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Full Length Research Paper

Assessment of the effect of salinity on the early growth stage of the common sunflower *(Sanay cultivar)* using spectral discrimination techniques

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Salinity is one of the main limiting factors for agricultural production. This is especially true in arid and semi-arid regions of the world like Turkey. The objective of this study was to determine if the effect of salt concentration on the physiological and physiological features of the sunflower (*Helianthus annuus* L) could be measured using remote sensing techniques. Sunflower seedlings were grown under controlled conditions and irrigated with ½ Hoagland Solution containing three different concentrations of NaCl (salt) (0.0, 0.5, 1.0 and 1.5%). The results showed that plant growth decreased proportionally with increasing levels of NaCl. Chlorophyll concentration and a Normalized Difference Vegetation Index (NDVI) were derived for the plants using a spectroradiometer. There was found to be a significant (r^2 = 0.76) correlation between chlorophyll and NDVI values. Therefore, factors that can be derived through remote sensing such as NDVI and chlorophyll can be used to indirectly demonstrate the impact salinity has on sunflower plants. Therefore, agriculturalists can assess growth rate changes caused by salinity using remote sensing techniques.

Key words: Salinity, plant growth, chlorophyll, NDVI, spectroradiometer.

INTRODUCTİON

Adequate water and nutrients are essential for successful crop production. Plants face adverse environmental stress such as salinity in the water and soil which inhibits growth, especially in arid and semi-arid regions of the world (Shannon et al., 1994). The inhibitory effects of salinity on plants are well documented (Leone et al., 2001; Turhan, 2005). Plants under salinity stress exhibit symptoms at physiological, physiological and even molecular levels.

Remote sensing techniques have been used to monitor crops throughout their growing period to help make decisions for good agricultural practices (Clevers et al., 1994; Vina et al., 2004). Data for vegetation and nature could be obtained using appropriate tools and techniques, which cannot be observed by naked eye (Jensen 2000; Lillesand and Kieffer, 2000). Chlorophyll in plants absorbs blue (400 – 500 nm) and red (600 – 700 nm) wavelength radiation, whereas it reflects green (500 – 600 nm) wavelength radiation (Rees, 2001). Therefore, plants exposed to environmental stress show a different reflection pattern compared to healthy plants (Clevers et al., 1994). Furthermore, plants showing symptoms of nutrient deficiency differ from healthy ones as changes in cell structure changes the amount of reflected radiation in the infrared part of the spectrum (Vina et al., 2004).

Chlorophyll content in plants correlates directly to the healthiness of plant (Rodriguez and Miller 2000; Zhang et al., 2005). Handheld chlorophyll meters have been used to rapidly detect plant status such as nutrition deficiency, environmental stress and disease or insect damage (Wood et al., 1992; Schepers et al., 1996). Although

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handheld chlorophyll meters are convenient for monitoring plant health, they are not sufficiently sensitive to derive detailed spectral information from the plant. Therefore, multi-spectral techniques for determination of plant status, obtaining geographical information, as well as crop yield estimation, were used in this study.

Spectral data can be enhanced by using vegetation indices which are mathematical combinations between bands or selected wavelengths, when multispectral data were used (Barragan et al., 2006). Vegetation indices are a basic form of the spectral characteristic of vegetation that can be calculated from multispectral data. These vegetation indices operate by contrasting intense chlorophyll pigment absorptions in the red against the high reflectivity of plant materials in the NIR (Tucker 1979; Elvidge and Chen 1995; Blackburn, 1999; Moran et al., 2000).

A number of spectral reflectance indices have been used mainly to estimate plant features related to the development of the total photosynthetic area of the canopy. The most widely used index by remote sensing researchers is the Normalized Difference Vegetation Index (NDVI), originally proposed as a means of estimating green biomass (Rouse et al., 1973) and ultimately grain yield (Penuelas et al., 1997; Aparicio et al., 2000; Shanahan et al., 2001). NDVI equation showing plant healthiness is calculated using the formula:

NDVI = (Near-infrared – Visible Red) / (Near-infrared + Visible Red) (1)

NDVI potential values range from –1 to 1 (Rees, 2001). NDVI values closer to 1 indicate that the plant contains more chemical substances responsible for green coloration or chlorophyll molecules. The NDVI generally varies between 0.5 and 0.7 in a healthy plant under normal atmospheric conditions (Jensen, 2000; Campbell, 2002).

Materials and Methods

The study was conducted in Agricultural Data Processing and Remote Sensing Laboratory of Agricultural Faculty, Canakkale Onsekiz Mart University in Turkey. Oil type hybrid sunflower seeds of the species *Sanay cultivar* were obtained from the Syngenta Seed Company. The growth room was illuminated with cool white fluorescent tubes at 23±2°C with a 16 h photoperiod and a photosynthetic photon flux density (PPFD) of 95 $\mu molm^{-2}s^{-1}$. The experimental design was a randomized block design with four replicates. The 6 L pots were filled in with washed and oven dried sand (3 kg) with an average bulk density of 1.53 gcm^{-3}. Then, the water retention by the sand in a pot was determined.

Preparation and application of solutions

Five seeds were sown in each pot and all pots were watered every day at the same time with ½ Hoagland solution containing different levels of NaCl (0, 0.5, 1.5 and 2.0 %). The modified Hoagland solution consisted of 5 mM KNO_3, 5 mM $Ca(NO_3)_24H_2O$, 2 mM $MgSO_47H_2O$, 1 mM KH_2PO_4, 0.1 mM EDTA, 0.1 mM Na_2SO_4, 0.1 mM Fe_2SO_4, 11.5 mM H_3BO_3, 4.6 mM $MnCl_2.4H_2O$, 0.2 mM $ZnSO_4.7H_2O$, 0.12 mM $Na_2MO_4.2H_2O$, 0,08 mM $CuSO_4.5H_2O$. The pH of all solutions was adjusted to 6.

De-ionized water was used exclusively for irrigation. Drained excess water was collected for each pot and, after bringing it up to the same volume with water, it was re-used for the next watering. This kept the concentration of salt constant.

NaCl, the most common compound in salt-affected soils (Szabolcs, 1994), was chosen to produce saline conditions in this experiment. Sunflower is a moderately salt tolerant crop (Turhan and Ayaz, 2004), therefore, 0, 0.5, 1.0 and 1.5% NaCl concentration were used. After emergence, one seedling was left in each pot and rests were discarded. Although the seeds were germinated at 1.5% NaCl, they could not survive and so were not included in the final analysis. All measurements were taken on plants after they formed real leaves. These measurements included (1) number of leaves per plant, (2) plant height, (3) total leaf area, (4) stem diameter, (5) fresh leaf weight, (6) dry leaf weight, (7) fresh root weight and (8) dry root weight. Total leaf area was determined by scanning all the leaves in a plant. For dry weights, the samples were dried in an oven for three days at 105°C and immediately weighed.

Spectral measurements

A FieldScout CM 1000 chlorophyll meter (FieldScout Spectrum Technologies, Inc.) was used to measure the chlorophyll content. Chlorophyll was measured on the same day that the spectral measurement made. The FieldScout CM 1000 chlorophyll meter detects and measures electromagnetic radiation at wavelengths between 700 and 840 nm to estimate the quantity of chlorophyll in leaves. A laser beam defines the target when activated. Chlorophyll measurements were made from a range of 30 cm with field of view of 1.10 cm in diameter. Three chlorophyll index measurements were taken from each leaf. The appropriate wavelengths of ambient and reflected radiation are used to calculate and display a chlorophyll index value. The recorded data was downloaded to a computer for further analysis using FieldScout CM1000TM software.

Canopy reflectance was detected with a narrow-bandwidth visible-near-infrared portable FieldSpec Handheld Spectroradiometer (ASD HH) manufactured by Analytical Spectral Devices, Inc. It was fitted with a 1° field of view optic (FOV) and 0.5cm diameter (Figure 1). The ASD HH instrument detects 512 continuous bands with a 1.5 nm sampling interval sensitive to the 325 to 1075 nm portion of the spectrum. The spectral resolution of the ASD HH spectroradiometer is 3.5 nm for the region 325 - 1075 nm. The sensor was placed above each plant at 30 cm. Three spectral reflectance measurements were taken for each plant, each being the average of five scans (Figure 1). The reflectance spectrum was calculated in real time as the ratio between the reflected and the incident spectra on leaf, where the incident spectrum was periodically obtained from the radiation reflected by a barium sulphate standard panel before and immediately after each measurement (ASD 2006). All corresponding measurements for were made on the same day under halogen light with a 45° FOV (Figure 1). Each scan was saved on a computer. Narrow bands spectral reflectance data were collected by the ASD HH for each of 16 days growth period and were used to calculate the NDVI.

Statistical analysis was carried out by using the SAS computer package (SAS 1999). The least significant difference (LSD) test was applied for means of separation at the 0.05 significance level. For the results, in addition to absolute values, relative decrease was also used. The relative decrease was calculated for each Salinity level using of the formula:

Relative decrease (%) = 100 - (Absolute value x 100 / Value at 0% NaCl) (2)

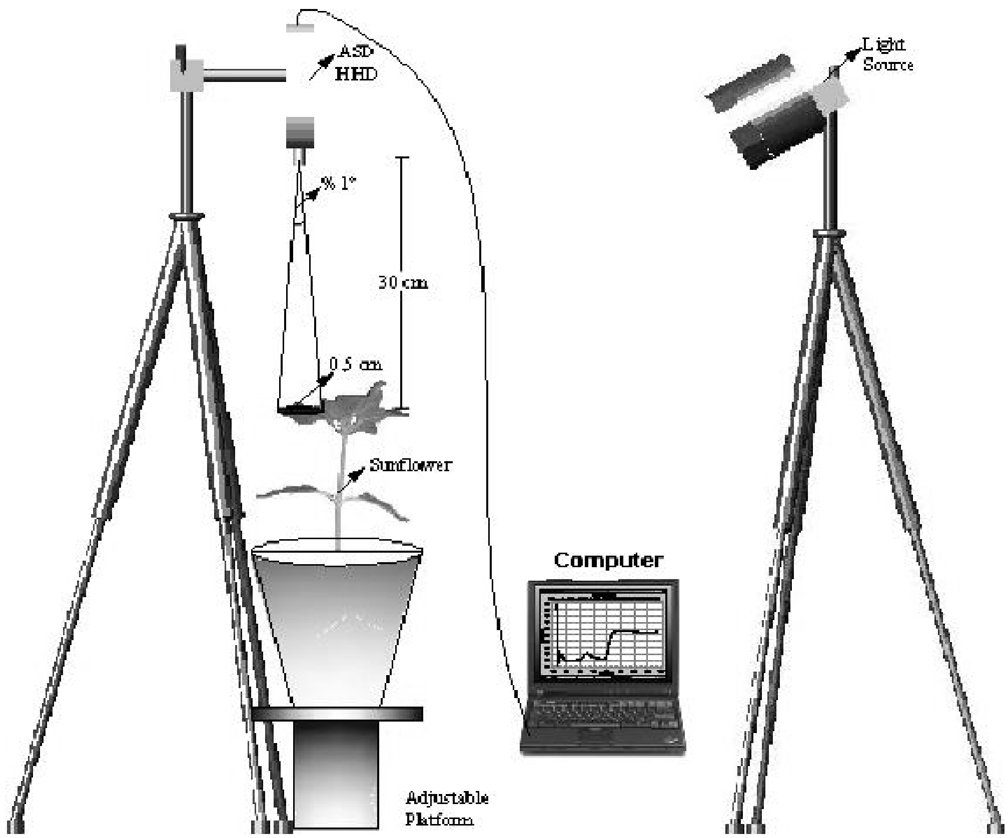

Figure 1. Data collection with handheld Spectroradiometer.

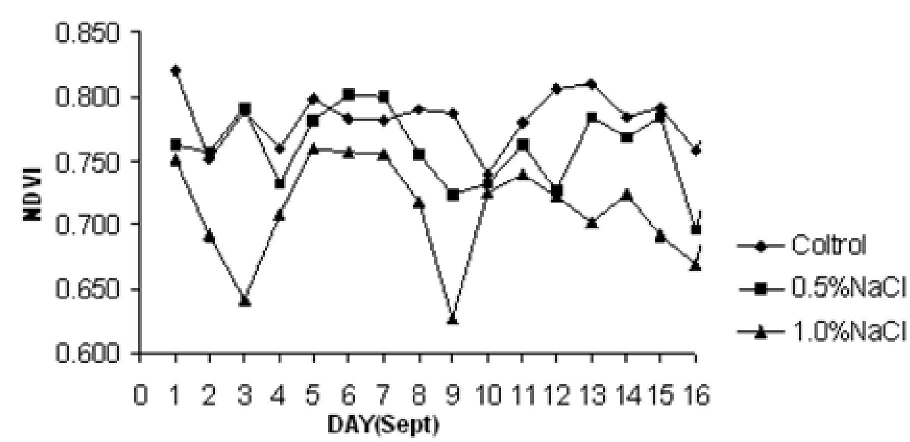

Figure 2. Daily change in NDVI values for sunflower at seedling stage. Each point represents the average of five readings.

Results and Discussion

Results of daily average NDVI and chlorophyll index value are shown in Figure 2 and Figure 3, respectively. The spectroradiometer and chlorophyll index value were taken when first leaf (except cotyledon) formed. For NDVI values, control and 0.5% NaCl treatments produced a similar pattern throughout growth period. However, NDVI values for 1.0% NaCl showed a noticeable lower trend compared to the control and 0.5% NaCl treatments (Figure 2). NDVI and chlorophyll index showed a similar pattern (Figure 3). However, the differences between treatments in terms of chlorophyll index values were more apparent than NDVI results. At the end of the

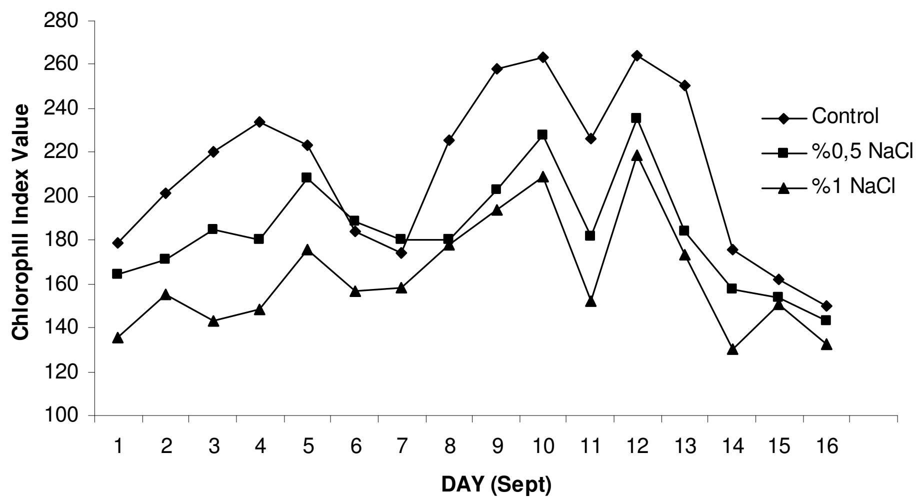

Figure 3. Daily change in chlorophyll index value for sunflower at seedling stage. Each point represents the average of five readings.

seedling stage, growth slowed down and so consequently did the chlorophyll index value.

Results of variance analysis indicated that the effect of NaCl on the plants were highly significant with the growth of sunflower decreasing with increasing levels of NaCl. Plants under NaCl stress produced fewer and smaller leaves and, consequently less total leaf area per plant (Figure 4). Moreover, salinity caused reductions in shoot and root growth. Increasing levels of NaCl caused a delay in seed germination. In previous studies, this inhibitory effect of salinity on plant growth was well documented (Zeng and Shannon, 2000; Turhan and Ayaz, 2004). As a result, the test method used in this study can be applied to determine response of the sunflower to salinity.

Similar to the plant's physiological characteristics, the average chlorophyll index value as a physiological character decreased with increasing salinity (Figure 4). The 0.5 and 1.0% NaCl treatments induced 41 and 58%, respectively, a reduction in the chlorophyll content as shown in Figure 4. Similarly, Netondo et al. (2004) found that 250 mM NaCl (1.46%) caused reductions up to 58 and 68% in chlorophyll a and chlorophyll b, respectively, in sorghum.

Netondo et al. (2004) found that the chlorophyll content reduction of leaves started to occur in plants grown at 100 mM NaCl and higher concentrations. These reductions could be attributed to the effect of salinity that causes inhibition of synthesis of chlorophyll or accelerating its degradation (Reddy and Vora 1986). It was also reported by Rodriguez and Miller (2000) that hand-held chlorophyll meter readings are positively correlated with chlorophyll and nitrogen concentrations in plants.

Spectral reflectance results of sunflower in controlled conditions were acquired using a spectroradiometer and a chlorophyll meter. NDVI results showed that control treatment did not significantly differ from the 0.5% NaCl although there was a reduction in NDVI value at 0.5% NaCl compared with the control (Figure 5). Similar results were demonstrated by Leone et al. (2001) on pepper plant grown under salinity stress. However, the 1.0% NaCl treatment was significantly different than the control and the %0.5 NaCl treatments.

Correlations between sunflower plant physiology under salt stress are presented in Table 1. It was found that all the correlations between the physiological characteristics were highly significant ($P < 0.01$ and $P < 0.001$) and also that the correlation coefficients were high. These high levels of correlations indicate that the physiological characteristics were highly dependent on each other. Similarly, in spectral measurements, there was a significant correlation between chlorophyll measured by handheld chlorophyllmeter and NDVI derived from the spectro-radiometer measurement (coefficient = 0.76). In addition, the correlation coefficients between chlorophyll and the physiological characteristics were higher than those between NDVI and the physiological characteristics. In other words, correlation coefficiency for NDVI ranged between 0.76 - 0.86, whereas for the chlorophyll index it ranged between 0.85 - 0.95 (Table 1).

The physiological characteristics used in this study required more destructive and time consuming measurements. The relationship between chlorophyll and NDVI values in the spectral aspect has been previously investigated by several researchers (Penuelas et al., 1995; Jacobsen et al., 1998; Sims and Gamon, 2002). This research indicated that simple spectral index such as NDVI provides some information on crop exposed to salinity at early growth stage such as seedling.

Conclusion

Salinity has a detrimental effect on sunflower seedlings

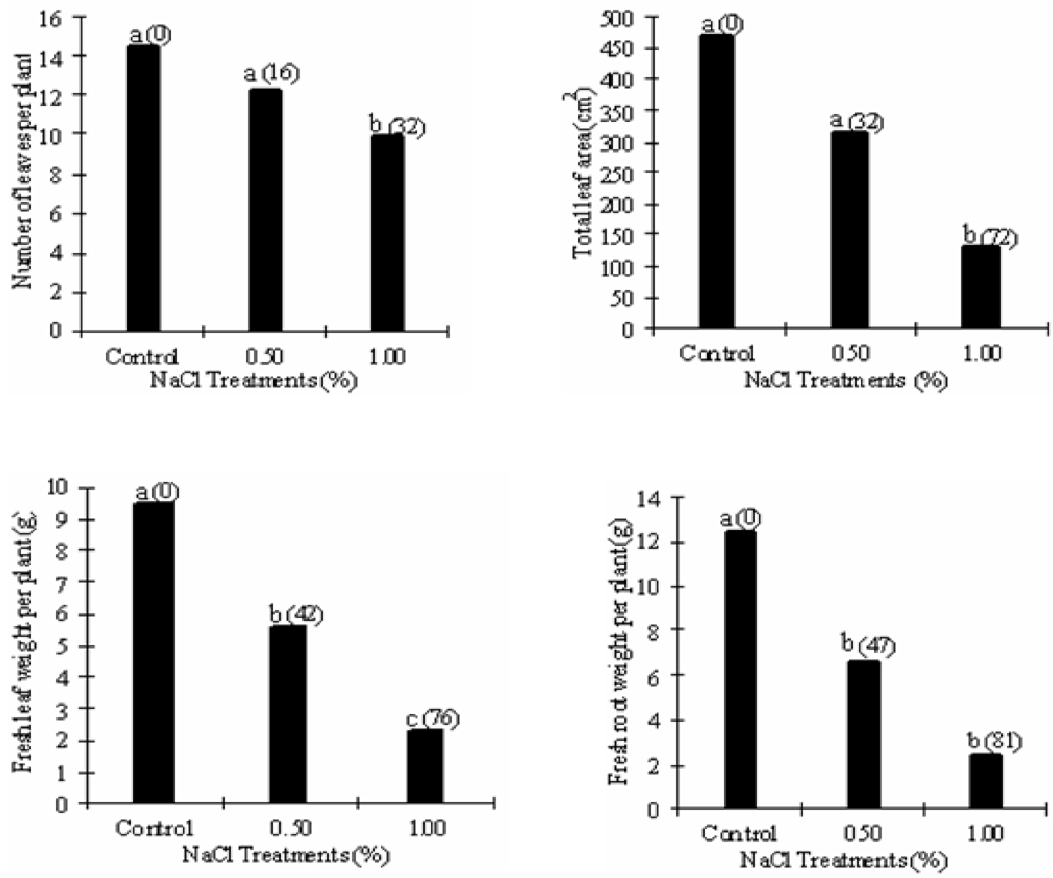

Figure 4. The effect of NaCl on some physiological characteristics in sunflower at early growth stage. Means with the same letter are not significant at P = 0.05. Values in brackets represent relative reductions (%).

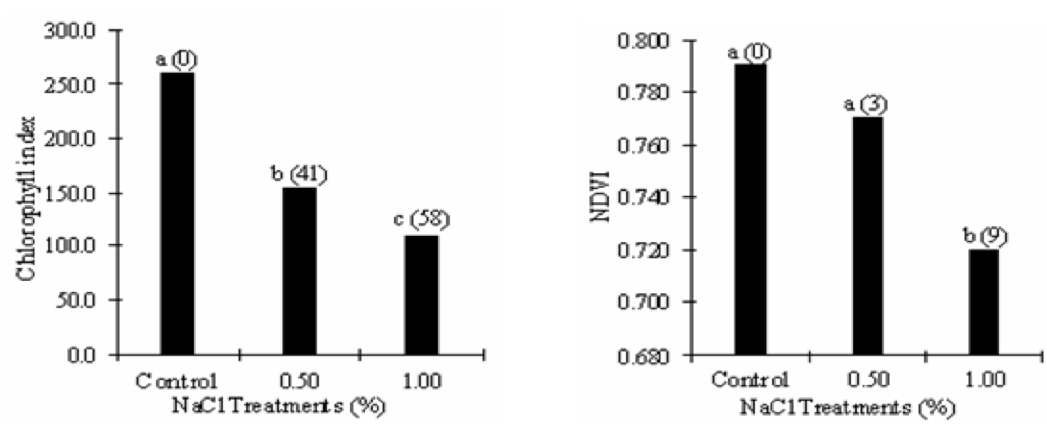

Figure 5. The effect of NaCl on chlorophyll index and NDVI values in sunflower at early growth stage. Means with the same letter are not significant at P = 0.05. Values in brackets represent relative reductions (%).

and chlorophyll index measurements can discriminate the effects of all NaCl concentrations on sunflower growth. Although the effect of salinity on the growth in terms of NDVI was clearly seen, there was no significant

Table 1. The correlation between the measured characteristics in sunflower seedlings grown under salt stress.

Characters	NL	PH	TLA	SFW	LFW	LDW	SD	RFW	RDW	CHL
PH	0.86***									
TLA	0.90***	0.93***								
SFW	0.88***	0.97***	0.95***							
LFW	0.93***	0.93***	0.95***	0.98***						
LDW	0.86***	0.93***	0.93***	0.99***	0.97***					
SD	0.91***	0.97***	0.96***	0.97***	0.98***	0.93***				
RFW	0.91***	0.83**	0.83**	0.93***	0.96***	0.91***	0.91***			
RDW	0.91***	0.89***	0.89***	0.97***	0.97***	0.96***	0.95***	0.98***		
CHL	0.84**	0.90***	0.88***	0.94***	0.95***	0.92***	0.94***	0.91***	0.93***	
NDVI	0.77**	0.76**	0.78**	0.86***	0.84**	0.84**	0.80**	0.84**	0.86***	0.76**

** and ***: Significant at 0.01 and 0.001 level, respectively.
NL: Number of leaves per plant, PH: Plant height, TLA: Total leaf area, SFW: Shoot fresh weight, LFW: Leaf fresh weight, LDW: Leaf dry weight, SD: Stem diameter, RFW: Root fresh weight, RDW: Root dry weight, CHL: Chlorophyll index value, NDVI: Normalized difference vegetation index.

difference between the control and 0.5% NaCl. Thus, it seems that chlorophyll index measurements more accurately determined the salinity effect than NDVI derived from the handheld spectroradiometer. However, in order to derive accurate results, one may use some other indices such as ratio index, water index, chlorophyll index, soil adjusted vegetation index to determine relationship between reflectance and physiological parameters. Both NDVI and chlorophyll index measurements provide capability to detect salt stress on sunflower plants at seedling stage prior to their visual symptoms. Therefore, farmers will have opportunity to evaluate problematic areas and take preventative steps for stress factors using these spectral techniques before damage occurs. However, before this approach is app-lied to agricultural fields, further experiments in the field must be carried out.

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