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Original article

Deciphering of Microbes × Nitrogen source fertilizers Interaction for improving nitrogen use efficiency in spring maize

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ABSTRACT

In this study the influence of combined use of organic and inorganic nitrogen (N) sources along with the beneficial micro-organisms on grain N uptake, N use efficiency and N utilization efficiency in maize was evaluated. Organic fertilizer (FYM) and inorganic N source (synthetic fertilizer) was applied in ratio *viz.* (0:100, 25:75, 50:50, 75:25 and 100:0) with and without beneficial micro-organism. Different levels *viz.* 100, 150 and 200 kg ha^{-1} of N was used to accelerate the efficiency of N. Results showed that combination of FYM and inorganic N (50:5) along with the application of beneficial micro-organism significantly increased the total N uptake, N utilization efficiency, highest stover grain and grain protein. However, maximum N uptake and protein contents were recorded with application of 150 kg N ha^{-1}. Application of N (100 kg ha^{-1}) gave the highest N-use efficiency and N utilization efficiency. In conclusion, N application level of 100 kg N ha^{-1}, beneficial micro-organism and 50:50 ratio of inorganic and organic N was proved better in enhancing N-use efficiency and grain quality of maize.

1. Introduction

Maize (*Zea mays* L.) is primary cereal playing vital role in meeting food requirement of millions people and act as nutritional source for livestock and provide raw material to industries (Khaliq et al., 2004). Moreover, its grain has high nutritional value as it contains 72%, 10%, 8.5%, 4.8% and 1.7% of starch, proteins, fiber, oil and ash (Chaudhary, 1993). This crop was cultivated on large area (1.32 million ha) in Pakistan with 6.31 million tons total production. Whereas in Khyber Pakhtunkhwa the total production of maize was 0.89 million tons produced from 0.47 million ha area with average yield 1943 kg ha^{-1} (GOP (Government of Pakistan), 2020).

In Khyber Pakhtunkhwa the productivity of maize is low because of untimely nutrients availability, imbalance input application and poor soil fertility. Over the last decades, exhaustive agricultural practices adversely affected the agricultural environment due to soil erosion and decreasing soil organic matter (SOM). Therefore, improving soil health is critical for the environmental quality and to increase crop yield on sustainable basis. Adoption of sustainable cropping pattern is crucial for economic, environmental and agronomic aspects (Zhao et al., 2009). The deteriorating sustainability regarding soil fertility and yield demand the new approaches such as application of farm yard manure (FYM) in combination with synthetic fertilizers. To minimize the negative

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effects on agriculture production system sustainable practices such as balanced fertilizers application are required to adopt (Bilalis et al., 2009). The main purpose of nutrients application is to increase the level of soil fertility and crop yield to fulfill the food requirement. The soils of Pakistan are low in organic matter and primary nutrients including N, phosphorus (P) (Ali et al., 2011).

Integration of synthetic and organic fertilizers helps to improve the soil structure by increasing water holding ability, total porosity and nutrients pool (Shaaban, 2006). Application of manures increases the concentration and availability of nutrients ultimately resulting in better soil structure and fertility (David et al., 2005). The use of microbes in crop growth favors the improvement in yield quality and production. In maize crop time and rate of nutrients application greatly affect the yield potential of hybrid and the basic goal of fertilizer management is to increase its use efficiency by reducing the losses. The uptake efficiency of N can be improved by increasing its recovery and minimizing losses occur through volatilization, leaching, and denitrification (Wortmann et al., 2011).

Globally, nitrogen use efficiency (NUE) in maize is approximately 33% and most of its concentration reach the beyond the rhizosphere due to volatilization and leaching (Sindelar et al., 2015). Accumulation of N in plant parts occur during particularly vegetative stage based on the N concentration in soil. In maize one half of N uptake at time when crop attain maximum one fourth of biomass (Abendroth et al., 2011). Thus, uniformity between nutrient application and its uptake decrease the total fertilizer while improving yield and NUE (Venterea et al., 2012). Different characteristics of soil are crucial to determine the accurate fertilizer amount to deliver. Thus, N is the essential element because of its significant effect on biota in root zone and plant water relations.

There are different pathways through which N lost from agricultural cropping systems and environment. Therefore, one of the balanced approach is to improve N use efficiency by applying N through different sources (organic and inorganic) along with the microbial application. Therefore, the specific objective of this study was to investigate the influence of integrated application of N application (applied through inorganic and organic sources) with the combination of microbes application on the grain N uptake, total N uptake and N use efficiencies.

Table 1a
Soil physico-chemical properties of experimental field over two years (averaged 2014 and 2015).

Texture	Silty loam
pH	7.60
EC (d Sm-1)	0.18
Total nitrogen (%)	0.07
Phosphorus (ppm)	3.21
Potassium (ppm)	122.00
Organic matter (%)	0.46

2. Materisld and methods

2.1. Site and soil

Two year (2014 and 2015) experiments were carried out at Agronomic Research Farm, University of Agriculture, Peshawar, Pakistan (latitude 34.015° N and longitude 71.581°E, asl 331 m). Experimental soil was well-drained and silt-loam in texture. The climate of experimental area was semi-arid with 450 mm annual rainfall (mean). Soil physico-chemical properties of experimental site are given in Table 1a. The data of temperature and rainfall obtained is presented in Fig. 1.

2.2. Experimental design, treatment combination and field preparation

The experiment was laid in randomized complete block design (RCBD) having 3 replications. Plot size 4.2 × 4 m^2 was maintained with 6 rows. Row to row (70 cm) and plant to plant (20 cm) distance was maintained. Total 93 plots were made with treatment combination including levels of beneficial micro-organisms (BM) (i) with BM, and (ii) without BM, three N levels *viz.* 100, 150 and 200 kg N ha^{-1} and five inorganic and organic N ratios *viz.* 0:100,

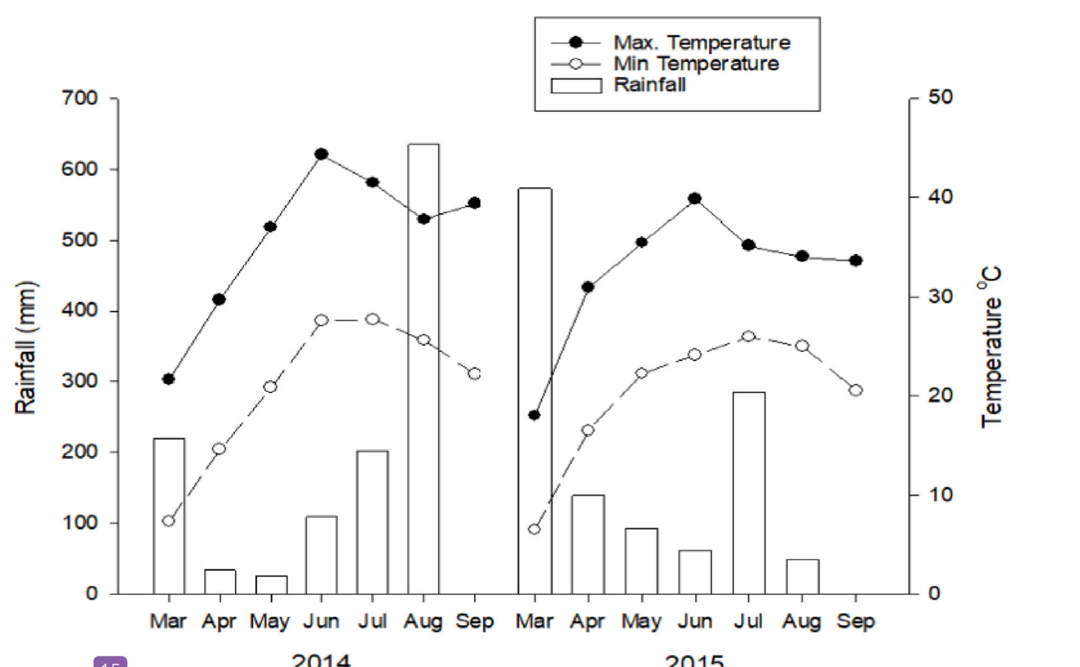

Fig. 1. Maximum temperature (°C), minimum temperature (°C) and total monthly rainfall (mm) of study area.

25:75, 50:50, 75:25 and 100:0. There was one control treatment in each replicate.

The BM culture is commercially called as effective micro-organisms (EM) Bioaab and it was collected from Nature Farming Research and Development Foundation, Faisalabad-Pakistan. There was rich population of Azotobacter (1×10^{11} cfu mL^{-1}) and Azospirrilum (1×10^{6} cfu mL^{-1}). Tap water was used to dilute stock solution to formulate solution of 0.2% which was immediately used. Fifteen days before and after sowing 0.2% diluted solution was used to irrigate plots with EM/BM treatment. Thereby, respective plot was delivered with 5 L (diluted EM solution) twice.

Before planting maize stale-seedbed technique was used for effective weeds management. Two weeks before maize plantation, FYM (six months decomposed) was soil incorporated as organic N source. As an inorganic N source, ammonium nitrate was used which was delivered in splits; half quantity while preparing seedbed and remaining at V3 stage (three-leaf). Chemical properties of FYM composite samples are given in Table 1b. BM solution (50 mL) was obtained and diluted using 5 L water. This prepared solution was applied on BM treatments in sprinkle form (using sprinkler) when crop achieved two-leaf stage (V2). Phosphorus and potassium were applied before sowing at recommended rate 90 kg ha^{-1} and 60 kg ha^{-1}. For other agronomic management (weeding, hoeing, and irrigation) recommended procedures were followed. Sowing of maize was done on March 1st and March 16th in 2014 and 2015, respectively and manually harvested on June 29th, 2014 and July 15, 2015.

2.3. Data recording

After harvesting the data regarding grain N uptake, grain protein contents, N utilization efficiency and stover N uptake was recorded following the recommended methods. The methods of determination of parameters are given below

$$\text{Grain protein contents} = \text{N\%} \times 6.25 \tag{1}$$

Determination of grain N uptake (GNU) was done following the method

$$\text{GNU} = \text{Grain N}(\text{g kg}^{-1}) \times \text{Grain yield}(\text{kg ha}^{-1})/1000 \tag{2}$$

Calculation of stover N uptake (SNU) was performed according to the formulae

$$\text{SNU} = \text{Stover N}(\text{g kg}^{-1}) \times \text{Stover yield}(\text{kg ha}^{-1})/1000 \tag{3}$$

Determination of total N uptake (TNU) was done according to below given formulae

$$\text{TNU} = \text{SNU} + \text{GNU} \tag{4}$$

NUE was calculated using formulae

$$\text{NUE} = \text{Gw}/\text{Ns} \tag{5}$$

Table 1b
Chemical properties of composite samples of FYM (averaged 2014–15).

pH	7.81
Organic carbon (g kg^{-1})	252.50
Nitrogen (%)	0.65
Phosphorus (%)	0.52
Potassium (%)	0.29
Extractable Cu (ppm)	0.70
Exchangeable Mg (ppm)	2.64
Extractable Zn (ppm)	131
Exchangeable Na (ppm)	5.64
Extractable Fe (ppm)	5.09
Exchangeable Ca (ppm)	11.18
Extractable Mn (ppm)	7.02

N utilization efficiency (NUtE) was determined with formulae

$$\text{NUtE} = \text{Grain yield}\,(\text{kg ha}^{-1})\,/\,\text{TNU by plants} \tag{6}$$

2.4. Statistical analysis

Collected data was statistically analysis by using fisher analysis of variance technique with Statistix 8.1 software. Whereas, treatments comparison was performed using least significant difference (LSD) test at 5% probability level (Steel et al., 1996).

3. Results

3.1. Grain protein content

Results of two years study revealed significant differences in protein content of grains among both years (Table 2). In 2015, protein content was higher (7.81%) recorded as compared with the year 2014 (7.25%). Beneficial microbes have astounding effects on protein contents of grains. Significantly higher protein content (10.23%) were noted where beneficial microbes were applied than control (no treatment) (9.86%). Similarly, application of N through inorganic and organic sources highly affected protein contents of grains. Nitrogen application (50:50 – organic, inorganic sources) increased protein contents (10.5%) than N applied (75:25 – organic, inorganic sources) (10.36%). Different levels of N greatly affected the protein content, nonetheless N application (200 kg N ha^{-1}) improved protein contents (10.33 %) compared with (150 kg N ha^{-1}) (10.19%). Considerably, lower protein content was recorded (5.01 %) with control treatment. Interaction for protein content among beneficial microbes, organic and inorganic nitrogen application (BM × R) and (BM × R × N) was significant. However, interaction among BM × R showed that increasing the level of organic N @ 50 % augmented the protein content with beneficial microbes and organic N @ 75 % increased protein content without beneficial microbes (Fig. 2a). Interaction among BM × R × N revealed that ris-

Table 2
Effect of beneficial microbes, N levels, organic and inorganic ratios on the protein content of grains, N uptake by stover and N uptake by grains of spring maize in 2014 and 2015.

Treatment	Protein Content of Grain (%)	Nitrogen Uptake by Stover (kg ha^{-1})	Nitrogen Uptake by Grain (kg ha^{-1})
Beneficial Microbes (BM)			
BM	10.23 a	76.31 a	89.84 a
BM (0)	9.86b	69.75b	73.83b
Probability	0.012	0.005	0.000
Organic and Inorganic Nitrogen % (R)			
0:100	9.89b	64.11c	69.80 d
25:75	9.57b	68.74 bc	79.63c
50:50	10.50 a	81.06 a	97.17 a
75:25	10.36 a	78.23 ab	85.16b
100:0	9.90b	73.01b	77.43c
$LSD_{(0.05)}$	0.46	7.11	5.28
Nitrogen Levels (N) (kg ha^{-1})			
100	9.62b	49.05c	72.07b
150	10.19 a	76.91b	85.82 a
200	10.33 a	93.13 a	87.63 a
$LSD_{(0.05)}$	0.35	5.51	4.09
Planned Mean Comparison			
Control	5.01b	25.96b	26.24b
Rest	10.05 a	73.03 a	81.84 a
Probability			
Years			
2014	7.25b	45.12b	48.74b
2015	7.81 a	53.88 a	59.34 a

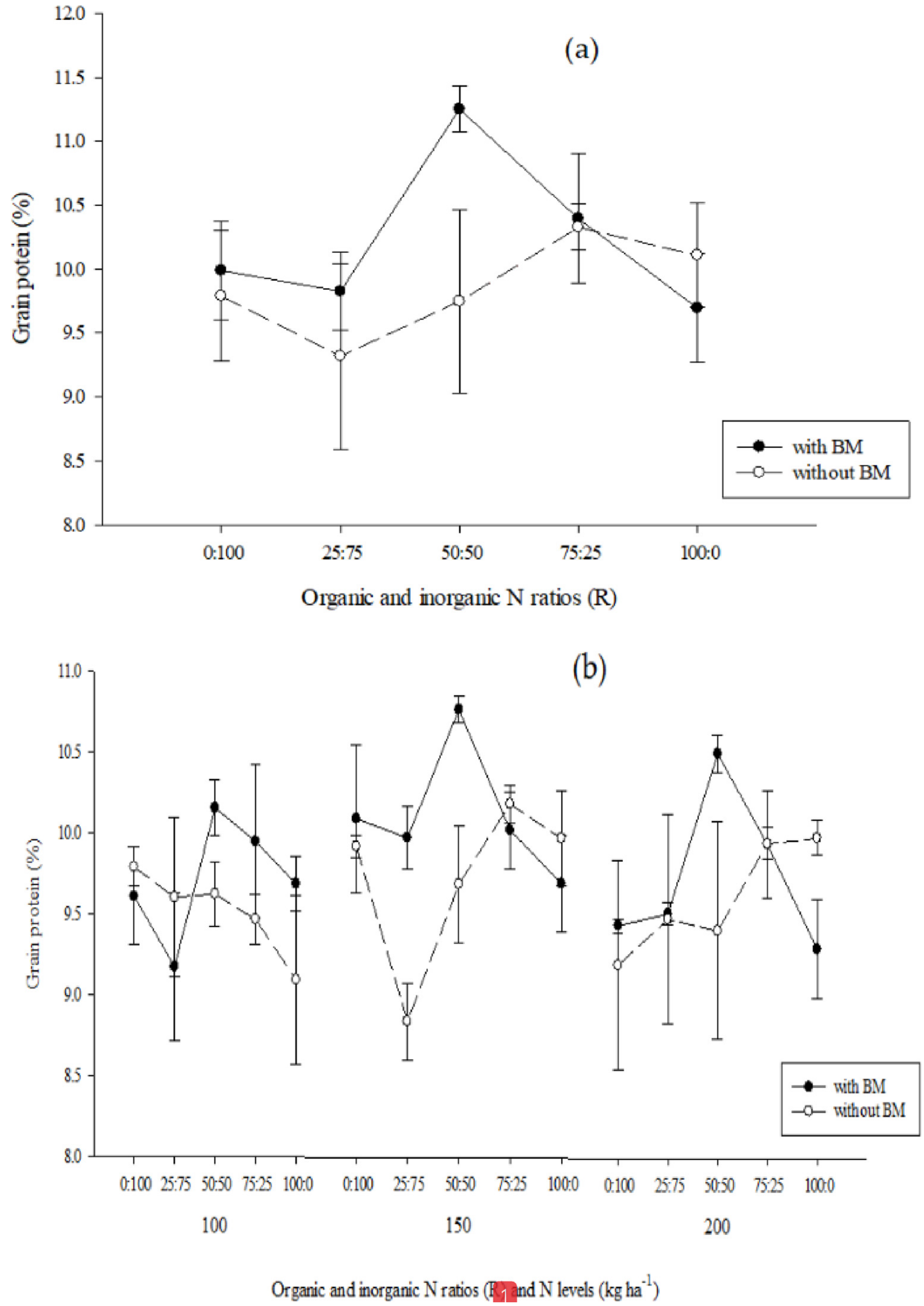

Fig. 2. Interaction between BM × R (a) and BM × R × N (b) for protein content (%) of spring maize. Means data of three replicates with standard deviations presented by vertical bars.

ing the organic N @ 50 % with beneficial microbes augmented the protein content but increment was higher with the application of N @ 200 kg N ha^{-1} (Fig. 2b).

3.2. Stover nitrogen uptake

Two years study depicted significant differences for the uptake of nitrogen by stover of spring maize (Table 2). In 2015, significant higher uptake of nitrogen by stover (53.88 kg ha^{-1}) was recorded in comparison with 2014 (45.12 kg ha^{-1}) respectively. Treatment of beneficial microbes greatly augmented the nitrogen uptake by stover (76.31 kg ha^{-1}) as compared with no application of microbes (69.75 kg ha^{-1}). Various proportions of organic and inorganic nitrogen also greatly affected the nitrogen uptake by stover of maize. Higher nitrogen uptake by stover was recorded (81.06 kg ha^{-1}) @ of 50:50 N. The effect of different N levels was also significantly affected the uptake of N (Fig. 3a). Increasing the levels of nitrogen increased the nitrogen uptake by stover of maize. Comparative effect of treated plots depicted significantly higher uptake of nitrogen (73.03 kg ha^{-1}) as compared with control plots (25.96 kg ha^{-1}). Interaction between R × N and BM × R × N significantly affected the SNU of maize. However, interaction among

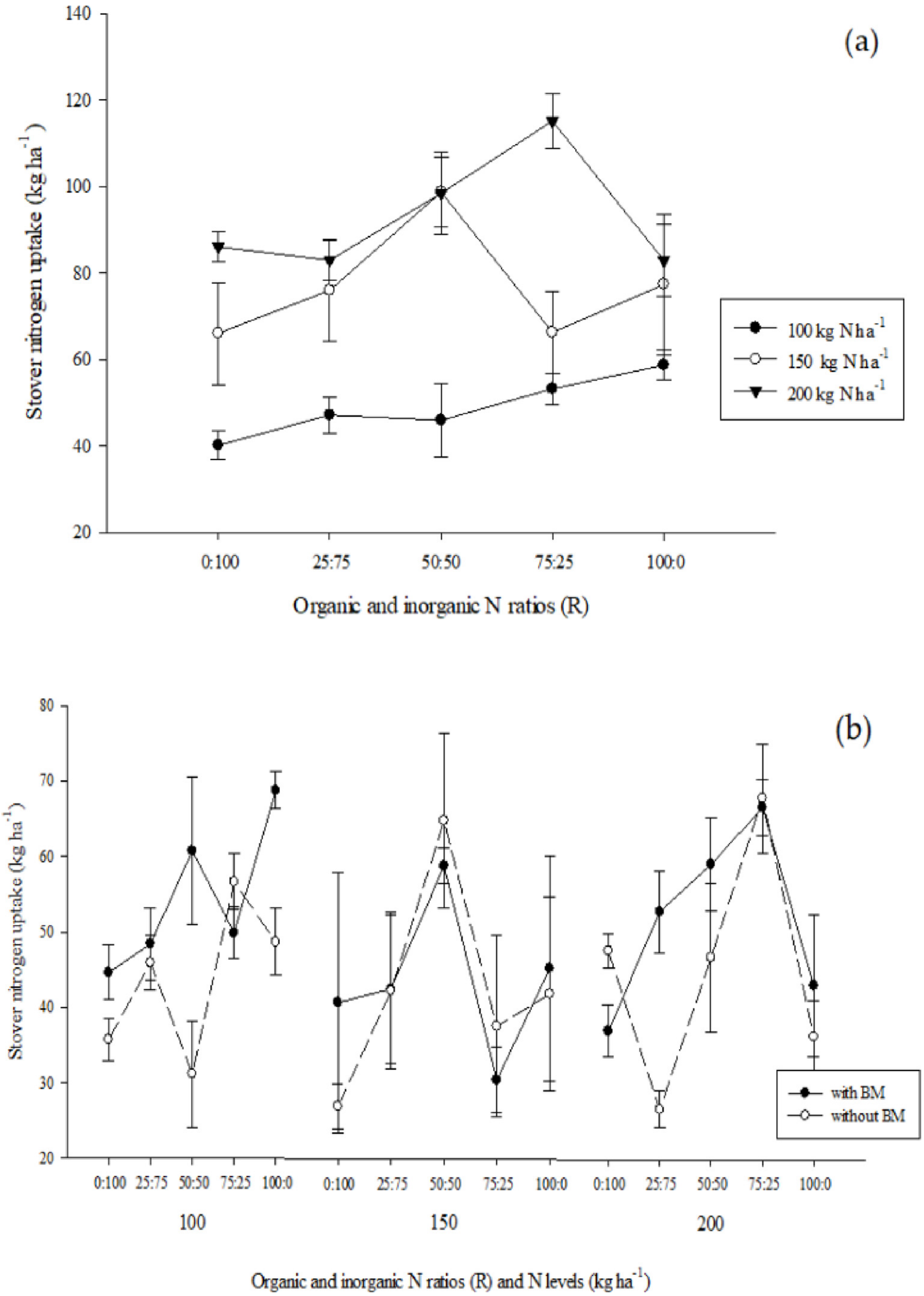

Fig. 3. Interaction of R × N (a) and BM × R × N (b) for uptake of nitrogen (kg ha^{-1}) by stover of spring maize. Means data of three replicates with standard deviations represented with vertical bars.

R × N depicted that organic N @ 50 % improved the nitrogen uptake with 150 kg N ha^{-1}, however nitrogen content of stover was elevated with organic nitrogen @75 % with 200 kg N ha^{-1} and 100 kg N ha^{-1}. Interaction among BM × R × N affirmed that application of organic N upto 50 % increased the N content of stover with the treatment of microbes @ 150 and 200 kg N ha^{-1}. Whereas, treatment of organic nitrogen @ 100 kg ha^{-1} with beneficial microbes increased the N content of stover linearly (Fig. 3b).

3.3. Uptake of nitrogen by grains of maize

Results related to nitrogen uptake by grains depicted significantly differences among both the years (Table 2). In 2015, higher nitrogen uptake (59.34 kg ha^{-1}) was recorded as compared with 2014 (48.74 kg ha^{-1}). In the presence of beneficial microbes, significantly higher content of nitrogen (89.84 kg ha^{-1}) was recorded in comparison with the absence of beneficial microbes (73.83 kg ha^{-1}). The proportion of organic and inorganic nitrogen also greatly affected the nitrogen uptake by grains of maize. However, maximum and higher GNU (97.17 kg ha^{-1}) was analyzed with the treatment of N @ 50:50 while least (69.80 kg ha^{-1}) were recorded with inorganic N only. Application of 200 kg N ha^{-1} increased the GNU (87.63 kg ha^{-1}) in comparison with 150 kg N ha^{-1} (85.82 kg ha^{-1}). Treated plots had significantly higher GNU (81.84 kg ha^{-1}) as compared with control plots (26.24 kg ha^{-1}). Interaction between different factors such as BM × R, BM × N and BM × R × N was found significantly for grains nitrogen uptake of maize. Interaction between BM × R depicted that with the increment of organic N ratios up to 50 % enhanced the nitrogen uptake by grains in the presence of microbes however further increment in N reduced the GNU of maize (Fig. 4a). Interaction among BM × N showed that GNU increased with the increase of N linearly in the presence of beneficial microbes (Fig. 4b). Interaction between three factors BM × R × N revealed that GNU was increased with the increment of N ratio upto 50% in the presence of beneficial microbes while GNU was recorded higher @ 150 and 200 kg N ha^{-1} (Fig. 4c).

3.4. Total nitrogen uptake

Data presented in Table 3 depicted significant differences among the total nitrogen uptake of maize during 2014 and 2015. Total nitrogen uptake was significantly higher (104.78 kg ha^{-1}) during the year 2015 as compared with 2014 where low TNU (96.02 kg ha^{-1}) was recorded. Addition of beneficial microbes significantly increased total nitrogen uptake (127.22 kg ha^{-1}). Whereas proportions of organic and inorganic N significantly increased the total uptake of nitrogen by maize. Total nitrogen uptake (131.96 kg ha^{-1}) was higher recorded with the ratios of 50:50 of organic and inorganic N sources however lower TNU (115.01 kg ha^{-1}) was recorded with inorganic N only. Various levels of nitrogen greatly affected the TNU and maximum nitrogen uptake (144.03 kg ha^{-1}) was recorded with the application of 200 kg ha^{-1}. Control plots revealed minimum TNU (76.87 kg ha^{-1}) as compared with treated plots. Interaction between R × N and BM × R × N was also found significantly for total nitrogen uptake. Interaction among R × N depicted that increment in the ratio of N upto 50 % enhanced the TNU @ 150 and 200 kg N ha^{-1}, while increment in organic ratio of N @100 kg increased linearly in TNU of maize (Fig. 5a). Interaction between BM × R × N showed that application of nitrogen @ 200 kg ha^{-1} in 50:50 enhanced total nitrogen uptake with microbes whereas in the absence of microbes TNU was reduced with 150 and 200 kg N ha^{-1} respectively (Fig. 5b).

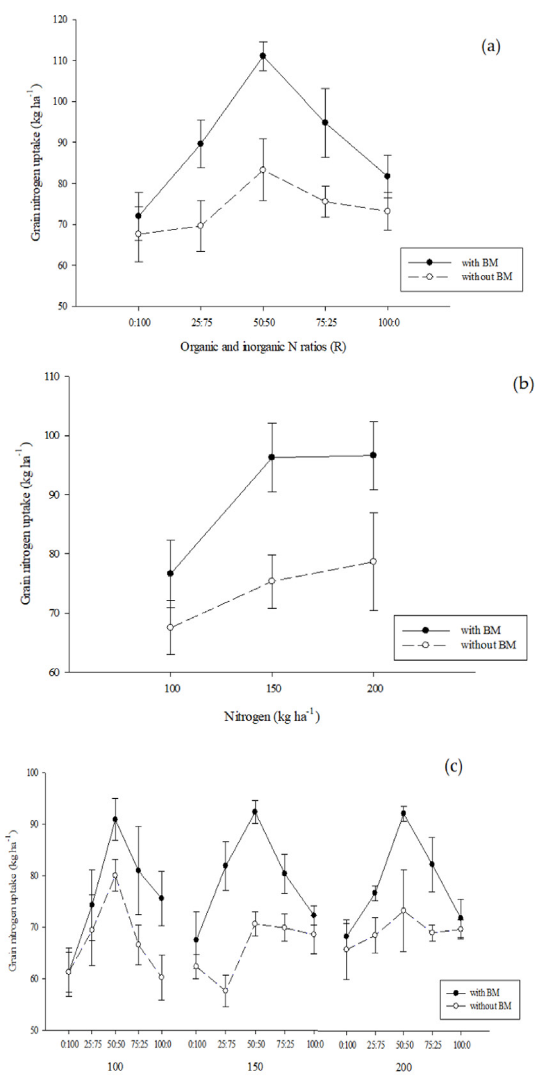

Fig. 4. Interaction among BM × R (a); BM × N (b) and BM × R × N (c) for uptake of nitrogen (kg ha^{-1}) of grains of spring maize. Mean data of three replicates are presented with standard deviations with vertical bars.

Table 3
Effect of various levels of organic and inorganic N proportions with beneficial microbes on the total N uptake, N use efficiency and N utilization efficiency of spring maize during 2014 and 2015.

Treatment	Total Nitrogen Uptake (kg ha^{-1})	Nitrogen Use Efficiency (%)	Nitrogen Utilization Efficiency (%)
Beneficial Microbes (BM)			
With BM	127.22	24.50 a	43.93 a
Without BM	120.65	21.25b	40.59b
Probability	0.005	0.000	0.005
Organic and Inorganic N Proportions (R)			
0:100	115.01c	19.97 d	39.56c
25:75	119.65 bc	23.35b	44.18 ab
50:50	131.96 a	25.92 a	46.04 a
75:25	129.13 ab	23.08 bc	41.38b
100:0	123.92b	22.04c	40.15 bc
$LSD_{(0.05)}$	7.11	1.12	3.66
Nitrogen (kg ha^{-1})			
100	99.96c	26.13 a	47.68 a
150	127.82b	23.30b	42.07b
200	144.03 a	19.19c	37.04c
$LSD_{(0.05)}$	5.51	0.87	2.84
Planned Mean Comparison			
Control	76.87b	42.79 a	42.79
Rest	123.94 a	22.87b	42.26
Probability			
Years			
2014	96.02b	31.16b	41.43b
2015	104.78 a	34.5 a	43.62 a

3.5. Nitrogen use efficiency

Results of experiments of both years eastablished the significant differences among various treatments on nitrogen use efficiency of spring maize (Table 3). In 2015, NUE was higher (34.50 kg grains kg^{-1}N supply) as compared with (31.16 kg grains kg^{-1}N supply) in 2014. Nitrogen use efficiency (24.50 kg grains kg^{-1}N supply) of maize was greatly affected with beneficial microbes. Significantly higher NUE (25.92 kg grains kg^{-1}N supply) was recorded with the treatment of ratio of organic and inorganic N @ 50:50 whereas lower (19.97 kg grains kg^{-1}N supply) was obtained in the plots with inorganic nitrogen only. Various levels of nitrogen.

Increased NUE and maximum (26.13 kg grains kg^{-1}N supply) was recorded with application of 100 kg N ha^{-1} and NUE decreased with further increment in levels of nitrogen. Interaction for NUE among BM × R and R × N was significant. However interaction among BM × R depicted that increment in organic N ratio at 50% augmented NUE despite the treatment of microbes, however increment was greater with the treatment of microbes (Fig. 6a). Interaction among R × N showed that increase in the ratio of organic N till

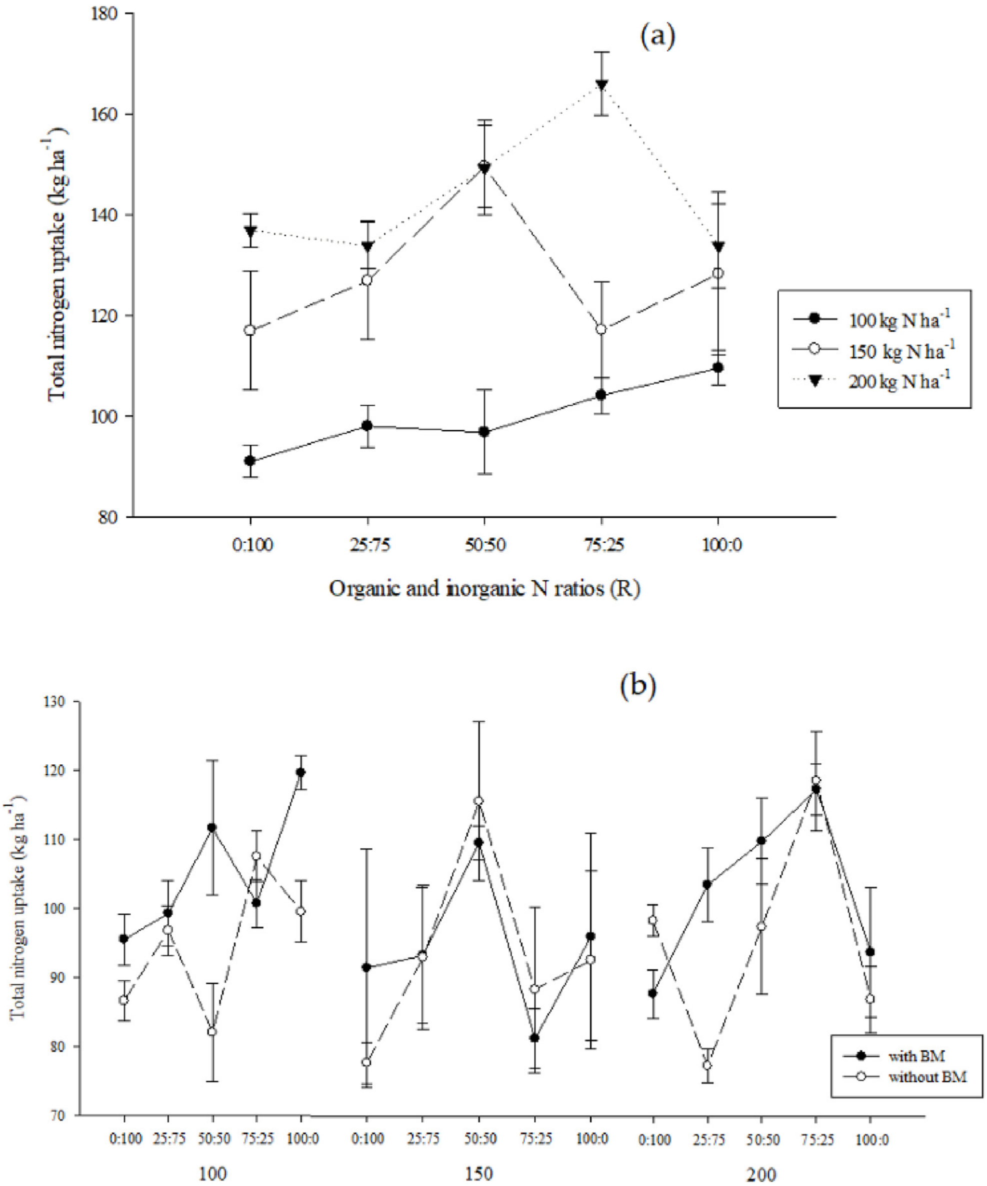

Fig. 5. Interaction among R × N (a) and BM × R × N (b) for total nitrogen uptake (kg ha^{-1}) of spring maize. Mean data of three replicates is presented with standard deviations by vertical bars.

50 % @ (100, 150 and 200 kg ha^{-1}) augmented the NUE whereas increment in ratio of N decreased NUE (Fig. 6b).

3.6. Nitrogen utilization efficiency

The year effect was found insignificant for nitrogen utilization efficiency (NUtE) of maize (Table 3). The beneficial microbes significantly exaggerated the NUtE of spring maize and NUtE was maximum (43.93) with beneficial microbes. Various proportions of organic and inorganic nitrogen greatly affected NUtE and greater NUtE (46.04) was found with the treatment of N (50:50 ratio) of organic and inorganic nitrogen, whereas NUtE was analyzed lower (39.56) in the plots treated with inorganic nitrogen only. Various treatments of N greatly affected the NUtE and maximum NUtE (47.68) was found with 100 kg N ha^{-1} and NUtE decreased linearly with increment in N ratios. Comparison between control vs rest was found non-significant for NUtE. Interaction between BM × R, BM × N and R × N were found greater for NUtE. The BM × R interaction showed that mounting organic N ratio up to 50% increased NUtE in spite of beneficial microbes. Further increment in organic nitrogen up to 75% enhanced NUtE with beneficial microbes and decreased NUtE without beneficial microbes, whereas decline was greater without beneficial microbes (Fig. 7a). Interaction of BM × N showed that increment in N level reduced the NUtE despite of beneficial microbes, however the decline was greater without beneficial microbes (Fig. 7b). The R × N interaction showed that augmenting the ratio of organic N up to 50% enhanced the NUtE, while further increasing the organic ratio of N reduced the NUtE at all three level of N, however increment in NUtE was gretaer @ 100 kg ha^{-1} (Fig. 7c).

4. Discussion

Nitrogen is the most limiting factor for crop growth and development. Nitrogen management is a very exigent task and numerous methods have been used to increase its efficiency. However, improvement in nitrogen use efficiency is still needed. Protein

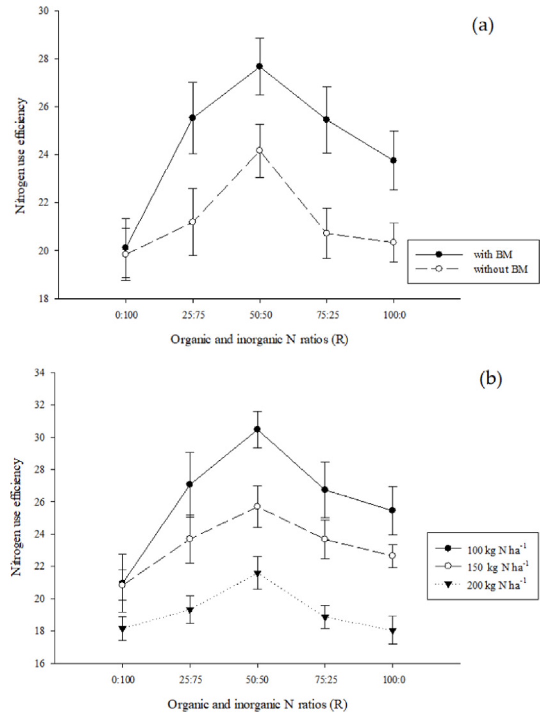

Fig. 6. Interaction between BM × R (a) and R × N (b) for nitrogen use efficiency of spring maize. Mean data of three replicates is presented with standard deviations by vertical bars.

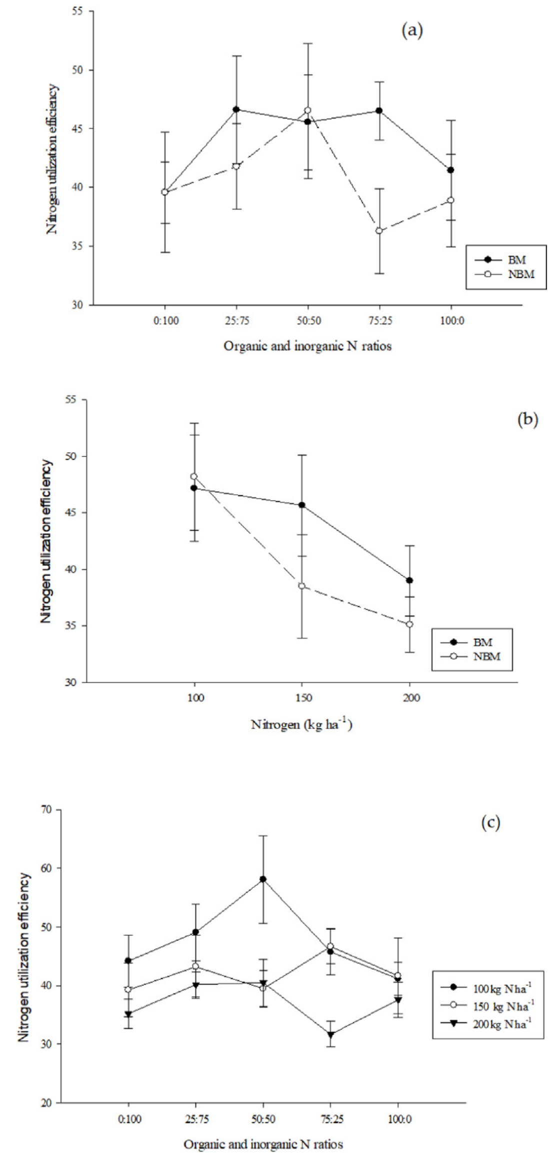

Fig. 7. Interaction among BM × R (a); BM × N (b); and R × N for nitrogen utilization efficiency of spring maize. Mean data of three replicates with standard deviations depicted through vertical bars.

contents of grains, nitrogen uptake by stover and grain, nitrogen use efficiency and nitrogen utilization efficiency was found lower in comparison with 2nd year, it may be due to more nutrients were available due to residual effects of treatments in 1st year.

Nutrient uptake efficiency increased in plants due to induced changes in mode of action of plants by beneficial microbes (Sangakkara et al., 1998). Nutrient uptake, shoot nitrogen, phosphorous and potassium contents increased under mineral and beneficial microbes amended soil by cereal crops (Khaliq et al., 2006). It has been reported that treatment of beneficial microbes astoundingly increased macro and micro nutrients such as N, P, K, Fe, Mn and Zn and also nutrient content in leaves (Gorski and Kleiber, 2010). Similar findings have been reported due to appliance of beneficial microorganism in cotton with increased nutrient contents in leaves (Khaliq et al., 2006). Nitrogen uptake by plants is highly inclined through rate and split application (Rahman et al., 2011). Nitrogen availability between flag leaf and milking stage enabled plants to build up greater amount of nitrogen in grains. Appliance of nitrogen at anthesis in late spring season is highly useful in increasing protein contents of grains (Jama and Ottman, 1993). Foliar application of urea with high level of N concentration from 2 to 10% increased absorption of $NH4^+$ (Altman et al., 1983). It has been reported that foliar appliance of urea enhanced nitrogen concentration in leaves of wheat (Vagen, 2003). Many scientists reported that application of urea in soil as well as foliar appliance increased nitrogen uptake in plants (Abad et al., 2004). Ever increasing nitrogen application enhanced the nitrogen uptake and contents in plants (Mattas et al., 2011). Presence of higher nitrogen contents in grains in response to better N application may be linked with mineralization-immobilization turnover (Strong and Bacon, 1995), as a result in higher nitrogen loses through immobilization in low level of nitrogen. The other probable cause is the better mineralization of organic matter (Aulakh et al., 2000) to available nitrogen resulted ultimately in nitrogen uptake in straw and grains. Rising rate of nitrogen improved the uptake of nitrogen (Fan et al., 2005) might be associated with increased availability of nitrogen owing to release of nitrogen from organic source (Shah and Ahmad, 2006). Higher rate of mineralization of FYM increased uptake of nitrogen, which may be reduced nitrogen losses with denitrification or immobilization in organic forms (Wells and Bitzer, 1984) found in plots with N and FYM. These results were in line with the findings of Russell and Olson (Russelle and Olson, 1983) reported that augmented efficiency of nitrogen from belated applications or mineralization. Plant species and soil factors highly influenced on increased uptake efficiency of nitrogen might be helpful in improved mineralization of manures to fulfill the demand of crop plants. Presence of higher nitrogen in the soil increased nitrogen uptake by plants (Mercedes et al., 1993). Appliance of organic nitrogen and urea increased the availability of nitrogen, resulted in enhanced uptake of nitrogen (Dhillon et al., 1998). Results of our experiment are in accordance with the findings of Parmar and Sharma (Parmar and Sharma, 2001), who accomplished that absorbance of nitrogen by wheat enhanced with increasing level of N and FYM. Integration of farm yard manure and nitrogen application increased grain protein as compared with control or FYM alone. Fertilization with nitrogen increased photosynthetic activity, therefore enhanced protein content of grains (Lopez-Bellido et al., 1998) and quality of grains. Soil applied nitrogen might be increased protein content of grains (Rathi and Singh, 1973). Soil nitrogen content increased uptake by plants resulted in higher protein content (Brejda et al., 1995). Amalgamation of nitrogen in soil increased accumulation and enhanced protein contents of grains (Pedersen and Jorgensen, 2007). Concentration of crude protein differ with moment (Muir, 2006) therefore, late application of nitrogen enhanced protein 12% without losses in yield from excessive nitrogen (Brown and Petrie, 2006). Excess mineralization in fertilized soil (Soon et al., 2001) increased N in the soil resulted in higher grain protein content. The process of mineralization of nitrogen should be constant with the better organic N, mineralizable nitrogen and recycling of nitrogen (Doran, 1987).

5. Conclusion

Beneficial microorganism and application of organic and inorganic N fertilizers @ 50:50 ratios increased the protein content of grains and stover, total uptake of nitrogen, nitrogen use and utilization efficiency. Protein content, grain nitrogen uptake was better with application of 150 kg N ha^{-1} while stover and total uptake was improved with appliance of 200 kg N ha^{-1} and nitrogen use efficiency and utilization efficiency was better in 100 kg N ha^{-1}.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abad, A., Lloveras, J., Michelena, A., 2004. Nitrogen fertilization and foliar urea effects on durum wheat yield and quality and on residual soil in irrigated Mediterranean conditions. Field Crops Res. 87 (2), 257–269.

Abendroth, L.J., Elmore, R.W., Boyer, M.J., Marlay, S.K., 2011. Corn growth and development. PMR 1009. Iowa State Univ. Ext., Ames.

Ali, K., Munsif, F., Zubair, M., Hussain, Z., Shahid, M., Din, I.U., Khan, N., 2011. Management of organic and inorganic nitrogen for different maize varieties. Sarhad J. Agric. 27 (4), 525–529.

Altman, D.W., McCuistion, W.L., Kronstad, W.E., 1983. Grain protein percentage, kernel hardness and grain yield of winter wheat with foliar applied urea. Agron. J. 75, 87–91.

Aulakh, M.S., Khera, T.S., Doran, J.W., Singh, K., Singh, B., 2000. Yield and nitrogen dynamics in a rice-wheat system using green manure and inorganic fertilizer. Soil Sci. Soc. Am. J. 64, 1867–1876.

Bilalis, D., Karkanis, A., Efthimiadou, A., Konstantas, A., Triantafyllidis, V., 2009. Effects of irrigation system and green manure on yield and nicotine content of Virginia (flue-cured) organic tobacco (*Nicotiana tabacum* L.), under Mediterranean conditions. Ind. Crops Prod. 29, 388–394.

Brejda, J.J., Brown, J.R., Hocnshell, C.L., 1995. India grass and Caucasian bluestem responses to different nitrogen sources and rates in the Ozarks. J. Range Manag. 48, 172–180.

Brown, B.D., Petrie, S., 2006. Irrigated hard winter wheat response to fall, spring, and late season applied nitrogen. Field Crops Res. 96, 260–268.

Chaudhary, A.R., 1993. Maize in Pakistan. Punjab Agri. Res. Cord. Board Univ. Agri, Faisalabad, Pakistan.

David, M., Meisner, C.A., Sadat, M.A., Hossain, M.I., 2005. Long-term effects of applied organic manures and inorganic fertilizers on yield and soil fertility in a wheat-rice cropping pattern. 19th World Congress of Soil Sci. Soil Solutions for a Changing World, Brisbane, Australia.

Dhillon, G.P.S., Dhanda, R.S., Dhillon, M.S., 1998. Performance of wheat under scattered trees of *Acacia nilotica* under rainfed condition of Punjab. India. The Indian Forester 124 (1), 48–53.

Doran, J.W., 1987. Microbial biomass and mineralizable nitrogen distributions in no-tillage and plowed soils. Biol. Fert. Soils. 5, 68–75.

Fan, M.F., Jiang, R., Liu, X., Zhang, F., Lu, S., Zeng, X., Christie, P., 2005. Interactions between non-flooded mulching cultivation and varying nitrogen inputs in rice–wheat rotations. Field Crop Res. 91, 307–318.

GOP (Government of Pakistan), 2020. Economic Survey of Pakistan. Economic Advisory Wing, Finance Division, Islamabad.

Gorski, R., Kleiber, T., 2010. Effect of effective microorganisms (EM) on nutrient contents in substrate and development and yielding of rose (*Rosa* x *hybrida*) and gerbera (*Gerbera jamesonii*). Eco. Chem. Eng. 17 (4), 505–512.

Jama, A.O., Ottman, M.J., 1993. Timing of the first irrigation in corn and water stress conditioning. Agron. J. 85, 1159–1164.

Khaliq, A., Abbasi, M.K., Hussain, T., 2006. Effect of integrated use of organic and inorganic nutrient sources with effective microorganisms (EM) on seed cotton yield in Pakistan. Bioresour. Technol. 97, 967–972.

Khaliq, T., Mahmood, T., Masood, A., 2004. Effectiveness of farm yard manure, poultry manure and nitrogen for corn productivity. Intl. J. Agri. Bio. 2, 260–263.

Lopez-Bellido, L., Fuentes, M., Castillo, J.E., López-Garrido, F.J., 1998. Effects of tillage, crop rotation and nitrogen fertilization on wheat-grain quality grown under rainfed Mediterranean conditions. Field Crop Res. 57, 265–276.

Mattas, K.K., Uppal, R.S., Singh, R.P., 2011. Effect of varieties and nitrogen management on the growth, yield and nitrogen uptake of durum wheat. Res. J. Agri. Sci. 2 (2), 376–380.

Mercedes, M.A., Frank, M.H., Vincent, A.H., 1993. Nitrogen fertilization timing effect on wheat production, nitrogen uptake efficiency, and residual soil nitrogen. Agron. J. 85 (11–12), 1198–1203.

Muir, J.P., 2006. Weight gains of meat goat kids on wheat pastures fertilized at different nitrogen levels. Small Ruminant Res. 66, 64–69.

Parmar, D.K., Sharma, V., 2001. Nitrogen requirement of single hybrid maize (*Zea mays* L.) -wheat (*Triticum aestivum* L.) system under rainfed conditions. Ind. J. Agric. Sci. 71 (4), 252–254.

Pedersen, L., Jorgensen, J.R., 2007. Variation in rheological properties of gluten from three biscuit wheat cultivars in relation to nitrogen fertilization. J. Cereal Sci. 46, 132–138.

Rahman, M., Fukai, S., Blamey, F.P.C., 2011. Forage production and nitrogen uptake of forage sorghum, grain sorghum and maize as affected by cutting under different nitrogen levels. In: Rowe, B. (Ed.), Proceedings of 10th Australian agronomy conference', Hobart. Available at www. Regional. Org. au/au/asa/2001/1/c/rahman. htm [Verified 10 January 2011].

Rathi, K.S., Singh, R., 1973. Study on the rate and method of nitrogen application of wheat. Ind. J. Agric. Res. 7, 111–114.

Russelle, M.P., Olson, R.A., 1983. Nitrogen accumulation rate of irrigated maize. Agron. J. 75, 593–598.

Sangakkara, U.R., Marambe, B., Attanayake, A.M.U., Piyadasa, E.R., 1998. Nutrient use efficiency of selected crops grown with effective microorganisms in organic systems. In: Proc. 4th Int. Conf. Kysei Nature Farming held in Paris, France, June 19-21 (Eds. JF Parr, SB Hornick), 1995. pp: 111-117.

Shaaban, S.M., 2006. Effect of organic and inorganic nitrogen fertilizer on wheat plant under water regime. J. App. Sci. Res. 2 (10), 650–656.

Shah, Z., Ahmad, M.I., 2006. Effect of integrated use of farmyard manure and urea on yield and nitrogen uptake of wheat. J. Agric. Boil. Sci. 1, 60–65.

Sindelar, A.J., Coulter, J.A., Lamb, J.A., Vetsch, J.A., 2015. Nitrogen, stover, and tillage management affect nitrogen use efficiency in continuous corn. Agron. Soil Environ. Qual. 107, 843–850.

Soon, Y.K., Clayton, G.W., Rice, W.A., 2001. Tillage and previous crop effects on dynamics of nitrogen in a wheat–soil system. Agron. J. 93, 842–849.

Steel, R.G.D., Torrie, J.H., Dickey, D.A., 1996. Principles and procedures of statistics: a biometric approach. McGraw Hill Book Co., Inc., New York.

Strong, W.M., 1995. Nitrogen fertilization of upland crops. In: Bacon, P.E. (Ed.), Nitrogen fertilization in the environment. Marcel Dekker, New York, pp. 129–169.

Vagen, I.M., 2003. Nitrogen uptake in a broccoli crop. I. Nitrogen dynamics on a relative time scale. Acta Hort. 627, 195–202.

Venterea, R.T., Halvorson, A.D., Kitchen, N., Liebig, M.A., Cavigelli, M.A., Grosso, S.J.D., Motavalli, P.P., Nelson, K.A., Spokas, K.A., Singh, B.P., Stewart, C.E., 2012. Challenges and opportunities for mitigating nitrous oxide emissions from fertilized cropping systems. Front Ecol. Environ. 10, 562–570.

Wells, K.L., Bitzer, M.J., 1984. Nitrogen management in the no-till system. In: Hauck, R.D. (Ed.), Nitrogen in crop production. ASA, CSSA, and SSSA, Madison, WI, pp. 535–549.

Wortmann, C.S., Tarkalson, D.D., Shapiro, C.A., Dobermann, A.R., Ferguson, R.B., Hergert, G.W., Walters, D., 2011. Nitrogen use efficiency of irrigated corn for three cropping systems in Nebraska. Agron. J. 103, 76–84.

Zhao, Y., Wang, P., Li, J., Chen, Y., Ying, X., Liu, S., 2009. The effect of two organic manures on soil properties and crop yields on a temperate calcareous soil under a wheat-maize cropping system. Eur. J. Agron. 31, 36–42.