RESPONSE OF MAIZE TO FOLIAR APPLICATION OF ZINC AND AZOTOBACTER INOCULATION UNDER DIFFERENT LEVELS OF UREA FERTILIZER

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Abstract: In order to investigate the effects of zinc and Azotobacter on maize production properties under different regime of urea as mineral fertilizer, an experiment was conducted at research field of Sari Agricultural Sciences and Natural Resources University during the cropping season of 2014. The nitrogen mineral fertilizer in 100, 200 and 300 amounts of kg urea ha⁻¹, Azotobacter (inoculation and non-inoculation) and foliar application of zinc (Zn1 = 1000 mg l⁻¹ zinc sulfate, Zn2 = 500 mg l⁻¹ zinc sulfate and Zn3 = 0 mg l⁻¹) were considered as the applied treatments. Grain yield, biological yield (dry matter), and total protein content of each treatment were assessed. Results indicated that foliar applications of Zn had a significant effect on all studied traits. The highest grain yield was related to Zn1 treatment in each urea application and also between biological fertilizer treatments the highest yield was observed in inoculation treatment. Between all studied treatments the maximum grain yield (with 10.23 ton ha⁻¹) was obtained through non-inoculation treatment × Zn1 for 300 kg urea ha⁻¹. The highest biological yield was observed in Zn1 + inoculation treatment. There were no significant differences between Azotobacter inoculation and non-inoculation for each level of zinc applications in 300 kg urea ha⁻¹ treatment. Finally, it might be concluded that using of mineral fertilizer could be reduced by combining some management strategies in maize production.

Key words: biofertilizer, dry matter, grain yield, protein content.

Introduction

Maize (Zea mays L.) is the third most important cereal grain worldwide after wheat and rice. It is referred to as the cereal of the future for its valuable nutritional facts in human diet (Enyisi et al., 2014). Annual production of cereals in 2014 was more than 2.4 billion tons. The 872 million tons of cereal production was maize production. According to the reports, Iran’s share of world maize production is

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only 1.250 million tons (FAO, 2014). Increasing the global demand for food with limited arable land for agriculture will be a major challenge for researchers. Accordingly, when the actual development of agriculture is not possible, consequently the farmers will compensate for increasing their production by taking more types of inputs, especially mineral fertilizers, which is neither environmentally acceptable nor agriculturally sustainable. For example, nitrogen fertilizer is one of the main applied nutrients in agricultural production for its key role in the formation of proteins and nucleic acids in plants. Thus, one of the basically alternative methods is the use of environmentally friendly biological fertilizers such as plant growth promoting *rhizobacteria*. Biofertilizers include mainly the nitrogen fixing and plant growth promoting microorganisms. These promoting biofertilizers consist of *Azotobacter*, *Azospirillum*, blue green algae, *Azolla*, *P*-solubilizing microorganisms, *Mycorrhizae* and *Sinorhizobium* (Selvakumar et al., 2009). Amongst them, *Azotobacter* is capable of fixing an average of 20 kg nitrogen per hectare during the cropping season (Kizilkaya, 2009). *Azotobacter* can also produce thiamin, riboflavin, indole acetic acid and gibberellins. When *Azotobacter* is used as a seed inoculant, the related properties of seed germination are enhanced. The exact mode of action by which *Azotobacteria* promote plant growth is not yet fully understood. But, the main possible proposed mechanism is N₂ fixation as well as producing of some phytohormones to alternate plant growth (Mrkovacki and Milic, 2001).

Meanwhile, the essential micronutrients required by the plant should not be overlooked. The role of micronutrients such as zinc involved from very simple to very complex reactions. Zn plays a very important role in plant metabolism by influencing the activities of hydrogenase and carbonic anhydrase and stabilization of ribosomal proteins (Tisdale, 1984). Zinc activates the plant enzymes by carbohydrate metabolism, maintaining the integrity of cellular membranes, protein synthesis and regulation of auxin synthesis (Marschner, 1995). Also, Zn is required for regulation and maintenance of the gene expression to induce tolerance of environmental stresses in plants (Cakmak, 2000).

Amongst crops, maize shows the high sensitivity to Zn deficiency for its physiological requirements (Marschner, 1995). By application of nitrogen fertilizers, zinc deficiency can be increased in maize. Also, nitrogen integrated with zinc improved plant height and yield in maize (Xia et al., 2004). Alloway (2004) reported that wheat grown on N-deficient soil with adequate levels of all nutrients except N and Zn, did not respond to Zn application in the absence of NH₄NO₃ fertilizer, however, a strong response to Zn application was observed in the presence of N fertilizer.

Therefore, the objective of this study was to evaluate the interaction effects of *Azotobacter* inoculation and foliar application of zinc on grain yield, dry matter and protein content of maize under field conditions.
Material and Methods

Study area and experimental setup

In order to evaluate the effects of zinc micronutrient spray and *Azotobacter* inoculation on maize (SC 704 hybrid) production parameters and seed protein content under different levels of nitrogen (urea) fertilizer, an experiment was conducted at the research farm of Sari Agricultural Sciences and Natural Resources University, Iran, during the cropping season of 2014. The field area was 600m$^2$ and was not cultivated during the previous year. The plots were irrigated using the tape irrigation method and the applied water amounts were controlled by water meters and the following equation [based on field capacity according to evapotranspiration (ET) losses during the silking stage (R1)]:

\[
\text{Equation 1: } d = \frac{(\text{FC}-0)}{D} \times 100
\]

where, FC$\%$ = field capacity; $\theta$% = soil moisture content; D$_{cm}$ = soil depth; d$_{cm}$ = irrigation water depth.

Soil samples were collected and its physicochemical properties were analyzed by the method described by Blakemore et al. (1987).

Table 1. Physical and mechanical properties of soil.

| Depth   | Texture    | EC ($d_{so}^{-1}$) | pH | T.N.V (%) | O.C% | P (mg l$^{-1}$) | K (mg l$^{-1}$) | Zn (mg l$^{-1}$) |
|---------|------------|---------------------|----|-----------|------|----------------|----------------|-----------------|
| 0–30cm  | Clay silt  | 1.4                 | 7.5| 19.3      | 3.48 | 12.3           | 367.3          | 1.12            |

Uniform maize seeds of single cross 704 hybrid were purchased from Agricultural and Natural Resources Center, deputy of Mazandaran. Selected intact seeds were manually planted in early June 2014 within 75-cm rows and 12.5-cm distance between them in row. The experiment was laid out in a split factorial arrangement based on a complete block design with three replications. The nitrogen mineral fertilizer was used as the main treatment (100, 200 and 300 kg urea ha$^{-1}$), *Azotobacter* (inoculation and non-inoculation) and zinc sulfate (0, 500, 1000 mg l$^{-1}$ as foliar application) were considered as the second and third factors in the factorial arrangement, respectively. Seed inoculation with the *Azotobacter chroococcum* spp. was carried out by using 10% sugar solution carrier. Maize seeds were soaked in the inoculum solution for 4 h. These seeds were kept in shade before sowing. The inoculation was done just few hours before seed sowing. Zinc micronutrient solution was prepared in 0, 500 and 1000 mg l$^{-1}$ concentrations and applied foliarly three times based on morpho-physiological characterization of maize, in ear and tassel initiations (V6), tassel emergence (VT) and silking (R1) stages, respectively. The urea fertilizer was applied as the topdressing method at
three growing stages (1/3 of amount after planting, 1/3 during the V9 stage and the final third was applied during the silking stage).

Plants were harvested from September 5 to September 15 at the physiological maturity stage. Grain yield, dry matter and seed protein content were determined after removing the border rows, two middle rows of each plot were harvested and weighed for further analysis.

Total protein determination

Estimation of total protein content was conducted using the Kjeldahl method as mentioned by Isaac and Johnson (1976) (Kjeltec Auto1030 Analyzer, Foss Tecator AB, Hoganas, Sweden). For nitrogen determination, the grain was dried (Fan AzmaGostar, 24060, Iran) for 72 h in an oven. Total nitrogen was determined by using a micro Kjeldahl procedure with sulfuric acid, digestion catalyst and conversion of organic nitrogen to ammonium form according to the Total Kjeldahl Nitrogen (TKN) method. The protein content was determined by the calculation: N content × convert factor (6.25) (King-Brink and Sebranek, 1993).

Statistical analysis

Analysis of variance was performed for studied traits by using the general linear model (PROC GLM) procedure in Statistical Analysis System (SAS) and the mean comparisons were evaluated based on the Least Significant Differences (LSD).

Results and Discussion

Foliar application of Zn had a significant effect on all studied traits (Table 2). Different levels of urea application also showed a significant effect on grain and biological yields. The biological fertilizer also had significant effects on biological yield and protein content.

Table 2. Mean squares of each treatment among studied traits (ANOVA table for a split-factorial design).

| S.O.V      | df | Grain yield | Biological yield | Protein content |
|------------|----|-------------|------------------|-----------------|
| Block      | 2  | 0.525       | 0.754            | 0.328           |
| Urea (a)   | 2  | 25.599**    | 34.30**          | 2.430ms         |
| Error a    | 4  | 0.271       | 0.508            | 2.411           |
| Zn (b)     | 2  | 22.80**     | 142.75**         | 3.699*          |
| Azotobacter (c) | 1  | 0.0021ns   | 2.436*           | 20.384**        |
| a*b        | 4  | 1.416**     | 0.387ns          | 12.906**        |
Response of maize to foliar application of Zn and *Azotobacter* inoculation

| Interaction | df | Mean Sq | F     | P-value |
|------------|----|---------|-------|---------|
| a*c        | 2  | 0.056ns | 38.74** | 9.158** |
| b*c        | 2  | 1.323** | 1.656*  | 2.83ns  |
| a*b*c      | 4  | 2.974** | 31.812** | 18.002** |
| Error      | 30 | 0.17    | 0.430  | 0.827   |
| CV %       | 5.57 | 5.41 | 10.068 |

ns, *, and ** indicate: not significant, significant at the 5% and 1% probability levels, respectively.

Grain yield

According to the results (Figure 1), the highest grain yield was related to Zn1 treatment in each urea application, and also between biological treatments the highest yield was observed in inoculation treatment. The maximum grain yield (with 10.23 ton ha⁻¹) was obtained through non-inoculation treatment × Zn1 in 300 kg urea ha⁻¹. According to the report of Mahmodi and Yarnia (2012), foliar application of zinc can increase maize grain yield up to 40% compared to control. Grzebisz et al. (2008) indicated that foliar treatment of zinc in the early stage of maize produced significantly higher yields. Grain yield is a final product of many yield-contributing components, from physiological processes to morphological development which take place in plants during growth and development stages. Both N and Zn applications increased the grain yield significantly. Therefore, the maximum grain yield was obtained when a foliar spray of Zn was applied at 300 kg urea ha⁻¹ treatment. Zinc is an important micronutrient needed by the maize plant and its deficiency reduces the grain yield, especially during the grain filling stage (Peck and McDonald, 2010). The results are in agreement with the findings of Dang et al. (2010), who reported that foliar application of ZnSO₄ significantly increased the grain yield of hybrid maize. These results are also in agreement with those obtained by Grzebisz et al. (2008), who showed that foliar application of ZnSO₄ increased the biomass of maize hybrids. Our results show that *Azotobacter* inoculant increased the yield in Zn1 under 100 and 200 amounts of kg urea ha⁻¹ applications, but not in all treatments.

On the other hand, nitrogen as the most frequently used macro element has the key role in increasing yield and utilization of potassium, phosphorus and other micro and macro elements in plants. A positive response of nitrogen fertilizers has been well documented by many authors (El Amin, 2003; Abdelgader, 2007; Nemati and Seyedsharifi, 2012; Kisetu et al., 2014). In our study, by increasing levels of urea fertilizer from 100 kg ha⁻¹ to 300 kg ha⁻¹, the grain yield was positively affected and increased (Figure 1). It suggests that C₄ photosynthesis pathway allows a very efficient conversion of CO₂ into carbohydrates and final grain yield, especially under high nitrogen input systems and also it is well known that plants require the highest amounts of nitrogen (Ghannoum et al., 2011).
Dry matter

The crop dry matter yield strongly correlated with the grain yield under optimal conditions (Abdalla et al., 2015). The various significant differences were observed between inoculant treatments and different levels of zinc in 200 kg urea ha\(^{-1}\) treatment for dry matter (Figure 2). So that, the highest dry matter was related to Zn1 + inoculation treatment (19.36 ton ha\(^{-1}\)). There was no effect of \textit{Azotobacter} inoculation on biological yield for each level of zinc applied in the 300 kg urea ha\(^{-1}\) treatment. Alloway (2004) summarized that zinc external supply is a primary factor accelerating plant root growth and in turn increasing zinc uptake. Our results are in
line with the findings of Malavolta (2006), who also reported that foliar application of micronutrients was a more efficient method compared with ground fertilization, and zinc should be applied as foliar application to the maize plant grown especially in zinc-deficient soils. Moreover, it has been reported that the maize biomass increased through the seed inoculation with *Azotobacter chroococcum* (Adjanohoun et al., 2011). Shaharoona et al. (2006) observed the biomass improved up to 68.4% in maize when the seeds were inoculated by *Azotobacter* compared to control. Vegetative growth and consequently biological yield are highly dependent on consumption of micro and macro chemical elements by the maize plant (Ehsanullah et al., 2015), so application of fertilizers that contain those elements leads to an increase in biological yield of maize.

Figure 2. The interaction effect of zinc and *Azotobacter* treatments between different urea levels on dry matter (A = 100 kg ha⁻¹ of urea; B = 200 kg ha⁻¹ of urea; C = 300 kg ha⁻¹ of urea).
Protein content (%)

The grain protein content has a very important role in nutritional value of the cereals (Shewry and Halford, 2002). Thus, based on the results amongst different levels of N treatment (urea) the highest protein content was attributed to 300 kg urea ha\(^{-1}\) treatment (with the average of 9.43%), but the maximum protein content was observed in Zn1 + inoculation (with 15.22%) in 100 kg urea ha\(^{-1}\) treatment (Figure 3).

![Figure 3](image)

Figure 3. The interaction effect of zinc and *Azotobacter* treatments between different urea levels on protein content (A = 100 kg ha\(^{-1}\) of urea; B = 200 kg ha\(^{-1}\) of urea; C = 300 kg ha\(^{-1}\) of urea).

Palmgren et al. (2008) found that nitrogen efficiency was more essential for Zn absorption and uptake in cereals especially in the maize plant. Since zinc has a direct effect on the increase of protein in grains, the deficiency of this element in
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crops will reduce the grain protein and thus amino acids could not be accumulated. Morshedi and Naghibi (2004) showed that zinc when applied foliarly increased the protein content of rapeseed. The increase of total nitrogen uptake induced by zinc application confirms the thesis of its primary effect on main physiological processes related to nutrients uptake (Potarzycki and Grzebisz, 2009). Changes in nitrogen supply levels affect the biosynthesis of all other nitrogen-containing compounds such as amino acids, proteins and hormones, especially in C₄ plants. In the maize plant, the availability of nitrogen can affect the chlorophyll content of the leaves which is positively correlated with leaf nitrogen content and its allocations into grains (Schlüter et al., 2012).

**Conclusion**

According to the results, it was found that the biofertilizer and foliar application of zinc could partially reduce the mineral N fertilizer inputs. Nevertheless, it may be impossible to reach maximum production by only using the biological fertilizers or plant micro elements, because it has been well proven that application of macro nutrient increases yield, growth, and quality of crops. Also, the results of our study showed that by combining some management strategies in maize production, the application of mineral fertilizers (the main causes of environmental and groundwater resources pollution) could be reduced. From the sustainability point of view, the importance of Integrated Nutrient Management (INM) has extended in recent years. This strategy covers the application of essential trace elements and biofertilizers, with the minimum use of chemical fertilizers to produce optimum crop yields. Consequently, the demand for biofertilizers can be expected to rise in the near future.

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Rezime

Kako bi se ispitao uticaj cinka i azotobaktera na proizvodna svojstva kukuruza pri različitim režimima korišćenja uree kao mineralnog dubriva, ogled je sproveden na istraživačkom polju Univerziteta za poljoprivredne nauke i prirodne resurse u Sariju tokom 2014. godine. Primjenjeni su sledeći tretmani: azotno mineralno dubrivo urea u kolичinama od 100, 200 i 300 kg ha⁻¹, azotobacter (sa i bez inokulacije) i folijarno primenjen cink (0, 500, 1000 mg l⁻¹: Zn₁ = 1000 mg l⁻¹ cink sulfata, Zn₂ = 500 mg l⁻¹ cink sulfata i Zn₃ = 0 mg l⁻¹ kao kontrola).

Ocenjivani su prinos zrna, biološki prinos (suva materija) i ukupan sadržaj proteina kod svakog tretmana. Rezultati su ukazali da folijarna primena cinka ima značajan uticaj na sve proučavane osobine. Najveći prinos zrna je bio povezan sa nižom koncentracijom cinka pri svakoj primeni uree, kao i među tretmanima sa mikrobiološkim dubrivom gde je najveći prinos uočen kod tretmana sa inokulacijom. Među svim proučavanim tretmanima maksimalni prinos zrna 10,23 t ha⁻¹ je dobijen na tretmanu bez inokulacije × Zn₁ sa ureom od 300 kg ha⁻¹. Najveći biološki prinos je bio uočen kod Zn₁ sa inokulacijom azotobakterom. Nije bilo značajnih razlika između tretmana sa i bez inokulacije azotobakterom za svaki nivo primene cinka kod tretmana sa ureom od 300 kg ha⁻¹. Na kraju, moglo bi se zaključiti da se korišćenje mineralnog dubriva može smanjiti kombinovanjem nekih upravljačkih strategija u proizvodnji kukuruza.

Ključne reči: biodubrivo, suva materija, prinos zrna, sadržaj proteina.

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