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# DEVELOPING A NOVEL COATED UREA WITH BIODEGRADABLE POLYMER AND UREASE INHIBITORS TO ENHANCE THE YIELD OF RICE GROWN UNDER AN *USTIC TORRIFLUENT*

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## ABSTRACT

The study deals with evaluating the viability of using biodegradable polymer palm stearin (PS) as a coating material for the provision of nitrogen (N), copper (Cu), and zinc (Zn) on slow-release mechanism. Nitrogen in conventional form results mainly in ammonia volatilization loss. Decelerating hydrolysis rate of urea with the help of certain inhibitors (Cu and Zn); can enhance N-uptake and accordingly increases rice production. A novel PS coated urea was developed manually in laboratory conditions. The effect of PS coated urea evaluated for N, Cu and Zn concentration under field conditions on rice yield and nutrients concentration (N-Cu-Zn). The experiment made use of 140 kg N ha$^{-1}$ coated with 10 and 15 kg Zn ha$^{-1}$ and 3 and 5 kg Cu ha$^{-1}$ applied separately or in combinations. Our results indicated that the applied polymer coated urea with 15 kg Zn ha$^{-1}$ and 3 kg Cu ha$^{-1}$ had affirmative response to increase the yield more than half as compared to control and one-fold increase in Zn and N contents in soil and plants ($p \leq 0.05$). This study has prospective to apply Zn and Cu coated urea as environment friendly fertilizer to reduce the N-losses and increase Zn contents.

**Keywords**: ammonia volatilization; slow-release urea; synchronizing nutrients; alkaline soils

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## INTRODUCTION

Fertilizers are *sine qua non* for sustainable crop production and food security. Low fertilizer use efficiency is the biggest threat not only for the sustainability of agriculture and environment but also for the economic stability of the farming community. Food security can be bearable by applying synthetic fertilizers, which contributes almost 50% improvement in crop yield (Sutton *et al.,* 2011). However, inconsequential fertilizer management results in poor fertilizer use efficiency which successively leads to acute environmental risks (González *et al*., 2015; Pereira *et al*., 2015). Agriculturists nowadays are engaged in introducing such fertilizers which can synchronize nutrients and make them available towards plants. In this regard, controlled release fertilizers (CRFs) can play a better role. Such fertilizers could release specific nutrients slowly and gradually, which consequently harmonize nutrient's demand throughout the crop growth. Hence, the CRFs indicates an abundant prospective to improve fertilizer use efficiency and proven to be an eco-friendly fertilizer (Qiao *et al*., 2016). Usually, for this purpose, conventional fertilizers are encapsulated with protective coating (biodegradable/polymer materials), which release the nutrients through physical barriers for controlling the hydrolysis and dissolution rate of fertilizers (Trenkel, 2010; Mohammad *et al.,* 2017).

Various CRFs are introduced in agriculture system, polymer coated fertilizers are extensively renowned as slow release fertilizers. Numerous polymer materials have been anticipated for CRFs encapsulation. Natural polymers have proven efficient and relatively cheap, non-toxic and exhibit excellent coating properties (Chen *et al.,* 2018). Palm stearin (PS) is the harder fraction of palm oil, containing a higher proportion of saturated fatty acids and triacylglycerols (TAGs) with a higher melting point 48–50°C. Palm stearin has iodine value (IV) of ~34 g I2/100 g, where IV reflects the concentration of unsaturation in fats (Podchong*, et al.,* 2018). Palm stearin can be fractionated into softer and harder PS (Gunstone, 2001). Harder palm stearin (HPS) was used under current study, produced by Sime Darby Berhad (Malaysian Trading conglomerate). Harder palm stearin was selected due to warmer weather conditions of Pakistan. However, the effects of palm stearin coating urea on retarding nutrients release with the help of urease inhibitors i.e. Cu and Zn are limited especially at field scale under alkaline soils. It has been advocated that urease and nitrification inhibitors significantly lower the hydrolysis and nitrfication and enhance the N uptake by maize which consequently increase maize yield (Noor *et al.,* 2018). Limited N absorption is a net product of low nitrogen use efficiency (NUE). Excess application of N-fertilizers consequently leads to N-losses rather being aborbed effectively. The NUF in rice production is generally believed to be half and even meager (Yao, *et al.,* 2018).

Rice (*Oryza sativa* L.) is renowned as a cash crop for its export of aromatic and fine quality indica rice in Pakistan which took a considerable contribution to national exchequer in foreign exchange earnings (Abdullah *et al.,* 2015). Rice sowing area is declining in the region which is particularly interrelated with the nutrient uptake effects due to sudden variations in local climatic conditions, the lessening of water periods in rice fields and trendy fertilizer application (Kirby *et al.*, 2017). Among all these issues, the primary one is an imbalanced fertilizer application that significantly impact on yield and yield components predominantly of cereals (Abbas *et al.,* 2013). Nitrogen losses upon application of conventional urea has been reported widely, there is a dire need to develop such a system that can increase N-efficiency, overcome N-losses as well as micronutrient deficiency and minimize environmental pollution (Chien *et al.,* 2009). Incorporation of micronutrient with macronutrient fertilizer can improve the nutrient deficiencies (Monreal *et al.,* 2016). There are various ways to incorporate the micro and macronutrient fertilizers through different natural coating materials (Azeem *et al.,* 2014). Coating synchronizes time, release of nutrients and sequential plant requirements, resulting to enhance nutrient efficiency followed by a profitable yield (Chauhan *et al.,* 2012). Zinc and Cu coating with biodegradable polymer (palm stearin) are the most suitable strategies to increase effects of urea fertilizer and to overcome the Zn and Cu deficiencies. Interruption in urease activity because of Zn and Cu improves crop yield by minimizing the hazardous emission of ammonia ($NH_3$) and nitrous oxide ($N_2O$) gases from the commercial urea (Purkayastha and Chhonkar, 2006). Selection of palm stearin as a parallel synthetic polymer facilitates a stable coating without any risk concerns (Babar *et al.,* 2016). Natural polymers like palm stearin and waxes have proven good adhesive agents by combining urea with Zn and Cu (Nasima *et al.,* 2013). Hence forth, the fertilizer uses according to the demand of soil and crops is an indispensable approach. Agricultural production system suffers mainly due to careless approaches and ignorance of importance of micronutrients during all fertilizer applications. Alkaline soils are mainly calcareous with low organic matter and coarse texture, that ultimately affects the solubility and availability of the Zn contents (Alloway, 2009; Nazif *et al.,* 2015).

Therefore, considering active role of Zn and Cu in rice production and significant losses of N, current study aimed to appraise the effects of coated urea with Zn and Cu (urease inhibitors) in combination with biodegradable polymers (palm stearin) on rice *cv* Shandar.

## MATERIALS AND METHODS

***Properties of soil and rice plant***: Analytical results (Table 1) categorized the soil of experimental area as heavy, fine-textured, free from salinity hazards, medium alkaline, moderately calcareous and low in Walkley-Black organic matter content, while deficient in Kjeldahl N and 0.5-M $NaHCO_3$ extractable phosphorus (Olsen-P), adequate in 1-M $NH_4HCO_3$ + 0.005 M DTPA (ABDTPA) extractable potassium (K) and Cu and deficient in ABDTPA-Zn. Random composite soil sampling was done at 20-cm depth, before starting the experiment and treatment administration, for analysis through standard methods (Ryan *et al*., 2001). The field experiment was conducted at Agriculture Research Institute (ARI), Tandojam, Sindh, Pakistan (25.415568 N and 68.539238 E), occuring in a semi-arid, subtropical climate (annual rainfall 175 mm per annum). To ensure the limited availability of nutrients, *Rustam* soil series (Riverine Alluvium), belonging to *Ustic Torrifluents* great group, was selected for this study. The rice variety Shandar (IR6 mutated with Gamma rays, 250 Gy), released in 2006 by the Nuclear Institute of Agriculture (NIA), Tandojam, was used in this study. It is moderately resistant against biotic and abiotic stresses and is characterized by non-chalkiness and narrow grains.

**Table 1. Properties of soil used for the study.**

| Soil Property | Unit | Value |
|---|---|---|
| Sand | % | 3.2% |
| Silt | % | 41.5% |
| Clay | % | 55.3 |
| Texture | Silty Clay | |
| EC | dS $m^{-1}$ | 0.13 |
| pH | … | 7.9 |
| $CaCO_3$ | g $kg^{-1}$ | 74.0 |
| Organic matter | g $kg^{-1}$ | 6.9 |
| Kjeldahl nitrogen | g $kg^{-1}$ | 0.40 |
| $NaHCO_3$ P | mg $kg^{-1}$ | 6.91 |
| $NH_4HCO_3$-DTPA K | mg $kg^{-1}$ | 207 |
| $NH_4HCO_3$-DTPA Cu | mg $kg^{-1}$ | 2.01 |
| $NH_4HCO_3$-DTPA Zn | mg $kg^{-1}$ | 0.40 |

**Experimental design and nursery transplantation:** The soil was prepared well to make a fine seed bed by ploughing and harrowing each once. Thirty five days old healthy seedlings of rice *cv*. Shandar were obtained from NIA, Tandojam and transplanted to the small sub-plots of 4m × 4m (16 $m^2$) size, at inter-row and inter-plant spacing of 20 x 20 cm, during first week of June, following randomized complete block design with three repeats. Blocks were made by observing the soil gradient (there was water channel and trees on one side). Treatments were placed in those blocks randomly. These blocks are actually the repeats (3 replications for each treatment). All the recommended cultural practices were followed throughout the experiment.

**Chemical properties of irrigation water:** Irrigation was applied after an interval of four days for a month and subsequently on weekly basis, as the temperature decreased. The water level in the field was maintained at 2-3 inches till maturity of the rice crop. The crop was irrigated using good quality canal water ($EC_{iw}$: 0.63 dS $m^{-1}$, pH: 7.9; SAR: 4.3; RSC: absent), as categorized following Qureshi and Barrett-Lennard (1998).

**Use of biodegradable polymer – Palm Stearin as a coating material:** For the first time, Malaysian palm stearin, extracted from oil palm, was tested to evaluate its efficacy as a coating material on alkaline-calcareous soils of Pakistan, due to its high temperature resistance quality. Sime Darby Berhad Malaysia was a producer for that HPS. It was sold for 450 US dollars per metric ton. The company claims that HPS can resist melting upto 50ºC. The aim behind the selection of HPS was the high summer temperature of Pakistan. As natural coating material its (HPS) efficacy has already been tested in previous experiments at glass house (Babar *et al.,* 2016).

**Development of novel controlled release nitrogen fertilizer by coating with palm stearin and urease inhibitors:** The coating of commercial urea $[CONH_2)_2]$ fertilizer (46% N-$NH_2$) was done manually in the laboratory by using two parts of urea and one part of PS, subsequently coated well with the urease inhibitors (Zn and Cu) using $ZnSO_4.7H_2O$ containing 22.7% Zn and 11% S, or with $CuSO_4.5H_2O$ containing 25.4% Cu and 13% S, or with both. The manufacturing of controlled release N- fertilizer in the laboratory involved coating of fertilizer urea with PS, Zn, and Cu on a water bath at ~100 °C temperature. Soon after the proper melting of palm stearin in the water bath, Zn and Cu sources were added followed by the addition of fertilizer urea. The mixture was then immediately taken out from the water bath and finely spreaded over a white paper sheet to let it cool and stablize at the laboratory temperature (~25 °C).

**Treatment Details:** In total, nine different batches/treatments of Zn and Cu coated urea fertilizer were prepared as per the requirements of this experiment. The rate of fertilizer urea was kept constant for all these nine batches/treatments, i.e. 140 kg $ha^{-1}$, coated with three rates of Zn @ 0, 10 and 15 kg $ha^{-1}$ and three rates of Cu @ 0, 3 and 5 kg Cu $ha^{-1}$, used in integration, making nine treatment combinations used in this study. The batch/treatment in which urea fertilizer was applied as 140 kg N $ha^{-1}$, without any Zn and/or Cu biofortification, served as the control treatment. All these treatments also involved a blanket dose of 70 kg $P_2O_5$ and 60 kg $K_2O$ $ha^{-1}$. Phosphorus was supplied in the form of calcium dihydrogen phosphate or monocalcium phosphate

$[Ca(H_2PO_4)_2.H_2O]$, commonly known as triple super phosphate (TSP) containing 45% $P_2O_5$ and 15% Ca. Potassium was given as potassium sulfate $[K_2SO_4]$ containing 50% $K_2O$ and 54% $SO_4$. All the Zn and Cu coated urea was applied at the time of land preparation (before the transplantation of nursery to the field), along with the full dose of K fertilizer. Full dose of P was applied after 3 weeks of nursery transplantation.

**Harvesting of experiment and recording of observations:** The crop was harvested from each sub-plot after 120 days of sowing to record plant height, number of tillers, number of panicles, panicle length, 1000-grain weight, coupled with the grain and straw yield (t $ha^{-1}$). Filled grains were separated from the unfilled grains as described earlier (Seizo, 1980).

**Plant analyses*:*** Five plants were selected from each replication at the time of maturity (exactly after 90 days of transplantation). The plants were separated into grains and straw by passing through an electrical thresher. Straw samples were thoroughly washed with the distilled water and dried in the laboratory at room temperature (25±2ºC). Both grain and straw samples were stored separately in paper bags and subsequently dried at 65ºC in an oven for 48 hours at a constant weight and ground (0.25 mm) on a Wiley Mill. Rice grain and straw concentration of N was determined by using Kjeldhal method, while Zn and Cu through $HNO_3$-$HClO_4$ wet acid digestion method (Ryan *et al.,* 2001). The Zn and Cu readings were obtained by using Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 400).

**Soil N, Cu, and Zn status after harvesting of rice crop:** Random composite soil samples were collected from each sub-plot at 20-cm depth after the harvesting of rice crop to analyze soil N, Zn and Cu status, following standard methods (Ryan *et al*., 2001).

**Statistical Analysis:** The statistical analyses were performed using Statistix© ver. 8.1 (Analytical Software 2005). F test was performed by using two-way ANOVA to know the impact of different treatments and blocks (coated urea with Cu and Zn). However, effect of Cu coated and Zn coated urea was estimated/identified using Tukey's HSD (Honest Significant Difference) Test. It was followed by comparing the significant ($p\leq0.05$) treatment means for each character.

## RESULTS

**Rice growth traits:** Applied coated urea with Zn and Cu either singly or in combination affected all growth parameters of rice plants significantly ($p\leq0.05$).. Plant height (cm), number of tillers $plant^{-1}$, number of panicles $plant^{-1}$ and panicle length increased in applied coated urea with Zn and Cu treated plots as compared to control (Table 2). There was no significant difference between treatments containing 15 kg Zn $ha^{-1}$ used singly or with 3 or 5 kg Cu $ha^{-1}$ for all growth parameters. It is assumed that positive enhancement in growth of rice was due to uptake of N, Cu and Zn under the influence of using coated urea which improved the induced improvements in these agronomic characters. The tallest plants, maximum number of tillers, concentrated number of panicle $plant^{-1}$, maximum panicle length were evidently increased under applied coated urea with 15 kg Zn $ha^{-1}$ coated with urea.

**Unfilled grain and 1000-grain weight:** This study proved that coating with Zn and Cu at the same time made N more available to plants with their synergistic effects on 1000 grain filling percentage. Unfilled grain percentage was significantly reduced after application of coated urea with Zn and Cu (Table 3). The maximum percentage of unfilled grains was observed from uncoated urea (without Zn and Cu coating). The results showed that 15 kg Zn $ha^{-1}$ with or without 3 or 5 kg Cu $ha^{-1}$ encouraged completion of physiological and biochemical processes leading to grain filling in rice plants. Highest grain weight was also noted in the plots where coated urea was applied with Cu and Zn. The maximum positive response in terms of unfilled grains and grain weight was observed from the treatment where coated urea was applied with Zn at the rate of 15 kg $ha^{-1}$.

**Table 2. Effect of Zn and Cu coated urea on plant height (cm), number of tillersplant$^{-1}$, number of paniclesplant$^{-1}$ and panicle length (cm) of rice.**

| Rate of Zn and Cu (kg $ha^{-1}$) coated to urea (applied @140 kg $ha^{-1}$) | | Plant height (cm) | Number of tillersplant$^{-1}$ | Number of paniclesplant$^{-1}$ | Panicle length (cm) |
|---|---|---|---|---|---|
| Zn | Cu | | | | |
| Control/0 | 0 | 72.2±1.10 e | 11.3±0.66 e | 8.17±0.33 d | 16.0±0.14 c |
| 10 | 0 | 103.3±1.18 b | 18.5±0.50 d | 17.3±0.88 c | 22.0±0.66 b |
| 15 | 0 | 116.8±0.38 a | 24.9±0.72 abc | 23.7±0.33 ab | 27.0±1.10 a |
| 0 | 3 | 88.9±0.69 c | 17.8 ±0.24d | 16.5±0.33 c | 21.3±0.63 b |
| 10 | 3 | 102.6±0.48 b | 22.4±0.55 c | 21.8±0.33 b | 22.3±0.49 b |
| 15 | 3 | 115.6±0.61 a | 25.9±0.14 ab | 24.4± 0.33ab | 25.7±1.06 a |
| 0 | 5 | 80.5±0.44d | 18.3±0.12 d | 17.2±0.33 c | 21.3±0.17 b |

| | | | | | |
|---|---|---|---|---|---|
| 10 | 5 | 116.1±0.93 a | 22.7±0.84c | 22.1±0.33 b | 22.3±0.89 b |
| 15 | 5 | 116.0±0.78 a | 26.5±0.28 a | 25.2±0.33 a | 25.3±0.37 a |
| F-value | | $476.3^{***}$ | $89.3^{***}$ | $57.8^{***}$ | $39.5^{***}$ |
| HSD | | 3.92 | 2.57 | 3.52 | 2.56 |

*$^{***}$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*

**Table 3. Effect of Zn and Cu coated urea on on unfilled grain (%) and 1000-grain weight (g), grain yield and straw yield of rice (t ha$^{-1}$).**

| Rate of Zn and Cu coated (kg ha$^{-1}$) to urea (applied @140 kg ha$^{-1}$) | | Unfilled grain (%) | 1000 grain weight (g) | Grain yield (t ha$^{-1}$) | Straw yield (t ha$^{-1}$) |
|---|---|---|---|---|---|
| Zn | Cu | | | | |
| Control/0 | 0 | 25.3±0.32 a | 25.0±0.05 g | 5.17±0.07 e | 6.14±0.16 de |
| 10 | 0 | 18.3±0.24 b | 35.3±0.33 de | 6.47±0.07 bc | 7.34±0.06 bc |
| 15 | 0 | 15.3±0.22 c | 43.7±0.57 a | 7.81±0.07 a | 9.06±0.05 a |
| 0 | 3 | 19.1±0.32 b | 32.7±1.20 ef | 6.07±0.31 d | 6.39±0.43 d |
| 10 | 3 | 18.0±0.06 b | 40.0±0.57 bc | 6.73±0.37 b | 7.55±0.06 b |
| 15 | 3 | 15.0±0.04 c | 44.3±0.33 a | 8.07±0.15 a | 9.33±0.06 a |
| 0 | 5 | 18.7±0.25 b | 31.0±0.57 f | 5.90±0.31 cd | 6.42±0.43 c |
| 10 | 5 | 18.9±0.20 b | 38.0±0.57 cd | 6.57±0.37 bc | 7.55±0.35 b |
| 15 | 5 | 18.5±0.25 c | 43.0±0.57 ab | 7.99±0.17 a | 9.25±0.02 a |
| F-value | | $200.6^{***}$ | $98.27^{***}$ | $90.4^{***}$ | $199.2^{***}$ |
| HSD | | 1.17 | 3.29 | 0.53 | 0.43 |

*$^{***}$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*

**Grain and straw yield of rice :** Depending on the concentration of applied coated urea with Zn and Cu, grain and straw yields were significantly increased as compared to the control treatment (Table 3). The lack of Zn (0.40 mg kg-1) nutrition in the experimental field and rice plants cultivated on this land might be causing this positive outcome. The maximum grain and straw yield was realized from coated urea application with 15 kg Zn +3 kg Cu ha$^{-1}$ followed by coated urea with 15 kg Zn +5 kg Cu ha$^{-1}$ and coated urea with 15 kg Zn ha$^{-1}$. However, the application of Zn alone was less effective numerically. There may be a connection between Zn and Cu playing an influential role in several metabolic processes that enhance the accumulation of assimilates in grains to improve weight. However, the exact mechanism has not been identified yet.

**N, Cu and Zn concentration in rice plants:** Nitrogen, Cu and Zn uptake of grains and straw in rice plants showed a significant response to coated urea (Table 4). The highest N, Cu and Zn concentration in grains were observed in treatments where urea was coated with urease inhibitors i.e., Cu and Zn. Biodegradable palm stearin served as a vital component of the coating to facilitate the gradual absorption of nutrients. Therefore, it was noticed a remarkable increase in N (70%), Cu (41.6%), and Zn (95%) contents in grains after coated urea application as compared to control. The nutrient contents were affected by all treatments which were coated, resulted in increased N, Cu and Zn accumulation in straw of rice plants. Following application of coated urea with Cu and Zn fertilizers to rice straw, N increased by 72.9%, Cu increased by 45.5%, and Zn increased by 95.4%, respectively. . Among all the treatments the maximum increase in the studied three nutrients (N, Cu and Zn) was observed where urea was coated with 5 kg Cu and 15 kg Zn ha$^{-1}$. These results are indicating the potential use of coated urea (combo of all essential nutrients like N, Cu and Zn coated with natural palm stearin) to ease the uptake of N, Cu and Zn in rice plants substantially and meet the nutrient demand than that of control.

**Table 4. Effect of Zn and Cu coated urea on N, Cu and Zn concentration in rice grain and straw.**

| Rate of Zn and Cu coated (kg ha$^{-1}$) to urea (applied @140 kg ha$^{-1}$) | | N (g kg$^{-1}$) | | Cu (mg kg$^{-1}$) | | Zn (mg kg$^{-1}$) | |
|---|---|---|---|---|---|---|---|
| Zn | Cu | Grains | Straw | Grains | Straw | Grains | Straw |
| Control/0 | 0 | 9.1±0.01e | 8.5±0.01c | 12.2±0.16f | 11.2±0.10d | 22.0±0.10 e | 19.9±0.14d |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 10 | 0 | 14.1±0.01d | 13.6±0.01b | 13.7±0.08e | 12.3±0.12c | 35.3±0.19 c | 32.8±0.26b |
| 15 | 0 | 15.2±0.01ab | 14.8±0.05a | 13.9±0.12e | 12.8±0.01c | 42.5±0.89 b | 39.7±0.54a |
| 0 | 3 | 14.0± 0.01d | 13.5± 0.01b | 17.4±0.08b | 16.7±0.11ab | 26.0±0.32 d | 21.9±0.27c |
| 10 | 3 | 15.0±0.02ab | 14.2±0.01ab | 17.8±0.10b | 16.7±0.17ab | 41.34±0.30b | 39.1±0.58a |
| 15 | 3 | 15.7±0.05a | 14.8± 0.05a | 16.7±0.57d | 16.0±0.05b | 43.3±0.21 a | 39.5±0.31a |
| 0 | 5 | 14.8±0.01c | 14.0±0.02ab | 18.3±0.09a | 17.3±0.15a | 25.9±0.37 d | 22.6±0.36c |
| 10 | 5 | 14.9±0.02bc | 14.2±0.01ab | 17.1±0.10b | 16.3±0.27ab | 41.3±0.31 b | 38.9±0.18a |
| 15 | 5 | 15.5±0.01ab | 14.7±0.01a | 17.0±0.06c | 16.3±0.33ab | 43.5±0.71ab | 38.9±0.63a |
| F-value | | $247.1^{***}$ | $143.1^{***}$ | $465.9^{***}$ | $178.9^{***}$ | $478.1^{***}$ | $678.7^{***}$ |
| HSD | | 0.08 | 0.06 | 0.509 | 0.86 | 2.21 | 1.77 |

*$^{***}$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*

**Status of N, Cu and Zn in soil after harvest*:*** The concentration of N, Cu and Zn in soil samples after harvesting the rice crop is summarized in Table 5. All combination of treatments (coated urea) significantly (*p*≤0.05) affected the nutrient concentrations in soil as compared to control. The effects of applying coated urea with Zn at the rate of 15 kg ha$^{-1}$ and Cu at the rate of 5 kg ha$^{-1}$ were significantly (*p*≤0.05) different as compared to control.

## DISCUSSION

This research expresses a simple fertilizer application method that combines Cu-Zn-N using a palm stearin (a biodegradable coating material) to load both macro (N) and micro nutrients (Cu and Zn) onto rice yield and nutrients availability.

**Growth traits:** The productivity of cereal crops is directly related to the prodcutive number of tillers (Li *et al*., 2012). Usually lower N content in stems and leaves may inhibit the development of tillers. As per reports rice tillers are developed significantly when N contents are more than 27 and 35 mg g$^{-1}$ whereas, tiller formation may curb where N contents are lower than 13 or 15 mg g$^{-1}$ (Zhong *et al.,* 2003). In current research the maximum numbers of tillers were related to the availability of N (Table 2), which indicated that N absorbed by plant leaves was distributed evenly to the rice shoots. Nowadays, most intimidate research is nitrogen use efficiency (NUE) for meeting the alarming increase of food demand (Fixen *et al.,* 2015). According to Babar et al. (2016), coated urea with Cu and Zn significantly increased NUE and rice yield. This study manifested that growth parameters were positively correlated with coated urea (Table 1). Enhanced NUE under coated urea were attributed to improve N uptake and reduced N losses. It is presumed that Zn and Cu are involved in a number of physiological processes in rice plants; therefore, their availability influenced growth parameters significantly under this research. The increment in growth parameters might be attributed to N, Zn and Cu, that contributes to enhanced enzymatic activities (Khan *et al.* 2012).

**Unfilled grains and 1000-grain weight:** Through this study we observed that the uptake of essential nutrients (Cu-N-Zn) by rice plants through coated urea allows them to compete in their life cycle accordingly and in a healthier way.. The number of unfilled grains was high under control and the maximum 1000-grain weight was observed from coated urea with Zn and Cu. The rice fields are flooded which makes $NH_4$ form of N incapable of absorbing or inhibiting its availability. Furthermore, $NH_4^+$-N can merely be resisted for a period of 5-10 days after fertilizer application and after this period addition of some fungi like Azola can enhance the BNF (Yao *et al.,* 2018). Similarly, current results showed that if fertilizer is amended with coated urea, it could benefit rice plants as synchronizing nutrients availability. After adding the contribution from coated urea with Cu and Zn treatments still resulted in greater grain filling and grain weight over the treatments with control and conventional urea. Results are in accordance with Babar *et al*., (2019), who observed the positive effect of coated urea on grain filling and grain weight in wheat plants as compared to conventional urea.

**Grain and Straw Yield:** Grain and straw yield after application of biodegradable polymer coated urea with Cu and Zn were markedly influenced as compared to control. These results are supported by Nasima *et al.* (2013) and Panhwar *et al.* (2015) who documented significant increase in maize yield and rice seeds weight by mixing Cu and Zn coating or Zn, Fe, Mn, Cu and B in combination in the same order. The results manifested that application of 15 kg Zn ha$^{-1}$ with 3 kg Cu ha$^{-1}$ had significant effects on grain and straw yield. Zn and Cu are nutrients and acidifying chemicals as well as urease inhibitors. The presence of Zn and Cu at urea microsite stabilizes soil pH and facilitates the absorption of nutrients (Ahmed *et al.,* 2006). Binding materials also play a key factor in the dissolution rate of Zn and Cu depending on their strength (Monreal *et al.,* 2018). Urea granules coated with Zn and Cu enable plants to absorb selected nutrients according to their requirements. It is assumed that limited diffusion of nutrients from coated fertilizers may be due to reactions of nutrients and coating materials at the surface of granules, mass flow of water in soil towards the fertilizer granules or pH effects (Milani *et al.,* 2015). The results of Guimarães *et al.* (2016) showed that the urea hydrolysis was inhibited over the intact range of evaluated Cu and/or Zn. They showed that 10 kg Zn $ha^{-1}$ and 5 kg Cu $ha^{-1}$ perceived significant effect on inhibition. In fact, immobilization of metals is influenced by soil organic C through adsorption or chelation and can be reduced by applying Zn and Cu.

**N, Cu and Zn concentration in rice plants:** In the present research at the same application rates of N with two different methods of application (conventional urea and coated urea layered with urease inhibitors; Cu and Zn bonded by biodegradable palm stearin), coated urea treatments provided a reliable and steady improvement in nutrient concentration. The nature of Zn and Cu has a synergistic effect on the uptake of N. When Zn was applied in combination with Cu, it increased N-uptake in plants. Nasima *et al.* (2013), Luo and Rimmer (1995); Panhwar *et al.* (2015); Wei *et al.* (2012) approved the positive interaction of Cu and Zn towards uptake of these metals in maize, spring barley, and rice plants respectively. Similarly, Khan *et al.* (2015) reported a significant effect of coated urea on N uptake. They highlighted the importance of coated urea due to its slow-release mechanism on N uptake in rice plants as compared to granular urea (GU). The poor N uptake in control treatment (conventioanl urea) is associated with urea alone treatments could be due to the large volatilization and leaching in alkaline calcareous soils under this study. These results are in line with the findings of Geng *et al.* (2016), they have reported the effectiveness of polymer coated urea on N uptake in cotton yeild.

**N, Cu and Zn content in soil after harvest:** Soil N, Cu and Zn concentration increased significantly after coated urea application (Table 5). The water absorbency and degradability of coating material (palm stearin as biodegradable) depict a true shape of its importance (Noppakundilograt *et al.,* 2015). Earlier research has shown that layers of coating material with lower water absorbency were more effective in controlling nutrient uptake and their release (Han *et al.,* 2009). The maximum nutrients concentration in soil after rice harvesting was recorded under this study from coated urea. This could be possible due to film up urea granule with palm stearin along with Cu and Zn. Copper and Zn have synergistic effect and been recognized since decade (Doelman and Haanstra, 1986; Luo and Rimmer, 1995). Presence of added Cu improves amount of extractable Zn by displacing the adsorbed Zn in soil reservoir. Copper inhibits soil urease activity by attracting amino groups in both urea and urease (Daif and Beusichem, 1981).

**Table 5. Effect of Zn and Cu coated urea on soil N, Cu and Zn status after harvesting rice crop.**

| Rate of Zn and Cu coated (kg $ha^{-1}$) to urea (applied @140 kg $ha^{-1}$) | | N (g $kg^{-1}$) | Cu (mg $kg^{-1}$) | Zn (mg $kg^{-1}$) |
|---|---|---|---|---|
| Zn | Cu | | | |
| Control/0 | 0 | 0.41±0.00 c | 2.19±0.07 d | 0.40±0.01 d |
| 10 | 0 | 2.1±0.00 b | 2.41±0.02 d | 1.32±0.04 b |
| 15 | 0 | 2.4±0.00 a | 2.41±0.01 d | 1.53±0.01 a |
| 0 | 3 | 2.4±0.00 a | 4.28±0.04 b | 0.54±0.01 c |
| 10 | 3 | 2.5±0.00 a | 3.99± 0.05 c | 1.24±0.01 b |
| 15 | 3 | 2.5± 0.00 a | 3.89±0.05 c | 1.55±0.00 a |
| 0 | 5 | 2.4±0.00 a | 5.08±0.08 a | 0.58±0.01 c |
| 10 | 5 | 2.3±0.00 ab | 4.03±0.04 c | 1.26±0.00 b |
| 15 | 5 | 2.4±0.00 a | 4.11±0.00 bc | 1.56±0.00 a |
| F-value | | $212.3^{***}$ | $434.0^{***}$ | $636.7^{***}$ |
| HSD | | 0.02 | 0.24 | 0.09 |

$^{***}$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

**Conclusıons:** The present study examined the development and compatibility of palm stearin coated urea. Besides, the N-transformation process from applied conventional urea showed that coated urea was significantly inhibiting the hydrolysis rate of urea. Urease inhibitors Zn and Cu incorporated with urea improved the availability of N due to their synergistic effect on N, which increased rice yield.According to a field study, coated urea using either Zn alone or in combination with Cu seemed toimprove rice grain yield and yield components compared to the control treatments. The treatment improved accumulation of Zn, Cu and N in rice grains and straws. Thus, this study reported a development of a new protocol and promising rice production system using a simple strategy for Zn/Cu as coated urea.

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