



1 **Improving Nitrogen Use Efficiency of Urea by using Urease and** 2 **Nitrification Inhibitors and Plant Growth Regulators** 3 **under Spinach Crop**

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5 **ABSTRACT:** An experiment was conducted to investigate the effect of applying different
6 rates of urea treated with nBT alone, or a combination of both nBTPT and D and
7 DM on the productivity of spinach crop at experimental area of Faculty of Agriculture,
8 Gomal University, Dera Ismail Khan during 2012-13. The experiment under glasshouse
9 conditions was laid out in Complete Randomized Design (CRD) with nine treatments and
10 four replications. Composite soil samples were collected before the experiment for various
11 physico-chemical analysis viz soil texture, soil pH, organic matter, bulk density, $\text{NH}_4\text{-N}$,
12 $\text{NO}_3\text{-N}$, extractable P and exchangeable K. The overall results revealed that N uptake in the
13 various forms of treated urea especially treatment (T_6) with dose of Urea@150 kg/ha + GA_3
14 @60 gram/ha enhanced the tested parameters significantly i.e tallest plant height (11.54cm),
15 maximum number of leaves (69), highest fresh weight (22.37g), highest dry weight (4.64g),
16 maximum biological yield (227g pot^{-1}) and highest net return (13.415 million) may be due to
17 availability of nitrogen for longer period and reduced loss on nitrogen by volatilization. The
18 results suggested that applying urea treated with Gibberellic acid (GA_3) or Agrotain
19 significantly gave profitable spinach growth due to better nitrogen use efficiency as compared
20 to urea alone.

21 **Key words:** Agrotain, Gibberellic acid, nBTPT, Spinach,

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INTRODUCTION

23 Spinach (*Spinacia oleracea*) is an annual member of Chenopodiaceae family and a valuable
24 vegetable which contains nutrients such as iron, calcium, phosphorus, sulfur, potassium etc
25 (Peivast, 2002). Urea is the predominant form of N fertilizer worldwide and also in Pakistan
26 (ca. 70%), principally because of its lower cost per unit of N, and the convenience for
27 transport, storage and spreading, butes which result from its higher N content (46% N), in
28 comparison to the 35, 26, 21, and 18% N of ammonium nitrate (AN), calcium ammonium
29 nitrate (CAN), ammonium sulphate (AS) and di-ammonium phosphate (DAP), respectively.
30 However, urea has been reported to have lower N response efficiency (NRE), where NRE is

defined as kg of additional dry matter (biomass and grain) produced per kg of applied N, when compared to other ammonium- and nitrate-based fertilizers. This is especially so if urea is applied under non-optimum soil moisture (very dry) and high temperature (above 20°C) conditions. The lower NRE of urea has always been attributed to the wide range of N losses (5% to 53% of the applied N) as ammonia (NH₃), when compared to AN, CAN, AS and DAP (Saggar *et al.*, 2012). Of course, some N losses, like gaseous emissions of NH₃, nitric oxide (NO), nitrous oxide (N₂O) and molecular N (N₂) and immobilization by microorganisms to organic N, will be unavoidable because they are part of the natural N cycle and would occur after application of any ammonium-based fertilizer or dairy farm effluents. Hence, controlling the rate of all of these N losses is critical for improving the response efficiency of all chemical fertilizers.

During the past decade, researchers have developed a variety of management practices and technologies to improve the NRE of urea. One such approach is to treat granular urea with the urease inhibitor N-(n-butyl) thiophosphoric triamide (nBTPT - trade-name Agrotain®). The nBTPT is typically applied at a range of 250 to 500 mg per kg of urea in order to delay urea's hydrolysis by 7 to 10 days (Zaman *et al.*, 2008). Several field and glasshouse studies have shown that treating urea with nBTPT (250 to 1,000 mg nBTPT per kg of urea-N) has the greatest potential to improve NRE in many cropping systems (Soares *et al.*, 2012). Similarly, urea can be treated with both nBTPT and nitrification inhibitors like dicyandiamide (DCD) or 3, 4-dimethylpyrazol-phosphate (DMPP) e gained commercial adoption in agricultural system.

Similarly, fertilizer use efficiency can be further improved if urea is applied with different additives like plant growth hormones or regulators (Sturm *et al.*, 1994). The word 'hormone' came from Greek language, where it means 'to stimulate' or 'to set in motion'.

Plant hormones/growth regulators (PGR) are a group of naturally occurring organic substances, which have the ability to influence physiological processes such as plant growth, differentiation and development, stress responses, cell division and reproductive activities when applied at a very low concentrations. Among the major classes of PGRs, auxins (generally represented by indole-3-acetic acid (IAA)), the gibberellins (GAs), and the cytokinins (CKs) have the most potential to be used as plant growth regulators.

However, limited information is available on the effect of urea treated with nBTPT alone versus a combination of nBTPT and DCD or DMPP, and PGRs on plant productivity in hot climate conditions in Pakistan. Therefore, the objectives of this study are to investigate the effect of applying different rates of urea treated with nBTPT alone, or a combination of both nBTPT and DCD and DMPP, on productivity of spinach. Owing to the importance of spinach, and the effect of soil mineral nutrient content on its growth, yield and quality, this experiment was conducted to evaluate the effect of manure and urea on yield, nutrient uptake and nitrate accumulation in spinach.

MATERIALS AND METHODS

An experimental study was undertaken to investigate the effect of applying different rates of urea treated with nBTPT alone, or a combination of both nBTPT and DCD and DMPP on productivity of spinach crop at experimental areas as the laboratory work of N use efficiency was done in Department of Soil and Environmental Sciences, Faculty of Agriculture, Gomal University, Dera Ismail Khan. The experiment was laid out in Complete Randomized Design with following nine treatments containing four replications;

Treatments: Description

- T1: No Nitrogen or GA₃ (Control)
- T2: Urea only @150 kg N/ha
- T3: Agrotain treated Urea @10 kg/ha

- 81 T4: Agrotain treated Urea + DCD@10 kg/ha
 82 T5: Agrotain treated urea @150 kg/ha + DMPP@2 kg/ha
 83 T6: Urea@150 kg/ha + GA₃ @60 gram/ha
 84 T7: Agrotain treated Urea + GA₃ @60 gram/ha
 85 T8: Agrotain treated urea@150 kg/ha + DCD @10 kg/ha + GA₃@60 gram/ha
 86 T9: Agrotain treated urea @150 kg/ha + DMPP@2 kg/ha + GA₃@60 gram/ha

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88 Composite soil samples were collected before the experiment for various physico-chemical
 89 analysis viz soil texture, soil pH, organic matter, bulk density, NH₄-N, NO₃-N, extractable P
 90 and exchangeable K. While in plant analysis, N uptake by the plant was determined other
 91 analysis like plant height, number of leaves and biological yield were also worked out. In the
 92 diagnostic and research laboratory, the chemicals were used such as DCD (Powder) 10 kg per
 93 hectare, DMPP (Powder) 2 kg ha⁻¹, 150 kg Nitrogen ha⁻¹, GA₃ 60 gram ha⁻¹ were used.
 94 Nitrogen uptake by the spinach plant was obtained. In terms of agronomic analysis plant
 95 height (cm), number of leaves per plant, fresh and dry weight (g) and biological yield (g pot⁻¹)
 96 In case of economic analysis, economic yield index as net return (Rs. in million) was
 97 calculated for its commercial cultivation extensively. Data recorded for tested parameters
 98 were statistically analyzed for Analysis of Variance (ANOVA) to find out the significance of
 99 the studied parameters. Means of the studied parameters were compared by least significant
 100 difference (LSD_{0.05}) using Advanced Statistix 10 computer program (Steel et al., 1997).

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RESULTS AND DISCUSSION

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Plant Height (cm)

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Perusal of data regarding Plant height of spinach as affected by applying different rates of urea treated with nBTPT alone, or a combination of both nBTPT and DCD and DMPP, on productivity of spinach are presented in Table I. The statistical analysis of data revealed that

plant height varied significantly ($P < 0.05$) among the treatments. The shortest plant height (5.88 cm) in spinach plants recorded from control treatment (T_1) where no fertilizer was applied, whereas tallest plant height (11.54 cm) was noticed from treatment (T_6) amended with Urea@150 kg/ha + GA_3 @60 gram/ha, followed by treatments (T_5 and T_8) respectively that differed significantly from all other treatments. The value of Least Significant Difference (LSD) as 2.5, indicated significant impact of Urea@150 kg/ha + GA_3 @60 gram/ha on the plant height of spinach in agro-climatic conditions of Dera Ismail Khan. Previous study conducted by Islam *et al.* (2011) pointed out that three types of organic manure alone produced moderate plant height and they were statistically similar with each other. Similar work undertaken by Tsai *et al.* (1997), who arrived at decision that Gibberellins helped in increasing internodal length, promote flowering, cellular division, and as detailed above, promote seed growth after germination.

The results revealed that N uptake in the form of Urea@150 kg/ha + GA_3 @60 gram/ha enhanced the plant height of spinach in agro-climatic conditions of Dera Ismail Khan as compared to urea alone and control. This may be due to availability of N for longer period and reduced loss on N by volatilization. Similar results findings were worked out by Zaman *et al.* (2009).

Number of Leaves per Spinach

Data regarding number of leaves per spinach affected by applying different rates of urea treated with nBTPT alone, or a combination of both nBTPT and DCD and DMPP, on productivity of spinach are presented in Table I. The statistical analysis of data revealed that number of leaves varied significantly ($P < 0.05$) among the treatments. The Minimum number of leaves (26) in spinach plants recorded from control treatment (T_1) where no fertilizer was applied, while, maximum number of leaves (69) were recorded in spinach plants treated with

Urea@150 kg/ha + GA₃ @60 gram/ha followed by treatments (T₇ and T₂) respectively that differed significantly from all other treatments. The value of Least Significant Difference (LSD) as 15, showed highly significant impact of Urea@150 kg/ha + GA₃ @60 gram/ha on the number of leaves of spinach in agro-climatic conditions of Dera Ismail Khan. The similar findings of Akhtar et al. (2008) revealed that interaction between concentration of GA₃ and different soaking times plants treated with ppm for 24 hours gave maximum number of leaves per plant

Table I indicated increased N uptake (69) in the form of Urea@150 kg/ha + GA₃ @60 gram/ha in hot climatic conditions of Dera Ismail Khan, as compared to urea alone and control. Results findings of Babakhanzade et al. (2012) revealed that maximum manure and urea application rates considerably enhanced yield of spinach; additionally the interaction of 300 kg urea/ha × 45 ton manure/ha resulted in enhanced yield by about 54% as compared to control.

Fresh Weight (g) of Spinach

Perusal of data regarding fresh weight have made an effort to determine significant role of tested treatments by applying different rates of urea treated with nBTPT alone, or a combination of both nBTPT and DCD and DMPP on the productivity of spinach are presented in Table 1. The statistical analysis of data revealed that fresh weight varied significantly (P<0.05) among the treatments. The lowest fresh weight (8.52g) in spinach plants recorded from control treatment (T₁) where no fertilizer was applied, while, highest fresh weight of 22.37 g was attained in spinach plants amended with Urea@150 kg/ha + GA₃ @60 gram/ha followed by treatments (T₇ and T₅) respectively that differed significantly from all other treatments. The value of Least Significant Difference (LSD) as 4.16, indicating highly significant impact of different combinations especially rates of Urea@150 kg/ha + GA₃ @60 gram/ha on the dry weight of spinach in agro-climatic conditions of Dera Ismail

Khan. Past study conducted by Wang et al. (2009) arrived at conclusion that any practice of maintaining a certain amount of $\text{NH}_4^+\text{-N}$ in soil condition is greatly useful to producing an enhanced fresh weight of spinach and optimized biological yield with high quality of spinach.

Similar studies by Min-na et al. (2012) concluded that the shoot fresh mass may be employed important index for the evaluation of NUE changes in spinach genotypes at the same N level due to high co-efficiency of variation recorded at low and high N levels in spinach production.

Dry Weight (g) of Spinach

Perusal of data recorded in Table 1 on dry weight of Spinach indicated that significant variation ($P<0.05$) existed among treatments as affected by applying different rates of urea treated with nBTPT alone, or a combination of both nBTPT and DCD and DMPP. The lowest dry weight (3.40g) in spinach plants recorded from control treatment (T_1) where no fertilizer was applied, whereas, significant highest dry weight of 4.64 g was achieved on the application of Urea@150 kg/ha + GA_3 @60 gram/ha followed by treatments (T_9 and T_5) respectively that differed significantly from each other. The value of Least Significant Difference (LSD) as 0.7, indicated low level significant impact of Urea@150 kg/ha + GA_3 @60 gram/ha on the dry weight of spinach in agro-climatic conditions of Dera Ismail Khan.

The findings of Wang et al. (2009) revealed a combination of N nutrition at a ratio of 25:75 in hydroponics cultivation of spinach would optimize the fresh and dry biomass yield and quality of spinach.

As far as increased N uptake is concerned, N use efficiency of urea by nitrification inhibitors as projected at treatment (T_6) amended with application of Urea@150 kg/ha + GA_3 @60 gram/ha, enhanced the growth of dry weight in spinach plants in hot climatic conditions of Dera Ismail Khan in comparison with urea alone. Bottom line in the experimental trial of

spinach conducted in study is that Urea@150 kg/ha + GA₃ @60 gram/ha considerably improved the dry weight in spinach plants over moderate rate of N or urea alone. GA₃ is

proved instrumental in promoting seed germination, elongation of stem, growth of flowering and fruit in various commercial plants. These materials have crucial role in improving nitrogen management of spinach plants. Similarly findings were worked out by Margreet et al. (2008) arrived at conclusion that net nitrate uptake rate (NNUR) efficiency considerably enhanced with increasing trend of RGR. Zaman *et al.*, (2008) also suggested reduced N losses via volatilization and enhanced pasture growth with combined application of urea and GA₃ as compared to urea alone.

Biological Yield (g pot⁻¹) of Spinach

Biological yield of spinach as affected by applying different rates of urea treated with nBTPT alone, or a combination of both nBTPT and DCD and DMPP, on productivity of spinach is reflected in Table I. The statistical analysis of data revealed that biological yield varied significantly ($P < 0.05$) among the treatments. The lowest biological yield (202 g plot⁻¹) in spinach plants recorded from control treatment (T₁) where no fertilizer was applied, whereas, highest biological yield of 277 g plot⁻¹ at treatment (T₆) was recorded in spinach plants amended with application of Urea@150 kg/ha + GA₃ @60 gram/ha followed by treatments (T₅ and T₃) respectively that differed significantly from all other treatments. The value of Least Significant Difference (LSD) worked out as 10.6, indicating overall significant impact of tested treatments especially highlighted dose of Urea@150 kg/ha + GA₃ @60 gram/ha on the biological yield of spinach grown in hot climatic environment of Dera Ismail Khan. Previous studies of Wang *et al.* (2009) were in association with present study which revealed a combination of N nutrition at a ratio of 25:75 in hydroponic cultivation of spinach would optimize the biomass yield and quality of spinach. Any practice of maintaining a certain amount of NH₄-N in soil condition is greatly effective in producing optimized yield with



high quality of spinach. Moreover, the past research conducted by Babakhanzade et al. (2012) pointed out highest application of manure and urea application rates resulted in increased spinach yield; furthermore, the interaction results (300 kg urea/ha × 45 ton manure/ha) also revealed enhanced yield by about 54% in comparison with control.

Economic Analysis of Spinach Yield

The computation of economic analysis of spinach production in Table 2 revealed that maximum Net Return (13.4 Million) was achieved by Treatment (T₆), followed by Treatments (T₅ and T₃) respectively. It has been suggested that treatment (T₆) with dose of Urea@150 kg/ha + GA₃ @60 gram/ha in terms of highest net return proved most economical and financially feasible treatments for profitable production of spinach in hot climatic conditions of Dera Ismail Khan. Economize nitrogen application indicated enhanced growth contributing factors of spinach and higher nutrient uptake, which may be attributed to its favourable effect on the environment. Additionally, such development holds a great promise to increase biological yield of spinach. These results are in accordance with the findings of Jeuffroy et al. (2002) who revealed that growers are faced with the task of producing crops that achieve maximum yield, while limiting production costs, in order to maximize profit, minimize the environment impact, and produce a crop of extremely high quality that is demanded by today's consumers. Similar research work undertaken by Sturm et al. (1994) mentioned that the cost of treating urea with the AGROTAIN® would be in the range of US\$ 66.00 to 68.00 per (metric) t of urea. The present studies were also on the analogy of Shah et al. (2009) worked out that Benefit Cost Ratio (BCR) either on total cost container⁻¹ bases (0.58: 1.00 and 0.76: 1.00) or on solution chemicals cost bases (1.25: 1.00 and 2.76: 1.00) were found significantly greater for Imai's recipe grown spinach crop.

Table 1. Plant Height (cm), Number of Leaves, Fresh Weight (g), Dry Weight (g) and Biological Yield (g pot⁻¹) of Spinach

Treatments	Plant Height (cm)	Number of Leaves per Spinach	Fresh Weight (g)	Dry Weight (g)	Biological Yield (g pot ⁻¹)
T ₁	5.88 d	25.5 d	8.52 d	3.40 c	202 d
T ₂	7.17 cd	62.75 ab	15.79 bc	4.30 ab	216 bc
T ₃	8.33 cd	53.50 bc	14.12 c	4.28 ab	223 ab
T ₄	7.79 cd	54.50 abc	14.32 c	3.90 bc	213 c
T ₅	10.99 ab	54.00 abc	17.63 bc	4.43 ab	225 abc
T ₆	11.54 a	68.75 a	22.37 a	4.64 a	227 a
T ₇	8.67 bc	65.50 ab	19.41 ab	4.29 ab	215 bc
T ₈	9.66 abc	36.25 c	16.59 bc	4.17 ab	216 bc
T ₉	9.53 abc	58.25 abc	16.57 bc	4.63 a	219 abc
LSD _{0.05}	2.5	15	4.16	0.7	10.6

Means followed by similar letter(s) do not differ significantly at P_{0.05}

Table 2. Economize Nitrogen Fertilizer in Spinach Production

Treatments	Value of Produce (Rs. in Million)	Cost of Production (Rs. in Million)			Net Return (Rs. in Million)
		Cost of produce	Cost of Chemicals	Total	
T ₁	12.120	0.012	-	0.012	12.108
T ₂	12.960	0.012	0.013	0.025	12.935
T ₃	13.380	0.012	0.050	0.062	13.318
T ₄	12.780	0.012	0.260	0.272	12.508
T ₅	13.500	0.012	0.079	0.091	13.409
T ₆	13.620	0.012	0.193	0.205	13.415
T ₇	12.900	0.012	0.255	0.267	12.633
T ₈	12.960	0.012	0.465	0.477	12.483
T ₉	12.140	0.012	0.259	0.271	11.869

CONCLUSION

The study arrived at conclusion that almost all tested parameters were found instrumental in improving the N use efficiency of urea by urease and nitrification inhibitors and plant growth regulators especially dose of Urea@150 kg/ha + GA₃ @60 gram/ha termed influential plant growth regulator for the sustained productivity of spinach in hot climatic conditions of Dera Ismail Khan.

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