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Estimation of Nitrogen Levels By Remote Sensing Method in Alfalfa (*Medicago sativa* L.)

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ABSTRACT
The aim of this study was to obtain spectral reflectance values by using remote sensing method in alfalfa plant and to estimate nitrogen levels by using these values. The treatments were carried out in both field and greenhouse conditions. Different nitrogen, phosphorus and potassium doses were applied to plots in the field and pots in greenhouse. Spectral reflectance measurements were made at both the canopy (general) level and the single leaf level when the plants were in pre-flowering stage. Reflectance measurements were undertaken using a portable spectroradiometer measuring the wavelength range of 325-1075 nm of the electromagnetic spectrum. Plant probe with artificial light source and leaf clip were used for leaf measurements. Statistical analyses were conducted using stepwise regression analysis implemented in MINITAB-13 statistical program. As a result of the study, significant relationships (Field-canopy: 0.94, Field-leaf: 0.23, Greenhouse-canopy: 0.55, Greenhouse-leaf: 0.92) were determined between the nitrogen levels in the plant and the reflectance values. Also results showed that changes in nitrogen levels affect reflectance in the visible region (400-700 nm) of the spectrum. When the wavelengths were evaluated separately, it was observed that the wavelengths of the blue, red and near infrared regions, which are important for photosynthesis, can be used to estimate the nitrogen levels.

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Yonca (Medicago sativa L.)'da Uzaktan Algılama Yöntemi İle Azot Düzeylerinin Tahmin Edilebilirliği

ÖZET
Bu çalışmada, yonca (*Medicago sativa* L.) bitkisinde uzaktan algılama yöntemi ile spektral yansıma değerlerinin elde edilmesi ve bu değerlerin kullanılmasıyla azot düzeylerinin tahmin edilmesi amaçlanmıştır. Çalışmalar hem tarla hem de sera koşullarında yürütülmüştür. Tarla koşullarında parsellere ve sera koşullarında saksılara farklı oranlarda azot, fosfor ve potasyum uygulaması yapılmıştır. Spektral yansıma ölçümleri bitkilerin çiçeklenmeye başladığı dönemde kanopi (genel) ve tek yaprak düzeyinde yapılmıştır ve yansıma ölçümleri elektromanyetik spektrumun 325-1075 nm dalga boyları arasında yansıma ölçümleri yapabilen taşınabilir bir spektroradyometre kullanılarak yapılmıştır. Yapraktan yapılan ölçümler için yapay ışık kaynağı bulunan yaprak ölçüm cihazı (plant probe) ve yaprak tutucu (leaf clip) kullanılmıştır. Elde edilen verilerin istatistiksel analizi MINITAB 13 istatistik programında stepwise (değişken ekleme-çıkarma) regresyon analizi kullanılarak yapılmıştır. Çalışma sonucunda, bitkideki azot düzeyleri ile yansıma değerleri arasında önemli (Tarla-genel: 0.94, Tarla-yaprak: 0.23, Sera-genel: 0.55, Sera-yaprak:0.92) ilişkiler belirlenmiştir. Sonuçlar ayrıca, azot düzeylerindeki değişimlerin özellikle spektrumun görünür bölgesindeki (400-700 nm) yansımaları etkilediğini göstermiştir. Dalga boyları ayrı ayrı değerlendirildiğinde ise özellikle fotosentez için önemli olan mavi, kırmızı ve yakın kızılötesi bölgelere ait dalga boylarının azot seviyelerinin tahmininde kullanılabileceğini göstermiştir.

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INTRODUCTION

Alfalfa (*Medicago sativa* L.) has an important place in animal nutrition all over the world and can grow in many areas thanks to its wide adaptability ability. Economic value of alfalfa is quite high and cultivation areas are around 660 thousand hectares in Turkey (Anonymous, 2018). The alfalfa plant is called "the queen of forage plants" because of its broad adaptability, nitrogen binding to the soil, high protein content from the unit side, and rich nutritional content in terms of mineral substances and vitamins (Manga et al. 1995; Elçi, 2005; Yılmaz and Albayrak, 2016).

Despite having a very large prescription in animal nutrition, there are some problems with forage crops in our country. Low forage crop area and poor quality of feed can be noted as leading problems. However, it is belived that the quality of feed as well as the high efficiency in the production of forage crops is at the desired level (Açıkgöz et al. 2005).

The quality concept of forage crops is closely related to nutritional value (Sulak and Aydın, 2005). The most important factor affecting the nutritional value of plants is the plant nutrients that plants received from the soil (Kacar and Katkat, 2007). For the cultivation of good forage crops, it is necessary that all of these elements be present in sufficient quantities (Ball et al. 1996). Nitrogen is one of the most important nutrients in forage crops cultivation as in many plants. Nitrogen is used most intensively in plant production (Schlemmer et al. 2005) and is very important for yield and quality in forage plants. It is an essential element for normal growth and development in plants with the reason that it is a building blocks of proteins and nucleic acids. The effect of nitrogenous fertilizers on the crude protein ratio in the forage crops is very significant. The ratio of raw protein to nitrogen and fertilized protein per unit area is increased with applied nitrogenous fertilizers (Açıkgöz 2001).

The lack of one or more nutrients in plants (in some cases excess) affects the yield and quality of the product negatively. One of the biggest causes of poor quality in forage crops is lack of plant nutrients as is in many plants. For this reason, to determine the nutrient content of the plant quickly and to intervene immediately are very important if there is a deficiency. Various chemical analysis (eg, the Kjeldahl method for nitrogen) are performed in laboratories to determine the nutrient status of plants. However, these chemical methods used in determining the quality characteristics of plants are intensive laboratory studies and so, they are expensive and time-consuming methods (Kokaly and Clark 1999, Graeff et al 2001, Li et al 2006, Zhao et al 2007). Therefore, alternative methods are needed to determine nutrient levels in plants.

The remote sensing method, which has been used in many areas in recent years, also has the potential to be used in nutrient diagnosis in plants. Remote sensing, which allows information to be obtained from objects at any distance without physical contact, is now widely used in agriculture and forestry (Wright et al. 2005). These systems are very advantageous because they are faster and cheaper than traditional methods.

In this study, it is aimed to estimate nitrogen levels in alfalfa plant by using spectral reflectance values obtained by remote sensing systems.

MATERIALS and METHODS

The study was carried out in both greenhouse and field conditions in the Akdeniz University, Faculty of Agriculture, Field Crops Department (Antalya). Alfalfa (*Medicago sativa* L.) was used as a plant material. The field applications of the experiment were carried out at a pH of 8.7, a strong alkaline, clayey and organic medium soil. Greenhouse experiments were performed in plastic pots with capacity of 5.5 L filled with a mixture of soil (pH: 8.5, strong alkaline, clay loam, low humus), peat (pH:6.5, buffered, high organic matter), and perlite.

In the study, Ammonium Nitrate fertilizer containing 33% nitrogen was applied to the parcels and pots at a ratio of 0, 10 and 20 kg da^{-1}. Diamonium Phosphate and Potassium sulphate fertilizers were applied to the plots and pots at the same doses and 27 different applications (N: P: K, 0:0:0, 0:0:10, 0:0:20, 0:10:0, 0:10:10, 0:10:20, 0:20:0, 0:20:10, 0:20:20, 10:0:0, 10:0:10, 10:0:20, 10:10:0, 10:10:10, 10:10:20, 10:20:0, 10:20:10, 10:20:20, 20:0:0, 20:0:10, 20:0:20, 20:10:0, 20:10:10, 20:10:20, 20:20:0, 20:20:10, 20:20:20) were performed with three replication. Thus, the experiment contained 81 plots in field and 81 pots in greenhouse. In the field experiments, 10 grams of seed were sown to each plot at a rate of 5 kg da^{-1}. In the pots, 15-20 seeds were applied and they were reduced to 5 plants per pot by thinning.

Reflectance measurements were made at the beginning of the flowering and a spectroradiometer was used to measure the reflectance of the electromagnetic spectrum between 325-1075 nanometers (nm) wavelengths (Albayrak 2008). However, since the reflectance values below 400 nm and above 900 nm were in extreme fluctuations, the values between 400 and 900 nm wavelengths were taken into account when the resultswere evaluated. This situation has been pointed out by some researchers (Han and Rundquist 2003; Lin and Liquan 2006; Johan et al., 2013; Kahriman et al., 2016).

In the study, both canopy (general) and single leaf reflectance measurements were made in plots and pots and measurements were performed between 10.00 and 11.30 during the day when the weather was clear. In canopy measurements, the distance between the sensor of the device and the upper surface of the plants was set at 1.5 m (Albayrak 2008) in the plots and 25 cm in the pots. Reflectance measurements were made in 5 replications of each of the 81 plots and pots and a reference panel (spectralon) measurement was performed for calibration in every three measurements (Beeri et al 2007).

For leaf measurements, 5 leaflets were chosen randomly from each plot and pot. Reflectance measurements were performed with plant probe which mounted 100-watt halogen lamp as artificial light source and leaf clip (Delalieux et al., 2008). After the leaf measurements, whole plants in the plots and in the pots were harvested and 150 g of plants in the plots and all of plants in the pots were dried in oven at 65°C for 48 hours (Brink et al., 2003). Wet digestion method was used for the nitrogen analysis of the samples (Karaca and Çimrin 2002). However, no nitrogen analysis was performed on leaves taken for leaf measurements.

For the statistical analysis of the data, stepwise regression analysis was used in the MINITAB statistical program. As a result of the statistical analysis, the wavelength related to the nitrogen level of the plant was determined and regression equations were formed by using this wavelength. When the results were evaluated, wavelengths were considered for four different spectral bands [blue: 400-500 nm, green: 501-600 nm, red: 601-700 nm, NIR (Near Infrared): 701-900 nm] (Summy et al., 2003).

RESULTS

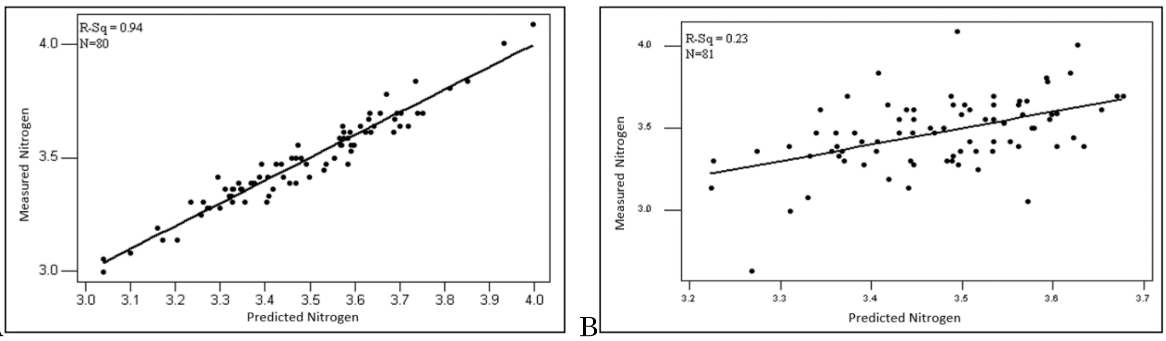
Field experiment

The regression equations and the R^2 values which show the relation between the nitrogen levels and the reflectance values of the field experiment results, are shown in Table 1. According to the regression analyzes applied to the field-canopy measurements, a total of 32 wavelengths including 10 from the blue region, 3 from the green region and 19 from the red region of the spectrum were included in the regression equation determined for nitrogen and a very high regression coefficient (0.94) was calculated. In the field-leaf measurements, 4 wavelengths were included in equation, but the regression coefficient of the equation produced by these wavelengths was low (0.23). Graphs showing the relationship between the values determined by the analyzes in the laboratory and the values calculated by the regression equations are shown in Figure 1 for field-canopy and field-leaf.

Table 1. The regression equations and R2 values showing the relationship between measured nitrogen levels and reflectance values (Field)

		Regression equations	R^2
Field	**Canopy**	$3.76 + (180xR_{609}) + (-483xR_{695}) + (-338xR_{449}) + (-334xR_{480}) + (533xR_{435}) + (324xR_{672}) + (93.9xR_{512}) + (323xR_{439}) + (196xR_{696}) + (-513xR_{438}) + (-154xR_{679}) + (-472xR_{625}) + (343xR_{603}) + (297xR_{636}) + (337xR_{614}) + (-109xR_{607}) + (-383xR_{423}) + (243xR_{485}) + (295xR_{422}) + (-289xR_{648}) + (-195xR_{585}) + (-233xR_{663}) + (145xR_{668}) + (-204xR_{631}) + (175xR_{602}) + (180xR_{694}) + (-155xR_{590}) + (126xR_{615}) + (243xR_{494}) + (-91.4xR_{612}) + (-191xR_{450}) + (101xR_{680})$	0.94^{**}
	Leaf	$3.71 + (-186xR_{647}) + (21.4xR_{417}) + (195xR_{648}) + (-29.0xR_{495})$	0.23^{**}

**: P<0.01



A B

Figure 1. Regression graphs showing the relation between the nitrogen values measured in the laboratory and the nitrogen values predicted by regression equations (A:Field-Canopy and B:Field-Leaf)

Greenhouse experiment

The regression equations and R^2 values showing the relationship between the nitrogen levels and the reflectance values of the greenhouse experiment results are shown in Table 2. As a result of the stepwise regression analysis applied to the greenhouse-canopy measurement, 9 wavelengths which located in the near infrared region (701-900 nm) were found to be associated with the nitrogen levels and the R^2 value of the regression equation generated with these wavelengths was 0.55.

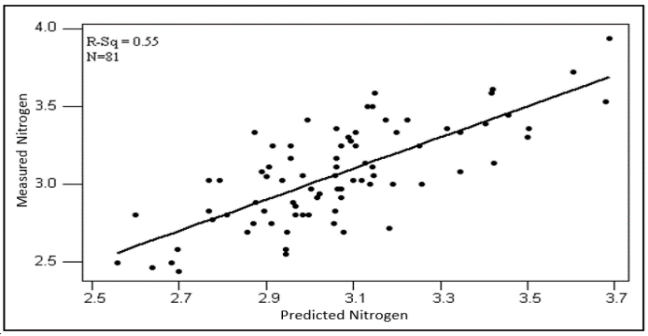
Table 2. The regression equations and R^2 values showing the relationship between measured nitrogen levels and reflectance values (Greenhouse)

		Regression equations	R^2
Greenhouse	**Canopy**	$3.18 + (-62.9xR_{820}) + (-168xR_{834}) + (295xR_{797}) + (62.3xR_{899}) + (-127xR_{845}) + (26.4xR_{896}) + (107xR_{877}) + (-70.2xR_{858}) + (-62.6xR_{891})$	0.55^{**}
	Leaf	$2.80 + (-226xR_{722}) + (-55.0xR_{439}) + (16.4xR_{425}) + (117xR_{467}) + (-28.4xR_{473}) + (-24.8xR_{442}) + (-32.7xR_{465}) + (13.2xR_{403}) + (-89.5xR_{502}) + (262xR_{514}) + (63.4xR_{487}) + (-143xR_{518}) + (80.5xR_{440}) + (224xR_{723}) + (-11.3xR_{406}) + (-25.4xR_{491}) + (24.3xR_{444}) + (128xR_{524}) + (-60.8xR_{535}) + (-148xR_{515}) + (-148xR_{513}) + (33.5xR_{496}) + (-19.8xR_{433}) + (6.73xR_{415}) + (79.1xR_{709}) + (135xR_{516}) + (-10.0xR_{420}) + (-49.6xR_{480}) + (-42.7xR_{686}) + (-69.1xR_{713})$	0.92^{**}

**: $P<0.01$

According to the results of the greenhouse-leaf measurement, 30 wavelengths (blue: 17, green: 8, red: 1, NIR: 4) which are related to the nitrogen values were determined. The R^2 value of the regression equation generated by using these wavelengths was recorded as 0.92. The relationship between the nitrogen values predicted by regression equations and the measured values of the laboratory analyzes are shown in Figure 2.

A



B

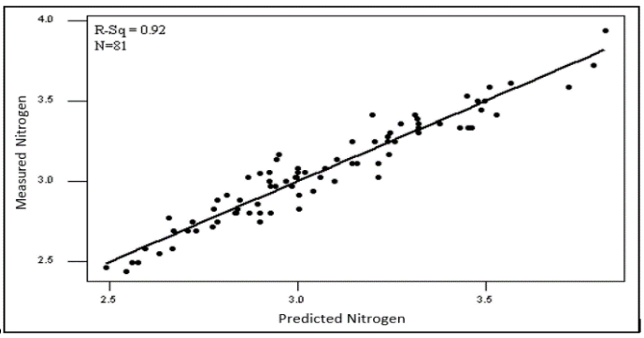


Figure 2. Regression graphs showing the relation between the nitrogen values measured in the laboratory and the nitrogen values predicted by regression equations (A:Greenhouse-Canopy and B: Greenhouse - Leaf)

DISCUSSION

In this study, the wavelengths of blue, red and NIR (Near Infrared) regions of the spectrum are mostly found in the regression equations for nitrogen in the alfalfa plant. Results indicated that there is a significant relationship between nitrogen levels and wavelength reflectance in these regions. Walburg et al. (1982), reported that nitrogen applications affected the reflectance in the red and infrared regions and the reflectance in the red region increased while the reflectance in the infrared region decreased in the case of nitrogen deficiency. St-Jacques and Bellefleur (1991) indicated that the reflectance values in the red region of the spectrum are strongly correlated with the leaf nitrogen concentration. While the reflectance is lower due to absorption of light by chlorophyll in the blue and red regions of the spectrum, reflectance is high in the green region. For this reason, plants appear to be green. Also, chlorophyll concentration reduces in the case of nitrogen deficiency. This leads to an increase in reflectance in the green and red regions (Daughtry et al., 2000). Thomas and Oerther (1972) concluded that there is no visual difference in the pepper when the leaf nitrogen ratio falls from 5% to below 4%, but that these differences can be determined with the help of reflectance values and reflectance values at 550 nm and 675 nm.

CONCLUSION

This study was conducted to investigate the estimation of nitrogen levels in alfalfa plants based on spectral reflectance values. Findings of the study showed that there was significant relationship between the nitrogen level of the plant and the wavelength reflectance in the blue, red and near infrared regions of spectrum. The results show that nitrogen levels in alfalfa plants can be estimated using reflectance values obtained by remote sensing systems, and that in studies, especially blue and red region of spectrum, must be taken into account.

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