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Effect of Zn Applied with or Without Palm Stearin Coated Urea on The Growth and Mineral Element Concentration of Maize (*Zea mays L.*)

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Abstract

Inhibition of ammonia (NH_3) volatilization by deaccelerating urea hydrolysis rate in Central Anatolian lands is the indispensable approach for eco-friendly fertilization and nitrogen use efficiency (NUE). Nitrogen (N) and zinc (Zn) are critically limited here in alkaline soils. An experiment was conducted under controlled conditions to determine the availability of Zn applied as a solution and bound with polymer palm stearin (PS) coating material as a urease inhibitor. The treatments consisted of urea as a commercial commodity, urea with PS only, urea impregnated with PS and Zn, Zn-coated urea, and Zn in solution (SOL) form. During winter, 2019-20, the experiment was conducted in the glasshouse of the department of Soil Science and Plant Nutrition at Ankara University, Türkiye. Data indicated that Zn with PS and in SOL form produced more growth traits i.e., plant height (130 cm), stem girth (13.2 mm), shoot dry matter (4.63 g plant^{-1}), root dry matter yield (0.61 g plant^{-1}), and chlorophyll (42.16 mg g^{-1}) content ($p \leq 0.01$). Similarly, we had higher concentration of N (3.19%) and Zn (50.46 mg kg^{-1}) content in maize plants ($p \leq 0.01$) as compared to control. In conclusion, Zn at the rate of 10 mg kg^{-1} either in solution or coated with urea seems highly effective to sustain better crop productivity and NUE. While concerning N and Zn content, coated urea with Zn markedly responded as compared to Zn in SOL. Synergism between N and Zn can lead to better fertilizer management.

Keywords: Zn solution; control release urea; synergistic effect; mineral contents; hybrid

Introduction

Urea is used in more than 40% of the world's crop production as it contains the largest amount of nitrogen (N) (460 g N kg^{-1}) compared to any other N-containing fertilizer. This is the only long-standing trendy fertilizer with satisfactory price and physical characteristics, as a result, making it convenient for the farmers' community (Gangon *et al*. 2012). Aligned with soil nitrogen (N) and phosphorous (P), zinc (Zn) is also an inadequate mineral element in alkaline soils and food nutrition (Choudhary *et al*. 2019). Application of N without any risk concern is a common practice throughout the world (Heffer and Prud'homme 2011). It is predicted that urea can increase by 1.6 to 2.0 % in annual growth worldwide, though it is produced at the rate of 107 million metric tons per year globally (Engineers SoP 2012-2018). Nitrogen losses are commonly observed predominantly as ammonia volatilization loss upon direct application of urea (conventional urea) in both acidic and alkaline soils (Babar *et al*. 2016). The rate of urea hydrolysis is accelerated as applied in conventional form due to urease enzymes, which bring about higher soil pH at the urea granules microsite (Chient *et al*. 2009); Such that ammonia (NH_3) losses can reach a level of 60% (Jiang *et al*. 2012) and varies with soil type and climatic conditions (warmer climate favors more volatilization losses). Moreover, this is not the only aspect of economic significance for farmers, but its implications towards deleterious ecological influence as the emission of nitrogenous gases, particularly nitrous oxide (N_2O), participate in stratospheric ozone around the earth's polar regions and consequently the greenhouse effect (Tian *et al*. 2015). Hence, minimizing N-losses is of course an indispensable approach to obviate environment and groundwater pollution as a part of saving the fertilizer cost to farmers. Currently, another course of action being practiced throughout the world is to enhance the nitrogen use efficiency (NUE) of applied urea fertilizers and improve its uptake by crops. Variable rates, method & time of application, placement, and split distribution are the primary measures being commonly observed. Agriculturists are trying to rotate or replace split doses, with band applications as broadcasting so can reduce ammonia volatilization losses (Arif, 2019). While such alterations (placement) result in unfeasible for some specific types of soil and plants due to their dispersing properties i.e. sandy soils and toxicity to plant roots respectively (Zhu *et al*. 2019).

Slow-releasing nitrogen fertilizers are embracing agricultural inputs. The central notion behind the surface coating of conventional urea prill with hydrophobic chemical is to provide a physical barricade contract to water and consequently reduces dissolution rate (Ibrahim *et al*. 2014). This gradual release of nutrients can synchronize the distribution of urea wisely and according to the plants' requirements. Proven from research before, coating with semi-permeable coating materials can hinder the initial absorbance and provide a steady supply of nutrients for a longer period of time (Naz and Sulaiman 2016). Coating alone can cause problems; hence inhibition of urease is brought into effect to minimize the hydrolysis rate of urea (Babar *et al*. 2016; Nasima *et al*. 2013; Du *et al*. 2012) by deactivating of soil urease. A urease inhibitor that is eco-friendly and feasible is the best.

The important and effective urease inhibitors in the literature are heavy metal ions (Affendi *et al*. 2020; Du *et al*. 2012; Dong *et al*. 2012; You *et al*. 2012; Zaborska *et al*. 2004). Different binders have been tried with different inhibitors to serve as an eco-friendly fertilizer and improve N-recovery; hence discovering the appropriate binder to hold urea along with suitable inhibitors on a fertilizer microsite is important, these include Natural phenolic aldehyde (Horta *et al*. 2016). Thus, the use of a biodegradable coating material or adhesive proves to be a fine alternative material to minimize N-losses and reduce environmental pollution. Palm stearin (PS) considered as coating the urea has not received satisfactory attention with the exception of a very rare quality work (Mathialagan, *et al*. 2020; Noor Affendi *et al*. 2018; Babar *et al*. 2016). Though PS is a by-product of palm oil and less valuable in the palm oil industry, its value has yet to be highlighted as a urea binder. However, to the best of the author's knowledge, the effect of PS-Zn coated urea has not been reported yet on Turkish soils. Therefore, keeping these specifics in mind, the current study aimed to evaluate the effect of Zn with or without PS-coated urea on the mineral concentration of maize plants. The objectives of this study were: (1) to evaluate the prominence method of Zn application as PS-ZnCU or Zn in solution form, and (2) to recognize the synergism of Zn with N and its effect on mineral concentrations in maize plants.

Material and Methods

Glasshouse study: A greenhouse experiment was conducted at the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Ankara University, Türkiye during the summer (June-July, 2019). The study aimed to appraise the effect of Zn application with and without PS-coated urea on maize production. Concerning with the current research, coating was done manually in the laboratory of the Department of Soil Science and Plant Nutrition, Ankara University, Türkiye. Necessary cautions were made regarding coating material and the selected coated material was a natural and eco-friendly i.e. palm stearin (extracted from oil palm) to skip the risks of affluence. The ratio of coating material and urea was set at 1:2 (this ratio can be adjusted according to climatic conditions), one part of urea and 2 parts of PS.

Treatments Regimes*:* The experimental package consisted of eleven treatments with one soil type and one maize cultivar. Treatments comprised of T1, without any fertilizer (control), T2, Zn applied as solution form (5 mg Zn kg^{-1}), T3, Zn applied as solution form (10 mg Zn kg^{-1}), T4, uncoated urea (150 mg N kg^{-1} + 0 mg Zn kg^{-1}), T5, uncoated urea with Zn solution (150 mg N kg^{-1} + 5 mg Zn kg^{-1}), T6, uncoated urea with Zn solution (150 mg N kg^{-1} + 10 mg Zn kg^{-1}), T7, coated urea (150 mg N kg^{-1} + 0 Zn), T8, coated urea (150 mg N kg^{-1} + 5 mg Zn kg^{-1}), T9, coated urea (150 mg N kg^{-1} + 10 mg Zn kg^{-1}), T10, coated urea with Zn solution (150 mg N kg^{-1} + 5 mg Zn kg^{-1}) and T11, coated urea with Zn solution (150 mg N kg^{-1} + 10 mg Zn kg^{-1}). Phosphorous and K were applied to each pot except for control at the rate of 80 mg P kg^{-1} and 100 mg K kg^{-1} in the form of potassium dihydrogen phosphate (KH_2PO_4). Total 33 pots were arranged in a randomized complete block design (RCBD) with 3 replications and 2 kg of soil. Irrigation of pots on a daily basis was made with distilled water (pure water) to avoid the interaction of available metals in tap water. The soil used in the study was collected from the Ayaş region of western Ankara, Turkey was clayey in texture with 41.8% clay, 25.3 % silt, and 32.9 % sand and was analyzed following Bouyoucos (1951).

Basic & Elemental analysis: Selection of soil for current research was made due to its limitations to the mineral elements N and Zn. Nitrogen (0.05 g kg^{-1}) and Zn (0.42 mg kg^{-1}) were deficient as per the guide of Ülgen & Yurtsever (1974) and Sillanpää (1990) respectively. The soil was moderately alkaline (pH 8.28) with no salinity problem (0.32 dS m^{-1}) referring to Richards (1954). Low O.M (10.5 g kg^{-1}) and high calcium carbonate (8.21 g kg^{-1}) were noted (Ülgen & Yurtsever, 1974). Of five seedlings, three were left after thinning for further agronomic observations such as plant height (cm), fresh and dry shoot weight (g plant-^{1}), root weight (g plant-^{1}) and stem girth (mm) at the time of harvesting and chlorophyll contents (mg kg^{-1}) after 42 days of plant growth were recorded. The tasseling stage was selected to cut the plants (65 days), then first washed with tap water and then re-washed with distilled water and dried in an oven 65°C for 48 hours. Dried plant samples from each pot were ground separately in a 3 velocity Thomas Wiley mini mills Scientific Grinder. Nitrogen and Zn concentrations in maize plants were analyzed with wet digestion (Sillanpää, 1990). Nitrogen was observed with the Kjeldhal procedure (Bremner, 1965) and Zn was evaluated from powdered plant samples digested in a mixture of HNO_3:$HClO_4$ on a hot plate and filtered then extracted ran on ICP-OES (Model: Perkin Elmer Optima 2100 DV).

Data analysis with Statistix 9.1*:* The significance of the effect of the treatments on the reported traits was evaluated by one-way analysis of variance (ANOVA). Then significant differences among the means were resolved by Tukey's HSD (Honest Significant Difference) test at alpha 5% ($p \leq 0.05$) on Statistix 9.1 software (Statistix, 2009).

Results

N and Zn concentration in plant biomass as affected by PS-Zn Coated urea and Zn-Solution: Elemental analysis was carried out to determine the effects of PS-Zn coated urea and Zn in solution form on N and Zn contents in maize plants Cv Helen. The experiment has shown that the effect of adding nitrogen and zinc to soil, in either case, has been positively recorded and had greater N and Zn contents in plant biomass (Table 1). There were different modes of application practiced. Urea as the main source of N was applied as it is available in the market (without any modification) as conventional urea, urea with coating material i.e. palm stearin by adding urease inhibitor Zn, and coating with only palm stearin. Whereas, Zn is applied in two modes: one is coated with urea and the other is in solution form. The highest rise in Zn concentration (135%) in plant biomass was perceived in T9 (ZnCU) followed by Zn content increase (128%) in maize biomass grown under T11 (PSCU+ZnSOL). The maize plants grown without adding any form of Zn source had the lowest Zn content. There were no significant differences between T1, T4, and T7 where no Zn was applied ($p \geq 0.05$) and the lowest Zn contents were recorded under those treatments. A similar trend was observed with N-contents, though the rate for N was kept the same throughout the entire treatment but the difference in its application method made it significantly different. The highest N-contents (3.19%) were recorded in T9 (ZnCU), where we coated the urea with the urease inhibitor Zn. However, the lowest N-contents were observed in T1, T2, and T3 respectively where no N was applied. The application of urea in the form of a coating enhanced N-contents. Zinc coated urea performed a distinguished role in N-content advancement as compared to only coated with palm stearin ($p \leq 0.05$). The major attribute of T9 in an increase in N-contents is the addition of Zn in coating material (PS), as Zn acts as a urease inhibitor and has a synergistic effect with N.

Table 1. Effect of PS-Zn coated urea and ZnSOL on N and Zn contents of maize plants

S.No	Treatments	N (%)	Zn (mg kg^{-1})
1	T1 (CONTROL)	0.77±0.11 d	21.42±0.48 f
2	T2 (0N+ZnSOL 5)	0.56±0.08 d	29.47±0.31 e
3	T3 (0N+ZnSOL 10)	0.70±0.00 d	35.84±0.37 cd
4	T4 (UCU 150 mg N kg^{-1}+ZnSOL 0)	1.87±0.08 c	21.42±0.34 f
5	T5 (UCU 150 mg N kg^{-1}+ ZnSOL 5)	1.92±0.07 c	34.03±1.96 d
6	T6 (UCU 150 mg N kg^{-1}+ZnSOL 10)	1.67±0.15 c	38.46±0.38 c
7	T7 (PSCU 150 mg N kg^{-1}+Zn 0)	1.95±0.03 c	23.5±0.55 f
8	T8 ZnCU (150 mg N kg^{-1}+5 mg Zn kg^{-1})	2.14±0.00 bc	45.93±0.33 b
9	T9 ZnCU (150 mg N kg^{-1}+10 mg Zn kg^{-1})	3.19±0.06 a	50.46±0.27 a
10	T10 (PSCU150 mg N kg^{-1}+ZnSOL 5)	2.08±0.04 bc	45.93±0.43 b
11	T11 (PSCU 150 mg N kg^{-1}+ZnSOL 10)	2.14±0.28 ab	48.95±0.42 ab
F-value		46.68***	228.95***
HSD		0.16	1.02

****highly significant p≤0.01. Means followed by the same letter in column are not significantly different using Tukey's HSD, Honest Significant Difference, test at p≤0.05*

**UCU= Un-Coated Urea*
**PSCU= Palm Stearin Coated Urea*
**ZnCU= Zinc Coated Urea*
**ZnSOL= Zinc in Solution form*
**0N= zero nitrogen*
NS= Non-Significant

Agronomic Parameters in response to different Treatments: As revealed by one-way ANOVA, dry shoot weight, root weight and stem girth were significantly affected (Table 2) by the various treatments of coated urea and Zn in solution form (T6, T7, T8, T9, T10, and T11); there was no effect of treatments that didn't contain nitrogen or had un-coated urea. Increasing the N supply in an appropriate way and addition of Zn either coated with urea or in solution form increased the dry shoot weight (g plant^{-1}), root weight (g plant^{-1}) and stem girth (mm) progressively in all ZnCU and ZnSoL treatments ($p \leq 0.01$). There was no significant difference in plant height (cm) between all ten treatments (T2 to T11) except T1 (control). But taller plants (148 & 142 cm) were recorded in T9 (ZnCU). However, the difference from T2 to T11 was non-significant ($p \geq 0.05$). There were significant differences between the zero/no N treatment (T1, T2, and T3) and N treatments (T4 to T11) in dry shoot weight (g plant^{-1}), root weight (g plant^{-1}) along with the addition of Zn either coated or in solution form. The ranking across the experiment was as follows: T9>T11>T10>T8>T6>T7. Treatment which consists of ZnCU, PSCU and ZnSoL consistently produced more dry weight and root weight. Narrower stem girth (7.41 mm) was recorded in control (T1). There were significant differences in stem girth (mm) between T9 and T8. The wider stem girth (13.21 mm) was noted in ZnCu with Zn 10 mg kg^{-1} (T9) followed by T8 ZnCu with Zn 5 mg kg^{-1}. The ranking across the experiment was as follows: T9>T11>T8. However, the trend showed a positive response to the addition of N (coated urea) and Zn (either in coated urea or in solution form). The mean comparison between treatments expressed a significant difference ($P \leq 0.05$) except those which had zero/no N.

Table 2. Effect of PS-Zn coated urea and ZnSOL on agronomic determinants of maize plants

S#	Treatments	Stem grith (mm)	Plant height (cm)	Shoot dry wt (g plant^{-1})	Root dry wt (g plant^{-1})
1	T1 (CONTROL)	7.41±0.19 f	113.3± 12.01	2.69±0.03 g	0.28±0.01 e
2	T2 (0N+ZnSOL 5)	7.99±0.19 ef	120.0 ±13.27	3.15±0.06 f	0.36±0.01 cde
3	T3 (0N+ZnSOL 10)	8.48±0.11 def	123.3±14.01	3.51±0.05 de	0.38±0.01 bcd
4	T4 (UCU 150 mg N kg^{-1}+ZnSOL 0)	8.89±0.00 cdef	98.00± 1.15	3.22±0.02 ef	0.30±0.00 de
5	T5 (UCU 150 mg N kg^{-1}+ ZnSOL 5)	9.02±0.03 cde	120.0±5.77	3.84±0.03 cd	0.37±0.01 cde
6	T6 (UCU 150 mg N kg^{-1}+ZnSOL 10)	10.3±0.38 bc	125.6±6.40	3.99±0.01 c	0.38±0.01 bcd
7	T7 (PSCU 150 mg N kg^{-1}+Zn 0)	9.68±0.31 bcd	118.3±4.40	4.08± 0.11 c	0.43±0.01 bc
8	T8 ZnCU (150 mg N kg^{-1}+Zn 5 mg kg^{-1})	10.8±0.48 b	124.0±2.64	4.84±0.09 ab	0.45±0.01 bc
9	T9 ZnCU (150 mg N kg^{-1}+ Zn 10 mg kg^{-1})	13.2±0.60 a	128.0±1.52	4.99±0.06 a	0.46±0.00 b
10	T10 (PSCU150 mg N kg^{-1}+ZnSOL 5)	10.4±0.33 bc	129.0±3.05	4.56±0.12 b	0.58±0.03 a
11	T11 (PSCU 150 mg N kg^{-1}+ZnSOL 10)	11.0±0.05 b	130.0±7.00	4.63±0.04 b	0.61±0.03 a
F-value		28.5***	3.71NS	118***	29.9***
HSD		0.438	10.98	0.351	0.094

****highly significant p≤0.01. Means followed by the same letter in column are not significantly different using Tukey's HSD, Honest Significant Difference, test at p≤0.05*

**UCU= Un-Coated Urea*
**PSCU= Palm Stearin Coated Urea*
**ZnCU= Zinc Coated Urea*
**ZnSOL= Zinc in Solution form*
**0N= zero nitrogen*
NS= Non-Significant

Chlorophyll contents were also significantly different in every treatment (Table 3). The PS-coated urea and Zn-coated urea had significantly ($p \leq 0.01$) increased the chlorophyll contents as compared to conventional application of urea. The lowest chlorophyll contents (28.07 mg g^{-1}) were recorded from control treatment. The chlorophyll produced by T8, T9, T10, & T11 (PSCU and ZnCU+ZnSOL) was non-significant ($p \geq 0.05$) according to Tukey's HSD range test. The highest chlorophyll (42.88 mg g^{-1}) was seen in ZnCU. However, PSCU and ZnSOL were also different as compared to no N and uncoated urea.

Table 3 Effect of PS-Zn coated urea and ZnSOL on Chlorophyll contents of maize plants

S.No	Treatments	Chlorophyll (mg g^{-1})
1	T1 (CONTROL)	28.07±0.498 f
2	T2 (0N+ZnSOL 5)	31.06±0.317 e
3	T3 (0N+ZnSOL 10)	33.73±0.366 d
4	T4 (UCU 150 mg N kg^{-1}+ZnSOL 0)	34.66±0.475 cd
5	T5 (UCU 150 mg N kg^{-1}+ ZnSOL 5)	36.24±0.265 bcd
6	T6 (UCU 150 mg N kg^{-1}+ZnSOL 10)	37.74±0.318 b
7	T7 (PSCU 150 mg N kg^{-1}+Zn 0)	37.00±0.555 bc
8	T8 ZnCU (150 mg N kg^{-1}+Zn 5 mg kg^{-1})	41.62±0.791 a
9	T9 ZnCU (150 mg N kg^{-1}+ Zn 10 mg kg^{-1})	42.88±0.057 a
10	T10 (PSCU150 mg N kg^{-1}+ZnSOL 5)	41.74± 0.825 a
11	T11 (PSCU 150 mg N kg^{-1}+ZnSOL 10)	42.16±0.534 a
F-value		93.7***
HSD		2.54

****highly significant p≤0.01. Means followed by the same letter in column are not significantly different using Tukey's HSD, Honest Significant Difference, test at p≤0.05*

Discussion

Nitrogen loss is a ubiquitous universal truth so does Zn deficiency in soil, plants and humans. In our study the progressive increase in fresh and dry shoot weight, root weight, stem girth and chlorophyll contents was in response to increasing N supply with Zn. All Zn treatment groups demonstrated that the selected N and Zn doses were suitable to cause wide differences in growth parameters under controlled glasshouse conditions (Table 2&3). Only one level of

N (150 mg N kg^{-1}) was used in all treatments with different modes of application (UCU, PSCU, and ZnCU). Nitrogen loss is drastic in all types of soils, then whether it is in ammonia volatilization, in leaching or in the form of denitrification (Chen *et al*. 2017). Slow or controlled release fertilizer is actually designed to coat the urea granules for reducing N-losses. Slow or controlled-release fertilizer can simply be defined by the Association of American Plant Food Control Officials as “A fertilizer containing a plant nutrient in a form that delays its availability for plant uptake and uses after uptake”. Such delay in initial availability or an extended time of continued availability may occur through a variety of mechanisms. These include controlling water solubility of the material by semi-permeable coatings, occlusion, protein materials, or other chemical forms, by slowing the hydrolysis of water-soluble low molecular weight compounds, or by other unknown means” (Trenkel 2010). Babar *et al*. (2019) reported that using controlled-release urea (CRU) in the form of encapsulated urea for wheat during the winter growing season significantly increased N-availability in soil and improved yield by 40.5%. A similar observation was also reported previously by Babar *et al*. in 2018 where they applied coated urea; used palm stearin as a binder which was enriched with Cu and Zn (urease inhibitors) observed a positive effect on all the growth parameters and N- contents along with the chlorophyll contents in rice plants under acidic soil conditions of Malaysia. They have documented a 40% increase over control in rice yield. Chlorophyll contents are mainly dependable on N-availability, as was proposed in our current study that coated urea makes N-more available to plants. In the current study, the mineral concentration of N and growth parameters are significantly increased in T9 (ZnCU) and T11 (PSCU+ZnSOL). This positive response of N and Zn is due to the synergistic effect of both mineral elements i.e. N and Zn. The significance of the Zn and N relationship depends highly on Zn availability to plants (Grujcic *et al*. 2018). Xue *et al*. (2014) witnessed this relationship; indicating that maize in the early growth stage has the highest accumulation of Zn. It is assumed that increased N nutrition has a positive effect on the group of transporter proteins responsible for the absorption and transport of Zn and Fe in the shoots. Furthermore, they can play a key role in the translocation of these two micronutrients from vegetative tissues into grains by nitrogenous compounds such as peptides or nicotianamine (Xue *et al*. 2012). The existing study revealed that Zn application in the form of coating with urea (T9 (ZnCU)) or in solution form (T11 (ZnSOL10)) both were very effective in the mineral concentration of Zn (Table 1) in maize plants. The difference between T9 and T11 was non-significant. Rasheed *et al*. (2019) conducted research on Zn application methods, and summed up the results as a solution applied to Zn proved better results than a foliar application because of lesser interaction and more availability to plants. Based on the results we obtained in this study, it can be suggested that combined ZnCU and ZnSOL applications should be taken into consideration when both Zn availability and N-losses are targeted. However, the N contents in maize plants under this research increased significantly in T9 (ZnCU); the statistical difference between T9 and T11 was non-significant. Our results are in accordance with Mathialagan *et al*. (2220), Pelster *et al*. (2019), and Soares *et al*. (2012) who had explained the property of coated urea which shows some retardation on urease activity toward urea which reduces the mineralization activity. The higher accumulation of ammonium around uncoated urea tends to increase the soil pH and thus raise its ammonia volatilization. This can help to confess that such circumstances favor the loss of urea-N from the soil surface. Therefore, higher recovery of urea-N in coated urea was reported compared to uncoated urea.

Conclusion

The glasshouse study proved the improvement and compatibility of palm stearin-coated urea with Zn application. Besides the application of ZnCU, ZnSOL also proved to be a potential source of nutrition for maize crop. The synergism of N with Zn significantly increased N and Zn contents in maize plants. The stable rate of N and Zn (150 mg N kg^{-1} and 10 mg Zn Kg^{-1}) was found sufficient for sustainable maize production. Consequently, the N contents in un-coated urea were the lowest compared to palm stearin-coated urea. This proved PSCU as the best coating system to reduce the hydrolysis rate of urea and successively improve N-efficiency.

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