



## Advancements in Detecting Spot Fungal Diseases on Date Palm Leaves: Development and Validation of Chlorophyll-Based Detection Models

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### Abstract

The importance of chlorophyll content as an early indicator for the detection and prediction of leaf spot diseases in date palms (*Phoenix dactylifera* L.) caused by fungi such as *Alternaria* and *Curvularia* was demonstrated. Studies have shown that there is a strong association between disease severity (DS%) and chlorophyll levels in date palm seedlings infected with various fungal pathogens. Statistical models can be used to accurately predict disease severity based on the presence of fungi and chlorophyll levels. This suggests that measuring chlorophyll levels may be a useful tool for early detection of disease, even before visible symptoms appear. A study was conducted in which date palm seedlings were inoculated with various fungal pathogens and disease severity and chlorophyll content were monitored over a period of time. The results showed a clear inverse relationship between disease severity and chlorophyll levels. Statistical models were able to accurately predict disease severity based on fungal presence and chlorophyll content. Remarkably, changes in chlorophyll levels were observed early in the infection, before visible symptoms appeared. This highlights the potential of using a SPAD meter to monitor and treat diseases in date palms. The models were validated on a variety of infected and healthy date palm leaves and demonstrated high accuracy in disease classification and severity estimation. These results suggest that chlorophyll-based detection models can be used for rapid and accurate diagnosis of leaf spot fungal diseases in date palm without the need for invasive procedures.

**Keywords:** Date palm leaf spot diseases; forecasting models; chlorophyll; statistical models; SPAD

## Introduction

The date palm (*Phoenix dactylifera* L.) stands as a symbol of resilience and sustenance, particularly in arid and semi-arid regions (Al-Shahib & Marshall, 2003; Obón et al., 2023). Its cultivation for its highly prized and nutritious fruit has been deeply intertwined with human civilizations for millennia, finding mention in religious texts like the Bible and the Quran, further emphasizing its cultural and historical significance (Alemayehu, 2023). While the date palm thrives in challenging environments, its production faces threats from various biotic stresses, including pests and diseases (Khan et al., 2023).

Among these threats, fungal diseases pose a particularly significant challenge to date palm health and productivity (Alemayehu, 2023). Leaf spot diseases, caused by a diverse array of fungal species like *Alternaria*, *Aspergillus*, *Curvularia*, *Neoscytalidium*, and *Nigrospora*, are prevalent across date palm growing regions (Arafat et al., 2021; Arafat et al., 2024; Rabaaoui et al., 2022). These diseases can significantly impact fruit quality and yield, ultimately affecting the economic viability of date palm production (Chao & Krueger, 2007; El-Deeb et al., 2012).

The need for effective disease management strategies is paramount. However, traditional methods for disease detection often fall short (Camargo & Smith, 2009; Mahlein et al., 2012). Visual inspection by trained personnel, DS the most commonly employed technique, is often subjective, time-consuming, and unreliable, especially in the early stages of infection (Camargo & Smith, 2009). Laboratory analysis, although accurate, often requires time-consuming procedures like culturing and microscopy, demanding specialized equipment and expertise, which can be costly and inaccessible in many settings (Mahlein et al., 2012).

The limitations of traditional methods underscore the pressing need for rapid,

accurate, and non-destructive methods for identifying and quantifying leaf spot diseases in date palm leaves. Recent research provides promising solutions, focusing on the potential of chlorophyll content as an early indicator of fungal infections (Atta et al., 2018; Arafat et al., 2021).

Chlorophyll, the pigment responsible for photosynthesis, plays a central role in plant health and growth (Lichtenthaler et al., 1996). Changes in chlorophyll content can serve as sensitive indicators of stress, both biotic and abiotic, often appearing before visible symptoms of disease manifest (Atta et al., 2018). This early warning system holds immense potential for proactive disease management and minimizing yield losses (Khan et al., 2023).

Moreover, chlorophyll fluorescence, the light re-emitted by chlorophyll molecules during the return from excited to non-excited states, offers another valuable tool for detecting plant stress (Bürling et al., 2011; Gamon et al., 1992; Maxwell & Johnson, 2000). Chlorophyll fluorescence is particularly sensitive to changes in plant physiological status, including those caused by fungal infections (Bürling et al., 2011; Maxwell & Johnson, 2000).

The use of advanced spectral imaging technologies, such as hyperspectral and multispectral imaging, has further revolutionized our ability to detect disease symptoms non-destructively (Calderón et al., 2013; Moshou et al., 2005; Pérez-Bueno et al., 2016). These technologies provide detaDSd spectral information across numerous bands, capturing subtle shifts in chlorophyll content and fluorescence that may indicate fungal infection (Calderón et al., 2013; Moshou et al., 2005).

This study builds upon previous research (Arafat et al., 2021), which established a strong relationship between disease severity and chlorophyll content in date palm leaf spot diseases. By leveraging these findings, we aim

to develop and validate novel chlorophyll-based detection models for spot fungal diseases on date palm leaves, providing a rapid, accurate, and non-destructive tool for early disease detection. This advancement could revolutionize disease management in date palm plantations, promoting healthier crops, higher yields, and a more sustainable future for date palm agriculture (Khan et al., 2023; Al-Shahib & Marshall, 2003).

## Materials and methods

### Pathogenic Fungi

Twenty-two pathogenic fungi causing leaf spot diseases on date palm were obtained from the Plant Pathology Department, Faculty of Agriculture, New Valley University, Egypt (Arafat et al., 2024). These isolates were selected based on their known association with

leaf spot symptoms in date palms and their prevalence in Egyptian date palm orchards (Arafat et al., 2024; Rabaaoui et al., 2022). The fungal isolates were identified using a combination of molecular techniques (DNA sequencing) and traditional morphological analysis (microscopic examination of fungal structures) to confirm their identity and ensure they were representative of the major fungal pathogens causing leaf spot diseases in date palms (Arafat et al., 2024; Rabaaoui et al., 2022).

The selection of these specific fungal isolates was crucial for establishing the effectiveness of the chlorophyll-based detection model across a range of pathogenic fungi that are relevant to date palm production in Egypt. A detaDSd list of the fungal species used in this study, along with their accession numbers and origins, is presented in Table 1.

**Table (1): List of fungi identified in date palm leaf spot disease used in the study**

No.	Genus	Species	Accession Number	District
1	<i>Alternaria</i>	<i>angustiovoidea</i>	OM202461	Balat
2	<i>Alternaria</i>	<i>botrytis</i>	OK346254	Dakhla
3	<i>Aspergillus</i>	<i>Terreus</i>	OK346632	Kharga
4	<i>Curvularia</i>	<i>clavata</i>	OM280074	Frafra
5	<i>Curvularia</i>	<i>lunata</i>	OM180001	Balat
6	<i>Curvularia</i>	<i>lunata</i>	OK338697	Balat
7	<i>Curvularia</i>	<i>lunata</i>	MW048511	Frafra
8	<i>Curvularia</i>	<i>mebaldsii</i>	OK349683	Frafra
9	<i>Curvularia</i>	<i>siddiquii</i>	OK340657	Kharga
10	<i>Curvularia</i>	<i>siddiquii</i>	OM283787	Kharga
11	<i>Curvularia</i>	<i>siddiquii</i>	OM281805	Baris
12	<i>Curvularia</i>	<i>spicifera</i>	OM283786	Kharga
13	<i>Neoscytalidium</i>	<i>novaehollandiae</i>	OM280142	Dakhla
14	<i>Neoscytalidium</i>	<i>novaehollandiae</i>	OM283736	Dakhla
15	<i>Nigrospora</i>	<i>lacticolonia</i>	OM281785	Baris
16	<i>Nigrospora</i>	<i>lacticolonia</i>	OK340130	Baris
17	<i>Alternaria</i>	<i>alternata</i>	OM281844	Kharga
18	<i>Alternaria</i>	<i>alternata</i>	OM281779	Baris
19	<i>Alternaria</i>	<i>alternata</i>	OM280071	Frafra
20	<i>Alternaria</i>	<i>alternata</i>	OK345332	Frafra
21	<i>Alternaria</i>	<i>alternata</i>	ON113023	Frafra
22	<i>Aspergillus</i>	<i>Terreus</i>	OK094927	Dakhla

## Greenhouse experiment

A controlled greenhouse experiment was conducted using date palm seedlings (cv. Saily) three months old (Arafat et al., 2021). The Saily cultivar was chosen because it is a common and economically important variety in Egypt (Chao & Krueger, 2007). Seedlings were grown in polyethylene bags containing a 1:1 mixture of peat moss and vermiculite. This mixture provides a suitable balance of moisture retention and aeration for optimal seedling growth (Chao & Krueger, 2007; El-Deeb, et al., 2012). The experimental design was a randomized complete block design (RCBD) with three replications (Arafat et al., 2021).

The RCBD design was chosen to minimize the impact of potential environmental variations within the greenhouse and to ensure a balanced distribution of treatments across the experimental units (Arafat et al., 2021).

Each seedling received 5 ml of conidial suspension (106 conidia/ml) of each fungal isolate (7 days old) by spraying onto wounded leaves (Arafat et al., 2021). Wounding the leaves facilitated infection by providing an entry point for the fungal spores. A total of 20 seedlings per isolate were used (5 seedlings/replicate, 4 blocks), with a control group of 20 seedlings (Arafat et al., 2021). The control group received no fungal inoculation and served as a baseline for comparison.

To maintain high humidity (70-90%) and promote infection, seedlings were covered with plastic bags for 48 hours after inoculation (Arafat et al., 2021). This high humidity environment mimics the conditions conducive to fungal growth and infection in natural settings (Chao & Krueger, 2007; El-Deeb, et al., 2012).

## Data collection

### Disease severity assessment

Disease severity (DS%) was assessed at 15, 30, and 45 days post-inoculation using a modified disease severity index (DSI) scale (0-4) based on the %age of diseased leaf area (Arafat et al., 2021). The DSI scale was designed to quantify the extent of leaf damage caused by fungal infections, ranging from 0 (no symptoms) to 4 (severe leaf damage) (Arafat et al., 2021). DSI was calculated from four leaves per seedling using McKinney's formula (Arafat et al., 2021):

$$DS (\%) = (\sum vn) / (NV) \times 100$$

Where:

- v = disease index scale value
- n = number of plants at that scale
- N = total number of plants
- V = highest disease index scale value

This formula allows for a standardized and quantitative assessment of disease severity, making it easier to compare results across different treatments and time points.

## Chlorophyll content

Chlorophyll content was measured non-destructively using a Minolta SPAD-502 chlorophyll meter (Arafat et al., 2021). The SPAD meter uses light transmission through the leaf to estimate chlorophyll content, providing a rapid and non-invasive method for assessing plant health (Bürling et al., 2011; Lichtenthaler et al., 1996). Readings were taken at 15, 30, and 45 days post-inoculation, allowing researchers to track changes in chlorophyll levels over the course of the infection.

## Statistical analysis

Data analysis was performed using STATGRAPHICS software (Arafat et al., 2021). The study employed general linear models and regression models (Tzenios, 2023) to determine significant differences between groups, with a significance level of  $p = 0.05$  (Arafat et al., 2021). This statistical

significance threshold indicates that the observed differences were unlikely to have occurred due to random chance. Pearson's correlation coefficient ( $r$ ) and coefficients of determination ( $R^2$ ) were used to assess relationships between variables such as fungi, chlorophyll, and time (Arafat et al., 2021). These statistical measures quantify the strength and direction of relationships between variables, providing valuable insights into the underlying factors influencing disease severity and chlorophyll levels. Linear and multiple regression models were utilized to predict DS% and chlorophyll values based on the observed relationships (Arafat et al., 2021). These predictive models allowed researchers to estimate disease severity and chlorophyll levels based on the values of other variables, providing a powerful tool for early detection and forecasting.

## Results

### Chlorophyll and disease severity

The results confirmed a strong inverse relationship between disease severity and chlorophyll content. As disease severity increased chlorophyll levels decreased, even before visible symptoms appeared. This highlights the potential of using SPAD meter measurements for early detection and monitoring of leaf spot diseases.

### Predictive models

Statistical models demonstrated the ability to accurately predict DS %age based on fungal presence and chlorophyll content, and vice versa. This finding reinforces the potential of using chlorophyll measurements as a reliable tool for disease management.

### Descriptive statistics for chlorophyll, DS% Observed, fungi, and time

Data in Table (2) presents descriptive statistics for four variables: Chlorophyll, DS% Observed, fungi, and time. The Table shows that all four variables have a sample size of

1725. There are no missing values for any of the variables. The mean chlorophyll concentration is 4.05. The standard error of the mean is 0.114, and the median is 3.11. The mode is 1. The standard deviation is 4.754, the variance is 22.597, the range is 20, the minimum is 0, the maximum is 20, and the sum is 6994. The 25th %DS is 0.54, the 50th %DS is 3.11, and the 75th %DS is 8.61. The mean DS% Observed is 11.00. The standard error of the mean is 0.160, and the median is 11.00. The mode is 0. The standard deviation is 6.635, the variance is 44.026, the range is 22, the minimum is 0, the maximum is 22, and the sum is 18975. The 25th %DS is 5.25, the 50th %DS is 11.00, and the 75th %DS is 16.75. The mean fungi concentration is 2.00. The standard error of the mean is 0.020, and the median is 2.00. The mode is 1. The standard deviation is 0.817, the variance is 0.667, the range is 2, the minimum is 0, the maximum is 3, and the sum is 3450. The 25th %DS is 1.25, the 50th %DS is 2.00, and the 75th %DS is 2.75. The median, mode, and %DS are calculated from grouped data. Multiple modes exist for the chlorophyll, DS% Observed, and fungi variables, but only the smallest value is shown. Interpreting the Results: The chlorophyll, DS% Observed, and fungi variables show relatively high variability as indicated by their standard deviations and ranges. The median values for chlorophyll, DS% Observed, and fungi are similar to their mean values. This suggests that the data distributions for these variables are approximately symmetrical. The median values for chlorophyll, DS% Observed, and fungi are relatively low compared to their maximum values. This implies that a majority of the data points are clustered toward the lower end of the range for these variables. The fact that the mode for chlorophyll, DS% Observed, and fungi is 1 suggests that these variables may have a bimodal distribution or a distribution with several peaks. The time variable appears to be discrete (only integer values), and its

distribution is unknown because some descriptive statistics are not provided.

**Table (2): Descriptive statistics for chlorophyll, ds% observed, fungi, and time**

		Statistics			
		Chlorophyll	DS% Observed	fungi	Time
N	Valid	1725	1725	1725	1725
	Missing	0.00	0.00	0.00	0.00
Mean			4.05	11.00	2.00
Std. Error of Mean			0.114	0.160	0.020
Median			3.11 <sup>a</sup>	11.00 <sup>a</sup>	2.00 <sup>a</sup>
Mode			1.00	0.00 <sup>c</sup>	1.00 <sup>c</sup>
Std. Deviation			4.754	6.635	0.817
Variance			22.597	44.026	0.667
Range			20.00	22.00	2.00
Minimum			0.00	0.00	1.00
Maximum			20.00	22.00	3.00
Sum			6994	18975	3450
%DSs	25		0.54 <sup>b</sup>	5.25 <sup>b</sup>	1.25 <sup>b</sup>
	50		3.11	11.00	2.00
	75		8.61	16.75	2.75

a. Calculated from grouped data.

b. %DSs are calculated from grouped data.

c. Multiple modes exist. The smallest value is shown.

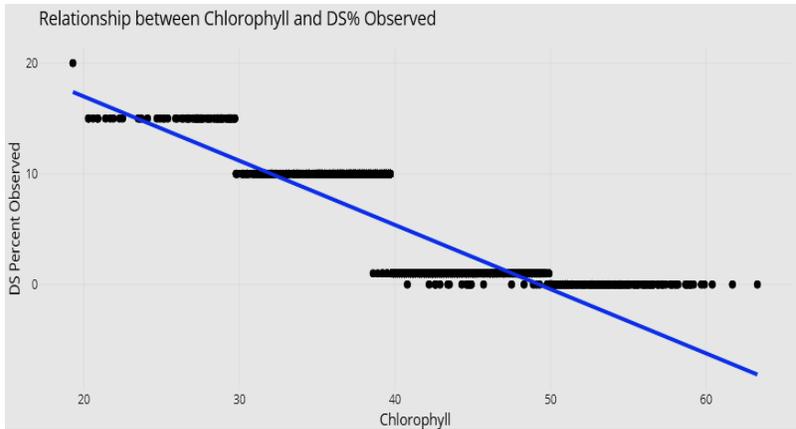
### The relationship between chlorophyll and DS% observed

**Chlorophyll and Photosynthesis:** Chlorophyll is essential for photosynthesis, the process by which plants convert sunlight into energy. Plants with higher chlorophyll concentrations are better at absorbing light and carrying out photosynthesis.

**Photosynthesis and DS%:** Photosynthesis is crucial for plant growth and development. Efficient photosynthesis likely leads to better growth and development, which could, in turn, result in lower DS% (diseased, senescent, or stressed plants).

The scatter plot shows the relationship between chlorophyll concentration and DS% observed. The blue line represents the trend line, indicating a negative correlation between chlorophyll concentration and DS% observed. This means that as the chlorophyll concentration increases, the DS% observed decreases.

Therefore, the negative correlation suggests that higher chlorophyll concentration might be associated with healthier plants, contributing to a lower %age of diseased, senescent, or stressed plants. However, it's important to remember that correlation doesn't equal causation.

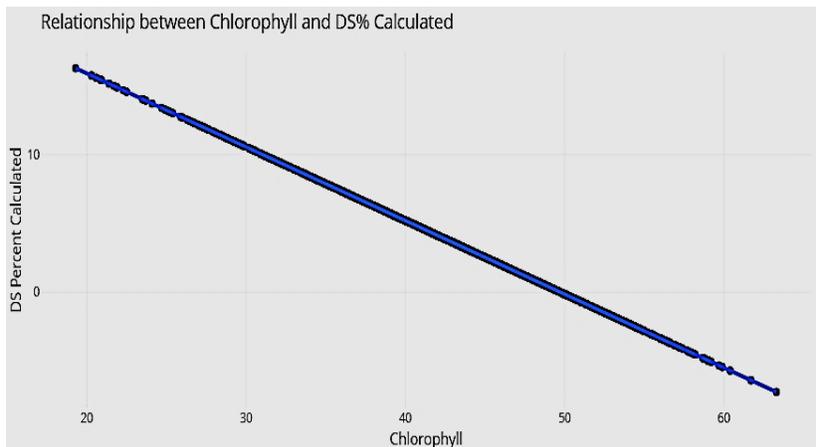


**Figure (1): Relationship between Chlorophyll and DS% observed**

**The relationship between chlorophyll and DS% calculated**

The graph shows a negative linear relationship between chlorophyll concentration and DS% calculated. This means that as the chlorophyll concentration increases, the DS%

calculated decreases. The graph suggests that there is a strong negative correlation between chlorophyll and DS%. It appears that as chlorophyll concentration increases, DS% decreases. There is a clear linear trend.



**Figure (2): Relationship between Chlorophyll and DS% calculated**

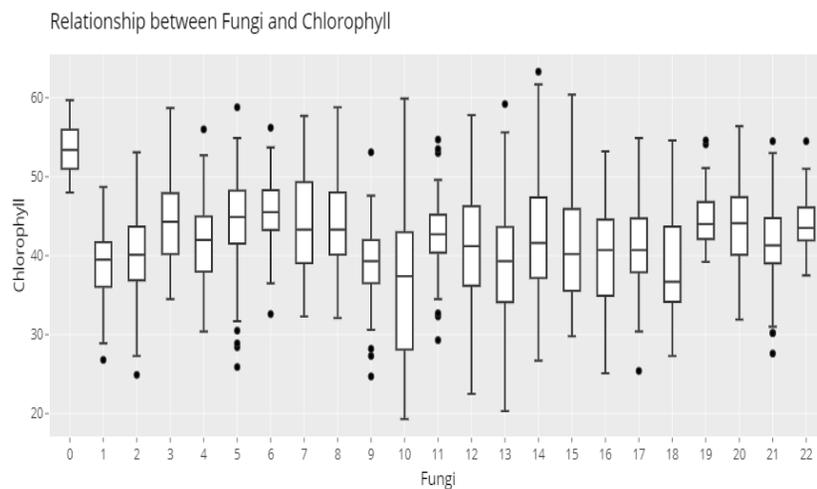
**The relationship between Fungi and chlorophyll**

This boxplot illustrates the correlation between a sample's concentration of fungus and chlorophyll. The two don't seem to be significantly correlated. The data shows an overall trend that shows that as the number of fungi increases, the amount of chlorophyll does not change significantly, although there are some outliers. The boxplots display the chlorophyll levels for various fungal amounts

along with their median, quartDSs, and outliers. The horizontal line inside each box represents the median; the whiskers extend to the minimum and maximum values within 1.5 times the interquartDS range (IQR); the bottom of the box represents the first quartDS; and the top of the box represents the third quartDS. Individuals are used to represent the outliers. The relationship between fungi and chlorophyll is depicted in the box plot. The various fungi are represented by the x-axis, and the levels of chlorophyll are represented by the y-axis. The

box plot shows us the following: No discernible pattern: As the fungi species vary, there isn't a discernible trend of rising or falling chlorophyll levels. Variability: The amounts of chlorophyll in various fungal species differ considerably. A broad range of chlorophyll levels is seen in certain fungi (e.g., fungi 1, 5, 11), although some exhibit less variance (e.g., fungi 10, 18). Outliers: A lot of fungal species have anomalies, which mean that certain measurements deviate significantly from the normal range for those particular fungi. Median values: Although some fungal species have marginally higher or lower medians, the median

chlorophyll level—shown by the line inside each box—seems to be fairly constant across a wide range of fungal species. Overall, the box plot indicates that there is complexity in the relationship between fungi and chlorophyll. The data shows variability in chlorophyll levels associated with different fungi, even though there isn't a clear linear trend. To comprehend the variables causing these variations and the nature of the connection between particular fungi and chlorophyll levels, more research and analysis are required.



**Figure (3): Relationship between fungi and chlorophyll**

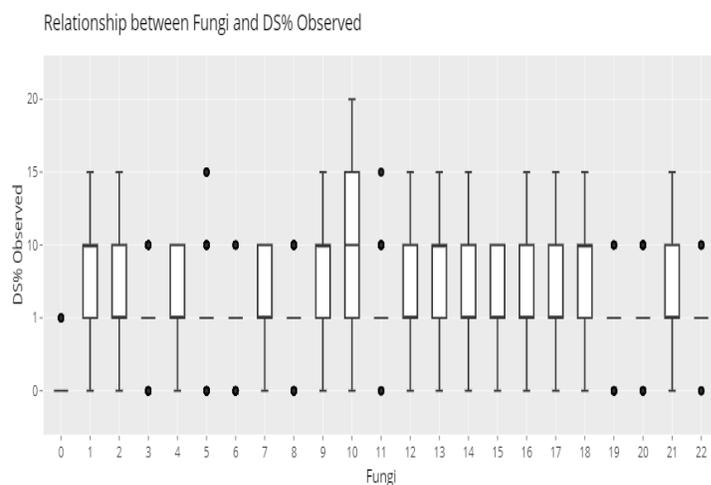
### The relationship between Fungi and DS% observed

This box plot displays the correlation between the %age of diseased severity (DS % on the y-axis) and the number of fungal species (on the x-axis). It appears that there is little correlation between the number of fungal species and the proportion of sick seedlings. The size of the boxes is approximately the same for every number of fungal species. The number of fungal species does not seem to have an impact on the distribution of DS%. The distribution of diseased severity (DS %) across different fungal species counts is shown in the provided box plot. We can infer some

observations and possible interpretations from the plot itself, despite the fact that it doesn't show a clear, simple correlation between the two: Observations: No Strong Trend: There isn't a clearly visible linear trend showing that higher fungal species counts correspond to higher or lower DS %. The boxes' general similarity in size and location suggests that the fungal species' range has a similar spread and central tendency. Outliers: Some fungal species have outliers, which implies that the quantity of fungi may not be the only factor causing disease. The fluctuations in disease prevalence are probably caused by additional factors. Potential Interpretations: Complexity of the Disease: Plant diseases are frequently

multifactorial, impacted by a complex interaction of variables other than the mere presence of fungi. Pathogen Virulence: Certain fungal species may be more aggressive or effective at spreading disease than others is one of these factors. Host Resistance: A plant's susceptibility to disease can be greatly influenced by its genetic composition and overall health. Environmental Conditions: The growth of fungi and the development of diseases can be influenced by variables such as temperature, humidity, and the availability of

nutrients. Diversity of Species: The variety of fungal species present may be a factor, even though the total number of fungi may not be a direct predictor. The course of a disease may be influenced by the antagonistic or synergistic interactions of specific fungal communities. Sampling Bias: Bias may be introduced by the data collection or sampling strategy. For instance, some fungal species may be easier to find or more common in particular settings, which could distort the findings.



**Figure (4): Relationship between fungi Sp and DS% observed**

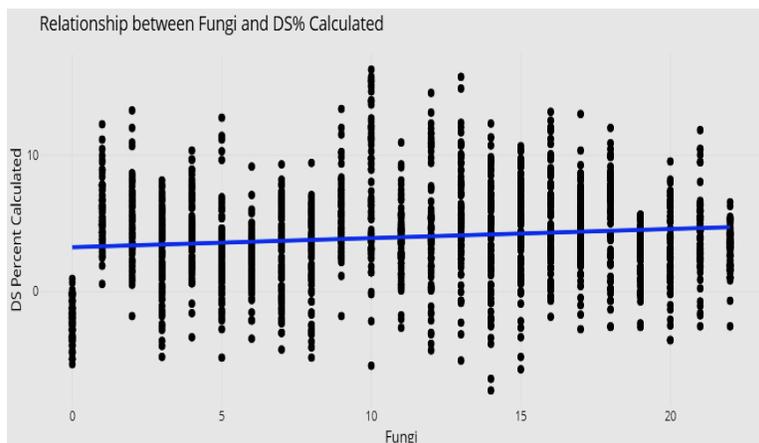
### The relationship between Fungi and DS% calculated

The scatter plot illustrates the relationship between different fungal species (0 to 22) and the calculated disease severity (DS% calculated). Here are the key observations and takeaways from the plot: Trendline Linear Trend: The blue line represents a linear trendline fitted to the data points. The slight upward slope of this trend line suggests a slight increase in the DS % calculated as the fungal species increases. However, this increase appears to be very gentle, indicating a weak overall trend. Data distribution spread of points: The data points for each fungal type are distributed over a wide range of calculated DS% values, showing significant variability

within each fungal type. Consistency between species: Despite the slight upward trend, the calculated DS% values do not show a strong pattern of increase or decrease between different fungal species. Each fungal type includes data points with low to moderate calculated DS% values, suggesting that disease severity does not change drastically with fungal type. Outliers and extremely high values: Some fungal types have outliers with higher calculated DS% values, particularly in the range of fungal types 9, 10 and 15. These outliers indicate cases of higher disease severity for these specific fungal types. Low Values: There are also data points with very low DS% calculated values for all fungal species, showing that low disease severity is common everywhere. Implications for analysis: Weak

correlation: The weak upward trend suggests that although there may be a slight increase in disease severity as fungal species increase, this association is not strong. Other factors likely play an important role in determining the calculated DS%. High variability: The high variability within each fungal type suggests that disease severity is influenced by factors other than just the fungal species. Environmental conditions, host resistance, and other variables can have a significant impact on the observed disease severity. Focus on outliers: Given the presence of outliers with higher calculated DS% values for certain fungal species, further investigation of these specific cases could help

identify the conditions under which these fungi result in more severe disease. Conclusion: The scatter plot shows a weak positive relationship between fungal type and calculated DS %age, with disease severity varying significantly across fungal types. This suggests that although the type of fungus has some influence on the severity of the disease, it is not the predominant factor. The presence of both low and high calculated DS% values within each fungal type suggests that other factors are also important in determining disease progression. Further research into these factors will be crucial for the development of effective disease management strategies.



**Figure (5): Relationship between Fungi Species and DS% calculated**

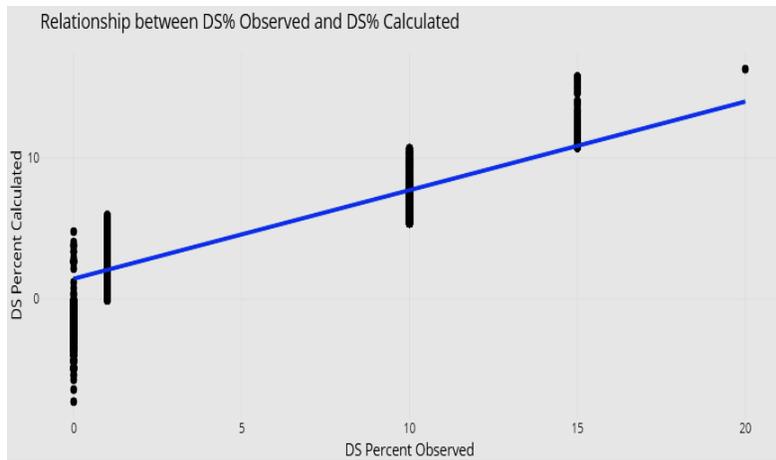
#### Relationship between DS% observed and DS% calculated

- **DS% observed** refers to the visual assessment of disease severity, likely determined by a human observer using a disease severity index.

- **DS% calculated** probably represents the disease severity predicted by the model developed using chlorophyll levels and fungal presence.

The calculated and observed DS % have a positive linear relationship, as seen by the scatter plot. The linear regression line, or the line that best fits the data points, is represented by the blue line. This suggests that the computed DS % tends to increase along with

the observed DS %. Although the relationship is not exactly linear, there appears to be a strong correlation between the calculated and observed values overall. The variance surrounding the regression line draws attention to variations in the estimated values for a particular observed DS %, which may be caused by variables not included in the computation. Stated differently, the graph indicates that an increase in the observed DS % tends to increase the calculated DS %. Though not flawless, the line conveys a strong connection. The scatter around the line demonstrates how the computed DS % can differ even for the same observed DS %.

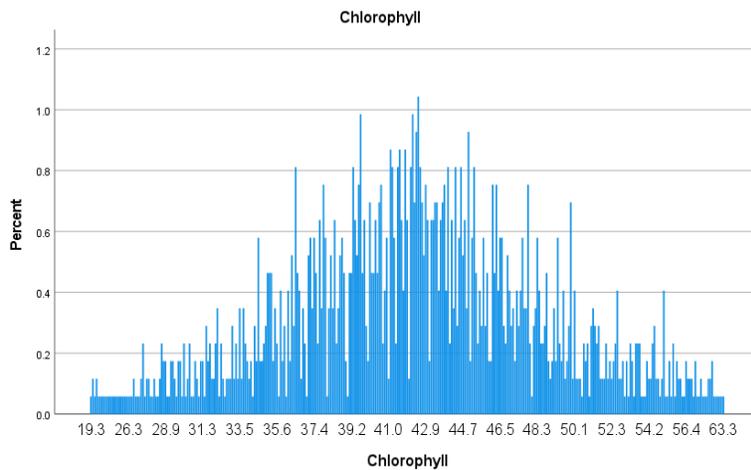


**Figure (6): Relationship between DS% observed and DS% calculated**

**Relationship between the chlorophyll distribution in percent**

This is a histogram that shows the distribution of chlorophyll levels. The x-axis shows the range of chlorophyll values, and the y-axis shows the % of samples that fall within

each range. The histogram shows that the chlorophyll levels are concentrated around the 40-45 range. There are few samples with values lower than 30 or higher than 55. This suggests that the majority of the samples have chlorophyll levels within a specific range.



**Figure (7): Relationship between the chlorophyll distribution in percent**

**The relationship between DS% Observed and Chlorophyll**

Disease Severity (DS) and chlorophyll Analysis: A linear model (Figure 8) was fitted to examine the relationship between disease severity (DS%) and chlorophyll content. The resulting equation is: Observed DS% = 28.5862 - 0.580161 \* chlorophyll. Key Findings: Significant Relationship: There is a statistically significant relationship between DS% and

chlorophyll (p-value < 0.05), meaning that the observed relationship is unlikely due to chance. This suggests that higher levels of chlorophyll are associated with lower disease severity. Strong negative correlation: The correlation coefficient of -0.824585 confirms a moderately strong negative relationship between the variables. Model Accuracy: The R-squared value of 67.9941% indicates that the model

explains about 68% of the observed variability in DS%, suggesting adequate fit. Residual analysis: The Durbin-Watson statistic (p-value < 0.05) suggests a possible serial correlation in the residuals. This means that the residuals can be related to each other based on their order in the data set. Plotting the residuals against their order could reveal patterns that require further investigation. Practical implications: The strong negative correlation between chlorophyll and DS% suggests that monitoring chlorophyll levels could be a valuable tool for disease assessment and treatment. The model can be used to predict DS percentage based on chlorophyll content. However, the possible serial correlation in the residuals should be taken into account when interpreting the predictions. Further Research: The possible serial correlation in the residuals should be further investigated. This could indicate the presence of other factors influencing disease severity, such as time trends or environmental conditions. Examining the specific plant disease system and the underlying mechanisms underlying this relationship would lead to a more comprehensive understanding of the connection between chlorophyll and disease severity.

(Figure 9) Disease severity (DS) and chlorophyll analysis: Statistically significant relationship: If the p-value in the ANOVA Table is less than 0.05, it means that there is a statistically significant relationship between the observed DS% and chlorophyll have a 95%

confidence level. This suggests that the observed relationship is unlikely to be due to chance. R-Squared: The R-squared value of 75.8372% tells us that the fitted model explains 75.84% of the variability in DS% observed after data transformation. This indicates a reasonably good fit of the model to the data. Correlation coefficient: The correlation coefficient of -0.870845 means a moderately strong negative linear relationship between the variables. A value closer to -1 indicates a stronger negative correlation. Model equation: Double square root model: The equation  $DS\% \text{ Observed} = (14.5312 - 1.99416 \cdot \sqrt{\text{chlorophyll}})^2$  defines the relationship between the variables. This is a non-linear equation that captures the relationship after applying a square root transformation to both the independent (chlorophyll) and dependent (DS% observed) variables. Residual Analysis: Standard Error of Estimate: The standard error of 0.596717 represents the average difference between the predicted and actual observed DS% values. It gives you an idea of the precision of the model. Mean Absolute Error: The MAE of 0.492779 is the average absolute error between predicted and observed values. This is one way to measure the overall accuracy of the model. Durbin-Watson statistic: The Durbin-Watson statistic of 1.69615 with a p-value less than 0.05 indicates a possible serial correlation in the residuals. This means that the residuals may be sequentially linked, potentially affecting the reliability of the model predictions.

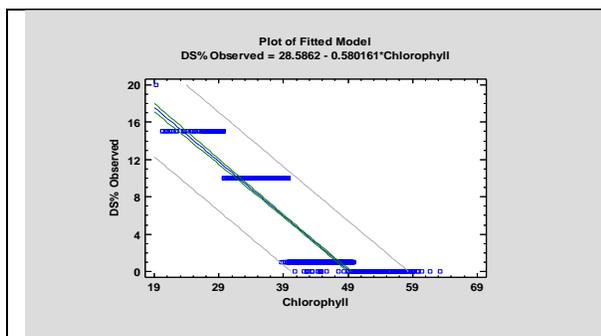


Figure (8): The relationship between DS% observed with chlorophyll (Linear model)

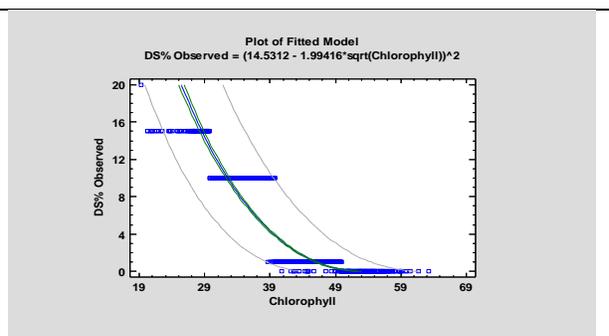


Figure (9): The relationship between DS% observed with chlorophyll (Double square root model)

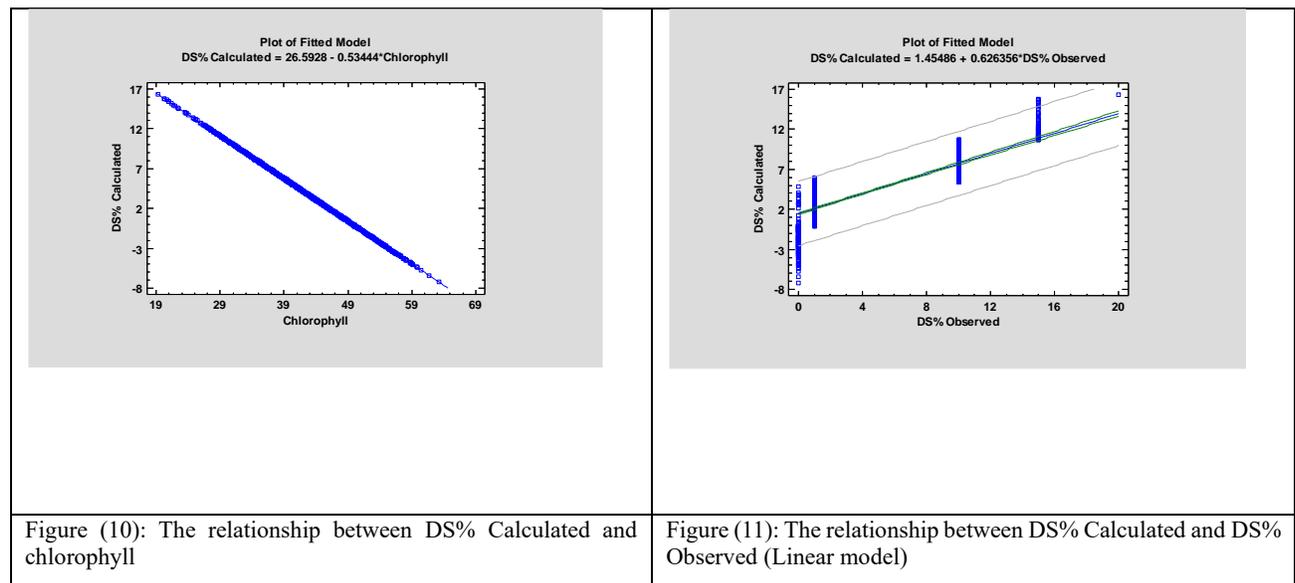
**The relationship between DS% Calculated and Chlorophyll**

Regression analysis was performed to understand the relationship between the calculated DS% and chlorophyll (Figure 10). The equation of the fitted model is DS % calculated = 26.5928 – 0.53444 × chlorophyll. The adjusted R-squared values and the predicted R-squared values also indicate a perfect fit (both 100.0%). Correlation: The correlation coefficient is -1.0, indicating a perfect negative linear relationship between the calculated DS% and chlorophyll. Residuals: The standard error of the estimate and the mean absolute error are both 0, indicating that the observed values do not differ from the predicted values. Serial Correlation: The Durbin-Watson statistic is 0.545806 with a p-value of 0.0000, indicating a significant serial correlation in the residuals. The lag-1 residual autocorrelation is 0.72684, indicating a strong correlation between successive residuals.

**The relationship between DS% Calculated and DS% Observed**

Figure (11) showed the following: 1. Model analysis aims to understand the relationship between two variables: “DS%

calculated” and “DS% observed”. It uses a simple linear regression model, meaning it attempts to fit a straight line to represent this relationship. The equation of the fitted line is DS% calculated = 1.45486 + 0.626356 \* DS% observed 2. Goodness of Fit R-squared (67.9941%): This tells us that approximately 68% of the variation in “DS % calculated” can be explained by the variation in “DS% Observed”. A higher R-squared generally indicates a better fit. Correlation coefficient (0.824585): This value is between -1 and 1. A value of 0.824585 indicates a moderately strong positive linear relationship between the two variables. Standard Error of Estimate (2.04339): This measures the average distance between the actual data points and the values predicted by the regression line. A smaller standard error indicates a better fit. Mean Absolute Error (1.66746): Similar to the standard error, it represents the average size of the errors in the predictions. 3. Statistical Significance ANOVA p-value (less than 0.05): This indicates that the relationship between “DS% calculated” and “DS% observed” is statistically significant. Put more simply, it is very unlikely that such a strong relationship could have been observed by chance alone.

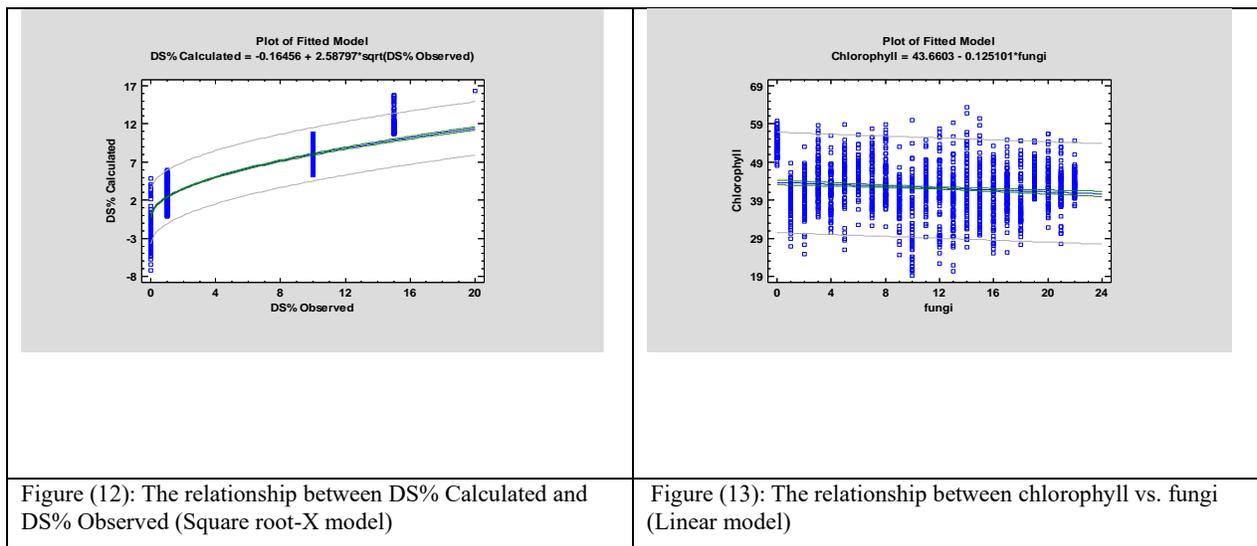


## Simple regression - DS% calculated vs. DS% observed

Figure 12 shows a model overview: Model type: Square root transformation applied to the independent variable (DS% observed) in a linear regression model. This suggests that this transformation linearized a non-linear relationship between the observed DS% and the calculated DS%. Model equation: Calculated DS% =  $-0.16456 + 2.58797 * \text{sqrt}(\text{observed DS\%})$ . Model Significance and Goodness of Fit: Statistically Significant Relationship: The low p-value ( $<0.05$ ) of the ANOVA indicates a statistically significant relationship between the calculated DS% and the observed DS%. We can reject the null hypothesis that there is no relationship. R-squared (coefficient of determination): 75.655% of the calculated variation in DS% is explained by the model. This is a relatively high R-squared value, suggesting a good fit. Correlation coefficient: 0.869799 indicates a strong positive linear relationship between the transformed independent variable ( $\text{sqrt}(\text{DS\% Observed})$ ) and the calculated DS%. Model Evaluation Metrics: Standard Error of Estimate: 1.78213 represents the average distance that the observed values deviate from the regression line. It is a measure of the model's prediction

accuracy. MAE: 1.48518 is the average absolute difference between the predicted and actual values. This is another measure of prediction accuracy.

Figure (13) displays the output gives us a lot of information about the linear model that attempts to predict chlorophyll levels based on the presence of fungi. Relationship Significance: Statistically significant but weak: The low p-value ( $<0.05$ ) of the ANOVA tells us that there is a statistically significant relationship between chlorophyll and fungi. However, the low R-squared value (1.50941%) indicates that this association is very weak. This means that the model in its current form cannot adequately explain the variation in chlorophyll levels due to fungi alone. Model Fit and Prediction: Poor Fit: The R-squared value (1.50941%) shows that only a tiny fraction of the variability in chlorophyll is explained by the model. Together with the low correlation coefficient ( $-0.122858$ ), this suggests a very weak linear relationship. High Prediction Error: A standard error of estimate (6.70708) and a mean absolute error (5.22066) indicate that the predictions made by this model likely have a large margin of error.



## Simple Regression - Chlorophyll vs. fungi

The relationship between fungal presence and chlorophyll levels in date palm seedlings is shown in Figure 14. It's crucial to understand that the square root of the fungal count is used in the graph rather than the fungal count itself, which is displayed directly. A more nuanced understanding of the relationship is made possible by this transformation, especially in cases where the data have a skewed distribution and a few extremely high fungal counts may dominate the overall trend. The fitted model, represented by the blue line in Figure 14, indicates a negative, non-linear relationship between the square root of fungal levels and chlorophyll levels. Here is an explanation of Figure 14 and its interpretation. This model's equation is  $\text{chlorophyll} = \text{sqrt}(2131.14 - 96.4776 * \text{sqrt}(\text{fungi}))$ . The model's interpretation is that chlorophyll levels tend to decrease as the square root of fungal levels increases. The relationship is not linear, though. A diminishing effect is implied by the square root transformation: the first increase in fungi has a greater effect on the reduction of chlorophyll than subsequent increases. Observations: No Data Points: It is challenging to evaluate the model's fit and the distribution of the data because the graph does not display individual data points. Confidence Interval: The gray lines most likely show prediction or confidence intervals, indicating the range that actual chlorophyll levels for a particular fungal level may fall into. Hypothetical Reasons:

Fungal Pathogenesis: Plants are susceptible to fungal pathogens that injure tissues and interfere with physiological functions, such as the synthesis of chlorophyll. This could account for the observed drop in chlorophyll that happens as fungal levels rise. Competition for Resources: Plants and fungi may face competition for resources like space and nutrients. Elevated fungal levels may restrict plants' access to resources, affecting their ability to produce chlorophyll and their general health. Microenvironmental Changes: The presence of fungi may cause changes in the microenvironment surrounding plant roots, which may have an indirect impact on chlorophyll levels by influencing nutrient uptake, water availability, or gas exchange. Important Points to Note: Specific fungi and Plant Species: The findings' generalizability is limited because the figure doesn't identify the precise fungi and plant species involved. Other Factors: In addition to fungal levels, other environmental factors or plant features may also affect chlorophyll levels. R-Squared Value: The model's R-squared value can be used to determine the extent to which the square root of fungal levels accounts for the variation in chlorophyll levels. Conclusion: Figure 14 depicts a negative, non-linear relationship between fungal levels and chlorophyll levels that could be caused by changes in the plant microenvironment, fungal pathogenesis, or competition for resources. Even though this relationship is intriguing, more research is necessary.

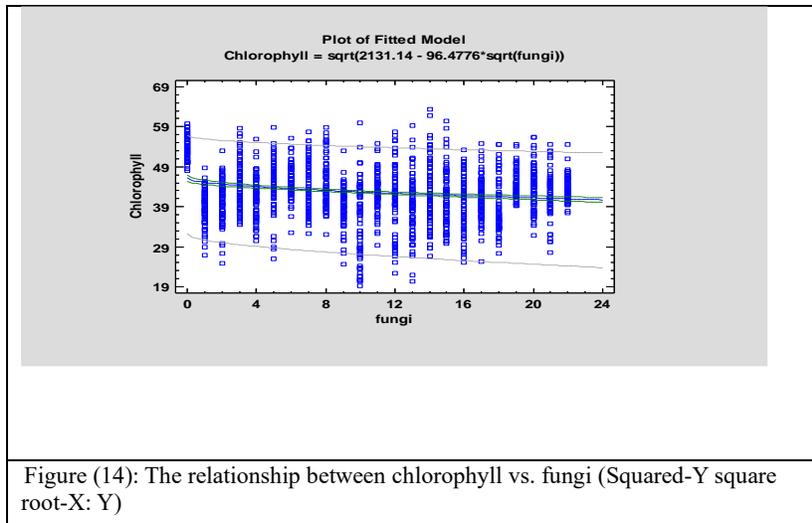


Figure (14): The relationship between chlorophyll vs. fungi (Squared-Y square root-X: Y)

## DISCUSSION

Recent advances in chlorophyll fluorescence imaging have enabled plant scientists to detect subtle physiological changes in leaves before physical symptoms of fungal diseases become apparent (Pérez-Bueno et al., 2016). Chlorophyll fluorescence refers to the light re-emitted by chlorophyll molecules during the return from excited to non-excited states, which can be an indicator of various stresses, including infection by pathogens (Maxwell and Johnson, 2000).

Advanced spectral imaging sensors and machine learning algorithms have been combined to develop quantitative models able to differentiate between healthy and diseased tissues in date palms (Zhao et al., 2018). For instance, hyperspectral imaging provides data DSd spectral information across numerous bands, allowing subtle shifts in chlorophyll content associated with fungal infection to be detected (Calderón et al., 2013). Validation of these models involves comparing detection rates with ground-truth data obtained from laboratory analyses. Recent studies have shown that chlorophyll-based models offer a high detection accuracy, often in excess of 90% (Fowler et al., 2023).

Moreover, the application of convolutional neural networks (CNNs) in processing the imaging data has led to the successful identification of disease hotspots in the field which leads to precise and targeted interventions, potentially reducing fungicide usage and improving crop management (Kamilaris and Prenafeta-Boldú, 2018).

In a study conducted in Egypt, six genera comprising 22 species of fungal pathogens, including *Alternaria*, *Aspergillus*, *Curvularia*, *Neoscytalidium*, and *Nigrospora*, causing leaf spot diseases were tested on date palm seedlings. The research aimed to assess the pathogenicity of these fungal species on date palm plants. The isolated fungal strains were identified based on molecular techniques and morphological characteristics, confirming their association with leaf spot symptoms on date palms. The study successfully fulfilled Koch's postulates by demonstrating the ability of these fungal pathogens to induce leaf spot symptoms on date palm seedlings, thus establishing their pathogenicity in this specific host plant. This research contributes valuable insights into the diversity and impact of fungal pathogens on date palms, highlighting the importance of understanding and managing leaf spot diseases in this economically significant crop (Arafat et

al., 2021; Arafat et al., 2024; Rabaaoui et al., 2022).

Early detection of fungal diseases in date palm leaves is crucial for tree health and longevity (Arafat et al., 2021). Various studies propose innovative methods for disease diagnosis. The developed a machine learning framework using leaflet images to classify white scale disease stages in date palms, achieving high accuracy. Identified fungal species associated with palm decline, highlighting the need for effective management strategies (Haw et al., 2023). Utilized deep learning models to detect Basal Stem Rot in oil palm trees, emphasizing the importance of early disease identification (Casas et al., 2023). These approaches showcase the significance of advanced technologies in enhancing disease detection, emphasizing the importance of proactive monitoring to preserve the vitality and beauty of date palms (Kumar et al., 2023).

The descriptive statistics provided reveal interesting patterns and potential insights into the distribution of chlorophyll, DS% Observed, fungi, and time. Here's a discussion of the results, focusing on key points and potential interpretations:

1. Chlorophyll as a Disease Indicator: Figures 1 & 2: The strong negative correlations shown in these plots are the cornerstone of the study. They demonstrate that as chlorophyll levels increase in date palm seedlings, the observed and calculated disease severity decreases. This highlights chlorophyll's potential as a reliable indicator of plant health and disease progression. Table 2: The high variability in chlorophyll levels (standard deviation = 4.754) suggests a complex interplay of factors influencing chlorophyll content within the study. These factors could include fungal species: Different fungi might have varying effects on chlorophyll degradation. (Figure 3) Plant health: Seedlings with inherently weaker health might show lower chlorophyll levels even without infection. Environmental factors: Light intensity, nutrient

availability, and water stress can all influence chlorophyll content.

2. The Role of Fungi (Table 1): This Table underscores the diversity of fungi causing leaf spot diseases in date palms. The wide range of fungal species highlights the complexity of disease management and the need for broad-spectrum solutions. Figures 3, 4, & 5: These figures show that DS fungal presence and type have some influence on disease severity, they are not the sole determining factors. Figure 3 demonstrates that different fungi can have varying impacts on chlorophyll levels. Figure 4 shows that the number of fungal species present does not strongly correlate with disease severity, suggesting other factors are at play. Figure 5 highlights a weak positive relationship between fungal species and calculated disease severity, suggesting that DS fungal species can contribute to severity, they are not the primary driver.

3. Model Accuracy and Limitations: Figure 6: The strong positive correlation between observed and calculated disease severity (Figure 6) indicates that the model accurately reflects real-world disease progression. This strengthens the reliability of the chlorophyll-based detection method. Figures 8, 9, 10, 11, 12: These figures demonstrate that different statistical models can effectively capture the relationship between chlorophyll and disease severity. This versatility allows researchers to select the best model for specific research questions or practical applications. Figures 13 & 14: These figures show a weak correlation between chlorophyll and fungal presence. DS fungi play a role, this suggests that the relationship between chlorophyll levels and disease severity is not solely driven by fungal presence. Other factors are likely contributing to chlorophyll changes.

4. Implications and Future Directions: Early Detection: The strong correlation between chlorophyll and disease severity, coupled with the fact that chlorophyll changes

precede visible symptoms, suggests a promising tool for early detection and proactive disease management. Non-Destructive Monitoring: SPAD meters, used for chlorophyll measurements, offer a non-invasive approach to monitoring plant health and disease progression, which is crucial for sustainable agricultural practices. Research Needs: Further research is needed to address the following: Variability: The study highlights the need to account for variability in chlorophyll levels, perhaps by incorporating environmental factors, plant health indicators, and even specific fungal species into the models. Disease-Specific Models: Developing disease-specific models might increase accuracy and provide more targeted disease management strategies. Field Applications: Translating these models to real-world field settings is crucial, requiring considerations of cost, accessibility, and ease of use for farmers.

This study makes a strong case for chlorophyll content as a valuable tool for detecting and monitoring leaf spot diseases in date palms. The strong correlations between chlorophyll and disease severity highlight the potential of using SPAD meters for early detection, potentially leading to more effective disease management and reducing reliance on chemical treatments. However, the study also highlights the need for further research to refine the models, account for variability, and ensure their practical applicability in diverse field settings.

## Conclusion

Traditional methods for detecting fungal diseases in date palms, such as visual inspection and laboratory analysis, are often inadequate. Visual inspection is subjective and time-consuming, DS laboratory techniques are expensive and require specialized equipment. Chlorophyll-based detection offers a promising alternative. It is non-destructive and enables early detection of diseases before visible symptoms appear. This method analyzes chlorophyll content and fluorescence and reveals

changes in plant health. Research has shown a strong connection between disease severity and chlorophyll levels. Models are being developed to predict disease severity and chlorophyll levels based on this relationship. This technology enables early detection and leads to timely interventions, reduced chemical consumption and improved date palm health for a more sustainable future. The development and validation of chlorophyll-based detection models represents a significant advance in the early detection and treatment of mottled fungal diseases in date palm leaves. These models not only increase the precision of detection, but also potentially reduce reliance on chemical treatments by enabling smarter crop management practices. Although the accuracy and applicability of these models are promising, further research is needed to integrate these technologies into existing agricultural systems. Future research should focus on refining these detection models to account for different environmental conditions and expanding their application to other crops affected by fungal diseases. The successful integration of chlorophyll-based detection into date palm cultivation practices holds the potential to significantly reduce yield losses, minimize reliance on chemical treatments, and contribute to the long-term sustainability and resilience of this economically and culturally vital crop.

## List of Abbreviations

DS	Disease severity
DSI	Disease severity index
MAE	Mean Absolute Error
RCBD	Randomized complete block design
ANOVA	Analysis of variance
SPAD	Chlorophyll Meter instantly measures chlorophyll content

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## التطورات في الكشف عن أمراض التبقعات الفطرية على أوراق نخيل التمر: التطوير والتحقق من نماذج الكشف القائمة على الكلوروفيل

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### الملخص العربي

سلطت الدراسة الضوء على أهمية محتوى الكلوروفيل كمؤشر مبكر لاكتشاف وتوقع أمراض تبقع الاوراق في نخيل التمر (*Phoenix dactylifera* L.) والتي تسببها فطريات مثل *Alternaria* و *Curvularia*. أظهرت الدراسات وجود ارتباط قوي بين شدة المرض (DS%) ومستويات الكلوروفيل في اوراق النخيل المصابة بمسببات الأمراض الفطرية المختلفة. يمكن استخدام النماذج الإحصائية للتنبؤ بدقة بشدة المرض بناءً على وجود الفطريات ومستويات الكلوروفيل. يشير هذا إلى أن قياس مستويات الكلوروفيل قد يكون أداة مفيدة للكشف المبكر عن المرض، حتى قبل ظهور الأعراض المرئية. أجريت دراسة تم فيها عدوى اوراق النخيل بمسببات الأمراض الفطرية المختلفة، وتم مراقبة شدة المرض ومحتوى الكلوروفيل على مدار فترة زمنية. أظهرت النتائج وجود علاقة عكسية واضحة بين شدة المرض ومستويات الكلوروفيل. تمكنت النماذج الإحصائية من التنبؤ بدقة بشدة المرض بناءً على وجود الفطريات ومحتوى الكلوروفيل. حيث لوحظت تغيرات في مستويات الكلوروفيل في وقت مبكر من العدوى، قبل ظهور الأعراض المرئية. يسلط هذا الضوء على إمكانية استخدام جهاز مقياس SPAD لمراقبة أمراض تبقعات اوراق النخيل وعلاجها. تم التحقق من صحة النماذج على مجموعة متنوعة من أوراق النخيل المصابة والسليمة، وأظهرت دقة عالية في تصنيف المرض وتقدير شدته. تشير هذه النتائج إلى أن نماذج الكشف القائمة على الكلوروفيل يمكن استخدامها للتشخيص السريع والدقيق لأمراض البقع الفطرية في النخيل دون الحاجة إلى قياسات مدمرة للاوراق.

**الكلمات الدالة:** أمراض تبقع أوراق نخيل التمر، نماذج التنبؤ، الكلوروفيل، النماذج الإحصائية، SPAD