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Assessment of Red Tip Disease in Pineapple (*Ananas comosus*) on Peat Soil Using NDVI

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Abstract: Red tip disease has emerged as a significant constraint in pineapple production on peat soils in Johor, Malaysia. Currently, detection relies on destructive diagnostic techniques such as RNA extraction. This study aimed to evaluate the suitability of a handheld Greenseeker® sensor for detecting red tip disease in pineapple by analyzing the Normalized Difference Vegetation Index (NDVI). Field experiments were conducted at Peninsular Pineapple Sdn. Bhd. (PPSB), Simpang Renggam, involving 7- and 11-month-old SR36 pineapple plots. NDVI readings and disease severity (%DS) were recorded systematically. Regression analysis revealed strong negative correlations between NDVI values and disease severity, with R^2 values of 0.68 (7-month) and 0.75 (11-month). Laboratory analyses confirmed phytoplasma presence in symptomatic plants using nested PCR. The study concludes that NDVI readings from Greenseeker® offer a reliable, non-destructive method to identify early-stage red tip disease in pineapple cultivation.

Keywords: Pineapple, NDVI, Red tip disease, Greenseeker, Remote sensing, Precision agriculture precision

1. Introduction

Pineapple (*Ananas comosus*), a member of the Bromeliaceae family, is a significant tropical fruit crop with considerable global demand. It ranks third in worldwide tropical fruit consumption after bananas and mangoes (FAO, 2021). Malaysia is among the leading producers of pineapple in Southeast Asia, with Johor being the primary state of cultivation due to its vast peat soil areas, which are particularly suitable for the Gandul variety commonly used in the canning industry (Malaysian Pineapple Industry Board [MPIB], 2020). However, the productivity of pineapple has seen a notable decline in recent years, largely due to biotic stresses including the emergence of red tip disease (Nik Masdek et al., 2005; Ismail et al., 2006).

Red tip disease manifests with distinctive symptoms such as reddening of leaf tips, downward curling, leaf tip dieback, and reduced fruit size. These symptoms often appear six months after planting and lead to substantial economic losses in large-scale plantations (Vijiandran et al., 2001). Unlike mealybug wilt of pineapple (MWP), which has been associated with viral pathogens transmitted by *Dysmicoccus* spp. (Sether & Hu, 2000), the causal agent of red tip remains unclear. Although nematodes, particularly *Paratylenchus* spp., have been implicated, comprehensive diagnostic confirmation is still lacking (Caswell et al., 1990; Ismail et al., 2006).

The conventional method of red tip disease detection involves destructive laboratory-based techniques such as RNA extraction and polymerase chain reaction (PCR), which are time-consuming, labor-intensive, and not practical for large-scale monitoring. This limitation highlights the need for rapid, non-invasive, and reliable tools for early detection. Remote sensing has emerged as a powerful technique for crop health monitoring and disease detection. Vegetation indices derived from reflectance data, such as the Normalized Difference Vegetation Index (NDVI), offer valuable insights into plant physiological status, chlorophyll content, and stress response (Sellers, 1985; Huete, 1988; Wang et al., 2022).

NDVI is calculated using the difference between near-infrared (NIR) and red reflectance, with healthy vegetation exhibiting high NIR and low red reflectance due to strong chlorophyll absorption and internal leaf structure (Gausman, 1974; Qi et al., 1994). Changes in leaf pigment composition and structure due to stress or disease often result in measurable changes in NDVI, thus making it an effective indicator of plant health (Adams et al., 1999; Chappelle et al., 1984). Recent advances in handheld optical sensors, such as the Greenseeker®, enable rapid, on-the-ground NDVI assessments with high spatial resolution and without damaging plant tissues (Mulla, 2013; Zhang et al., 2021).

Precision agriculture integrates technologies like remote sensing and NDVI-based tools to optimize crop management decisions, reduce yield variability, and enhance disease surveillance (Balasundram et al., 2006; Liu et al.,

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2023). In the context of pineapple cultivation, few studies have assessed the utility of NDVI for disease detection. Therefore, this study aimed to evaluate the potential of a handheld Greenseeker® NDVI sensor to differentiate between healthy and red tip-infected pineapple plants and to develop predictive models that support early and non-destructive diagnosis.

2. Materials and Methods

2.1 Study Area and Plant Material

The study was carried out at Peninsular Pineapple Sdn. Bhd. (PPSB), Simpang Renggam, Johor, Malaysia, covering approximately 2,000 hectares of pineapple cultivation. Four one-hectare plots of SR36 variety pineapples were selected two plots each for 7-month and 11-month plantings. The SR36 cultivar is a hybrid between Gandul and Sarawak types.

2.2 NDVI Data Collection

Systematic sampling was employed by selecting every fifth planting bed, with two random plants per selected bed. A total of 80 plants per plot were evaluated. NDVI readings were collected using the Greenseeker® handheld sensor at a fixed distance of 20 cm from the plant canopy. Each NDVI value was the average of 10 readings per plant.

2.3 Disease Severity Index

Disease severity was calculated by the percentage of symptomatic leaves (reddening, tip dieback, curling) to the total number of leaves per plant. Symptoms were visually assessed and recorded concurrently with NDVI readings.

2.4 Laboratory Confirmation

To validate the presence of the red tip disease causal agent in symptomatic pineapple plants, laboratory-based molecular diagnostics were conducted. Leaf samples were collected from a subset of visually identified symptomatic and asymptomatic plants, cleaned, and preserved at −20 °C prior to analysis. A total of ten samples were processed, comprising eight symptomatic and two asymptomatic plant leaves.

DNA was extracted from the leaf tissues using the Cetyl Trimethyl Ammonium Bromide (CTAB) method, which is widely employed for isolating high-quality nucleic acids from plant tissues, particularly those rich in polysaccharides and polyphenolic compounds (Doyle & Doyle, 1987). The extracted genomic DNA was visualized via gel electrophoresis using 5% polyacrylamide gels stained with silver nitrate. Clear bands of approximately 8 kilobases were observed, confirming successful DNA extraction from both symptomatic and asymptomatic samples.

Subsequently, nested polymerase chain reaction (nested PCR) was employed to detect phytoplasma-like organisms. Nested PCR is a highly sensitive technique that involves two successive rounds of PCR amplification using two sets of primers, which increases specificity and minimizes false negatives (Lee et al., 1993). The universal primer pairs used were P1/P7 for the first round and R16F2n/R16R2 for the second round, which target the 16S rRNA gene of phytoplasmas a conserved region widely used for phytoplasma identification in plant pathology studies (Gundersen & Lee, 1996).

In the second round of amplification, four out of eight symptomatic samples produced visible amplicons of approximately 1,500 base pairs (bp), which is consistent with the expected size of phytoplasma-specific fragments. No amplification was detected in the asymptomatic samples, supporting the hypothesis that the red tip symptoms are associated with a phytoplasma-like organism.

These molecular results suggest a potential phytoplasma involvement in red tip disease expression in pineapple. Although phytoplasmas have not been definitively identified as the sole causal agent, the presence of a 16S rRNA gene fragment in symptomatic plants indicates the likely presence of a phloem-limited pathogen contributing to the observed stress symptoms (Bertaccini & Lee, 2018). Further confirmatory studies such as sequencing and phylogenetic analysis are warranted to characterize the specific pathogen strain.

2.5 Statistical Analysis

Descriptive statistics, correlation, and regression analyses were performed using Statistix 8.1 and Minitab 14. Data normality was assessed via the Shapiro-Wilk test, and outliers were identified using Grubb's test (Moore et al., 1999). Linear regression was used to develop prediction models for %DS based on NDVI.

3. Results and Discussion

3.1 NDVI and Disease Severity Descriptive Statistics

Descriptive analysis was conducted on NDVI values and percentage of disease severity (%DS) collected from 7-month and 11-month-old pineapple plants (Table 1). The mean NDVI for 7-month-old plants was 0.724 (SD = 0.107), and for 11-month-old plants, it was slightly higher at 0.766 (SD = 0.097). In contrast, the mean %DS was 47.2% for 7-month-old plants, indicating higher symptom intensity, while the 11-month-old plants showed a lower %DS at 43.5%.

These findings suggest that younger plants exhibited more visible disease symptoms, potentially due to higher physiological sensitivity during the early vegetative stage. This aligns with research indicating that early-stage plants tend to have higher susceptibility to biotic stresses due to underdeveloped structural and metabolic defense mechanisms (Pieterse et al., 2014; Hassan et al., 2020).

Table 1: Descriptive statistics for NDVI and percentage of disease severity (%DS) for 7-month and 11-month-old pineapple plants.

Plant Age	Variable	Mean	Std. Dev.	Min	Median	Max	CV (%)
7-month	NDVI	0.713808	0.047874	0.589013	0.713652	0.812614	6.706868
7-month	%DS	47.08637	9.176112	28.01229	47.84397	71.83242	19.48783
11-month	NDVI	0.77512	0.041187	0.688994	0.777193	0.924109	5.313675
11-month	%DS	43.99726	8.674603	14.32859	43.90863	62.6973	19.71623

3.2 Distribution Analysis and Data Normality

Histograms plotted for NDVI and %DS datasets confirmed normal distribution after outlier removal (Figure 1). The data's adherence to normality assumptions validated the use of parametric statistical analyses for correlation and regression. Normal distribution in NDVI datasets reflects consistent sensor readings across sampled plants, reinforcing the reliability of the Greenseeker® in heterogeneous field conditions.

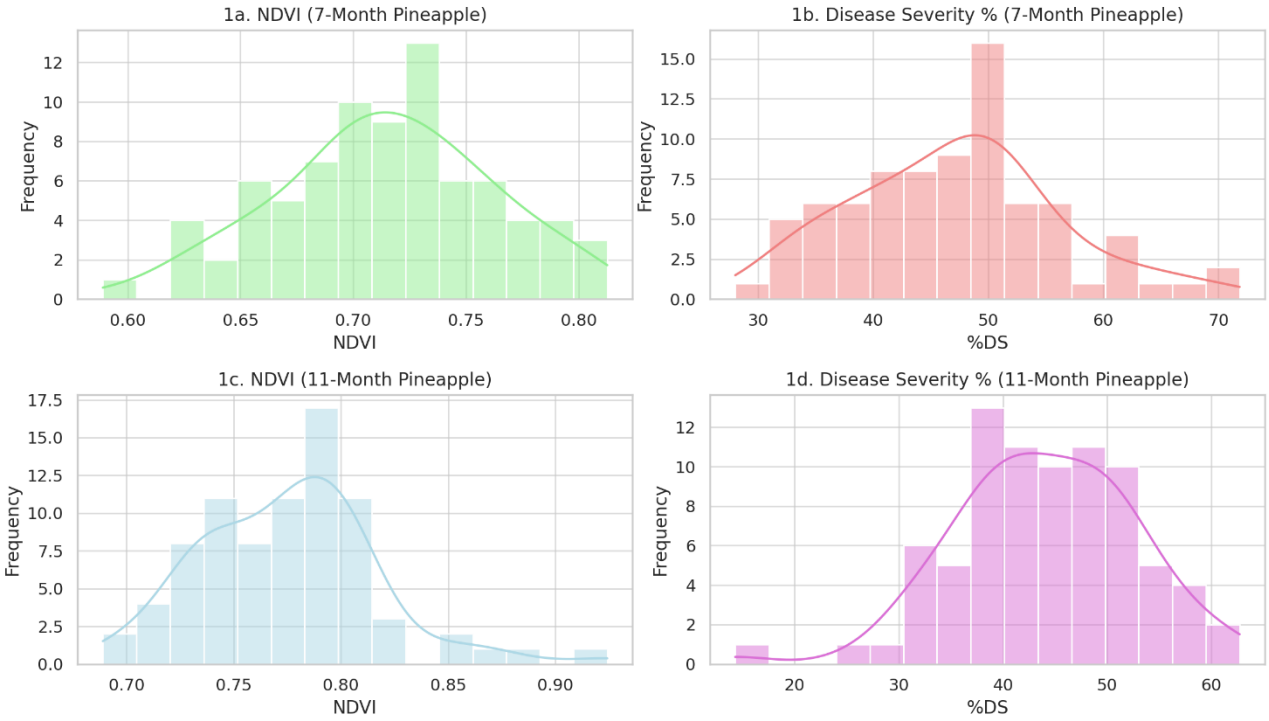


Fig. 1: Distribution of NDVI and disease severity (% DS)

3.3 Correlation Between NDVI and Disease Severity

Pearson correlation analysis revealed a strong negative correlation between NDVI values and %DS in both plots. For 7-month-old plants, the correlation coefficient was $r = -0.825$ ($p < 0.001$), and for 11-month-old plants, it was even stronger at $r = -0.876$ ($p < 0.001$). These values indicate that as disease severity increases, NDVI values decline significantly (Table 2).

Table 2: Correlation between NDVI and %DS

PLOT	R-VALUE	P-VALUE
PLOT 7	-0.825	0
PLOT 11	-0.876	0

This inverse relationship corroborates previous studies where NDVI was shown to decrease under biotic stress due to chlorophyll degradation, reduced photosynthetic capacity, and disrupted canopy structure (Sellers, 1985; Gitelson et al., 2003; Wang et al., 2022). Specifically, red tip disease appears to impair photosynthetic machinery, resulting in altered reflectance properties, particularly in the red and NIR spectra, which are key to NDVI computation.

3.4 Regression Modeling for NDVI–Disease Relationship

Linear regression models were constructed to quantify the relationship between NDVI and disease severity (Figure 2 and 3). The regression equations were:

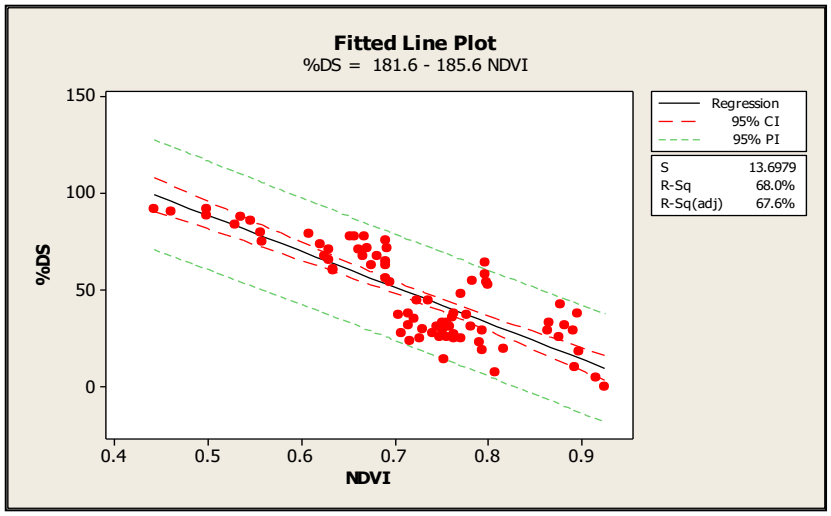


Fig 2: Regression of % DS vs NDVI (7-month pineapple)

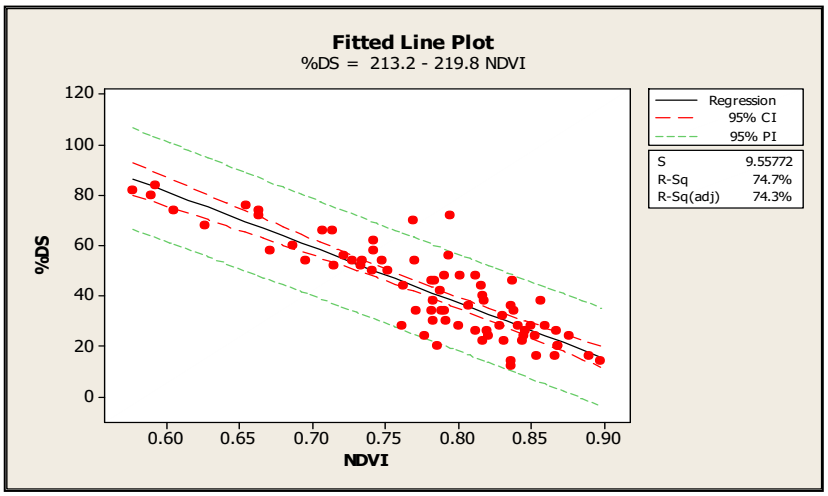


Fig 3: Regression of % DS vs NDVI (11-month pineapple)

Both models were statistically significant ($p < 0.001$) and showed high coefficients of determination, indicating that 68% and 75% of the variability in disease severity could be explained by NDVI readings for 7- and 11-month-old plants, respectively. The higher R^2 in older plants may reflect more stable physiological and structural traits that enhance spectral detection of disease-related changes.

This modeling approach is consistent with findings in precision agriculture where NDVI-based regression models have been successfully used for predicting disease severity in wheat rust, tomato late blight, and rice blast (Zhang et al., 2021; Aravind et al., 2022; Liu et al., 2023). The effectiveness of NDVI as a predictor reinforces its utility as a non-destructive, rapid field assessment tool for disease surveillance.

3.5 Validation of Predictive Models

To evaluate the accuracy of the regression models, predicted %DS values were plotted against observed values. Both models exhibited strong linear fits (R^2 = 0.71 for Model 1 and R^2 = 0.84 for Model 2), indicating a good agreement between measured and estimated disease severity (Figure 4). The tighter confidence and prediction intervals in Model 2 further support the robustness of NDVI readings in more mature plants, where vegetative structure is better developed and more responsive to spectral reflectance-based monitoring (Mulla, 2013).

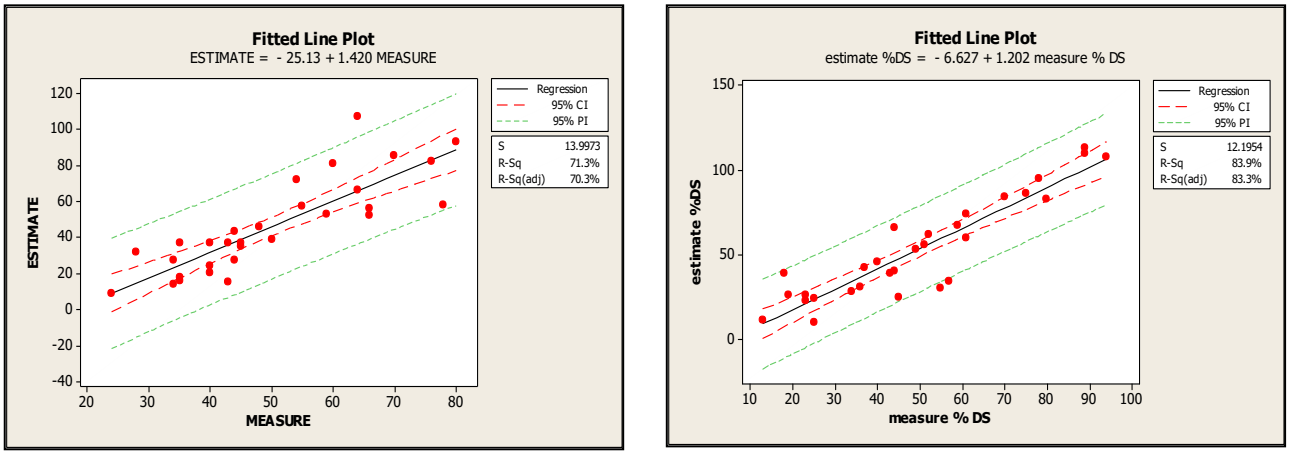
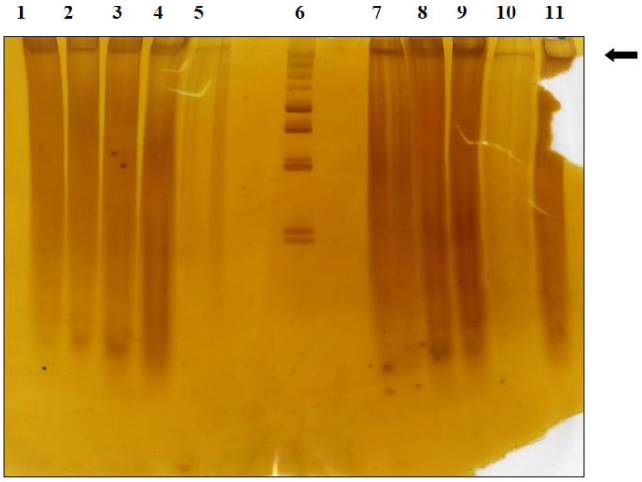


Fig. 4: Model fit between measured and predicted % disease severity

3.6 Laboratory Confirmation

DNA extracted from symptomatic and asymptomatic leaves showed successful amplification of a ~1.5 kb phytoplasma band in four of eight symptomatic samples via nested PCR (Figure 5). No amplification occurred in asymptomatic samples (Figure 6), validating field-level symptom observations and Greenseeker® readings.



Lane 1 asymptomatic sample 1 (H1)
Lane 2 symptomatic sample 1 (D1)
Lane 3 symptomatic sample 2 (D2)
Lane 4 symptomatic sample 3 (D3)
Lane 5 symptomatic sample 4 (D4)
Lane 6 1kb DNA ladder
Lane 7 asymptomatic sample 2 (H2)
Lane 8 symptomatic sample 5 (D5)
Lane 9 symptomatic sample 6 (D6)
Lane 10 symptomatic sample 7 (D7)
Lane 11 symptomatic sample 8 (D8)

Fig. 5: Analysis of CTAB extracted DNA for all the leaf samples. Bands of 8kb were observed as indicated by arrow

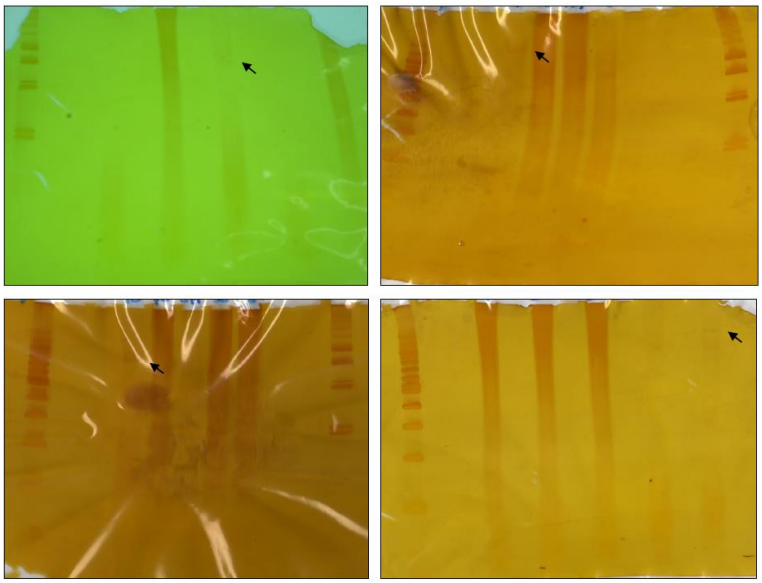


Fig. 6: Nested PCR analysis of DNA extracted by CTAB. Amplification of four set of universal primer (P1, P7; R16F2n, R16R2), and analyzed with 5% Polyacrylamide gel electrophoresis with silver staining

The association between red tip symptoms and phytoplasma presence suggests that this pathogen plays a role in disease etiology, although further confirmation through sequencing and pathogenicity testing is warranted. This is in line with recent studies identifying phytoplasmas as common but under-detected causal agents in monocot diseases (Bertaccini & Lee, 2018; Kaminska et al., 2021).

The integration of Greenseeker® NDVI sensing into disease monitoring workflows offers a practical, scalable alternative to destructive and time-consuming laboratory techniques. Real-time NDVI data could support precision input management, targeted field scouting, and temporal tracking of disease progression. Adoption of such technologies may significantly reduce yield losses in commercial pineapple plantations and improve sustainability in peat-based cropping systems (Balasundram et al., 2006; Liu et al., 2023).

5. Conclusion

This study successfully demonstrated the application of the Greenseeker® handheld NDVI sensor for assessing red tip disease in pineapple cultivated on peat soils. NDVI readings were strongly correlated with visually assessed disease severity and supported by molecular confirmation. The generated models can be used to differentiate between healthy and diseased plants at early growth stages, contributing to precision management and reducing reliance on destructive diagnostic methods. Future work should focus on validating these models across different environments and cultivars and integrating NDVI mapping into farm-level disease management systems.

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Conflict of Interest

The authors declare no conflicts of interest.

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