Original Research Article

Raising the efficiency of mineral fertilization using Azolla with microelements

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 and 2022 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 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_2xF_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 and sustainable agriculture.
Abbreviation: A-N, available nitrogen; A-P, available phosphorus; A-K, available potassium**.**

INTRODUCTION

Synthetic nitrogen fertilizers can contribute to environmental hazards like water pollution and greenhouse gas emissions **(Hashimi and Hashimi, 2020).** Excess nitrogen from synthetic fertilizers can run off into waterways, leading to harmful algal blooms and oxygen-deprived "dead zones" in aquatic ecosystems **(Randive *et al.* 2021).** Additionally, the usage of mineral nitrogen fertilizers can contribute to potentially harming plant growth **(El-Shamy *et al.* 2022).** 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 **(El-Sherpiny *et al.* 2022)**. 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 **(El-Zemrany and Faiyad, 2021).**

It is possible to use organic sources such as Azolla as a partial substitute for synthetic nitrogen fertilizers in cultivation **(Taha *et al.* 2017).** 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 **(Setiawati *et al.* 2018).** Azolla can be used as a green manure, where it is grown in fields and ploughed into the soil to improve soil fertility **(Taha *et al.* 2018).** 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 **(Malyan *et al.* 2019).** Additionally, Azolla can be used as a feed supplement for livestock as it is high in protein **(Abou Hussien *et al.* 2020).** The usage of Azolla can reduce the cost of fertilizer inputs, and promote sustainable agriculture **(Abou Hussien *et al.* 2021).**

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 **(El-Ramady *et al.* 2018 and Elbehiry *et al.* 2021).** Zn and Cu are essential micronutrients required by higher plants for their normal development and growth **(Tsonev and Cebola-Lidon, 2012).** 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 **(Eteng *et al.* 2014).** Also, it is necessary for the synthesis of growth hormones and regulation of the stomatal aperture (**Sturikova *et al.* 2018 and Ewais, *et al*. 2023).** While Cu is essential for the formation of plant cell walls and the synthesis of chlorophyll, which is necessary for photosynthesis (**Yruela, 2005).** Also, the deficiency of Cu causes reduced crop yield **(Kumar *et al.* 2009 and Abbaszadeh-Dahaji *et al.* 2019).**

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 **(Evans and Wardlaw, 2017 and El-Mantawy *et al.* 2022).**

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.

MATERIALS AND METHODS

A field trial was carried out during the two successive seasons (2021-2022) 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.

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. 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.

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.

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, (1970).** 2. **Hesse, (1971).** 3. **Gee and Baudet (1986).** 4. **Tandon (2005).**
Chemical characteristics		Ash content, g Kg^{-1}	100	
pH	8.00	Crude protein, g Kg^{-1}	260	
$CaCO_3$ %	1.40	Starch, g Kg^{-1}	69.0	
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				

4. Experimental design and treatments

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

5. Experimental Setup

Seeds were sown on the 10th 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 26th of April during the two studied seasons.

6. Measurement traits.

- **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*. (2013)**. Chemical content in wheat tissues (leaves, D.W.) were determined according to the stander methods reported by **Walinga *et al*. (2013)**, 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 (1968).** The apparatuses used were kjeldahl (for N), spectrophotometric (for P), flame photometer (for K) and atomic adsorption (for Zn and Cu).

- **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) $\times$ 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, (1990) and Cipollini Jr *et al.* (1994),** respectively. As Protein content was calculated by using the following formula: Protein % = (N) × 5.75.

- **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.

7. Statistical Analysis.

It was done according to **Gomez and Gomez, 1984, [**using **CoStat version 6.303 copyright (1998-2004)]**.

RESULTS AND DISCUSSION.

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 $\mathbf{T_2}$ 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 $\mathbf{T_1}$ treatment (100% of NRD as urea, control) came in the second order followed by $\mathbf{T_3}$ treatment (50% of NRD as urea+50% of NRD as Azolla), while $\mathbf{T_4}$ (25% of NRD as urea +75% of NRD as Azolla) and $\mathbf{T_5}$ (100% of NRD as Azolla) treatments came in the last order, respectively. The superiority of $\mathbf{T_2}$ 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 **(Taha *et al.* 2017; Setiawati *et al.* 2018; Abou Hussien *et al.* 2020 & 2021).**

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. **(Kumar *et al.* 2009; Tsonev and Cebola-Lidon, 2012; Eteng *et al.* 2014; Sturikova *et al.* 2018 and Abbaszadeh-Dahaji *et al.* 2019).**

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

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.	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
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
	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}	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
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											
	F_1	3.24	3.29	0.390	0.397	3.14	3.17	40.89	41.34	7.92	7.98
T_1	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
	F_1	3.33	3.38	0.393	0.401	3.16	3.19	41.47	41.90	7.98	8.05
T_2	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
	F_1	2.95	3.00	0.356	0.363	2.89	2.92	37.88	38.22	7.58	7.68
T_3	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
	F_1	2.89	2.94	0.346	0.349	2.82	2.87	37.26	37.71	7.51	7.61
T_4	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
	F_1	2.70	2.74	0.330	0.336	2.61	2.65	35.50	35.85	7.28	7.37
T_5	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															
	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
T_1	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
	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
T_2	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
	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
T_3	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
	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
T_4	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
	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
T_5	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		N, %		P, %		K, %		Zn, mg kg^{-1}		Cu, mg kg^{-1}		Carbohydrates %		Protein %	
		Grains chemical constituents										**Biochemical traits**			
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
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															
	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
T_1	**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
	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
T_2	**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
	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
T_3	**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
	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
T_4	**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
	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
T_5	**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})

2. *Soil analysis after wheat harvest.*

Fig1 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.* (2022) and El-Sherpiny *et al.* (2022).**

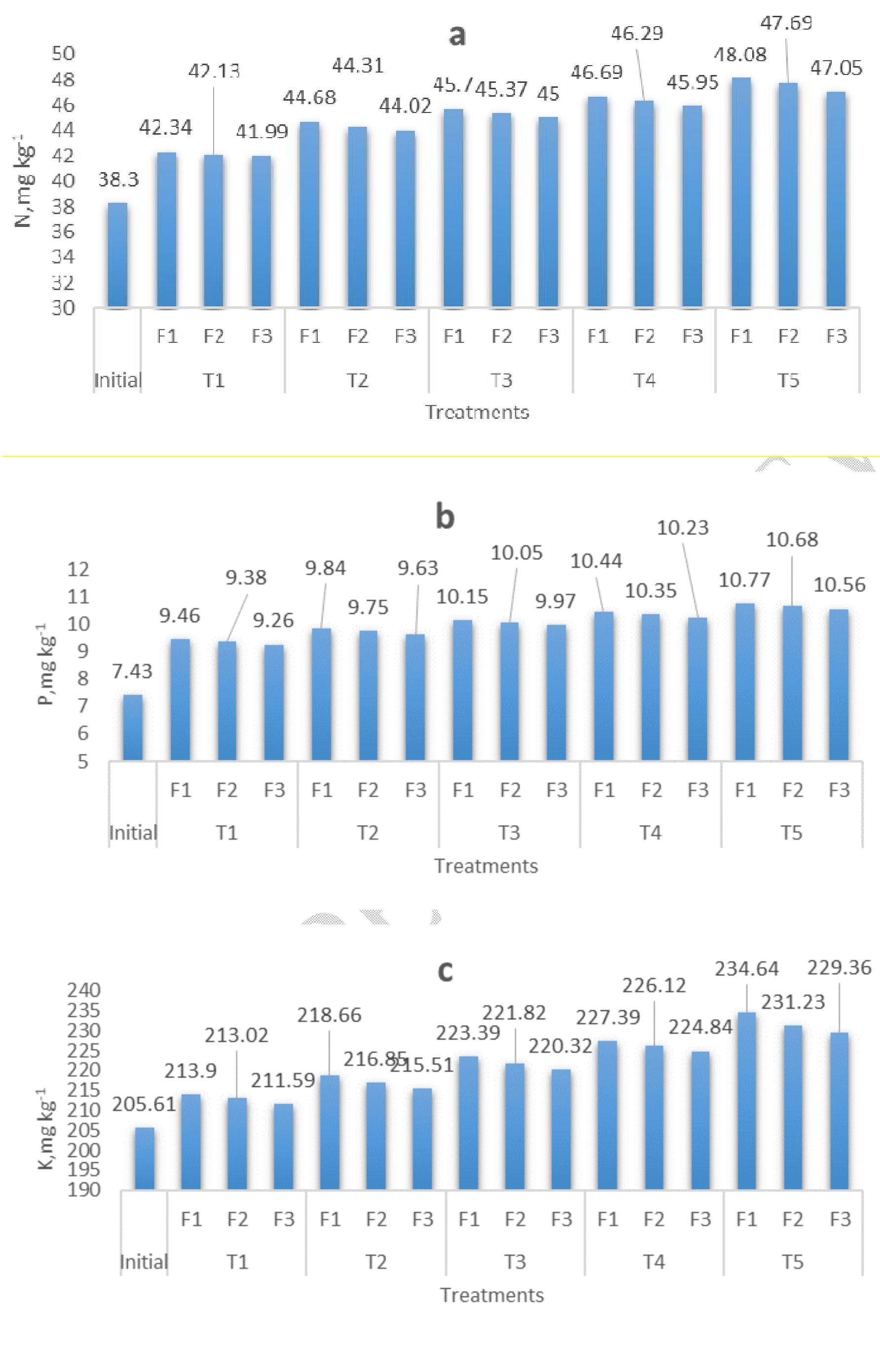

Fig 1. Effect of different ratios of mineral and organic nitrogen sources as combined treatments and foliar application of Zn and Cu on some soil fertility parameters like A-N (Fig1,a), A-P (Fig1,b) and A-K (Fig1,c) at harvest stage (combined data over both seasons)

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

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.

REFERENCES

Abbaszadeh-Dahaji, P., Baniasad-Asgari, A., & Hamidpour, M. (2019). The effect of Cu-resistant plant growth-promoting rhizobacteria and EDTA on phytoremediation efficiency of plants in a Cu-contaminated soil. *Environmental Science and Pollution Research*, *26*(31), 31822-31833.

Abou Hussien, E. A., Ahmed, B. M., & Elbaalawy, A. M. (2020). Efficiency of Azolla and biochar application on Rice (*Oryza sativa* L.) productivity in salt-affected soil. *Egyptian Journal of Soil Science*, *60*(3), 277-288.

Abou Hussien, E., Nada, W. M., & Mahrous, H. (2021). Improving chemical and microbial properties of calcareous soil and its productivity of Faba Bean (*Vicia faba* L.) plants by using compost tea enriched with humic acid and azolla. *Egyptian Journal of Soil Science*, *61*(1), 27-44.

Anonymous, (1990). "Official Methods of Analysis of the Association of Official Analytical Chemists". 15^{th} Ed. Vol. 11. Helrich (Ed.) Assoc. off. Ana. Chemists. Inc., Virginia, USA.

Cipollini Jr, D. F., Newell, S. J & Nastase, A. J. (1994). Total carbohydrates in nectar of *Sarracenia purpurea* L.(northern pitcher plant). American Midland Naturalist, 374-377.

CoStat version 6.303 copyright (1998-2004). CoHort Software 798 Lighthouse Ave. PMB 320, Monterey, CA, 93940, USA.

Dewis, J., & Freitas, F. (1970). Physical and chemical methods of soil and water analysis. FAO soils Bulletin, (10).

Elbehiry, F., Elbasiouny, H., Cappuyns, V., & Brevik, E. C. (2021). Available concentrations of some potentially toxic and emerging contaminants in different soil orders in Egypt and assessment of soil pollution. *Journal of Soils and Sediments*, *21*, 3645-3662.

El-Mantawy, R. F., Mokhtar, N. A. Y., & El-Sherpiny, M. A. (2022). Identifying tolerance of some wheat genotypes to water stress conditions. Journal of Global Agriculture and Ecology, 13-24.

El-Ramady, H., Alshaal, T., Bakr, N., Elbana, T., Mohamed, E., & Belal, A. A. (Eds.). (2018). The soils of Egypt. Springer.

El-Shamy, M. A., El-Naqma, K. A., & El-Sherpiny, M. A. (2022). Possibility of using clover residues, green manures as a partial substitute of mineral nitrogen fertilizer to wheat plants grown on normal and saline soils. *Journal of Global Agriculture and Ecology*, 51-63.

El-Sherpiny, M. A., Kany, M. A., & Sakara, H. M. (2022). Enhancement of growth and yield quality of onion plant via foliar application of bio-stimulants under different nitrogen sources. Journal of Global Agriculture and Ecology, 13-24.

El-Zemrany, H. M., & Riham M. N. Faiyad (2021). Maximizing use efficiency of mineral fertilizers using K fulvate and *Azotobacter chroococcum* DSM 2286 and their effect on wheat production and nutrients uptake. Egyptian Journal of Soil Science, 61(1): 13-25.

Eteng, E. U., Asawalam, D. O., & Ano, A. O. (2014). Effect of Cu and Zn on maize (*Zea mays* L.) yield and nutrient uptake in coastal plain sand derived soils of southeastern Nigeria. *Open Journal of Soil Science*, *2014*.

Evans, L. T., & Wardlaw, I. F. (2017). Wheat. In *Photoassimilate distribution in plants and crops* (pp. 501-518). Routledge.

Ewais, M. A., Faiyad, R. M. N. and Zakaria, S. M. (2023). Enhancing adapting maize to climate changes using different nitrogen and zinc sources and their effects on growth, yield and quality under Nile Delta conditions. Menoufia J. Soil Sci., Vol. 8 (1): 1-20.

Gee, G.W. & J.W. Bauder. (1986). Particle-size Analysis. p 383-411 In A. Klute (ed.) Methods of Soil Analysis Part 1. Soil Science Society of America Book Series 5, Madison, Wisconsin, USA.

Gomez; K. A & Gomez, A.A (1984). "Statistical Procedures for Agricultural Research". John Wiley and Sons, Inc., New York.pp:680.

Hashimi, R., & Hashimi, M. H. (2020). Effect of Losing Nitrogen Fertilizers on Living Organism and Ecosystem, and Prevention Approaches of their Harmful Effect. *Asian Soil Res J*, *4*, 10-20.

Hesse, P. R. (1971). "A textbook of soil chemical analysis". Joon Murry (Publishers) Ltd, 50, Albemarle Street, London.

Kumar, R., Mehrotra, N. K., Nautiyal, B. D., Kumar, P., & Singh, P. K. (2009). Effect of copper on growth, yield and concentration of Fe, Mn, Zn and Cu in wheat plants (*Triticum aestivum* L.). *Journal of Environmental Biology*, *30*(4), 485-488.

Malyan, S. K., Bhatia, A., Kumar, S. S., Fagodiya, R. K., Pugazhendhi, A., & Duc, P. A. (2019). Mitigation of greenhouse gas intensity by supplementing with Azolla and moderating the dose of nitrogen fertilizer. *Biocatalysis and Agricultural Biotechnology*, *20*, 101266.

Peterburgski, A. V. (1968)."Handbook of Agronomic Chemistry". Kolos Puplishing House, Moscow,(in Russian, pp. 29-86).

Picazo, A., Rochera, C., Vicente, E., Miracle, M. R., & Camacho, A. (2013). Spectrophotometric methods for the determination of photosynthetic pigments in stratified lakes: a critical analysis based on comparisons with HPLC determinations in a model lake. *Limnetica*, *32*(1), 139-158.

Randive, K., Raut, T., & Jawadand, S. (2021). An overview of the global fertilizer trends and India's position in 2020. *Mineral Economics*, 1-14.

Setiawati, M. R., Damayani, M., Herdiyantoro, D., Suryatmana, P., Anggraini, D., & Khumairah, F. H. (2018). The application dosage of Azolla pinnata in fresh and powder form as organic fertilizer on soil chemical properties, growth and yield of rice plant. In *AIP conference proceedings* (Vol. 1927, No. 1, p. 030017). AIP Publishing LLC.

Sturikova, H., Krystofova, O., Huska, D., & Adam, V. (2018). Zinc, zinc nanoparticles and plants. *Journal of hazardous materials*, *349*, 101-110.

Taha, A., El-Zehery, T., El-Aal, A., & El-khadrwy, T. (2017). Comparitive of Some Microorganismus on Sandy Soil Fertility, and Wheat Productivity. *Journal of Soil Sciences and Agricultural Engineering*, *8*(5), 203-208.

Taha, N., Shakweer, N., & El-Shahat, R. M. (2018). Impact of Different Sources of Natural, Mineral and Bio-Fertilizers on Apple Trees Performance, Growth and Yield on Sandy Soil. *Egyptian Journal of Soil Science*, *58*(1), 113-126.

Tandon, H. L. S. (2005). Methods of analysis of soils, plants, waters, fertilisers & organic manures. Fertiliser Development and Consultation Organisation.

Tsonev, T., & Cebola-Lidon, F. J. (2012). Zinc in plants-an overview. *Emirates Journal of Food & Agriculture (EJFA)*, *24*(4).

Walinga, I., Van Der Lee, J. J., Houba, V. J., Van Vark, W. & Novozamsky, I. (2013). Plant analysis manual. Springer Science & Business Media.

Yruela, I. (2005). Copper in plants. *Brazilian Journal of Plant Physiology*, *17*, 145-156.