Utilizing Hyper-Spectral No-Image Measurement to Assess the Development of disease severity of Cercospora Leaf Spot disease in sugar beet canopy

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Abstract: Sugar beet (*Beta vulgaris*) in Egypt is second important source sugar. In Egypt sugar beet is attacked by Cercospora leaf spot (CLS) caused by *Cercospora beticola* fungus. Remote sensing and GIS represent spectral signature and vegetation indices were used in the present study to determine the ability of these technique in the plant pathology sector.spectral signature and vegetation indices associated with infection of sugar beet crops by CLS disease using Hyperspectral No-image measurements SpectraPen SP100. Depend on the reaction between selecting wavelength values (R490, R560, R655, R705, R740 and R775) with canopy of sugar beet plants. Using spectral signature and vegetation indices (NDVI, SR, OSAVI, AR1 and AR2) result revealed that succeeded in determining the change in plant infected canopy causing by CLS disease of sugar beet. The result showed that spectral reflectance in selecting wavelength can discriminating between healthy and infected plant which represented (visible, red-edge and near-infrared) which have the best result to detect CLS and high correlation with disease severity of CLS. Result showed that. Result showed that vegetation indices NDVI have the highest correlation followed by AR1 and AR2.

Keywords: Sugar beet, Cercospora leaf spot, Spectral signature, Vegetation indices, Remote sensing

INTRODUCTION

Sugar beet (*Beta vulgaris*) is the most important crops, which represents 30% of the world's sugar production (FAO, 2016). In Egypt, it is considered the main important sources for its high content of sucrose and the second important crop for sugar production after the sugar cane crop (Gobarah *et al.,* 2019). Moreover, under the environmental condition of Egypt, it is sugar beet is attacked by several foliar pathogens fungal causing serious diseases such as Cercospora leaf spot (CLS), powdery mildew, and sugar beet rust (El-Kholi, 2000; Ziedan *et al.,* 2011).CLS disease caused by *Cercospora beticola* fungal and considered the most damaging foliar disease due to lead to reduction yield of sugar beet with rate 30 to 42% (Smith and Martin 1978; Shane and Teng, 1992; Wolf *et al.,* 1995; Wolf and Verret, 2002; El-Mansoub *et al.,* 2017). CLS disease destroys and destruction the leaf tissue by causing leaf spots to appear as individual, circular spots that are tan to light brown with reddish-purple borders sometimes and sometimes with a reddish-brown margin (Wolf and Verret, 2002; Weiland and Koch, 2004; Franc, 2010). The leaf spots merge to form large necrotic areas (Vereijsssen *et al.,* 2004). As the disease progresses heavily infected leaves first become yellow and eventually turn brown and necrotic Blighted leaves soon fall and fall to the ground but remain attached to the crown. In recent times, CLS disease monitoring methods rely on occasional field surveys by teams of specially trained and skilled workers (Dutta, 2006). It is considered the approach used in the detection and identification of plant diseases to the present (Gogoi *et al.,* 2018). Therefore, it must apply a monitoring plan to assess CLS within short term periods to suggest a suitable mitigation measure for decreasing the loss of sugar beet due to infection with CLS disease. Therefore, there are many methods are utilized for detecting the plant infective according to if the diseases don't have any visibility symptoms associated or those emerge when action can longer be taken (Buja *et al.,* 2021). Furthermore, the symptoms of plant diseases can only be detected in specific electromagnetic spectrum bands which are in visible or in don't visible to humans (Barbed, 2013). The suitable approach to detect and explore spectral signature and vegetation indicesareapplying remote sensing (RS) and geographic information system (GIS) tools to detect and monitoring of CLS diseases in many crops Mahlein *et al.* (2013). Reflectance in visible, Near-infrared, and short waves regions can recognize any type of alteration or modification in content , internal leaf structure and process which effected by observation radiation .Furthermore or in addition to detect any plant disease or other stress that due to physiological stress in plant (Coops *et al.,* 2003; Eitel *et al.,* 2011). These detected variations are related with alteration in the quantity of chlorophyll and chemical properties of the affected leaves (Carter and Knapp, 2001; Mutanga and Skidmore, 2007). In the present study, spectral signature and vegetation indices techniques is used to eliminate spectral reflectance and vegetation indices for determining the ability of this techniques to measure and determine the alteration (symptoms) which appears in the canopy of the sugar beet plant as result to infect by CLS.

MATERIALS AND METHODS

Field data

Four varieties of sugar beet (Panthir (poly), Misrbal (poly), Sibel (mono), and Puma (mono) were infected by of CLS disease. The experimental was conducted in two seasons 2017/2018 at the faculty of Agricultural Farm of Suez Canal University. Varieties were culture in three plots each plot size plot 3.7 × 2.75 m² in averages and consisted of 4 rows, 2m long and 50 cm apart. The experimental was design in randomized

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complete block design in three replications. To maintain crop vigor normal agronomic practices including recommended fertilization dose and irrigation schedule were applied, Plants were watered as necessary and fertilized weekly with NPK 19:19:19 and Urea. Plants were used for the experiments after reaching the growth stage (GS 16). Sixty-day-old plants were sprayed with 3×10 \ml conidia (spore/ml) of *Cercospora beticola* using an anatomizer. Before inoculation, plants were sprayed with water to make a thin film of water on the leaf surface. Suspension of spores was sprayed onto the upper and lower side of the leaves. After inoculation, the plants were covered with plastic bags to create 100% RH for two days. One raw in each plot was left as control plants without fungal inoculation. The disease severity data for four varieties were measured weekly using scale 0 to 8 as showed in Table (1) and equation as following:

$$\text{DS\%} = \frac{\sum(P \times Q)}{M \times N} \times 100$$

Where P =Severity score Q = Number of infected plants having the same score M = Total number of plants observed N = Maximum rating scale number

Table (1): Disease severity scale

Grade	Count of spot	Description
0	0	No spots on the leaf
1	1-5	Number of spots on the leaf
2	6-12	Number spots on the leaf
3	13-25	Number spots on the leaf
4	26-50	Number spots on the leaf
5	51-75	Number spots on the leaf
6	76-85	Number spots on the leaf
7	86-95	Number spots on the leaf
8	>95	Number spots on the leaf

Hyperspectral No-image measurements data

For the acquisition of hyperspectral data from sugar beet canopy, a non-imaging sensor was used. Spectral reflectance was measured by SpectraPen SP100. The spectral range of the SpectraPen SP100 is from 340 nm to780 nm, only values between 400 to 780 nm reflectance. The spectral sampling interval was automatically interpolated from 0.5 nm to 1 nm steps. SpectraPen SP100 was used to measure the spectral reflectance from plant canopies at a specific height 0.5m from the canopy of the plant and with keeping a constant distance of 0.5m between the SpectraPen SP100 and the canopy of the plant surface by using an iron stand. Reflectance spectra of the plant canopies were acquired with solar radiation during clear days (free-cloud days) from 12:00 PM to 2:00 PM. Spectra collection was started after the inoculation plant of sugar beet with *Cercospora beticola* pathogen as mentioned before. Four scans for each plant were acquired to obtain the average of reflectance canopy of the Sugar beet plant. In each treatment (inoculated and non-inoculated sugar beet plant), spectra from 12 plants inoculated and healthy plants were taken to realize a multi-temporal measurement. The objectives of this technique were to evaluate the property of distinguishing between infected and healthy plants Spectral reflectance.

Spectral signatures analysis

Changes in spectral signature during pathogenesis as well as between healthy and the infected plant have been analyzed by simple linear correlation analyses to extract the wavelength(s) suitable for the differentiation among healthy and infected plant. Correlations between disease severity and reflectance data were tested by computing Pearson’s coefficient of correlation (r) using the Superior Performing System SPSS 17.0 (SPSS Inc., Chicago, USA). With correlation curves, the intensity and direction of the relationship of each narrow band of the spectrum was visualized and specific wavebands of the spectral signature, closely related to disease infestation were selected.

Vegetation analysis

Correlation between vegetation indices and disease severities were conducted and tested to classify different disease situations more specifically and discrimination between healthy and infected plant. five of vegetation indices in present study were used from No-image hyperspectral (Spectra Pen 100) represent NDVI,SR,OSAVI ,AR1 and AR2 indices to find the VIs which have a unique characteristic and determine their ability to discriminate between healthy and infected of CLS disease in sugar beet.

RESULTS AND DISCUSSION

Spectral signatural data

The Results showed that Fig (1, 2, 3 and 4) spectral reflectance of healthy sugar beet canopy which not inoculated for all varieties (Panthir, Misbal, Sible and Puma) were characteristic for strong absorption on wavelength (R490, R560 and R655-R705which effective with greenness, vigor, and pigment (chlorophyll) of leaves plant and by photosynthetic efficient. These are showing increase in reflectance. In wavelength (R780) at NIR spectrum revealed that increasing in reflectance as result to interaction with internal structure these result consideration as spectral signature for phenology of sugar beet plant and varieties in study area .For inoculated plant (infected) by CLS disease the characteristic reflectance curve Fig (5, 6, 7 and 8). Showed that changes in reflectance were strongly correlated to the occurrence of disease specific symptoms and progress of disease severity. Spectral signatures of canopy of plant which inoculated by CLS changed in reflectance values with progress and development of disease symptoms. Where results showed that the reflectance for wavelength R490, R560, R665 and R705 which represent visible light blue, green and red spectrum were increasing with increasing of disease severity of CLS disease in canopy of plant that for all varieties Misrbal, Panthir, Sibel and Puma in wavelength (R740 and R775) which similar to red-edge2 and red-edge3 spectrum illustrated that the reflectance decreased by increasing disease severity of CLS disease. While for wavelength (R780) which represent NIR spectrum, reflectance was decreased when the disease severity of CLS disease is increasing .The peak of disease severity was recorded (35.89%, 43.12%, 48.26%, and 45.55%) for Puma, Sibel, Misrbal and Panthir varieties respectively, reflectance were reached to peak increased in visible wavelength R490, R560, R665 and R705 and decreasing in (R740, R775 and R780) .There were represent red-edge2, red-edge3 and NIR spectrum due to sever infection with CLS disease which lead to degradation pigment and died leaves of plant and decay the cell structural.

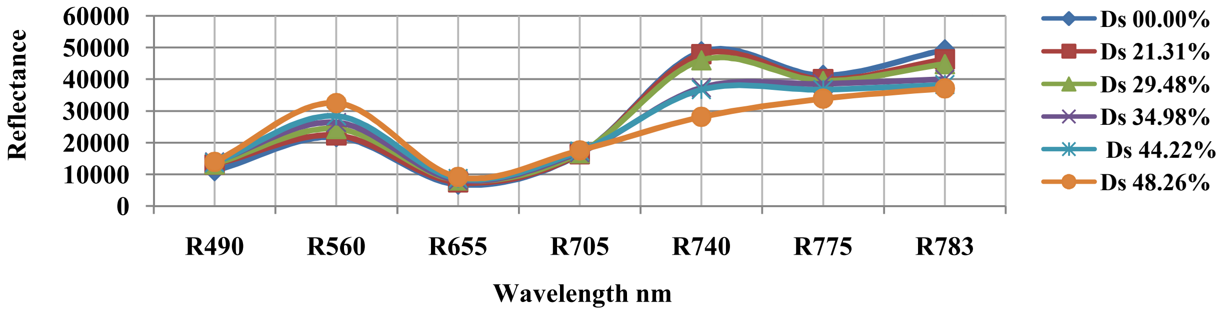

Fig. (1): Spectral reflectance of inoculated sugar beet during development of disease severity in Misrbal variety

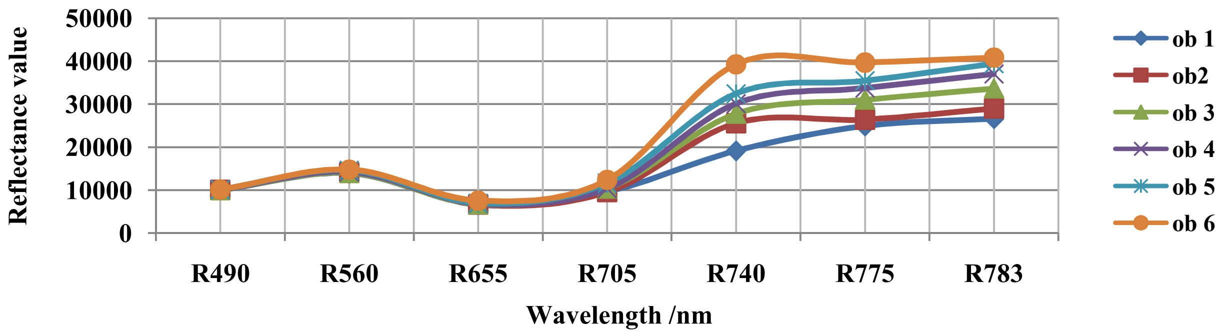

Fig. (2): Spectral reflectance of healthy sugar beet during development of growing in Misrbal variety

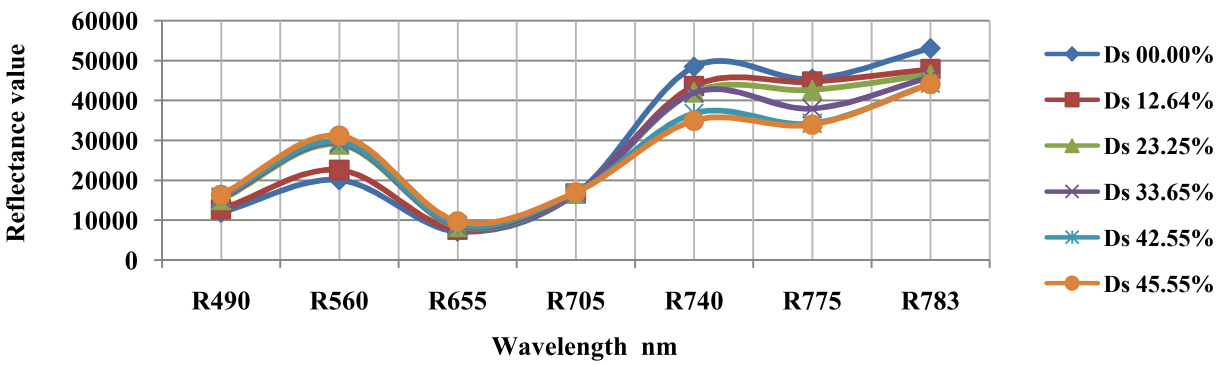

Fig. (3): Spectral reflectance of inoculated sugar beet during development of disease severity in Panthir variety

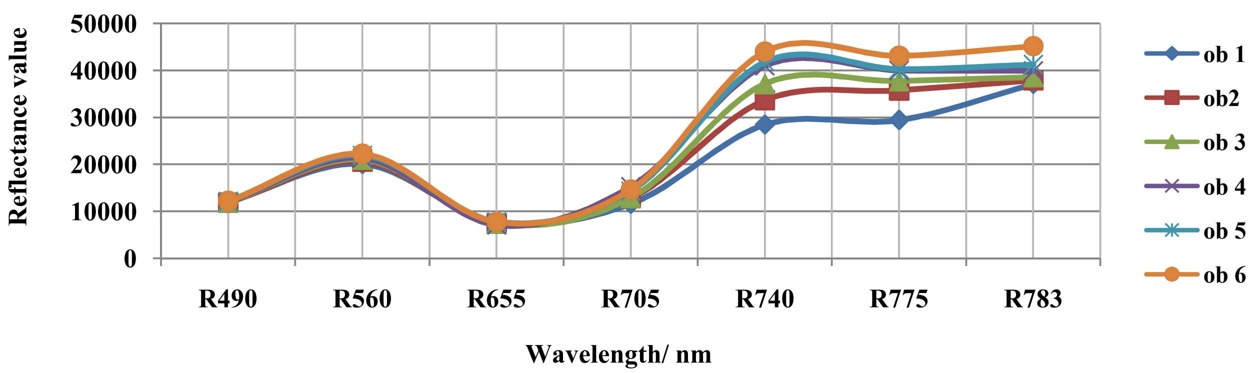

Fig. (4): Spectral reflectance of healthy sugar beet during development of disease severity in Panthir variety

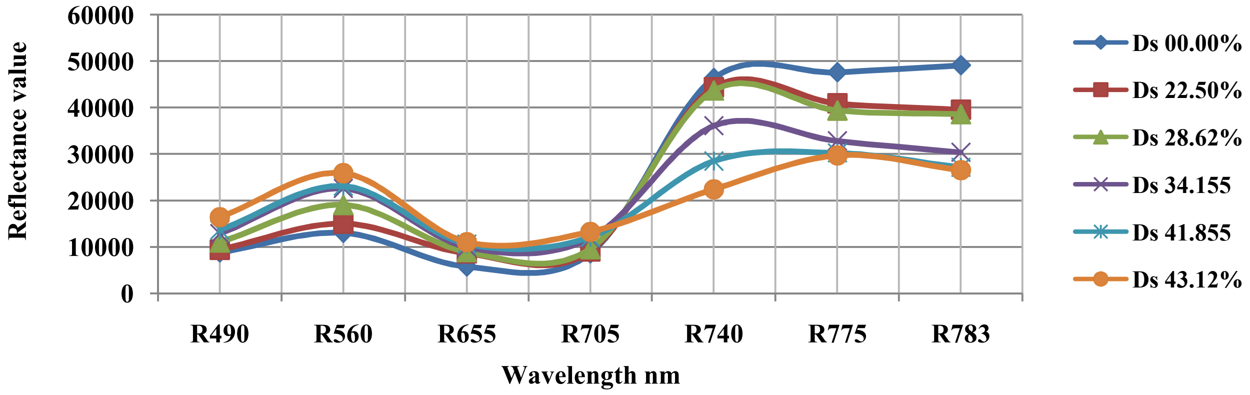

Fig. (5): Spectral reflectance of inoculated sugar beet during development of disease severity in Sibel variety

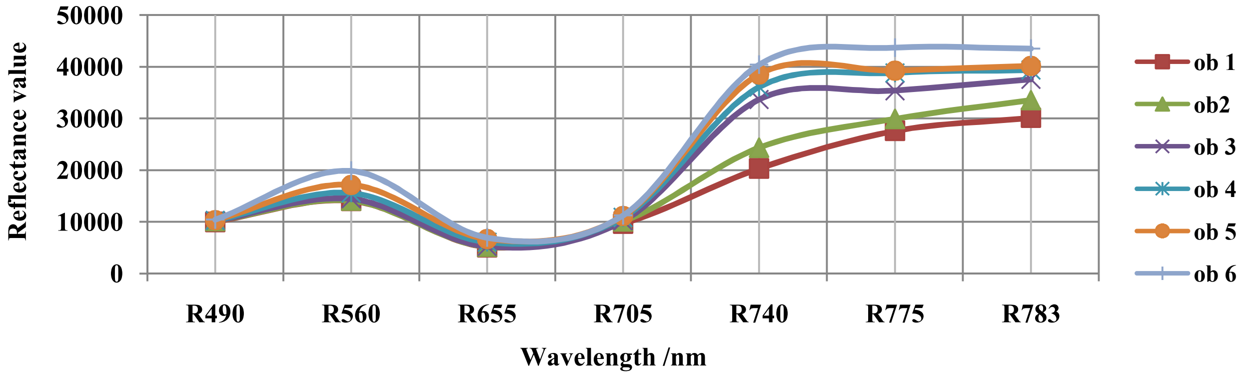

Fig. (6): Spectral reflectance of healthy sugar beet during development of disease severity in Sibel variety

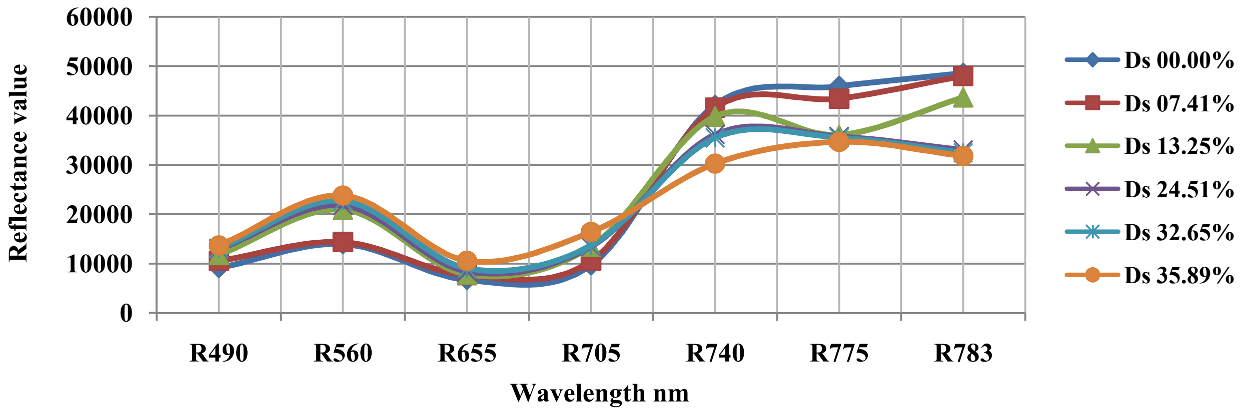

Fig. (7): Spectral reflectance of inoculated sugar beet during development of disease severity in Puma variety

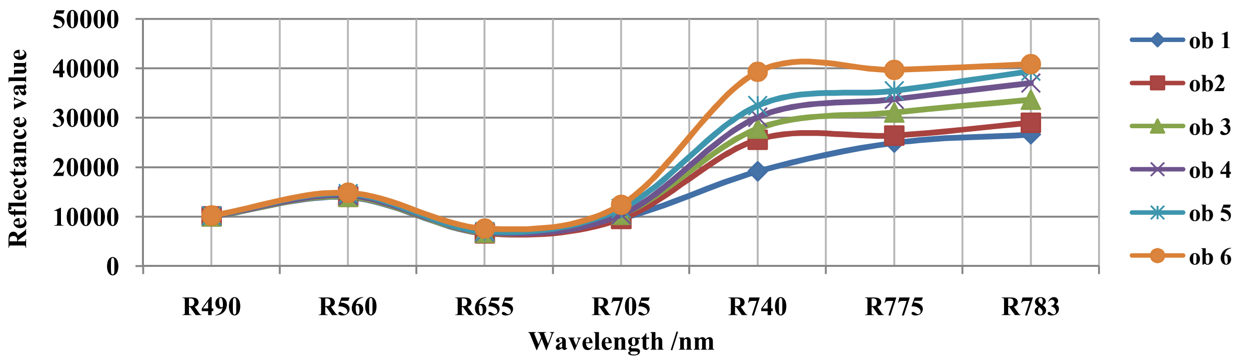

Fig. (8): Spectral reflectance of healthy sugar beet during development of disease severity in Puma variety

Correlation between spectral signatures and disease severity

The correlation coefficients (R) for disease severity of CLS disease and reflectance wavelength were carried out in the four varieties Puma, Sibel, Misrbal and Panthir (Table 2). The correlation result between CLS disease severity and reflectance for all varieties have positive in wavelength R490, R560, R665 and R705 that were represent visible light blue, green and red spectrum. In wavelength (R740, R775 and R780) were equivalent to red-edge 2, red-edge 3 and NIR spectrum result showed that correlation was negative. That mean for wavelength R490, R560, R665 and R705 each increasing in the levels of disease severity associated with increasing in reflectance on those wavelengths. While in case of wavelength (R740, R775 and R780) each increasing in level of disease severity associated with decreasing in reflectance. The visible spectrum range is characterized by low reflectance, due to absorption by photoactive plant pigments. The chlorophyll amount in the parenchyma and spongy mesophyll controls the level of light absorption Chlorophyll absorbs blue (400 to 495 nm) and red light (620 to 700 nm) and transfers the absorbed energy into the photosynthetic electron chain.

Table (2): Correlation coefficient between disease severity of CLS disease and reflectance wavelength using non-image hyperspectral SpectraPen SP100

Wavelength nm	Correlation coefficients (R)						
	R490	**R560**	**R665**	**R705**	**R740**	**R775**	**R780**
Misrbal	0.937	0.883	0.979	0.896	-0.856	-0.885	-0.958
Panthir	0.965	0.944	0.949	0.940	-0.957	-0.963	-0.946
Puma	0.970	0.922	0.932	0.908	-0.946	-0.880	-0.966
Sibel	0.868	0.929	0.996	0.866	-0.828	-0.969	-0.974

High reflection in this region is influenced by direct reflection on the leaf surface and multiple internal scattering processes within the leaf tissue. CLS increased reflectance in the VIS between 450 and 700 nm. A shift of the red edge position was monitored. Reflectance in the NIR decreased with increasing disease. In general, of spectral reflectance data in field experimental showed that there were slightly different between reflectance in four varieties that due to the differential in vigor of plant and leaf coverage from variety to other were showed in healthy plant variety Panthir was the highest vigor and leaf coverage (leaf area index) therefore showed that the highest reflectance value in NIR spectrum follow Misbal then Sible variety and finally variety Puma the lowest reflectance. While for infected showed reaction of varieties were differential with CLS disease were showed Puma, variety was the lowest disease severity which due to the different in reflectance. From previous data spectral reflectance may be useful for discrimination between varieties and their reaction with disease severity and selection the best resistance variety for disease.

Vegetation indices

There are five of VIs in present study were used to extract from No-image hyperspectral (Spectra Pen 100) represent NDVI, SR, OSAVI, AR1 and AR2 indices to find the VIs which have a unique characteristic and determine their ability to discriminate between healthy and infected of CLS disease in sugar beetwhere VI for healthy plant and plant inoculated (infected) by *Cercospora beticola* pathogen was recorded for 60 days after inoculation. Result showed that VI of healthy sugar beet canopy was characteristic for high values on NDVI, SR and OSAVI for all varieties. The highest value war recorded by Panthir which have the highest leaf coverage (LAI) and vigor follow by Misbal which less than Panthir in leaf coverage (LAI), then Sible variety and finally variety Puma was recorded the lowest values in NDVI, SR and OSAVI .Where increasing in growing and development plant leaf coverage of plant and greenness which lead to increasing in values of index. Where these indices are sensitive to leaves chlorophyll concentration, canopy leaf area, foliage clumping, and canopy architecture Table . The combination of its normalized difference formulation and use of the highest absorption and reflectance regions of chlorophyll makes it robust over a wide range of conditions. It can, however, saturate in dense vegetation conditions when LAI becomes high. This index is best used in areas with relatively bare vegetation where soil is visible through the canopy. The simple equation is easy to understand and is effective over a wide range of conditions. As with the NDVI, it can saturate in dense vegetation when LAI becomes very high. For Anthocyanin Reflectance Indices (AR1 and AR2) provide a measure of stress-related pigments, which are present in higher concentrations in weakened vegetation. Result showed that the value of these indices is very low where there is not stressed vegetation this. Increases in ARI indicate canopy changes in foliage via new growth or death. This index uses reflectance measurements in the visible spectrum to take advantage of the absorption signatures of stress-related pigments. In low level of disease severity result showed that NDVI, SR and OSAVI VI have ability to discriminate between healthy and non-healthy (infected) where result showed in infected plants showed decreasing in values compare with healthy plant Fig. (9, 10, 11 and 12). In of low disease severity all varieties showed slightly decreasing in value of these indices except SR index where value of NDVI and OSAVI decreasing with increasing of degree disease severity of CLS disease while SR index value decreasing when disease severity recorded average 34 % for Panthir , Misbal and Sible respectively while Puma at 24.51%. For Anthocyanin Reflectance Index (AR1 and AR2) as mentioned before providing a measure of stress-related pigments, which are present in higher concentrations in weakened vegetation. Result showed increasing of value with increasing in disease severity were development and growing of CLS in leaves and tissues of plant lead to decay and degradation of pigment of leaves plant which led to increasing in present in higher concentrations in weakened vegetation of these pigment.

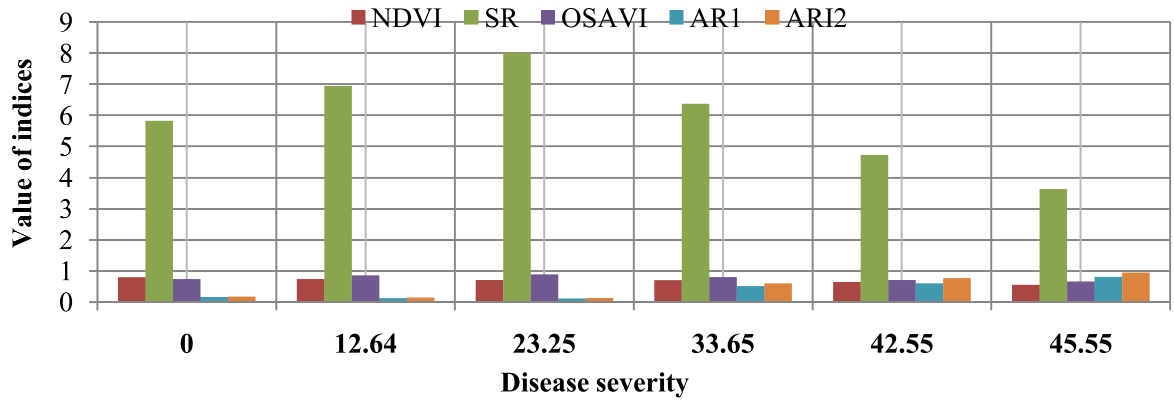

Fig. (9): Vegetation indices of sugar beet during development of disease severity in Panthir variety

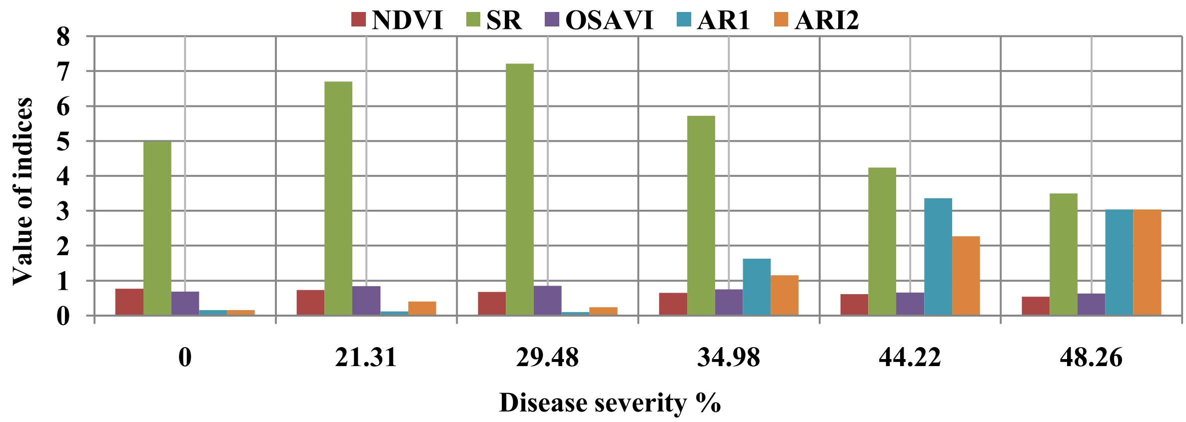

Fig. (10): Vegetation indices of sugar beet during development of disease severity in Misbal variety

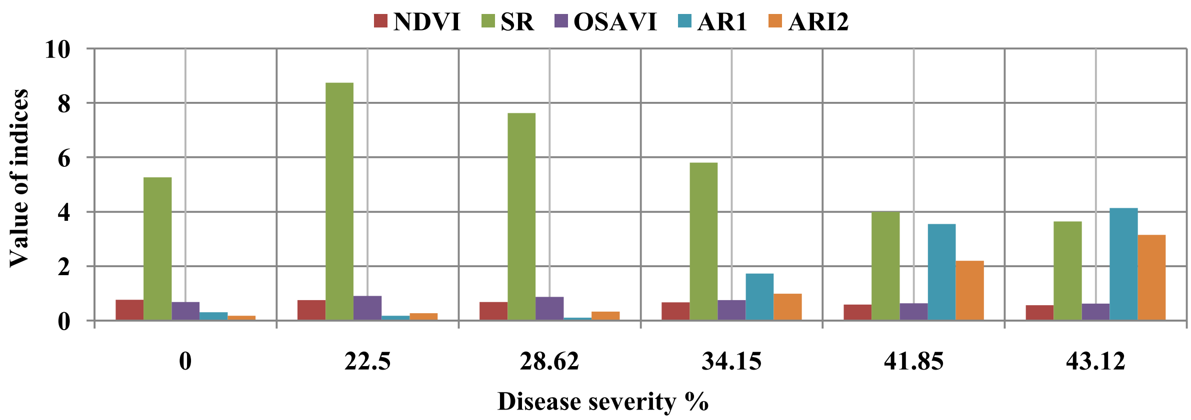

Fig. (11): Vegetation indices of sugar beet during development of disease severity in Sibel variety

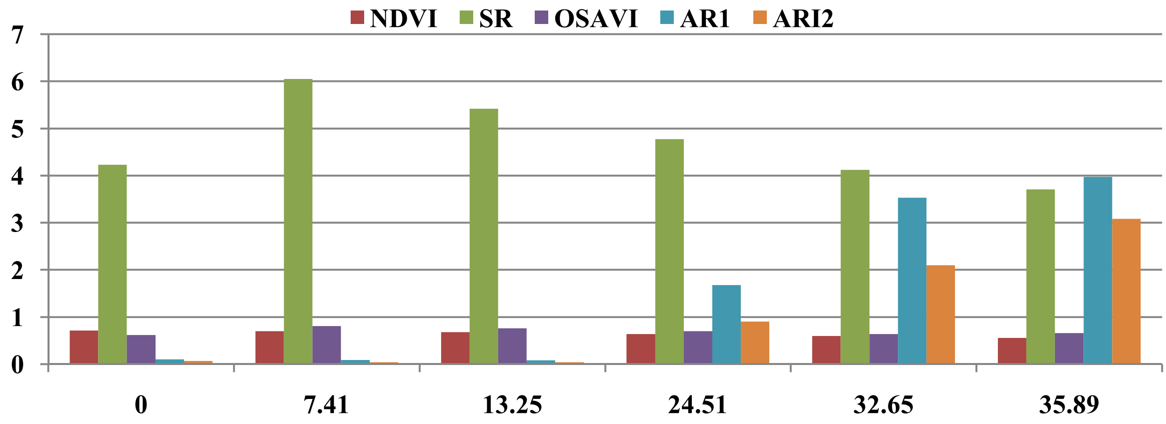

Fig. (12): Vegetation indices of sugar beet during development of disease severity in Puma variety

Correlation between VI and disease severity of CLS

According to the previous study result of the Vis showing that, the best VIs was (NDVI, SR, OSAVI, AR1 and AR2) used for discriminating between healthy and infected plant. Therefore, correlation coefficient between disease severity of CLS on sugar beet in all degree of disease severity as mentioned before were calculated In Table (3) the results of correlation revealed that there is relation between disease severity NDVI, AR1 and AR2 These have negative and significant and recorded average for four varieties (-0.945,-0.916,-0.972 and -0.882), respectively.

That is mean each decreasing in those indices associated with increasing in levels of disease severity of CLS disease that is due to effective of disease in pigment, greenness of vegetation , quality of photosynthetic and measurements in the NIR this spectrum allows these indices to be more sensitive to smaller changes in vegetation health than the broadband greenness VIs. For Anthocyanin Reflectance Index (AR1 and AR2) were correlation betweendisease severity and indices were positive and significant where Correlation coefficient (R) recorded for varieties AR1 (0.814, 0.873, 0.841 and 0.760) respectively at 0.01 level probabilities.That is means with each increasing in value of indices associated with increasing in disease severity degree that due to decay the of canopy and died of leaves which lead to increasing Anthocyanin pigment. The use of reflectance in the visible NIR and NIR spectrum, therefore, can be effectively used to detect disease symptoms even before they are visible.

Table (4): Correlation coefficient between disease severity of CLS disease and VI using non-image hyperspectral SpectraPen SP100

	Varieties	Correlation coefficients (R)				
		NDVI	SR	OSAVI	AR1	ARI2
Disease severity	Misrbal	-0.945	-0.393	-0.307	0.814	0.831
	Panthir	-0.916	-.0561	-0.457	0.873	0.885
	Puma	-0.972	-0.555	-0.301	0.941	0.911
	Sibel	-0.882	-0.360	-0.250	0.760	0.769

The result showed that spectral reflectance in selecting wavelength can discriminating between healthy and infected plant which represented (visible , red-edge and near-infrared , that is agreement with Steinkamp *et al.* (1979), Feindt *et al.* (1981) due to tissue degradation and the accumulation of brown and reddish brown pigments, reflectance spectra of CLS significantly increase in the complete visible spectrum, especially between 600 and 700 nm Leaf reflectance of sunlight in the visible (VIS, 400 to 700 nm), near infrared (NIR, 700 to 1100 nm) are driven by multiple interactions radiant energy absorption induced by leaf chemistry, scattering of light as a result of leaf surface and internal cellular structures. (Curran, 1989; Govaerts *et al.,* 1999; Carter and Knapp, 200) were reported that the visible spectrum range is characterized by low reflectance, due to absorption by photoactive plant pigments. The chlorophyll amount in the parenchyma and spongy mesophyll controls the level of light absorption Chlorophyll absorb blue (400 to 495 nm) and red light (620 to 700 nm) and they reported that the visible spectrum range is characterized by low reflectance, due to absorption by photoactive plant pigments. The chlorophyll amount in the parenchyma and spongy mesophyll controls the level of light absorption Chlorophyll absorbs blue (400 to 495 nm) and red light (620 to 700 nm) and transfers the absorbed energy into the photosynthetic electron chain. (Sims and Gamon, 2002) were reported that Carotenoids (stress pigment) absorb blue light (400 to 495 nm). Jensen (2002) was reported that the transition from visible to NIR is specified by the so-called red edge, the reflectance slope between 680 and 750 nm. The reflectance in the NIR is mainly dominated by leaf internal structure, leaf anatomy, and by the characteristics of the epidermal surface. High reflection in this region is influenced by direct reflection on the leaf surface and multiple internal scattering processes within the leaf tissue. Correlation between Vegetation Indices and disease severity of CLS disease According to the previous results using by hyperspectral non-imaging sensors in the VIS and NIR (SpectraPen SP100) using for discriminating between healthy infected plant were founded that VI of healthy sugar beet canopy were characteristic forhigh values on NDVI for all varieties. That agreement with (Delalieux *et al.,* 2009; Graeff *et al.,* 2006; Steddom *et al.,* 2005; Thenkabail *et al.,* 2000) they reported that the potential of NDVI for early disease detection has been investigated in several studies . Most of the developed indices are highly correlated to the content of pigments, biomass, or leaf area. Different changes in spectral reflectance not only denoted the occurrence of a disease, but also provided information on the developmental stage and severity of the disease. (Penuelas *et al.,* 1995; Mahlein *et al.,* 2013) have been used VI based on visible is one of these indices used to assess radiation use efficiency by plants, and some others have used VIs based on the NIR. Correlation between vegetation indices and disease severity of CLS from the previous topic the result showed that the best Vegetation indices NDVI for discriminating between healthy and no-healthy (infected) plant. That agreement with Mahlein *et al.,* 2013 reported that the index of NDVI that is sensitive to changes in chlorophyll concentration.

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استخدام القياس الطيفي المتعدد غير التصويري لتقييم تطور شدة المرضية لمرض التبقع السركسبورى في الغطاء النباتي بنجر السكر

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بنجر السكر (Beta vulgaris) في مصر هو ثاني أهم مصدر للسكر في مصر، يتعرض بنجر السكر للهجوم من قبل مرض تبقع الأوراق السركسبورى التييسببها *فطر Cercospora beticola*. ويمثل الاستشعار عن بعد ونظم المعلومات الجغرافية التوقيع الطيفي واستخدمت مؤشرات الغطاء النباتي في هذه الدراسة لتحديد قدرة هذه التقنية في قطاع أمراض النبات. مؤشرات التوقيع الطيفي والغطاء النباتي المرتبطة بعدوى نباتات بنجر السكر بمرض CLS باستخدام قياسات Hyperspectral بدون صورة SpectraPen SP100. تعتمد على التفاعل بين اختيار قيم الطول الموجي (R490، R560، R655، R705، R740، R775) الغطاء الخضريلنباتات بنجر السكر. باستخدام التوقيع الطيفي ومؤشرات الغطاء النباتي (NDVI، SR، OSAVI، AR١ و AR٢) كشفت النتائج التي نجحت في تحديد التغير في الغطاء الخضري لنباتات المصابة من بنجر السكر. أظهرت النتيجة أن الانعكاس الطيفي في اختيار الطول الموجي يمكن أن يميز بين النبات السليم والمصاب والتي لديها أفضل نتيجة للكشف عن مرض التبقع السركسبورى كم اوجد وارتباط عال مع شدة مرض CLS. وأظهرت النتائج ذلك. أظهرت النتائج أن مؤشرات الغطاء النباتي NDVI لها أعلى ارتباط تليها AR1 وAR2.

الكلمات المفتاحية: بنجر السكر تبقع الاوراق السركسبورى، التوقيع الطيفي، مؤشرات الغطاء النباتي، الاستشعار عن بعد