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Research article

Modulated Ammonia Volatilization from Coated Nitrogen Fertilizer and Wheat Productivity on Phosphorus Amended Alkaline Soils Involving ^{15}N Tracer Technique

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Abstract

The nitrogen (N) fertilizers are markedly lost as ammonia volatilization from alkaline soils and hence is deemed as major cause of lower N utilization by field crops. The current study explored possible measures to reduce N loss from applied fertilizer and enhance its availability to wheat crop grown on alkaline soil. The treatments involved Zinc-coated (1% Zn) urea [ZnU] and sole urea applied/incubated with soil at recommended rate (RR) and 80% RR after further coating with inhibitors [NBPT at 1% or ATC at 2% urea]. Compared to sole urea, ZnU showed lower N loss in laboratory environment; whereas in field trials on wheat crop, ZnU (at 80% of RR) along with NBPT produced similar wheat yield as produced by RR of sole urea application. Phosphorus application further enhanced wheat biomass (1.03 and 1.04 kg m^{-2}) and grain yield (0.362 and 0.407 kg m^{-2}) at half (23 kg ha^{-1}) and full P rate (46 kg ha^{-1}) as applied with ZnU, respectively. Whereas, the ZnU_{80} coated with NBPT produced highest NUE (62.8%) when applied with full P rate, followed by half P application rate (NUE=58.7%). Another collateral study proved the significance of P application (0, 15, 30 and 45 mg P kg^{-1}) in improving NUE (15-20%) in wheat at similar N application as $(^{15}NH_4)_2SO_4$. Overall, the studies clearly suggest that appropriate N management reduces N losses, improves nutrient uptake and yield parameters of wheat crop grown on alkaline soil.

Keywords

Alkaline Soil, Ammonia N Losses, Nitrogen Use Efficiency, Wheat Productivity, Zinc-Coated Urea and Inhibitors

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1. Introduction

Synthetic fertilizers have been found a key contributors to productivity of food crops during green revolution and they are still largely applied with the aim to achieve higher crop yields [1]. N fertilizers largely contributes to crop production, yet its excessive use encounters N losses, exceeding 50% either as nitrate leaching or gaseous N losses, i.e. N_2O emissions or ammonia volatilization [2]. Wheat (*Triticum aestivum* L.), a staple food crop, occupies almost 41% of the total cultivated land (22.8 million ha) and consumes ~45% of the fertilizers in Pakistan. Nevertheless, wheat yield has remained low in this region compared to others, mainly due to imbalanced fertilization and lower fertilizer use efficiency [3, 4]. The average N use efficiency in cereals is very low, i.e. 33 to 36% in Pakistan [5] compared to the global efficiency of 57%. Consequently, a huge share of the applied N fertilizer is lost to the environment, originating from a total annual fertilizer consumption of 2.8 million tons in Pakistan; the fertilizer losses account for about US$ 600 million per annum [6]. A wise N management could curtail N losses and enhance crop responses to applied fertilizer, which is however challenging. [7, 8]. Ammonia volatilization is the main pathway of N losses in alkaline soils. Urea and/or ammonium containing fertilizers result in higher ammonia volatilization rates, but lower NUE in crop grown on alkaline soils [1]. Urea-coating with chemicals, polymers, metal elements and inhibitors potentially curtail N losses and increase crop N utilization. Furthermore, zinc-coated urea seems beneficial for its potential of zinc biofortification [10-12]). The use of Sulphur and urease inhibitor (Agrotain) coated urea was found to reduce ammonia losses by 50% from sandy loam soils [13]. Ammonia losses from the soil surface also decrease by the addition of P fertilizer of acidic nature [14]. Acidic P fertilizers may have the added benefit of promoting plant P nutrition besides controlling ammonia volatilization through lowering the soil pH [15, 16]. Phosphoric acid may also supply/mobilize calcium, capable of reducing urea hydrolysis and hence resulting in lower ammonia volatilization [14]. Humic acids are also capable to retain more ammonium than ammonia, thus improving crop N utilization and reducing environmental losses [17].

Combined application of N and P fertilizer proved beneficial in winter wheat in terms of higher crop productivity and higher utilization of the applied nutrients. For instance, increases in grain yield and grain protein were observed with increasing rates of N fertilizer amendment alongside with P application [18]. Another study showed beneficial effects of appropriate applications of N and P fertilizer, where a $N:P_2O_5$ ratio of 2:1 produced optimum yield of wheat crops grown on calcareous soils. The N form, i.e. the application of ammonium (NH_4^+) and nitrate (NO_3^-) also seems important as they variably affect biochemical processes in plants or in soil environments. The absorption of nitrate is accompanied by the uptake of inorganic cations and the release of OH^-, whereas ammonium favours the absorption of inorganic anions and the release of H^+ into the rhizosphere environment [19].

Nitrate utilization may infer higher energy costs than ammonium assimilation, due to greater ATP requirements for the reduction of nitrate by nitrate and nitrite reductase to ammonium in plant cells, to form the variety of amino acids, a process mediated by glutamine synthetase (GS) and glutamate synthase (GOGAT) [20]. Besides other factors, cereal yield is therefore also influenced by N forms [21]. An appropriate ammonium/nitrate ratio leads to optimum growth of apple (*Malus domestica* Borkh.) [22]), tomato (*Solanum lycopersicum* L.) [23] and rice (*Oryza sativa* L.) [24]. Predominant ammonium source has negative effects on the growth of Arabidopsis thaliana [25], while yet another study reported stimulating impacts of ammonium on glucosinolate metabolism in A. thaliana [26]. An appropriate ammonium/nitrate ratio enhances plant tolerance to abiotic stresses and helps alleviate light stress in Chinese cabbage (*Brassica pekinensis*) [27].

The crop N utilization of slow-release fertilizers can be precisely determined using ^{15}N tracer techniques [28]. Despite the different mass, ^{15}N displays similar chemical reactivity as the more abundant ^{14}N, and hence ^{15}N labelled fertilizers can be used to trace and quantify its uptake by the plant and to quantify N losses and their pathways. Fertilizer use efficiency is a quantitative measure of actual uptake of fertilizer N by the crop plant in relation to the amount of N added to the soil as fertilizer. The crop usually responds more to the application of nutrients such as N and P when the soil is deficient in such nutrients [29].

An effective use of fertilizers ensures higher crop productivity, the best N management involving nutrient sources, time of application, mode of application, and the interactions with different farming systems including irrigation, residue management etc. Effective N fertilizer management requires better information on the processes and dynamics of N from the applied fertilizer and the contribution of soil N to crop growth [30].

Therefore, in this study, a set of experiments were carried out in the laboratory, in pot cultures and under field conditions, to gain this information. Different agronomic approaches were tested to identify N fertilizers for better plant availability and lower ammonia volatilization losses from alkaline soils to achieve higher wheat productivity under natural field conditions. Overall, these experiments aimed at determining a) the impact of chemo-amended urea (coated with zinc and/or inhibitors) on reducing ammonia volatilization and consequently improving wheat productivity and its nutritional quality on alkaline soil, and b) the influence of appropriate N plus P application on vegetative growth and yield of wheat. The ^{15}N tracer technique was employed to differentiate crop N deriving from fertilizer N or native soil

N and determine NUE in wheat crops.

2. Materials and Methods

The studies were conducted to elaborate the impact of chemo-amended urea on ammonia volatilization in a controlled lab environment, and the contribution of ammonium and nitrate on N and P uptake, and wheat productivity with variable phosphate rates. The field trials also conducted to determine the impact of N fertilization with P application at sufficient P application (recommended rate, RR) and its half and consequently observation were recorded for ammonia volatilization, nutrient uptake, NUE and yield parameters of wheat grown on alkaline calcareous soils.

Experimental site and soil description

The experiments were conducted at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan (North 31° 23’, East 73° 01’, 184 m msl). Faisalabad has a hot desert climate with high evapotranspiration, i.e. termed BWh according to the Köppen-Geiger classification [31]. The temperature in the region ranges between −2 ℃ in wintertime to +50 ℃ in summer time. The summer season starts in April, the winter season in November. The average yearly rainfall ranges between 300 and 350 mm, however half of the yearly rainfall occurs in July and August.

The soil (0-15 cm) was collected at the experimental field area of NIAB, Faisalabad (geographic coordinates as mentioned above), was air-dried, passed through a 2-mm sieve and then analyzed for ECe (Electrical Conductivity of saturated paste extract) and pH (U.S. Salinity Lab. Staff, 1954) [32], $CaCO_3$ content [33], soil texture [34], organic matter concentration [35], amount of mineral N [36], and Olsen P [37]. The soil was found intermediate in electrical conductivity (EC=1.2 dSi m^{-1}), alkaline in nature (pH=7.8) calcareous in reaction ($CaCO_3$ equivalent = 1.7%), [38, 39] and have clay loam texture, low amounts of organic matter (0.98%), nitrate (4 mg kg^{-1} soil), ammonium (5 mg kg^{-1} soil) and Olsen P (9.80 mg kg^{-1} soil). Soil used in incubation study and pot experiments was also collected from the same experimental field.

Experiment 1: Quantifying changes in ammonia volatilization from chemo-amended urea applications (150 mg N kg^{-1} soil) in P amended soil with incubation time

An incubation study was performed by employing a system (fitted on the very first day) for collecting ammonia emitted from treated soil. The ammonia volatilized was measured at desired days, i.e. 4, 7 and 14 after application of commercial-urea, zincated-urea and their coatings with NBPT and ATC to the soil containing sufficient P (46 mg P kg^{-1} soil) and native P (No addition of P or Zero P content). The ZnO (1% Zn coating) was coated on commercial urea to form zinc coated urea (ZnU) and then both commercial and ZnU were then incubated in controlled laboratory conditions. Fertilizer granules were then further coated with inhibitors [urease inhibitor at 1% urea, i.e. N-(n-butyl) thiophosphoric triamide (NBPT) and nitrification inhibitor at 2% urea, i.e. 4-Amino-1,2,4-triazole (ATC)] or not as shown in Table 1 thus creating six different treatments.

Table 1. *Experimental treatments for Lab studies (applied as given below) and field trials.*

Nr.	Treatment description	Lab Code	Field Code
1	Urea applied at recommended rate (RR), 150 mg N kg^{-1} soil	Urea	Urea
2	*ATC coated urea applied at RR	U+ATC	U_{80}+ATC
3	**NBPT coated urea applied at RR	U+NBPT	U_{80}+NBPT
4	Zincated Urea applied at RR, 150 mg N kg^{-1} soil	ZnU	ZnU
5	ATC coated ZnU applied at RR	ZnU+ATC	ZnU_{80}+ATC
6	NBPT coated ZnU applied at RR	ZnU+NBPT	ZnU_{80}+NBPT

*ATC= 4-Amino-1,2,4-triazole; **NBPT= N-(n-butyl) thiophosphoric triamide;
Note: Field trial contained all the six treatments with little modification in N & P application. Actually, Lab studies produced higher NH_3 loss in inhibitor treatment (ATC), so both inhibitor treatments were applied at 80% of recommended rate (RR) in field trial and denoted as U_{80}+ATC and U_{80}+NBPT for commercial urea while ZnU_{80}+ATC and ZnU_{80}+NBPT for zincated urea. Moreover, field trails also contained sufficient P application (RR) and half of RR to observe the differences of P level as well on wheat productivity.

The incubation experiment in completely randomized design (CRD) was conducted to determine ammonia losses for a period of 14 days. Aliquots of soil (300 g air dry weight) were placed in glass vessels and were treated with chemo- amended urea as mentioned in Table 1 at 150 mg N kg^{-1} soil. The phosphate amendments viz. phosphoric acid PA) were applied in solution at an P application rate of 46 mg P kg^{-1} soil, while the same N treatments were also incubated without P application. Deionized water was regularly added to maintain the soil moisture at 20% (almost equivalent to field capacity). The flasks were further fitted with a device generally air tight and with acid trap to absorb ammonia evolved during incubation. The device also consisted of a rubber stopper, a central hole which is fitted with a glass tube having a glass vial with 5 ml 0.3 M H_2SO_4 attached to its lower end. The vial bottom was almost 1.5 cm above the soil surface in the flask while the other end of the glass tube was around 1 cm above the top of the stopper. The stoppered flasks were then placed in an incubator maintained at 30 ℃ for 4, 7 and 14 days. After each incubation time, acid vial contents were retrieved and fresh acid was replaced for the next incubation. Ammonia absorbed in the acid traps was calculated by an acid- base titration method [40].

Experiment 2: Impact of chemo-amended urea on ammonia volatilization, and the nutritional quality and agronomic parameters of wheat grown in phosphorus amended soil under field conditions.

In this field trial, commercial and zinc-coated (zincated) urea were applied at the recommended rate (RR= 150 kg N ha^{-1}), while further coating with inhibitors, the coated urea being applied at 80% of recommended rate. Therefore, six urea treatments as indicated in Table 1 with half and full dose of P were evaluated in the experiment. In a CRBD, chemo-amended urea with half and full P application were compared for ammonia volatilization, wheat yield, growth and N uptake. The experiment was performed at the NIAB Research area with three replicate plots. Due to the better performance of wheat cultivar Faisalabad-2008 in the greenhouse experiment, same was sown in field at 100 kg seed ha^{-1} using tractor mounted drill. Commercial and zincated urea was applied at 150 kg N ha^{-1} (100% of the recommended rate), while both urea fertilizers coated with inhibitors were applied at 80% of RR. The N fertilizer was applied in two halfs, i.e. 50% at 22 days after sowing (DAS) and the rest at booting stage. Phosphorus (46 kg P ha^{-1} as H_3PO_4) was applied in two splits, i.e. half of the P was applied at seed bed preparation and the remaining with the second split of urea application. The recommended doses of K (45 kg ha^{-1} as K_2SO_4) and Zn (5 kg ha^{-1} as $ZnSO_4$) were also applied at the time of seed bed preparation. To achieve more precision in NH_3 volatilization peak, more samplings were taken and hence ammonia loss was recorded at 0, 2, 4, 7, 10 & 14 days after the second split of urea was applied to the experimental plots maintained without and with phosphorus application. Sample device consisted of polyvinyl chloride (PVC) hard plastic bucket, PVC pipe chambers (basal area 0.0324 m^2) and two sponges having thickness of 2 cm and diameter of 16 cm. Both sponges were homogeneously soaked with 15 mL of glycerol solution (50 mL of phosphoric acid + 40 mL of glycerol, constant volume to 1000 mL). The lower sponge is 5 cm away from the bottom and the upper sponge is flat with the top of the bucket. Bucket was then inserted into the soil soon after the application of fertilizer to capture ammonia losses. Upper sponges absorb ammonia in the air prevent it from entering into the lower. The lower sponge absorbs the ammonia of the soil. After each absorption, sponges were extracted with 1N KCl solution and replaced with set of sponges. KCl extract was then analyzed for ammonia losses by titration method by Kjeldahl distillation apparatus. At crop maturity, an area of 1 m^2 from three different locations in each plot was harvested and pooled to determine the wheat yield and the 1000-grain weight. Yield components like spike length, spike weight and grains per spike were recorded from 10 randomly selected plants of each plot and also pooled to get mean value for each plot.

Experiment 3: Impact of variable P fertilizer rates on wheat NUE in inhibitor-$(NH_4)_2SO_4$ amended soil as assessed by ^{15}N tracer technique.

A pot experiment was carried out to determine the NUE in wheat– soil systems with ^{15}N labelled fertilizer $(NH_4)_2SO_4$ applied at 120 mg N kg^{-1} soil with variable P rates (0, 15, 30 and 45 mg P kg^{-1} soil as phosphoric acid). The ^{15}N labelled $(NH_4)_2SO_4$ was applied as such and also after coating with nitrification inhibitor, i.e. ATC (2% of fertilizer on w/w basis) to limit its conversion to nitrate and to predominantly supply ammonium during crop growth. Pots were filled with 3 kg soil and two wheat cultivars (cv. Faisalabad 2008 and cv. Lasani) were grown up to maturity. Pots were placed in completely randomized block design with three replicates. The N fertilizer was applied in two splits, i.e. half at sowing and half at 40 days after seedling emergence; whereas P as phosphoric acid (variable rates as mentioned above), K (45 mg K kg^{-1} soil) as K_2SO_4 and Zn (5 mg kg^{-1} soil) as $ZnSO_4$ were applied at sowing. Three uniform seedlings were maintained after germination. The effect of the treatments was tested for plant growth, grain yield and grain quality of wheat. Grains were digested to determine total N concentration, and the titrant of the analysis for total N was concentrated to determine ^{15}N using isotope ratio mass spectrometry.

Measurements applied in ^{15}N tracing experiments

For all the experiments with ^{15}N labelled materials, the following basic data is needed:

1) Dry matter (D.M.) yield for the whole plant or plant parts
2) Total N concentration of the whole plant or plant parts
3) Plant atom%^{15}N abundance analyzed by emission spectroscopy or isotope ratio mass spectrometry
4) Fertilizer atom%^{15}N abundance
5) ^{15}N labelled fertilizer(s) used and N rate(s) of application

Calculations for experiments with ^{15}N

The first parameter when studying fertilizer uptake by a crop using isotope techniques is to determine the fraction of the nutrient derived from the labelled fertilizer, i.e.: fdff, expressed as a percentage, i.e.:

1. % dff = fdff x 100

Then, atom%^{15}N abundance is changed into atom%^{15}N excess by subtracting the natural abundance of ^{15}N (0.3663 atom%^{15}N) from the atom%^{15}N abundance of the sample. After that the following calculations are made [41].:

2. % Ndff = atom%^{15}N excess plant / atom%^{15}N excess fertilizer * 100

Dry Matter (DM) yield per unit area:

3. DM yield (kg/ha) = FW (kg) * 10000 (m^2/ha) / area harvested (m^2) * SDW (kg) / SFW (kg)

where FW is fresh weight and SDW and SFW are subsample dry and fresh weight, respectively.

4. N yield (kg/ha) = DM yield (kg/ha) * %N/100

5. Fertilizer N yield (kg/ha) = N yield (kg/ha) * % Ndff / 100

6. % Fertilizer N utilization = Fertilizer N yield (kg/ha) / Rate of N Application * 100

Experiment 4: Influence of ammonium and nitrate nutrition on N and P uptake, early growth and yield of wheat under variable phosphate application rates under greenhouse conditions.

During 2020-2021, two wheat cultivars (cv. Faisalabad 2008 and cv. Lasani) were grown under greenhouse conditions to assess the influence of ammonium and nitrate nutrition on N and P uptake, growth and yield of wheat under variable phosphate rates. Pots filled with 5 kg soil were placed in completely randomized block design with three replicates for each treatment, to which ammonium and nitrate sources of N were applied at 150 mg N kg^{-1} soil: In this experiment urea coated with a nitrification inhibitor, i.e. ATC (2% of urea) was added as an ammonium source designated as U+ATC, while $Ca(NO_3)_2$ was used as a nitrate source. The N application was carried out in two splits, i.e. half at sowing and the other half at early booting stage. The phosphate amendments via phosphoric acid were applied in solution form, equivalent to P application rates of 0, 15, 30 and 45 mg P kg^{-1} soil). The basal doses of K (45 mg K kg^{-1}soil) as K_2SO_4 and Zn (5 mg Zn kg^{-1} soil) as $ZnSO_4$ were applied at sowing. Twelve wheat seeds per cultivar were sown into each pot filled with 5 kg soil after maintaining the moisture of soil at 75% of the maximum water holding capacity throughout the experiment. Thinning was done to keep nine uniform seedlings in each pot per cultivar after germination. During experimentation, three seedlings per pot were harvested after 28 days and three after 48 days of sowing. After each harvest, fresh weights were recorded by washing the samples using deionized water and then blotting them dry with tissue papers. Thereafter, samples were dried in an oven at 85 ℃ for three days to record their dry weights. The relative growth rate was calculated using the formula.:

$$RGR = (LN\ w2 - LN\ w1)/ (t2 - t1)$$

Where w2 and w1 are dry weights of plants (in grams) at times t2 and t1 (in days), respectively, and LN is the natural logarithm.

The wheat plants were harvested at physiological maturity for measuring yield parameters. The effect of the treatments was tested for plant growth, yield and grain quality. During growth, physiological parameters (Net photosynthesis rate, transpiration rate & stomatal conductance) were noted, whereas the harvested crop material was analyzed for yield parameters and for mineral NP contents in the grain.

Statistical analysis:

Effectiveness of treatments were tested employing the ANOVA technique with factorial design (two way & three way interaction depending upon factors, i.e. N management, P levels, time interval and wheat genotype) using statistical programme [42] (Statistix8.1) for Microsoft windows and significant differences in the treatment mean were compared using LSD at 5% level of probability [43].

3. Results

Ammonia losses from chemo-amended Urea with and without P application

The ammonia losses from commercial and chemo amended urea applied at RR as shown in Figure 1 was measured with and without P application under controlled laboratory conditions at different days i.e. 4, 7 and 14 after incubation. Maximum ammonia losses were observed after 4 days of incubation in all the treatments and thereafter ammonia losses gradually decreased. However, a significant reduction in ammonia losses was observed in soils amended with phosphoric acid as P source compared to soils without P application. After 4 days of incubation, commercial urea produced higher (U, 12.5%) ammonia losses compared to zincated urea (ZnU, 10.0%) in zero-P control soils, with commercial urea and zincated urea coated with ATC proving similar and non-significantly higher results. When compared to the others, NBPT treatments showed significantly ($p<0.05$) lower ammonia losses (3.74 to 6.54%) after 4 days of incubation, which further declined to negligible levels after 14 days of incubation, with values of 0.52 and 1.25% with and without phosphorus application, respectively.

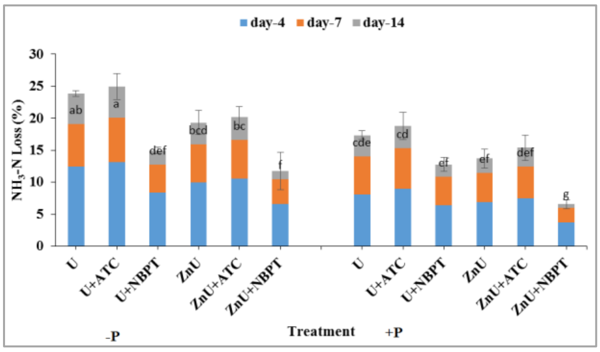

Figure 1. *Ammonia losses from conventional urea and chemo-amended-urea applications (150 mg N kg^{-1} soil) without and with P amendment at different days of incubation (day 4, 7 and 14).*

Mean values of treatments are represented as stacked bars, where the bars sharing the same letter(s) do not differ significantly at P<0.05; vertical lines (I) on each bar denote standard errors. Abbreviations: -P = without phosphorus; +P = with phosphorus; U = urea; ATC = 4-Amino-1,2,4-triazole; NBPT = N-(n-butyl) thiophosphoric triamide; Zn-U = zinc coated urea.

Commercial urea exhibited higher cumulative ammonia losses (23.9%) in soils without P application compared to 17.3% in soils with P application. In comparison with commercial urea, ZnU produced lower cumulative loss (13.7 and 19.3%) with and without P application, respectively. However, ATC treated urea exhibited higher losses (20.1 to 24.9%) over sole application of commercial urea fertilizers. A significant variance ($p<0.05$) in ammonia losses was observed when U or ZnU was applied with or without the inhibitor NBPT to P amended soils. U+NBPT showed 12.8% cumulative ammonia losses, whereas ZnU+NBPT exhibited significantly ($p<0.05$) lower ammonia losses (6.54%) in P treated soils. An almost similar trend was observed in soils without P treatment, though at higher levels of ammonia losses. Overall, a significant reduction in ammonia losses as shown in Figure 1 was observed with the application of P in all fertilizer treatments When compared to no-P application, ammonia losses were decreased by 37.7 and 40.9% in U and ZnU treatments in soils with P amendment.

Ammonia volatilization from wheat cropped soil with urea and phosphate application under natural field conditions

Fertilizer application to wheat grown in alkaline soil under field conditions (Figure 2) showed variable levels and time kinetics of N losses via ammonia volatilization

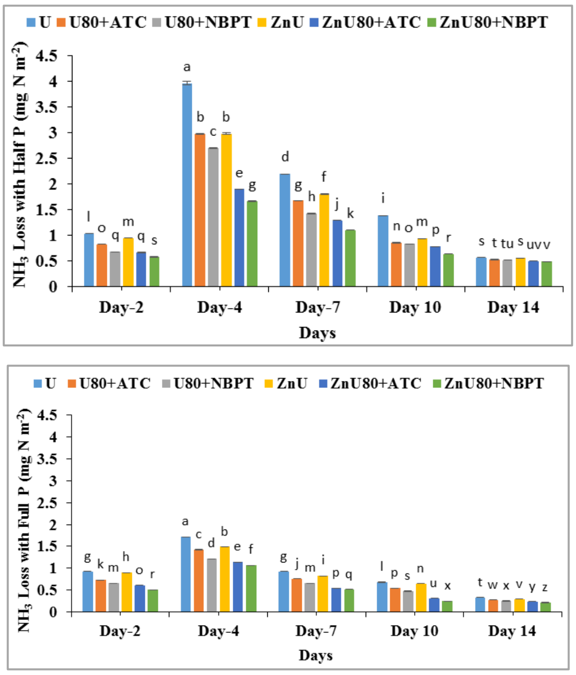

Figure 2. *NH_3 volatilization losses at different days after application of conventional and chemo-amended urea with half (left side) and full (right side) dose of P addition.*

Mean values of treatments are represented as a set of bars, where the bars sharing the same letter(s) do not differ significantly at $P<0.05$. Vertical lines (I) on each bar denote standard errors. U = urea; ATC = 4-Amino-1,2,4-triazole; NBPT = N-(n-butyl) thiophosphoric triamide; Zn-U = zinc coated urea. U = urea applied at recommended rate (100% RR, 150 mg N kg^{-1} soil); U80+ATC = ATC-coated urea applied at 80% RR; U80+NBPT = NBPT-coated urea applied at 80% RR; ZnU = zincated urea applied at 100% RR; ZnU80+ATC = ATC-coated ZnU applied at 80% RR; ZnU80+NBPT = NBPT_coated ZnU applied at 80% RR; Half P =23 kg P ha^{-1}, Full P = 46 kg P ha^{-1}

Similar to the laboratory findings, the highest ammonia losses occurred during the first week of fertilizer application, while the losses declined during the second week, reaching a minimum 14 days after fertilizer application. Overall, the commercial urea produced highest ammonia volatilization losses in soils applied at both P rates, i.e. at half and full level of the recommended dose of P as phosphoric acid, while ZnU produced lower ammonia losses. The maximum cumulative ammonia loss (By summing all the losses at different intervals of time) of 744 mg N m^{-2} was found with the application of commercial urea with no further coating and at half of the recommended P rate, while the minimum loss of 208 mg N m^{-2} was observed in the ZnU+NBPT treatment showed in Figure 3 with full P application Almost similar trends of ammonia volatilization were observed for fertilizer applications with half and full dose of P application (Figures 2 & 3), though at significantly lower levels at the full P rate. ATC-coated urea produced higher ammonia volatilization losses compared to NBPT-coated urea in soils applied either at half or full level of P application. Overall, ammonia losses were lower in any urea treatment amended as showed in Figure 3 with the full dose of P application as compared to those applied with half of the P dose.

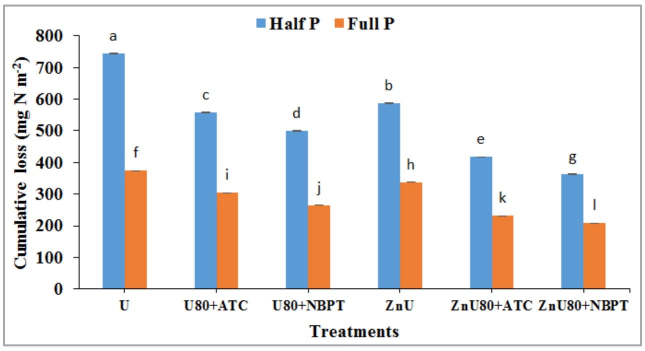

Figure 3. *Cumulative ammonia losses from conventional and chemo-amended urea applications with half and full dose of phosphorus addition.*

Mean values of treatments are represented as a set of bars, where the bars sharing the same letter(s) do not differ significantly at $P<0.05$. Vertical lines (I) on each bar denote standard errors. U = urea; ATC = 4-Amino-1,2,4-triazole; NBPT = N-(n-butyl) thiophosphoric triamide; Zn-U = zinc coated urea. U = urea applied at recommended rate (100% RR, 150 mg N kg^{-1} soil); U80+ATC = ATC-coated urea applied at 80% RR; U80+NBPT = NBPT-coated urea applied at 80% RR; ZnU = zincated urea applied at 100% RR; ZnU80+ATC = ATC-coated ZnU applied at 80% RR; ZnU80+NBPT = NBPT_coated ZnU applied at 80% RR; Half P =23 kg P ha^{-1}, Full P = 46 kg P ha^{-1}

Wheat productivity as affected by urea and phosphate application

At harvest, plant height and yield components like spike length, spike weight, grains per spike and 1000-grain weight were observed for 10 randomly selected wheat plants. Total biomass as well as grain yield was noted from one square meter from three subsites within each plot. Data on grain yield and other yield parameters of wheat grown under field conditions was showed in Figures 4 and 5. Maximum plant height as showed in Figure 4 was observed at the recommended application rate of ZnU (101.1 and 98.4 cm) and urea (95.8 and 93.8 cm) with half and full dose of P application, respectively. Yield and yield related traits of wheat (spike length and spike weight) showed in Figure 4 did not significantly respond to the different urea treatments. Maximum spike length was observed in ZnU and U treatments (9.82 and 9.74 cm), respectively, with full dose of P application, and a similar trend was observed with half dose of P application. A non-significant change (p>0.05) was detected in spike length between the treatments ZnU_{80}+NBPT and ZnU_{80}+ATC (9.64 and 9.61 cm), respectively, with full dose of P application when compared with ZnU. Similar spike length was observed for the treatments U, U_{80}+ATC and U_{80}+NBPT with half and full dose of P application. Maximum spike weight was observed in ZnU (2.55 and 2.17 g) with full and half dose of P application, respectively. Spike weight did not differ between ZnU_{80}+NBPT andZnU_{80}+ATC when compared with ZnU and U at both doses of P application. Maximum grain numbers per spike and 1000-grain weights were observed in ZnU and U, respectively. Overall, the results highlight the superior performance of ZnU compared to commercial urea as an N fertilizer regarding its positive effect on wheat productivity. The 80% dose of N applied as chemo-amended urea produced as much biomass as was produced by the full dose (recommended rate) of commercial urea. Maximum biomass was produced by the recommended application of ZnU (1.160 and 1.157 kg m^{-2}) and urea (1.037 and 1.010 kg m^{-2}) with full and half dose of P application, respectively. We found no significant variance (p<0.05) in total wheat biomass (1.143 and 1.047 kg m^{-2}) with 80% of chemo-amended fertilizer such as ZnU_{80}+NBPT and U_{80}+NBPT, respectively, with full dose of P application. Almost similar trends were observed with ZnU_{80}+ATC and U_{80}+ATC with half and full dose of P application.

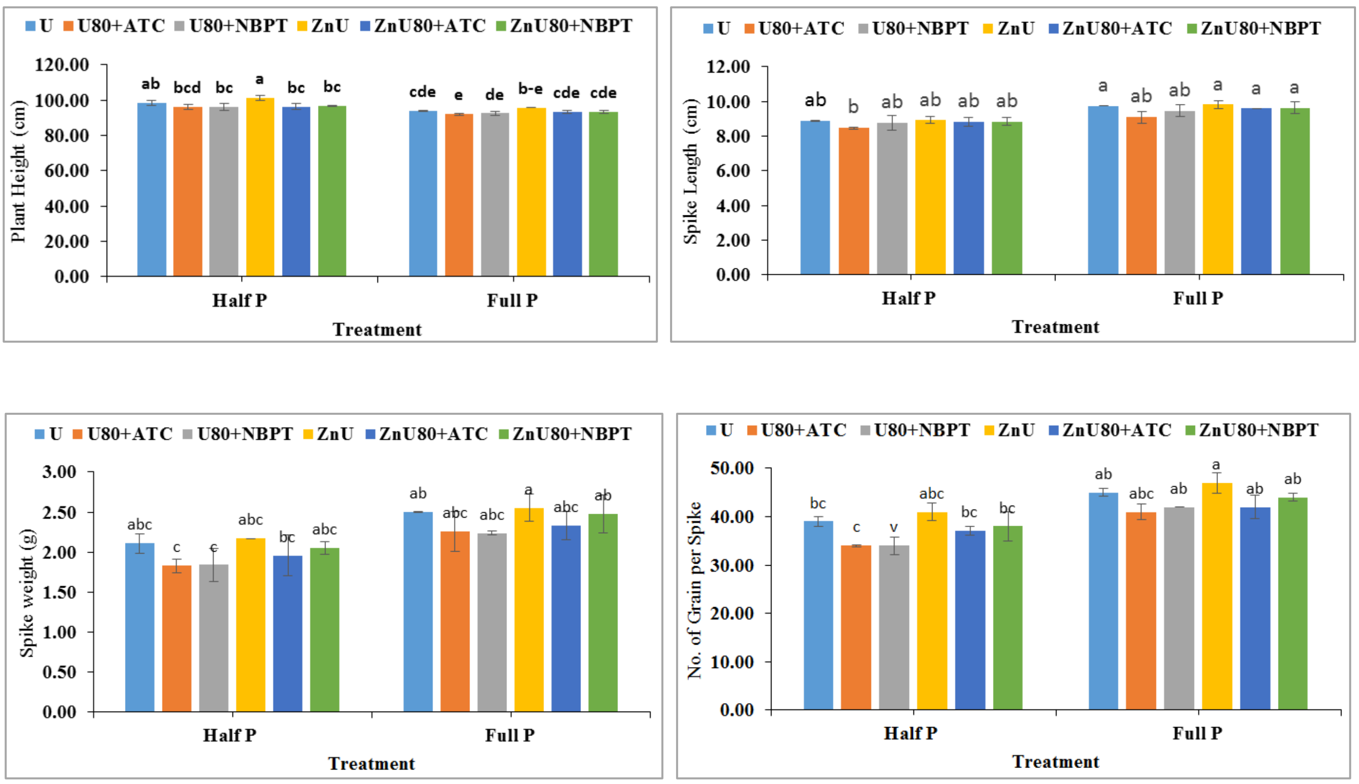

Figure 4. Effect of conventional and chemo-amended urea applications on plant height and yield-related traits of wheat with half and full dose of P addition.

Mean values of treatments are represented as a set of bars, where the bars sharing the same letter(s) do not differ significantly at P<0.05. Vertical lines (I) on each bar denote standard errors. U = urea; ATC = 4-Amino-1,2,4-triazole; NBPT = N-(n-butyl) thiophosphoric triamide; Zn-U = zinc coated urea. U = urea applied at recommended rate (100% RR, 150 mg N kg^{-1} soil); U80+ATC = ATC-coated urea applied at 80% RR; U80+NBPT = NBPT-coated urea applied at 80% RR; ZnU = zincated urea applied at 100% RR; ZnU80+ATC = ATC-coated ZnU applied at 80% RR; ZnU80+NBPT = NBPT_coated ZnU applied at 80% RR; Half P =23 kg P ha^{-1}, Full P = 46 kg P ha^{-1}

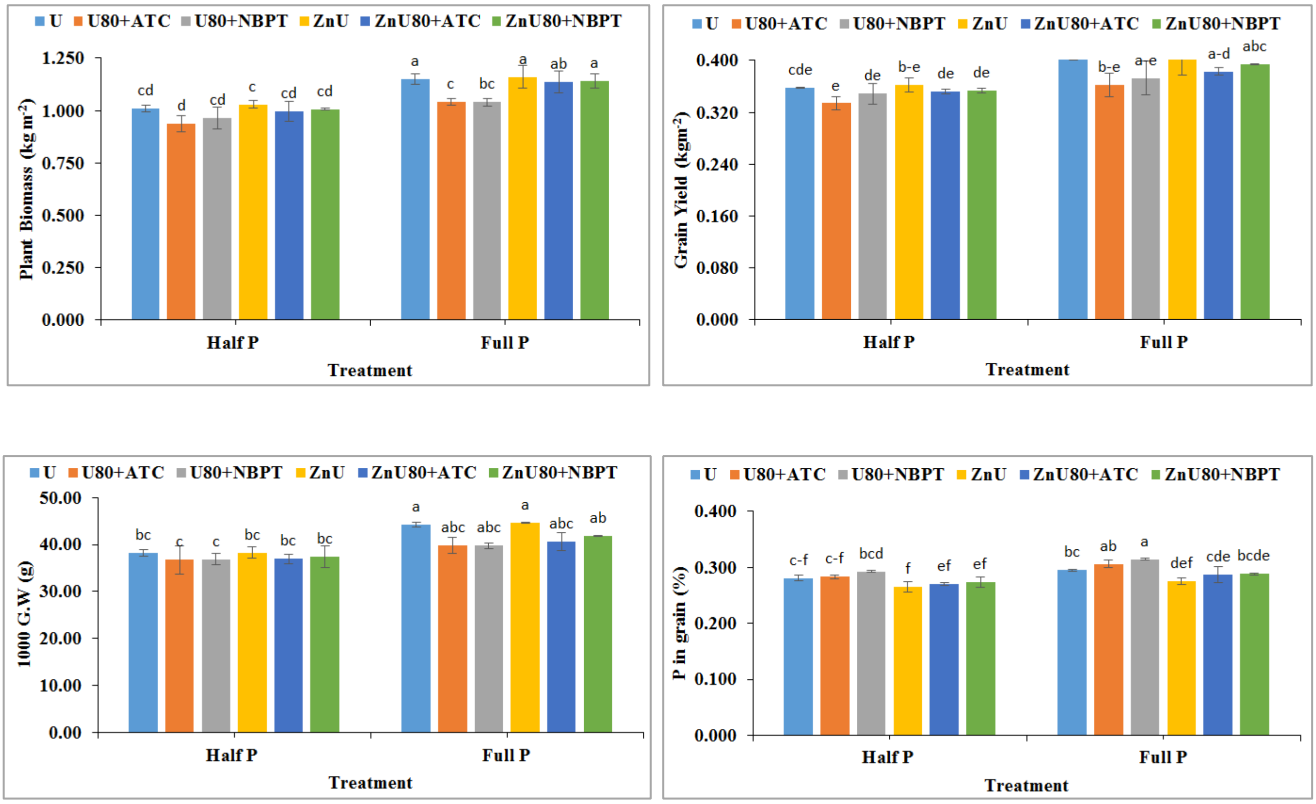

Figure 5. *Effect of conventional and chemo-amended urea applications on plant biomass, grain yield, 1000-grain weight and grain P concentration in wheat with half and full dose of P addition.*

Mean values of treatments are represented as a set of bars, where the bars sharing the same letter(s) do not differ significantly at P<0.05. Vertical lines (I) on each bar denote standard errors. U = urea; ATC = 4-Amino-1,2,4-triazole; NBPT = N-(n-butyl) thiophosphoric triamide; Zn-U = zinc coated urea. U = urea applied at recommended rate (100% RR, 150 mg N kg^{-1} soil); U80+ATC = ATC-coated urea applied at 80% RR; U80+NBPT = NBPT-coated urea applied at 80% RR; ZnU = zincated urea applied at 100% RR; ZnU80+ATC = ATC-coated ZnU applied at 80% RR; ZnU80+NBPT = NBPT_coated ZnU applied at 80% RR; Half P =23 kg P ha^{-1}, Full P = 46 kg P ha^{-1}

Overall, Figures 4 and 5 showed that plant height was lower in all treatments with full dose compared to half dose of P application (. Similarly, maximum grain yield was observed in ZnU and U (0.407 and 0.401 kg m^{-2}), respectively, with full dose of P application. Grain yield did not differ between ZnU$_{80}$+NBPT and ZnU$_{80}$+ATC (0.395 and 0.383 kg m^{-2}) compared to ZnU applied at the recommended rate. Grain yield produced in ZnU and U was 0.362 and 0.359 kg m^{-2}, respectively, with half dose of P application. A non-significant change (p>0.05) as showed in Figure 5 was observed in grain yield between U$_{80}$+NBPT and U$_{80}$+ATC with 0.350 and 0.334 kg m^{-2}, respectively, with half dose of P application. The results ratify that 80% of the recommended rate of N applied as chemo-amended urea is sufficient to produce comparable grain yield to the recommended rate of N applied as commercial urea. Maximum P concentration in wheat grain was observed in U$_{80}$+NBPT, followed by U$_{80}$+ATC at the full dose of P application when compared with ZnU and U as showed in Figure 5 whereas lower grain P contents but almost similar trends were observed at half dose of P application. Moreover, across all inhibitor treatments ZnU caused lower grain P contents compared to commercial urea. Almost similar trends were observed for the fertilizer treatments in terms of grains per spike and 1000-grain weights at half and full dose of P application. No significant difference was observed in both parameters when ZnU and U at recommended rates were compared with 80% of the recommended rate of chemo-amended fertilizers. Maximum N uptake and NUE (Figure 6) was recorded for fertilizers at full dose of P application N uptake in grain was similar for U and ZnU, but declined with inhibitor treatments by 0.5 to 1.0 g N m^{-2}. Higher wheat NUE was found in ZnU$_{80}$+NBPT and ZnU$_{80}$+ATC (62.8 and 60.6%, respectively) when compared with ZnU and U (52.4 and 51.6%, respectively). Wheat NUE in U$_{80}$+NBPT and U$_{80}$+ATC was intermediate, with 58.8 and 56.7%, respectively, with full dose of P application. Almost similar trends in NUE were observed for all urea treatments with half dose of P application.

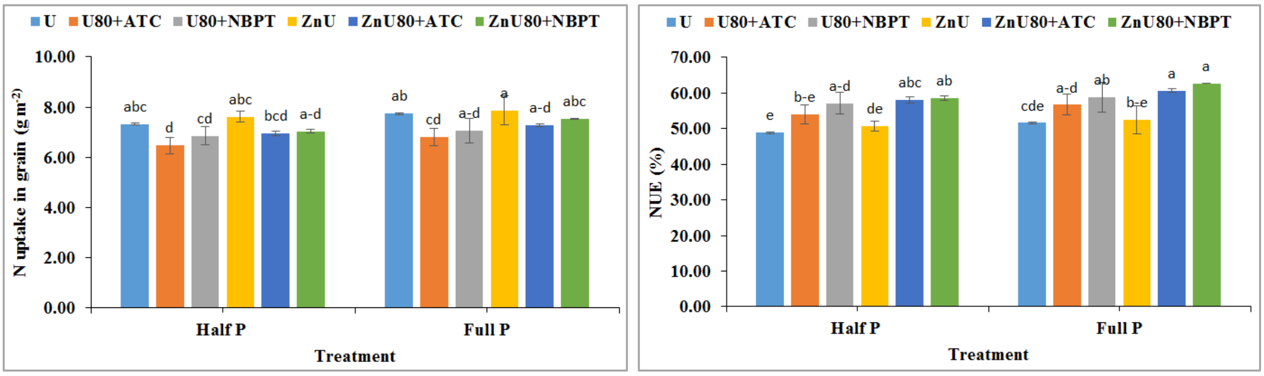

Figure 6. Effect of conventional and chemo-amended urea applications on N uptake and NUE in wheat with half and full dose of P addition.

Mean values of treatments are represented as a set of bars, where the bars sharing the same letter(s) do not differ significantly at $P<0.05$. Vertical lines (I) on each bar denote standard errors. U = urea; ATC = 4-Amino-1,2,4-triazole; NBPT = N-(n-butyl) thiophosphoric triamide; Zn-U = zinc coated urea. U = urea applied at recommended rate (100% RR, 150 mg N kg^{-1} soil); U80+ATC = ATC-coated urea applied at 80% RR; U80+NBPT = NBPT-coated urea applied at 80% RR; ZnU = zincated urea applied at 100% RR; ZnU80+ATC = ATC-coated ZnU applied at 80% RR; ZnU80+NBPT = NBPT_coated ZnU applied at 80% RR; Half P =23 kg P ha^{-1}, Full P = 46 kg P ha^{-1}. N = nitrogen; NUE = nitrogen use efficiency; Half P =23 kg P ha^{-1}, Full P = 46 kg P ha^{-1}

Change in Wheat NUE with increasing P rates as assessed by ^{15}N tracer technique

A pot experiment to determine the fractions %Ndff, %Ndfs, crop N yield, fertilizer N yield, and plant N utilization as shown in Table 2 was carried out.

Table 2. Yield parameters and nitrogen use of wheat supplied with $(NH_4)_2SO_4$ fertilizer with and without the nitrification inhibitor 4-Amino-1,2,4-triazole (ATC) at variable phosphate addition rate (0-45 mg P kg^{-1} soil).

Variety	Treatment	P Application	Biomass	Grain yield	1000-G.W	Ndff	Ndfs	Crop N yield	Fertilizer N yield	Fertilizer N utilization
		mg P kg^{-1} soil		g pot^{-1}			%	g N pot^{-1}		%
Fsd-2008	$^{15}NH_4(SO_4)_2$ + ATC	0	66.46f-i*	23.39fgh	40.5g	46.72ab	53.27fg	0.385h	0.18cde	49.94d-g
		15	69.61def	23.66e-h	42.1ef	49.25a	50.74g	0.397fgh	0.196ab	54.37bc
		30	71.72bcd	26.62ab	44.55bcd	42.39efg	57.61abc	0.461bc	0.195bc	54.16bc
		45	75.31a	27.67a	46.35a	42.81c-g	57.19a-e	0.494a	0.211a	58.73a
	$^{15}NH_4(SO_4)_2$	0	65.65hi	23.09gh	38.1h	44.5b-e	55.5-f	0.374h	0.167ef	46.28gh
		15	67.21e-i	23.70d-h	41.15fg	45.27b-e	54.73c-f	0.392gh	0.177cde	49.26efg
		30	70.34cde	25.04cd	43.45cde	41.48fg	58.52ab	0.423def	0.175de	48.67fg
		45	74.03ab	27.04a	44.8bc	40.51g	59.48a	0.468ab	0.189bcd	52.51c-f
Lasani	$^{15}NH_4(SO_4)_2$ + ATC	0	66.13ghi	23.3gh	40.4g	45.74bc	54.26ef	0.381h	0.174e	48.32g
		15	67.87e-h	23.88d-g	41.7fg	43.74b-f	56.25b-f	0.399e-h	0.174e	48.47fg
		30	71.41bcd	25.5bc	44.35cd	43.87b-f	56.12b-f	0.435cd	0.191bcd	53bcde
		45	74.19ab	27.4a	45.85ab	42.65d-g	57.34a-d	0.48ab	0.203ab	56.86ab
	$^{15}NH_4(SO_4)_2$	0	64.42i	22.41h	37.6h	45.47bcd	54.52def	0.339i	0.154f	42.75h
		15	66.02hi	23.62e-h	40.7fg	44.10b-f	55.89b-f	0.385h	0.169e	47.01g

Variety	Treatment	P Application	Biomass	Grain yield	1000-G.W	Ndff	Ndfs	Crop N yield	Fertilizer N yield	Fertilizer N utilization
		mg P kg^{-1} soil		g pot^{-1}		%		g N pot^{-1}		%
		30	69.32d-g	24.715c-f	43.25de	42.92c-g	57.08a-e	0.411d-g	0.179de	48.95fg
		45	73.44abc	24.96cde	44.65bcd	45.08bcde	54.91cdef	0.426de	0.193bc	53.34bcd
	LSD (p<0.05%)		3.269	1.347	1.49	3.00	3.00	0.029	0.016	4.049

*Mean values in columns sharing the same letter(s) do not differ significantly at *P* <0.05. $^{15}NH_4(SO_4)_2$+ATC = ^{15}N labelled ammonium sulphate coated with a nitrification inhibitor, ATC = 4-Amino-1,2,4-triazole; G.W. = grain weight; Ndff= nitrogen derived from fertilizer; Ndfs = nitrogen derived from soil; N = nitrogen.

We observed that plant biomass increased with increasing P application rate in both varieties, when ^{15}N-labelled fertilizer was applied with and without the nitrification inhibitor ATC. In general, both cultivars (cv. Faisalabad-2008 and cv. Lasani) showed maximum plant biomass when ^{15}N-labelled fertilizer was applied with the nitrification inhibitor ATC (designated as $^{15}NH_4(SO_4)_2$+ATC). Maximum plant biomass (75.3 g pot^{-1}) was observed in cv. Faisalabad-2008 at 45 mg P kg^{-1} application rate and this decreased with decreasing P rates, e.g. to 71.7 g pot^{-1} plant biomass at 30 mg P kg^{-1} application and further to 66.5 g pot^{-1} at zero-P application. An almost similar trend was observed in cv. Lasani when it was supplied with $^{15}NH_4(SO_4)_2$+ATC as well as $^{15}NH_4(SO_4)_2$ without inhibitor at different levels of P application. Similarly, maximum plant biomass was observed in cv. Faisalabad-2008 (75.3 g pot^{-1}) followed by cv. Lasani (73.4 g/pot) when $^{15}NH_4(SO_4)_2$ was applied without ATC at the highest rate of P application. Minimum grain yield was observed in both cultivars with zero-P application. Maximum grain yield (27. 7 and 27.0 g pot^{-1}) was observed in cv. Faisalabad-2008 followed by cv. Lasani, respectively, with 45 mg P kg^{-1} application in the $^{15}NH_4(SO_4)_2$+ATC treatment. In general, no significant variance (p>0.05) was observed in plant biomass and grain yield of both varieties in the application of N fertilizer with ATC inhibitor, and almost the similar trend was seen when N fertilizer was applied without ATC. However, significant differences (p<0.05) were observed in plant biomass and grain yield of wheat cultivars between N fertilizer treatments with and without ATC inhibitor. Maximum percentage N derived from fertilizer (%Ndff) was recorded in both, cv. Faisalabad-2008 and cv. Lasani, the former being higher, with $^{15}NH_4(SO_4)_2$+ATC however, this was non-significant when N fertilizer was applied alone. %Ndff ranged between 40.5 and 49.3%, and overall %Ndff declined with increasing P rate (except cv. P application in $^{15}NH_4(SO_4)_2$+ATC. Overall, fertilizer N utilization in both varieties increased with increasing P rate as shown in Table 3 and by co-amendment of the nitrification inhibitor ATC. Fertilizer N yield of the ATC-N treatment increased with increasing P application rate (from 0 to 45 mg kg^{-1}) in cv. Faisalabad-2008 and cv. Lasani from 50 to 59% and from 43 to 57%, respectively. An almost similar trend was observed as shown in Table 2 when N fertilizer was applied without inhibitor, fertilizer N utilization increasing from 46 to 59% and from 43 to 57% in cv. Faisalabad-2008 and cv. Lasani, respectively..

Table 3. *Influence of ammonium and nitrate nutrition on early growth (28 and 48 days after sowing) of wheat under variable P application rates.*

Variety	Treatment	Fresh & dry weight after 28 and 48DAS					
		P Application	F.W	D.W	F.W	D.W	RGR
		ppm	g/plant				g/ day
Fsd-2008	U+ATC	0	1.385ef	0.166fg	3.8efg	0.62efg	0.065a
		15	1.464def	0.182cdef	4.5bcdef	0.69cde	0.067a

Variety	Treatment	P Application ppm	F.W g/plant	D.W	F.W	D.W	RGR g/ day
	Fresh & dry weight after 28 and 48DAS						
		30	1.56bcde	0.198abcd	4.83abcd	0.767bc	0.068a
		45	1.754a*	0.211a	5.56a	0.88a	0.072a
	$Ca(NO_3)_2$	0	1.357f	0.156g	3.66fg	0.58g	0.066a
		15	1.4405def	0.170efg	3.88defg	0.673def	0.069a
		30	1.5115def	0.183bcdef	4.5bcdef	0.73cd	0.069a
		45	1.700abc	0.2015abc	5abc	0.85ab	0.072a
	U+ATC	0	1.377f	0.163fg	3.66fg	0.59fg	0.064a
		15	1.447def	0.175defg	4cdef	0.682cde	0.068a
		30	1.531cdef	0.19abcde	4.73abcde	0.752cd	0.069a
		45	1.712ab	0.206ab	5.13ab	0.859a	0.071a
Lasani	$Ca(NO_3)_2$	0	1.106g	0.128h	2.96g	0.48h	0.067a
		15	1.406def	0.1685efg	3.83defg	0.631efg	0.066a
		30	1.468def	0.18cdef	4.16bcdef	0.723cd	0.069a
		45	1.569bcd	0.198abcd	4.6abcdef	0.767bc	0.068a
	LSD (p<0.05%)		0.177	0.023	1.012	0.088	8.4E-03

*Mean values in columns sharing the same letter(s) do not differ significantly at $P < 0.05$. U+ATC = urea coated with ATC (4-Amino-1,2,4-triazole); F.W= fresh weight; D.W= dry weight; RGR= relative growth rate; DAS= days after sowing

Influence of N forms on wheat productivity under variable phosphate application rates

Application of increasing P amounts with two different sources of nitrogen (ammonium or nitrate) significantly increased fresh and dry mass of wheat showed in Table 3 harvested at two different growth stages. The effect of P rate with U+ATC on fresh and dry mass was evident in both varieties from the very 1st harvest onwards where higher P rates increased the fresh and dry mass accumulation in U+ATC over that of the nitrate treatment. These differences were also evident ($p<0.05$) at the 2nd harvest. The rate of P application in the U+ATC and $Ca(NO_3)_2$ treatments also positively though non-significantly influenced relative growth rates shown in Table 3, measured between 28 and 48 days of growth, which ranged narrowly between 0.65 and 0.72 g d-1. It was observed (Figure 7) that Increasing P supply also increased the foliar chlorophyll content, grain N and P concentrations, and grain yield. The behaviour of the two cultivars was diverse for fresh mass, dry mass, and grain N and P concentration when treatments U+ATC and $Ca(NO_3)_2$ were considered under different rates of P application. Overall, cv. Faisalabad-2008 and cv. Lasani showed higher fresh and dry mass when treated with an indirect ammonium source of N, i.e. U+ATC compared to the nitrate source, i.e. $Ca(NO_3)_2$. At 1st harvest, cv. Faisalabad-2008 showed lowest fresh and dry mass (1.386 and 0.166 g plant-1), respectively, with zero-P application and fresh and dry mass (1.755 and 0.211 g plant^{-1}) increased significantly ($p<0.05$) at 45 mg P kg^{-1} application along with the ammonium source. An almost similar trend was observed with cv. Lasani. Both cultivars, treated with $Ca(NO_3)_2$, showed lower fresh and dry mass (1.357 and 0.156 g plant-1) as shown in Table 3, and lower grain N and P concentrations (Table 4) compared to the ammonium treatment. Maximum grain N and P concentrations were observed with 45 mg P kg^{-1} application along with ammonium as an N source.

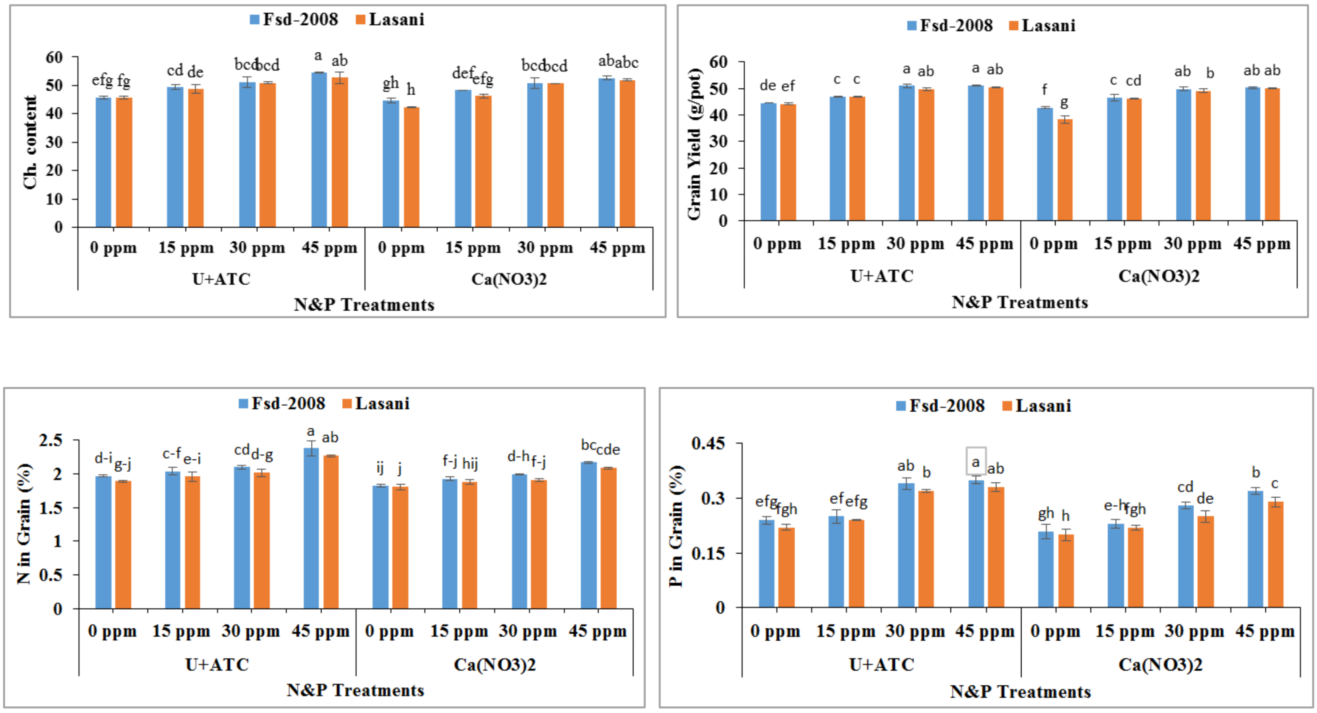

Figure 7. Impact of ammonium and nitrate nutrition on Chlorophyll content (unit value), grain yield and nutrient uptake of wheat under variable P application rates.

Mean values of treatments are represented as a set of bars, where the bars sharing the same letter(s) do not differ significantly at $P<0.05$. Vertical lines (I) on each bar denote standard errors. U+ATC = urea coated with ATC (4-Amino-1,2,4-triazole); Chl. Content = leaf chlorophyll content; N = nitrogen; P = phosphorus.

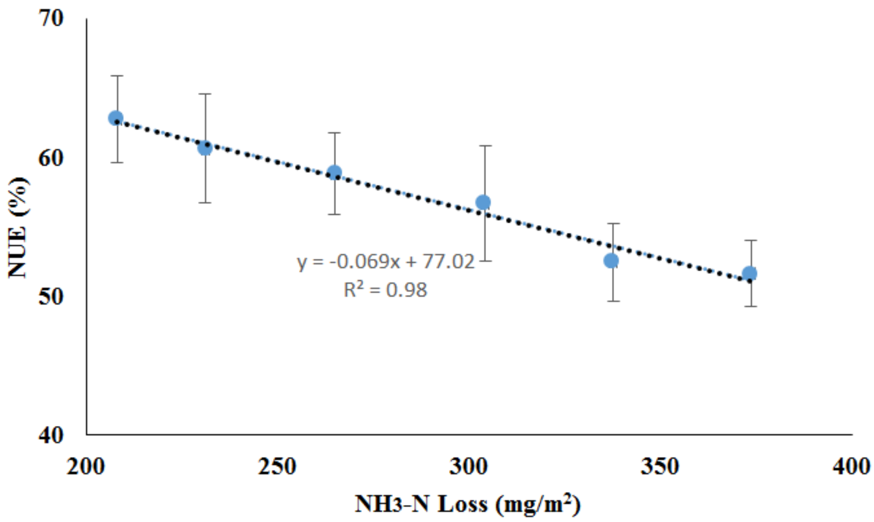

Figure 8. Correlation of N loss vs NUE (%) regardless of experimental treatments (each dot in the linear regression showing mean of 3-replicate and error bar).

Table 4. *Influence of ammonium and nitrate nutrition on N and P uptake at early growth (28 and 48 days after sowing) of wheat under variable P application rates.*

Variety	Treatment	P Application	N content	P content	N content	P content
	N&P content after 28 and 48 DAS					
		ppm		**%**		
Fsd-2008	U+ATC	0	4.48bcde	0.26bcd	3.79c	0.249hi
		15	4.74abcd	0.31abcd	3.85bc	0.27gh
		30	4.97ab	0.36abcd	3.99bc	0.35ab
		45	5.14a	0.416a	4.45a	0.37a
	$Ca(NO_3)_2$	0	4.25de	0.235cd	3.68c	0.207j
		15	4.56abcde	0.27bcd	3.74c	0.243hi
		30	4.78abcd	0.344abcd	3.88bc	0.287efg
		45	5.02ab	0.39ab	4.03bc	0.327bcd
Lasani	U+ATC	0	4.32cde	0.24cd	3.71c	0.237i
		15	4.68abcd	0.30abcd	3.75c	0.257ghi
		30	4.89abc	0.344abcd	3.92bc	0.303def
		45	5.09ab	0.401ab	4.16ab	0.336bc
	$Ca(NO_3)_2$	0	4.05e	0.233e	3.29d	0.207j
		15	4.54abcde	0.267bcd	3.71c	0.236i
		30	4.77abcd	0.33abcd	3.87bc	0.281fg
		45	4.98ab	0.38abc	3.96bc	0.31cde
	LSD (p<0.05%)		0.629	0.141	0.36	0.029

*Mean values in columns sharing the same letter(s) do not differ significantly at $P<0.05$. U+ATC = urea coated with ATC (4-Amino-1,2,4-triazole); N = Nitrogen; P= Phosphorus; DAS= days after sowing

4. Discussion

Reasons of low ammonia losses in alkaline soils amended with acidic phosphate

The cumulative ammonia losses from urea ranged from 4.8% to 12.5% and 3.3% to 8.1% with and without P application as phosphoric acid, respectively. Previously, it was observed that ammonia losses decreased in alkaline soils by H_3PO_4. Acidic P application reduced ammonia volatilization losses by 12–43% from commercial urea and this reduction was further increased by 29 to 50% with ZnU. Phosphate sources generally reduce ammonia losses, but H_3PO_4 has been found to be most effective in reducing ammonia losses from alkaline soil. Our results are in accordance with earlier reported results by scientists [2], who elucidated the mechanism of the reduction in ammonia losses and reported lower ammonia losses from urea applied with phosphoric acid, followed by single superphosphate and triple superphosphate when compared to urea alone. They highlighted that the addition of phosphate sources, particularly of phosphoric acid, significantly decreased soil pH, which shifts the pH-dependent chemical equilibrium of $NH_4^+ \rightleftharpoons NH_3$ towards the protonated form, reducing the concentration of free ammonia and therefore the rate of ammonia volatilization. Moreover, the decrease in soil pH, by providing acidic soil microenvironments, can subsequently decrease urea hydrolysis at lower soil pH [44].

This is based on strong alterations of the enzyme kinetics of urease enzymes with pH, i.e. substrate affinity (the inverse of Km values) decreases linearly with decreasing soil pH whereas maximum enzyme catalytic rates (Vmax) increase with rising soil pH, to reach a maximum at pH 6.0 and then decline at higher soil pH [44, 14, 45]. Greater concentrations and stability of ammonium over ammonia at lower soil pH and the formation of meta-stable products, such as ammonium calcium phosphate [$Ca(NH_4)_2(PO_4)_2$], may further promote the reduction in ammonia losses. It was also reported that addition of phosphate may supply or mobilize Calcium, which could lesser urea hydrolysis and decrease ammonia volatilization [46].

Highest ammonia losses were detected during initial days of fertilizer application, which then declined gradually with passage of time, irrespective of fertilizer amendments with or without P application. Similar findings were also reported previously [47], i.e. that maximum N losses from urea arisen during initial stages of application, and accredited these losses to strong ammonium accumulation due to rapid hydrolysis of urea and hence greater ammonia volatilization losses early after urea application. Previously it was also found maximum losses up to 7 days from non-saline soils and up to 10 days from salt-affected soils. Thereafter, ammonia losses decreased exponentially until the 4th week of incubation [48]. Actually, strong ammonium accumulation in soils due to rapid hydrolysis of urea induced ammonia emissions shortly after urea application and these ammonia losses reached the maximum in U+ATC applied to alkaline soil studied here. Similar results were reported previously [9]. Synthetic nitrification inhibitors (SNIs) normally inhibit the nitrification process and cause accumulation of ammonium on urea hydrolysis, thus increase ammonia volatilization from alkaline soils. In this experiment, U+NBPT and ZnU+NBPT showed lower losses as NBPT inhibitor delays the immediate hydrolysis of urea and thereby decreases the soil ammonium / ammonia concentration. Urea hydrolysis consumes protons (H^+) and increases soil pH, driving the equilibrium ($NH_4^+ \rightleftharpoons NH_3$) towards the gaseous form, ammonia [49].

The inhibitor NBPT is found to be more stable under alkaline conditions. In the present study ZnU caused negligible ammonia losses when compared to commercial urea, due to the down-regulation of the release of ammonium in soils with and without P application. However, zincated urea alone acted as slow-release fertilizer, while its further coating or amendment with SNIs enhanced its effectiveness in reducing ammonium formation and ammonia losses during the early days of incubation, because of the joint inhibitory effects of Zn coating and SNIs [12, 50]. Almost similar cumulative N losses were observed in both, commercial and chemo-amended urea after 14 days of incubation, but a significant reduction of ammonia losses was observed with compared to without P application. The laboratory experiment showed higher ammonia losses at higher N application rates, irrespective of the applied fertilizer treatments, as also observed previously [51].

As in the field experiment, in the laboratory experiment chemo-amended urea was applied at 80% of RR and consequently encountered lower N-losses and conferred higher benefits in terms of plant growth and N uptake. Almost similar trends of ammonia losses were observed in the field trial, where commercial urea as well as and zincated urea at RR exhibited higher ammonia losses when compared to chemo-amended fertilizer applied at 80% of RR. Minimum losses were examined with the second split of fertilizer application, the reason of lower ammonia volatilization losses being higher root accumulation of N by the actively growing crop at this stage.

Our results are in accordance with earlier reported results by scientists [52], who also elucidated the effect of various fertilization regimes on yield, NUE and N loss in long term experiments carried out at three rice-wheat rotation sites with the amendment of sole chemical N, N+P (NP), and N+P+K (NPK) fertilizer. Nitrogen loss/input ratios were ~60, ~40 or ~30% under N, NP or NPK, indicating significant decreases in N losses by P or P+K additions, as witnessed in the current study for both, the improvement of NUE and the reduction in N losses.

Significance of acidic phosphate on improving NUE in crop production

The appropriate management of fertilizer application is considered an important tool in enhancing the efficacy of the fertilizer applied at farm level. Phosphate addition along with urea, especially acidic phosphate, helps avoid ammonia volatilization losses, thereby increasing the plant availability of the applied N fertilizer. Previous studies also reported a great potential of P amendments to improve NUE in crop production [53]. In a previous study, annual NUE under N, NP or NPK treatments was 22, 36 or 48% for wheat, which is similar to our findings for N versus NP, whereas NUE was much higher in rice, i.e. 64% [54]. Compared with sole N fertilization in a long term experiment, P addition significantly improved NUE in both, rice and wheat crops. Long term P fertilization in Asia improved rice yield and NUE which may be attributed to decreased fertilizer N losses [55].

In West Africa, increasing P application was reported to increase NUE in maize crops and to decrease fertilizer N losses [56] and current findings as shown in Figure 7 also proved positive effect of lower N losses on NUE in wheat irrespective of fertilizer treatments. Similar to these current findings, earlier studies also elaborated on the significance of input management at the farm level to improve nutrient utilization for crop production, hence suggesting the essentiality of P co-application in sustainable agricultural systems. Compared to sole N fertilization, wheat grain yields increased significantly by NP amendment, representing the importance of P in order to improve crop productivity and fertilizer N use efficiency [52].

Our results are alike to those of earlier results by scientists [57], who also reported that P application enhanced grains spike^{-1}, spike length, spike weight, 1000-grain weight, grain yield, due to the promotion of the accumulation of photosynthates and accelerated grain ripening, which resulted in greater grain mass. Our results also highlight that a balanced ratio of N and P fertilizers is crucial to obtain high wheat yield, which is against the practice of common farmer's in the area, who do not bother about the balance of applied fertilizers at sowing [58].

Significance of inhibitors on N availability in alkaline soils

Overall, the results elucidate the significance of chemo-amended fertilizers at a rate of 80% of RR, showing their greater effectiveness compared to commercial and zincated urea applied at 100% RR regarding their effect on wheat biomass and grain quality. The 80% dose of N applied as chemo-amended fertilizer produced as much wheat biomass and grain as produced by the full dose (100% RR) of commercial and zincated urea. Similarly, yield and yield-related traits of wheat were also statistically non-significantly different between urea treatments differing in RR (100% RR without inhibitors versus 80% RR with inhibitors). This demonstrates that 80% of the recommended dose of N applied as chemo-amended fertilizer is sufficient to produce grain yields and qualities comparable to the recommended rate of N applied as commercial and zincated urea. However, both ZnU_{80}+NBPT & U_{80}+NBPT behaved similarly regarding their effects on growth and yield attributes of wheat, justifying the use of urease and/or nitrification inhibitors at lower fertilizer N application rates to obtain higher agronomic benefits and increased NUE. The here reported evaluations of chemo-amended fertilizer under laboratory and field conditions provided a sufficient data-set, which is required for achieving reliable information farm level application of these altered fertilization regimes. The better efficiency of the chemo-amended fertilizer at 80% of RR was been accredited to lower losses of ammonia compared to the full dose of commercial and zincated urea fertilizer, both at half and at the full dose of P fertilizer. In short, lower doses of chemo-amended fertilizer at 80% of RR are as much effective as the full dose of N applied as commercial and zincated fertilizer.

Role of N forms (ammonium versus nitrate) on wheat productivity

Much research has been devised to the effect of ammonium versus nitrate on plant nutrition. While some studies concluded that nitrate is usually superior to ammonium for plant and more specifically for crop growth, the effect N speciation may vary with plant species, the environment and with soil conditions such as soil pH and other factors [59]. Our results are in accordance with earlier [60], who also reported that ammonium outperformed than nitrate in terms of total yield and of yield components of wheat, which might thus have resulted in non-significant differences between both N forms for many wheat yield parameters. In our pot experiment, with either ammonium and nitrate sources, we observed that increasing the application of P significantly increased the fresh mass, dry mass, and grain N and P content of wheat across three growth periods, as previously reported [3]. The rate of P application also influenced (non-significantly) the relative growth rates during the vegetative stage of crop [61]. An almost similar trend was observed in the greenhouse experiment shown in Table 3 involving the ^{15}N tracer technique. The inhibitor treatment with ATC here caused as light, non-significant increase in %Ndff and a corresponding inverse response of %Ndfs, as previously reported [30]. However, maximum %Ndfs was observed in the treatment with ^{15}N labelled fertilizer without inhibitor, which might be due to active nitrification and nitrate accumulation in soils without ATC. The ammonium source performed better in the current study with increasing rates of P application than the nitrate source, eventually due to the competitive impact of anion uptake between phosphate and nitrate anions and the overall imbalance developing between cations and anions when switching between ammonium and nitrate nutrition. However, optimum NP supply in the growth medium caused an increase in grain yield, 1000-grain weight, and N and P uptake in wheat, as reported previously [62], though different cultivars behaved differently to the applied N sources and rates of fertilizer application, which depends on their morphological characteristics [59].

5. Conclusions

The collateral studies in laboratory, greenhouse as well as in field condition provides sufficient data-set mandatory in order to get reliable information on efficacy of chemo-amended urea vs commercial fertilizer. The studies explored possible ways to improve N availability (either as ammonium and nitrate) for wheat production in phosphorus (P) amended alkaline soils. The use of inhibitor coated urea even at lower rate (80% of RR) may produce statistically similar wheat grain yield and yield parameters to that observed in commercial fertilizers applied at recommended rate.

The coated fertilizers produced lower NH_3-N losses and hence proved more efficient over other treatments. Ammonium application favours more NP accumulation by plant than nitrate fertilization, probably due to ion-competition and differences in N assimilation processes within plant. Overall, this study clearly suggests that appropriate N management reduces ammonia losses and improves nutrient uptake and yield parameters of wheat grown on alkaline soil.

Abbreviations

N	Nitrogen
P	Phosphorus
NH_3	Ammonia
N_2O	Nitrous Oxide
NUE	Nitrogen Use Efficiency
P_2O_5	Phosphorus Pentoxide
NH_4	Ammonium
NO_3	Nitrate
OH^-	Hydroxyl Ion
ATP	Adenosine Tri Phosphate
GS	Glutamine Synthetase
GOGAT	Glutamate Synthase
RR	Recommended Rate
NIAB	Nuclear Institute for Agriculture & Biology
ECe	Electrical Conductivity of Extract
$CaCO_3$	Calcium Carbonate

NBPT	n-butyl Thiophosphoric Triamide
ATC	4- Amino-1,2,4-triazole
Zn	Zinc
ZnO	Zinc Oxide
ZnU	Zincated Urea
U	Urea
CRD	Complete Randomized Design
CRBD	Complete Randomized Block Design
PA	Phosphoric Acid
M	Molar
N	Normal
H_2SO_4	Sulphuric Acid
H_3PO_4	Phosphoric Acid
K	Potassium
K_2SO_4	Potassium Sulphate
$ZnSO_4$	Zinc Sulphate
KCl	Potassium Chloride
$Ca(NO_3)_2$	Calcium Nitrate
$(NH_4)_2SO_4$	Ammonium Sulphate
$Ca(NH_4)_2 SO_4$	Ammonium Calcium Sulphate
DAS	Days After Sowing
PVC	Polyvinyl Chloride
fdff	Fraction of Nutrient Derived from Fertilizer
Ndff	Nitrogen Derived from Fertilizer
Ndfs	Nitrogen Derived from Soil
FW	Fresh Weight
DW	Dry Weight
SFW	Shoot Fresh Weight
SDW	Shoot Dry Weight
RGR	Relative Growth Rate
Ln	Logarithm Natural
W	Weight
T	Time
SNIs	Synthetic Nitrification Inhibitors
ANOVA	Analysis of Variance
LSD	Least Significant Difference

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Conflicts of Interest

The authors declare no conflicts of interest.

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