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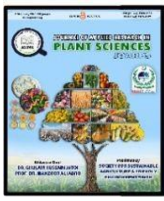

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Response of bread wheat (*Triticum aestivum L.*) to Different Tillage Practices and Nitrogen Levels in a Clay Loam Soil

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Abstract

We can overcome food security this global challenge by minimizing input costs and optimizing yields by employing appropriate tillage techniques and balanced fertilizer application. Appropriate tillage practices together with balanced fertilizer may be a promising practice of soil management to improve soil properties and crop production. The present study was carried out in wheat-maize cropping pattern (in the year 2019-20) at agricultural research farm of Gomal University, Dera Ismail Khan, Pakistan. The experiment was set up in a split-plot layout with a randomized complete blocks design. The nitrogen levels of 0, 90, 120, 150, and 180 kg N ha^{-1} were assigned to subplots. Tillage and nitrogen treatments separately had a significant impact on growth and yield characteristics, but the interaction between them had minimal effect, according to the findings. The tallest heights were found in the conventional tillage plots and the high N input (N180) plots, with heights of 101 and 104 cm, respectively. The spikes per m^2 were found 242 in zero tillage plots, while the highest number of spikes per plot (265) was recorded in N180. The yield contributing factor number of grain spike^{-1} was 48.7 in zero tillage, while the nitrogen fertilizer @ 180 kg ha^{-1} yielded 52, 1000-grain weight of 35g was measured in conventional tillage and 38.8g in nitrogen fertilizer plots. Deep tillage where the nitrogen @ 150 kg ha^{-1} was applied gave the maximum grain yields, with 3113 kg ha^{-1} and 3415 kg ha^{-1}, respectively. The benefit-cost ratio of different treatments was highest in zero tillage plots, with nitrogen @ 150 kg ha^{-1}, at 1.65. Hence, it may be concluded from the study that both tillage and nitrogen levels influenced the agronomic properties of wheat, influenced the economics of farmers, and soil fertility.

Keywords: Triticum aestivum; Tillage; Nitrogen; Economics; Yield; Soil Fertility

Introduction

Wheat (*Triticum aestivum* L.) is a highly regarded staple food all over the world (Mishra *et al.*, 2019). Wheat is cultivated more than rice, maize, and potatoes all over the world due to its high nutritional need (Zohary *et al.*, 2000). It has higher food value over rice as it contains excellent amount of nutrition *i.e.* 70.10% carbohydrates, 11-12% protein, 2.10% fat and 2.10% mineral (Katayan, 2019). Its demand has increased over the year due to the increase in population. The next century will be very different from the previous one, as another 3 billion or so humans join the current population of 6.5 billion, so as in near future it will create food insecurity in those countries where wheat is utilized as staple food (Jury and Vaux, 2007). It becomes imperative to increase the area under wheat crop or enhance wheat yield by management practices (Abbas *et al.*, 2007). Amongst the soil management practices tillage operations and appropriate fertilizer application have played a significant role among the different elements impacting wheat crop productivity. With appropriate tillage, soil physical condition and tilth are improved (Khan *et al.*, 2010), increasing crop nutrient usage efficiency and eventually leading to good crop yields (Bahadur *et al.*, 2015). Deep and conventional tillage operations, according to Caroca *et al.* (2011), are helpful in enhancing soil characteristics and conserving nutrients and moisture in the soil for plants and microbes. But others think that deep and conventional tillage results in loss of organic matter due to rapid decomposition and release of carbon into the atmosphere. They considered zero and mulch tillage as most effective and environmentally friendly (Allmaras *et al.*, 2018; Ussiri and Lal, 2009). Wheat improper inputs management is one of the many reasons for its lower productivity. Amongst the fertilizers, nitrogen is the primary major nutrient required by plants for their growth, physiological functions, and crop yield. Amongst the nutrients Nitrogen is the macro – nutrient which is utilized in greater amount by the plants and has soil losses greater than the other nutrients, hence its importance has increased over the years. Fageria (2014) reported that using nitrogen at an appropriate rate increases the wheat grain yield and harvest index. It has become important to study the availability of this nutrients under different tillage operations. Tillage operations manipulate the soil and usually influence soil structure, its impact on the availability of nutrients specially nitrogen is rarely studied (Kladivko, 2001). Some of the researcher consider the conservation tillage or zero tillage as the most effective means for rendering nitrogen in soil (Bartaula *et al.*, 2020). It is hypothesized that optimum nitrogen level for yield of wheat is influenced by the tillage operations. Considering the importance of tillage and fertilizer in wheat yield, a field experiment was carried out utilizing various implements to pulverize soil and apply inorganic nitrogen fertilizer. The study was carried out to investigate the influence of tillage and nitrogen fertilizer on wheat growth and yield, as well as the economics of farmers, which is critical for Pakistan's subsistence farmers.

Materials and Methods

Field experimentation was done at the Agricultural research farm of Gomal University, Dera Ismail Khan, Pakistan, in the year 2019-20 (31º49.696'N and 70º54.919'E) to study the effect of tillage operations and nitrogen levels on soil physical and chemical properties and yield components of the wheat crop, in wheat-maize cropping pattern. Climatically this region is characterized as subtropical arid. The soil used in the study was Typic Torrifluvents, Zindani series. The study was designed in a randomized complete block design with five treatments, replicated four times. In the main plots, tillage practices including mulch tillage (MT), deep tillage (DT), conventional (CT), and zero tillage (ZT) were allocated, while in subplots, the size of sub plot 1.8 x 3 m^{-2} with different nitrogen levels (i.e., 0, 90, 120, 150, and 180 kg ha^{-1}) were applied. The source of nitrogen fertilizers was urea applied into two splits, one at seed spreading and the second half was given at the time of 1st irrigation on crown root initiation stage and other irrigations were given with interval of 25 to 30 days. The phosphatic fertilizer, single super phosphate, was given at the time of second irrigation. The Wheat (Faisalabad-2008) was sown in rows (distance between two plants and rows was kept as 10 cm and 30 cm) on the 1st week of December 2019 by drill method.

Soil Sampling:

The soil physio-chemical characteristics were determined prior to the experiment by collecting a composite sample from the field and the sample was analyzed for various physicochemical Characteristics. The Soil pH and electrical conductivity was measured by pH meter and conductivity meter using the saturation extract method (Ryan *et al.*, 2001), while organic matter was determined titrimetrically (Nelson and Sommer, 1982), total nitrogen using Kjeldhal apparatus (Bremner, 1996), Olsen extractable phosphorus using spectrophotometer (Olsen *et al.*, 1954) and extractable potassium through Flame photometer (Richard, 1954). The detail of the physical and chemical analysis is given in Table 1

Table 1. Soil analysis of pre – sowing and post-harvest data at 0-30 cm depth

Parameters	Units	Values
pHe		8.07
ECe	$mScm^{-1}$	2.82
Organic matter	%	0.59
Soil organic carbon	%	0.34
Available nitrogen	mg kg^{-1}	6
Extractable phosphorus	mg kg^{-1}	6.70
Extractable potassium	mg kg^{-1}	160
Soil water retention	%	54
Bulk density	g cm^{-3}	1.12
Saturated hydraulic conductivity	cm/day	24
Textural class	Clay Loam	
Microbial activity	Soil phosphatase: 20.4 ng g^{-1}soil min^{-1} and Soil glucosidase: 2.42 ng g^{-1}soil min^{-1}	

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All the management practices include irrigation normal cultural practices such as (hoeing, weeding etc.) during the growing season. The harvesting of the crop was done on 2nd week of April. The seasonal weather conditions during the crop season are presented in Fig. 1. Agronomic parameters of wheat crop like plants height (cm), number of spikes m^{-2}, length of spikes (cm), grain spikes^{-1}, 1000- grains weight (g), leaf area (cm), crop yield (kg ha^{-1}), harvest index (HI) (%) and grain yield (kg ha^{-1}) were noted. Data were statistically analyzed (statistics 8.1) to determine the difference between the applied treatments by calculating the Least significant difference (LSD).

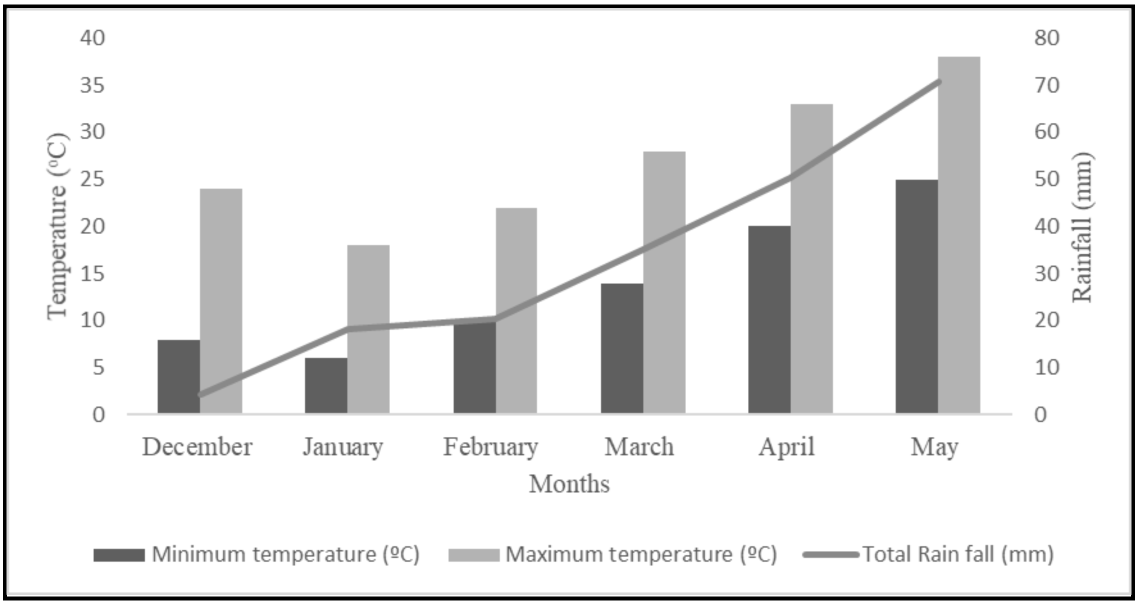

Fig. 1. Seasonal weather data

Table 2. Agronomic factors as affected by tillage and nitrogen levels.

Parameters	Plant height (cm)	Spikes m^{-2}	Grains spike^{-1}	1000 grain weight (g)	Grain yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (%)
T_1 Mulch tillage	94 b	197 b	44.5	34.4	2880 ab	5622 b	51.3 a
T_2 Deep tillage	99 a	224 a	42.5	35.9	3113 a	6518 a	47.9 ab
T_3 Conventional Tillage	101 a	242 a	45.3	34.7	2876 ab	6760 a	42.7 bc
T_4 Zero tillage	99 a	241 a	48.7	34.4	2386 b	6476 a	37.3 c
$LSD_{0.05}$	4.375	23.183	Ns	Ns	572.17	624.49	6.9046
N levels							
N0	95 d	182 d	38.9 d	34.2 bc	2280 e	5558 c	42.0 c
N90	95 cd	208 c	42.3 c	31.8 c	2568 d	5499 c	47.0 a
N120	98 bc	232 b	44.8 c	34.0 bc	2798 c	6196 b	45.2 ab
N150	100 b	244 b	48.5 b	38.8 a	3415 a	7847 a	44.0 bc
N180	104 a	265 a	51.9 a	35.5 b	3008 b	6622 b	45.7 ab
$LSD_{0.05}$	2.3909	18.260	2.7341	2.7757	162.39	440.94	2.6785
Interaction (T × N) $LSD_{0.05}$	NS	NS	NS	NS	NS	NS	NS

*-significant, ns-non significant, means in each column followed by the same letter do not differ significantly at $P_{0.05}$.

Layout of experiment

<table>
<tr><td></td><td></td><td colspan="22">MOST IMPORTANT WAY</td><td></td></tr>
<tr><td rowspan="3">R1</td><td rowspan="9">SUB WATER CHANEL</td><td colspan="22">WATER CHANEL</td><td rowspan="9">SUB WATER CHANEL</td></tr>
<tr><td colspan="6">T_1</td><td colspan="5">T_2</td><td colspan="5">T_3</td><td colspan="6">T_4</td></tr>
<tr><td>NEA</td><td>N1</td><td>N2</td><td>N3</td><td>N4</td><td>N5</td><td>N2</td><td>N3</td><td>N1</td><td>N5</td><td>N4</td><td>N1</td><td>N4</td><td>N5</td><td>N2</td><td>N3</td><td>N5</td><td>N4</td><td>N1</td><td>N3</td><td>N2</td><td>NEA</td></tr>
<tr><td rowspan="3">R2</td><td colspan="22">WATER CHANEL</td></tr>
<tr><td colspan="6">T_1</td><td colspan="5">T_2</td><td colspan="5">T_3</td><td colspan="6">T_4</td></tr>
<tr><td>NEA</td><td>N5</td><td>N4</td><td>N1</td><td>N2</td><td>N3</td><td>N4</td><td>N1</td><td>N5</td><td>N3</td><td>N2</td><td>N3</td><td>N5</td><td>N2</td><td>N1</td><td>N4</td><td>N2</td><td>N1</td><td>N5</td><td>N4</td><td>N3</td><td>NEA</td></tr>
<tr><td rowspan="4">R3</td><td colspan="22">CENTRAL CHECK WAY</td></tr>
<tr><td colspan="6">T_1</td><td colspan="5">T_2</td><td colspan="5">T_3</td><td colspan="6">T_4</td></tr>
<tr><td>NEA</td><td>N3</td><td>N5</td><td>N4</td><td>N1</td><td>N2</td><td>N5</td><td>N2</td><td>N3</td><td>N4</td><td>N1</td><td>N4</td><td>N1</td><td>N3</td><td>N2</td><td>N5</td><td>N3</td><td>N5</td><td>N2</td><td>N1</td><td>N4</td><td>NEA</td></tr>
<tr><td colspan="22">WATER CHANEL</td></tr>
</table>

Results

Effect of tillage and nitrogen levels on plant height (cm): Tillage (T) and nitrogen levels had a substantial effect on plant height; however, the T x N interaction was not significant (Table 2). Tillage mean values demonstrated that mulch tillage resulted in reduced plant height compared to deep tillage, conventional tillage, and zero tillage. The mean values for N levels exposed that plant height improved gradually with the incremental increase in N level and peaked at the highest N level (180 kg N ha^{-1}). The data show that, while the T x N interaction had no effect on plant height, the primary impacts of tillage and N levels had a considerable impact on plant height. DT and CT had profound effect on plant height, while MT showed the least effect on plant height. The Supreme plant height (104 cm) was observed at nitrogen level 180 kg $ha^{-1,}$ while zero kg N ha^{-1} showed the lowermost plant height (95 cm). MT did not influence plant height, probably due to the negligible effect of the previous crop cotton stalks incorporation with tillage operation. DT and CT had a profound impact on plant height; this might be on account of rapid mineralization due to excessive tillage.

Effect of tillage and nitrogen levels on spikes m^{-2}: The T and N levels had a substantial effect on spikes m^{-2}; however, the T x N interaction was not significant (Table 2). Tillage mean values demonstrated that MT had lower spikes m^{-2} than DT, CT, and ZT. Spikes m^{-2} grew progressively with the incremental rise in N level and peaked at the highest N level, according to mean values for N levels (180 kg N ha^{-1}). The findings show that T x N interaction had little effect on spikes m^{-2}, while the main impacts of tillage and N levels had a substantial impact on spikes m^{-2}. DT and CT had a profound effect on spikes m^{-2}. In CT the maximum number of Spike m^{-2} (242) was recorded, while (265) were recorded at 180 kg N ha^{-1}. In control, the lowest number of Spike was seen. The MT had little effect on spikes m^{-2}, presumably because the previous crop cotton stalks were not included with the tillage process. The DT and CT had a profound effect on spikes m^{-2}; this might be on account of rapid mineralization due to excessive tillage. Increasing doses of N Increased Spikes m^{-2}.

Effect of tillage and nitrogen levels on grains spike^{-1}: The T and N levels ensured a substantial effect on grain spike^{-1}; however, the T x N interaction was not significant (Table 2). Tillage mean values demonstrated that MT had a smaller grain spike^{-1} than DT, CT, and ZT. Grain spike^{-1} rose progressively with the incremental rise in N level and peaked at the highest N level, according to mean values for N levels (180 kg N ha^{-1}). The data show that, while the T x N interaction had no effect on grain spike^{-1}, the main effects of tillage and N levels had a substantial impact on grain spike^{-1}. The DT and CT had a profound impact as compared to MT on grain spike^{-1}. Increasing doses of N Increased Grains spike^{-1}. The MT did not influence grain spike^{-1} (Table 2), probably due to the negligible effect of the previous crop cotton stalks incorporation with tillage operation. The DT and CT had profound effect on grain spike^{-1}; this might be on account of rapid mineralization due to excessive tillage. Increasing doses of N increased plant height.

Effect of tillage and nitrogen levels on thousand grains weight (g)

The T and N levels had a substantial effect on thousand-grain weight (g); however, the T x N interaction was not significant (Table 2). Tillage mean values demonstrated that MT had a lower 1000 grain weight (g) than DT, CT, and ZT. Plant height increased progressively with the incremental rise in N level and peaked at the highest N level, according to mean values for N levels (180 kg N ha^{-1}). The results point out that although T x N interaction did not influence 1000 grains weight (g), however main impact of tillage and N levels had a significant influence on 1000 grains weight (g). The DT and CT had a profound impact on 1000 grains weight (g), while MT showed the most negligible effect on 1000 grains weight (g). Furthermore, increasing doses of N increased 1000 grains weight (g).

Effect of tillage and nitrogen levels on grain yield (kg ha^{-1})

The T and N levels had a substantial effect on grain production (kg ha^{-1}); however, the T x N interaction was not significant (Table 2). Tillage mean values demonstrated that MT produced less grain yield (kg ha^{-1}) than DT, CT, and ZT. The grain yield (kg ha^{-1}) grew gradually with the incremental rise in N level and peaked at the highest N level, according to the mean values for N levels (180 kg N ha^{-1}). The results show that, while the T x N interaction had no effect on grain yield (kg ha^{-1}), the primary impacts of tillage and N levels had a considerable influence on grain yield (kg ha^{-1}). The DT and CT had a profound effect on grain yield (kg ha^{-1}), while MT showed the most negligible effect on grain yield (kg ha^{-1}). Furthermore, increasing doses of N increased grain yield (kg ha^{-1}). The higher grain yield in DT and CT as compared to MT could be due to higher 1000- grain weight and higher number of grains per spike in DT and CT.

Effect of tillage and nitrogen levels on biological yield (kg ha^{-1})

The T and N levels had a substantial effect on biological yield (kg ha-1); however, the T x N interaction was not significant (Table 2). Tillage mean values demonstrated that MT produced less biological yield (kg ha^{-1}) than DT, CT, and ZT. The mean N levels showed that biological yield (kg ha^{-1}) grew progressively with increasing N levels and peaked at the highest N level (180 kg N ha^{-1}). Although the T x N interaction had no effect on biological yield (kg ha^{-1}), the primary effects of T and N levels had a considerable impact on biological yield (kg ha^{-1}). The DT and CT had a profound effect on grain yield (kg ha^{-1}); while MT showed the least effect on biological yield (kg ha^{-1}). Furthermore, increasing doses of N increased biological yield (kg ha^{-1}):

Effect of tillage and nitrogen levels on harvest index (%): The T and N levels had a substantial effect on HI (%); however, the T x N interaction was not significant (Table 2). Tillage means data demonstrated that MT had a greater HI (%) than DT, CT, and ZT. The HI (%) increased progressively with the incremental rise in N level and peaked at the highest N level, according to the mean values for N levels (180 kg N ha^{-1}). Although the T x N interaction did not show any effect on harvest index (%), the primary impacts of tillage and N levels had a substantial impact on HI (%). The DT and CT had a profound effect on harvest index (%), while MT showed the least impact on biological yield (kg ha^{-1}). Furthermore, increasing doses of N increased HI (%).

The cost-benefit ratio (BCR) calculated after the harvest of the wheat crop showed that the highest BCR was recorded in 1.65 in zero tillage plots, applied with nitrogen @ 120 kg ha^{-1}. This could be due to reduced expenditure on ZT practices. The least BCR of 0.92 was found in the MT plots along with nitrogen used @ 180 kg ha^{-1} (Table 3).

Table 3. Economics of the treatments of tillage and nitrogen level

Treatments	Mulch Tillage			Deep Tillage			Conventional Tillage			Zero Tillage		
	Total cost (Rs)	Total income (Rs.)	BCR	Total cost (Rs.)	Total income (Rs.)	BCR	Total cost (Rs.)	Total income (Rs.)	BCR	Total cost (Rs.)	Total income (Rs.)	BCR
N_0	37459	50339	1.34	36234	49720.4	1.37	34989	54326.7	1.55	33754	50744.2	1.50
N_{90}	44866	65463.3	1.45	43641	74968	1.59	42396	61034	1.43	41161	58282	1.41
N_{120}	47384	63518	1.34	46159	59089	1.28	44914	62114	1.38	43679	66527	1.52
N_{150}	49847	77885	1.56	48622	76870	1.58	47377	75401	1.59	46142	76536.3	1.65
N_{180}	59199	54660	0.92	57974	66370	1.14	56720	60925	1.07	55494	64706	1.16

BCR mean benefit-cost ratio

Discussion

Tillage operation enhances the rate of nitrogen and organic matter (OM) mineralization in soil, thereby effect the growth and yield of crop. The current study showed that the growth parameters including plant height, spike^{-2} etc. were significantly influenced by DT and CT as compared to MT. Other researchers have reported profound effect of ZT over CT on the plant height, probably due to conservation of plant material and conversion into soil organic matter Bartaula *et al.* (2020). Working on different tillage systems, Maqsood and co-worker (1998) verified that ZT gave maximum plant height. On the other hand, increasing doses of N increased plant height might be due to more vigorous growth at the higher dose of N. These results match with the study of Usman (Usman *et al.,* 2003), who also reported that plant height increased with increasing nitrogen levels.

As shown in Table 2, DT and CT had a significant effect on spikes m^{-2} when compared to MT. the ZT also performed better regarding spikes m^{-2}, probably due to more accumulation of resources. Our findings match with those of Khan and Rahman (Khan *et al.*, 2010; Rahman *et al.*, 2011)

As shown in Table 2, the DT and CT had a profound effect on grain spike^{-1}. The ZT also performed better regarding grain spike^{-1}, probably due to more accumulation of resources as reported by Donald and Khan (1992) and Mohammadi *et al.* (2013). On the other hand, increasing doses of N increased grain spike^{-1}, probably due to more vigorous growth at a higher dose of N as communicated by Shehab-Eldeen *et al.* (2021).

As shown in Table 2, The DT and CT had a profound effect on 1000 grains weight (g) as compared to MT. This might be because of rapid mineralization due to excessive tillage. The ZT also performed better regarding 1000 grains weight (g), probably due to more accumulation of resources as reported by Ali *et al.* (2011). On the other hand, increasing doses of N increased 1000 grains weight (g), probably due to more vigorous growth at the higher dose of N as communicated by Shehab-Eldeen *et al.* (2021).

As shown in Table 2, mulch tillage did not influence grain yield (kg ha^{-1}), probably due to the negligible effect of the previous crop cotton stalks incorporation with tillage operation. The DT and CT had profound effect on grain yield (kg ha^{-1}); this might be on account of rapid mineralization due to excessive tillage. The ZT also performed better regarding plant height, probably due to more accumulation of resources as reported by Khan and Hassan (2017). These findings contrast those of Rahman *et al.* (2011) and Xue *et al.* (2019). On the other hand, increasing doses of N increased grain yield (kg ha^{-1}), probably due to more vigorous growth at the higher dose of N.

As shown in Table 2, MT did not influence biological yield (kg ha^{-1}), probably due to the negligible effect of the previous crop cotton stalks incorporation with tillage operation. The DT and CT had a profound effect on biological yield (kg ha^{-1}); this might be on account of rapid mineralization due to excessive tillage. The ZT also performed better regarding biological yield (kg ha^{-1}), probably due to more accumulation of resources as reported by Nasri (Nasri *et al.*, 2014). On the other hand, increasing doses of N increased biological yield (kg ha^{-1}), probably due to more vigorous growth at the higher dose of N as reported by Ullah *et al.* (2018).

The MT did not affect harvest index (%), probably due to the negligible effect of the previous crop cotton stalks incorporation with tillage operation. The DT and CT had profound effect on harvest index (%); this might be on account of rapid mineralization due to excessive tillage. The ZT also performed better regarding harvest index (%), probably due to more accumulation of resources as reported by Uhart and Andrade (1995). On the other hand, increasing doses of N increased harvest index (%), probably due to more vigorous growth at the higher dose of N as communicated by Yousaf and co-workers (2014).

As shown in Table 3, maximum BCR was obtained when N was applied @ 120 kg ha^{-1}. These findings are similar to Lakho *et al.* (2004), who reported the highest BCR for nitrogen fertilizers applied @ 120 kg ha^{-1}. Furthermore, Khan *et al.* (2010) reported a higher BCR value for conventional tillage over the deep ploughs.

Soil analysis data showed that soil fertility improved with different tillage operations and inorganic fertilize nitrogen except for no-tilled soil. Organic matter % and micronutrients content increased in soil over control. Eventually, soil fertility improvement was observed in all treatments where different tillage practices were used. Xue *et al.* (2021) also observed improvement in soil fertility while applying different tillage operations. Thus, it can be inferred that the improvement of soil might be responsible for the increase in the yield of wheat (Khan and Hassan, 2017).

Conclusions

This is the first study which was conducted to study the effect of tillage and nitrogen fertilizer on growth and yield of wheat. It was observed that T and N levels had a substantial effect on plant height, Spikes m^{-2}, Thousand grains weight (g) and biological yield (kg ha^{-1}) but the T x N interaction was not significant for all the parameters. According to the data tillage practices, including DT and CT have shown the highest grain yield (35.9 g, 34.7 g respectively) as compared to ZT and MT (34.4 g, 34.4 respectively). Because the DT and CT made the soil porous break the hard pan due to the roots of crop are easily penetrate into the soil and obtained their nutrients easily, which in ZT soil were not mix thoroughly and also some restriction in the root penetration. While from different nitrogen levels, the highest grain yield (38.8 g) was obtained from nitrogen level @150 kg per hectare. The DT and CT and nitrogen level @ 150 kg ha^{-1} have also shown better results on crop growth parameters along with higher BCR. Hence, intensive tillage operations and 150 kg nitrogen per hectare could be recommended as compared to ZT, mulch, and different nitrogen level to get maximum yield in the agro-ecological zone of Pakistan Dera Ismail Khan.

Declaration of competing interest

The authors declare that they have no competing interests.

Authors Contributions statement:

Sanaullah as a main author conceived the idea, designed, implemented and analyzed the study and wrote the first draft. Said Ghulam and Qudrat Ullah Khan supervised the study and revised the manuscript. Muhammad Azeem, Abdul Latif, Muhammad Arsalan and Madeeha Khan helped in writing, editing and statistical analysis.

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