Detection, Disease Severity and Chlorophyll Prediction of Date Palm Leaf Spot Fungal Diseases

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Abstract

Date palm leaves are infected with the fungal pathogens genus viz., *Alternaria*, *Curvularia*, *Aspergillius* and *Neoscytalidium* causing leaf spot diseases. The evaluation of chlorophyll content in the infected seedlings possibly could provide a good indicator for a degree of disease or infection, and changes during pathogenesis. Date palm seedlings at three-month-old were infected with 6 pathogenic fungal inoculums were tested. Disease severity% (DS%) and chlorophyll (Chl) contents using a single-photon avalanche diode (SPAD) meter were recorded at 15. 30 and 45 days after inoculation. Pearson's correlation analysis, Durbin Watson and regression analysis were performed to evaluate the relationship between the variables. It was found that the relationship between DS% with fungi, chlorophyll and days were in multiple regression models (R^2 =91.88 and 91.87%, respectively). While, the relationship between chlorophyll with fungi, DS% and days were in multiple regression models (R^2 =92.22 and 92.20%, respectively). The SPAD chlorophyll value could be considered as a better alternative over the DS% as the SPAD chlorophyll value was strongly related to DS%, as well as able to detect physiological changes in the infected date palm at the early stages of leaf spot pathogenesis. The aim of this study was to examine the possibility of the relationship between disease severity % with fungi, chlorophyll and days for the detection and quantification of date palm leaf spot diseases This is the first research study done to study the relationship between DS%, chlorophyll and time on date palm leaf spot fungal diseases.

Keywords: date palm, leaf spot, fungal diseases, chlorophyll- SPAD

Introduction

The Date palm (*Phoenix dactylifera* L., Family Arecaceae) is the concept to be the oldest tree fruit grown in the arid regions for its edible fruit, is considered as the "tree of life". Date palm is an important crop in terms of the number of trees and their distribution in the Egypt and worldwide. Date palm is a perennial, diploid, monocotyledonous, dioecious, tall and evergreen palm (Barrow, 1998). Date palm tree is mostly cultivated for fruit, it is also grown in many countries as an ornamental plant or as a landscape tree (Biglari et al., 2008). Depending on the host pathogen system and disease specific symptoms, different regions of the reflectance spectrum are affected, resulting in specific spectral signatures of diseased plants. Establishment of leaf spot diseases in date palm seedlings through artificial inoculation of several pathogenic fungi are widely used for studies of various aspects of plant pathology, including epidemiology, etiology, disease resistance, host-parasite interaction, and disease control (De Castro Megías et al., 2021). Date palms are commonly influenced by several leaf spotting phytopathogenic fungi. Leaf spots can be circular to elongated, brown, black, and possibly oily in appearance. It is difficult to differentiate among the leaf spotting fungi by visual disease symptoms alone (Holliday, 1995). Leaf spot disease significantly affects the quantity and quality of date palm fruits. The literature on leaf spot diseases has highlighted several pathogenic fungi, viz., *Biopolaris australiensism*, *Nigrospora oryzae* and *N. sphaerica*, *Alternaria chlamydospora*, *A. alternata*, *A. radicina*, *A. tomato*, *A. arborescens, Cladosporium cladospoiroides*, *C. herbarum*, *Diplodia* sp., *Drechslera* sp., *Epicoccum purpurascens*, *Fusarium oxysporum*, *F. solani*, *Mycosphaerella* sp., *Helminthosporium* sp., *Pestalotia* sp., *Ulocladium atrum*, *Nigrospora* sp., *Thieloviopsis* sp., *Curvularia subpapendrofii*, *Tilletiopsis minor*, *Ulocladium* sp. *Phoma leveillei*, *Phoma glomerata* and *Thielaviopsis*

paradoxa in Iraq (Abass et al., 2013; ABASS et al., 2007; Abass & Mohammed, 2014; Al-Asad, 2010; Al-Nadabi et al., 2021; Al-Nadabi et al., 2018; Alasadi & Alnajim, 2014; El Badawy et al.; Khudhair et al., 2015; Muhsin & Madi, 2011). *Nigrospora sphaerica* in Pakistan (Alam et al., 2020) *Mycosphaerella tassiana*, *Alternaria* spp., and *Dreshcleri* sp. in Sultanate of Oman (Livingston et al., 2002) *Pseudopestalotiopsis theae* in China (Tao et al., 2021). *Alternaria* sp. *Aspergillus* sp. and *Helminthosporium* sp. fungi are the main causal agents of leaf spot diseases in Qatar (El Badawy & Elkharbotly, 2014; Manzelat, 2019). Occurrence of leaf spot disease on date palm caused by *Neopestalotiopsis clavispora* in Iran (Basavand et al., 2020). *Alternaria alternata* and *Xylohypha nigrescens* were isolated from Saudi Arabian date palms with leaf spot symptoms (Sheir et al., 1981). *Alternaria*, *Botryodiplodia*, *Chaetosphaeropsis*, *Diplodia*, *Fusarium*, *Graphiola*, *Gliocladium*, *Mycosphaerella*, *Phoma*, *Phomopsis* and *Thielaviopsis* in Egypt (Atallah et al., 2008; El-Deeb et al., 2006; Farrag & Abo-Elyousr, 2011). Artificial inoculation is essential for various studies in plant pathology, including epidemiology, etiology, disease resistance, host-parasite interaction, and disease control. External disease symptoms are the common marker used to evaluate the establishment of disease. However, there are other reciprocate physiological characteristics of plant that could be used to evaluate the disease establishment, such as assessment of the chlorophyll content in the leaf of a plant measured using an SPAD chlorophyll meter (Uddling et al., 2007). Mentioned that expanding the range of the investigated cultivars of date palm to assure the accuracy of the species-specific calibration equation used to estimate chlorophyll concentration in date palm as an independent plant species.(Almansoori et al., 2021). The similar findings also reported by (Chang et al., 2015), where the chlorophyll content reduced as the disease progressed in different stages of cucumber growth. Evaluation of disease severity based on the

external disease signs and symptoms which correspond to disease scales require tedious work and careful observation for data recording, as well as time consuming. The use of the SPAD chlorophyll meter device could provide better alternative to evaluate disease severity in a plant. Thus, the objective of this study was to evaluate the relationship between the values of disease severity % with fungi, chlorophyll and days in date palm seedlings accompanied by leaf spot diseases.

Materials and Methods
Date palm leaf spot pathogenic fungi:
The six pathogenic fungal causing leaf spot diseases of date palm, viz., *Alternaria botrytis*, *A. alternata*, *Curvularia mebaldsii*, C. *spicifera*, *Aspergillus terreus* and *Neoscytalidium novaehollandiae* were brought from Plant Pathology Department, Faculty of Agriculture, New Valley University, Egypt.

Greenhouse experiments
The artificial inoculation experiment carried out in the greenhouse of Plant Pathology Department, Faculty of Agriculture, New Valley University, Egypt on date palm leaves cv. Saidy. The seedlings were placed in a polyethylene bag containing a mixture of peat moss and vermiculite (1:1). The assays for all pathogenic fungi were carried out by spraying the leaves of each seedling (3 months old) by 5 ml of conidial suspension (10^6 conidia per ml) for each leaf (wounded) of each fungus (7 days old). Each isolate tested with 5 seedlings per replicate in four blocks for a total of 20 seedlings per each isolate, with 20 seedlings as control. All seedlings covered with a plastic bag to ensure high humidity of 70-90% during 48 hrs. after inoculation to ensure the infection occurred. The total of 140 seedlings were arranged in a randomized complete block design (RCBD). The experiment was repeated three times.

Collected data

Disease severity assessment%
In greenhouse experiments, disease severity assessed three times after inoculation at 15, 30 and 45 days. The disease severity index (DSI) was assessed according to (Ilias, 2000) with modification. For measurements on the leaf scale, the percentage of diseased leaf area of the measured leaf in relation to healthy leaf tissue was estimated visually. Area of observed external disease symptoms of infected seedlings, were scored for disease index using a scale from 0 to 4 (Table 1). The severity data were processed by McKinney's formula, which generates a numeric disease severity index (DSI): DS (%) $=(\Sigma vn)/(NV)\times100$, where v represents the numeric value of the disease index scale, n is the number of plants assigned to the disease index scale, N is the total number of the plants and V is the numeric value of the highest disease index scale. DSI was calculated from four leaves of each date palm seedlings (Rakib et al., 2019).

Chlorophyll Content
SPAD-meter measurements A Minolta SPAD-502 meter (Minolta Camera Ltd., Osaka, Japan), was used for non-destructive assessment of leaf chlorophyll content (Chl). The instrument determines the relative amount of chlorophyll present, by measuring the transmittance of the date palm leaf. The dimensionless SPAD-units are proportional to the amount of chlorophyll (Almansoori et al., 2021). The experiments were repeated three times (Oehlert, 2010).

Statistical Analysis
Statistical analyses were performed using the STATGRAPHICS - Version 19 – Stat Point, Inc. The obtain Data were analyzed using a general linear model and the regression model test to determine statistically significant differences (p = 0.05). Correlations between disease severity with fungi, chlorophyll, and days, respectively, were tested by computing Pearson's coefficient of correlation (r), and coefficients of determination R^2 were

Table 1: Disease numerical scale and their corresponding disease symptoms

Scale	Disease symptoms
0	No spots = Healthy
1	1-3 spots = Gradual spot occurred on 1-25 % of date palm leaves.
2	4-6 spots = Gradual spot occurred on 26-50 % of date palm leaves.
3	7-9 spots = Gradual spot occurred on 51-75 % of date palm leaves.
4	<10 spots = Gradual spot occurred on 76-100 % of date palm leaves.

superscript by a linear and multiple regression models.

Results and Discussion

The present work focuses on the potential of relationship between DS% with fungi, chlorophyll and days for the detection, differentiation, and quantification of leaf spot diseases of date palm. The hypothesis was that the six-causal agent of leaf spot diseases influence the optical and physiological properties of a plant in different ways.

Pathogenicity test

All pathogenic fungal inoculation plants showed initial symptoms of leaf spot disease within 2 weeks. The disease severity of infection increased gradually. To monitor the chlorophyll changes during leaf spot disease on date palm, the disease severity development was rated on a five point scale (0-4) based on disease severity of infection. Leaf spot disease establishes gradually in leaves of the plant with distinctive changes in chlorophyll composition. Data in table (2) show that, the highest mean DS% of leaf spot diseases for different pathogenic fungi was a record (7.60 and 7.50 % for *A. terreus* and *C. spicifera*, respectively), while the lowest mean DS% was (5.52% for *A. alternata*). Furthermore, the highest mean DS% of leaf spot diseases for days were a record (6.74 and 5.85 % at 45 and 30 days, respectively), while the lowest mean DS% was (4.51% at 15 days).

Whereas, the highest mean chlorophyll of leaf spot diseases for different pathogenic fungi was a record (Chl= 40.50 for *A. alternata*), while the lowest mean chlorophyll was (Chl=35.89 for *A. terreus*). Furthermore, the highest mean chlorophyll of leaf spot diseases for days was a record (Chl= 40.41 and 39.63 at 15 and 30

days, respectively), while the lowest mean chlorophyll was (Chl= 38.67 at 45 days). Concerning to the results in Table 2, these results confirm the association between DS% and chlorophyll, when DS% increased the chlorophyll decreased in all pathogenic fungi tested (inversely proportional). Total chlorophyll content was drastically reduced in diseased leaf, with (Chl= 35.89) for *A. terreus* as compared with healthy leaves (Chl= 48.50). This drop in chlorophyll may be attributed to the disorganization of the plastid membrane upon infection as mentioned by (Alwadi & Baka, 2001; Sharma et al., 2011; Yang & Luo, 2021). A strong relationship between DS% and chlorophyll has been reported and agreement with (Chang et al., 2015; Rakib et al., 2019). The current study found a strong relationship between DS% and chlorophyll to detect and prediction of date palm leaf spot fungal diseases.

Relationship between DS% with fungi, chlorophyll, and Days

The six pathogenic fungi tested , showed that positive symptoms of leaf spot diseases infection on date palm. Leaf spot symptoms were observed as the infection progressed over fungi and time. Furthermore, external symptoms appeared at 15 days after inoculation according to different isolates of pathogenic fungi. Prediction models for disease severity % (DS%) were dependent on the data measurement from the interaction between fungi, chlorophyll, days, and disease severity in greenhouse.

Forecasting Disease Severity % Models

A- Relationship between DS% with fungi, chlorophyll, and Days

Table 2: Pathogenicity test for pathogenic fungi causing leaf spot diseases in date palm.

Pathogenic fungi/ No.	Days/ DS%			Mean	Days/ Chlorophyll			Mean
	15 [b]	30 [a]	45 [a]		15 [a]	30 [ab]	45 [b]	
Control (0)	0.00	0.00	0.00	0.00[c]	42.43	51.90	51.18	48.50[a]
Curvularia spicifera (1)	7.19	7.50	7.81	7.50[a]	37.11	37.07	35.52	36.57[cd]
Aspergillus terreus (2)	7.19	7.50	8.13	7.60[a]	36.18	36.14	35.35	35.89[d]
Curvularia mebaldsii (3)	3.75	6.25	8.75	6.25[ab]	41.81	38.62	33.97	38.13[bcd]
Alternaria alternata (4)	3.44	6.25	6.88	5.52[b]	43.74	39.40	38.36	40.50[b]
Neoscytalidium novaehollandiae (5)	6.25	6.25	6.56	6.35[ab]	40.16	38.88	38.42	39.15[bc]
Alternaria botrytis (6)	3.75	7.50	9.06	6.77[ab]	42.80	36.87	35.11	38.26[bcd]
Mean	4.51	5.85	6.74		40.41	39.63	38.67	

Relationship between DS% with fungi (Models 1 & 2)

The results of the correlational analysis are shown in Table (3) linear model was given the lowest percentage of predict the DS% expected with fungi ($r^2 = 7.47\%$) and (r^2 adjusted for d.f.= 6.34%) in the model No. 1, and as shown in (Fig. 1). The equation of the fitted linear model is DS% Average = 4.17039 + 0.420387*Fungi. The correlation coefficient equals 0.273247, indicating a relatively weak relationship between the variables. While, the Double square root model was given the highest percentage of predict the DS% expected with fungi (r^2= 39.07%) and (r^2 adjusted for d.f.= 38.32%) in the model No.2, and as shown in (Fig. 2). The equation of the fitted Double square root model is DS% Average = $(0.89589 + 0.787424*sqrt(Fungi))^{2}$. The correlation coefficient equals 0.625035, indicating a moderately strong relationship between the variables.

Table 3: Relationship between DS% with fungi

| No.	Model	Equation	Output	Correlation Coefficient	R²%	R² (adjusted for d.f.)%	Standard Error of Est	Mean absolute error	Durbin-Watson statistic	Lag 1 residual autocorrelation	Model No.
1	Linear	Y = a + b*X	DS% Average = 4.17039 + 0.420387*Fungi	0.273247	7.46638	6.33792	2.99576	2.63366	0.850876 (P=0.0000)	0.562302	1
	Double square root	Y = (a + b*sqrt(X))^2	DS% Average = (0.89589 + 0.787424*sqrt(Fungi))^2	0.625035	39.0669	38.3238	0.774526	0.665269	0.660567 (P=0.0000)	0.661483	2
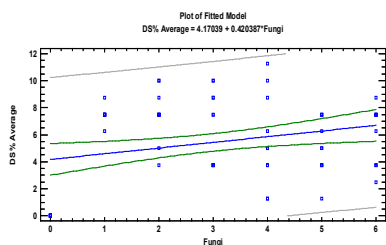
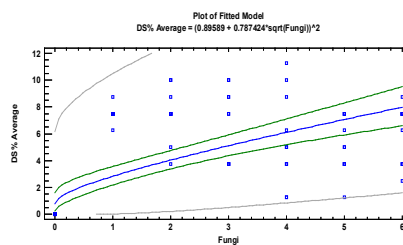**Figure 1: Relationship between DS% with fungi (model 1)**

Figure 2: Relationship between DS% with fungi (model 2)

Relationship between DS% with chlorophyll Models 3 & 4)

The results, as shown in Table (4) indicate that linear model was given the lowest percentage of predict the DS% expected with Chlorophyll (r^2 = 91.62%) and (r^2 adjusted for d.f.= 91.52%) in the model No. 3, and as shown in (Fig. 3). The equation of the fitted linear model is DS% Average = 26.5928 - 0.53444*Chlorophyll. The correlation coefficient equals -0.957207, indicating a relatively strong relationship between the variables. While, the Squared-X model given the higher percentage of predict the DS% expected with Chlorophyll (r^2 = 91.95%) and (r^2 adjusted for d.f.= 91.85%) in the model No. 4, and as shown in (Fig. 4). The equation of the fitted Squared-X model is DS% Average = 15.9242 - 0.0065655*Chlorophyll$^{\wedge 2}$. The correlation coefficient equals -0.958909, indicating a relatively strong relationship between the variables. A positive correlation was found between DS% and chlorophyll.

Table 4: Relationship between DS% with chlorophyll

| No.	Model	Equation	Output	Correlation Coefficient	R^2%	R^2 (adjusted for d.f.)%	Standard Error of Est	Mean absolute error	Durbin-Watson statistic	Lag 1 residual autocorrelation	Model No.
2	**Linear**	**Y = a + b*X**	**DS% Average = 26.5928 - 0.53444*Chlorophyll**	**-0.957207**	**91.6245**	**91.5223**	**0.901286**	**0.710574**	**1.46515 (P=0.0051)**	**0.244413**	**3**
	Squared-X model	**Y = a + b*X$^{\wedge 2}$**	**DS% Average = 15.9242 - 0.0065655*Chlorophyll$^{\wedge 2}$**	**-0.958909**	**91.9506**	**91.8524**	**0.883566**	**0.721057**	**1.57526 (P=0.0205)**	**0.195174**	**4**
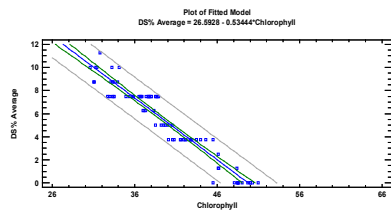
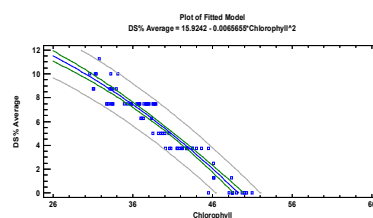Figure 3: Relationship between DS% with chlorophyll (model 3).

Figure 4: Relationship between DS% with chlorophyll (model 4)

Relationship between DS% with days (Models 5 & 6)

Data in Table (5) show that linear model was given the lowest percentage of predict the DS% expected with days (r^2 = 5.90%) and (r^2 adjusted for d.f.= 4.75%) in the model No. 5, and as shown in (Fig. 5). The equation of the fitted linear model is DS% Average = 3.60119 + 0.0610119*Days. The correlation coefficient equals 0.242849, indicating a relatively weak relationship between the variables. While, the Squared-Y reciprocal-X model given the higher percentage of predict the DS% expected with days (r^2 = 91.95%) and (r^2 = 10.49%) and (r^2 adjusted for d.f.= 9.40%) in the model No. 6, and as shown in (Fig. 6). The equation of the fitted Squared-X model is DS% Average = sqrt(60.3516 - 524.833/Days). The correlation

coefficient equals -0.323929, indicating a relatively weak relationship between the variables.

Relationship between DS% with fungi, chlorophyll, and Days (Models 7 & 8)

Data in Table (6) show that multiple regression model between DS% with fungi, chlorophyll and Days was given the lowest percentage of predict the DS% expected with fungi (r^2 = 91.88%) and (r^2 adjusted for d.f.= 91.57%) in the model No. 7, and as shown in (Fig. 7). The equation of the fitted multiple regression model is DS% Average = 27.1025 - 0.0800682*Fungi - 0.542954*Chlorophyll + 0.00225651*Days. The R-Squared statistic indicates that the model as fitted explains 91.8801% of the variability in DS% Average. The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 91.5757%. In determining whether the model can be simplified, notice that the highest *P*-value on the independent variables is 0.7859, belonging to Days. Since the P-value is greater or equal to 0.05, that term is not statistically significant at the 95.0% or higher confidence level. Consequently, we should remove Days from the model. While, multiple regression model between DS% with fungi and chlorophyll given the higher percentage of predict the DS% expected with fungi (r^2 = 91.87%) and (r^2 adjusted for d.f.= 91.67%) in model No. 8, and as shown in (Fig. 8). The equation of the fitted multiple regression model is DS% Average = 27.2277 - 0.0813198*Fungi - 0.544312*Chlorophyll. The R-Squared statistic indicates that the model as fitted explains 91.8726% of the variability in DS% Average. The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 91.6719%. In determining whether the model can be simplified, notice that the highest *P*-value on the independent variables is 0.1197, belonging to Fungi. Since the *P*-value is greater or equal to 0.05, that term is not statistically significant at the 95.0% or higher confidence level. Consequently, we should consider removing Fungi from the model.

Table 5: Relationship between DS% with days

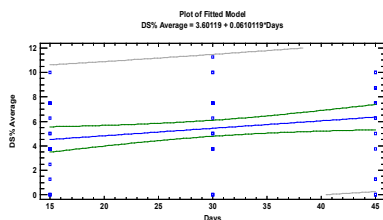| No.	Model	Equation	Output	Correlation Coefficient	R^2%	R^2 (adjusted for d.f.)%	Standard Error of Est	Mean absolute error	Durbin-Watson statistic	Lag 1 residual autocorrelation	Model No.
3	**Linear**	Y = a + b*X	**DS% Average = 3.60119 + 0.0610119*Days**	0.242849	5.89756	4.74997	3.02105	2.42878	0.793692 (P=0.0000)	0.588638	5
	Squared-Y reciprocal-X	Y = sqrt(a + b/X)	**DS% Average = sqrt(60.3516 - 524.833/Days)**	-0.323929	10.493	9.40145	29.2992	23.6009	1.26611 (P=0.0002)	0.361971	6

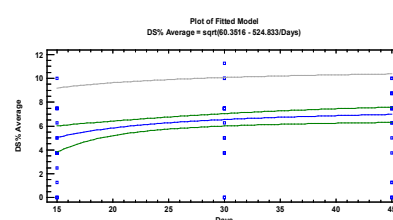**Figure 5: Relationship between DS% with Days model 5)**

Figure 6: Relationship between DS% with Days model 6)

Table 6: Relationship between DS% with fungi, chlorophyll, and days

| No.	Model	Equation	Output	R^2%	R^2 (adjusted for d.f.)%	Standard Error of Est	Mean absolute error	Durbin-Watson statistic	Lag 1 residual autocorrelation	Model No.
4	Multiple	$Y = a + b_1X_1 + b_2X_2 + b_3X_3$	DS% Average = 27.1025 - 0.0800682*Fungi - 0.542954*Chlorophyll + 0.00225651*Days	91.8801	91.5757	0.898448	0.701909	1.55204 (P=0.0098)	0.202278	7
	Multiple	$Y = a + b_1X_1 + b_2X_2$	DS% Average = 27.2277 - 0.0813198*Fungi - 0.544312*Chlorophyll	91.8726	91.6719	0.893299	0.700833	1.55644 (P=0.0126)	0.200415	8
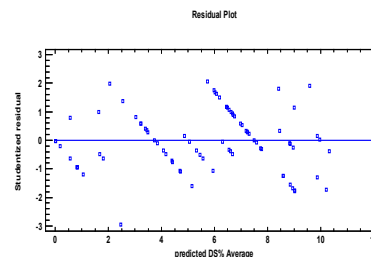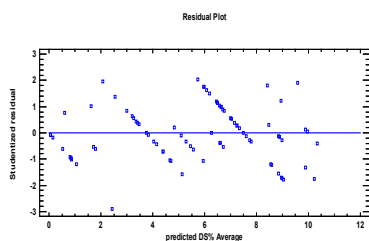
Figure 7:: Relationship between DS% with fungi, chlorophyll, and Days (model 7). Figure 8:: Relationship between DS% with fungi and chlorophyll (model 8)

B- Relationship between chlorophyll with fungi, DS% and Days

Relationship between chlorophyll with fungi (Models 9 & 10)

Data in Table (7) show that linear model was given the lowest percentage of predict the chlorophyll expected with fungi (r^2 = 7.00%) and (r^2 adjusted for d.f.= 6.78%) in model No. 9, and as shown in (Fig. 9). The equation of the fitted linear model is Chlorophyll = 41.9095 - 0.77*Fungi. The correlation coefficient equals -0.264664, indicating a relatively weak relationship between the variables. While, Squared-Y square root-X model given the higher percentage of predict the chlorophyll expected with fungi (r^2 = 20.09%) and (r^2 adjusted for d.f.= 19.90%) in model No. 10, and as shown in (Fig. 10).

The equation of the fitted Double square root model is Chlorophyll = sqrt(2037.81 - 281.655*sqrt(Fungi)). The correlation coefficient equals -0.448284, indicating a relatively weak relationship between the variables.

Relationship between chlorophyll with DS% (Models 11 & 12)

Data in Table (8) show that linear model was given the lowest percentage of predict the chlorophyll expected with DS% (r^2 = 91.62%) and (r^2 adjusted for d.f.= 91.52%) in model No. 11, and as shown in (Fig. 11). The equation of the fitted linear model is Chlorophyll = 48.9071 - 1.7144*DS% Average. The correlation coefficient equals -0.957207, indicating a relatively strong relationship between the variables. While, Squared-Y model given the higher percentage of predict the chlorophyll expected with fungi (r^2 = 91.95%) and (r^2 adjusted for d.f.= 91.85%) in model No. 12, and as shown in (Fig. 12). The equation of the fitted Squared-X model is Chlorophyll = sqrt(2358.85 - 140.051*DS% Average). The correlation coefficient equals -0.958909, indicating a relatively strong relationship between the variables.

Relationship between chlorophyll with days (Models 13 & 14)

Data in table (9) show that linear model was given the lowest percentage of predict the chlorophyll expected with days (r^2 = 2.43%) and (r^2 adjusted for d.f.= 2.20%) in the model No. 13, and as shown in (Fig. 13). The equation of the fitted linear model is Chlorophyll= 41.8224 - 0.0740952*Days. The correlation coefficient equals -0.155959, indicating a relatively weak relationship between the variables. While, Reciprocal - Y squared -X model given the higher percentage of predict the chlorophyll expected with fungi (r^2 = 4.52%) and (r^2 adjusted for d.f.= 4.30%) in model No. 14, and as shown in (Fig. 14). The equation of the fitted Squared-X model is Chlorophyll= 1/(0.0246947 + 0.00000102277*Days^2). The correlation coefficient equals 0.212724, indicating a relatively weak relationship between the variables.

Relationship between chlorophyll with fungi, DS% and Days (Models 15 & 16)

Data in table (10) show that multiple regression model between chlorophyll with fungi, DS% and Days was given the lowest percentage of predict the chlorophyll expected with fungi (r^2 = 92.22%) and (r^2 adjusted for d.f.= 91.93%) in the model No. 15, and as shown in (Fig. 15). The equation of the fitted multiple regression model is Chlorophyll = 49.5127 - 0.220034*Fungi - 1.66916*DS% Average - 0.00637577*Days. The R-Squared statistic indicates that the model as fitted explains 92.2184% of the variability in Chlorophyll. The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 91.9266%. In determining whether the model can be simplified, notice that the highest *P*-value on the independent variables is 0.6614, belonging to Days. Since the *P*-value is greater or equal to 0.05, that term is not statistically significant at the 95.0% or higher confidence level. Consequently, we should remove Days from the model. While, multiple regression

model between chlorophyll with fungi and DS% given the higher percentage of predict the chlorophyll expected with fungi (r^2 = 92.20%) and (r^2 adjusted for d.f.= 92.01%) in the model No. 16, and as shown in (Fig. 16). The equation of the fitted multiple regression model is Chlorophyll = 49.3492 - 0.217234*Fungi - 1.67582*DS% Average. The R-Squared statistic indicates that the model as fitted explains 92.1996% of the variability in Chlorophyll. The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 92.007%. In determining whether the model can be simplified, notice that the highest *P*-value on the independent variables is 0.0167, belonging to Fungi. Since the *P*-value is less than 0.05, that term is statistically significant at the 95.0% confidence level. Consequently, we probably don't want to remove any variables from the model.

Conclusion

This study set out to develop a prediction model for detection date palm leaf spot fungal diseases. The disease severity of date palm leaf spots evaluation based on external symptoms, which correspond to disease scales require tedious work and careful observation for data recording, as well as time consuming. The use of the SPAD chlorophyll meter device could provide the better alternative to evaluate disease severity in a plant. The relationship between DSI and chlorophyll value was inversely proportional in a linear and multiple models trends with confidence level of 91.62 and 91.95% respectively. The declines in the chlorophyll values were detected on the early stages of pathogenesis (0 to 15 days after infection) although the obvious change was recorded in the DS% during those early stages. This concluded that chlorophyll value could be used as an alternative as well as a better alternative as rapid indicator and nondestructive method to detection and evaluate the disease establishment in the date

palm leaf spot fungal diseases. The current study confirmed the validity of exploiting SPAD-502 estimation of total chlorophyll concentration in date palm leaflets. Accordingly, SPAD-502 can be considered as potential tools for monitoring the physiological status and assessing the health of date palm trees under various environmental conditions. The generic conversion equation derived for SPAD indices, indicates that the SPAD can be used inter change ably to estimate chlorophyll concentration in date palm leaflets. By predicting detection DS%, chlorophyll, and time, it is possible to protect the date palm leaves before or in the first-stage infection, period occurs. In advanced research, we should be monitoring date palm trees and offshoots in fields at the various locations in Egypt for validation of disease prediction models of primarily leaf spot diseases.

Conflicts of Interest: The authors declare that they have no competing interests.

Table 7: Relationship between chlorophyll with fungi

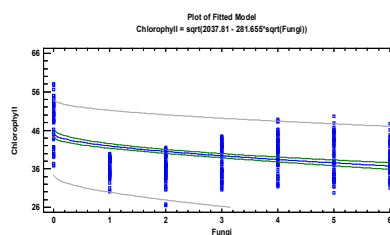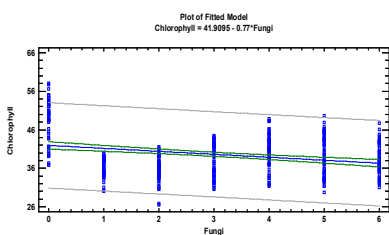| No.	Model	Equation	Output	Correlation Coefficient	R^2%	R^2 (adjusted for d.f.)%	Standard Error of Est	Mean absolute error	Durbin-Watson statistic	Lag 1 residual autocorrelation	Model No.
5	Linear	Y = a + b*X	Chlorophyll = 41.9095 - 0.77*Fungi	-0.264664	7.00472	6.78224	5.62461	4.69651	0.384257 (P=0.0000)	0.806211	9
	Squared-Y square root-X	Y = sqrt(a + b*sqrt(X))	Chlorophyll = sqrt(2037.81 - 281.655*sqrt(Fungi))	-0.448284	20.0959	19.9047	438.084	370.171	0.406422 (P=0.0000)	0.796172	10

Figure 9: Relationship between chlorophyll with fungi (model 9). **Figure 10: Relationship between chlorophyll with fungi (model 10)**

Table 8: Relationship between chlorophyll with DS%

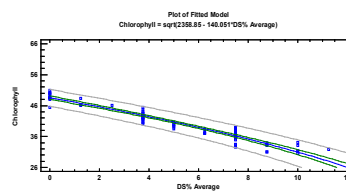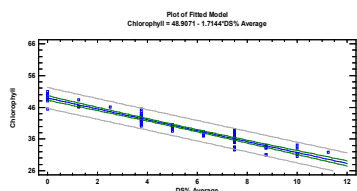| No.	Model	Equation	Output	Correlation Coefficient	R^2%	R^2 (adjusted for d.f.)%	Standard Error of Est	Mean absolute error	Durbin-Watson statistic	Lag 1 residual autocorrelation	Model No.
6	Linear	Y = a + b*X	Chlorophyll = 48.9071 - 1.7144*DS% Average	-0.957207	91.6245	91.5223	1.61424	1.28957	1.59935 (P=0.0266)	0.173691	11
	Squared-Y model:	Y = sqrt(a + b*X)	Chlorophyll = sqrt(2358.85 - 140.051*DS% Average)	-0.958909	91.9506	91.8524	129.047	103.214	1.6384 (P=0.0402)	0.15984	12

Figure 11: Relationship between chlorophyll with DS% (model 11). **Figure 12: Relationship between chlorophyll with DS% (model 12)**

Table 9: Relationship between chlorophyll with days

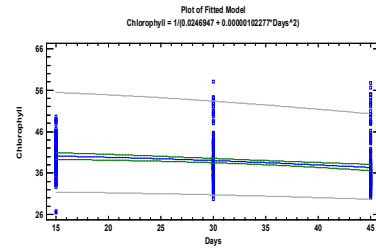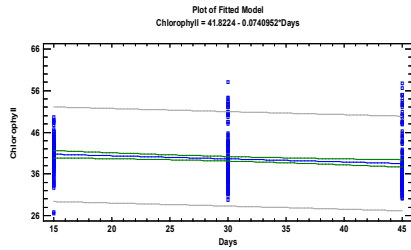| No.	Model	Equation	Output	Correlation Coefficient	R²%	R² (adjusted for d.f.)%	Standard Error of Est	Mean absolute error	Durbin-Watson statistic	Lag 1 residual autocorrelation	Model No.
7	Linear	Y = a + b*X	Chlorophyll = 41.8224 - 0.0740952*Days	-0.155959	2.43232	2.19891	5.76123	4.40113	0.351791 (P=0.0000)	0.82179	13
	Reciprocal-Y squared-X	Y = 1/(a + b*X^2)	Chlorophyll = 1/(0.0246947 + 0.00000102277*Days^2)	0.212724	4.52516	4.29675	0.00349638	0.00270602	0.447411 (P=0.0000)	0.77445	14

Figure 13: Relationship between chlorophyll with days (model 13)   **Figure 14: Relationship between chlorophyll with days (model 14)**

Table 10: Relationship between chlorophyll with fungi, DS% and days

| No.	Model	Equation	Output	R²%	R² (adjusted for d.f.)%	Standard Error of Est	Mean absolute error	Durbin-Watson statistic	Lag 1 residual autocorrelation	Model No.
8	Multiple	Y = a + b₁X₁+ b₂X₂+ b₃X₃	Chlorophyll = 49.5127 - 0.220034*Fungi - 1.66916*DS% Average - 0.00637577*Days	92.2184	91.9266	1.57529	1.24081	1.75486 (P=0.0822)	0.102268	15
	Multiple	Y = a + b₁X₁+ b₂X₂	Chlorophyll = 49.3492 - 0.217234*Fungi - 1.67582*DS% Average	92.1996	92.007	1.56742	1.24104	1.7493 (P=0.0903)	0.104161	16
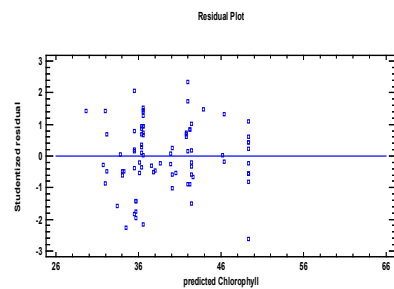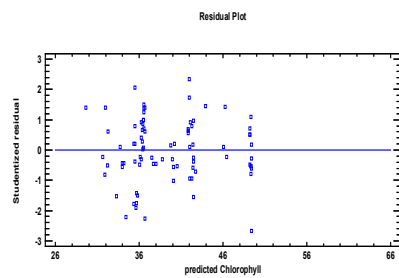
Figure 15: Relationship between chlorophyll with fungi, DS% and days (model 15).   **Figure 16: Relationship between chlorophyll with fungi and DS% (model 16)**

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