



Research Article

Management of Nitrogen Sources with and without Beneficial Microbes and its Application Timing for Wheat Crop stand and Phenology Improvement

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Abstract | To manage nitrogen sources i.e. farmyard manure (FYM), poultry manure (PM) and urea with and without beneficial microbes (BM) for improving phenology and crop stand of wheat, research was conducted at the Agronomy Research Farm, The University of Agriculture Peshawar, during 2019-20. The experiment comprised of nitrogen application from integrated sources (50% FYM + 50% urea + BM, 50% FYM + 50% PM + BM, 50% PM + 50% urea + BM, 50% FYM + 50% urea – BM, 50% FYM + 50% PM – BM and 50% PM + 50% urea – BM) and N sources application timing (1, 15 and 30 days before sowing). Experiment also consists of two controls: one was 100% N from urea and other was without fertilizer. Organic sources (FYM and PM) added in the field above mentioned days before sowing, while urea applied in split form after sowing. These ratios compensated total of 120 kg N ha⁻¹. Nitrogen application at the rate of 120 kg ha⁻¹ compensated as 50% from FYM and 50% from urea without beneficial microbes resulted in delayed emergence (15.4 days) as compared to rest of treatments, while delayed heading (114.8 days), anthesis (124.7 days) and physiological maturity (158.9 days) were recorded with application of 120 kg N ha⁻¹ applied as 50% from FYM and 50% from PM with beneficial microbes as compared to remaining treatments. Among the nitrogen sources application timing, delayed emergence (15.5 days) was recorded when nitrogen organic sources applied one day before sowing, while delayed heading (115.1 days), anthesis (125.3 days) and physiological maturity (159 days) were recorded when these sources applied one month before sowing. Maximum leaf area tiller⁻¹ (118.9 cm²) and plant height (92.4 cm) were noted with addition of 120 kg N ha⁻¹ applied as 50% from FYM and 50% from PM with beneficial microbes. Likewise, nitrogen sources application one month before sowing has increased leaf area tiller⁻¹ (121.6 cm²) and plant height (91.3 cm). Therefore, it is recommended that 120 kg N ha⁻¹ compensated as 50% each from FYM and PM with the combination of beneficial microbes and its application in the field one month before sowing can enhance phenology and crop stand.

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Keywords | Application timing, Beneficial microbes, Crop stand, Nitrogen sources, Phenology



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Introduction

Wheat (*Triticum aestivum* L.) belongs to family Poaceae and is a main source of food for most of the people on the earth (Khan *et al.*, 2021a). In Pakistan, wheat crop is cultivated on 8,734 thousand hectares area with production of 25.492 million tonnes, while in Khyber Pakhtunkhwa (KP), Pakistan, cultivated on 749 thousand hectares area with production of 1.365 million tonnes (MNFSR, 2017-18). Wheat contributes about 9.1% to the agriculture and 1.7% to total GDP of the Pakistan (Pakistan Economic Survey, 2017-2018). Average yield of wheat in the country is about 3 tonnes ha⁻¹, which is very low. This low productivity is due scarcity of water, calcareous soil, shrinking in cultivated area, changing rainfall pattern, conventional farming, imbalance nutrients availability, weeds entry and fog and smog in the months of November to January which may decrease up to 25-30% of yield (Pakistan Economic Survey, 2017-2018).

Nitrogen fertilizer application is key component in crop production for well establishment of the crop. Among the primary macro nutrients, nitrogen is important element for plant nutrition, which increases wheat yield, crude protein and gluten content in grain and overall improves quality of the seed (Litke *et al.*, 2018). Nitrogen fertilizer demand in the world is increasing at the rate of 1.7% per annum which is 7.6 million tons and especially more in Asia, which is 68% of it. In 2015, total nitrogen need projected at 106 million tonnes (Maqsood *et al.*, 2016). In Pakistan, annual fertilizer need is about 8 million tonnes in which urea are 5.6 million tonnes. Due to volatilization, denitrification and leaching, about 3.4 million tonnes urea is lost in the world annually, which causing economic loss of 40.5 billion US dollar and serious ecological problems (Pakistan Economic Survey, 2012-13). Chemical fertilizers

enhance fertility of soil for a short-term and left long-term negative consequence on soil health (Yang *et al.*, 2015). Instead of mineral fertilizers, sustainable crop production should rely on organic fertilizers. Addition of crop residue enhanced soil organic matter (SOM) and also work as an energy source and nutrients for growth of microorganisms (Fang *et al.*, 2018). Fertilizer organic sources like FYM, straw, compost or green manures can increase SOM in cultivated lands which have long-term positive influence on soil fertility and also useful to offer ecosystem services (Liu *et al.*, 2019). Manures available essential nutrients for crop growth; but, they are not sufficient to achieve the production requirements. Hence, integrated nutrient management is best option to improve production and soil health.

Organic and chemical fertilizers are used in combination to achieve the nutrient requirements of crop through integrated nutrient management (Bharti *et al.*, 2016). Nitrogen also obtained from organic sources and that must be transformed into inorganic i.e. ammonium (NH₄⁺) or nitrate (NO₃⁻) form before consuming by plant. The supply of animal manures along with chemical sources is productive for maximizing crop productivity. Integrated use of organic and inorganic fertilizer can help in the management of soil health and sustainable crop production (Meena *et al.*, 2019). Poultry manure is an enriched source of macro and micro nutrients which have great influence on soil health and it use as an organic matter for the soil, increases biological life of the soil and improved water holding capacity of the soil (Jan *et al.*, 2018b). Nutrients release from organic manures is very slow, therefore, organic fertilizers interaction with beneficial microbes aided quickly in the nutrients release (Shaheen *et al.*, 2017). The microorganisms cultured in beneficial microbes are lactic acid bacteria, photosynthetic bacteria, yeast and actinomycetes and its help in production of

hormones and enzymes, encourage photosynthesis, soil disease prevention and start lignin degradation in the soil (Shah *et al.*, 2019). Lactic acid bacteria are plant growth promoting bacteria which promote growth by enhancing availability of nutrients from organic sources and also avoid plant from abiotic and biotic stresses (Lamont *et al.*, 2017). Photosynthetic bacteria are capable of nitrogen fixation (Ge and Zhang, 2015), secrete growth hormones and antiviral factors (Katsuda *et al.*, 2004), improve plant roots growth (Kuo *et al.*, 2012), enhance photosynthesis and chlorophyll content (Lee *et al.*, 2008) and reduce toxic effects of heavy metals (Nunkaew *et al.*, 2014).

The selection of fertilizer, the application timing and the application method and the rate of fertilizer are significant characteristics for reducing losses of nitrogen from the soil (Rhezali and Lahlali, 2017). Optimum dose of nitrogen and its timely application important for boosting wheat grain yield and protein content, as well as preserving ecological sustainability. Nitrogen fertilizer splitting application increases economic yield, grains protein content and nitrogen uptake and decreasing the production losses (Walsh *et al.*, 2018). Cereal crops yield parameters and N uptake influences by the application method and timing of nitrogenous fertilizer (Duan *et al.*, 2015). Keeping in view the importance of integrated use of nitrogen and its application timing, the current experiment was conducted to evaluate the influence of nitrogen application from organic and inorganic fertilizer with and without beneficial microbes and its application timing for higher wheat productivity. The objectives of the experiment were (1) To examine the impact of different nitrogen sources with and without beneficial microbes on phenology and crop stand of wheat, (2) To investigate the impact of application timing of fertilizer on wheat phenology and crop stand (3) finding the its interaction response on wheat productivity.

Materials and Methods

Research site

To manage the influence of integrated nitrogen with and without beneficial microbes and its application timing on wheat phenology and crop stand, a field experiment was conducted at Agronomy Research Farm, The University of Agriculture Peshawar (34°N, 71°E) during 2019-20. Before experiment, five randomly soil sample of 20 cm depth taken for finding physico-chemical properties. Site soil was alkaline

with pH (8.3), SOC (0.53%), total N (0.051%), mineral N (17.94 mg kg⁻¹), extractable P (2.8 mg kg⁻¹), K (88.3 mg kg⁻¹) and soil bulk density (1.25 g cm⁻³). Experimental location soil was silt loam with silt (54.9%), sand (28.2%) and clay (16.1%) piedmont alluvium and classified as Ustochrepth based on USDA classification (Anonymous, 2007). During the experiment, minimum temperature ranged from 5.8 °C (December, 2019) to 21.8 °C (May, 2020), while maximum temperature ranged from 18.9 °C (December, 2019) to 36.7 °C (May, 2020). Due to high rainfall (446.9 mm) during experiment, total three irrigations (first at 21 December 2019 and other two in March 2020) were applied at initiation of crown root, tillering and anthesis stage using flood irrigation.

Treatments and experimental design

For managing nitrogen sources i.e. farmyard manure (FYM), poultry manure (PM) and urea with and without beneficial microbes (BM), this experiment was conducted. The experiment was arranged in randomized complete block (RCB) design with four replications. Experiment was consisting of two factors: one was different ratios of nitrogen (50% FYM + 50% urea + BM, 50% FYM + 50% PM + BM, 50% PM + 50% urea + BM, 50% FYM + 50% urea – BM, 50% FYM + 50% PM – BM and 50% PM + 50% urea – BM) and other is application timing (1 day before sowing (27 November 2019), 15 days before sowing (12 November 2019) and 30 days before sowing (28 October-2019)). There were two controls: one was 100% N from urea and other was without fertilizer. Organic sources (FYM and PM) added in the field above mentioned days before sowing, while urea applied in split form after sowing. These ratios compensated total of 120 kg N ha⁻¹ to all experimental plots, except control plot. The FYM and PM were collected from the Dairy Farm, The University of Agriculture, Peshawar before starting experiment. Before applying of organic manures their nutrient analysis was done in laboratory for finding NPK (nitrogen, phosphorus and potassium) concentration and then applied according to the recommended rate (120:90:60 kg ha⁻¹). FYM consist of 0.64% N, 0.53% P and 0.40 % K, while PM consists of 0.75% N, 0.62% P and 0.49 % K. The urea contains 46% N. The phosphorus and potassium were compensated from manures (FYM and PM) and remaining from single super phosphate and sulphate of potash, respectively. The beneficial microbes were provided from "BIOAAB" containing *Rhodospseudomona* ssp. (photosynthetic bacteria),

Lactobacillus spp. (lactic acid bacteria) and *Saccharomyces spp.* (yeast), manufactured locally by Nature Farming Research and Development Foundation (NFRDF), non-government organization (NGO) in association with The University of Faisalabad-Pakistan. Microbes in the bioaab are dormant and need to be stimulated to form an extended solution. In the recommended method, microbes (5%) were added to molasses solution (5%) and water (90%) to make 100% solution according to APNAN manual (APNAN, 1995) for the use of beneficial microbes. For our experiment, to prepare the needed amount of 9 liters solution, 0.45 liters microbes solution was added to 0.45 liters molasses solution and 7.2 liters water. This solution was kept sealed at a cool and dark place for 7 days until its pH fell to 4.0. The lid of the container was unlocked each day for a moment to release the gases produced due to fermentation. After one week the solution containing the extended population of microbes was ready to use. The prepared bioaab solution (10%) was applied at the rate of 250 ml plot⁻¹. The prepared bioaab solution was applied at the time of manures application as mentioned above. However, in case of the without beneficial microbes plots, solution was not applied. Field was ploughed two times using common cultivator and treatments were assigned to the experimental plots after field layout. The plot size was 3m x 1.8m with row to row distance of 30 cm having six rows. Rotavator was used for preparing seed bed and sowing done on seed drill. Wheat variety Pirsabak-2015 was sown on 29th November 2019. All the agronomic methods were kept constant for the all plots. The crop was harvested on 7th May 2020.

Observations and measurements

Random manures samples were taken from dairy farm and soil samples (20 cm depth) were taken from five locations from the whole experimental plot and brought to laboratory for elemental analysis. Samples were air dried and grinded with grinder (Kinematic, Switzerland) of 2 mm mesh size, and then determined N, C, pH and EC in the sample. The determination of mineral N was found according to Keeney and Nelson (1982) and total nitrogen was measured by the Kjeldahl principle of Bremner and Mulvaney (1982) and phosphorus was determined by the technique of Soltonopour and Schaswab (1997). Soil organic carbon was measured by the protocol of Nelson and Sommers (1982). The soil pH was measured using the Mclean (1982) procedure of 1:5 (soil: water) suspension, whereas the soil bulk density was found with procedure of Black and Hartge (1984).

Days were noted from sowing till the 75% emergence of the seed in each experimental plot and considered as days to emergence data. After emergence of all seedling in the experimental plot, the emergence was find by measuring the three rows through meter rod and counted all plants occupied in one meter and then changed into emergence (m⁻²). Days to heading, anthesis and physiological maturity stage were noted from sowing till the 75% plants reached to heading, anthesis and physiological maturity stage in each plot, respectively. Three leaves in five tillers were taken in each plot and their length and width was noted through measure tap and number of leaves tiller⁻¹ also recorded. Then leaf area tiller⁻¹ was determined by following formula:

$$\text{Leaf area tiller}^{-1} = \text{leaf length} \times \text{leaf width} \times C.F \times \text{no. of leaves tiller}^{-1}$$

C.F = correction factor = 0.65 (Jalal *et al.*, 2020)

The leaf area index (LAI) was find by using the following formula:

$$LAI = \text{leaf area/ground area}$$

Plant height was measured from the base of plant to the tip of the spike including awns via meter rod by choosing five plants in each plot at physiological maturity stage and then averaged. Tillers was noted by counting the number of tillers in one-meter selecting three rows and then changed into number of tillers (m⁻²) using the following formula:

$$\text{Tillers (m}^{-2}\text{)} = \frac{\text{No. of tiller counted n rows}}{\text{No. of rows} \times R - R \text{ distance} \times \text{row length}} \times 1$$

Statistical analysis

The data was statistically analyzed by analysis of variance techniques applicable for randomized complete block design and means was compared by using LSD test at 0.05 level of probability (Steel *et al.*, 1997).

Results and Discussion

Days to emergence

Days to emergence was significantly (p<0.05) influenced by nitrogen sources ratios with and without beneficial microbes (BM) and their application timing, while their interaction (N × AT) was found non-significant (Table 1). Among the application timing, delayed emergence (15.5 days) was recorded with application of nitrogen organic sources one day

before sowing as compared to 15 and 30 days before sowing (14.5 and 14.4 days, respectively). In case of the N sources ratios, delayed emergence (15.4 days) was recorded in plot which received 50% N each from FYM and urea without the amendment of BM. In comparison, unfertilized plot and plot fertilized 100% from urea had significantly delayed emergence (16 days) as compared to plots fertilized from integrated nitrogen (14.8 days).

Table 1: Effect of nitrogen (N) application timing and nitrogen sources on the days to emergence, days to heading and days to anthesis of wheat.

Treatments	Parameters		
	Days to emergence	Days to heading	Days to anthesis
N application timing (AT)			
1 DBS	15.5 a	111.1 c	120.8 c
15 DBS	14.5 b	113.8 b	124.0 b
30 DBS	14.4 b	115.1 a	125.3 a
LSD _(0.05)	0.25	0.74	0.72
Nitrogen sources (N) @ 120 kg ha⁻¹			
50% FYM + 50% Urea + BM	14.6 bc	113.5 b	123.8 ab
50% FYM + 50% PM + BM	14.5 c	114.8 a	124.7 a
50% PM + 50% Urea + BM	14.4 c	114.3 ab	124.4 a
50% FYM + 50% Urea -BM	15.4 a	111.9 c	121.7 c
50% FYM + 50% PM -BM	14.9 b	112.8 bc	122.7 bc
50% PM + 50% Urea -BM	15.1 ab	112.7 bc	122.9 b
LSD _(0.05)	0.35	1.05	1.03
Control vs rest	*	*	*
Control 1 (unfertilized)	16.0 a	108.0 b	118.0 b
Rest treatments	14.8 b	113.3 a	123.3 a
Significance	*	*	*
Control 2 (100% Urea)	16.0 a	107.8 b	119.0 b
Rest treatments	14.8 b	113.3 a	123.3 a
Significance	*	*	*
Interaction			
AT × N	ns	*	*

DBS: days before sowing; FYM: farm yard manure; PM: poultry manure; BM: beneficial microbes.

Days to heading

Nitrogen sources with and without beneficial microbes and its application timing significantly ($p \leq 0.05$) affected heading days of wheat crop (Table 1) and its interaction (N × AT) was also significant. Among nitrogen sources, delayed heading (114.8 days) was recorded with application of 50% N each from FYM and PM with beneficial microbes, while early heading (111.9 days) was with 50% N each from FYM and urea without beneficial microbes.

Organic manures application 30 days before sowing observed late heading (115.1 days) as compared to 1 and 15 days before sowing (111.1 and 113.8 days, respectively). Plots treated with integrated nitrogen significantly delayed heading (113.4 days) as compared to non-fertilized and 100% fertilized from urea (107.8 and 108 days, respectively). Interaction of nitrogen sources and its application timing (Figure 1) shows that application of nitrogen organic sources before sowing, days to heading delayed with addition of nitrogen from integrated sources with beneficial microbes. Heading delayed with application of 50% N each from FYM and PM with beneficial microbes and applied 30 days before sowing.

Days to anthesis

Anthesis in wheat significantly ($p \leq 0.05$) influenced by integrated nitrogen with and without beneficial microbes and its application timing, while their interaction was also found significant (Table 1). Delayed anthesis (125.3 days) was reported with nitrogen sources application 30 days before sowing as compared to 1 day before sowing (120.8 days) and 15 days before sowing (124 days). In case of the nitrogen ratios, late anthesis (124.7 days) was noted when nitrogen applied as 50% N each from FYM and PM in combination with beneficial microbes, while early anthesis (121.7 days) was recorded when applied as 50% N each from FYM and urea without beneficial microbes. Plots fertilized from integrated nitrogen took more days to anthesis (123.3 days) as compared to untreated plot (118 days) and treated 100% with urea (119 days). The interaction between nitrogen and its application timing (Figure 2) indicates that addition of organic nitrogen sources before sowing, days to anthesis delayed with addition of nitrogen from integrated sources with beneficial microbes. Delayed anthesis recorded with addition of 50% N each from FYM and PM along with beneficial microbes and applied in the field 30 days before sowing.

Days to physiological maturity

Days to physiological maturity was significantly ($p \leq 0.05$) influenced by nitrogen sources with and without beneficial microbes and its application timing, while their interaction was found insignificant (Table 2). Among nitrogen sources application timing, late physiological maturity (159 days) was observed when applied 30 days before sowing in comparison with the 15 days before sowing (158.8 days) and 1 day before sowing (157.5 days). Delayed physiological maturity

(158.9 days) was noted in plots which received nitrogen 50% each from PM and urea and 50% each from FYM and PM with the inoculation of beneficial microbes, while earlier physiological maturity (157.9 days) was noted with addition of 50% N each FYM and PM without beneficial microbes. Integrated nitrogen treated plots significantly late physiological maturity (158.4 days) in comparison with non-fertilized plot and plot 100% fertilized from chemical source (157.3 days).

Table 2: Effect of nitrogen (N) application timing and nitrogen sources on the days to physiological maturity, emergence (m^{-2}) and leaf area tiller $^{-1}$ (cm^2) of wheat.

Treatments	Parameters		
N Application timing(AT)	Days to physiological maturity	Emergence (m^{-2})	Leaf area tiller $^{-1}$ (cm^2)
1 DBS	157.5 b	97	108.6 c
15 DBS	158.8 a	98	117.7 b
30 DBS	159.0 a	98	121.6 a
LSD _(0.05)	0.41	ns	2.88
Nitrogen sources (N) @ 120 kg ha $^{-1}$			
50% FYM + 50% Urea + BM	158.9 a	98	117.2 ab
50% FYM + 50% PM + BM	158.9 a	98	118.9 a
50% PM + 50% Urea + BM	158.7 ab	99	118.8 a
50% FYM + 50% Urea - BM	158.1 b	98	112.1 c
50% FYM + 50% PM - BM	157.9 b	98	114.4 bc
50% PM + 50% Urea - BM	158.2 b	97	114.4 bc
LSD _(0.05)	0.58	ns	4.08
Control vs rest	*	ns	*
Control 1 (unfertilized)	16.0 a	97	88.9 b
Rest treatments	14.8 b	98	116.0 a
Significance	*	ns	*
Control 2 (100% Urea)	16.0 a	97	105.2 b
Rest treatments	14.8 b	98	116.0 a
Significance	*	ns	*
Interaction			
AT × N	ns	ns	ns

DBS: days before sowing, FYM: farm yard manure, PM: poultry manure, BM: beneficial microbes.

Emergence (m^{-2})

Different integrated nitrogen ratios with and without beneficial microbes and their application timing had non-significant ($p \leq 0.05$) effect on the emergence (Table 2). Likewise, interaction between nitrogen sources ratios and its application timing was also found non-significant.

Leaf area tiller $^{-1}$ (cm^2)

Leaf area tiller $^{-1}$ of wheat was significantly ($p \leq 0.05$) varied with nitrogen sources with and without beneficial microbes (BM) and its application timing, while their interaction was found insignificant (Table 2). Maximum leaf area tiller $^{-1}$ ($121.6 cm^2$) was recorded when organic sources of nitrogen applied 30 days before sowing as contrast to 1 day before sowing ($108.6 cm^2$) and 15 days before sowing ($117.7 cm^2$). In case of the nitrogen ratios, highest leaf area tiller $^{-1}$ ($118.9 cm^2$) was recorded with addition of nitrogen 50% each from PM and FYM with BM, which is statistically same with 50% nitrogen each from PM and urea with BM ($118.8 cm^2$). Lowest leaf area tiller $^{-1}$ ($112.1 cm^2$) was noted when applied half nitrogen each from FYM and urea without BM. Plots treated with integrated nitrogen produced significantly maximum leaf area tiller $^{-1}$ ($116 cm^2$) as compared to non-treated plot ($88.9 cm^2$) and 100% fertilized from urea ($105.2 cm^2$).

Number of tillers (m^{-2})

Integrated nitrogen with and without beneficial microbes (BM) and its application timing had significantly ($p \leq 0.05$) affected tillers of wheat, while their interaction was found not significant (Table 3). Among the application timing, maximum tillers ($285 m^{-2}$) was noted in those plots when applied nitrogen sources 30 days before sowing as compared to 1 day ($275 m^{-2}$) and 15 days before sowing ($281 m^{-2}$). More tillers ($286 m^{-2}$) was produced with application of recommended nitrogen half each from PM and FYM with the inoculation of BM, while less tillers ($275 m^{-2}$) was reported with addition of 50% N each from FYM and urea without BM. Plots treated with integrated nitrogen had produced significantly more tillers ($280 m^{-2}$) as compared to non-treated ($266 m^{-2}$) and treated 100% with urea ($275 m^{-2}$).

Plant height (cm)

Plant height (cm) significantly varied by different nitrogen ratios with and without BM and its time of application, whereas their interaction was found not significant (Table 3). Organic sources of nitrogen added in the field one month before sowing recorded taller plants ($91.3 cm$), while dwarf plants were recorded when added 1 and 15 days before sowing (88.1 and $90.3 cm$, respectively). Among sources of nitrogen, taller plants ($92.5 cm$) were measured in the plot when applied 50% N each from FYM and PM with the interaction of BM, while dwarf plants ($88 cm$) were noted with application of half nitrogen each from FYM and urea without BM. Plots treated with

integrated nitrogen produced taller plants (89.9 cm) as compared to untreated and 100% treated from urea (84 cm and 87.7 cm, respectively).

Leaf area index

Integrated nitrogen with and without beneficial microbes (BM) and its time of application had significantly ($p \leq 0.05$) varied leaf area index (LAI) of wheat, while their interaction was found not significant (Table 3). Among the application timing of nitrogen sources, highest LAI (3.5) was noted when applied 30 days before sowing as compared to 1 day (3.0) and 15 days before sowing (3.3). Highest LAI (3.4) was produced with application of 50% N each from PM and FYM with BM and 50% N each from PM and urea with BM. Lowest LAI (3.1) was reported with addition of 50% N each from FYM and urea without BM. In comparison, plots treated with integrated nitrogen had produced significantly maximum LAI (3.3) as compared to non-treated (2.4) and treated 100% with urea (2.9).

Table 3: Effect of nitrogen (N) application timing and nitrogen sources on the tillers (m^{-2}), plant height (cm) and leaf area index of wheat.

Treatments	Parameters		
N application timing (AT)	No. of tillers (m^{-2})	Plant height (cm)	Leaf area index
1 DBS	275 c	88.1 c	3.0 c
15 DBS	281 b	90.3 b	3.3 b
30 DBS	285 a	91.3 a	3.5 a
LSD _(0.05)	2.97	0.72	0.09
Nitrogen sources (N) @ 120 kg ha ⁻¹			
50% FYM + 50% Urea + BM	280 b	90.3 c	3.3 ab
50% FYM + 50% PM + BM	286 a	92.5 a	3.4 a
50% PM + 50% Urea + BM	283 ab	91.4 b	3.4 a
50% FYM + 50% Urea - BM	275 c	88.0 e	3.1 b
50% FYM + 50% PM - BM	280 b	89.2 d	3.2 b
50% PM + 50% Urea - BM	276 bc	88.2 d	3.2 b
LSD _(0.05)	4.20	1.02	0.13
Control vs rest	*	*	*
Control 1 (unfertilized)	266 a	84.0 b	2.4 b
Rest treatments	280 b	89.9 a	3.3 a
Significance	*	*	*
Control 2 (100% Urea)	275	87.7 b	2.9 b
Rest treatments	280	89.9 a	3.3 a
Significance	ns	*	*
Interaction			
AT × N	ns	ns	ns

DBS: days before sowing, FYM: farm yard manure, PM: poultry manure, BM: beneficial microbes.

Phenological observations

Emergence of wheat influenced by food stored in the cotyledon (Saharan *et al.*, 2016), optimal temperature (Zavattaro *et al.*, 2017) and soil moisture (Saikia *et al.*, 2015). Nitrogen application at the rate of 120 kg ha⁻¹ applied as 50% each from PM and urea with beneficial microbes took less days to emergence (Table 1). It might be due to organic manures application before sowing which increased water holding capacity of soil. Other possible reason is rapid decomposition of poultry manure by beneficial microbes and also application of urea which readily available nutrients to the plants for absorption (Muhammad *et al.*, 2014). Other probable reason is the increasing of soil permeability by manures which soften the soil surface for emergence and increase soil moisture (Saikia *et al.*, 2015). Poultry manure contains phosphorus which is a vital component of ATP, the energy unit of plants which help in early emergence of crop (Khan *et al.*, 2013).

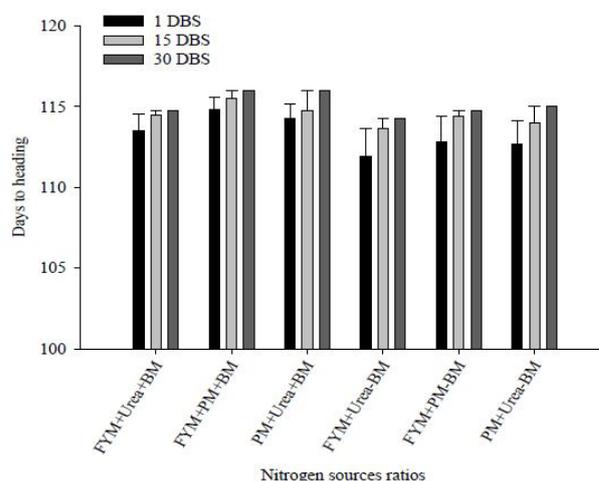


Figure 1: Interaction of nitrogen sources and application timing for days to heading of wheat. (DBS= days before sowing, BM= beneficial microbes). Each of the farmyard manure (FYM), poultry manure (PM) and urea were applied to provide 50% of 120 kg N ha⁻¹.

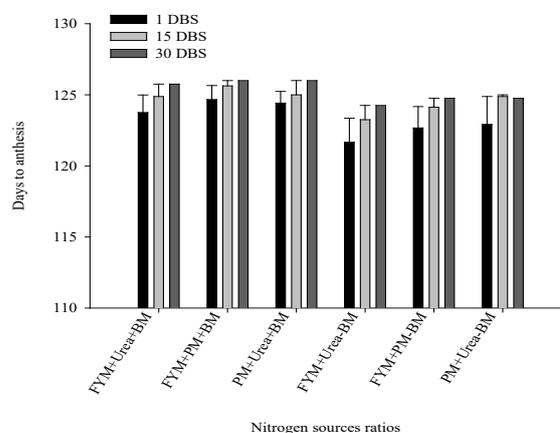


Figure 2: Interaction of nitrogen sources and application timing for days to anthesis of wheat. (DBS= days before sowing, BM= beneficial microbes). Each of the farmyard manure (FYM), poultry manure (PM) and urea were applied to provide 50% of 120 kg N ha⁻¹.

Vegetative growth (Zhang *et al.*, 2015), availability of nutrients (Khan *et al.*, 2019a) and stand of crop (Ibrahim and Khan, 2017) influenced the wheat heading, anthesis and physiological maturity. Environmental temperature directly affected the physiological maturity (Olesen *et al.*, 2012) and vegetative growth in directly affects it (Khan *et al.*, 2008). Heading, anthesis and physiological maturity of wheat crop delayed with nitrogen application 30 days before sowing at the rate of 120 kg ha⁻¹ compensated as 50% each from PM and FYM with the inoculation of beneficial microbes as compared to unfertilized and 100% fertilized from urea (Tables 1 and 2). This is due to addition of organic manures which enhanced the availability of mineral nitrogen (Khan *et al.*, 2019a), providing macro and micronutrients (Bowles *et al.*, 2014), and consequently delayed the wheat phenology. It might be due to constant cell multiplication and division with suitable quantity of nitrogen and potassium from organic sources which prolonged vegetative stage (Khan *et al.*, 2021b). Nitrogen itself is also responsible for late phenology (Anjum and Khan, 2020) and manures application reduced nutrients losses as compared to chemical fertilizers (Geng *et al.*, 2019) which timely available nutrients to the plants which later delayed phenology. Integrated nitrogen delayed anthesis in wheat (Shafi *et al.*, 2018). Manures (farmyard and poultry manures) contain greater amount of essential nutrients which hastens vegetative growth and delayed wheat maturity (Jan *et al.*, 2018b). Management of nitrogen application timing delayed heading stage in wheat (Nakano *et al.*, 2008). Sharifi and Namvar (2016) and Hammad *et al.* (2013) also confirmed that nitrogen application timing had great influence on the crop phenology. The result is similar with the Assefa and Mekonnen (2019) who reported that 40% nitrogen application each at sowing and tasseling and 20% at 40 days after sowing delayed maize physiological maturity and concluded that application timing and levels of nitrogen should be consider in crop yield.

Plots treated beneficial microbes delayed phenology (heading, anthesis and physiological maturity) as compared to the untreated plots (Tables 1 and 2). It might be due to the beneficial microbes which accelerate the decomposition rate of manures and rapidly available essential nutrients to the plants which helping the plants to prolonged their vegetative growth. Application of beneficial microbes had stimulated manures decomposition and later

enhanced the availability of nutrients (Liu *et al.*, 2010), prolonged the asexual growth (Khan *et al.*, 2019b) due to shifting of the metabolites and its transferring to the vegetative tissues (Khan *et al.*, 2014) and consequently delayed the phenology. Population of soil microbes increased with the addition of beneficial microbes (Ali *et al.*, 2019), increased release of nutrients (Khan *et al.*, 2019a) and hence late the physiological maturity.

Crop growth and stand establishment

Emergence (m⁻²) is depending upon seed quality (Abati *et al.*, 2017), soil moisture (Saikia *et al.*, 2015), temperature stress and availability of oxygen (Balla *et al.*, 2019). Emergence (m⁻²) of wheat was non-significantly affected by different nitrogen sources with and without beneficial microbes and its application timing, while their interaction also found non-significant (Table 2). It is due to the endosperm of grain that provide nutrients to seed and that is why external nutrients have no influence on emergence (m⁻²) of the crop (Saharan *et al.*, 2016). Our results are in accordance with Liaquat *et al.* (2019) who reported that poultry manure, urea and beneficial microbes had no significant effect on the emergence (m⁻²) of wheat. For that reason, nitrogenous fertilizers sources either organic or inorganic have no important role in the emergence.

The wheat plant height depends on environmental conditions and agronomic practices. Obtaining optimum height is of more advantages for the stability, productivity and yield safety of the genotypes (Bognar *et al.*, 2007). Integrated nitrogen application with beneficial microbes recorded taller plants as compared to sole urea application and control plot (Table 3). The possible cause for taller plant with beneficial microbes inoculation might be the enriched availability of plant nutrients especially nitrogen (Fang *et al.*, 2018). Beneficial microbes have the capability to undergo hasty decomposition of manures and make nutrients available to crop and later helps in up taking more nutrients (Akhtar *et al.*, 2019c; Wen *et al.*, 2016). Other possible reasons are plant photosynthesis proficiency (Saikia *et al.*, 2015) and nitrogen role in cell expansion and division that ultimately influence vegetative growth (Singh and Agarwal, 2001). These results are also reported by Ali *et al.* (2019) and Shah *et al.* (2019). Organic sources of nitrogen applied 30 days before sowing in the field recorded maximum plant height as compared to the 1 and 15 days before sowing (Table 3). Adekiya and Agbede (2017) also

reported taller plants with addition of poultry manure three weeks before sowing.

Light, temperature, genotype, soil moisture, plant spacing and nutrition practices influence tillering (Pawar *et al.*, 2016). Nitrogen application one month before sowing at the rate of 120 kg ha⁻¹ supplied as 50% each from FYM and PM with beneficial microbes produced significantly more tillers (Table 3). The application of N from manures in addition to the other nutrients (Foster *et al.*, 2016) increase the plant growth (Xie *et al.*, 2016), number of leaves and leaf size (Hariadi *et al.*, 2016), formation of photo-assimilate (Saikia *et al.*, 2015), and thus the development of tillers. This also might be due to the application of organic manures with beneficial microbes which contain certain bacteria which help in faster decomposition of manures. Organic manures improve characteristics of soil, uptake of nutrients, maintaining sustainable environment and less risk of nutrients leaching (Farooq *et al.*, 2020). Quicker decomposition adds macro and micro-nutrients to the soil which made readily available to the plant that helps in crop growth (Khan *et al.*, 2018). Other possible reason is the addition of these manures 30 days before sowing (Table 3). Therefore, application of nitrogen at suitable time has high potential and vigor which helps in maximum quantity of assimilates transportation from source to sink organs and ultimately resulted in maximum tillers (m⁻²) in wheat (Kibe *et al.*, 2006).

Leaf area is an imperative factor to assess several attribute of crop plants like canopy, photosynthesis and evapotranspiration (Ahmad *et al.*, 2015). The leaf area index (LAI) directly affects crop photosynthetic efficiency and ultimately influence biological yield (Tan *et al.*, 2020). Nitrogen application from organic sources (FYM and PM) in 50:50 ratios with the inoculation of beneficial microbes enhanced leaf area tiller⁻¹ and LAI as compared to sole urea and control (Tables 2 and 3). This might be due to beneficial microbes which contain photosynthetic bacteria and it also accelerated the mineralization of nitrogen organic sources and ultimately enhanced leaf area (Shaheen *et al.*, 2017; Pan *et al.*, 2018). Maximum leaf area and LAI could be due to higher availability of nutrients (Yao *et al.*, 2018) and residual effect (Reeve *et al.*, 2012) due to addition of manures which increase the net N. Maximum N can consider to improve the plants photosynthetic efficiency (Dabin

et al., 2016), and hence increase the size and number of the cell (Akhtar *et al.*, 2019b; Keller and Koblet, 2015), and consequently the leaf area and LAI. These results confirmed by (Anjum and Khan, 2020) and (Ali *et al.*, 2019). Organic sources of nitrogen when applied 30 days before sowing increased leaf area as compared to 1 and 15 days before sowing (Table 2). This is due to the manures slow release nature of nutrients from manures which reduces losses and prolonged nutrients availability for plant growth (Adekiya and Agbede, 2017). Panison *et al.* (2019) also determined that timing of nitrogen fertilizers application influenced leaf area. Feng *et al.* (2017) also documented that addition of nitrogen in ratios at different timing recorded higher wheat vegetative growth which also linked with the LAI.

Conclusions and Recommendations

It is concluded that application of 120 kg N ha⁻¹ applied as half each from farmyard manure (FYM) and poultry manure (PM) with the interaction of beneficial microbes (BM) improved crop stand, leaf area and delayed wheat phenology. In case of the application timing of nitrogen, nitrogen organic sources applied 30 days before sowing increased plant height, leaf area and prolonged phenological parameters of wheat. Hence, it is recommended that application of 120 kg N ha⁻¹ as half each from FYM and PM combined with BM and applied 30 days before sowing is best production practice to improve crop stand and delayed wheat phenology in agro-ecological condition of Peshawar.

Novelty Statement

Phenological observations delayed and crop stand improved with 120 kg N ha⁻¹ applied as 50% from FYM and 50% from PM with combination of BM and its application in the field 30 days before sowing.

Author's Contribution

Faiq Ahmad: Carried out research and drafted the manuscript.

Shahen Shah: Conceived the idea and supervised the research.

Muhammad Amin: Helped in data analysis

Ikram Ullah: Results and discussion interpretation

Sajid Ali: Helped in data collection

Maaz Khan: Helped in data collection and tabulation

Muhammad Shakur: Assisted in format setting, editing and final draft improvement

Conflict of interest

The authors have declared no conflict of interest.

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