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Estimating Nitrogen and Chlorophyll Content in Corn Using Spectral Vegetation Indices Derived From UAV Multispectral Imagery

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ABSTRACT

Remote sensing is a unique and cost-effective tool that provides information about the nitrogen status of plants in a non-destructive way. The objective of this study is to evaluate the effectiveness of aerial multispectral imagery captured by UAV for estimating corn nitrogen (N) and chlorophyll (Chl) content at different growth stages. The study used a fully randomized experimental design with four treatments of nitrogen fertilizer (0, 50%, 100%, and 150%). Ten plants were randomly selected in each plot at the phenological stages of 8 leaves (V8) and tasseling growth stages (VT) for sampling. Leaf samples were taken to measure total nitrogen (N) and chlorophyll (Chl) content. Mathematical models were created using vegetation indices extracted from aerial multispectral images to estimate the amount of nitrogen and chlorophyll. The models were evaluated using the leave-one-out cross-validation method. The results showed that there is a significant positive relationship between the leaf dry weight (LDW), the Chl and N content with the amount of nitrogen fertilizer used. So, the results indicated that the REIP index is suitable for estimating chlorophyll content in both the V8 (R^2 of 0.997) and VT (R^2 of 0.980) growth stages. Additionally, the REIP index was found to be an appropriate index for estimating N content in both growth stages (R^2 of 0.980). It can be concluded that aerial multispectral remote sensing technology is a reliable method for estimating corn nitrogen and chlorophyll content.

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INTRODUCTION

Nitrogen (N) is an essential corn nutrient (Santana et al., 2021; Vleugels et al., 2017). Providing a sufficient amount of nitrogen increases photosynthesis and chlorophyll content, seed quality, and crop yield (Santana et al., 2021; Xu et al., 2023).

The traditional method for determining plant N content involves destructive methods by the cutting of fresh biomass, subsequent drying and the measurement of N concentration, which is labor-intensive, tedious, time-consuming and therefore impractical under normal farm conditions (Wang et al., 2019). Also, the SPAD[1] meter is the most commonly used tool applied for chlorophyll content diagnosis (Ban et al., 2022; Lin et al., 2010). With this method, measurements were conducted at specific points, and readings were affected by different leaf positions (Krienke et al., 2017).

Recent research into detecting N and Chl variability has focused on non-destructive, timely and more effective in-season management techniques (Shanahan et al., 2008). A variety of sensors and technologies including hyperspectral and RGB sensors, LiDAR systems (Escalante et al., 2019), the GreenSeeker (Xia et al., 2016), the Crop Circle (Cao et al., 2015), and satellite remote sensing (Bagheri et al., 2022; Xu et al., 2021) have been used to develop a non-destructive and quick method to estimate the N and chlorophyll content in crops (Wang et al., 2019).

Remote sensing is a unique tool for providing information linked to plant N and chlorophyll status in a rapid, cost-effective, and non-destructive way (Liu et al., 2018). Satellite imagery is known as one of the most popular types of remote sensing images (Wang et al., 2019). Satellite imagery has shown the ability to diagnose N and chlorophyll status in corn (Bagheri et al., 2013), rice (Huang et al., 2015) and maize (Gabriel et al., 2017). The use of satellites for precise monitoring of crop growth in smallholder fields is very extensive but limited due to its high cost, time consumption, and low resolution (Escalante et al., 2019; Xu et al., 2021).

In recent years, new opportunities for crop monitoring have been opened up by the use of Unmanned Aerial Vehicles (UAVs). UAVs are more flexible in scheduling field surveys than satellite remote sensing techniques (Pádua et al., 2017). In comparison with satellite remote sensing or aircraft-based imaging, UAV-based low-altitude remote sensing could acquire timely images with higher resolution and lower cost (Krienke et al., 2017; Narmilan et al., 2022). UAVs have the potential as a platform for detecting and managing crop stress during the growing season, and they provide unique advantages compared with other platforms (Colomina & Molina, 2014; Xu et al., 2021). In the last decade, there has been an increase in remote sensing applications with UAVs for precision agriculture rather than conventional satellite imagery (Escalante et al., 2019; Huang et al., 2013; Tripicchio et al., 2015; Valente et al., 2011; Zhang & Kovacs, 2012). UAV-based vegetation indices have been successfully regressed against leaf chlorophyll (Lebourgeois et al., 2008; Miao et al., 2009; Narmilan et al., 2022; Noh & Zhang, 2012) and N content (Barzin et al., 2021; Geipel et al., 2016; Lebourgeois et al., 2012; Reyniers & Vrindts, 2006) of different crops such as wheat (Chen et al., 2023), corn (Xu et al., 2021), maize (Bagheri et al., 2022; Corti et al., 2019; Jaberi-Aghdam et al., 2024; Krienke et al., 2017), rice (Ban et al., 2022; Li et al., 2015), canola (Liu et al., 2018), grass seed (Wang et

[1]. Chlorophyll meter soil plant analysis

al., 2019) and barley (Escalante et al., 2019). The summary of previous research shows that aerial images taken by UAV have a good ability to estimate the biochemical characteristics of crops. Considering the importance of nitrogen and chlorophyll in examining plant growth and yield estimation, the objectives of this research are as follows:

- Investigating the ability of aerial multispectral images taken by UAV to estimate the amount of nitrogen and chlorophyll of corn plants in V8 and VT growth stages.
- Introduction of appropriate vegetation indices extracted from aerial multispectral images to estimate nitrogen and chlorophyll in different stages of crop growth.
- Providing mathematical models with acceptable accuracy to estimate the studied variables.

MATERIALS AND METHODS

Field Experiments

The study area was a research field at Varamin, Iran (35° 8′N and 51° 40′E) during the 2018 growing season (Fig1). Soil sampling was carried out before corn planting. Five samples from the soil depth of 0-30 cm were collected and then sent to the laboratory for chemical analysis. Based on the results of soil experiments, the soil was classified as a sandy loam with N: 1.1%, Phosphorus (P): 10.4 ppm, Potassium (K): 410 ppm, Electrical Conductivity (EC): 3.65 Ds/m[1] and pH: 7.69.

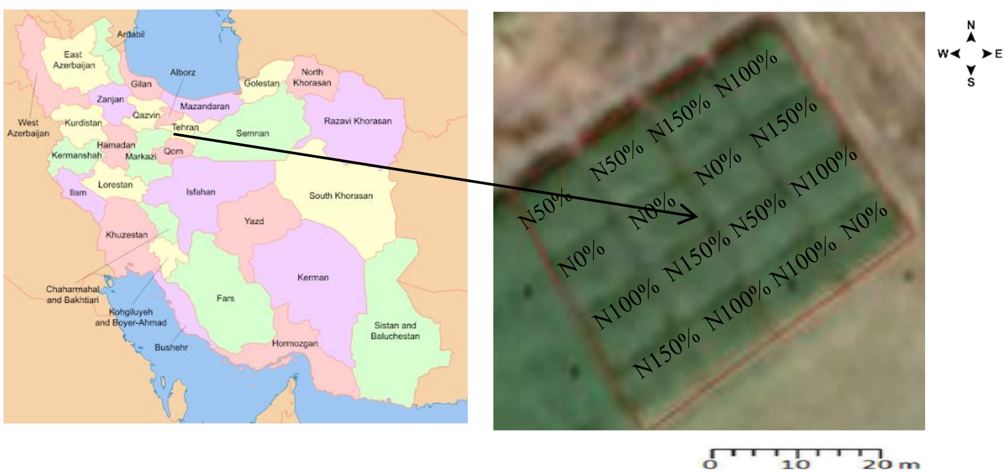

Figure 1. The study area and experimental plots of the research

The experimental field trial design was a fully randomized design with four N fertilizer treatments and four replications. The area of each plot was 22.5m^2. Corn seed (Gazda MTC 450) was planted in all plots by seeder in 5-8 cm soil depth with a 75 cm row distance. Four N fertilizer levels of 0, 50%, 100% (71.3 kg/ha N), and 150% were applied by water irrigation in the V8 [2] and the VT [3] growth stages. Plant sampling and aerial imaging were performed in one day. Ten plants were randomly sampled per plot between 12:00- 14:00 to measure Chl, N contents and Leaf dry weight (LDW). Chl content of plant samples was measured in the V8, and VT[4] corn growth stages using a Minolta SPAD 502 Chlorophyll meter

[1]. Desi Siemens per Meter
[2]. The 8 leaves on the corn plant
[3]. Tasseling stage
[4]. The silking stage (silk is visible outside the husk)

(Minolta Corp., Osaka, Japan). SPAD values calculate relative Chl content based on the amount of light transmitted by the leaves at two different wavelengths: red (650 nm) and near-infrared (940 nm) (Maresma et al., 2016). Measurements were carried out from all leaves of selected plants. Three readings were taken from the base, middle and top of each leaf, and the averaged data were used for processing. The Kjeldahl method is used to determine leaf N content. First, the leaves were separated from the stems, oven-dried at 70°C for 48 hours, weighed on a digital scale with 0.1 g accuracy, ground to pass a 1 mm mesh screen, then stored in plastic bags, and sent to the plant analysis laboratory (Bagheri et al., 2013). LDW of samples was obtained by weighting dried samples with a digital scale with 0.1 g accuracy. Data of Chl reading, N, and LDW for each treatment (and in each replication) were used to construct mathematical models.

Aerial imagery acquisition

Aerial multispectral imagery of corn crops was carried out using a UAV before collecting leaf samples. The developed UAV by Bagheri et al. (2017) is used for aerial imaging. It was an eight-rotor aerial platform with a robust carbon-fiber flight frame capable of vertical take-off and landing. This system consisted of onboard and ground-station subsystems. The onboard subsystem was equipped with body and wings, 8 D.C. brushless motors, 8 control speeds, chargeable 3-cell Lithium polymer battery (3300mAh- 11.1V, Shanghai Danlions International Co., Shanghai, China), carbon-fiber camera mount, and autopilot intelligent navigation system (NAZA MV2). The ground station was equipped with 8-frequency radio control and laptop and flight monitor software. In the present study, the aerial images were taken in the V8 (2 October 2018) and VT (18 November 2018) corn growth stages between 11:00 to 12:00 in sunny weather, cloud-free and clear sky. Aerial images were obtained at a flight height of 100m above the ground level with a spatial resolution of 4cm. Therefore one static image shot was enough to cover the whole area.

ADC-Micro multispectral camera (Tetracam, Inc, Gainesville, FL, USA) with 520-900 nm wavelengths in Green (520-600 nm), Red (630-690 nm), and Near-infrared (760-900 nm) channels were used to collect imagery. This camera has a 3.2 megapixel CMOS sensor (resolution of 2048 * 1536 pixels), and a fixed lens with a focal length of 8.43 mm. A Teflon calibration target was used for image calibration. The Teflon target exhibits a reflectivity of approximately 99% in the wavelength range of 520-900 nm. DCM File format was selected for image saving and the camera was set to the automatic exposure time (5s).

The calibration image was captured at the height of the canopy level so that the surface of the Teflon target was inside the image frame. Aerial multispectral imagery of the corn farm in the V8 and the VT growth stages is shown in Fig 2.

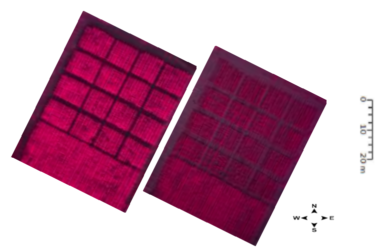

Figure 2. Aerial multispectral imagery of the corn farm in a: V8 (Right) and b: VT growth stages (Left) (NIR-R-G bands)

Multispectral aerial images analysis

Preprocessing and processing of the collected aerial imagery were performed after collecting images and extracting data from the SD memory card of the camera. The PixelWrench2 (Tetracam Co, USA) and the environment for visualizing images software (ENVI 5.4, L3Harris Geospatial) were used for the preprocessing and processing of imagery, respectively. For preprocessing, the color reconstruction of the raw images was carried out. The Tagged Imagery File Format (TIFF) was produced for saving images. Then, the false-color composite of the collected imagery with the NIR-Red-Green band composite was prepared. Also, radiometric calibration is performed by using a white Teflon calibration plate. Processing images was carried out by the ENVI 5.4 software. The vegetation indices (VIs) based on green, red, and near-infrared spectral bands as effective predictors of plant greenness as well as the N and Chl content in plants were used (Liu et al., 2018; Shanahan et al., 2008) (Table 1). After extracting the spectral value of G, R, and NIR bands for each pixel of the image, vegetation indices were calculated for each image pixel. Then the values of VIs were averaged (16 average VIs for four treatments and four replications). The mean values of VIs data were used to construct mathematical models for estimating N and Chl content.

The relationship between calculated vegetation indices with Chl reading and corn N content of treatments was obtained by second-order polynomial regression models. The Leave-one-out cross-validation method was used for the validation of vegetation indices-based models. In the method, all but one sample was used for training the model (15 samples), then the trained model was used to predict the variable of interest in the leaved-out (test) instance. This process was repeated 15-times, each time changing the test sample. These values were averaged and reported following the procedure used by Escalante Escalante et al., 2019. The root means square error (RMSE) and the coefficient of determination (R^2) was used to evaluate the performance of models by Excel 10 software (Microsoft, USA). The best equations fit all models were second-order polynomial equations.

Table 1. Vegetation indices for estimation of N and Chl contents

Vegetation Index	Equation	References
RDVI[1]	$\frac{NIR-R}{\sqrt{NIR+R}}$	(Haboudane et al., 2004)
MSR[2]	$\frac{\left(\frac{NIR}{R}-1\right)}{\sqrt{\left(\frac{NIR}{R}+1\right)}}$	(Cao et al., 2015)
MCARI1[3]	$1.2 \times [2.5(NIR-R) - 1.3(NIR-G)]$	(Daughtry et al., 2000)
MCARI2[4]	$\frac{1.5 \times [2.5(NIR-R) - 1.3(NIR-G)]}{\sqrt{-0.5 + (2NIR+1)^2 - (6NIR - 5\sqrt{R})}}$	(Haboudane et al., 2004)
TCARI[5]	$3\left[(NIR-R) - 0.2(NIR-G) \times \left(\frac{NIR}{R}\right)\right]$	(Elvanidi et al., 2018)
ARI[6]	$\left(\frac{1}{G}\right) - \left(\frac{1}{NIR}\right)$	(Gitelson et al., 2001)
REIP[7]	700 + 40((R + NIR)/2 -R)/(NIR-R)	(Mistele & Schmidhalter, 2008)

[1]. Randomized Difference Vegetation Index
[2]. Modified Simple Ratio
[3]. Modified Chlorophyll Absorption Ratio Index1
[4]. Modified Chlorophyll Absorption Ratio Index2
[5]. Transformed Chlorophyll Absorption and Reflectance Index
[6]. Anthocyanin Reflectance Index
[7]. Red Edge Inflection Point

RESULTS AND DISCUSSION

The relationship between corn N content and applied N fertilizer

The relationship between the applied N fertilizer and the LDW of treatments is shown in Fig 3. Based on the figure, the LDW of treatments increased by increasing the amount of N fertilizer applied to the farm in both V8 and VT growth stages. The LDW of the crop in the VT stage was more than the LDW of the V8 stage because, in the VT stage, the plant received N fertilizer once more than in the V8 stage and the plant biomass and nitrogen content were higher in the VT stage in comparison with the V8 stage. Santana et al (2021) and Xu et al (2023) found that there is a positive and significant relationship between nitrogen content and plant weight and increasing the dry weight of the plant increases yield.

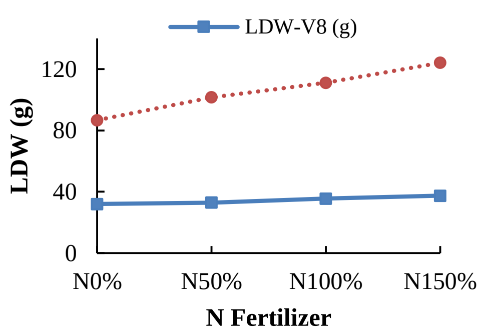

Figure 3. The relationship between applied N fertilizer and LDW

The relationship between applied N fertilizer and corn N content (g) of treatments is shown in Fig 4. The content of N is obtained by multiplying the percent of N content by LDW. Based on Fig 4, N content increased by increasing N fertilizer dosage in both the V8 and VT stages. Increasing the nitrogen content with increasing the amount of fertilizer in the V8 growth stage is not very significant, but the increasing trend is evident in the VT growth stage. Since the plant in the VT growth stage has received N fertilizer once more than the V8 growth stage and LDW has increased, so the amount of nitrogen content of the plant has increased in this growth stage.

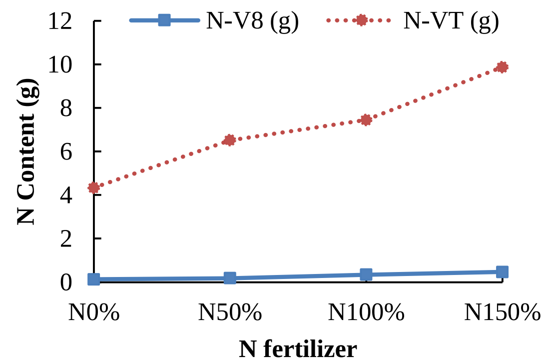

Figure 4. The relationship between corns N content (g) and applied N fertilizer

The relationship between Chl and N content of leaves

The relationship between the Chl and N content of leaves is shown in Fig 5. Based on the figure, the higher the N content of the leaves, the greater the Chl reading for both V8 and VT growth stages. Results showed a high correlation between leaves Chl reading and N content in the V8 (R^2=0.97) and VT (R^2=0.99) growth stages. According to the report of Xu et al. (2023), nitrogen is one of the important components of chlorophyll and there is a positive and significant relationship between them.

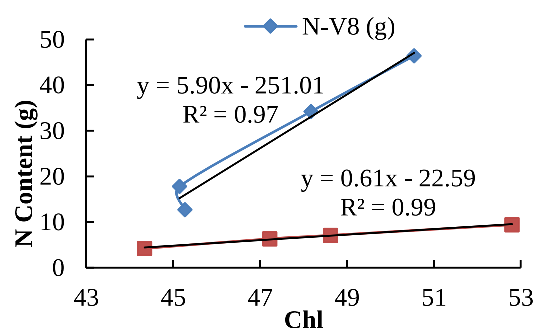

Figure 5. The relationship between Chl reading of leaves and N content

The relationship between leaves Chl and vegetation indices

To determine the correlation between Chl and vegetation indices in V8 and VT growth stages, the mathematical models of predicting Chl based on VIs were obtained. The results of predicted models and their validation by leave-one-out validation test are presented in Table 2. Second-order polynomial equations were used for modeling because of better fitting in comparison with other equations. Based on the results, REIP is predictable by Chl with R^2 values of 0.997, 0.990, 0.997, 0.970 and 0.997, respectively in the V8 growth stage and R^2 values of 0.982, 0.966, 0.900, 0.970 and 0.985, respectively in the VT growth stage. ARI and MCARI1 indices are good predictors of Chl in the V8 stage with R^2 values of 0.910 and 0.932, respectively and also TCARI and MCARI2 are good predictors of Chl in the VT stage with R^2 values of 0.961 and 0.971, respectively. By comparing R^2 values, it could be concluded that REIP and ARI are good predictors of Chl with R^2 of 0.99 in the V8 stage. The REIP index is a good predictor of Chl in the VT stage with an R^2 of 0.98. Ben et al. (2022) reported the correlation between the actual and estimated chlorophyll values with this method from 0.76 to 0.86. Narmilan et al. (2022) also found the coefficients of determination (R2) between the actual and estimated chlorophyll value of sugarcane, according to the type of algorithm used, from 0.68 to 98.

Table 2. Validation results of VIS models for estimating Chl

VI	Growth Stage	Predicted	Validated	
		R^2	R^2	RMSE
RDVI	V8	0.357	0.990	0.006
	VT	0.450	0.966	0.002
MSR	V8	0.620	0.025	0.132
	VT	0.999	0.609	0.365
MCARI1	V8	0.570	0.932	4.57
	VT	0.888	0.749	50.40
MCARI2	V8	0.920	0.584	0.007
	VT	0.440	0.971	0.006
TCARI	V8	0.921	0.174	169.3
	VT	0.990	0.961	135.8
ARI	V8	0.999	0.91	0.000
	VT	0.830	0.1	0.000
REIP	V8	0.984	0.997	0.03
	VT	0.978	0.985	0.004

The relationship between vegetation indices and applied N

The relationship between investigated vegetation indices and the level of applied N is shown in Fig 6. It is found that VIs had different responses to the N at the low, optimal and excessive N fertilizer. Most VIs showed a higher correlation with amounts of nitrogen in the V8 growth stage than in the VT growth stage because there was not much difference between treatments due to the small amount of nitrogen distributed in the V8 growth stage. MCARI2, and TCARI, indices were correlated more with the N in the VT growth stage than the V8 growth stage because more variations in plant N concentration could be explained by VIs at later growth stage when the canopy was fully closed compared to earlier stages when the field was partially covered by the canopy (Cao et al., 2015).

Based on the figure MSR indices values increased by increasing the applied N fertilizer in both V8 and VT growth stages. For the RDVI index, there is an increasing trend with increasing applied N fertilizer in the V8 stage and for the VT stage, the index value increased by increasing N fertilizer level in N50% and N150%. For the MCARI1 index, an increasing trend was obtained by increasing the N fertilizer level with a low slope in the V8 stage. In the VT stage, the MCARI1 index value decreased with increasing the N fertilizer level in N100% but it showed increasing trends in N50% and N150%. For the MCARI2 index, increasing trends were observed by increasing the N fertilizer level in the V8 stage except for N50%. These indices showed increasing trends by increasing the N fertilizer level except for N100% in the VT stage. The TCARI index in the V8 and VT stages decreased and increased by increasing the N fertilizer level, respectively. For the ARI index, a decreasing trend was observed by increasing the applied N fertilizer in the V8 stage. In the VT stage, the ARI index value decreased to N50% and N150% levels. The REIP index value decreased by increasing the applied N fertilizer level for both V8 and VT stages but in the V8 stage, the decreasing trend had a low slope. Santana et al. (2021) found a positive and significant relationship between corn nitrogen content and some vegetation indices.

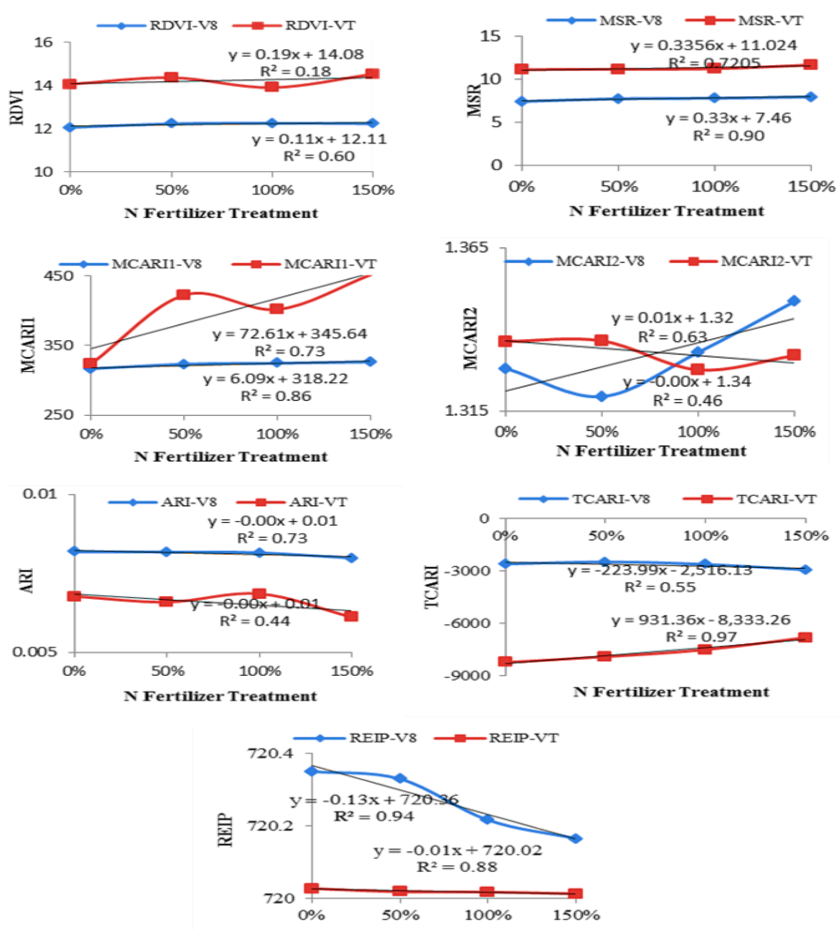

Figure 6. The relationship between vegetation indices and applied N

The results of the validation of obtained models for estimating N content (g) of leaves by VIs are presented in Table 4. In this table, R^2 and RMSE values of evaluated models by the Leave-one-out method are shown. The results demonstrated that strong relationships exist between the REIP indices and corn N content in both V8 and VT growth stages. Among the studied VIs ARI and TCARI indices are good predictors of N content in the V8 and VT growth stages, respectively. Therefore, by comparison of the R^2 and RMSE values of models, it could be concluded that the REIP index was the best vegetation index for modeling N content in the V8 growth stage. The TCARI index was the best vegetation index among investigated indices for modeling N content in the VT growth stage. Chen et al obtained correlation coefficients of 0.47, 0.83, 0.86 and 0.77 between the actual and estimated nitrogen values of the wheat crop for booting, heading, flowering and filling growth stages, respectively. Xue et al (2023) obtained similar results for monitoring leaf nitrogen content in rice.

Table 4. The results of the validation of models for estimating N content

VI	Growth Stage	R^2	RMSE	VI	Growh Stage	R^2	RMSE
RDVI	V8	0.65	0.15	TCARI	V8	0.88	0.11
	VT	0.22	3.48		VT	0.97	0.78
MSR	V8	0.65	0.18	ARI	V8	0.97	0.52
	VT	0.99	6.40		VT	0.35	2.83
MCARI1	V8	0.68	0.29	REIP	V8	0.98	0.03
	VT	0.88	1.52				
MCARI2	V8	0.75	0.10		VT	0.98	1.53
	VT	0.19	3.82				

CONCLUSIONS

In this paper, the application of precision agriculture and the performance of aerial multispectral imagery taken by the UAV were evaluated to estimate the N and Chl content of corn at different growth stages. The following conclusions are drawn from the results of the study:

- The amount of LDW, N and Chl contents of corn leaves increased by increasing the amount of applied N fertilizer in both V8 and VT growth stages.

- Among investigated indices, REIP was the best vegetation index for estimating the Chl content in both growth stages.

- Among investigated indices, the REIP index was the best vegetation index for estimating corn N content in both V8 and VT growth stages.

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