords: agronomic traits, biochemical traits, irrigation, seed yield.

Efeito de nano-quelado de nitrogênio e ureia fertilizante em plantas de trigo sob condições de estresse hídrico

RESUMO: Este estudo foi realizado para avaliar os efeitos de nitrogênio nano-quelatado e ureia fertilizante em plantas de trigo sob condição de estresse hídrico. Foram realizados experimentos em dois locais na província de Fars, no Irã. O delineamento experimental adotado foi em parcelas subdivididas no modelo RCBD. O primeiro fator incluiu os tratamentos de irrigação (irrigação normal e retenção na fase de floração), o subfator foi o tratamento com nitrogênio (0, 37, 74 e 110 kg ha\(^{-1}\)) na forma de fertilizante ureia e sub-subfator foi nitrogênio (0, 14, 27 e 41 kg ha\(^{-1}\)) na forma de adubo nitrogenado nano-quelatado. As características morfométricas estudadas foram peso da haste, peso da espiga, comprimento da espiga, altura da planta, número do perfilho, diâmetro do caule, antocianina, flavonoides, prolinha, carboidratos solúveis, clorofila a e b, carotenoides, peso de 1.000 sementes, rendimento biológico, produtividade e índice de colheita. De acordo com a análise de variações, o estresse hídrico, o nitrogênio (ureia) e o nitrogênio nano-quelatado apresentaram efeitos significativos em todas as características agronômicas estudadas. De acordo com comparações de médias, o estresse hídrico levou a uma redução de 51% no rendimento de sementes em comparação com a irrigação normal. A aplicação de 37, 74 e 110 kg ha\(^{-1}\) de ureia levou a um aumento de 9, 19 e 27% no rendimento de sementes em relação ao controle, respectivamente. A aplicação de 14, 27 e 41 kg ha\(^{-1}\) de nano-quelatado levou a um aumento de 31, 44 e 98% no rendimento de sementes em comparação com o controle, respectivamente. Na interação entre estresse, ureia e nano fertilizante na produtividade das sementes, verificou-se que nas condições normal e de estresse a maior média de produtividade de grãos (7591 e 4091 kg ha\(^{-1}\)) foi obtida em 110 kg ha\(^{-1}\) de ureia e 41 kg ha\(^{-1}\) adubo nano. Em conclusão, substituindo a ureia por nitrogênio nano-quelatado pode aumentar o rendimento, mesmo no estresse hídrico e diminuir a quantidade de fertilizante necessário.

Palavras-chave: características agronômicas, características bioquímicas, irrigação, produtividade de sementes.

1. INTRODUCTION

Wheat is one of the widely cultivated crops in Iran, where drought is the main limiting factor for its production. Drought is a major environmental stress threatening crops such as wheat productivity worldwide. Although drought impedes wheat performance at all growth stages, it is more critical during the flowering and grain-filling phases (terminal drought) and results in substantial yield losses (FAROOGH
The severity and duration of the stress determine the extent of the yield loss. The principal reasons for these losses are reduced rates of net photosynthesis owing to metabolic limitations (Ali et al., 2018) - oxidative damage to chloroplasts (Abid et al., 2018) and stomatal closure-and poor grain set and development (FAROOGH et al., 2014).

The response of plants to water stress depends on several factors such as development stage, severity and duration of stress and cultivar genetics (BELTRANO; MARTA, 2008). The adaptation strategies of the plants to drought stress include drought escape, drought avoidance and drought tolerance (LEVITT, 1980). Allahverdiyev et al. (2015) reported that drought caused reduction in photosynthesis rate, stomatal conductance, transpiration rate, mesophyll conductance, pigments content, area, dry weight, relative water content of flag leaves.

Efficient nitrogen nutrition has the potential to alleviate drought stress in crops by maintaining metabolic activities even at low tissue water potential. Wang et al. (2018) reported that nitrogen fertilization improved water-use efficiency of winter wheat through increasing water use during vegetative rather than grain filling. Wu et al. (2018) suggested that nutrient application has the potential to mitigate the drastic effects of water stress on Moso bamboo by improving photosynthetic rate, water-use efficiency, and increasing of membrane integrity.

In last decade, nanotechnology as a novel technology has solved many problems in different fields of science and industry and it has found its position and functions in agriculture. Nanotechnology has various functions in all stages from production, processing, storage, packing and transportation of agricultural products (SCOTT; CHEN, 2003). Nanofertilizers are the most important part of nanotechnology in the production phase of agriculture. Application of nanofertilizers instead of common fertilizers, nutrients are provided to plants gradually and in a controlled manner.

In relation to environmental effects, the nanotechnology increases the application efficiency of fertilizers, reduces soil pollution and environmental risks of chemical fertilizers (NADERI et al., 2011). In the previous study, Zareabayan; Bayatvarkeshi et al. (2015) showed that using nano chelated nitrogen could increase potato yield in comparison to urea. In the present study we compared the effects of nano chelated nitrogen, which is synthesized based on novel Nanochelating technology, with urea and the aim of this study was to investigate whether nutrition supply in the form of a nano structure can improve the drought tolerance of wheat under dry conditions.

2. MATERIALS AND METHODS

Experiments were carried out in two locations including Khodayan (52° 20’ E, 29° 8’ N) and Nasrabad (52° 64’ E and 29° 58’ N), Fars province, Iran. Table 1 shows some physicochemical properties of the soil at 0 to 30 cm.

### Table 1. Some physicochemical properties of the soil at 0 to 30 cm.

|          | Khodayan | Nasrabad | Khodayan | Nasrabad |
|----------|----------|----------|----------|----------|
| pH       | 7.6      | 7.4      | 7.5      | 7.7      |
| EC (dS/m)| 1.2      | 1.3      | 4        | 3.7      |
| P (ppm)  | 34.86    | 36.75    | 47.23    | 47.55    |
| K (ppm)  | 370      | 380      | 18.6     | 21.21    |

2.2. Field operations

The field was as fallow in the last year. Field operation was done according to usual of region methods (plow, disc, land leveling and furrow). Fertilizer levels were determined after soil analysis (Table 1). Each plot contained 6 rows with 4 meters length and 0.2 m distance, with a constant density of 450 plants per square meter. In stem extension stage of wheat, weeds chemical control were carried out using Granstar herbicide (for the control of grassy weeds) and Puma super (for broadleaf weeds).

Final harvest was carried out at about 14% moisture content. At the end of the growth period, some properties measured such as stem weight, spike weight, spike length, plant height, tiller number, stem diameter, Anthocyanin, flavonoid, proline, soluble carbohydrates, Chlorophyll a and b, carotenoid, 1000 seed weight, biological yield, seed yield. Chlorophyll Content Assay - Leaf samples (1.0 g) were extracted in 10 mL 100% acetone using a mortar and pestle. The homogenate was filtered and centrifuged at 2000 rpm for 2 min. A 1 mL aliquot of the supernatant was pipetted and mixed with 9 mL of 80% acetone. The absorbance of the diluted samples was obtained using a spectrophotometer at 663 and 646 nm (IANCULOV et al., 2005): Total Soluble Carbohydrate Assay - Soluble carbohydrate was estimated according to the method proposed by Dubois et al. (1956). Leaf samples were homogenized in a mortar and pestle with 3 mL distilled water and the homogenate was filtered using a filter paper. Phenol (0.5 mL, 5%) and 2.5 mL 663 and 646 nm (IANCULOV et al., 2005):

Total Soluble Carbohydrate Assay - Soluble carbohydrate was estimated according to the method proposed by Dubois et al. (1956). Leaf samples were homogenized in a mortar and pestle with 3 mL distilled water and the homogenate was filtered using a filter paper. Phenol (0.5 mL, 5%) and 2.5 mL of 98% sulfuric acid were added to the homogenate. After the reaction of the materials, the test tubes were allowed to cool to room temperature. The carbohydrate content was determined from the absorbance measured at 483 nm and calculated based on a standard curve.

2.3. Data analysis

Data were analyzed using the general linear model (GLM) procedure of the statistical analysis system, SAS software. When analysis of variance showed significant treatment effects, Duncan’s multiple range tests were applied to compare the means at P < 0.05.
3. RESULTS AND DISCUSSION

3.1. Stem and spike weight

According to analysis of variances it was found that all treatments and their interaction had significant effects on stem weight at 1% or 5% statistically levels. Also spike weight affected by stress*urea*Nano interaction at 1% statistically level. Liu et al. (2016) mentioned that mild stress during all of the growth stages improved the distribution of assimilate to the grain prior to anthesis and increased the yield. Although moderate stress during all growth stages could improve dry matter translocation, the resulting yield was not high, as the accumulation of dry matter decreased after anthesis. Therefore, mild soil water stress can improve grain yields and WUE.

According to mean comparisons, drought stress led to 26 and 31% reduction in stem and spike weight compared to normal irrigation, respectively. Application of 37, 74 and 110 kg ha\(^{-1}\) urea led to 5, 9 and 3% increase in stem weight and 15, 15 and 8% increase in spike weight compared to control, respectively. Application of 14, 27 and 41 kg ha\(^{-1}\) nano fertilizer led to 16, 37 and 52% increase in stem weight and 12, 40 and 56% increase in spike weight compared to control, respectively. In relation to interaction between stress, urea and nano fertilizer on stem weight, it was found that at normal condition the highest mean of stem weight (1.37 kg\ m\(^{-2}\)) was obtained by 110 kg ha\(^{-1}\) urea and 41 kg ha\(^{-1}\) nano fertilizer, at stress condition the highest mean (1.05 kg\ m\(^{-2}\)) obtained by 110 kg ha\(^{-1}\) urea and 27 kg ha\(^{-1}\) nano fertilizer. In relation to interaction between stress, urea and nano fertilizer on spike weight, it was found that at normal condition the highest mean of spike weight (1.14 kg\ m\(^{-2}\)) was obtained by 110 kg ha\(^{-1}\) urea and 41 kg ha\(^{-1}\) nano fertilizer, at stress condition the highest mean (0.83 kg\ m\(^{-2}\)) obtained by 110 kg ha\(^{-1}\) urea and 27 kg ha\(^{-1}\) nano fertilizer.

Anjum et al. (2011) reported that drought stress disrupts photosynthetic pigments and reduces the gas exchange leading to a reduction in plant growth and productivity.

Table 2. Analysis of variances for studied traits.

| Location | Stem weight | Spike length | Plant height | Tiller number | Stem diameter | Anthocyanin | Flavonoid | Proline |
|----------|-------------|--------------|--------------|---------------|---------------|-------------|-----------|---------|
| Location*Stress | 13.35** | 0.021** | 154.09** | 0.335** | 4.859** | 42029** | 5.029** | 1.951** |
| Error | 96 | 0.006 | 3.13E-05 | 0.259 | 0.001 | 14.656 | 0.005 | 0.003 | 0.004 | 0.046 |
stress condition the highest mean of stem diameter (3 and 2.85 per m² respectively) were obtained by 74 kg ha⁻¹ urea and 41 kg ha⁻¹ nano fertilizer.

3.4. Anthocyanin and flavonoid

These traits affected by drought stress, urea and nano fertilizer at 1% statistically level. According to mean comparisons, drought stress led to 9 and 5% increase in anthocyanin and flavonoid compared to normal irrigation, respectively. Application of 37, 74 and 110 kg ha⁻¹ urea led to 10, 20 and 27% increase in anthocyanin and 8, 13 and 22% increase in flavonoid compared to control, respectively. Application of 14, 27 and 41 kg ha⁻¹ nano fertilizer led to 21, 35 and 51% increase in anthocyanin and 12, 24 and 35% increase in flavonoid by the compared to control, respectively. In relation to interaction between stress, urea and nano fertilizer on anthocyanin, it was found that at normal and stress condition the highest mean of anthocyanin (1.5 and 1.6 respectively) were obtained by 110 kg ha⁻¹ urea and 41 kg ha⁻¹ nano fertilizer. In relation to interaction between stress, urea and nano fertilizer on flavonoid, it was found that at normal and stress condition the highest mean of flavonoid (1.85 and 1.98 respectively) was obtained by 110 kg ha⁻¹ urea and 41 kg ha⁻¹ nano fertilizer.

Table 3. Means comparison between interaction of treatments on studied traits.

| Urea (kg/ha) | Nano (kg/ha) | Stem weight (kg.m⁻²) | Spike weight (kg.m⁻²) | Spike length (cm) | Plant height (cm) | Tiller number | Stem diameter (mm) |
|--------------|--------------|----------------------|----------------------|-------------------|------------------|---------------|-------------------|
| 0            | 0.6667       | jk 0.517 q           | 6.335                | q 50.89           | q 306 p         | 1.533 n       | Normal            |
| 14           | 0.8337       | efgh 0.733 j          | 8.5 klm              | 351.7 k           | 2.45 j          |               |                   |
| 27           | 1.05 c       | 0.850 f              | 9.333 hj             | 59.27 l           | 370 i           | 2.583 h       |                   |
| 41           | 1.317 a      | 0.983 c              | 10.33 efg            | 64.30 c           | 404.2 d         | 2.85 c        |                   |

3.5. Proline and soluble carbohydrates

These traits affected by drought stress, urea and nano fertilizer at 1% statistically level. According to mean comparisons, drought stress led to 1.95 and 1.49 increase in proline and soluble carbohydrates compared to normal irrigation, respectively. The results of Keyvan (2010) showed that with an increase in the intensity of drought stress on wheat cultivars, there was a decrease in relative water content, total chlorophyll content and increased proline content, but was not observed on trend relating to soluble carbohydrates content. Xu et al. (2015) reported that drought stress condition significantly increased leaf sucrose-phosphate synthase.

Application of 37, 74 and 110 kg ha⁻¹ urea led to 14, 14 and 31% increase in proline, also 74 and 110 kg ha⁻¹ urea led to 8 and 23% increase in soluble carbohydrates compared to control, respectively. Application of 14, 27 and 41 kg ha⁻¹ nano fertilizer led to 14, 24 and 44% the increase in proline and 17, 24 and 34% increase in soluble carbohydrates compared to control, respectively.

In relation to interaction between drought stress, urea and nano fertilizer on proline, it was found that at normal and stress condition the highest mean of proline (15.65 and 31.5, respectively) were obtained by 110 kg ha⁻¹ urea and 41 kg ha⁻¹ nano fertilizer. In relation to interaction between stress, urea and nano fertilizer on soluble carbohydrates, it was found that at normal and stress condition the highest mean of soluble carbohydrates (2.05 and 2.88, respectively) were obtained by 110 kg ha⁻¹ urea and 41 kg ha⁻¹ nano fertilizer.

3.6. Chlorophyll a and b

Chlorophyll a and b affected by drought stress, urea and nano fertilizer at 1% statistically level, according to mean comparisons, drought stress led to 16 and 11% reduction in chlorophyll a and b compared to normal irrigation, respectively. Water deficit can destroy chlorophyll and prevent making it (Lessani; Mojtahedi, 2002). Also, some researchers have reported damage to leaf pigments as a result of water deficit (Arjenaki, 20102). Mensah et al. (2006) found that subjecting sesame to drought stress caused leaf chlorophyll was increased and then remained unchanged.

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Application of 37, 74 and 110 kg ha⁻¹ urea led to 1.2 and 31% increase in chlorophyll a and 7, 11 and 34% increase in chlorophyll b compared to control, respectively. Application of 14, 27 and 41 kg ha⁻¹ nano fertilizer led to 32, 55 and 96% increase in chlorophyll a and 37, 54 and 96% increase in chlorophyll b compared to control, respectively.

In relation to interaction between drought stress, urea and nano fertilizer on chlorophyll a, it was found that at normal and stress condition the highest mean of chlorophyll a (2.51 and 2.11, respectively) were obtained by 110 kg ha⁻¹ urea and 41 kg ha⁻¹ nano fertilizer. In relation to interaction between drought stress, urea and nano fertilizer on chlorophyll b, it was found that at normal and stress condition the highest mean of chlorophyll b (1 and 0.82, respectively) were obtained by 110 kg ha⁻¹ urea and 41 kg ha⁻¹ nano fertilizer.

| Urea (kg/ha) | Nano (kg/ha) | Anthocyanin | Flavonoid | Proline | Soluble carbohydrates | Chlorophyll a | Chlorophyll b |
|--------------|--------------|-------------|-----------|---------|----------------------|--------------|--------------|
| 0            | 0            | 0.76        | 1.1       | p       | 7.05                 | 0.85         | 0.82         | 0.365        |
| 14           | 0.93         | g           | k         | l       | 10.47                | 1.27         | 1.56         | 0.617        |
| 27           | 1.05         | f           | g         | h       | 11.58                | 1.633        | 1.823        | 0.675        |
| 41           | 1.1          | i            | j         | k       | 13.55                | 1.633        | 2.250        | 0.845        |
| 37           | 0.87         | l            | 1.167     | o       | 10.78                | 1.2          | 1.072        | 0.42          |
| 14           | 0.98         | i            | j         | l       | 11.20                | 1.45         | 1.202        | 0.5017        |
| 27           | 1.1          | f            | 1.55      | h       | 11.37                | 1.567        | 1.883        | 0.71          |
| 41           | 1.3          | d            | 1.7       | d       | 13.50                | 1.7          | 2.450        | 0.9417        |

Normal

| 74           | 0.88         | k            | l          | o       | 9.32                 | 1.083        | 1.187        | 0.425         |
| 14           | 1.16         | f            | 1.567      | g       | 10.90                | 1.383        | 1.687        | 0.6333        |
| 27           | 1.26         | e            | 1.667      | d       | 12.48                | 1.608        | 1.900        | 0.59          |
| 41           | 1.41         | c            | 1.8        | b       | 14.35                | 1.717        | 2.300        | 0.8667        |

Stress

| 110          | 0.103        | h            | 1.483      | k       | 11.60                | 1.49        | 1.767        | 0.6833        |
| 14           | 1.15         | f            | 1.517      | g       | 12.90                | 1.692        | 1.970        | 0.808         |
| 27           | 1.33         | d            | 1.667      | d       | 14.03                | 1.8          | 2.083        | 1.085         |
| 41           | 1.5          | b            | 1.85       | b       | 15.65                | 0.205        | 2.517        | 1.002         |

Table 3. Means comparison between interaction of treatments on studied traits.

3.7. Carotenoid

This trait affected by drought stress, urea and nano fertilizer at 5% statistically level, according to mean comparisons, drought stress led to 10% the reduction in carotenoid by the compared to normal irrigation, respectively. Application of 37, 74 and 110 kg ha⁻¹ urea led to 16, 5 and 54% increase in carotenoid and application of 14, 27 and 41 kg ha⁻¹ nano fertilizer led to 26, 42 and 62% increase in carotenoid. In relation to interaction between stress, urea and nano fertilizer on carotenoid, it was found that at normal and stress condition the highest mean of carotenoid (2.25 and 1.86, respectively) were obtained by 110 kg ha⁻¹ urea and 41 kg ha⁻¹ nano fertilizer.

3.8. 1000-seed weight and seed yield

These traits affected by stress, urea and nano fertilizer at 1% statistically level. According to mean comparisons, drought stress led to 7 and 51% reduction in 1000-seed weight and seed yield compared to normal yield under irrigation, respectively. Application of 37, 74 and 110 kg ha⁻¹ urea led to 4, 5 and 12% increase in 1000-seed weight and 9, 19 and 27% increase in seed yield compared to control, respectively. Application of 14, 27 and 41 kg ha⁻¹ nano fertilizer led to 8, 14 and 20% increase in 1000-seed weight and 31, 44 and 98% increase in seed yield compared to control, respectively.

In relation to interaction between stress, urea and nano fertilizer on biological yield, it was found that at normal and stress condition the highest mean of biological yield (27000 kg/ha) was obtained by 110 kg ha⁻¹ urea and 41 kg ha⁻¹ nano fertilizer.
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and 15470 kg ha⁻¹) were obtained by 110 kg ha⁻¹ urea and 41 kg ha⁻¹ nano fertilizer. In relation to interaction between stress, urea and nano fertilizer on harvest index, it was found that at normal and stress condition the highest mean of harvest index (28%) were obtained by 110 kg ha⁻¹ urea and 41 kg ha⁻¹ nano fertilizer.

Table 3. Means comparison between interaction of treatments on studied traits. (continuing...)

Tabela 3. Comparação entre as interações das características agronômicas avaliadas. (continuação...)

| Urea     | Nano   | Carotenoid | 1000 seed weight | Biological yield | Seed yield | Harvest index |
|----------|--------|------------|-------------------|------------------|------------|---------------|
| (kg/ha)  | (kg/ha)|            |                   |                  |            |               |
|          |        |            |                   |                  |            |               |
|          | c      | d          | e                  |                  |            |               |
| 110      | 1.78   | cdef       | 41.5 f             | 17170 h          | 4583 f     | 0.25 e        |
| 00       | 0.65   | o          | 32.17 o            | 9167 q           | 917 o      | 0.10 s        |
| 0        | 0.11   | kl         | 35.67 m            | 11330 np         | 2067 kl    | 0.17 n        |
| 27       | 1.22   | jk         | 37.5 kl            | 13330 lm         | 2411 j     | 0.17 lm       |
| 1.47     | h      | 40.83 gh   | 15500 g            | 3092 h           | hi 0.19    | kl            |
| 37       | 0.10   | m          | 34.17 n            | 9500 q           | 1300 n     | 0.12 r        |
| 1.17     | kl     | 36.83 l   | 13830 kl           | 1958 lm          | 0.13 q     |               |
| 27       | 1.53   | h          | 39.83 i            | 13830 kl         | 2267 k     | 0.15 p        |
| 1.68     | fg     | 41.83 li  | 16170 jh           | 11830 h          | 0.19 l     |               |
| 74       | 0.83   | n          | 34.5               | 10670 p          | 1808 lm    | 0.17 mn       |
| 1.22     | jk     | 37.83 k   | 12330 n            | 2071 kl          | 0.16 o     |               |
| 1.28     | ij     | 39.83 i   | 13920 kl           | 2383 j           | 0.16 o     |               |
| 1.50     | h      | 41.83 def | 169600 lij         | 3898 g           | 0.22 l     |               |
| 110      | 0.68   | fg         | 37.83 k            | 12500 mn         | 2042 kl    | 0.15 p        |
| 1.75     | defg   | 40.17 hi  | 11720 no           | 2179 k           | 0.18 mn    |               |
| 27       | 1.63   | cde        | 42.17 de           | 13830 kl         | 2458 j     | 0.17 n        |
| 27       | 1.87   | bc         | 44.17 bc           | 15470 j          | 4092 f     | 0.29 a        |

4. CONCLUSIONS

This study was performed to evaluation of irrigation interaction with nitrogen fertilizer as urea and nano chelated nitrogen, according to results found that all traits were affected by drought stress and application of nitrogen led to reduction of negative effects of stress on yield traits. Also it was founded that of Nano Chelated nitrogen is best fertilizer for urea substitution in wheat cultivation, due to significant yield increase in lesser doses.

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