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Evaluating the impacts of nitrogen on the growth stages of cucumber

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ABSTRACT- Monitoring physiological parameters of plant and fertilizer requirements are the basic principles in precision farming. Non-destructive and accurate remote sensors make this process feasible and light-handed. The present study evaluates the efficiency of GreenSeeker (GS) and Soil-Plant Analyses Development (SPAD) in Nitrogen (N) fertilizer management. Normalized difference vegetation index (NDVI) was measured with GS and compared to SPAD. Fertigation with 5 different N treatments was applied to 100 pots. The first treatment (N1) had no N concentration, while the other treatments (i.e., N2, N3, N4, and N5) received 2, 9, 22, and 37 mmol.L^{-1} weekly, respectively. Next, the effect of volumetric fertilizing was investigated by adding supplemental fertilizer to N1 to N3 pots 71 days after planting. Nitrogen concentration in the leaf and first growing fruit was tested using the Kjeldahl method. The results of applied sensors confirmed with visible-near infrared spectroscopy at 200-1100 nm wavelength. NDVI, soil-adjusted vegetation index, and chlorophyll index were calculated from the available spectra and compared to the sensor outputs. Strong correlations were obtained between NDVI and all indices derived from spectra, especially in the vegetative phase. The results showed a strong correlation of NDVI with N rate, especially after supplemental fertilizing. Since the vegetation indices from spectra almost correlated well with NDVI and SPAD in all treatments, spectroscopy monitoring of cucumber could be a precise alternative technique. Linear and nonlinear regressions were applied to model variations of NDVI and SPAD. This study demonstrated the feasibility of using GS for N management according to its sensitivity to cucumber N status.

INTRODUCTION

Cucumbers are among the most commonly cultivated vegetables worldwide, and nitrogen is one of the most important macronutrients for plant growth and development. However, excessive nitrogen application can cause various negative effects on the growth and yield of cucumbers. Therefore, it is important to evaluate the impact of nitrogen on the growth stage of cucumbers to optimize its use.

Several studies investigated the impact of nitrogen on the growth of cucumbers at different growth stages. In a study, researchers evaluated the effects of different nitrogen levels (0, 80, 160, and 240 *kg* ha^{-1}) on the growth and yield of cucumber plants at different growth stages (vegetative, flowering, and fruit setting). The results showed that excessive nitrogen application (240 *kg* ha^{-1}) significantly decreased the yield of cucumber plants compared with the control treatment (0 *kg* ha^{-1}). However, the application of 160 *kg* ha^{-1} nitrogen resulted in the highest yield of cucumber plants, indicating that nitrogen application at an appropriate level can promote the growth and yield of cucumbers (Padilla et al., 2017). Another study evaluated the effects of different nitrogen application rates (0, 75, 150, and 225 *kg* ha^{-1}) on the growth and yield of cucumber plants at the flowering and fruiting stages. The results showed that nitrogen application at a rate of 150 *kg* ha^{-1} significantly increased the yield of cucumber plants compared with the control treatment (0 *kg* ha^{-1}). Nevertheless, excessive nitrogen application (225 *kg* ha^{-1}) significantly decreased the yield of cucumber plants compared with the optimal nitrogen treatment (150 *kg* ha^{-1}) (Basyouni et al., 2015). In addition, the impact of nitrogen on the growth of cucumbers can also be influenced by other factors such as soil type, climate, and management practices. For instance, another study found that nitrogen application increased the yield of cucumber plants in sandy soil but had no significant effect on yield in loamy soil. Therefore, the application of nitrogen should be tailored to the specific conditions of the cultivation environment (Basyouni et al., 2015).

Nitrogen management is a subject of precision agriculture. Potential yield and intensive vegetation depend on quantities of N fertilizer. Besides, excess use of nitrogen fertilizer causes groundwater contamination or N concentration on fruits. In addition, nitrogen production factories cause air pollution due to the emission of carbon dioxide into the atmosphere (Lemaire et al., 2008). Therefore, determination of N requirement for growth of crops is necessary and it depends on the amount of N soil supply. Monitoring crops, soil, and environmental effects allows farmers to control and correct N needs continuously (Padilla et al., 2016).

Remote sensing technology is among the non-destructive and precise methods to evaluate agricultural inputs. CropCircle sensor (Holland Scientific, Lincoln,

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NE) was applied to measure the canopy reflectance of cucumber (Padilla et al., 2017) and muskmelon (Padilla et al., 2014) to predict N status. Spectroscopy and digital or multispectral cameras were also available and easy methods (using different filters) to evaluate the N content of plants widely (Baresel et al., 2017; Yang et al., 2008). Since chlorophyll is a criterion to show the appropriate supply of nitrogen, a chlorophyll meter (Soil-Plant Analyses Development, SPAD) as a reliable sensor was applied to estimate leaf greenness and N content of different crops, including rice (Larijani and Farokhi-Teymorlou, 2012), muskmelon (Padilla et al., 2014), and maize (Schmidt et al., 2011). Biochemical chlorophyll and some vegetation reflectance of lettuce, leaf mustard, radish, and cabbage were determined using an atLEAF Chlorophyll meter (Ft Green LLC, Wilmington, DE) and a miniature leaf spectrometer (Alsina et al., 2016). In another study, the nitrogen concentration of potted poinsettia was assessed using atLEAF, SPAD, and GreenSeeker (GS) (Basyouni et al., 2016). GS sensor was used in N evaluation and yield estimation of cereal fields such as wheat (Cao et al., 2015), rice (Ali et al., 2014), and corn (Sharma et al., 2014, 2016). Such sensors with low measurement radia are commonly used in greenhouses; besides, monitoring large fields is possible with satellite continuously (Wu et al., 2007; Liaqat et al., 2017). Sensors act in a variety of wavelength ranges and measure different indices. Vegetation indices (VIs) are valuable parameters to assess physiological parameters, especially the N content of crops. The most commonly applied index is the normalized difference vegetative index (NDVI). This index is very useful for deriving two-dimensional vegetation parameters, such as the fraction of absorbed photosynthetically active radiation and green cover percentage (Tucker, 1979). NDVI has been widely used because of its strong correlation with the physiological and quality parameters of plants (Li *et al.*, 2013; Meng *et al.*, 2013; Ozdemir, 2014; Yuan et al., 2014).

Overall, the impact of nitrogen on the growth of cucumbers is complex and can be influenced by various factors. Excessive nitrogen application can have negative effects on the growth and yield of cucumber plants, while an appropriate amount of nitrogen can promote their growth and yield. Therefore, it is important to carefully evaluate the impact of nitrogen on the growth stage of cucumbers and optimize its application to achieve the best results.

The main objectives of this study are three-fold. First, it is tried to evaluate the ability of GreenSeeker as a portable and non-contact instrument to estimate N requirements of greenhouse cucumber. Second, this research compares the consistency of NDVI and its relationship with SPAD. Third, it suggests the sufficiency value of urea fertilizer with less N concentration in fruits.

MATERIALS AND METHODS

Experimental design

During the spring of 2017, cucumber crops were transplanted in 100 trays filled with original loam soil in a plastic greenhouse. After three weeks, the young seedlings were planted in bigger pots with 3 kg of soil and a layer of gravel underneath each pot for better ventilation. The soil composition was examined before putting it in pots (Table 1). Pots were placed in an experimental polycarbonate greenhouse in five rows, with each row representing a treatment. During the seedlings transfer, 9 roots and stems of plants were damaged such that the fifth treatment had 11 pots. An electrical fan and a heater were used as actuators a flap roof window for exchanging air and a simple matting shade for sunny hours. The greenhouse with southeast orientation and 34 47 N and 48 28 E coordination was located in the Agricultural Department of Bu-Ali Sina University, Hamadan, Iran.

Table 1. Soil features

Soil feature	Amount
pH	6.89
Electrical conductivity (EC)	0.2 $ds\ m^{-1}$
Ca	15 $mg\ lit^{-1}$
Mg	3 $mg\ lit^{-1}$
Cl	35.4 $mg\ lit^{-1}$
K	27.89 $mg\ lit^{-1}$
P	2.8 $mg\ lit^{-1}$
N	0.155 $mg\ lit^{-1}$

Growth conditions

In this research, 100 plants of each variety in 5 treatments with 20 samples were planted in pots. Then, the treatments were separated from each other by receiving different percentages of 46% urea fertilizer with weekly repetition (Table 2). Gianquinto et al. (2011) and Padilla et al. (2014 and 2017) suggested five fertilizer treatments in their studies on cucumbers.

Table 2. Amounts of fertilizer for cucumber

Amount of fertilizer	Treatment
1	noun
2	0.028 $g\ lit^{-1}$
3	0.138 $g\ lit^{-1}$
4	0.359 $g\ lit^{-1}$
5	0.607 $g\ lit^{-1}$

The deficiency of other elements in the plant was prevented using 0.5 g/L of full specialty humic fertilizer for all treatments. This fertilizer treatment (i.e., control fertilizer) was also used in other studies (Padilla et al., 2017; Gianquinto et al., 2011).

Each pot was irrigated 50 mL every day using a crop sprayer. Nutrients were supplied to crops by applying humic acid to every pot before the urea N treatment started. The main contents of humic acid were 10%, 5%, 4%, and 3% of nitrogen, phosphor, potassium, and sulfur, respectively. Four N concentrations (i.e., N2 = 2, N3 = 9, N4 = 22, and N5 = 37 $mmol.L^{-1}$) were applied using urea (NH_2CONH_2) fertigation with 46% nitrogen (N) every week and at six replications. There was a very N-deficient treatment (N1) with no urea fertilizer. Also, there were 20 samples in every treatment, except N5 that had 11 pots. The NDVI and SPAD measurements were applied one week after fertigation. The crop behaviors were studied by supplementing the fertilizer with pots in the 1st, 2nd, and 3rd treatments. Before determining the treatments (especially the control treatment), humic acid fertilizer was given to all pots for one week, so that the conditions of all the pots were the same. From the second week, the control treatment was separated and no more nitrogen fertilization was given to this treatment (N1). Also, from the 10th day onward, the rest of the pots (treatments) received the nitrogen fertilizer. The amount of supplemental N was calculated based on receiving fertilizer of N5 during the growth (Eq. (1)).

$$Supplemental\ fertilizer\ = \sum received\ N\ with\ N5 - \sum\ received\ N\ with\ Ni, i\ = 2,3,4 \quad Eq. (1)$$

Sensors reading

Vegetation indices were measured one week after fertilizing. The first fertigation was 12 days after transferring seedlings to an experimental greenhouse with a polycarbonate roof and walls. During the first week, humic acid was applied and 10 days later the N treatment was started. Furthermore, the first measurements were 22 days after planting (DAP). Afterwards, GS (Trimble Navigation Limited, Sunnyvale, California, USA) and SPAD (Minolta Camera Co. Ltd., Japan) were applied weekly to each pot. The VNIR spectrometer (AvaSpec-ULS 2048- UV-Vis, Avente, Netherland) was also used and vegetation indices were derived from spectra (Wavelength range was 600-690 nm). Leaves and fruits' nitrogen content was evaluated with the destructive but trusted experiment of Kjeldahl on 78 DAP. This test was performed before and after applying the N supplementation to N1, N2, and N3. In addition, the number of newly grown leaves was calculated every week, before the next fertilization.

Statistics

In vegetative and reproductive phases, the correlation of GS-NDVI with VNIR-NDVI (Eq. (2)), SPAD, Chlorophyll Index-CI (Eq. (3)), and Soil Adjusted Vegetation Index-SAVI (Eq. (4)) was determined in all available treatments. Besides, effect of N rate variation on sensor measurements was evaluated with the interface of supplemental fertilizer. To find more accurate relationships between SPAD and NDVI measured by GS (GS-NDVI) with NDVI derived from spectra (VNIR-NDVI), another vegetation index (VI) was derived from VNIR wavelength bands: VI = NIR/R (Gianquinto et al., 2011).

$$NDVI\ = \frac{NIR - R}{NIR + R}, R = average\ of\ 600\ to\ 690\ nm \qquad Eq. (2)$$

NDVI: Normalization Different Vegetation Index
R: Ration

$$CI\ = NIR/_{G} - 1, \quad G\ = green\ wavelength \qquad Eq. (3)$$

CI: Chlorophyll Index

$$SAVI\ = 1.5 \times (\frac{NIR - R}{NIR + R - 0.5}) \qquad Eq. (4)$$

SAVI: Soil Adjusted Vegetation Index

Linear, quadratic, cubic (Eq. (5)), and power (Eq. (6)) regressions were applied to evaluate the relationships between NDVI and SPAD during the growing period. Moreover, the multiple regression of SPAD was fitted to VIs to validate the model. The coefficient of determination (R^2) and *P*-value were calculated to measure the accuracy of regression models.

$$SPAD\ = ax_i + bx_i^2 + cx_i^3\ , x_i\ = GS.NDVI \qquad Eq. (5)$$

$$SPAD\ = a \times x^b\ x\ = GS.NDVI \qquad Eq. (6)$$

a, b, and c are the amounts of chlorophyll.

Table 3 shows the so-called thematic bands in the NASA LANDSAT satellite. The primary function of LANDSAT is to obtain and transmit images of the Earth from space to monitor the effect of environmental conditions on the planet. The bands are expressed in terms of wavelength, with 1 μm being equal to 10^{-6} *m*. For further information about the characteristics and uses of each band, see Muñoz-Huerta et al. (2013).

Table 3. Thematic bands in the NASA LANDSAT satellite

Band No.	Name	Wavelength (μm)	characteristic and use
1	Visible blue	0.45-0.52	Maximum water penetration
2	Visible green	0.52-0.6	Good for measuring plant vigor
3	Visible red	0.63-0.69	Vegetation discrimination
4	Near-infrared	0.76-0.90	Biomass and shoreline mapping
5	Middle infrared	1.55-1.75	Moisture content of soil and vegetation
6	Thermal infrared	10.4-12.5	Soil moisture; thermal mapping
7	Middle infrared	2.08-2.38	Mineral mapping

At the end, the correlation coefficient in SPSS software was applied to find out whether the measurement methods were efficient for the purpose of this research.

Correlation

Since the control treatment did not receive fertilizer, it is necessary to obtain and compare the correlation coefficients of the fertilizer content and the NDVI reading with the chlorophyll content of treatments 2 to 5.

RESULTS AND DISCUSSION

The relationship between N concentration and supplemented N

The average of NDVI and SPAD readings of pots in every treatment was ascending up to 43 DAP (Fig. 1).

According to Fig. 1a, a sudden decline occurs in NDVI after 43 DAP simultaneous as flowering. From 51 DAP, the chart returns to the previous state gradually until the first fruit grows from 64 to 71 DAP. The same results were obtained for NDVI of the fall and spring cucumbers (Padilla et al., 2017). A significant increase was observed after applying supplemental fertilizer in treatments N1, N2, and N3 such that their NDVI almost went beyond N4 and N5 as nitrogen-rich treatments. This result suggests that N addition had a direct impact on the measured NDVI of a canopy. There is an obvious difference between treatments throughout the crop growth stage. Differences observed for nitrogen-rich treatments (i.e., N4 and N5) in those NDVI were always greater than 0.4 during the growth even in reduction periods (flowering and reproductive stages).

The variation of SPAD was almost similar to NDVI in the growing stages of cucumber (Fig. 1b). The lowest value of SPAD belonged to N1 from 29 to 71 DAP (Fig. 1b), while it reached the highest point at 78 DAP. There was a slight difference between the behavior of GS-NDVI and SPAD measurements in treatments N1 to N3. These treatments showed the minimum value of SPAD in 71 DAP, thenceforth increased suddenly by adding supplemental fertilizer (78 DAP). Afterwards, SPAD values in N1, N2, and N3 reached 60, which exceeded those of N4 and N5. The same as GS-NDVI, SPAD increased after appending supplemental fertilizer in N1, N2, and N3.

Cucumbers of each treatment were also examined to find out the amount of nitrogen accumulation in fruits (Fig. 1c and Fig. 1d). Between 5 to 7 pots in every treatment fruited around 71 DAP. Results of the Kjeldahl test showed N concentration in fruits before supplemental fertigation at 71 DAP (Fig. 1c). The cucumber from N5 had the maximum N concentration, although there was not much difference between N5 and N4. Evaluation of fruits one week after supplementation (78 DAP) showed incredible results. Compared to the gradual fertilizing, receiving the required N entirely increased the N content of the fruit suddenly (Fig. 1d). The amounts of N concentration in fruits of N2 and N3 were even higher than N5, as the most rigorous urea-fertilizer treatment. Fig. 2 presents the relationship between GS-NDVI and SPAD during the growth stages of cucumbers.

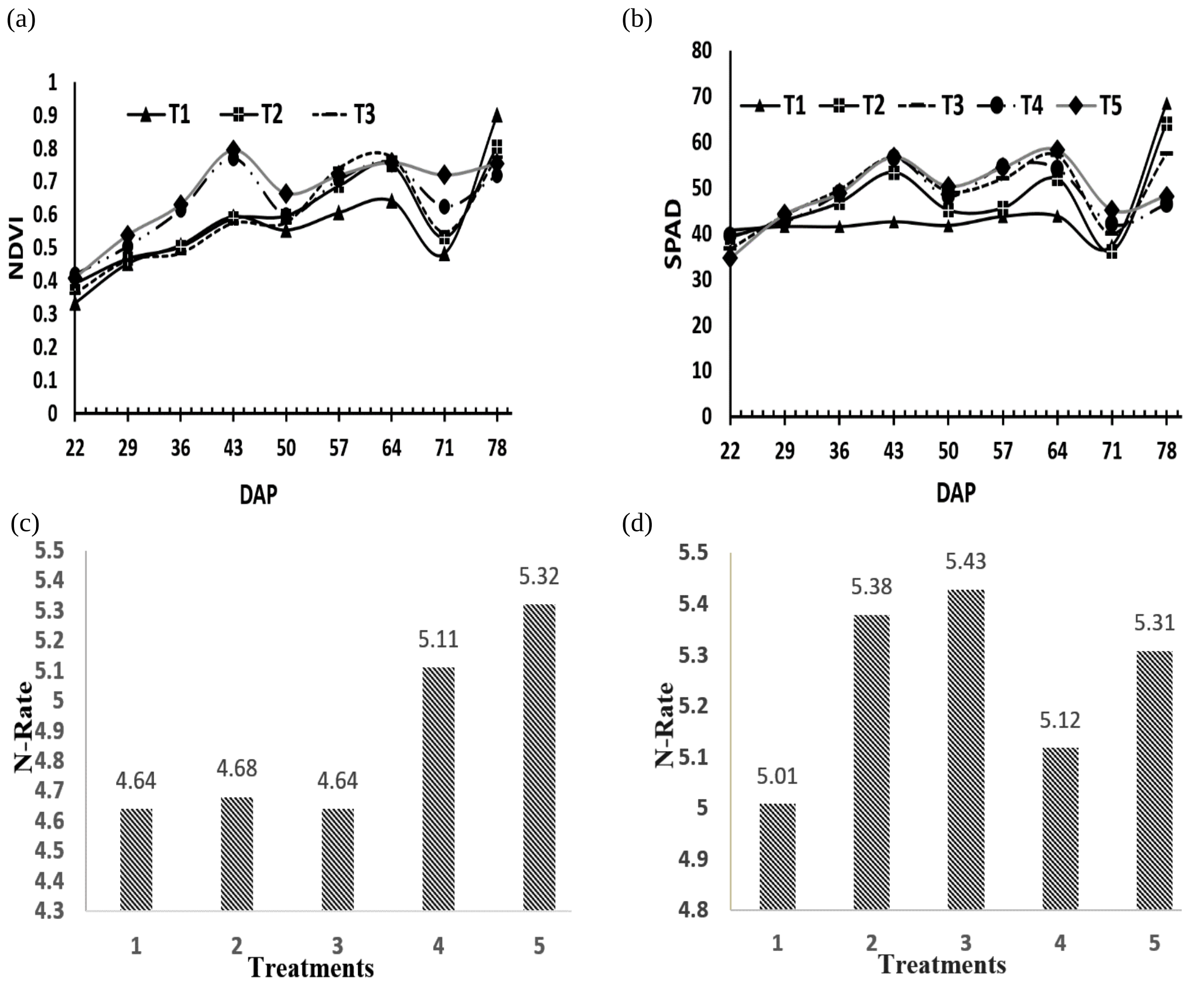

Fig. 1. Variation of (a) NDVI (Normalization Different Vegetation Index), (b) SPAD (Silicon Photodiode Arrester directly, Chlorophyll Meter) from 22 to 78 DAP (Date After Plant) and N rate (Kjeldahl) in cucumbers, (c) before supplemental, and (d) after supplemental fertilizer.

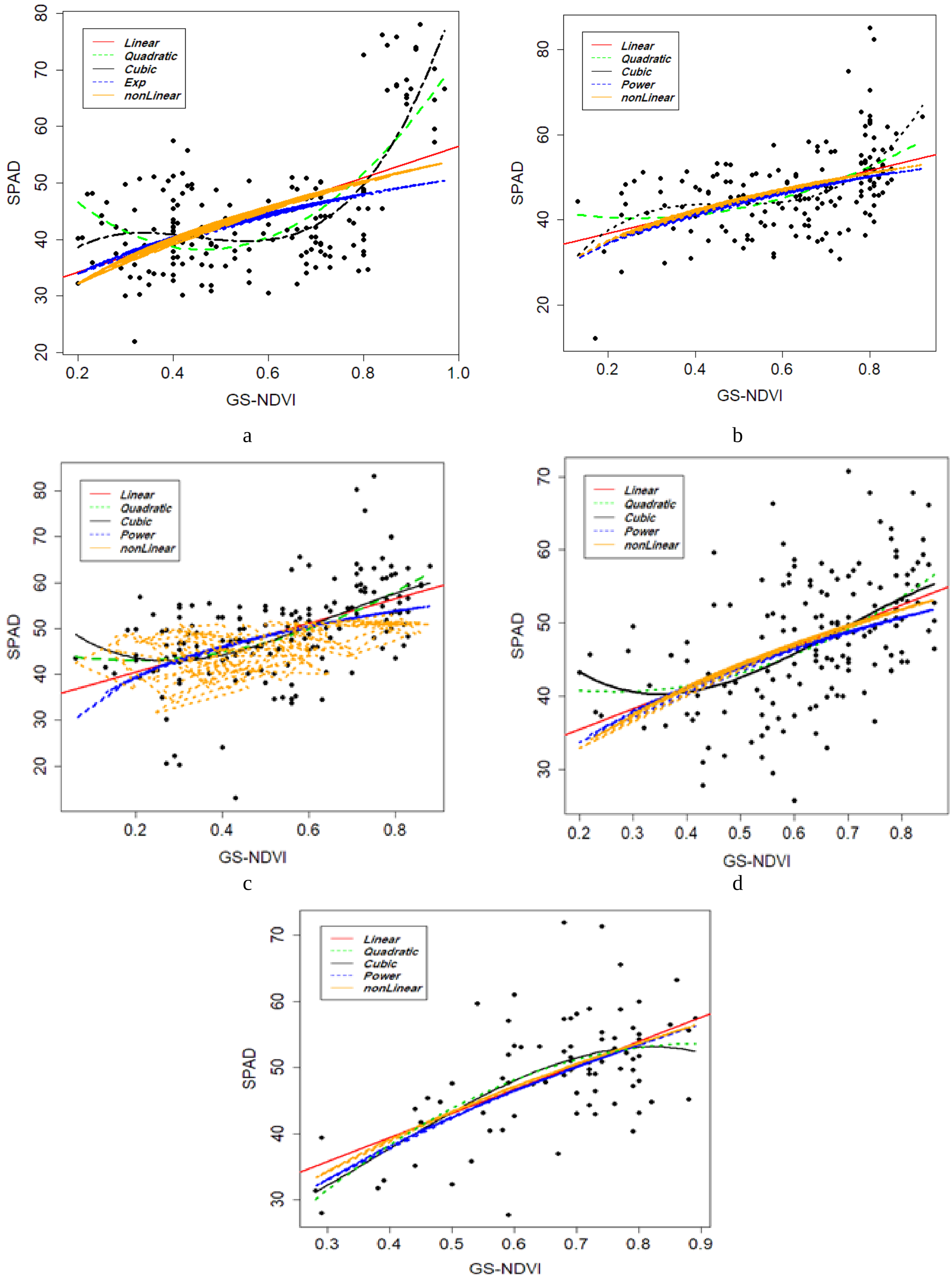

Fig. 2. The relationship between GS-NDVI (GreenSeeker measuring device Normalization Different Vegetation Index) and SPAD (Chorolopyll Meter) during the growth stages of cucumbers, (a) to (e) is related to N1 to N5, respectively.

Fig. 3 and Fig. 4 show the linear regression model. Nonlinear models did not indicate this difference accurately. The best model for each index was linear and in a few cases quadratic using the Akaike Information Criterion (AIC, Akaike, 1974). This criterion represents the best compromise between the goodness of fit and the complexity of a model.

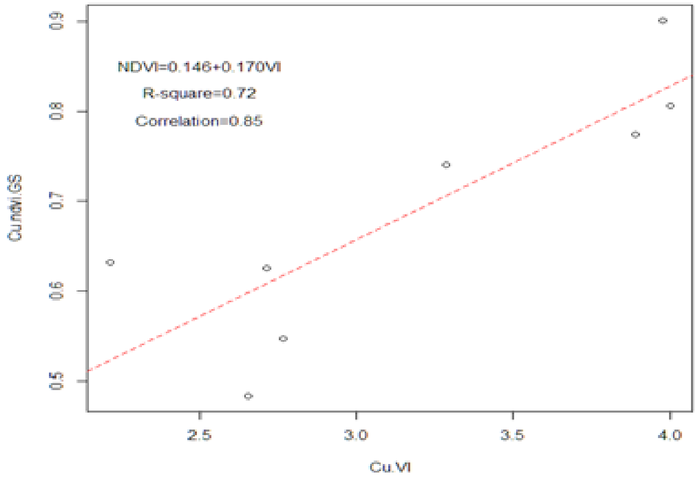

Fig. 3. Regression model and correlation of Vegetation index (VI) and NDVI (Normalization Different Vegetation Index).

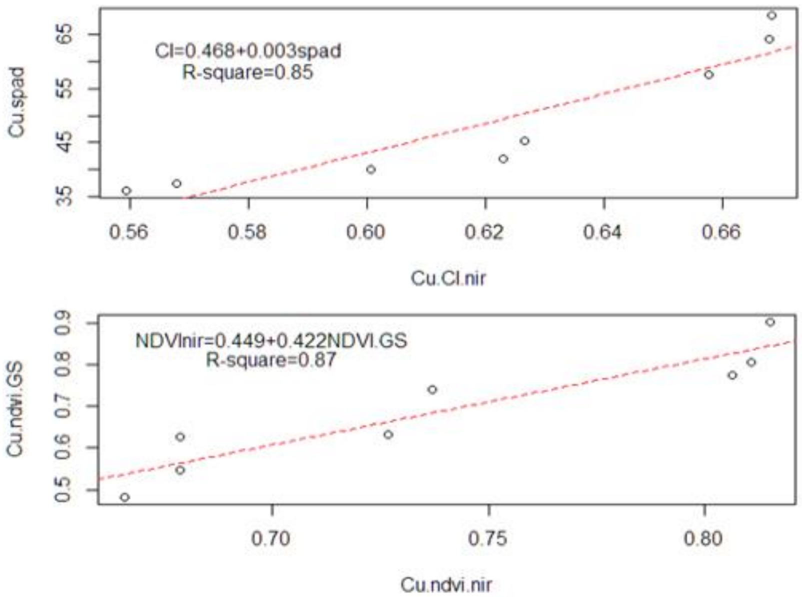

Fig. 4. Regression model for predicting chlorophyll index (CI) and Normalization Different Vegetation Index (NDVI) in cucumbers.

Effects of N on sensor readings

Sensors were used from 22 DAP when the crops had enough leaves to cover adequately. The correlation of GS-NDVI measurement with VNIR-NDVI, SPAD, CI, and SAVI was calculated during the growth of cucumbers (Table 4). In addition, there was a significant correlation between GS-NDVI and SPAD during the vegetative period. However, GS-NDVI and SPAD showed less correlation in the reproductive stage especially in N1, N2, and N3, which received lower N fertilizer. According to Table 4, a significant correlation in the flowering stage was observed in N4 and N5, while it was lower in N1 to N3. This difference can be related to the fertilizer richness of N4 and N5. In addition, VNIR-NDVI correlated well with GS-NDVI even in the reproductive phase. In other words, NDVI shows fertilizer poverty of cucumbers in fructiferous. Also, CI and SAVI had a significant correlation with NDVI in both vegetative and productive stages despite a slight decline in the reproductive period. Another result of Table 4 was the ascending trend of sensor measurements from N1 to N4 and N5.

To find the effect of N rate, the correlation of NDVI, SPAD, and leaf numbers was obtained with N fertilizer variation for all treatments (Table 5), except for N1 (since it had no fertilizer). Several correlations were obtained according to the N rate in available treatments. The relationship between N rates and NDVI was highly different compared to SPAD. Contrary to SPAD, NDVI correlated more significantly with the N rate in all treatments. Even though leaf numbers increased by increasing fertilizer, supplemental fertilizer had a reversed effect on N2 and N3. Gradual fertigation causes cucumber with more leaves (correlation with leaf numbers increase from 0.766 in N2 to 0.917 in N5). Since the pots in treatments N4 and N5 were denser and bushier, the correlation of leaf numbers was stronger with the N rate compared with N2 and N3. Meanwhile, bushier crops would not guarantee whether fertilizer was enough. The most independent factor with the N rate was SPAD. Moreover, supplemental nitrogen had a great effect on the correlation of NDVI and N rate in N2 and N3. In other words, the vegetation index depended on the fertilizer rate (Basyouni *et al*., 2015). According to Fig.

1 and Table 5, gradual and sudden fertilizer had the same effect on the vegetation of cucumber.

In this study, VI was compared to SPAD and NDVI throughout the crop cycle to obtain a precise estimation of plant condition (Table 6). VI1, VI2, and VI3 were calculated in 560, 660, and 760 nm, respectively (Gianquinto et al., 2011). According to Table 5, VIs correlated well with sensor reading, especially with both calculated NDVIs. Meanwhile, there was not a big different correlation between VIs in available treatments, except for VI3 with GS-NDVI in N3 (bolded in Table 6).

Estimating SPAD with vegetation indices

Polynomial regression with a wide application was used to fit vegetation indices (Wang et al., 2017). The first-, second-, and third-degree polynomial and exponential functions were used to estimate the relationships of GS-NDVI with SPAD (Table 7). In comparison, linear to exponential functions were significant at $^{*}P < 0.05$ in N1 to N5. *P*-values of quadratic and cubic functions were low enough (N1, N2, and N3), but they were not always significant. In linear, quadratic, and cubic functions, R^2 values of N1 to N5 were almost the same. In contrast, the cubic model showed a non-significant *P*-value ($P > 0.1$) in N3, N4, and N5. Unlike the acceptable R^2 value, the quadratic model showed significant results with $^{**}P < 0.01$ at most treatments. Despite a significant *P*-value, the exponential function was not statistically significant with such low R^2 values (0.23-0.31). The regression lines of different models are displayed in Fig. 2a-e. According to Padilla et al. (2016), moderate and low determination coefficients could be acceptable.

Table 4. Correlation coefficients of GS-NDVI (GreenSeeker measuring device Normalization Different Vegetation Index) with canopy parameters of greenhouse cucumber

Treatment	Vegetative phase				Productive phase			
	SPAD	VNIR-NDVI	CI	SAVI	SPAD	VNIR-NDVI	CI	SAVI
N1	0.797	0.849	0.819	0.981	0.494	0.747	0.651	0.698
N2	0.829	0.874	0.854	0.865	0.586	0.788	0.683	0.793
N3	0.853	0.956	0.855	0.867	0.496	0.902	0.553	0.962
N4	0.996	0.925	0.971	0.940	0.872	0.871	0.799	0.928
N5	0.993	0.855	0.912	0.922	0.817	0.840	0.885	0.919

Table 5. Correlation coefficients of N-rate with canopy parameters of greenhouse cucumber

Treatment	After supplemental fertilizer				Before supplemental fertilizer			
	GS-NDVI	VNIR-NDVI	SPAD	Leaf No.	GS-NDVI	VNIR-NDVI	SPAD	Leaf No.
N2	0.847	0.934	0.101	0.757	0.423	0.501	0.147	0.766
N3	0.896	0.951	0.190	0.764	0.529	0.604	0.182	0.867
N4	-*	-	-	-	0.499	0.617	0.034	0.875
N5	-	-	-	-	0.473	0.475	0.034	0.917

* There was no supplemented fertilizer for N4 and N5
GS-NDVI: GreenSeeker measuring device Normalization Different Vegetation Index
VNIR-NDVI: wavelength bands of Normalization Different Vegetation Index
SPAD: Chorolopyll Meter

Table 6. Correlation coefficients between Vis (Vegetation index) and sensor readings

Parameter	Stage	N1	N2	N3	N4	N5
GS-NDVI	VI1	0.952	0.993	0.907	0.991	0.998
	VI2	0.891	0.953	0.690	0.881	0.991
	VI3	0.825	0.868	0.414	0.874	0.991
SPAD	VI1	0.865	0.726	0.807	0.849	0.802
	VI2	0.708	0.601	0.804	0.589	0.828
	VI3	0.519	0.660	0.804	0.563	0.779
VNIR-NDVI	VI1	0.968	0.909	0.956	0.905	0.830
	VI2	0.942	0.853	0.756	0.889	0.812
	VI3	0.838	0.864	0.494	0.888	0.826

GS-NDVI: GreenSeeker measuring device Normalization Different Vegetation Index
SPAD: Chorolopyll Meter
VNIR-NDVI: wavelength bands of Normalization Different Vegetation Index

Table 7. R^2 values of polynomial models in growth stages

	Model	N1	N2	N3	N4	N5
R^2	Linear	0.58	0.52	0.57	0.54	0.54
	Quadratic	0.66	0.64	0.69	0.56	0.66
	Cubic	0.72	0.68	0.61	0.56	0.66
	Exponential	0.32	0.23	0.28	0.25	0.31
P-value	Linear	8.82e-14***	2.15e-10***	5.85e-13***	1.54e-10***	1.54e-10***
	Quadratic	2.20e-16***	2.66e-10**	4.61e-13*	1.61e-10*	3.33e-08.
	Cubic	2.20e-16***	2.78e-11**	1.72e-12	6.17e-10	1.58e-07
	Exponential	4.55e-16***	5.83e-11***	1.53e-13***	5.14e-11***	7.59e-08***

Some multiple regressions were applied to find the more accurate relationship between SPAD and VIs and leaf number and VIs. The models were evaluated using AIC and the best prediction model was defined in each treatment. The following four models (Eqs. (7-10)) were applied to validate VIs. According to Eqs. (7-10), leaf numbers or SPAD could be the successor as Y. AIC results showed that the lowest AIC belongs to the model in Eq. (9) in all treatments with 8 degrees of freedom. In these models, Y was predicted with $R^2 > 0.93$, indicating that new growing leaf and SPAD values will be determined by knowing VIs.

$$Y = a + b1 * VI1 + b2 * VI2 + b3 * VI3 \quad \text{Eq. (7)}$$

$$Y = a + b1 * VI1 + b2 * VI2 + b3 * VI3 + b4 * VI1 * VI + b5 * VI1 * VI3 + b6 * VI2 * VI3 + b7 * VI1 * VI2 * VI3 \quad \text{Eq. (8)}$$

$$Y = a + b1 * VI1 + b2 * VI2 + b3 * VI3 + b4 * VI1 * VI2 + b5 * VI1 * VI3 + b6 * VI2 * VI3 \quad \text{Eq. (9)}$$

$$Y = b1 * VI1 + b2 * VI2 + b3 * VI3 \quad \text{Eq. (10)}$$

On days 78 and 57, cucumber leaves were subjected to spectrometry by AvaSpec-ULS 2048-UV-Vis spectrometer (Avante Co. Nrtherland & Perkin Elmer, USA) in the spectral range of 200 to 1100 nm. The accuracy of Greenseeker and Spad sensors was evaluated by extracting NDVI and CI from the spectrum, respectively. Fig. 4 shows the linear regression model for predicting NDVI and chlorophyll of cucumber. Since the R^2 obtained in all cases was higher than 70%, the accuracy of GS and SPAD was confirmed as non-destructive methods in measuring the greenness of greenhouse cucumbers.

CONCLUSION

In this research, NDVI and chlorophyll data were collected in two stages without fertilization, one stage for control fertilizer, one stage for humic acid fertilizer, four stages for application of fertilizer program, and one stage for treatment fertilizer at a time interval of 7 days for cucumbers. The data collection interval of 7 days was considered as this interval shows the highest performance. Due to the same amount of soil nitrogen in all treatments, a control fertilizer step was applied to all treatments of cucumber to make the nitrogen of the plants the same. Due to the low amount of control fertilizer, NDVI changes occurred at the same level. Humic acid fertilizer was used to meet the plant's need for other nutrients. The reason for the changes in the NDVI index at the time of humic acid fertilizer application was the presence of 10% nitrogen in this fertilizer. The NDVI index had an ascending trend until the third stage of data collection when implementing the experiment and applying nitrogen fertilizer. In the fourth stage of applying nitrogen fertilizer, the NDVI reading declined due to the plant fertilization and nitrogen saturation in the plant. The reason for the decrease in the diagram is nitrogen saturation in the plant. In the fifth stage of data collection, fertilizers were applied in treatments N1 to N3.

Nitrogen is one of the essential macronutrients required by plants for their growth and development. Cucumbers, like other plants, require nitrogen for their vegetative growth, chlorophyll synthesis, and protein formation. However, the impact of nitrogen on the growth stage of cucumber plants can be both positive and negative, depending on the amount and timing of application. The positive impacts of nitrogen on cucumber growth can be summarized:

- Increased leaf and stem growth: Nitrogen is a primary constituent of chlorophyll. This green pigment in plants enables them to photosynthesize. When cucumbers receive adequate nitrogen, they produce more chlorophyll, leading to more leaf and stem growth.
- Improved fruit yield and quality: Nitrogen is essential for synthesis of protein and proteins, as the building blocks of fruits and vegetables.
- Adequate nitrogen supply can enhance cucumber fruit yield and quality. Earlier maturity: nitrogen application can accelerate cucumber plant development, resulting in earlier maturity and harvest.

On the other hand, the negative impacts of nitrogen on cucumber growth are as follows:

- Delayed fruit formation: Excessive nitrogen application can delay cucumber fruit formation, resulting in reduced fruit yield.
- Reduced fruit quality: Excessive concentration of nitrogen can also affect cucumber fruit quality, making them softer and more prone to disease and pest infestation.
- Susceptibility to environmental stress: Cucumbers with an excess of nitrogen are more susceptible to environmental stress, such as drought and temperature extremes.

In conclusion, although nitrogen is essential for the growth and development of cucumber plants, it should be applied judiciously to avoid negative impacts on fruit yield and quality. Cucumber growers should supply carefully their plants with the appropriate amounts of nitrogen at the right time, depending on the specific growth stage of the plant.

According to the results of this study, NDVI is a potential vegetation index for monitoring N requirements of greenhouse cucumber. GS showed accurate results compared to SPAD, especially after the vegetation phase. When pots had a green dense canopy, GS would have accurate performance, while SPAD was useful from the first growing leaf. Spectroscopy provided more accurate results at the early stage of the growing phase compared to GS and SPAD. A linear model could be efficient enough to find the relationship between the sensor readings. It is suggested to do the same study with a portable spectrometer with a wide range of wavelengths to support the results of these optical sensors.

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CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review and editing, visualization, supervision, project administration, and funding acquisition, by Behnam Sepehr.

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

This work is not related to experimental animals or specific human diseases that requires publication and approval of publication ethics.

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request."

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