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THE EFFICIENCY OF NITROGEN FERTILIZER IN WHEAT AS INFLUENCED BY DIFFERENT NITRIFICATION INHIBITORS

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ABSTRACT

The nitrification inhibitors are very important in plant nutrition because they prevent the losses and volatilization of nitrogen fertilizers from the soil surface. In this study, the coated nitrogen fertilizers with Dicyandiamide and 4-amino-l,2,4-triazole inhibitors were used and the results from these coated fertilizers was compared with their respective control treatments (without coating). Two wheat varieties Ujala16 and NA1530 was evaluated. The field experiment was arranged in randomized complete block design under split plot arrangement with three replications. Nitrogen treatments which are urea, urea+4-amino-l,2,4-triazole (ATC) inhibitors and urea+ Dicyandiamide (DCD) inhibitor as sub factor and varieties (Ujala16 and NA1530) were maintained as main factor. A control without urea application and a positive control of urea application without coating were maintained for comparison. Data were collected for different agronomic and biochemical related parameters. The data collected was analyzed by Fisher's analysis of variance technique to determine the variability among the data and mean values of treatments were compared by using least significant difference (LSD) test at 5% probability level. The results showed that nitrification inhibitors had increasing impact on different physiological and biochemical parameters related N treatments including (chl a, chl b, carotenoids, and catalase activity) as well as positive impact on total soluble protein. NI also contributes positively by increasing different yield and yield contributing parameters including (number of tiller per plant, grain weight per spike, 1000 grain weight, plant height, grain yield and harvest index). There is also increasing impact related to varieties with the application of NI on different biochemical parameters including (chl b, chl a+b, carotenoids, proteins, Pod and catalase activity). Different yield and yield components also enhance positively in varieties which are, (number of grains/spike, total plant height, grain yield and biological yield). Hence NI play a positive role in increasing different physiological and biochemical parameters as well as yield and yield components and ultimately wheat yield also increase.

***Keywords*:** Wheat, Food Security, Zero Hunger

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1. INTRODUCTION

Agricultural sector plays a vital role in uplifting the economy of Pakistan. Almost 70% population is linked with agriculture sector, and it comprises about 42.3% of labor force of the country. Agriculture contributes about 18.9% in gross national product of Pakistan. Wheat (*Triticum aestivum* L.) has been one of Pakistan's leading grains as well as traditional food plants. Wheat flour has been used to make bread as well as bakery foodstuffs as well as its bran and straw as livestock feed. In Pakistan, wheat is planted on an area of 9 million ha with annual grain production of 26.3 million tons. The average yield of wheat in Pakistan is 2,893 kg ha^{-1} (Faheem et al. 2019). Pakistan is still far behind in annual wheat production from its neighboring countries i.e., Bangladesh (4357 kg ha^{-1}) and India (2,962 kg ha^{-1}). Different biotic (insect pest and weeds) and abiotic factors (varieties, sowing time, irrigation and fertilizers) contributed to the low yield of wheat crop in Pakistan. Certain crop management practices had a significant role in increasing the yield of different field crops including wheat (Faheem et al. 2019). Adequate and time provision of fertilizer nutrients play a major role in enhancing crop growth and productivity. Among these

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nutrients, nitrogen in the form nitrogenous fertilizers is of great economic importance. It not only improves plant growth and productivity but also plays a vital role in assuring grain quality. Nitrogen is also the most mobile plant nutrient present in the soil and its losses in the form of leaching and volatilization from soil have also been reported (Myrbeck 2014).

Nitrogen (N) is indeed an important macronutrient and seems to be largely inadequate in arable land. It is one of the significant supplements which diminish the yield of plant if not connected in appropriate sum as it is required for quick development of plants and to get high yield per hectare. It plays significant role in all the metabolic processes of the plants. Nitrogen is the principal part and real constituent of plants particularly in living tissues. All the bio-chemical procedures happening in plants are for the most part represented by nitrogen and its related compounds which make it fundamental for the development and advancement of plants (Erenoglu et al. 2011). The growth, development, productivity and quality of wheat grains have been largely affected in the field conditions due to inadequate supply of nitrogen (Ansar et al. 2010). It has been reported that certain biological and growth parameters of wheat crop were also affected significantly by the nitrogen application (Abedi et al. 2011).

Diminishing the loss of ammonia by various techniques of implementation to ground transmissions is a reality and is linked to the availability of a physiological obstacle in the form of a soil layer to capture released ammonia. Nitrification as well as denitrification, however, are the primary procedures of failure when manure is pushed to depth (Freney et al. 1993). The efficiency of different nitrogenous fertilizers in field conditions can be improved with the exogenous application of different nitrification inhibitors (NI). The primary role of these NIs is to slow down the activity of nitrifiers bacteria in soil which are actively involved in the conversion of ammonium to nitrate. The integrated application of different nitrification inhibitors with fertilizers not only decrease nitrogen losses in soil but also enhance nitrogen use efficiency and increased the synchronization between applied N fertilizers and crop demand (Ladha et al. 2005).

Although a lot of experiments done to check the efficacy of nitrification inhibitors but a comprehensive effect of different NI on morphological growth and productivity of wheat is not reported still. So, considering this research gap, the present study was conducted with the following objectives.

- To investigate the effect of nitrification inhibitors on morpho-physiological growth and biochemical attributes of wheat.
- To compare the efficacy of different nitrification inhibitors in enhancing grain yield and yield components of two wheat cultivars.

2. MATERIALS AND METHODS

2.1. Experimental Location

A plot trial was carried out to evaluate the performance of nitrification inhibitors at research area of Nuclear Institute of Agriculture and Biology during Rabi season of 2018 (31.26° N latitude, 73.09° E longitude).

2.2. Soil Analysis

Soil analysis (physico-chemical) was conducted prior to sowing of crop. The composite and representative soil samples from field were collected from where soil was taken to fill up pots from depths of 15 and 30 centimeters with soil auger and made composite samples of each plot. Analysis of sampled soil was conducted in Soil and Environmental Sciences Division Nuclear Institute of Agriculture and Biology and results are given in Table 1.

2.3. Planting Material

Two varieties Ujala16 and NA1530 was acquired from Nuclear Institute of Agriculture and Biology Faisalabad, Pakistan.

2.4. Experimental Design

This research trial was carried out in Randomized Complete Block Design (RCBD) with split plot arrangement with three repetitions. Plot size in this experiment was 2 m × 2 m. Four treatments allocated in sub plots with two varieties (Ujala16 and NA 1530) sown in main plots.

2.5. Treatments

Factor A: Varieties

- Ujala16
- NA1530

Factor B: Nitrification Inhibitors

- Control (no urea application)
- Urea
- Urea + 4-amino-l,2,4-triazole (ATC) inhibitor
- Urea + Dicyandiamide (DCD) inhibitor

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Table 1: Analysis of soil (physico-chemical) before sowing

Characteristics	Unit	Value obtained	Status
Texture	-	-	Sandy loam
Ph	-	8.22	Alkaline
Silt	%	32.76	-
Sand	%	33.14	-
Clay	%	31.26	-
EC	d Sm^{-1}	1.4	Normal
Organic matter	%	0.93	Low
Nitrogen (N)	%	0.059	Low
Phosphorus (P)	Ppm	8.8	Low
Potassium (K)	Ppm	170	Sufficient

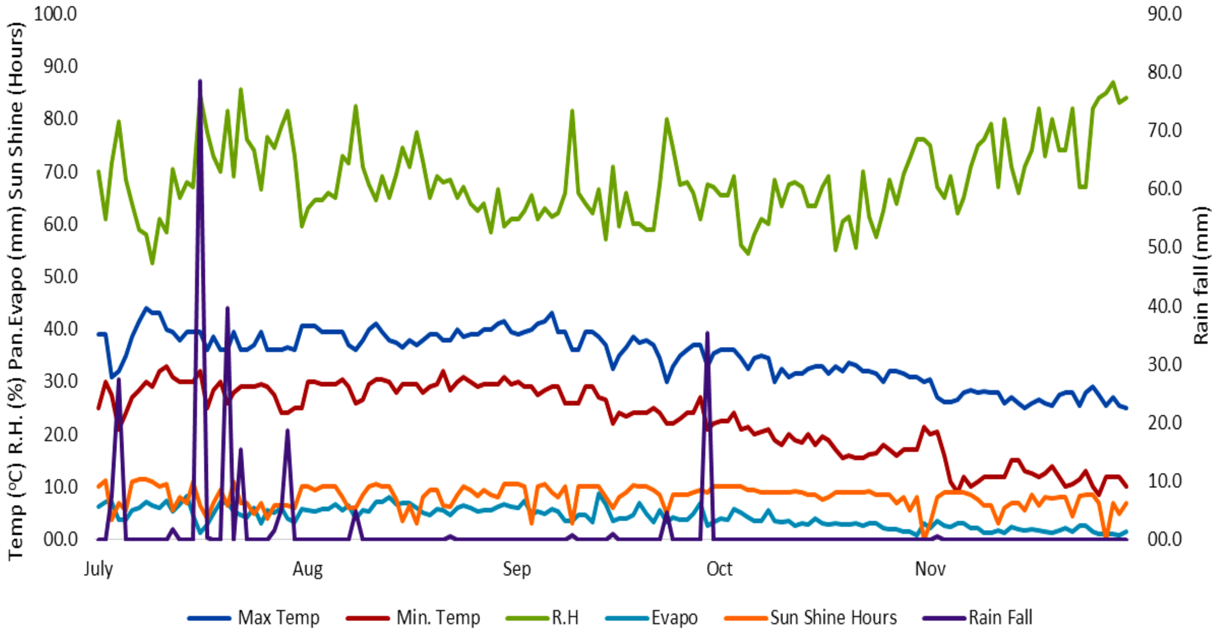

Fig.1: Weather data during crop growth season.

2.6. Agronomic Parameters:

Number of Total Tillers

5 random plants were selected from each plot after final harvesting, then average number of tillers of each plot was recorded to calculate number of total tillers.

Spike Length (cm)

Spikes of five random plants were selected from each plot then calculate the length of each spike of those plants. Calculate average spike length from each plot.

Plant Height (cm)

Five random plants were selected from each plot after final harvesting, then calculate the length of each plant. Calculate average plant length from each plot.

1000-Grain Weight (g)

The plant's production was counted then their weight was recorded with the help of an electric balance to get 1000-grain weight.

Harvest Index (%)

It was recorded for each pot by using the formula:

$$HI = \frac{\text{Economic yield (grain yield)}}{\text{Biological yield (grain + straw)}} \times 100$$

2.7. Total Soluble Sugar and Antioxidant Enzymes Measurement:

(a) Extraction:

Three tillers per plot were chosen randomly and their fully extended young leaves from the upper portion were taken and out of them 0.5g sample was crushed through pestle and mortar in the presence of chilled conditions under the pH of 7.8 with 1mL of phosphate buffer to get enzyme extract. Enzyme extract was centrifuged at 4°C for 10min. After centrifugation, enzyme determination was done from supernatant and the remaining residues were wasted and storing of extracted material at 4°C (Terras et al. 1993). Ninety-six well plates were used for pipetting of samples into it for measuring enzymatic antioxidants. At different wavelengths, microplate reader (ELX800, Bio-Tek Instruments, Inc., Winooski, VT, USA) was used for reading plates.

Total soluble proteins (mg g^{-1} FW):

Bradford reagent method was used to determine total soluble proteins. Five mL of Bradford reagent was mixed with 100 µL of sample enzyme extract and then at 595 nm wavelength absorbance was taken (Bradford 1976).

Catalase (unit mg^{-1} protein):

Determination of CAT was done with the method described by Liu et al. (2009) with some modifications. The reaction mixture (3mL) having 50 mM phosphate buffer (pH 7) and 5.9 mM H_2O_2 for 0.1mL enzyme extract was used. At 240 nm absorbance enzyme activity was determined.

Carotenoids (mg g^{-1} FW):

To determine the carotenoids contents, we used 0.5g grinded leaf samples to extract chlorophyll. The absorbance of filtrate was calculated at 663 and 480 nm. The carotenoids were calculated with the following formula.

Carotenoids = 4.16(A_{680})-[0.89(A_{663}) (V/1000 × W)]

In these formulae's' represent the volume of extract in ml. and 'W' represent weight of sample in g.

Chlorophyll contents (mg g^{-1} FW)"

With minor modifications in formerly described method (Arnon 1949), 0.5 g wheat leaves were crushed in 10 ml of 80% cold acetone and these tubes were placed in dark overnight at 20°C. Whatman No.1 paper was used for filtration of mixture. Through a spectrophotometer, a blank with acetone (80%) was run; the absorbance was taken at 645 and 663 nm. Using given formula chlorophyll contents was determined:

Chl a (mg per gram FW) = [12.70 (A 663) – 2.690 (A 645)] × V/1000 × W
Chl b (mg per gram FW) = [22.90 (A 645) – 4.680 (A 663)] × V/1000 × W
Chl (a + b) = [20.2 (A_{645}) +[8.02(A_{663}) (V/1000 × W)]
Where:
A = Absorbance
W = leaf sample weight
V = sample volume
These both W and V used in spectrophotometer (U-2001, Hitachi, Japan).

2.8. Statistical analysis

Fisher's analysis of variance technique (Johnson and Bhattacharyya 2019) was used to analyze recorded data statistically ($P \leq 0.05$) and Least Significant Difference (LSD) test was used to compare the means at 5% probability level. STATISTIX 8.1 software (Gomez and Gomez 1984) was employed for analysis of variance and comparison of treatments' means.

3. RESULTS

The analysis of variance (ANOVA) for studied traits in bread wheat indicated that the effects of varieties for plant height, Chlb, carotenoids, catalase, total soluble proteins, grain weight per spike, were significant. The treatment affects were significant for all studied traits whereas interaction of varieties with N treatments were significant for only plant height and chla (Table 2).

3.1. Chlorophyll 'a' (mg g^{-1} FW)

The effects of N treatments on Chlorophyll 'a' content was observed in terms of increased Chlorophyll 'a' content as compared to control (Table 3). The maximum four times increase in Chlorophyll 'a' content for wheat was observed for urea + DCD treatment 33.85 (mg g^{-1}) as compared to control 8.75 (mg g^{-1}) in (Ujala16). Similarly,

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Table 2: Mean square value regarding the effect of different nitrification inhibitors on agronomic and physiological traits of two wheat varieties

Source	DF	pH	Chla	Chlb	Car	Cat	TSP	T/P	SL	G/S	1000GW	HI
Block	2	66.36	0.63	2.26	0.089	15.36	0.0129	8.0083	1.08975	0.18442	0.9250	0.18601
Variety (V)	1	1235.21*	1.43	1346.60*	31.765**	103.68**	26.645**	5.2083	0.33075	0.1006*	0.0750	0.10800
Error I	2	348.26	5.73	7.94	0.233	4.01	0.037	7.5583	0.18525	0.1161	1.0750	0.11575
Treatment (T)	3	49.30*	1906.57**	5697.33**	187.022**	3801.17**	8.668*	52.6972*	2.7149*	2.4567*	25.3417*	2.4562*
V × T	3	54.70*	1.67	5.44	0.297	2.35	0.172	6.9417	0.91408	0.1130*	2.2083	0.11267
Error II	60	11.24	1.59	5.43	0.272	5.46	0.025	2.3772	0.22096	0.1064	0.7926	0.10849
Total	71											

**= Highly significant (P< 0.01); *= Significant (P< 0.01).

urea and urea + ATC application enhanced the Chlorophyll ‘a’ content approximately two 16.08 (mg g^{-1}) and three times 24.12 (mg g^{-1}) as compared to control. A similar pattern was observed for the (NA1530) as maximum increase in Chlorophyll ‘a’ was observed for combined application of urea and DCD 30.39 (mg g^{-1}) as compared to control 8.16 (mg g^{-1}) while the minimum two times increase in Chlorophyll ‘a’ was observed for urea application alone (Table 3). On the other hand, combined application of urea and ATC yielded three times 25.39 (mg g^{-1}) higher chlorophyll content as compared to control.

Taken together, it is evident that application of urea and its combined use with ATC and DCD had positive effect on Chlorophyll ‘a’ and all the treatments significantly enhanced the Chlorophyll A content while urea + DCD being the most effective one. However, there was no significant difference for varieties as depicted by mean values of 20.7 (mg g^{-1}) and 20.24 (mg g^{-1}) for (Ujala16, NA1530), respectively.

Table 3: Treatments’ comparison regarding the effect of different nitrification inhibitors on chlorophyll ‘a’ content (mg g^{-1}) of two wheat varieties

Treatments	Ujala16	NA1530	Mean
Control	8.75	8.16	8.46 D
Urea	16.08	16.50	16.29 C
Urea + ATC	24.12	25.39	24.74 B
Urea + DCD	33.85	30.89	32.37 A
Mean	20.7	20.24	

LSD value @5% probability level for treatments were (5.08). 4-amino-l,2,4-triazole (ATC) inhibitor. Dicyandiamide (DCD) inhibitor.

3.2. Chlorophyll ‘b’ (mg g^{-1} FW)

The effects of N treatments on chlorophyll ‘b’ content were observed in terms of increased Chlorophyll B content in wheat as compared to control (Table 4). Maximum 2.5 times increase in Chlorophyll ‘b’ content for wheat was observed for urea + DCD treatment (66.92) (mg g^{-1}) as compared to control 25.18(mg g^{-1}) in (Ujala16). Similarly, urea and urea + ATC application enhanced the Chlorophyll ‘b’ content approximately two times (43.74 and 57.24, respectively) compared to control. A similar pattern was observed for the (NA1530) as more than three times increase in Chlorophyll b was observed for combined application of urea and DCD 60.29 (mg g^{-1}) as compared to control (18.31) while approximately two times increase in Chlorophyll b was observed for urea application alone (Table 4). On the other hand, combined application of urea and ATC yielded 2.5 times 48.08 (mg g^{-1}) higher Chlorophyll b content as compared to control. Taken together, it is evident that application of urea and its combined use with ATC and DCD had a positive effect on Chlorophyll ‘b’ content in wheat and all the treatments significantly enhanced the Chlorophyll ‘b’ content while urea + DCD being the most effective one. Additionally, significant difference between both varieties was observed with mean values of 48.27 (mg g^{-1}) and 40.07 (mg g^{-1}) for (Ujala16, NA1530), respectively.

Table 4: Treatments’ comparison regarding the effect of different nitrification inhibitors on chlorophyll ‘b’ contents (mg g^{-1} FW) of two wheat varieties

Treatments	Ujala16	NA1530	Mean
Control	25.18	18.31	21.75D
Urea	43.74	33.58	38.66C
Urea + ATC	57.24	48.08	52.66B
Urea + DCD	66.92	60.29	63.61A
Mean	48.27A	40.07B	

LSD value @5% probability for treatments and varieties were (7.25, 4.34) respectively. Remaining footnote same as of Table 3.

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3.3. Total Carotenoids (mg g^{-1} FW)

The significant effects of N treatments on total carotenoids were observed in terms of increased total carotenoids in wheat as compared to control (Table 5). Maximum two times increase in total carotenoids for wheat was observed for urea + DCD treatment 12.70 (mg g^{-1}) as compared to control 5.85 (mg g^{-1}) in (Ujala16). Similarly, urea and urea + ATC application enhanced the Chlorophyll content approximately 50% and two times (7.7 and 10.84, respectively) compared to control. A similar pattern was observed for (NA1530) as almost three times increase in total carotenoids was observed for combined application of urea and DCD 10.15 (mg g^{-1}) as compared to control (3.88) (mg g^{-1}) while approximately 150% increase in total carotenoids was observed for urea application alone (Table 5). On the other hand, combined application of urea and ATC yielded 2.5 times 9.87 (mg g^{-1}) higher total carotenoids as compared to control 3.88 (mg g^{-1}). Application of urea and its combined use with ATC and DCD had a positive effect on total carotenoids in wheat and all the treatments significantly enhanced the total carotenoids while urea + DCD being the most effective one. Additionally, significant difference between both varieties was observed with mean values of 9.27 mg g^{-1} and 7.6 (mg g^{-1}) for (Ujala16, NA1530), respectively.

Table 5: Treatments' comparison regarding the effect of different nitrification inhibitors on total carotenoids (mg g^{-1} FW) of two wheat varieties

Treatments	Ujala16	NA1530	Mean
Control	5.85	3.88	4.87D
Urea	7.70	6.50	7.1C
Urea + ATC	10.84	9.87	10.36B
Urea + DCD	12.70	10.15	11.43A
Mean	9.27A	7.6B	

LSD value @5% probability for treatments and varieties were (1.5, 1.29) respectively. Remaining footnote same as of Table 3.

3.4. Catalase (unit mg^{-1} protein)

The effects of N treatments on catalase were observed in terms of increased catalase in wheat as compared to control (Table 6). Maximum increase in catalase for wheat was observed for urea + DCD treatment 67.40 (mg g^{-1}) as compared to control plants 35.60 (mg g^{-1}) in (Ujala16). Similarly, urea + ATC and urea application alone enhanced the catalase to 66.2 and 51.60, respectively) compared to control. A quite similar pattern was observed for (NA1530) as most significant increase in catalase was observed for urea + ATC application 64.8 (mg g^{-1}) as compared to control 33.20 (mg g^{-1}) while increase up to 64.00(mg g^{-1}) in catalase was observed for urea + DCD application (Table 6). Similarly, urea also increased the catalase to 47.2 (mg g^{-1}) as compared to 33.20 (mg g^{-1}) in control plants. Taken together, it is evident that application of urea and its combined use with ATC and DCD had a positive effect on catalase in and all the treatments significantly enhanced the catalase in both varieties while urea + DCD and urea + ATC being the most effective ones. Additionally, significant differences between both varieties were observed with mean values of 55.2 (mg g^{-1}) and 52.3 (mg g^{-1}) for (Ujala16 and NA1530), respectively.

Table 6: Treatments' comparison regarding the effect of different nitrification inhibitors on catalase activity (unit mg^{-1} protein) of two wheat varieties

Treatments	Ujala16	NA1530	Mean
Control	35.60	33.20	34.4C
Urea	51.60	47.20	49.25B
Urea + ATC	66.20	64.80	65.35A
Urea + DCD	67.40	64.00	65.7A
Mean	55.2A	52.3B	

LSD value @5% probability for treatments and varieties were (9.32, 2.13) respectively. Remaining footnote same as of Table 3.

3.5. Protein contents (mg g^{-1} FW)

Maximum two times increase in protein contents for wheat was observed for urea + DCD treatment 2.9 (mg g^{-1} FW) as compared to control 1.5 (mg g^{-1} FW) in (Ujala16). Similarly, urea and urea + ATC application enhanced the protein contents approximately and two times (2.17 and 2.8, respectively) compared to control. A similar pattern was observed for (NA1530) as almost 8 times increase in protein contents was observed for combined application of urea and DCD as compared to control 0.25 (mg g^{-1} FW) while approximately increase in protein contents was observed for urea application alone (Table 7). On the other hand, combined application of urea and ATC yielded six times 1.45 (mg g^{-1} FW) higher protein contents as compared to control 0.25 (mg g^{-1} FW).

Taken together, it is evident that application of urea and its combined use with ATC and DCD had a positive effect on protein contents in wheat and all the treatments significantly enhanced the protein contents while urea +

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DCD being the most effective one. Additionally, significant difference between both varieties was observed with mean values of 2.34 (mg g^{-1} FW) and 1.11 (mg g^{-1} FW) for (Ujala16, NA1530), respectively.

Table 7: Treatments' comparison regarding the effect of different nitrification inhibitors on protein contents (mg g^{-1} FW) of two wheat varieties

Treatments	Ujala16	NA1530	Mean
Control	1.50	0.25	0.87D
Urea	2.17	0.75	1.46C
Urea + ATC	2.80	1.45	2.13B
Urea + DCD	2.90	2	2.45A
Mean	2.34A	1.11B	

LSD value @5% probability for treatments were (0.04, 0.1), respectively. Remaining footnote same as of Table 3.

3.6. Number of tillers

Maximum increase in number of tillers for wheat was observed for urea + ATC treatment (10.33) as compared to control 6.33 in (Ujala16). Similarly, urea and urea + DED application enhanced the number of tillers to 8.66 and 9.33, respectively) compared to control. A similar pattern was observed for the (NA1530) as almost 1.5 times increase in number of tillers was observed for combined application of urea and DCD (10.66 tillers) as compared to control (7 tillers) while no significant increase in number of tillers was observed for urea application alone (7.33 tillers as compared to 7 in control plants) (Table 8). On the other hand, combined application of urea and ATC yielded 8.35 tillers as compared to seven tillers in control plants.

Taken together, it is evident that application of urea and its combined use with ATC and DCD had a positive effect on number of tillers in wheat and all the treatments slightly or significantly enhanced the number of tillers while urea + DCD and urea + ATC being the most effective ones. Additionally, non-significant difference between both varieties was observed with mean values of 8.66 and 8.34 for (Ujala16, NA1530), respectively.

Table 8: Treatments' comparison regarding the effect of different nitrification inhibitors on total number of tillers/ plant of two wheat varieties

Treatments	Ujala16	NA1530	Mean
Control	6.33	7.00	6.67C
Urea	8.66	7.33	7.80B
Urea + ATC	10.33	8.35	9.34A
Urea +DCD	9.33	10.66	9.99A
Mean	8.66	8.34	

LSD value @5% probability for treatments were (1.1). Remaining footnote same as of Table 3.

3.7. Spike length (cm)

Maximum increase in spike length for wheat was observed for urea + DCD treatment 5.13 (cm) as compared to control 4.36 (cm) in (Ujala16). Similarly, urea and urea + ATC application enhanced the spike length to 4.46 and 4.63, respectively) compared to control. A similar pattern was observed for the (NA1530) as maximum increase in spike length was observed for combined application of urea and ATC 4.96 (cm) as compared to control 4.16 (cm) while no significant increase in spike length was observed for urea application alone 4.26(cm) (Table 9). On the other hand, combined application of urea and DCD yielded 4.80 (cm) as compared to 4.16(cm) in control plants. Taken together, it is evident that application of urea and its combined use with ATC and DCD had a positive effect on spike length in wheat and all the treatments slightly enhanced the number of tillers while urea + DCD and urea + ATC being the most effective ones. Additionally, non-significant difference between both varieties was observed with mean values of 4.65(cm) and 4.53(cm) for (Ujala16, NA1530), respectively.

Table 9: Treatments' comparison regarding the effect of different nitrification inhibitors on total spike length (cm) of two wheat varieties

Treatments	Ujala16	NA1530	Mean
Control	4.36	4.16	4.26C
Urea	4.46	4.26	4.36C
Urea + ATC	4.63	4.96	4.79B
Urea + DCD	5.13	4.80	4.96A
Mean	4.65	4.53	

LSD value @5% probability for treatments were (0.4). Remaining footnote same as of Table 3.

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3.8. Grain weight/spike (g)

Maximum increase in grain weight/spike for wheat was observed for urea treatment 4.93 (g) as compared to control 3.53 (g) in (Ujala16). Similarly, urea + DCD and urea + ATC application enhanced the grain weight/spike to 4.03 and 4.00, respectively) compared to control. A quite similar pattern was observed for the (NA1530) as maximum increase in grain weight/spike was observed for combined application of urea and DCD 4.67 (g) as compared to control 3.53 (g) while no significant increase in grain weight/spike was observed for urea application alone (Table 10). On the other hand, combined application of urea and ATC yielded 4.13 (g) as compared to 3.53 (g) in control plants. Taken together, it is evident that application of urea and its combined use with ATC and DCD had a positive effect on grain weight/spike in wheat and all the treatments slightly or significantly enhanced the grain weight/spike while urea + DCD and urea + ATC being the most effective ones. Additionally, non-significant differences between both varieties were observed with mean values of 4.12 (g) and 4.07 (g) for (Ujala16, NA1530), respectively.

In short, a positive synergetic role of ATC and DCD along with urea for enhanced grain weight/spike was established and their combined use can help to increase the grain weight/spike and ultimately enhance the wheat grain yield.

Table 10: Treatments' comparison regarding the effect of different nitrification inhibitors on total grain weight (g)/ spike of two wheat varieties

Treatments	Ujala16	NA1530	Mean
Control	3.53	3.53	3.53C
Urea	4.93	3.93	4.43A
Urea +ATC	4.00	4.13	4.07B
Urea + DCD	4.03	4.67	4.35B
Mean	4.12	4.07	

LSD value @5% probability for treatments were (0.04). Remaining footnote same as of Table 3.

3.9. 1000 grain weight (g)

The effects of N treatments on thousand grain weight were observed in terms of increased thousand grain weight in wheat as compared to control (Table 11). Maximum increase in thousand grain weight for wheat was observed for urea + DCD treatment 10.33 (g) as compared to control 8.00 (g) in (Ujala16). Similarly, urea and urea + ATC application enhanced the thousand-grain weight to 8.33 and 9.33, respectively). A quite similar pattern was observed for (NA1530) as maximum increase in thousand grain weight was observed for combined application of urea with ATC and DCD separately (10) as compared to control 8.66 (g) while no significant increase in thousand grain weight was observed for urea application alone (Table 11). Taken together, it is evident that application of urea and its combined use with ATC and DCD had a positive effect on 1000 weight in wheat and all the treatments slightly or significantly enhanced the 1000 grain weight while urea + DCD and urea + ATC being the most effective ones. Additionally, non-significant differences between both varieties were observed with mean values of 8.99 (g) and 9.16 (g) for (Ujala16, NA1530), respectively. In short, a positive synergetic role of ATC and DCD along with urea for enhanced thousand grain weight was established and their combined use can help to increase the thousand-grain weight and ultimately enhance the wheat grain yield.

Table 11: Treatments' comparison regarding the effect of different nitrification inhibitors on 1000 grain weight (g) of two wheat varieties

Treatments	Ujala16	NA1530	Mean
Control	8.00	8.66	8.33C
Urea	8.33	8.00	8.17C
Urea + ATC	9.33	10.00	9.67B
Urea+ DCD	10.33	10.00	10.17A
Mean	8.99	9.16	

LSD value @5% probability for treatments were (0.5). Remaining footnote same as of Table 3.

3.10. Plant height (cm)

Maximum increase in plant length for wheat was observed for urea + ATC treatment (103.57) (cm) as compared to control (99.33) (cm) in (Ujala16). Similarly, urea + DCD and urea application enhanced the plant length to 102.67 and 102.33, respectively compared to control. A quite similar pattern was observed for the (NA1530) as maximum increase in plant length was observed for combined application of urea and ATC (98.33) (cm) as compared to control (97) (cm) while no significant increase in plant length was observed for urea application alone. On the other hand, combined application of urea and ADC yielded 95 cm as compared to 97 cm in control plants (Table 12). Taken together, it is evident that application of urea and its combined use with ATC and DCD had a positive effect on plant length in wheat and all the treatments slightly or significantly enhanced the plant length while urea + DCD and urea + ATC being the most effective ones.

Table 12: Treatments' comparison regarding the effect of different nitrification inhibitors on plant height (cm) of two wheat varieties

Treatments	Ujala16	NA1530	Mean
Control	99.33	97	98.17B
Urea	102.33	96.66	99.49B
Urea + ATC	103.57	98.33	100.95A
Urea + DCD	102.67	95	98.33B
Mean	101.98A	96.75B	

LSD value @5% probability for treatments, varieties and interaction between treatments and varieties were 0.3, 3.78, 1.1) respectively. Remaining footnote same as of Table 3.

3.11. Harvest index (%)

Maximum increase in harvest index for wheat was observed for urea + DCD treatment (1.43) as compared to control plants (0.90) in (Ujala16). Similarly, urea + ATC and urea application enhanced the harvest index to 1.40 and 1.20, respectively) compared to control. A quite similar pattern was observed for the (NA1530) as significant increase in harvest index was observed for combined application of urea and DCD (1.50) as compared to control (1.00) while non-significant increase (1.05) in harvest index was observed for urea application alone (Table 13). On the other hand, combined application of urea and ATC also increased the harvest index to 1.40 as compared to 1.00 in control plants. Taken together, it is evident that application of urea and its combined use with ATC and DCD had a positive effect on harvest index in and all the treatments slightly or significantly enhanced the harvest index in both varieties while urea + DCD and urea + ATC being the most effective ones.

Table 13: Treatments' comparison regarding the effect of different nitrification inhibitors on Harvest Index (%) of two wheat varieties

Treatments	Ujala16	NA1530	Mean
Control	0.90	1.00	0.95C
Urea	1.20	1.05	1.13B
Urea + ATC	1.40	1.40	1.4A
Urea + DCD	1.43	1.50	1.47A
Mean	1.23	1.24	

LSD value @5% probability for treatments were (0.07). Remaining footnote same as of Table 3.

4. DISCUSSION

For Chlorophyll 'a & b' content, effects of nitrification inhibitors were highly significant ($P<0.01$) and were observed in terms of increased Chlorophyll a content as compared to control. In this way, both the nitrification inhibitors (ATC and DCD) increased wheat photosynthetic performance. This can be linked to the ability of nitrification inhibitors to enhance the availability of soil inorganic N_2 and soluble organic carbon and conversion of nitrate to ammonium and increased N uptake by plants in wheat-maize crossing system. After the urea application, DCD has been reported to reduce N_2O emission up to 70% (Misselbrook et al. 2014) and N uptake by 13% (Liu Wang, & Zheng, 2013) thus helping to improve the N use efficiency. Sakamoto et al. (2006) found that early application of ATC along with urea increased chlorophyll content in wheat leaves, while late application of ATC decreased chlorophyll content. The authors suggested that the timing of application is an important factor in determining the effect of ATC on chlorophyll content (Sakamoto et al. 2006). The effects of nitrification inhibitors on catalase activities were observed in terms of increased catalase in wheat as compared to control. Maximum increase in catalase for wheat was observed for urea + DCD treatment as compared to control plants. Similarly, urea + ATC application enhanced the catalase compared to control. Ruser and Schulz (2015) reported that the application of urea with DCD, which is an inhibitor of nitrification, increased catalase activity in wheat leaves compared to urea alone. The inhibition of nitrification by DCD resulted in a lower level of oxidative stress in the plant, which may have contributed to the increased catalase activity (Ruser and Schulz 2015).

The significant effects of nitrification inhibitors on protein contents were observed in terms of increased protein contents in wheat as compared to control. Maximum 2.5 to 8 increases in protein contents for wheat was observed for urea + DCD treatment as compared to control. Similarly, urea and urea + ATC application enhanced the protein contents approximately 2 to 6 times compared to control. The application of urea with DCD increased total soluble protein content in wheat leaves compared to urea alone. The authors suggested that the reduction in oxidative stress

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due to the inhibition of nitrification by DCD may have contributed to the increase in total soluble protein content (Khan et al. 2013)

The significant effects of nitrification inhibitors on number of tillers were observed in terms of increased number of tillers in wheat as compared to control. An increase of 1.5 times in number of tillers for wheat was observed for urea + ATC treatment as compared to control. Similarly, urea and urea + DCD application enhanced the number of tillers significantly as compared to control. The NIs are reported to increase wheat protein content and grain yield thus validating our findings (Ruser and Schulz 2015). Similarly, the DCD application along with urea enhanced the wheat grain yield and biomass production by 8.5 and 8.6%, respectively (Liu et al. 2013) in wheat-maize cropping system. The effects of nitrification inhibitors on spike length were observed in terms of increased spike length in wheat as compared to control. Maximum increase in spike length for wheat was observed for urea + DCD treatment. Raza et al. (2019) reported that urea and urea + ATC application enhanced the spike length as well compared to control. The application of urea with DCD increased spike length in wheat compared to urea alone. The authors suggested that the reduction in nitrogen losses due to the inhibition of nitrification by DCD may have contributed to the increase in spike length (Raza et al. 2019). He et al. (2018) also found that the application of urea with DCD had no significant effect on spike length in wheat.

The effects of nitrification inhibitors on thousand grain weight were observed in terms of increased thousand grain weight in wheat as compared to control. Maximum increase in thousand grain weight for wheat was observed for urea + DCD treatment as compared to control. Similarly, urea and urea + ATC application enhanced the 1000 grain weight. The effects of nitrification inhibitors on plant length were observed in terms of increased plant length in wheat as compared to control. Maximum increase in plant length for wheat was observed for urea + ATC treatment as compared to control. Similarly, urea + DCD application enhanced the plant length compared to control. The effects of nitrification inhibitors on thousand grain weight were observed in terms of increased thousand grain weight in wheat as compared to control. Maximum increase in thousand grain weight for wheat was observed for urea + DCD treatment as compared to control. Similarly, urea and urea + ATC application enhanced the 1000 grain weight.

The effects of nitrification inhibitors on harvest index were observed in terms of increased harvest index in wheat as compared to control. Maximum increase in harvest index for wheat was observed for urea + DCD treatment as compared to control plants. Similarly, urea + ATC application enhanced the harvest index compared to control. He et al. (2018) found that the application of urea with DCD significantly increased grain yield in wheat compared to urea alone. The authors suggested that the reduction in nitrogen losses due to the inhibition of nitrification by DCD may have contributed to the increase in grain yield (He et al. 2018). According to Li et al. (2021), the application of urea with DCD significantly increased grain yield in wheat compared to urea alone, and also improved nitrogen use efficiency (NUE). The authors suggested that the use of DCD can be a sustainable way to increase grain yield and improve NUE in wheat (Dimkpa et al. 2020). Overall, the application of urea with DCD has consistently been found to increase grain yield in wheat, while the effect of urea with ATC on grain yield is more variable and may depend on the specific experimental conditions. However, further research is needed to optimize application rates and timing, and to assess the potential long-term impacts of using these inhibitors on soil health and ecosystem sustainability.

5. Conclusion

In conclusion, the use of inhibitors such as DCD and ATC in combination with urea fertilizer has been found to have a significant impact on wheat yield and physiology. The application of urea with DCD has consistently been found to increase grain yield and improve nitrogen use efficiency in wheat, while the effect of urea with ATC on grain yield is more variable and may depend on the specific experimental conditions. However, both of these inhibitors have been shown to have beneficial effects on wheat physiology, such as increasing the activity of antioxidant enzymes, improving photosynthetic efficiency, and increasing the accumulation of osmolytes. Overall, the use of these inhibitors can be a sustainable way to increase grain yield and improve nitrogen use efficiency in wheat, and can also help to mitigate the environmental impacts of excessive fertilizer use. However, further research is needed to optimize application rates and timing, and to assess the potential long-term impacts of using these inhibitors on soil health and ecosystem sustainability.

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