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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet

Asian Network for Scientific Information

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 6 (6): 539-543, 2003
ISSN 1028-8880

Effect of Integrated Use of Mineral, Organic N and *Azotobacter* on the Yield, Yield Components and N-nutrition of Wheat (*Triticum aestivum,* L.)

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Abstract: The effect of mineral N, organic N and *Azotobacter* alone and in various combination was investigated on the yield and N nutrition of wheat in an experiment. The results revealed that mineral, organic fertilizers and *Azotobacter* applied alone and in various combinations significantly improved the yield, yield components and N uptake by wheat crop as compared to control. The effect of fertilizers treatments in improving the biomass yield of wheat was statistically higher ($P \leq 0.05$) as compared to mineral N or produced biomass yield statistically ($P \leq 0.05$) at par with mineral N applied at 60 mg kg^{-1} soil. Mineral N (30 mg N kg^{-1} soil+ *Azotobacter*) organic N (30 mg N kg^{-1} soil+ *Azotobacter*) and organic N (60 mg kg^{-1} soil) resulted in significantly (($P \leq 0.05$) lower biomass yield as compared with mineral N applied (60 mg kg^{-1} soil). *Azotobacter* of wheat alone also produced lower biomass yield as compared to mineral N applied at 60 mg kg^{-1} soil. Application of mineral N, organic N and *Azotobacter* alone and in various combinations either improved statistically ($P \leq 0.05$) or produced an equivalent N uptake response as compared to mineral N applied at 60 mg kg^{-1} soil. *Azotobacterization* alone did not produce any significant improvement in biomass N uptake as compared to mineral N applied at 60 mg kg^{-1} soil. At last 50% of mineral N can be saved as a result of judicious combination of mineral N, organic N and or biofertilization of wheat with *Azotobacter* while obtaining an equivalent or higher biomass yield as compared to mineral N applied at 60 mg kg^{-1} soil.

Key words: Mineral N, organic N, *Azotobacter*, wheat, yield, biofertilization

Introduction

Inorganic or mineral fertilizer N is the rich and readily available source of N as plant nutrient (Lampe, 2000) contributing to environmental hazards in a varied of way (Idris *et al.,* 2001). Organic manures and residues though bulky in nature generally comprise cellulose 15-60%, hemicellulose 10-30%, water soluble fraction (sugars, amino acids, aliphatic acids) 5-30%, ether and alcohol soluble fraction (fats, oils, waxes, resins pigments) and proteins containing N and S, mineral constituents (Alexander, 1961). Fertilizer N and organic N have their own merits and demerits and the drawbacks associated with either sources of these plant nutrients are often overcome when these are mixed in judicious combinations (Lampe, 2000). Mineral N is immobilized into organic from and the immobilized from then acts as a slow release fertilizer N (Alexander, 1961). Interaction occurs and the yield increases are sometimes more as a result of synergistic effect than those obtainable from the use of equivalent quantities of nutrient alone from either source (Lampe, 2000).

Azotobacter is a heterotrophic, aerobic micro-organism, fixing nitrogen as non-symbiont (Alexander, 1961) which is a good source of biofertilizer to improve the growth and yield of cereals and many other crops (Bhandari and Somani, 1990).

Integrated plant nutrient management in crop nutrition is the integrated use of inorganic, organic and or/biosources of plants nutrients in judicious combinations to improve the efficiency of applied nutrients and soil fertility for sustaining increased crop productivity while respecting for environmental ecology or ecosystem by reducing environmental hazards attributed to the continued use and heavy rates of mineral fertilizer.

Fertilizer product consumption in Pakistan has crossed 5.5 million tones and 2.8 million nutrients tones by the year 1999-2000 accounting for about 123 kg ha^{-1} of nutrients on cropped area (Anonymous, 2000). However, the use pattern at farm level is skewed in favour N with share of 78.3% compared to 21.0% of P and negligible use of K (Anonymous, 2000). These indicated that mineral N consumption for crop production in Pakistan occupies the major position. The integrated plant nutrient management of mineral N would be the most desirable practice in the crop husbandry of Pakistan. It is, therefore, imperative to judiciously integrate and manage organic and biosources of N with inorganic N fertilizer to prevent the mining depletion of soil and sustaining crop yields respecting the environment and ecology.

Integrated plant nutrition approaches have never been extensively practiced in Pakistan (Anonymous, 1998a). However, the status, and opportunities of integrated plant nutrient management in Pakistan has been reported by (Twyford *et al.*, 1995; Ahmed, 1998 and 2000).The recent survey conducted by Anonymous, (2000) indicate that major nutrients like nitrogen and phosphorus are the most commonly used fertilizers followed by farmyard manure. The use of green manures (2.0%), crop residues (3.2%) and biofertilizer (0.2%) is small. Ploughing of cotton sticks is major crop residues. Jantar and guar are main green crops. Biofertilizers containing *Rhizobium* and non-legume bacteria are in the introductory stage. However, about 50% farmers are using farmyard manure. The use of mineral fertilizers, organic manures and biofertilizers in judicious combinations has to be promoted, if integrated plant nutrition approaches are to be adopted on large scale in Pakistan.

The objective of the present investigation was to study the effect of inorganic N (urea), organic N (*Sesbania aculeate* L.) and *Azotobacter* applied alone and in various combinations on the yield and nitrogen of wheat so as to select the most appropriate combinations doses of these fertilizers in wheat production saving as much fertilizer N as possible.

Materials and Methods

The experiment was conducted at NIFA-Peshawar during wheat growing season 2000-2001 in pots each containing 20 kg soil. The soil (RAJAR-QUTBAL soil series) was clay loam in texture, calcareous in nature, which was alkaline in pH, free of excessive salts, low in organic matter, total N and Olsen P (Table 1). Organic N used in the experiment was ground shoots of *Sesbania aculeate* L. (3.0% N). *Sesbania* plants at two months growth stage were chopped in small pieces dried to a constant weight at 70^0C in oven and ground to pass through a 40 mesh screen. Mineral N used was commercial urea (46.0% N). Mineral N at 60 mg kg^{-1} soil, organic N at 60 mg kg^{-1} soil and *Azotobacter* as biofertilizer were applied alone and in various combinations including control receiving no fertilizers making 10 treatments.

Azotobacter chroococcum isolated from soil of NIFA (Idris and Iqbal, 1999) was used as biofertilizer in the experiment. The bacterium was grown in semi solid yeast extract medium (Cleyet-Marel, 1993) to a viable count of 10^5 ml^{-1} suspension of 30°C in vibratory shaker for inoculation purpose.

The fertilizer doses were applied to soil at the time of sowing. One ml of the bacterial cultural suspension was used to inoculate one seedling of wheat. Three healthy wheat plants (cv. Fakhar-e-Sarhad) were maintained in each pot and harvested at maturity. Yield components like plant height, number of tillers and spike $plant^{-1}$ number of days taken to hading and maturity were recorded through visual observation. *Azotobacter* population (N. g^{-1} soil) in rhizospheric soil was performed from one replication according to Clark (1965) using N free sodium benzoate mannitol agar medium (Idris, 1973). serial dilutions upto 10^8 in 6 replications of each soil samples were made and mean number of colonies $plate^{-1}$ from infected plates were calculated to enumerate *Azotobacter* g^{-1} of soil on dry weight basis. Standard deviation was calculated according to usual procedure.

Table 1: Physico-chemical analysis of soil

A) Mechanical analysis		
i)	Sand %	24
ii)	Silt %	40
iii)	Clay %	36
Texture		Clay loam
B) Chemical analysis		
i)	pH =	8.1 (1:2.5)
ii)	EC (dSm^{-1}) =	1.8 (1:2.5)
iii)	Nitrogen (%) =	0.04
iv)	Olsen available P (mg Kg^{-1} soil)	4.0
v)	NH_4OAC extractable K (as K_2O) mg kg^{-1} soil	153.0
vi)	Organic matter (%)	0.8
vii)	$CaCO_3$ (%)	15.0

Soil and plant samples were analyzed either according to Jackson (1965) or AOAC (1984). The statistical analysis (ANOVA and DMRT) of the data were performed according to Steel and Torrie (1980) using MSTAT software package.

Results

The results (Table 2) revealed that except for number of days taken to heading and maturity, application of mineral N at 60 mg kg^{-1} soil, organic N at 60 mg kg^{-1} soil and *Azotobacterization* alone and in various combination (T_2 to T_{10}) significantly ($P \leq 0.05$) improved the yield components such as plant height, number of tillers and spike $plant^{-1}$ as compared to control receiving no fertilizer (T_1). The effect of mineral N at 30 mg kg^{-1} soil + organic N at 30 kg^{-1} soil + *Azotobacter* (T_9) and mineral N at 60 mg kg^{-1} soil + *Azotobacter* (T_{10}) significantly ($P \leq 0.05$) improved the plant height than the rest of the fertilizer doses (T_2 to T_7). In case of number of tillers and spike $plant^{-1}$ the effect of these fertilizer applied alone and in combinations was non significant.

Table 3 revealed that application of mineral N at 60 mg kg^{-1} soil, organic N at 60 mg kg^{-1} soil and *Azotobacter* applied alone and in various combination (T_4 to T_{10}) significantly ($P \leq 0.05$) out yielded the grain yield by 13.0 to 53.0% straw yield by 6.0 to 52.0% and biomass yield by 9.0 to 56.0% as compared to control (treatment receiving no fertilizer) T_1. The effect of fertilizer treatment T_8, T_9, T_{10} in improving the grain, straw and biomass yield was statistically ($P \leq 0.05$) the grain, straw and biomass yield of wheat as compared to fertilizer treatment T_2.

Table 2: Effect of integrated use of mineral N, organic N and *Azotobacterization* on the yield components of wheat

	Yield components				
	Plant height	No. of tiller	No. of spike	No. Of days taken to	
Treatments	(cm)	plant^{-1}	plant^{-1}	Heading	Maturity
T_1	65.0e	9.0d	8.0e	127.0a	155.0a
T_2	78.0bc	14.3ab	12.0c	127.0a	154.0a
T_3	76.0cd	12.0c	11.0cd	127.0a	13.0a
T_4	74.0d	13.0bc	10.1d	128.0a	154.0a
T_5	78.0bc	15.0a	12.1bcd	127.0a	155.0a
T_6	79.0b	14.0abc	13.3bc	128.0a	154.0a
T_7	78.0bc	14.0abc	14.0b	127.0a	155.0a
T_8	85.0a	15.0a	15.0ab	127.0a	154.0a
T_9	85.0a	15.0a	15.0ab	127.0a	155.0a
T_{10}	85.0a	15.0a	15.0ab	127.0a	154.0a

Figures within parentheses indicate % increase over control

Table 3: Effect of integrated use of mineral. N, Organic N and *Azotobacterization* on yield of wheat

	Yield (g kg^{-1} soil)					
Treatments	Grain		Shoot		Biomass	
T_1	1.5f	(-)	3.1e	(-)	4.5e	(-)
T_2	2.2bc	(47.0)	4.2bc	(35.0)	6.4b	(42.0)
T_3	2.0c	(33.0)	4.2bc	(35.0)	6.1c	(36.0)
T_4	1.7e	(13)	3.3d	(6.0)	4.9d	(9.0)
T_5	2.1bc	(4.0)	4.4b	(42.0)	6.4b	(42.0)
T_6	1.9d	(270)	4.1c	(32.0)	6.0c	(33.0)
T_7	1.8d	(20.0)	4.4b	(42.0)	6.1c	(33.0)
T_8	2.3a	(53.0)	4.7a	(52.0)	7.0a	(36.0)
T_9	2.3a	(53.0)	4.6a	(48.0)	7.0a	(56.0)
T_{10}	2.2a	(53.0)	4.7a	(52.0)	7.0a	(56.0)

Foot notes are generally the same as given in Table 2.
Figures within parentheses indicate % increase over contrl\ol.

Table 4: The effect of integrated use of mineral. N, Organic N and Azotobacterization on N concentration and uptake by wheat

	N concentration (%) and uptake (mg kg^{-1} soil)					
Treatments	Grain		Shoot		Biomass	
T_1	35.0d	(-)	40.0d	(-)	80.0d	(-)
T_2	50.0ab	(43.0)	60.0b	(50.0)	115.0b	(44.0)
T_3	45.0b	(29.0)	55.0b	(38.0)	110.0b	(38.0)
T_4	40.0c	(14.0)	47.0c	(18.0)	90.0c	(13.0)
T_5	50.0ab	(43.0)	55.0b	(38.0)	115.0b	(44.0)
T_6	50.0ab	(43.0)	55.0b	(38.0)	105.0b	(31.0)
T_7	45.0b	(29.0)	60.0b	(50.0)	110.0b	(38.0)
T_8	55.0a	(57.0)	65.0a	(63.0)	125.0a	(56.0)
T_9	55.0a	(57.0)	65.0a	(63.0)	125.0a	(56.0)
T_{10}	55.0a	(57.0)	65.0a	(63.0)	125.0a	(56.0)

Foot notes are generally the same as given in Table 2.
Figures within parentheses indicate % increase over contrl\ol.

Table 5: The effect of integrated use of mineral. N, Organic N and Azotobacterization on the Azotobacter population (No. g^{-1} dry soil) in wheat rhizospheric soil

Treatments	Azotobacter population (g^{-1} dry soil)
T_1	$3 \times 10^3 \pm 4.25$
T_2	$4 \times 10^3 \pm 8.45$
T_3	$4 \times 10^4 \pm 6.4$
T_4	$3 \times 10^4 \pm 5.4$
T_5	$4 \times 10^4 \pm 3.8$
T_6	$5 \times 10^4 \pm 4.5$
T_7	$6 \times 10^5 \pm 3.5$
T_8	$5 \times 10^5 \pm 8.5$
T_9	$6 \times 10^5 \pm 4.5$
T_{10}	$10^3 \times 10^5 \pm 3.5$

Counts are made through plate dilution method (Clark, 1965).
Values followed by ± indicate standard deviation

Table 4 revealed that application of mineral N, organic N and Biofertilization of wheat with *Azotobacter* alone and in combination (T_4 to T_{10}) increased significantly ($P \leq 0.05$) the grain N uptake by 14.0 to 57.0% straw N uptake bu 18.0 to 63.0% and biomass N uptake by 13.0 to 56.0%.

The effect of fertilizer treatments T8, T9, T10 in improving the biomass N uptake was statistically ($P \leq 0.05$) was more promising than the rest of fertilizer treatments (T_2, T_7). T_4 (*Azotobacterization* of wheat alone) significantly ($P \leq 0.05$) decreased the biomass N uptake than the rest of the fertilizer treatments. Mineral N, organic N and *Azotobacter* applied in various combination (T_3, T_5, T_6 and T_7) produced biomass yield at per with T_2.

The studies reveal that application of mineral N at 60 mg kg^{-1} soil, organic N at 60 mg kg^{-1} soil and *Azotobacterization* alone and in various combinations significantly ($P \leq 0.05$) improved the yield and N uptake by wheat. The integrated use of mineral N, organic N and biofertilizer N in various proportions (mineral N at 30 mg kg^{-1} soil + organic N at 30 mg kg^{-1} soil + Azotobacter; mineral N at 45 mg kg^{-1} soil + organic N at 15 mg kg^{-1} soil + Azotobacter; mineral N at 30 mg kg^{-1} soil + organic N at 30 mg kg^{-1} + Azotobacter) were the most appropriate doses in this experiment, which gave an equivalent or improved yield N uptake responses by wheat and caused a substantial reduction in the use of mineral N in wheat production. However, mineral N at 60 mg kg^{-1} soil + Azotobacter cannot be recommended as a good dose as this combination could not save mineral N.

Table 5 revealed that fertilizer treatments receiving organic N alone or in combination with mineral N (T_2, T_5, T_7) significantly ($P \leq 0.05$) improved the *Azotobacter* population (No. g^{-1} dry soil) in the wheat rhizospheric soil as compared to control receiving no fertilizer (T_1). Fertilizer treatments receiving Azotobacter alone (T4) or in combination with mineral N and organic N (T_4, T_7, T_8, T_9, T_{10}) further improved significantly ($P \leq 0.05$) the

Azotobacter population (No. g^{-1} dry soil) in the rhizospheric soil of wheat than the rest of the fertilizer treatments.

The results revealed that mineral N at 30 mg kg^{-1} soil + organic N at mg kg^{-1} soil + Azotobacter and mineral N at 30 mg kg^{-1} soil + organic N at 30 mg kg^{-1} soil were the doses of fertilizers in this experiment saving 50% mineral N while producing higher or equivalent biomass yield as compared to mineral N applied at 60 mg N kg^{-1} soil.

The beneficial effect of integrated plant nutrition system (IPNS) involving of different sources of organic and inorganic N to improve the crop yield has been reported (Anonymous, 1998b). More recently the status, opportunities and beneficial effects of integrated plant nutrition management in Pakistan has been described in Pakistan. The results of various workers have, however, revealed the beneficial effects of integrated plant nutrition system to improve the yield of various crops (Bhatti *et al*., 1985; Rashid *et al*., 1998; FAO, 1998). The beneficial effect of green manuring in sustaining rice wheat has been reported (Dargan *et al*., 1975). The prospects, limitations and beneficial effects of integrated plant nutrient management in North West Frontier Province of Pakistan with variable yield responses of wheat and maize crop have been reported by Bhatti and Khan (2000). Recently, while studying the effect of integrated use of mineral and organic N on the yield and nitrogen nutrition of wheat, (Idris and Mohammad, 2001) observed that combination of mineral and organic N at 70+10; 60+20; 50+30; 40+0 being of equal statistical rank order were producing yield significantly equivalent to 80 kg ha^{-1} of mineral N, saving 13.0 to 50.0% fertilizer nitrogen. Similarly while studying the integrated use of organic and mineral nitrogen and organic N of Sesbania in 1:1 ratio was producing yield statistically ($P \leq 0.05$) equivalent of 80 kg ha^{-1} of mineral N saving 50.0% fertilizer N. The results by and large confirm our present findings. Our present results further reveal that the judicious combination of mineral, organic N and or/biofertilizer (*Azotobacter*) can improve the yield of wheat crop, while saving 50% mineral N. Lampe (2000) is also similar same opinion.

The results of the experiment revealed that it is possible to save not only mineral N upto 50.0% but to shift yield plateau to higher level with the complementary use of organic manures and biofertilizer (*Azotobacter*) together with mineral fertilizer than with mineral fertilizer alone. It is, therefore, recommended to address the problem of plant nutrition in an integrated may through efficient and balanced use of nutrients, seeking maximum nutrient efficiency. While the main emphasis has to be on increasing use of mineral fertilizer in the right and balanced manner, the role of organic manure, biofertilizers and recycling of organic residue has to be supplementary and not substitutive.

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