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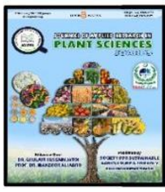

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Enhancing Wheat Productivity in Pakistan through Nitrogen Management and NDVI Monitoring with Green Seeker

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Abstract

Enhancing sustainability in modern farming systems, it is crucial to minimize environmental pollution from nitrogenous fertilizers by optimizing their application rates. The cost of production for wheat is increasing in Pakistan due to irrational use of nitrogen fertilizers by farming community which are costly and sometimes lead to high vegetative growth causing severe lodging thereby reducing yield. Optimum use of nitrogen (N) fertilizer is therefore empirical to improve the productivity of wheat at a reasonable cost. A field study for two-year was conducted at Ayub Agricultural Research Institute, Faisalabad to manage N fertilizers to enhance nitrogen use efficiency and improve financial return. A sensor-based nitrogen application using Normalized Difference Vegetation Index (NDVI) was compared with farmer practice by using *T*-test. Results revealed that there was 29% reduction in the use of N and an increase of 12 % in wheat grain yield. Increase in yield with sensor base fertilization improved nitrogen uptake efficiency (39%) and nitrogen fertilizer productivity (59 %) compared to farmer-based N application. The sensor-based management system and the farmer's practices had nitrogen-use efficiencies of 49% and 44%, respectively. The highest net benefits (US$789), benefit cost ratio (1.92) and resource use efficiency (0.864) in sensor-based application of N revealed that the nitrogen management with the help of Green Seeker could be a viable option for enhancing NUE, financial returns and reduction of environmental contamination.

Keywords: Fertilization. Financial returns. Nitrogen efficiency. Productivity. Wheat

Introduction

Worldwide, nitrogen (N) application is about 80 million tonnes, half of which are used in developing countries and remaining in developed world (Heffer & Prud'homme, 2016). By 2025, nitrogen consumption will increase up to 60-90%, of which about two-third would be used in developing countries (Jadhav & Ramappa, 2023). This trend in fertilizer use is driven primarily due to the needs of developing countries to maintain food supplies for growing population. Normally, farmers in Indo-gangetic plains of South Asia use N based on expected yields (yield target) which may not be feasible from field to field and time to time. In addition, farmers usually apply N fertilizer in high doses which are higher than recommendations with an expectation to get high grain yields. Several studies suggested that Nitrogen application should not be random and needs to be based on soil analysis, water availability, environmental conditions, previous crops and expected grain yield (Bredemeier, Vian, & Pires, 2016). Excessive use of N can lead to weed problems, increase the risk of lodging, delay maturity, and make wheat more susceptible to diseases and pests (Wato, Negash, & Bonga, 2020). As a dynamic element in soil, nitrogen goes through a number of transformations, including de-nitrification, immobilization, and mineralization. Furthermore, the uptake of N by crops varies by region and year of the same field (Chavarria, Rosa, Hoffmann, & Durigon, 2015). Consequently, nitrogen utilization efficiency will decrease if a consistent N rate is applied in the field without accounting for regional variations in soil nutrient supply capacity (Sharma & Bali, 2017).

Because of temporal and site-to-site variation in the field N application does not allow high N fertilizers use efficiency (NUE), even when recommended dose is applied (Dhital & Raun, 2016). In such situations, seasonal nitrogen management at specific locations can effectively replace nitrogen fertilizer recommendations to achieve high NUE. The use of N fertilizer according to the spatial variability should not only increase the efficiency of N use but also reduce the likelihood of nitrogen pollution associated with fertilizers (Martínez-Dalmau, Berbel, & Ordóñez-Fernández, 2021). It is evident that field-wise N fertilizer management, which accounts for both spatial and temporal variability in the amount of nitrogen available in the soil, can increase the high efficiency of applying

N fertilizers. This method of fertilizing wheat fields is also known as site-specific management or precision agriculture, and it offers a mutually beneficial way to enhance crop yields and environmental quality (Rao, Dey, & Reddy, 2021). It has been demonstrated that spectral vegetation indices, including the Normalized Differential Vegetation Index (NDVI), are helpful for gaining information indirectly about photosynthetic efficiency, productivity potential, and potential output (Báez-González, Chen, Tiscareño-López, & Srinivasan, 2002; Sellami *et al.*, 2022). To estimate NDVI values, optical reflective canopy sensors with high spatial resolution, such as Green Seeker, N-Sensor, and Crop-Circle, allow for real-time reflectance readings. The NDVI readings evaluated by canopy sensors can be used as a tool for N management. Using NDVI measurements, concepts of response index and potential yield could be developed to determine N fertilizer demand in wheat crop. Measured N response and improved N-use efficiency can be positively connected with the prediction of wheat response to N applications guided by optical sensors (Arnall *et al.*, 2013; Bushong, Mullock, Miller, Raun, & Brian Arnall, 2016). Nitrogen application managed using Green seeker sensor for wheat crop can improve N use efficiency by 15 % (Ratanoo, Kumar, Dhaka, & Singh, 2016). However, to use optical sensors or green seekers as a tool for N management and to apply N dose at a variable rate based on various vegetation indices (NDVI), it is necessary to develop an algorithm scale for estimation. A general approach for N management in wheat crop as recommended by NDVI reading by using Green Seeker is as below:

NDVI < 0.25; bare soil or soil with wheat stands poor

NDVI 0.25 to 0.57; the crop benefits from additional N

NDVI 0.57 to 0.73; Adding more fertilizer can increase grain production to its highest possible level.

Beyond NDVI 0.73; No benefit for additional fertilizer N

This experiment was intended to assess the effects of NDVI sensor-based nitrogen management on wheat because the general guideline is deemed insufficient. The aim is to achieve high yields and improved nitrogen use efficiency (NUE) while minimizing nitrogen loss from the soil. We anticipate that using the NDVI sensor for nitrogen application in wheat could enhance yield performance, NUE, and net returns.

Materials and Methods

***Site, Soil and climate*:** To evaluate the impact of nitrogen management by using NDVI value with Green Seeker, field experiments were conducted at Wheat Research Institute, Faisalabad (31°15′0 N, 73°03′0 E and altitude 184.4 masl) for two consecutive years, 2019-20 and 2020-21. The experimental soil was classified under the Lyallpur soil series, specifically as aridisol-fine-silty, mixed, hyperthermic Ustalfic Haplargid according to the USDA classification. Before the experiment, representative soil samples from two depths 0-15 cm and 15-30 cm were taken to study physico-chemical properties of the soil (Table 1). The region has a semi-arid climate with hot, humid summers and mild, dry winters. January's average temperature is 13.0°C, June's is 39°C, and there is 532.5 mm of precipitation on average each year. Weather conditions during the study period are given in Figure. 1

Table 1. Physico-chemical properties of experimental site

Depth (cm)	EC (ms/cm)	Soil pH	Organic matter (%)	Available P (ppm)	Available K (ppm)	Saturation % age	Texture
0-15	20.87	8.2	1.05	10.6	220	36	Loam
15-30	20.84	8.2	0.77	8.1	180	34	Loam

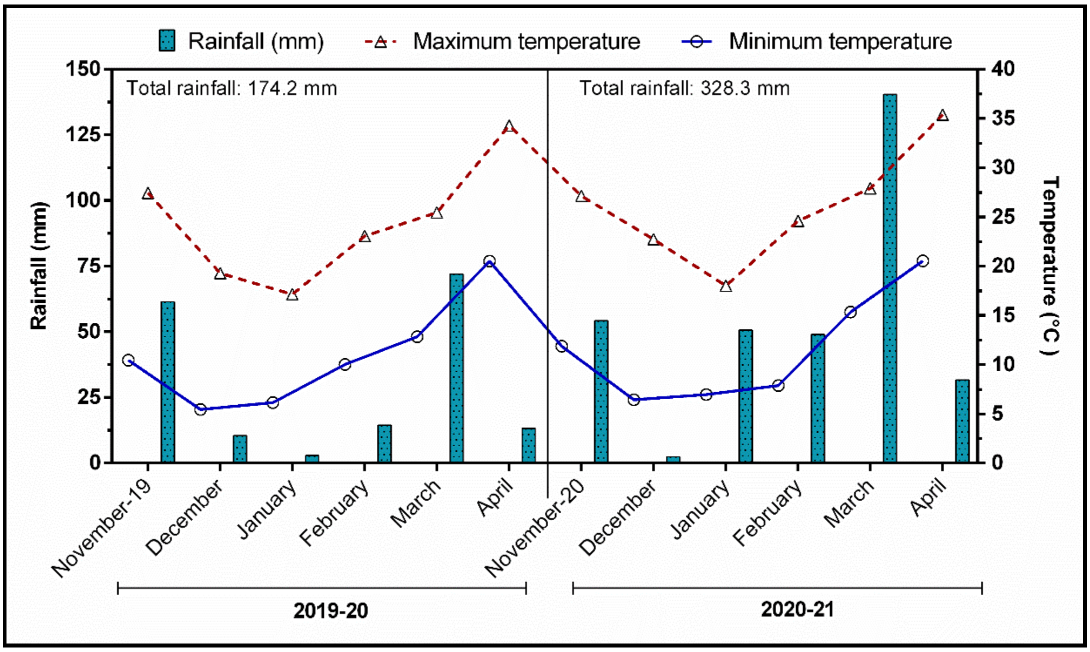

Figure 1. Temperature (°C) and rainfall (mm) data during 2019-20 and 2020-21 of wheat growing season.

***Experimental details*:** Faisalabad-2008 cultivar with 95% germination was used for the study. Experiment was conducted at four fields at Ayub Agricultural Research Institute, Faisalabad, Pakistan. Each field trial consisted of an N-rich strip, a sensor-managed area, and an area subject to farmer practice or traditional N management. The nitrogen-rich strip, measuring 10 × 10 meters, was established by applying 184 kg of nitrogen per hectare in split doses to ensure nitrogen availability. The sensor area and farmer practice area in each field was 0.2 hectare each. The NDVI data of both N-Rich strip and sensor was recorded and entered into a software program. The highest NDVI values were seen in the N-rich areas. This program is also available as a mobile application called 'Urea Calculator'. After entering information into Urea Calculator it calculates the quantity of N required for sensor area to make it equal in N availability to that of N-Rich strip. The details of the amount of N applied to each strip based on sensor reading and farmer practice during both years are given in Table 2.

Table 2. Nitrogen application rates according to sensor based and farmers practice during 2019-20 and 2020-21.

2019-20				
Location	**NDVI Data**		**Total urea used (kg ha^{-1})**	
	NRP	**SP**	**SP**	**FP**
Field-1	0.76	0.81	215	325
Field-2	0.76	0.80	220	325
Field-3	0.77	0.83	220	325
Field-4	0.75	0.79	215	325
2020-21				
Field-1	0.79	0.67	275	325
Field-2	0.79	0.74	236	325
Field-3	0.76	0.65	265	325
Field-4	0.79	0.77	214	325

NRP= N-Rich Plot, SP= Sensor Plot, FP= Farmer practice plot

***NDVI measurements*:** The Green seeker handheld crop sensor is an active light source optical sensor that is used to measure plant biomass and display as NDVI (Normalized Difference Vegetation Index). The device was held at a height of approximately one meter above the plant canopy in order to capture the readings. The sensor unit includes built-in illumination for both the red (656 nm with approximately 25 nm full width at half maximum (FWHM)) and near-infrared (NIR) (774 nm with about 25 nm FWHM) bands.
The Green Seeker calculates NDVI as;

$$\text{NDVI} = \frac{FNIR - FRed}{FNIR + FRed},$$

Where;
F_{NIR} = the percentages of NIR radiation that are reflected back from the detected area
F_{RED} = the fractions of Red radiation reflected back from the sensed area

The sensor takes samples at an extremely high rate of approximately 1000 measurements per second and averages the readings between outputs. It provides NDVI data at a frequency of 10 readings per second, with travel speeds around a slow walking pace of 0.5-1 m/sec (Singh, Singh, & Singh, 2022).

***Crop Management*:** Prior to seeding, the land was ploughed with cultivator followed by planking to get pulverized seed bed. At sowing time, recommended doses of phosphorus (P) @ 87 kg ha^{-1} and potassium (K) @ 57 kg ha^{-1} were drilled below the seed except N. At first irrigation, when the crop was at crown root initiation (CRI) stage, the first dose of N was applied as per recommendation @ 57 kg ha^{-1}. At the time of second irrigation, the spectral reflectance readings i.e. NDVI were taken and N was applied according to urea calculator in sensor plot. However, in farmers' practice plot N was applied conventionally. Three to four irrigations were applied (depending upon rainfall and climate) at various stages of crop, like, crown root initiation, booting, and grain-filling (Murad Ali *et al.*, 2023). Weeds were removed using selective herbicides for both broad and narrow leaved weeds.

***Statistical Analysis*:** Two-Sample T-test was used to check the significant difference between farmer based and sensor based nitrogen application (Steel, Torrie, & Dickey, 1997).

Traits Examined

***Yield and Yield Related Parameters*:** At the time of physiological maturity number of productive tillers in each plot from three random field sites each having 1×1m area were taken and averaged. Every plot's crop was carefully gathered, bundled, and left on the field to dry in the sun. After sun drying, biological yield of each plot was recorded with electrical weighing balance. The crop was threshed with mini thresher, cleaned with chuff and straw to record grain yield with weighing balance. After recording grain yield thousand grains from each harvested plots were taken and weighed for taking the data of 1000-grain weight.

***Nitrogen Utilization Efficiencies*:** Crop was harvested, threshed and both grain and straw were dried at 75ºC in an oven until their weight remained consistent. The procedure outlined by (Bremner, 1996) was used to determine the nitrogen content of wheat plants. The obtained value of N concentration both from grain and straw were multiplied with total biomass of plant to determine N uptake. Nitrogen uptake was determined with the formula adopted by (Billah, Ahmad, & Ali, 2019). N uptake efficiency (NupE), N fertilizer productivity (NfP), NUE, and nitrogen harvest index (NHI) were used to evaluate the effectiveness of N fertilizer utilization (Guo, Zhang, Zhao, Shi, & Yu, 2014), which were calculated according to the following equations (Koutroubas, Fotiadis, & Damalas, 2012; Montemurro, Maiorana, Ferri, & Convertini, 2006).

- Nup E (kg kg^{-1}) = total N uptake / N application
- NfP (kg kg^{-1}) = Grain yield / N application
- NUE (kg kg^{-1}) = Grain yield / Total N uptake

***Economic Analysis and Resource Use Efficiency*:** Economic analysis was conducted in order to ascertain the comparative net benefits (Cimmyt & Cimmyt, 1988). To produce adjusted biological and grain yield for economic analysis, the actual biological and grain yield was subtracted by 10%. For every wheat tillage system, the variable cost (tillage cost) was computed. All treatments had a predetermined total permanent cost, which comprised planting, fertilizing, watering, protecting plants, and harvesting. The whole cost of each therapy was deducted from the gross income to determine the net benefits. Net benefits to total costs were used to calculate resource use efficiency.

RESULTS

Yield and yield related parameters: Yield and yield related parameters such as productive tillers, 1000-grain weight and grain yield were significantly affected by sensor-based nitrogen application and farmer-based nitrogen application in both years. However, grains per spike and biological yield were not significantly affected by nitrogen application techniques in both years (Table 3). Number of tillers per unit area, 1000-grains weight and grain yield were highest in both years where N was applied based on NDVI data i.e. sensor-based nitrogen. In case of sensor-based nitrogen application, tillers m^{-1}, 1000-grains weight and grain yield were respectively 365 tillers, 38.8 g and 4.67 t ha^{-1} in 2019-20 and 346 tillers, 44.66 g and 4.65 t ha^{-1} in 2020-21. Similarly in plots with conventional nitrogen application practice, tillers m^{-1}, 1000-grains weight and grain yield were respectively, 326 tillers, 33.2 g and 4.17 t ha^{-1} in 2019-20 and 325 tillers, 34.72 g and 4.15 t ha^{-1} in 2020-21.

***Nitrogen Utilization Efficiencies*:** Nitrogen use efficiency (NUE), an index of grain yield per unit of N absorbed and nitrogen fertilizer productivity (Nfp) an index of grain yield per unit of N application rate differed significantly between sensor-based N application and farmers' practice in both years. The highest values for NUE and Nfp were detected where nitrogen was applied based on NDVI readings in both years (2019-20 and 2020-21), whereas the lowest values for NUE and Nfp were observed for plots with conventional nitrogen application in both years. NUE and Nfp values were 49.373 and 46.627 kg kg^{-1} in 2019-20 season and 49.519 and 41.18 kg kg^{-1} in 2020-21 season, respectively. For plots with nitrogen application as per farmer/conventional practice, the NUE and Nfp were 46.331 and 27.94 kg kg^{-1} in 2019-20 season and 44.746 and 27.242 kg kg^{-1} in 2020-21 season, respectively.

Total nitrogen uptake was not significantly affected by different nitrogen application rates however the nitrogen uptake efficiency (NupE) was significantly differed between sensor-based nitrogen application and farmer-based nitrogen application during both years (Table 4). NupE was highest in sensor-based plots and the lowest in farmers practiced plots in both 2019-20 and 2020-21seasons. In 2019-20 and 2020-21 NupE was respectively, 0.95 and 0.83 in sensor-based plots whereas in conventional plots it was 0.60 and 0.61 in 2019-20 and 2020-21 seasons

Table 3. Yield and yield related parameters as affected by sensor based and conventional or farmer based nitrogen application.

Treatments	2019-20 Mean	2019-20 P value	2020-21 Mean	2020-21 P value	Over all Mean	Over all P value
Grains per spike						
Farmer Practice N application	48.98 ± 0.57	0.1280^{NS}	45.60 ± 1.13	0.8799^{NS}	47.27 ± 0.86	0.4727^{NS}
Sensor Based N application	47.54 ± 0.59		45.35 ± 1.11		46.45 ± 0.71	
1000- grain weight (g)						
Farmer Practice N application	33.2 ± 0.64	0.0003^{**}	34.72 ± 0.74	0.0004^{**}	33.96 ± 0.54	0.0002^{**}
Sensor Based N application	38.8 ± 0.42		44.66 ± 1.20		41.72 ± 1.25	
Productive tillers (m^{-2})						
Farmer Practice N application	325.75 ± 1.32	0.0000^{**}	324.50 ± 1.76	0.0001^{**}	325.12 ± 1.04	0.000^{**}
Sensor Based N applic	364.50 ± 1.71		346.25 ± 1.32		355.36 ± 3.59	
Biological yield (t ha^{-1})						
Farmer Practice N application	10.62 ± 0.29	0.2155^{NS}	10.10 ± 0.53	0.3716^{NS}	10.36 ± 0.30	0.179^{NS}
Sensor Based N application	11.17 ± 0.48		10.82 ± 0.53		11.00 ± 0.34	
Grain yield (t ha^{-1})						
Farmer Practice N application	4.17 ± 0.08	0.0012**	4.15 ± 0.04	0.0001**	4.16 ± 0.04	0.000^{**}
Sensor Based N application	4.67 ± 0.04		4.65 ± 0.04		4.66 ± 0.03	

Highly Significant (**) and non-significant (NS) differences between treatments.

Table 4. Total N uptake (kg kg^{-1}), N uptake efficiency (kg kg^{-1}), N use efficiency (kg kg^{-1}), N fertilizer productivity of wheat at maturity as affected by Sensor base and Farmer based N application**.**

Treatments	2019-20 Mean	2019-20 P value	2020-21 Mean	2020-21 P value	Over all Mean	Over all P value
Total N uptake (kg kg^{-1}),						
Farmer Practice N application	89.95 ± 1.31	0.1467^{NS}	92.67 ± 0.52	0.0562^{NS}	91.31 ± 0.82	0.087^{NS}
Sensor Based N application	94.61 ±2.47		93.84 ± 0.50		94.22 ± 1.35	
N uptake efficiency (kg kg^{-1})						
Farmer Practice N application	0.60.± 0.015	0.0001^{**}	0.61 ± 0.13	0.0293^{*}	0.62 ± 0.018	0.0000^{**}
Sensor Based N application	0.95 ± 0.030		0.83 ± 0.48		0.86 ± 0.016	
N use efficiency (kg kg^{-1}),						
Farmer Practice N application	46.33 ± 0.32	0.0498^{*}	44.75 ± 0.29	0.0001**	45.54 ± 0.36	0.0000^{**}
Sensor Based N application	49.37 ± 1.00		49.52 ± 0.44		49.44 ± 0.5	
N fertilizer productivity						
Farmer Practice N application	27.94 ± 0.78	0.0000^{**}	27.24 ± 0.56	0.0241*	27.59 ± 0.46	0.0000^{**}
Sensor Based N application	46.63 ± 0.63		41.18 ± 0.24		43.90 ± 1.49	

Significant (*), Highly significant (**) and Non-significant (NS) differences between treatments

***Economic analysis and resource use efficiency*:** Economic analysis performed for the experiments for both years reveled that the highest net benefits, benefit cost ratio and resource use efficiency were with sensor based nitrogen application and these parameters were lowest with farmer based or conventionally applied nitrogen. The analysis revealed that the net benefits of 704.2 and 673.5 $ were obtained in 2019-20 and 2020-21 seasons, respectively (Table 5). Similarly benefit cost ratio and resource use efficiency were 1.94 and 1.90, and 0.88 and 0.84 in 2019-20 and 2020-21 seasons, respectively (Table 5).

Table 5. Economic analysis for sensor based and farmer based nitrogen application during 2019-20 and 2020-21.

Treatments	**2019-20**								
	Grain Yield	**Adjust. GY**	**Straw Yield**	**Adjust. Straw yield**	**Gross Income**	**Total cost**	**Net benefits**	**BCR**	**RUE**
Farmer Practice N application	4.17	3.75	6.47	5.82	1394.4	825.7	528.6	1.69	0.640
Sensor Based N application	4.66	4.20	6.86	6.17	1545.6	797.0	704.2	1.94	0.883
	2020-21								
Farmer Practice N application	4.15	3.73	5.72	5.15	1359.5	825.7	494.8	1.65	0.599
Sensor Based N application	4.65	4.18	6.18	5.56	1514.0	797.0	673.5	1.90	0.845
	Overall for 2019-20 and 2020-21 seasons								
Farmer Practice N application	4.16	3.74	6.09	5.48	1376.95	825.7	511.7	1.67	0.619
Sensor Based N application	4.66	4.19	6.52	5.86	1529.80	797.0	788.8	1.92	0.864

BCR=Benefit cost ratio; RUE= Resource use efficiency; GY= Grain yield

DISCUSSION

It is widely acknowledged that using a single N rate throughout the field is not sustainable from an environmental or economic standpoint. The efficiency with which N fertilizers are used is reduced by both over- and under-application, and the risk of N losses through leaching or volatilization is raised (Yang, Lu, Ding, Yin, & Raza, 2017). Farmers may be able to control N fertilization with favorable effects on their financial returns and environmental contamination by using precision fertilization, which is based on the reflected spectrum from canopy. In this two years field study, sensor-based N management was compared with conventional farmer practice to assess the economic efficiency of precision N management approach applied on wheat crop. The results of the two years study revealed significant increase in wheat yield, nitrogen utilization and financial returns. Sensor based nitrogen application produced the best efficiency in terms of productive tillers (9 %), grain weight (23 %) and number of grains per spike compared with farmer based nitrogen application (Table 3). Increase in the yield related parameters with sensor-based N fertilization increased grain yield by 12 % and biological yield by 6 %. The present findings are corroborated with the conclusion of (Walsh, Shafian, & Christiaens, 2018). Similarly, (A. M. Ali, 2020) found that yields rose when they switched from general fertilizer recommendations to an optical sensor-based N control method. Therefore, reducing N losses from the soil-plant system, increasing crop yield, and improving nitrogen-use efficiency can all be achieved by using vegetation indices like the NDVI to guide fertilization. According to (MM Ali, Al-Ani, Eamus, & Tan, 2017), the NDVI is a quick, simple, and non-destructive way to measure how a plant is growing and developing.

When NE-based solutions were applied instead of farmer's practices, the results showed a significant increase in nitrogen-use efficiency. Increase in yield with model base fertilization lead to improved nitrogen uptake efficiency (39 %), nitrogen fertilizer productivity (59 %) compared with farmer based N application. The sensor-based management system and the farmer's practices had nitrogen-use efficiencies of 49% and 44%, respectively (Table 4). Reduced production costs and increased nitrogen-use efficiency are two major benefits of sensor-based nitrogen (N) management directed by vegetation indices like the normalized difference vegetative index (NDVI). Farmers can improve resource usage by applying the optimum amount of nitrogen required for optimal crop growth by using NDVI as a guidance tool. The promise of this strategy was illustrated by (Diacono, Rubino, & Montemurro, 2013), who highlighted how it could improve nitrogen-use efficiency and lower manufacturing costs. According to our research, applying nitrogen in two or three splits at a constant rate may not always be as efficient as applying nitrogen based on NDVI sensor readings. The key advantage of NDVI-based nitrogen application lies in its ability to ensure better synchronization and timely delivery of nitrogen at appropriate doses. Therefore, reducing N losses from the soil-plant system, increasing crop yield, and improving nitrogen-use efficiency can all be achieved by using vegetation indices like the NDVI to guide fertilization. Sensor guided application of nitrogen to wheat crop can enhance NUE as well as reduce the cost of production by fixing N application to the minimum required quantity needed by the crop (Mitra *et al.*, 2023). In our study, the application of N by using sensors saved 44 kg N ha^{-1} (Table 2) along with increase in yield and nitrogen use efficiency. Higher nitrogen doses may not always produce yields that are profitable. (Mondal, Mitra, & Das, 2018) indicated that using an NDVI sensor has the potential to attain both higher yields and improved nitrogen use efficiency (NUE).

Farmers rely on the benefit-cost ratio (BCR) as a key tool to assess net returns and efficiently control total production costs (Ahmed & Basir, 2024). Average savings in N use of about US$ 26.10 per hectare while increased income of US$ 18.10 per hectare from enhanced yield, elevated net benefits (789 $) in sensor-based nitrogen management compared to farmer-based N application (512 $). The net return from non-uniform farmer-based N application is significantly impacted by fluctuating fertilizer costs, crop prices, and sampling expenses (Samborski, Gozdowski, Stępień, Walsh, & Leszczyńska, 2016). The cost of fertilizer and grains goes up with this benefit. Moreover, no crop sampling or laboratory tissue analysis is required by sensor base N application method resulting in higher benefit cost ratio (1.92). Upper limit of resource use efficiency (0.864) in sensor based N application also saved time and labor (Thomason *et al.*, 2011).

Conclusions

Based on two years results, it can be concluded that using a normalized difference vegetation index (NDVI) sensor can be a valuable tool for the efficient management of nitrogen fertilizer in wheat cultivation in Pakistan. It also well-established that use of NDVI values from Green Seeker in agriculture production systems results in high net returns. Moreover, fertilization by using information obtained by a vegetation index (NDVI) system enables farmers to manage N fertilization with positive outcomes of yield, nitrogen utilities and financial returns. Agricultural extension services need to create awareness and build capacity regarding this important technology to rationalize nitrogen application in Pakistan.

Authors Contribution

IH and AA designed the study. AA and MA conducted the experiment and wrote the manuscript. AB and AA analyzed the data. TPT reviewed the manuscript before submission.

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Disclosure statement

No conflict of interest was reported by the author (s).

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