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# Raising the Efficiency of Mineral Fertilization Using Azolla with Microelements

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Synthetic nitrogen fertilizers can contribute to environmental hazards. Moreover, their manufacturing is an energy-intensive process which causes greenhouse gas emissions, which can lead to climate change. Therefore, it should evaluate the possibility to use organic sources such as Azolla as a partial substitute for synthetic nitrogen fertilizers in cultivation, as this may reduce the cost of fertilizer inputs, and promote sustainable agriculture. So, a field trial was carried out in the Tag El-Ezz Experimental Farm (30°56' 12.88" E longitude and 31°31' 47.64" N latitude) during seasons of 2021/2022 and 2022/2023 to assess the additive of nitrogen recommended dose (NRD=178.6 kg N $ha^{-1}$) as combined treatments of urea (46.5%N) as mineral nitrogen source and Azolla (4.0 %N) as organic nitrogen source. Also, the exogenous application of zinc (zinc sulfate, 22.8% Zn) and copper (copper sulfate, 21.8 %Cu) was evaluated. The tested plant was wheat (Cv. Giza 171). The nitrogen treatments [$T_1$: 100% of NRD as urea (control),$T_2$:75% of NRD as urea+25% of NRD as Azolla,$T_3$:50% of NRD as urea+50% of NRD as Azolla, $T_4$: 25% of NRD as urea +75% of NRD as Azolla and $T_5$: 100% of NRD as Azolla] represented the main plots, while

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exogenous application of the studied elements [$F_1$: control (without spraying),$F_2$:Zn (at rate of 200 $mgL^{-1}$) and $F_3$: (Cu at rate of 20 $mgL^{-1}$)] represented the sub main plots. The findings illustrate that wheat plants grown under $T_2$ treatment had the highest values of growth performance (*e.g.,* fresh and dry weights and total chlorophyll), yield and its components (*e.g.,* grain yield, spike length and weight of 1000 grain) and biochemical traits (*e.g.,* carbohydrates and total protein) compared to the corresponding wheat plants grown under other studied N treatments, as $T_1$ treatment (control) came in the second order followed by $T_3$ treatment, while $T_4$ and $T_5$ treatments came in the last order, respectively. Regarding the external applications, the cu foliar application was the superior treatment followed by Zn treatment, while the control treatment came in the last order. Generally, the maximum values were recorded under combined treatment of ($T_2$x$F_3$). On the other hand, some soil fertility parameters like A-N, A-P and A-K were affected due to all studied treatments. Finally, it can be concluded the possibility of using Azolla as a partial substitute for synthetic nitrogen fertilizers. Also, the obtained results confirm the vital role of both Zn and Cu in wheat plants.

*Keywords: Azolla; urea; environmental hazards; sustainable agriculture.*

## ABBREVIATIONS

*A-N, available nitrogen; A-P, available phosphorus; A-K, available potassium.*

## 1. INTRODUCTION

Synthetic nitrogen fertilizers can contribute to environmental hazards like water pollution and greenhouse gas emissions [1]. Excess nitrogen from synthetic fertilizers can run off into waterways, leading to harmful algal blooms and oxygen-deprived "dead zones" in aquatic ecosystems [2]. Additionally, the usage of mineral nitrogen fertilizers can contribute to potentially harming plant growth [3]. On the other hand, the production of synthetic nitrogen fertilizers is an energy-intensive process that contributes to greenhouse gas emissions, which can contribute to climate change [4]. Excessive use of synthetic nitrogen fertilizers can cause several issues for both plants and humans. Synthetic nitrogen fertilizers can lead to soil degradation over time. They can reduce the soil's natural fertility and decrease its ability to retain water, leading to soil erosion and nutrient depletion. Excess nitrogen in the soil can leach into groundwater and surface water. Nitrate, a byproduct of nitrogen fertilizer use, can contaminate groundwater and cause health issues when consumed by humans, particularly infants. Excessive use of synthetic nitrogen fertilizers can cause damage to plant roots and leaves. This can make plants more susceptible to pests and diseases, as well as reduce their overall health and productivity [5].

It is possible to use organic sources such as Azolla as a partial substitute for synthetic nitrogen fertilizers in cultivation [6]. Azolla is a type of aquatic fern that can be used as an organic fertilizer in the agriculture sector. It is a rich source of nitrogen, which is an essential nutrient for plant growth [7]. Azolla can be used as a green manure, where it is grown in fields and ploughed into the soil to improve soil fertility [8]. It can also be used as a biofertilizer, where it is added to the soil or used as a foliar spray to supply plants with nitrogen [9]. Additionally, Azolla can be used as a feed supplement for livestock as it is high in protein [10]. The usage of Azolla can reduce the cost of fertilizer inputs, and promote sustainable agriculture [11].

In Egypt, most fertilization programs don't include zinc (Zn) and copper (Cu) as significant elements. However, fertilization with Zn and Cu is necessary due to the decline of their availability in Egyptian soils due to the high pH values [12,13]. Zn and Cu are essential micronutrients required by higher plants for their normal development and growth [14]. The deficiency of Zn causes interveinal chlorosis, stunted growth and reduced crop yield. Zn is involved in many biochemical processes, including respiration, photosynthesis, enzymatic reactions and the metabolism of proteins and carbohydrates [15]. Also, it is necessary for the synthesis of growth hormones and regulation of the stomatal aperture [16,17]. While Cu is essential for the formation of plant cell walls and the synthesis of chlorophyll, which is necessary for photosynthesis [18]. Also, the deficiency of Cu causes reduced crop yield [19,20].

Wheat (*Triticum aestivum*) is a commonly used test plant for agricultural experiments related to crop management and soil fertility because it is a major crop worldwide and is important for food security [21,22].

Therefore, the specific aim of the current research work was to evaluate the possibility of using Azolla as a partial substitute for synthetic nitrogen fertilizers in addition to evaluating the vital role of both Zn and Cu in wheat plants simultaneously.

## 2. MATERIALS AND METHODS

A field trial was carried out during the two successive seasons (2021/2022-2022/2023) aiming to assess the additive of nitrogen recommended dose (NRD =178.6 kg N $ha^{-1}$) as combined treatments of urea (46.5%N) as mineral nitrogen source and Azolla (4.0 %N) as organic nitrogen source. Also, the exogenous application of zinc (zinc sulfate, 22.8% Zn) and copper (copper sulfate, 21.8 %Cu) was evaluated.

### 2.1 Tested Crop

The wheat plant (*Triticum aestivum* Cv. Giza 171) was chosen as a model crop to realize the aim of the current study. Wheat grains were obtained from Agricultural Research Center (ARC).

### 2.2 Location of the Studied Area

The current study was implemented in the Tag El-Ezz Experimental Farm, ARC, Egypt, which located at 31°31' 47.64" N latitude and 30°56' 12.88" E longitude.

### 2.3 Soil Sampling and the Substances Studied

Table 1 points out the properties of the initial soil before sowing as well as the properties of used Azolla. The Azolla were obtained from Micro. Res., dep., Soil, Water and Environment Research Institute, ARC. While zinc sulfate ($ZnSO_4.7H_2O$) and copper sulfate ($CuSO_4.7H_2O$) were purchased from Agro Egypt for Agricultural Development Company.

### 2.4 Experimental Design and Treatments

The nitrogen treatments [$T_1$: 100% of NRD as urea (control),$T_2$:75% of NRD as urea+25% of NRD as Azolla,$T_3$:50% of NRD as urea+50% of NRD as Azolla, $T_4$: 25% of NRD as urea +75% of NRD as Azolla and $T_5$: 100% of NRD as Azolla] represented the main plots, while exogenous application of the studied micro-elements [$F_1$: control (without spraying),$F_2$:Zn (at rate of 200 $mgL^{-1}$) and $F_3$: (Cu at rate of 20 $mgL^{-1}$)] represented the sub main plots.

### 2.5 Experimental Setup

Seeds were sown on the $10^{th}$ of November during both seasons at a rate of 150 kg $ha^{-1}$. The sub plot area was 12.6 $m^2$ (3.0 m × 4.2 m). Before sowing, the plots received the Azolla fertilizer depending on the studied treatments. Urea was added depending on the studied treatments in two equal portions i.e. after 30 and 50 days of cultivation. Zinc sulfate and copper sulfate were sprayed according to the studied treatments in two times (after 45 and 60 days from sowing) during the experiment period with a volume of 960 L $ha^{-1}$. Calcium superphosphate (6.6%P) was added before ploughing at rate of 240 kg $ha^{-1}$, while potassium sulfate (48 % $K_2O$) was added at a rate of 122 kg $ha^{-1}$. Irrigation process was done with 5 irrigations under the flood system. The other normal agricultural practices were done as traditional. Harvesting was done on the $26^{th}$ of April during the two studied seasons.

### 2.6 Measurement Traits

#### 2.6.1 At a period of 70 days after wheat sowing

Ten wheat plants were taken to determine the following criteria:

a) Growth criteria (fresh and dry weights, g $plant^{-1}$).
b) Photosynthetic pigments (total chlorophyll and carotene, mg $g^{-1}$F.W.).
c) Leaves chemical constituents [N, P, K (%), Zn and Cu (mg $kg^{-1}$)].

Photosynthetic pigments (leaves, F.W) were determined according to the stander methods reported by Picazo et al. [27]. Chemical content in wheat tissues (leaves, D.W.) were determined according to the stander methods reported by Walinga et al. [28], as the samples of wheat leaves were digested according to the stander method [using mixture of $HClO_4$ and $H_2SO_4$ (1:1)] as described by Peterburgski [29]. The apparatuses used were kjeldahl (for N), spectrophotometric (for P), flame photometer (for K) and atomic adsorption (for Zn and Cu).

**Table 1. The properties of both initial soil and Azolla (the combined data over both studied seasons)**

| Initial soil | | Azolla | | References used |
|---|---|---|---|---|
| **Parameters and unit** | **Values** | **Parameters and unit** | **Values** | 1. Dewis and Freitas, [23]. |
| Chemical characteristics | | Ash content, g $Kg^{-1}$ | 100 | 2. Hesse, [24]. |
| pH | 8.00 | Crude protein, g $Kg^{-1}$ | 260 | 3. Gee and Baudet [25]. |
| $CaCO_3$ % | 1.40 | Starch, g $Kg^{-1}$ | 69.0 | 4. Tandon [26]. |
| EC, $dSm^{-1}$ | 6.15 | OM, g $Kg^{-1}$ | 800 | |
| O.M, % | 1.75 | N, % | 4.00 | |
| N, $mgKg^{-1}$ | 38.3 | K,% | 2.65 | |
| P, $mgKg^{-1}$ | 7.43 | Ca,% | 0.80 | |
| K , $mgKg^{-1}$ | 205.6 | Mg,% | 0.50 | |
| Particle size distribution (%) | | P,% | 0.90 | |
| Sand | 15 | Fe,% | 0.60 | |
| Silt | 37 | | | |
| Clay | 48 | | | |
| Textural class is clayey | | | | |

#### 2.6.2 At harvest stage

Ten wheat plants were taken to estimate the wheat yield and its components as follows:

a- Yield and its components [grain, straw and biological yield (Mg $h^{-1}$), harvest index (%), spike length (cm), spike weight (g), weight of 1000 (g)] were determined.
HI= (Economic yield/ Biological yield)×100

b- Nutrient status of grains [N, P, K (%), Zn, Cu (mg $kg^{-1}$)] were determined as formerly mentioned with leaves.

c- Qualitative traits of grains (protein and carbohydrates, %) were determined according to Anonymous, [30] and Cipollini Jr et al. [31], respectively. As Protein content was calculated by using the following formula: Protein % = (N) × 5.75.

#### 2.6.3 Soil analysis

Soil available nutrients like N, P and K were determined after wheat plants harvest (as average of both the studied seasons) using kjeldahl, spectrophotometric and flame photometer, respectively.

### 2.7 Statistical Analysis

It was done according to Gomez and Gomez, [32], [using CoStat version 6.303 copyright [33]].

## 3. RESULTS AND DISCUSSION

### 3.1 Growth Performance and Productivity

Data of Tables (from 2 to 5) illustrate the impact of different ratios of mineral and organic nitrogen sources as combined treatments and foliar application of Zn and Cu and their interactions on growth criteria *i.e.,* fresh and dry weights, (g $plant^{-1}$) and photosynthetic pigments *i.e.,* total chlorophyll and carotene (mg $g^{-1}$ F.W.) (Table 2) and leaves chemical constituents *i.e.,* N, P, K(%),Zn, Cu (mg $kg^{-1}$) (Table 3) as well as on wheat yield and its components *i.e.,* grain, straw and biological yield (Mg $h^{-1}$), harvest index (%), spike length (cm), spike weight (g), weight of 1000 grain (g) (Table 4) and nutrient status of grains and quality *i.e.,* N, P, K (%), Zn, Cu (mg $kg^{-1}$), protein and carbohydrates, (%)(Table 5). The data illustrate that all studied treatments significantly affected all aforementioned traits.

The findings illustrate that wheat plants grown under $T_2$ treatment (75% of NRD as urea+25% of NRD as Azolla) had the highest values of growth performance, yield and its components and biochemical traits compared to the corresponding wheat plants grown under other studied N treatments, as $T_1$ treatment (100% of NRD as urea, control) came in the second order followed by $T_3$ treatment (50% of NRD as urea+50% of NRD as Azolla), while $T_4$ (25% of NRD as urea +75% of NRD as Azolla) and $T_5$ (100% of NRD as Azolla) treatments came in the last order, respectively. The superiority of $T_2$ treatment compared to others N treatments may be attributed that the Azolla had a vital role in supplying nutrients to wheat plants, where this fertilizer contained many nutrient elements *e.g.,* Fe, Ca, N, P, K that are associated with improving photosynthetic efficiency as well as physiological and meristematic activities in the wheat plants. Moreover, its high content of organic matter. On other hand, Azolla is a rich source of nitrogen, which is an essential nutrient for plant growth. Nitrogen is a component of chlorophyll, the molecule that allows plants to photosynthesize and produce energy. Without enough N, plants may have stunted growth, yellowing of leaves, and reduced crop yield. N is also a necessary building block for producing amino acids, which are the building blocks of proteins. In addition, N is essential for producing nucleic acids, which are the building blocks of RNA&DNA. These molecules are essential for cell elongation and cell division which reflect in increasing plant growth. Generally, Azolla can be used as a green manure. Thus fertilizing with 75% of NRD as urea+25% of NRD as Azolla provided the wheat plant with all its nitrogen requirements. While the decrease in the ratio of urea at the expense of Azolla in the rest of the treatments was not feasible in achieving the wheat plant's nitrogen requirements [6,7,10,11].

Regarding the external applications, the cu foliar application was the superior treatment followed by Zn treatment, while the control treatment came in the last order. Concerning the comparison among foliar treatments, it could be concluded that copper and zinc treatments go one better than control treatment and this may be due to their vital role in many biochemical processes, including respiration, photosynthesis, and the metabolism of proteins and carbohydrates. The superiority of Cu treatment over Zn treatment may be due to Cu is considered more critical for plant nutrition than Zn, despite the importance of both Cu and Zn, because Cu is required in smaller quantities than Zn, and plants can easily become deficient in Cu due to its low availability in soils. Additionally, Cu is more tightly bound to soil particles than Zn, making it less available for plant uptake. Copper can play a key role in photosynthetic and respiratory electron transport chains, cell wall metabolism, ethylene sensing and oxidative stress protection as well as the biogenesis of molybdenum cofactor. Therefore, a deficiency in the copper supply leads to alter essential functions in the metabolism of higher plants. in addition, in wheat, copper helps to increase the proportion of gluten proteins, which are critical for the dough-forming and baking properties of wheat flour. Gluten proteins are responsible for the elasticity and stretchiness of dough, and they play an important role in determining the quality of wheat flour for bread-making and other baking applications. [14-16,19,20].

**Table 2. Effect of different ratios of mineral and organic nitrogen sources as combined treatments and foliar application of Zn and Cu on growth parameters and photosynthetic pigments at period of 70 days from sowing during two successive seasons (2021-2022)**

| Treatments | | Growth parameters | | | | Photosynthetic pigments | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Fresh weight, g plant$^{-1}$ | | Dry weight, g plant$^{-1}$ | | T. Chlorophyll, mg g$^{-1}$ F.W. | | Carotene, mg g$^{-1}$F.W. | |
| | | 1$^{st}$ | 2$^{nd}$ | 1$^{st}$ | 2$^{nd}$ | 1$^{st}$ | 2$^{nd}$ | 1$^{st}$ | 2$^{nd}$ |
| **Nitrogen treatments** | | | | | | | | | |
| $T_1$ | | 40.42b | 40.85b | 13.64b | 13.82b | 1.133b | 1.155b | 0.438b | 0.444b |
| $T_2$ | | 42.00a | 42.57a | 13.92a | 14.07a | 1.172a | 1.194a | 0.455a | 0.463a |
| $T_3$ | | 39.12c | 39.59c | 12.26c | 12.43c | 1.045c | 1.059c | 0.407c | 0.414c |
| $T_4$ | | 38.16d | 38.64d | 12.00d | 12.20d | 1.008d | 1.028d | 0.396d | 0.404d |
| $T_5$ | | 35.20e | 35.64e | 9.86e | 9.99e | 0.962e | 0.981e | 0.364e | 0.371e |
| LSD at 5% | | 0.11 | 0.11 | 0.16 | 0.03 | 0.005 | 0.009 | 0.004 | 0.005 |
| **Foliar applications** | | | | | | | | | |
| $F_1$ | | 38.09c | 38.51c | 11.75c | 11.90c | 1.028c | 1.049c | 0.397c | 0.404c |
| $F_2$ | | 38.81b | 39.27b | 12.45b | 12.64b | 1.073b | 1.091b | 0.414b | 0.420b |
| $F_3$ | | 40.04a | 40.60a | 12.82a | 12.96a | 1.091a | 1.110a | 0.426a | 0.433a |
| LSD at 5% | | 0.13 | 0.13 | 0.14 | 0.04 | 0.003 | 0.006 | 0.002 | 0.004 |
| **Interaction** | | | | | | | | | |
| $T_1$ | $F_1$ | 39.93 | 40.37 | 13.28 | 13.44 | 1.095 | 1.117 | 0.425 | 0.434 |
| | $F_2$ | 40.55 | 40.97 | 13.79 | 14.00 | 1.142 | 1.164 | 0.438 | 0.439 |
| | $F_3$ | 40.76 | 41.22 | 13.85 | 14.00 | 1.162 | 1.184 | 0.451 | 0.459 |
| $T_2$ | $F_1$ | 40.52 | 40.97 | 13.40 | 13.53 | 1.120 | 1.142 | 0.430 | 0.439 |
| | $F_2$ | 41.65 | 42.23 | 14.00 | 14.17 | 1.184 | 1.203 | 0.457 | 0.466 |
| | $F_3$ | 43.84 | 44.50 | 14.37 | 14.51 | 1.211 | 1.236 | 0.480 | 0.485 |
| $T_3$ | $F_1$ | 38.20 | 38.58 | 11.58 | 11.77 | 0.995 | 1.015 | 0.392 | 0.396 |

| Treatments | | Growth parameters | | | | Photosynthetic pigments | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Fresh weight, g plant$^{-1}$ | | Dry weight, g plant$^{-1}$ | | T. Chlorophyll, mg g$^{-1}$ F.W. | | Carotene, mg g$^{-1}$F.W. | |
| | | 1$^{st}$ | 2$^{nd}$ | 1$^{st}$ | 2$^{nd}$ | 1$^{st}$ | 2$^{nd}$ | 1$^{st}$ | 2$^{nd}$ |
| | $F_2$ | 39.28 | 39.79 | 12.56 | 12.74 | 1.056 | 1.066 | 0.411 | 0.419 |
| | $F_3$ | 39.88 | 40.40 | 12.64 | 12.77 | 1.083 | 1.095 | 0.419 | 0.427 |
| $T_4$ | $F_1$ | 37.53 | 37.98 | 11.22 | 11.41 | 0.977 | 0.996 | 0.383 | 0.390 |
| | $F_2$ | 38.25 | 38.67 | 12.24 | 12.49 | 1.020 | 1.040 | 0.400 | 0.408 |
| | $F_3$ | 38.70 | 39.28 | 12.56 | 12.69 | 1.028 | 1.048 | 0.406 | 0.415 |
| $T_5$ | $F_1$ | 34.27 | 34.66 | 9.25 | 9.36 | 0.954 | 0.973 | 0.357 | 0.364 |
| | $F_2$ | 34.31 | 34.69 | 9.66 | 9.80 | 0.961 | 0.982 | 0.364 | 0.371 |
| | $F_3$ | 37.01 | 37.57 | 10.67 | 10.81 | 0.971 | 0.988 | 0.372 | 0.379 |
| LSD at 5% | | 0.30 | 0.30 | 0.31 | 0.09 | 0.007 | 0.012 | 0.005 | 0.009 |

*Means within a row followed by a different letter (s) are statistically different at a 0.05 level*
*$T_1$: 100% of NRD as urea (control),$T_2$:75% of NRD as urea+25% of NRD as Azolla,$T_3$:50% of NRD as urea+50% of NRD as Azolla, $T_4$: 25% of NRD as urea +75% of NRD as Azolla, $T_5$: 100% of NRD as Azolla, $F_1$: control (without spraying),$F_2$:Zn (at rate of 200 mgL$^{-1}$) and $F_3$: (Cu at rate of 20 mgL$^{-1}$)*

**Table 3. Effect of different ratios of mineral and organic nitrogen sources as combined treatments and foliar application of Zn and Cu on straw chemical constituents at period of 70 days from sowing during two successive seasons (2021-2022)**

| Treatments | | N, % | | P, % | | K, % | | Zn, mg kg$^{-1}$ | | Cu, mg kg$^{-1}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1$^{st}$ | 2$^{nd}$ | 1$^{st}$ | 2$^{nd}$ | 1$^{st}$ | 2$^{nd}$ | 1$^{st}$ | 2$^{nd}$ | 1$^{st}$ | 2$^{nd}$ |
| **Nitrogen treatments** | | | | | | | | | | | |
| $T_1$ | | 3.34b | 3.40b | 0.398b | 0.406b | 3.26b | 3.30b | 41.86b | 42.33b | 8.00b | 8.08b |
| $T_2$ | | 3.45a | 3.50a | 0.407a | 0.415a | 3.34a | 3.37a | 42.87a | 43.45a | 8.11a | 8.20a |
| $T_3$ | | 3.07c | 3.11c | 0.371c | 0.378c | 3.02d | 3.06d | 39.25c | 39.73d | 7.74d | 7.85d |
| $T_4$ | | 2.97d | 3.02d | 0.359d | 0.364d | 2.90c | 2.95c | 38.23d | 38.72c | 7.63c | 7.73c |
| $T_5$ | | 2.77e | 2.81e | 0.335e | 0.341e | 2.67e | 2.65e | 36.07e | 36.51e | 7.37e | 7.46e |
| LSD at 5% | | 0.004 | 0.03 | 0.003 | 0.003 | 0.03 | 0.07 | 0.11 | 0.12 | 0.07 | 0.08 |
| **Foliar applications** | | | | | | | | | | | |
| $F_1$ | | 3.02c | 3.07c | 0.363c | 0.369c | 2.92c | 2.96b | 38.60c | 39.00c | 7.65b | 7.74c |
| $F_2$ | | 3.13b | 3.18b | 0.374b | 0.382b | 3.06b | 3.11a | 39.87b | 40.37b | 7.80a | 7.88b |
| $F_3$ | | 3.21a | 3.26a | 0.385a | 0.392a | 3.13a | 3.13a | 40.50a | 41.07a | 7.86a | 7.97a |
| LSD at 5% | | 0.03 | 0.04 | 0.002 | 0.004 | 0.04 | 0.07 | 0.14 | 0.14 | 0.10 | 0.08 |
| **Interaction** | | | | | | | | | | | |
| $T_1$ | $F_1$ | 3.24 | 3.29 | 0.390 | 0.397 | 3.14 | 3.17 | 40.89 | 41.34 | 7.92 | 7.98 |
| | $F_2$ | 3.36 | 3.41 | 0.397 | 0.405 | 3.28 | 3.33 | 42.08 | 42.54 | 8.02 | 8.10 |
| | $F_3$ | 3.43 | 3.50 | 0.408 | 0.416 | 3.37 | 3.40 | 42.62 | 43.12 | 8.07 | 8.16 |
| $T_2$ | $F_1$ | 3.33 | 3.38 | 0.393 | 0.401 | 3.16 | 3.19 | 41.47 | 41.90 | 7.98 | 8.05 |
| | $F_2$ | 3.45 | 3.51 | 0.410 | 0.419 | 3.39 | 3.43 | 43.25 | 43.91 | 8.15 | 8.24 |
| | $F_3$ | 3.56 | 3.61 | 0.419 | 0.427 | 3.47 | 3.51 | 43.89 | 44.55 | 8.20 | 8.32 |
| $T_3$ | $F_1$ | 2.95 | 3.00 | 0.356 | 0.363 | 2.89 | 2.92 | 37.88 | 38.22 | 7.58 | 7.68 |
| | $F_2$ | 3.09 | 3.14 | 0.370 | 0.377 | 3.07 | 3.11 | 39.60 | 40.17 | 7.79 | 7.90 |
| | $F_3$ | 3.16 | 3.20 | 0.386 | 0.394 | 3.10 | 3.15 | 40.28 | 40.79 | 7.85 | 7.97 |
| $T_4$ | $F_1$ | 2.89 | 2.94 | 0.346 | 0.349 | 2.82 | 2.87 | 37.26 | 37.71 | 7.51 | 7.61 |
| | $F_2$ | 2.99 | 3.05 | 0.363 | 0.370 | 2.92 | 2.98 | 38.41 | 38.82 | 7.64 | 7.72 |
| | $F_3$ | 3.03 | 3.09 | 0.369 | 0.373 | 2.96 | 3.00 | 39.03 | 39.64 | 7.73 | 7.84 |
| $T_5$ | $F_1$ | 2.70 | 2.74 | 0.330 | 0.336 | 2.61 | 2.65 | 35.50 | 35.85 | 7.28 | 7.37 |
| | $F_2$ | 2.75 | 2.79 | 0.333 | 0.340 | 2.65 | 2.69 | 36.02 | 36.43 | 7.38 | 7.46 |
| | $F_3$ | 2.86 | 2.90 | 0.342 | 0.349 | 2.74 | 2.61 | 36.68 | 37.23 | 7.45 | 7.55 |
| LSD at 5% | | 0.08 | 0.09 | 0.004 | 0.009 | 0.08 | 0.15 | 0.31 | 0.31 | 0.21 | 0.19 |

*Means within a row followed by a different letter (s) are statistically different at a 0.05 level*
*$T_1$: 100% of NRD as urea (control),$T_2$:75% of NRD as urea+25% of NRD as Azolla,$T_3$:50% of NRD as urea+50% of NRD as Azolla, $T_4$: 25% of NRD as urea +75% of NRD as Azolla, $T_5$: 100% of NRD as Azolla, $F_1$: control (without spraying),$F_2$:Zn (at rate of 200 mgL$^{-1}$) and $F_3$: (Cu at rate of 20 mgL$^{-1}$)*

**Table 4. Effect of different ratios of mineral and organic nitrogen sources as combined treatments and foliar application of Zn and Cu on wheat yield and its components during two successive seasons (2021-2022)**

| Treatments | | Grain yield, Mg $h^{-1}$ | | Straw yield , Mg $h^{-1}$ | | Biological yield , Mg $h^{-1}$ | | Harvest index, % | | Spike length, cm | | Spike weight, g | | Weight of 1000 grain, g | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| **Nitrogen treatments** | | | | | | | | | | | | | | | |
| $T_1$ | | 6.25b | 6.38b | 10.17b | 10.37b | 16.42b | 16.74b | 38.03b | 38.09a | 19.47b | 19.69b | 4.19b | 4.28b | 59.98a | 60.92b |
| $T_2$ | | 6.48a | 6.57a | 10.39a | 10.56a | 16.87a | 17.13a | 38.42a | 38.33a | 20.04a | 20.21a | 4.35a | 4.43a | 60.94a | 61.82a |
| $T_3$ | | 5.76c | 5.86c | 9.49c | 9.62c | 15.25c | 15.48c | 37.78c | 37.84ab | 18.03c | 18.25c | 3.88c | 3.94c | 58.76b | 59.68c |
| $T_4$ | | 5.60d | 5.69d | 9.32d | 9.52d | 14.92d | 15.21d | 37.53d | 37.40bc | 17.32d | 17.54d | 3.74d | 3.81d | 58.09b | 59.20c |
| $T_5$ | | 4.96e | 5.07e | 8.41e | 8.59e | 13.37e | 13.66e | 37.10e | 37.10c | 15.32e | 15.50e | 3.32e | 3.40e | 56.76c | 57.6 d |
| LSD at 5% | | 0.01 | 0.10 | 0.06 | 0.09 | 0.05 | 0.10 | 0.18 | 0.54 | 0.29 | 0.23 | 0.04 | 0.06 | 0.82 | 0.45 |
| **Foliar applications** | | | | | | | | | | | | | | | |
| $F_1$ | | 5.57c | 5.69c | 9.24c | 9.42b | 14.82c | 15.11c | 37.60b | 37.61b | 17.41c | 17.55b | 3.74c | 3.81c | 58.17b | 59.04b |
| $F_2$ | | 5.84b | 5.93b | 9.66b | 9.83a | 15.50b | 15.76b | 37.64b | 37.57b | 18.24b | 18.47a | 3.93b | 4.00b | 59.18a | 60.13a |
| $F_3$ | | 6.02a | 6.12a | 9.77a | 9.94a | 15.79a | 16.07a | 38.08a | 38.08a | 18.46a | 18.70a | 4.02a | 4.10a | 59.37a | 60.38a |
| LSD at 5% | | 0.07 | 0.06 | 0.10 | 0.12 | 0.14 | 0.15 | 0.32 | 0.31 | 0.19 | 0.25 | 0.02 | 0.03 | 0.39 | 0.26 |
| **Interaction** | | | | | | | | | | | | | | | |
| $T_1$ | $F_1$ | 6.03 | 6.17 | 10.00 | 10.21 | 16.03 | 16.38 | 37.63 | 37.69 | 18.94 | 19.19 | 4.02 | 4.11 | 59.66 | 60.46 |
| | $F_2$ | 6.28 | 6.40 | 10.20 | 10.38 | 16.48 | 16.77 | 38.09 | 38.14 | 19.57 | 19.79 | 4.19 | 4.27 | 60.00 | 60.99 |
| | $F_3$ | 6.43 | 6.57 | 10.32 | 10.51 | 16.75 | 17.08 | 38.38 | 38.45 | 19.90 | 20.10 | 4.37 | 4.46 | 60.27 | 61.28 |
| $T_2$ | $F_1$ | 6.18 | 6.29 | 10.12 | 10.32 | 16.29 | 16.61 | 37.91 | 37.88 | 19.24 | 19.16 | 4.13 | 4.20 | 60.00 | 60.93 |
| | $F_2$ | 6.53 | 6.55 | 10.46 | 10.63 | 16.99 | 17.18 | 38.43 | 38.12 | 20.38 | 20.70 | 4.44 | 4.53 | 61.39 | 62.16 |
| | $F_3$ | 6.75 | 6.86 | 10.59 | 10.74 | 17.34 | 17.60 | 38.92 | 38.99 | 20.51 | 20.79 | 4.48 | 4.56 | 61.45 | 62.37 |
| $T_3$ | $F_1$ | 5.50 | 5.61 | 9.04 | 9.16 | 14.54 | 14.77 | 37.82 | 37.96 | 17.61 | 17.78 | 3.75 | 3.83 | 57.34 | 58.27 |
| | $F_2$ | 5.85 | 5.95 | 9.68 | 9.82 | 15.53 | 15.77 | 37.68 | 37.72 | 18.12 | 18.39 | 3.91 | 3.97 | 59.30 | 60.25 |
| | $F_3$ | 5.93 | 6.01 | 9.75 | 9.88 | 15.68 | 15.90 | 37.83 | 37.83 | 18.36 | 18.57 | 3.98 | 4.04 | 59.66 | 60.56 |
| $T_4$ | $F_1$ | 5.39 | 5.48 | 8.78 | 8.99 | 14.18 | 14.47 | 38.05 | 37.87 | 16.19 | 16.38 | 3.54 | 3.60 | 57.21 | 58.14 |
| | $F_2$ | 5.56 | 5.65 | 9.56 | 9.74 | 15.12 | 15.39 | 36.75 | 36.70 | 17.73 | 17.92 | 3.82 | 3.88 | 58.42 | 59.60 |
| | $F_3$ | 5.85 | 5.93 | 9.62 | 9.84 | 15.47 | 15.77 | 37.81 | 37.62 | 18.03 | 18.32 | 3.87 | 3.94 | 58.64 | 59.90 |
| $T_5$ | $F_1$ | 4.77 | 4.88 | 8.26 | 8.44 | 13.03 | 13.31 | 36.58 | 36.63 | 15.08 | 15.24 | 3.25 | 3.33 | 56.62 | 57.39 |
| | $F_2$ | 4.99 | 5.09 | 8.41 | 8.60 | 13.40 | 13.69 | 37.22 | 37.17 | 15.39 | 15.56 | 3.30 | 3.37 | 56.78 | 57.62 |
| | $F_3$ | 5.13 | 5.25 | 8.56 | 8.75 | 13.70 | 13.99 | 37.48 | 37.49 | 15.49 | 15.70 | 3.42 | 3.50 | 56.85 | 57.79 |
| LSD at 5% | | 0.15 | 0.12 | 0.23 | 0.26 | 0.30 | 0.32 | 0.72 | 0.68 | 0.42 | 0.57 | 0.04 | 0.07 | 0.87 | 0.59 |

*Means within a row followed by a different letter (s) are statistically different at a 0.05 level*

*$T_1$: 100% of NRD as urea (control),$T_2$:75% of NRD as urea+25% of NRD as Azolla,$T_3$:50% of NRD as urea+50% of NRD as Azolla, $T_4$: 25% of NRD as urea +75% of NRD as Azolla, $T_5$: 100% of NRD as Azolla, $F_1$: control (without spraying),$F_2$:Zn (at rate of 200 $mgL^{-1}$) and $F_3$: (Cu at rate of 20 $mgL^{-1}$)*

**Table 5. Effect of different ratios of mineral and organic nitrogen sources as combined treatments and foliar application of Zn and Cu on wheat grain quality traits after harvest process during two successive seasons (2021-2022)**

| Treatments | | Grains chemical constituents | | | | | | | | | | Biochemical traits | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N, % | | P, % | | K, % | | Zn, mg $kg^{-1}$ | | Cu, mg $kg^{-1}$ | | Carbohydrates % | | Protein % | |
| | | $1^{st}$ | $2^{nd}$ | $1^{st}$ | $2^{nd}$ | $1^{st}$ | $2^{nd}$ | $1^{st}$ | $2^{nd}$ | $1^{st}$ | $2^{nd}$ | $1^{st}$ | $2^{nd}$ | $1^{st}$ | $2^{nd}$ |
| **Nitrogen treatments** | | | | | | | | | | | | | | | |
| $T_1$ | | 2.16b | 2.20b | 0.263b | 0.268b | 1.88b | 1.90b | 23.33b | 23.75b | 3.18b | 3.23b | 71.33b | 72.44b | 12.44b | 12.64b |
| $T_2$ | | 2.26a | 2.29a | 0.271a | 0.275a | 2.00a | 2.03a | 24.31a | 24.71a | 3.28a | 3.33a | 72.16a | 73.27a | 12.99a | 13.19a |
| $T_3$ | | 1.91c | 1.94c | 0.239c | 0.243c | 1.67c | 1.69c | 20.72c | 21.05c | 2.91c | 2.97c | 69.75c | 70.78c | 10.96c | 11.13c |
| $T_4$ | | 1.74d | 1.77d | 0.232d | 0.236d | 1.58d | 1.60d | 19.75d | 20.19d | 2.81d | 2.86d | 69.09d | 70.20d | 10.01d | 10.20d |
| $T_5$ | | 1.53e | 1.55e | 0.211e | 0.214e | 1.37e | 1.38e | 17.87e | 17.96e | 2.59e | 2.65e | 68.12e | 69.18e | 8.80e | 8.93e |
| LSD at 5% | | 0.06 | 0.03 | 0.002 | 0.002 | 0.02 | 0.05 | 0.44 | 0.09 | 0.08 | 0.09 | 0.49 | 0.32 | 0.36 | 0.19 |
| **Foliar applications** | | | | | | | | | | | | | | | |
| $F_1$ | | 1.81c | 1.84c | 0.232c | 0.237c | 1.59c | 1.61c | 20.22c | 20.44c | 2.84c | 2.90c | 69.34b | 70.40c | 10.42c | 10.58c |
| $F_2$ | | 1.95b | 1.98b | 0.246b | 0.250b | 1.73b | 1.75b | 21.39b | 21.77b | 2.98b | 3.03b | 70.32a | 71.44b | 11.20b | 11.37b |
| $F_3$ | | 2.00a | 2.03a | 0.251a | 0.255a | 1.78a | 1.80a | 21.98a | 22.38a | 3.04a | 3.09a | 70.61a | 71.68a | 11.50a | 11.70a |
| LSD at 5% | | 0.03 | 0.01 | 0.001 | 0.002 | 0.04 | 0.03 | 0.031 | 0.07 | 0.06 | 0.06 | 0.74 | 0.22 | 0.18 | 0.09 |
| **Interaction** | | | | | | | | | | | | | | | |
| $T_1$ | $F_1$ | 2.08 | 2.11 | 0.250 | 0.256 | 1.79 | 1.81 | 22.30 | 22.72 | 3.09 | 3.15 | 70.76 | 71.92 | 11.96 | 12.13 |
| | $F_2$ | 2.19 | 2.23 | 0.267 | 0.272 | 1.92 | 1.94 | 23.57 | 23.97 | 3.18 | 3.24 | 71.64 | 72.75 | 12.59 | 12.80 |
| | $F_3$ | 2.22 | 2.26 | 0.272 | 0.277 | 1.95 | 1.97 | 24.13 | 24.56 | 3.26 | 3.31 | 71.59 | 72.65 | 12.77 | 13.00 |
| $T_2$ | $F_1$ | 2.13 | 2.16 | 0.259 | 0.264 | 1.85 | 1.87 | 22.90 | 23.36 | 3.13 | 3.18 | 71.10 | 72.01 | 12.23 | 12.40 |
| | $F_2$ | 2.31 | 2.34 | 0.275 | 0.280 | 2.05 | 2.08 | 24.71 | 25.08 | 3.32 | 3.37 | 72.60 | 73.84 | 13.26 | 13.44 |
| | $F_3$ | 2.34 | 2.39 | 0.279 | 0.282 | 2.10 | 2.13 | 25.33 | 25.68 | 3.39 | 3.44 | 72.77 | 73.96 | 13.47 | 13.72 |
| $T_3$ | $F_1$ | 1.74 | 1.77 | 0.227 | 0.231 | 1.56 | 1.58 | 19.33 | 19.64 | 2.77 | 2.83 | 68.76 | 69.73 | 10.01 | 10.16 |
| | $F_2$ | 1.95 | 1.98 | 0.244 | 0.246 | 1.70 | 1.72 | 21.10 | 21.44 | 2.96 | 3.02 | 69.86 | 70.84 | 11.19 | 11.39 |
| | $F_3$ | 2.03 | 2.06 | 0.247 | 0.252 | 1.76 | 1.78 | 21.73 | 22.06 | 3.01 | 3.07 | 70.62 | 71.77 | 11.67 | 11.85 |
| $T_4$ | $F_1$ | 1.65 | 1.68 | 0.223 | 0.227 | 1.45 | 1.47 | 18.73 | 19.18 | 2.71 | 2.75 | 68.63 | 69.65 | 9.49 | 9.66 |
| | $F_2$ | 1.78 | 1.81 | 0.232 | 0.237 | 1.61 | 1.63 | 19.97 | 20.37 | 2.83 | 2.88 | 69.16 | 70.44 | 10.22 | 10.39 |
| | $F_3$ | 1.80 | 1.84 | 0.240 | 0.245 | 1.68 | 1.71 | 20.55 | 21.01 | 2.90 | 2.95 | 69.49 | 70.52 | 10.33 | 10.56 |
| $T_5$ | $F_1$ | 1.47 | 1.49 | 0.203 | 0.205 | 1.32 | 1.33 | 17.84 | 17.31 | 2.53 | 2.61 | 67.44 | 68.72 | 8.43 | 8.57 |
| | $F_2$ | 1.52 | 1.54 | 0.211 | 0.216 | 1.38 | 1.40 | 17.60 | 17.99 | 2.60 | 2.65 | 68.33 | 69.31 | 8.74 | 8.86 |
| | $F_3$ | 1.61 | 1.63 | 0.218 | 0.222 | 1.41 | 1.43 | 18.18 | 18.58 | 2.66 | 2.70 | 68.58 | 69.50 | 9.24 | 9.37 |
| LSD at 5% | | 0.07 | 0.04 | 0.003 | 0.007 | 0.08 | 0.07 | 0.70 | 0.15 | 0.13 | 0.13 | 1.65 | 0.48 | 0.40 | 0.20 |

*Means within a row followed by a different letter (s) are statistically different at a 0.05 level*

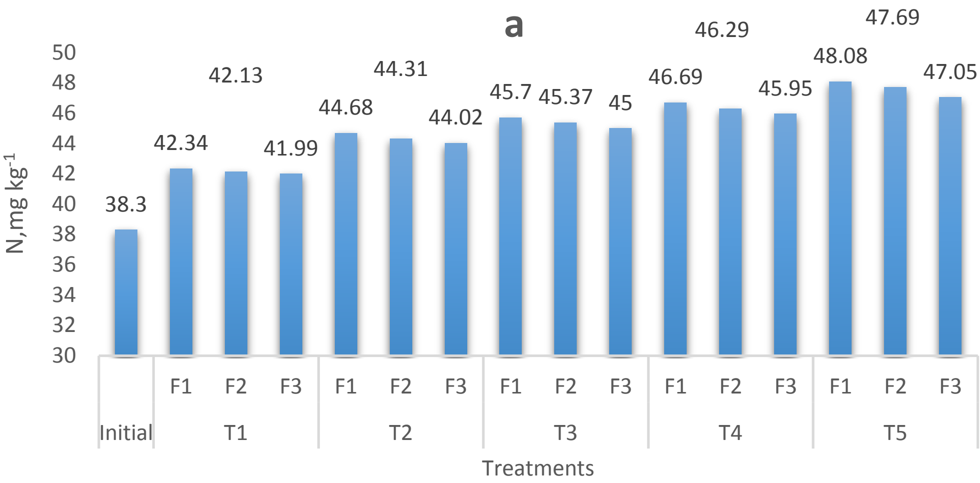
*$T_1$: 100% of NRD as urea (control), $T_2$: 75% of NRD as urea+25% of NRD as Azolla, $T_3$: 50% of NRD as urea+50% of NRD as Azolla, $T_4$: 25% of NRD as urea +75% of NRD as Azolla, $T_5$: 100% of NRD as Azolla, $F_1$: control (without spraying), $F_2$: Zn (at rate of 200 $mgL^{-1}$) and $F_3$: (Cu at rate of 20 $mgL^{-1}$)*

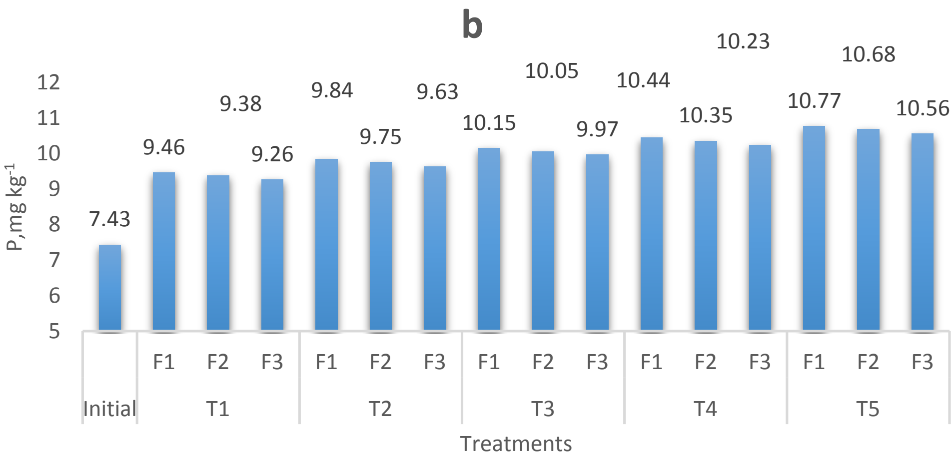
Generally, the maximum values of all parameters expressing growth performance and productivity were recorded under combined treatment of ($T_2$ x $F_3$).

### 3.2 Soil Analysis after Wheat Harvest

Fig. 1 indicate the effect of different ratios of mineral and organic nitrogen sources as combined treatments and foliar application of Zn and Cu on some soil fertility parameters as average values like available N, available P and available K (mg $kg^{-1}$) at harvest stage (combined data over both seasons). Fig 1 illustrates that using Azolla as a partial substitute for urea fertilizer led to increase soil available NPK (mg $kg^{-1}$) after harvest and this may be due to that Azolla fertilizer had a vital role in supplying the soil with different nutrients *e.g.,* N, P, K and organic matter. Also, it worth mentioning that the mean values of soil available NPK (mg $kg^{-1}$) increased as the ratio of Azolla increased. Also, soil addition of Azolla may have led to a relative decrease in the pH of the soil, and this positively reflected on the availability of nutrients in the soil.

The same Fig illustrates that external applications of Zn and Cu led to a decline in the mean values of soil available NPK (mg $kg^{-1}$) compared with the mean values of the corresponding soil containing wheat plants grown without exogenous applications. This may be owing to the role of Zn and Cu in improving wheat plant status via raising wheat plants' absorption of N, P, and K from the soil more than untreated wheat plants. Taking into consideration that wheat plant uptake with Cu was more than Zn, thus the mean values of available soil NPK (mg $kg^{-1}$) were less with Cu than Zn. The findings are in agreement with the obtained results of El-Shamy et al. [3] and El-Sherpiny et al. [4].



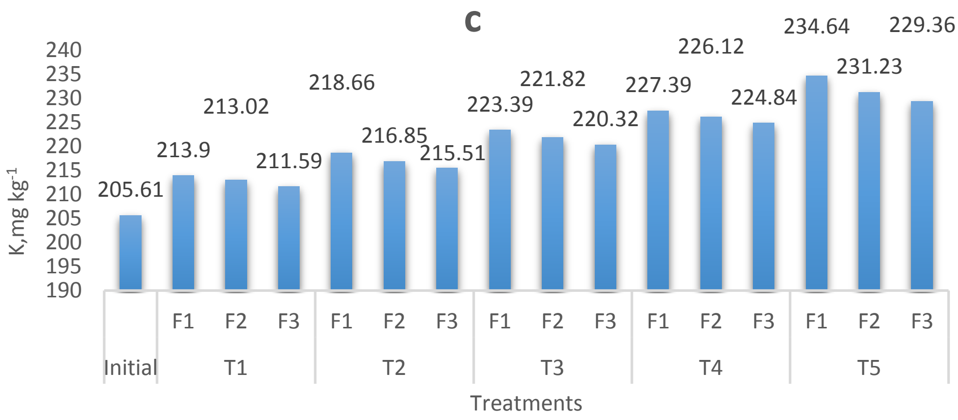



**Fig 1. Effect of different ratios of mineral and organic nitrogen sources as combined treatments and foliar application of Zn and Cu on some soil fertility parameters like A-N (Fig. 1,a), A-P (Fig. 1,b) and A-K (Fig. 1,c) at harvest stage (combined data over both seasons)**

*$T_1$: 100% of NRD as urea (control),$T_2$:75% of NRD as urea+25% of NRD as Azolla,$T_3$:50% of NRD as urea+50% of NRD as Azolla, $T_4$: 25% of NRD as urea +75% of NRD as Azolla, $T_5$: 100% of NRD as Azolla, $F_1$: control (without spraying),$F_2$:Zn (at rate of 200 mgL$^{-1}$) and $F_3$: (Cu at rate of 20 mgL$^{-1}$), A-N, available nitrogen, A-P, available phosphorus and A-K, available potassium*

## 4. CONCLUSION

Based on the obtained results of this study it could be concluded that the best growth performance and productivity for wheat plants under both studied conditions and other similar conditions were recorded under nitrogen fertilizing with 75% of NRD as urea+25% of NRD as Azolla and spraying with copper at a rate of 20 mg L$^{-1}$ during the wheat life period. Thus, it can be concluded the possibility of using Azolla as a partial substitute for synthetic nitrogen fertilizers. Also, the obtained results confirm the vital role of both Zn and Cu in wheat plants.

In conclusion, it can be said that the current research work is so important to add strength to researchers to improve strategic crops such as wheat and simultaneously reduce the usage of synthetic nitrogen fertilizers that positively affect the reduction of environmental pollution and climate change.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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