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Effects of the nitrification inhibitor nitrapyrin and the plant growth regulator gibberellic acid on yield-scale nitrous oxide emission in maize fields under hot climatic conditions

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ABSTRACT

Nitrification inhibitors are widely used in agriculture to mitigate nitrous oxide (N_2O) emission and increase crop yield. However, no concrete information on their mitigation of N_2O emission is available under soil and environmental conditions as in Pakistan. A field experiment was established using a silt clay loam soil from Peshawar, Pakistan, to study the effect of urea applied in combination with a nitrification inhibitor, nitrapyrin (2-chloro-6-tri-chloromethyl pyridine), and/or a plant growth regulator, gibberellic acid (GA_3), on N_2O emission and the nitrogen (N) uptake efficiency of maize. The experimental design was a randomized complete block with five treatments in four replicates: control with no N (CK), urea (200 kg N ha^{-1}) alone, urea in combination with nitrapyrin (700 g ha^{-1}), urea in combination with GA_3 (60 g ha^{-1}), and urea in combination with nitrapyrin and GA_3. The N_2O emission, yield, N response efficiency, and total N uptake were measured during the experimental period. The treatment with urea and nitrapyrin reduced total N_2O emission by 39%–43% and decreased yield-scaled N_2O emission by 47%–52%, relative to the treatment with urea alone. The maize plant biomass, grain yield, and total N uptake increased significantly by 23%, 17%, and 15%, respectively, in the treatment with urea and nitrapyrin , relative to the treatment with urea alone, which was possibly due to N saving, lower N loss, and increased N uptake in the form of ammonium; they were further enhanced in the treatment with urea, nitrapyrin, and GA_3 by 27%, 36%, and 25%, respectively, probably because of the stimulating effect of GA_3 on plant growth and development and the reduction in biotic and abiotic stresses. These results suggest that applying urea in combination with nitrapyrin and GA_3 has the potential to mitigate N_2O emission, improve N response efficiency, and increase maize yield.

Key Words: fertilizer use efficiency, greenhouse gas emission mitigation, N response efficiency, N uptake efficiency, N_2O flux, plant growth hormone, urea

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INTRODUCTION

The global climate is changing rapidly, leading to food insecurity and increasingly extreme weather events. A major cause of these extreme weather events is the rising temperature of the atmosphere, driven by increasing emissions of greenhouse gases (GHGs), which absorb heat in the atmosphere. Sustainable intensification of cropping systems through conservation agriculture practices (Li *et al*., 2016) and strategic use of soil nutrients and water resources has been identified as a potential solution to increase food production with less environmental impact (Garnett, 2011). In Pakistan, impacts of an increase in the human population from 164 to 194 million over the past decade (SDGP-PBS, 2016), urbanization, and climate change on agro-ecosystems are putting increasing pressure on food production systems and on dwindling land and water resources. National arable cropping systems receive most of their nitrogen (N) input from the application of synthetic fertilizers, predominantly urea, farm yard manure, plant residues, and some municipal wastes (MINFAL, 2012).

Urea has been shown to have lower N use efficiency than other types of N fertilizers under many soil conditions, with only 5%–56% of the applied N absorbed by crop. Rather, it is lost to the atmosphere as ammonia (NH_3) (Dawar *et al*.,

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2011; Zaman *et al.*, 2013), nitrous oxide (N_2O) (Zaman *et al.*, 2008; Schlesinger, 2009; Zaman and Nguyen, 2012), nitric oxide (NO), and nitrogen gas (N_2) (Sanz-Cobena *et al.*, 2012; Saggar *et al.*, 2012). Unless urea is immediately incorporated into the topsoil mechanically or with the proper irrigation (Sanz-Cobena *et al.*, 2011; Zaman *et al.*, 2013), N loss of up to 50% can occur. This leads to both environmental and agronomic losses (Galloway *et al.*, 2008; Schlesinger, 2009; Zaman *et al.*, 2013).

New management practices and technologies therefore need to be developed in order to optimize N utilization and avoid N loss to the atmosphere, streams, and lakes. One of the most promising new methods of reducing N loss is applying urea coated with N process inhibitors (Zaman *et al.*, 2008). Urea applied together with nitrification inhibitor (NI) has been shown to be highly effective in reducing N fertilizer loss (Majumdar *et al.*, 2002; Zaman *et al.*, 2008; Cui *et al.*, 2010, 2011; Moir *et al.*, 2012) and increasing productivity under a range of cropping systems (Linquist *et al.*, 2013; Zhang *et al.*, 2015; Sun *et al.*, 2015) and pasture systems (Zaman *et al.*, 2009).

Many NIs, such as dicyandiamide (DCD) and 3,4-dimethylpyrazol-phosphate (DMPP), are commonly used in agricultural systems, especially pasture and arable cropping systems. The NIs such as nitrapyrin or N-serve (2-chloro-6-(tri-chloromethyl) pyridine) effectively reduce N_2O emission and leaching nitrate (NO_3^-) loss while also increasing yield and fertilizer use efficiency (Ma *et al.*, 2013; Zhang *et al.*, 2015; Sun *et al.*, 2015) at relatively low levels (approximately 0.24% the rate of urea N applied).

Similarly, fertilizer use efficiency could be further improved by co-application of urea and plant growth regulators (PGRs) (Aulak *et al.*, 1991; Sturm *et al.*, 1994; Bronson and Fillery, 1998; Díaz-Zorita *et al.*, 2001; Kurepin *et al.*, 2014; Zaman *et al.*, 2014). Nitrogen applied to the soil is not fully utilized by plant, and it is estimated that about half of the applied N often remains unavailable (Greenwood, 1982), which causes increased NO_3^- leaching losses and enrichment of water bodies (Carvalho and Basch, 1995). It is thought that gibberellic acid (GA_3) affects N metabolism and N redistribution in plant and improves fertilizer use efficiency by increasing the utilization of soil-derived N. There are also several other theories as to how GA_3 affects N metabolism. For example, GA_3 enhances plant growth, which then leads to greater utilization of soil N, and spraying fertilizer during the pre-flowering stages may help to redistribute photoassimilates towards seeds. These processes may enhance N utilization, leading to increased crop yield.

Developing best soil, nutrient, and water management practices is key to enhancing crop production as well as to mitigating N_2O emission. However; no published work is available on the effect of urea treated with NI and/or plant growth hormone (GA_3) on plant productivity and N_2O emission in an arable agricultural system under hot climatic conditions as in Pakistan. Therefore, the objectives of this study were to investigate the effect of applying urea together with NI alone or in combination with GA_3 on N_2O emission, crop productivity, and N response efficiency.

MATERIALS AND METHODS

Experimental site

A field experiment was established at the National Institute of Food and Agriculture near Peshawar, Pakistan (34°01 N, 71°71 E, 350 m above sea level) in June 2015. The soil was silt loam, with a low organic matter content (4.70 g C kg^{-1}), pH of 8, total N of 1.0 g N kg^{-1}, and electrical conductivity of 0.16 dS m^{-1}. The study area is classified as semiarid, with an average annual air temperature of 23 °C and average annual rainfall of 384 mm, most of which occurs in July and August. May and June are the hottest months, with mean temperatures of 39 and 41 °C, respectively. This arable site has been under an irrigated maize-wheat crop rotation system for more than 10 years.

Experimental design and management

The soil was cultivated with a mould board plough to a depth of 0.30 m, followed by two cultivations and planking. Seeds of the maize variety Pioneer 3025 were sown on June 24, 2015. During the maize growing season, six irrigation events were applied, with each event being equivalent to 75 mm, except the first (pre-planting) which was equivalent to 100 mm. The plot size was 5 m × 3 m, and each plot contained six rows, with each row being 5 m long, a distance of 75 cm between rows, and a distance of 20 cm between plants. In each plot, a 0.5-m^2 area was allocated to soil sampling in order to measure ammonium (NH_4^+) and nitrate (NO_3^-) contents.

Before sowing, basal doses of phosphorus (P) at 90 kg P_2O_5 ha^{-1} in the form of single superphosphate and potassium (K) at 60 kg K_2O ha^{-1} in the form of potassium sulphate were applied and incorporated into the soil. The experimental design was a randomized complete block, consisting of the following five treatments in four replicates: control with no N (CK), urea (200 kg N ha^{-1}) alone, urea (200 kg N ha^{-1}) in combination with nitrapyrin (700 g ha^{-1}), urea (200 kg N ha^{-1}) in combination with GA_3 (60 g ha^{-1}), and urea (200 kg N ha^{-1}) in combination with nitrapyrin (700 g ha^{-1}) and GA_3 (60 g ha^{-1}). Urea was applied in two split applications, one half during first irrigation (July 7, 2015) and the other half when the maize plants were at knee height (August 15, 2015). Nitrapyrin and GA_3 were applied at a rate of 0.35% and 0.03% of the applied N (weight/weight), respectively, and the mixtures were obtained by dissolving urea with nitrapyrin and GA_3 in water. For a uniform application and to reduce NH_3 volatilization in the applied urea, nitrapyrin and GA_3 were dissolved in water 30 min before application, and the solutions were surface applied by hand at 90 L per plot. All other practices such as hoeing, weeding, and insect control were carried out on all plots uniformly. Soil moisture (water-filled pore space (WFPS), %) and temperature (°C) were measured in the top 10 cm of soil by inserting appropriate probes; precipitation (mm) was measured using an on-site rain gauge.

Soil and plant analyses

Before starting experiment, four composite soil samples (0–10 cm depth) were taken using a soil core from the experimental site and passed through a 2-mm sieve. This was to analyse key soil properties including mineral N (Mulvaney, 1996), organic matter (Nelson and Sommers, 1982), and texture (Gee and Bauder, 1986). Total N in soil and plant samples were determined by the Kjeldahl method described in Bremner and Mulvaney (1982).

Crop harvesting, yield measurement and N response efficiency calculation

Mature maize plants were harvested on October 15, 2015, and various agronomic parameters including plant height, number of leaves per plant, number of grains per ear, 100-grain weight, biomass yield, grain yield, and stover yield were recorded in two sub-plots (2 m × 2 m). Plant biomass yield was separated into two components: grains and above-ground plant tissues (*i.e.*, shoot and leaves). Plant samples were washed with tap water and deionized water, after which they were dried at 65 °C for seven days.

Nitrogen response efficiency was calculated by subtracting the biomass yield of CK from those of individual fertilizer treatments and dividing the result by the amount of fertilizer N applied.

Measurements of N_2O emission

The N_2O fluxes were measured in each plot using the modified static chamber method of Saggar *et al.* (2004, 2007). After closing the chambers, three gas samples taken after 0, 30, and 60 min were taken from each chamber using a 50-mL polypropylene syringe. The temperature inside the chamber was recorded by placing a thermometer inside the chamber. Gas samples from syringes were transferred to pre-evacuated 20-mL vials (with gray molded PTFE/black butyl septum, Agilent Technologies, Santa Clara, USA), and were then analysed using an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped with an electron capture detector. The equipment was calibrated with certificated analytical grade standard N_2O, CH_4, and CO_2 and with N as a balance gas as well as pure nitrogen gas (ultra-high purity). The concentrations of the standard gases had an uncertainty of 5%. Concentrations were evaluated by a linear fit curve with four points from 0.3–3.0 μL L^{-1}. The third standard was prepared by diluting a specific volume of the more concentrated standard. The volume was measured with a gastight syringe which was injected into a vial filled with nitrogen gas at the atmospheric pressure. A vial filled with another standard gas with a concentration around 1 μL L^{-1} was placed in each group of 15 vials. These standards were analyzed as if they were samples, and deviations of the obtained concentration from the certified value must be less than 20% in order for the equipment to be utilized. If this requirement was not fulfilled, a new calibration curve was measured. On average, the actual deviations were found to be 8.5% ± 4.5%. In addition, a new set of standards for every concentration used in the first calibration curve and a blank filled with urea-ammonium phosphate were placed after every 48 h of continuous injection, as well as in the end of the injection.

Basal levels of N_2O emission were measured one day before N fertilizer application, and then N_2O measurements were made after every fertilizer application from July 7 to October 7, 2015. The accuracy of the gas chromatographic data at ambient concentrations was 1% or better. The N_2O concentrations within the chamber headspace have been reported to increase linearly ($R^2 > 0.90$) with time (Zaman *et al.*, 2009). The average rate of change in N_2O concentration was, therefore, determined using linear regression, and N_2O fluxes (F, mg m^{-2} h^{-1}) were calculated using the ideal gas law:

$$F = \rho \frac{V}{A} \frac{\Delta c}{\Delta t} \frac{273}{T + 273} \tag{1}$$

where ρ is the density of N_2O (mg m^{-3}), V is the volume of the chamber (m^3), A is the base area of the chamber (m^2), $\Delta c/\Delta t$ is the average rate of change of concentration with time (mL L^{-1} h^{-1}), and T is the temperature (°C) in the chamber.

The yield-scaled N_2O emission was calculated as the amount of N emitted as N_2O divided by the total N uptake by the aboveground biomass (Van Groenigen *et al.*, 2010).

Statistical Analyses

Analysis of variance (ANOVA) was calculated to compare fertilizer treatments with respect to various measured parameters. When significant effects of treatments were found, adjusted LSD values of Turkey's test were calculated to make comparisons among the different fertilizer treatments. Minitab (version 12) was used to perform statistical analyses.

RESULTS

Soil and weather variables

Soil temperature and moisture data at a depth of 10 cm, as well as precipitation during the experimental period, are shown in Fig. 1. The average air temperature was approximately 34 °C, and soil temperature ranged from 23 to 35 °C from June to September. Soil moisture content changed temporally with rainfall and irrigation events. The minimum amount of precipitation was 8 mm and occurred in June. In August, the precipitation peaked, averaging 68 mm.

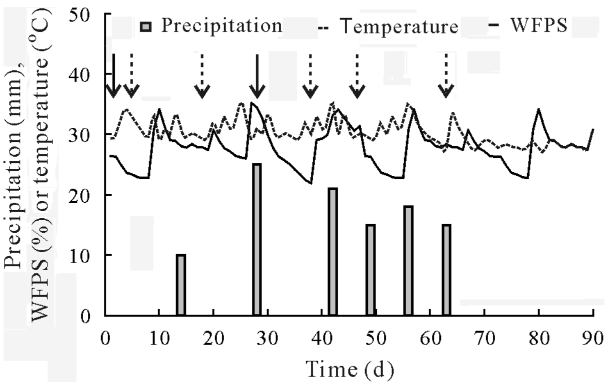

Fig. 1 Soil temperature (0–10 cm depth) and moisture (water-filled pore space (WFPS), 0–10 cm depth) and monthly average precipitation during the experimental period. The solid and dotted arrows indicate the timing of N fertilization and irrigation events, respectively.

Soil NH_4^+ content increased significantly ($P < 0.05$) after urea application (Fig. 2a). Urea applied alone or together with nitrapyrin produced significantly ($P < 0.05$) higher (7–9 mg N kg^{-1}) soil NH_4^+ content relative to CK on day 1. Soil NH_4^+ content in the treatment with urea alone reached its maximum on day 7, after which it decreased. Soil NH_4^+ content was significantly ($P < 0.05$) higher (60 mg N kg^{-1}) on day 14 in the treatments with nitrapyrin, compared to the treatment with urea alone. The first application of urea yielded a higher soil NH_4^+ content than the second application. Soil NO_3^- content was significantly ($P < 0.05$) lower (13 mg N kg^{-1}) in treatments with nitrapyrin, relative to treatment with urea alone (Fig. 2b).

N_2O emission

Nitrous oxide fluxes varied temporally throughout the maize growing season and were significantly ($P < 0.05$) higher in the treatments with urea, regardless of nitrapyrin application, relative to CK (Fig. 3). Three substantial N_2O flux peaks appeared in the treatment with urea alone on days 15, 35, and 49. The N_2O fluxes were always lower in the treatment with urea and nitrapyrin than in the treatment with urea alone, and these differences were significant. The application of urea in combination with GA_3 had no effect on N_2O flux.

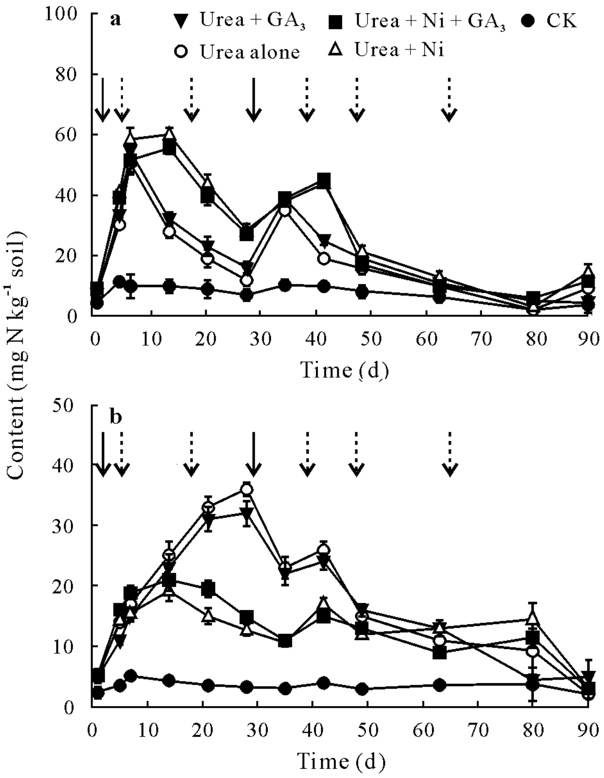

Fig. 2 Soil NH_4^+ (a) and NO_3^- (b) (0–10 cm depth) contents as affected by application of urea alone and in combination with nitrapyrin (Ni) and/or gibberellic acid (GA_3). Values are means with standard errors shown by vertical bars ($n = 4$). The solid and dotted arrows indicate the timing of N fertilization and irrigation events, respectively. CK = control with no N.

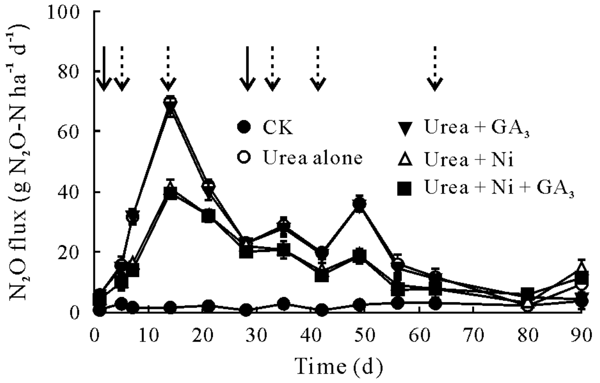

Fig. 3 Fluxes of N_2O as affected by application of urea alone and in combination with nitrapyrin (Ni) and/or gibberellic acid (GA_3). Values are means with standard errors shown by vertical bars ($n = 4$). The solid and dotted arrows indicate the timing of N fertilization and irrigation events, respectively. CK = control with no N.

The total N_2O emission was significantly ($P < 0.05$) higher (2.3 ± 0.41 kg N_2O-N ha^{-1}) from the treatment with urea alone than from the treatment with urea and nitrapyrin (1.4 ± 0.21 kg N_2O-N ha^{-1}) (Table I). The addition of urea in combination with nitrapyrin significantly ($P < 0.05$)

reduced cumulative N_2O emission, with a 39% reduction relative to the treatment with urea alone.

Yield-scaled N_2O emission

The yield-scaled N_2O emission, which was based on the cumulative N_2O emission and the aboveground N uptake (*i.e.*, uptake by grain and straw), ranged from 17.1 to 8.1 g N_2O-N kg^{-1} over the entire experimental period (Table I). Urea applied in combination with nitrapyrin significantly ($P <$ 0.05) decreased the yield-scaled N_2O emission by 47%–52% relative to urea alone across the entire experimental period.

Maize biomass and grain yields, N response efficiency, and N uptake

Maize biomass and grain yields, N response efficiency, and total N uptake all varied significantly ($P < 0.05$) when urea was applied in combination with nitrapyrin and also with GA_3 (Table II). Maize biomass and grain yields were significantly ($P < 0.05$) greater (23% and 17%) when urea was applied in combination with nitrapyrin relative to the treatment with urea alone. The yields increased further in the treatment with urea, nitrapyrin, and GA_3 in combination by 27% and 36%, respectively, compared to the treatment with urea alone. Total N uptake by maize was also significantly ($P < 0.05$) greater (14% or 25%) in the treatments with urea in combination with nitrapyrin or with GA_3 relative to the treatment with urea alone (Table II). Over the growing period, the aboveground maize biomass constituted 155 and 169 kg N ha^{-1} under the treatments with urea in combination with nitrapyrin and urea in combination with nitrapyrin and GA_3, respectively, compared to 135 kg N ha^{-1}under the treatment with urea alone. Similarly to total N uptake, N response efficiency was also significantly ($P < 0.05$) greater in the treatment with urea in combination with nitrapyrin and was further increased in the treatment with urea, nitrapyrin, and GA_3 in combination (Table II). The N response efficiency values were 9, 16, 24, and 28 kg kg^{-1} in the treatments with urea alone, urea in combination with GA_3, urea in combination with nitrapyrin, and urea in combination with nitrapyrin and GA_3, respectively. Plant height, number of leaves per plant, number of grains per ear, 100-grain weight, and leaf area were also influenced by the application of nitrapyrin and GA_3 (Table III). Urea applied in combination with nitrapyrin and/or GA_3 significantly enhanced growth, yield, and yield components in maize.

TABLE I

Total N_2O emission, percentage of N emitted as N_2O of the applied N, and yield-scaled N_2O emission as affected by application of urea alone and urea in combination with nitrapyrin (Ni) and/or gibberellic acid (GA_3)

Treatment	N_2O emission	Percentage of N emitted as N_2O of the applied N		Yield-scaled N_2O emission[a]	
		Amount	Change[b]	Amount	Change[b]
	kg N_2O-N ha^{-1}	%		g N_2O-N kg^{-1}	%
Control with no N	0.8 ± 0.12[c]c[d]				
Urea alone	2.3 ± 0.41b	1.1		17.1 ± 1.13c	
Urea + GA_3	2.2 ± 0.17b	1.1	0	15.0 ± 0.9c	−2
Urea + Ni	1.4 ± 0.21a	0.7	−39	9.1 ± 0.89b	−7
Urea + Ni + GA_3	1.3 ± 0.19a	0.6	−43	8.1 ± 0.81ba	−2

[a] Calculated as the amount of N emitted as N_2O divided by the total N uptake by the aboveground biomass.

[b] Relative to the treatment with urea alone.

[c] Means ± standard errors ($n = 4$).

[d] Means followed by the same letter within a column are not significantly different at $P < 0.05$.

TABLE II

Biomass and grain yields, total N uptake, and N response efficiency as affected by application of urea alone and in combination with nitrapyrin (Ni) and/or gibberellic acid (GA_3)

Treatment	Biomass yield		Grain yield		Total N Uptake		N response efficiency[a]	
	Amount	Change[b]	Amount	Change	Amount	Change	Amount	Change
	kg ha^{-1}	%	kg ha^{-1}	%	kg ha^{-1}	%	kg kg^{-1}	%
Control with no N	11 750 ± 231[c]c[d]		4 019 ± 53c		79 ± 13d			
Urea alone	13 521 ± 259bc		4 542 ± 51b		135 ± 11c		9 ± 2d	
Urea + GA_3	14 875 ± 238b	10	5 008 ± 77ab	10	146 ± 15b	08	16 ± 5c	20
Urea + Ni	16 667 ± 306a	23	5 332 ± 68ab	17	155 ± 19b	14	24 ± 4b	27
Urea + Ni + GA_3	17 205 ± 280a	27	6 219 ± 59a	36	169 ± 17a	25	28 ± 3a	38

[a] Calculated by subtracting the amount of the control from that of individual treatment and dividing the result by fertilizer N applied.

[b] Relative to the treatment with urea alone.

[c] Means ± standard errors ($n = 4$).

[d] Means followed by the same letter(s) within a column are not significantly different at $P <$0.05.

TABLE III

Yield components of maize as affected by application of urea alone and in combination with nitrapyrin (Ni) and/or gibberellic acid (GA_3)

Treatment	Plant height	Number of leaves per plant	Number of grains per ear	100-grain weight	Leaf area
	cm			g	cm^2
Control with no N	148.5 ± 9[a)]c[b)]	11.8 ± 0.8c	262.7 ± 15d	30.6 ± 1.8c	361.5 ± 17d
Urea alone	191.7 ± 13ab	12.6 ± 0.5bc	351.7 ± 19c	34.4 ± 2.7bc	404.7 ± 21c
Urea + GA_3	211.8 ± 11ab	12.9 ± 0.3b	373.3 ± 17b	36.9 ± 4.2ab	405.7 ± 15c
Urea + Ni	219.4 ± 16b	13.0 ± 0.3ab	389.1 ± 12ab	38.9 ± 3.4ab	431.2 ± 19b
Urea + Ni + GA_3	224.4 ± 19a	14.0 ± 0.6a	400.2 ± 14a	41.5 ± 4.2a	469.7 ± 18a

[a)] Means ± standard errors ($n = 4$).
[b)] Means followed by the same letter(s) within a column are not significantly different at $P < 0.05$.

DISCUSSION

During the experimental period, an impact of treatment type on N_2O emission trend was recorded (Fig. 2). Urea applied alone or in combination with nitrapyrin was rapidly hydrolysed immediately after application (Zaman *et al.*, 2008, 2013; Dawar *et al.*, 2011; Sanz-Cobena *et al.*, 2011), as exhibited by the significantly higher content of soil NH_4^- from urea, regardless of nitrapyrin application, during the first seven days (Fig. 2). From days 7 to 28, urea applied in combination with nitrapyrin created significantly ($P < 0.05$) more soil NH_4^+ than urea alone, which is evidence of the inhibitory effect of nitrapyrin on nitrification, as observed in previous studies using the same inhibitor (Li *et al.*, 2015; Sun *et al.*, 2015; Wang *et al.*, 2015; Zhang *et al.*, 2015). Retention of NH_4^+ reduces the risk of soil N being lost *via* NO_3^- leaching and N_2O emission (Gioacchini *et al.*, 2002; Macadam *et al.*, 2003; Chen *et al.*, 2010; Abalos *et al.*, 2012), but could increase the risk of NH_3 volatilization.

Across all treatments, the first prominent N_2O peak was observed on day 14, probably due to nitrification (Zaman and Nguyen, 2012), since nitrification produces N_2O as a byproduct (Inubushi *et al.* 1996) and the emission is directly related to the amount of NO_3^- in soil (Ding *et al.*, 2015). In contrast, the N_2O emission peaks on days 35 and 49 may be attributed to the combination of abundant NO_3^- (Fig. 2b) and high soil moisture from rainfall or irrigation events (Fig. 1); both are conducive to high denitrification rates (Tiedje, 1988; Bremner, 1997; Scholefield *et al.*, 1997; Delaune *et al.*, 1998). Lower NO_3^- and higher NH_4^+ contents in soil after the addition of nitrapyrin (Fig. 2a, b) thus likely reduced N_2O losses from both nitrification (Zaman and Nguyen, 2010) and denitrification (Firestone and Davidson, 1989).

Total N_2O emissions ranged from 0.80 to 2.3 kg N_2O-N ha^{-1} under CK and the treatment with urea alone. Applying urea in combination with nitrapyrin significantly reduced total N_2O losses by 39%–43% compared to the treatment with urea alone (Table. I). These results suggest that under nitrifying conditions such as those in the present study with WFPS ranging from 40% to 50%, nitrapyrin reduced nitrification, possibly by suppressing NH_3-oxidizing bacteria and other relevant soil enzymes, thus effectively reducing N_2O emission. This agrees with the findings of other studies (Ma *et al.*, 2013; Xiong *et al.*, 2013; Li *et al.*, 2015; Zhang *et al.*, 2015; Martins *et al.*, 2017) focusing on vegetable and arable fields, which have shown that nitrapyrin can significantly reduce N_2O emission (by 32%–49%).

The yield-scaled N_2O emission in the present study was within 5–15 g N_2O-N kg^{-1} estimated by others (Van Groenigen *et al.*, 2010; Cai *et al.*, 2013). In the present study, application of urea in combination with nitrapyrin significantly reduced yield-scaled N_2O emission by 47%–52% compared to application of urea alone (Table I). These results are in accordance with previous studies on yield-scaled N_2O emission in cropping systems (arable and vegetable) (Van Groenigen *et al.*, 2010; Wei *et al.*, 2010; Venterea *et al.*, 2011; Ma *et al.*, 2013; Bell *et al.*, 2015; Li *et al.*, 2015; Zhang *et al.*, 2015; Guardia *et al.*, 2017). Yield-scaled N_2O emission was significantly negatively correlated with N response efficiency, indicating that agronomic practices aiming to increase fertilizer N response efficiency can be directly linked to minimizing N_2O emission.

The application of urea in combination with nitrapyrin or GA_3 significantly increased yield, total N uptake, and N response efficiency compared to the treatment with urea alone. These results are in line with those of other studies (Liu *et al.*, 2013; Ma *et al.*, 2013; Zhang *et al.*, 2015). These increases are likely due to increases in mineral N in the form of NH_4^+ rather than NO_3^- for several days after urea and nitrapyrin application, thus increasing N uptake and crop yield (Aulakh *et al.*, 2001).

The observed improvements in maize yield caused by coating urea with NI could be due to N saving because of lower N loss. In less fertile soils with low organic matter, as in the present experimental field, N retained in soil is used by plants, which leads to increased crop yield. Additionally, increases in crop yield could be due to NH_4^+ retention by nitrapyrin and its subsequent uptake by maize, which leaves plants with additional energy from a nutritional view point. Ammonium retention in soil as a result of urea and nitrapyrin applied in combination not only provides environmental benefits by reducing N_2O emission and NO_3^- leaching (Gooding and Davies, 1992; Kettlewell and Juggins, 1992; Janzen *et al*., 1999), but also offers agronomic and economic benefits by increasing N response efficiency, especially in N-deficient soils.

Similarly, N response efficiency was further improved when GA_3 was applied. Pant growth regulators such as GA_3 are known to improve plant growth through stimulating both cell division and elongation, reducing biotic and abiotic stresses, and enhancing crop production and N uptake (Bose *et al*., 2013; Kurepin *et al*., 2014; Zaman *et al*., 2014). Thus, maize production was significantly increased in the treatments with GA_3.

CONCLUSIONS

Under the semiarid and hot climatic conditions of Pakistan, which are favourable to gaseous N loss from urea-based fertilizers, direct N_2O and also yield-scaled N_2O emissions were effectively reduced by the addition of nitrapyrin. On the other hand, urea applied in combination with nitrapyrin and/or GA_3 improved both biomass and grain yields of maize. The combination of nitrapyrin and/or GA_3 enhanced N response efficiency and N uptake compared to the use of urea alone. In conclusion, combining urea with nitrapyrin and/or GA_3 was likely to be a significant step toward mitigating N_2O emission from typical N fertilization practices in maize-producing areas in Pakistan, while improving N response efficiency as well as biomass and grain yields. Further long-term field research is, however, required under a wide range of soil and environmental conditions to evaluate the performance of GA_3 and to better understand the effect of nitrapyrin addition on N_2O emission and crop biomass production in Pakistan.

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