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Spectral Analysis of Pink Mealy Bug Damaged Mulberry Plants Using Hyperspectral Radiometry

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mulberry *(Morus spp.)* foliage forms a sole food to silkworms, *Bombyx mori* L. Its quality plays a pivotal role in superior silk fiber production. Like any other plants, mulberry is susceptible to detrimental diseases and infested by insect pests. Many sucking pests cause damage to mulberry among which, pink mealybug causes severe damage leading to significant loss in mulberry leaf yield (12-25 %). Conventional approaches to assess and manage damage caused by pink mealybugs demand substantial manpower and expertise, resulting in time-consuming and

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inefficient processes. However, leveraging modern tools and technologies like remote sensing has emerged as a remarkably effective strategy for crop protection and management.Specifically, hyperspectral remote sensing proves invaluable by furnishing insights into the biophysical and biochemical traits of crops. This is achieved through the recording of narrow wave bands that reflect specific plant characteristics. The main objectives were to understand the spectral reflectance characteristics of healthy and pink mealy bug damaged mulberry crops, to identify the optimal spectral bands and vegetative indices for detecting pink mealy bug damage in mulberry and to explore the feasibility of estimating pest damage levels based on the spectral properties of mulberry crops. At the mulberry garden of the Department of Sericulture, Tamil Nadu Agricultural University (TNAU), Coimbatore, studies were conducted on spectral analysis of pink mealy bug damaged mulberry plants using hyperspectral radiometry during 2018. In this trial, at 15-day intervals during the active damage stage, the percentage damage was observed in plots infested with pink mealy bugs and those that were healthy. Measurements of spectral reflectance across various wavelengths and vegetation indices (VIs) were taken using a hyperspectral radiometer. The analysis included evaluating the sensitivity of different spectral bands and VIs to pink mealybug damage, along with employing correlation and regression analyses between the extent of pest damage and the VIs. The findings revealed distinct differences in the spectral reflectance profiles of mulberry plants compared to their healthy counterparts. Typically, affected plants exhibited increased reflectance in the red (620–680 nm) and green (520–590 nm) bands and reduced near-infrared (NIR) reflectance (770–860 nm). The average values of the Normalized Difference Vegetation Index (NDVI), Green Red Vegetation Index (GRVI), and Ratio Vegetation Index (RVI) were significantly lower in the pink mealybug-damaged plants across all measurements. Notably, red reflectance demonstrated the greatest sensitivity to pink mealybug-induced damage. The study highlighted the Simple Ratio (SR) index as particularly effective for identifying pest damage. Damage estimation by pink mealybugs was refined using linear regression models based on spectral indices, specifically NDVI, RVI, and GRVI. The correlation intensity analysis pinpointed the green region at 516.73 nm as showing the most significant negative correlation (r = -0.02), whereas the highest positive correlation with pink mealybug damage was observed in the NIR region (r = 0.77), underscoring the precise wavelengths and indices most indicative of pest impact. Thus, using hyperspectral radiometry identification of damage caused by pink mealy bug possible, through analysis of spectral bands and indices.

Keywords: Pink mealy bug; spectral reflectance; vegetative indices; spectral band.

1. INTRODUCTION

Mulberry *(Morus indica* L.) is a perennial plant known for its lush foliage, which makes it a magnet for various insects and non-insect pests. Over time, as part of continuous crop improvement programs, numerous new mulberry varieties have been developed to enhance the quality and quantity of leaf production. However, these enhancements have also made the plants more susceptible to attacks by pests and diseases. Nearly 100 species of insects have been identified as threats to mulberry, with a few such as the pink mealy bug, thrips, and spiralling whitefly (*Aleurodicus dispersus)* causing significant damage and yield loss [1].

The pink mealy bug*, Maconellicoccus hirsutus* (Green), commonly referred to as mealy bug or Hibiscus mealy bug, is particularly notorious as a difficult-to-control pest. Recent years have seen substantial qualitative and quantitative losses, especially in regions like Karnataka, Andhra Pradesh, and Tamil Nadu, which collectively produce over 80 percent of India's raw silk. Mealy bug infestation has resulted in estimated losses of up to 4500 kg/ha/yr in leaf yield, sometimes exceeding 30 percent [2]. Incidences of pink mealy bug damage occur throughout the year, peaking between July and August [3] with high occurrences noted in March and decreased levels by August, reaching their lowest in December [4].

Early detection of pest infestations is crucial for effective management. Remote sensing techniques offer a promising approach for assessing crop damage across large areas in a short time. However, it is imperative to conduct ground-level studies to validate the relationship between actual infestations and remote sensing data. Utilizing multispectral or hyperspectral spectroradiometers allows for the detection, identification, and estimation of various crop stresses through spectral analysis [5,6]. Hyperspectral data, with its numerous narrow spectral bands, enables the formation of spectral curves for scene components [7] offering the potential for rapid pest and disease diagnosis and non-destructive sampling [8].

The methodology involves recording spectral reflectance at canopy level using hyperspectral spectroradiometers and comparing data from healthy and infested plants. Analysis of reflectance data from different bands, including blue, green, red, and near-infrared (NIR), through mathematical techniques helps calculate vegetation indices for detecting and estimating crop damages [9,10].

With the objective of harnessing advancements in remote sensing using hyperspectral spectroradiometry to assess crop health status, studies were conducted in mulberry fields with the following objectives:

- To understand the spectral reflectance characteristics of healthy and pink mealy bug damaged mulberry crops.
- To identify the optimal spectral bands and vegetative indices for detecting pink mealy bug damage in mulberry.
- To explore the feasibility of estimating pest damage levels based on the spectral properties of mulberry crops.

2. MATERIALS AND METHODS

Field experiments were conducted using hyperspectral radiometry to detect damage caused by sucking pests in mulberry, specifically the pink mealy bug (*Maconellicococus hirsutus*), thrips (*Pseudodentrothrips mori*), and spiralling whitefly (*Aleurodicus disperses*). Naturally occurring pest infestation was observed in two plots, each measuring 5x10 sq.m, designated as T1 (damaged) and T2 (undamaged) within an existing V1 mulberry variety. Ten plants were selected and labelled to represent the healthy (undamaged) and damaged categories in both the protected and unprotected plots. These ten tagged plants served as replications for each treatment. In the undamaged plot, plants were shielded from insect damage by applying suitable insecticides at regular intervals. Conversely, in the damaged plot, no protective measures were implemented to allow the insect population to proliferate. However, both plots were carefully monitored and treated with fungicides or bactericides as needed to prevent plant diseases [9,10,11].

2.1 Per Cent Shoot Damage

A spectroradiometer can measure the surface of the plant canopy and identify insect pest damage based on vegetation indices and spectral reflectance. Estimating insect damage using correlation and regression studies would be a more scientific approach than estimating pest population from spectral indicators. After pruning mulberry plants, ten tagged plants were monitored, and the percentage of damage was computed using a formula at regular intervals of 15, 30, 45, and 60 days [9,10,2].

Percent damage = Number of affected shoots / Total number of shoots X100

2.2 Per Cent Spectral Reflectance

A field portable spectroradiometer, model GER 1500, from the Department of Remote Sensing and Geographic Information System, TNAU, Coimbatore, was used to record the spectrum reflectance. On bright, sunny days between 10 a.m. and 1 p.m. local time, the instrument was aimed 30 cm above the leaf to gather the leaf spectral data. The equipment was optimized and calibrated before to the first measurement and then every five minutes thereafter to accommodate changing atmospheric conditions [12,13]. Before every set of measurements, the incoming spectrum was regularly taken from the light reflected by a standard panel made of barium sulphate. Using the following formula, the ratio of the target's reflected spectrum to the canopy's incoming spectrum (reference) was determined to determine the percent reflectance.

Per cent reflectance = Reflectance from target (Plant canopy) / Reflectance from reference (Barium sulphate panel) X 100

Using special software included with the device, the spectroradiometer's spectral reflectance data both the absolute and percentage reflectance values were transmitted to a personal computer as ASCII files with an ASC extension. Later, these files were viewed in a spreadsheet application, and more analysis was done. For every plant, and for ten plants in each treatment, the 512 values of % spectral reflectance at around 1.5 nm bandwidth interval, ranging from 276.86 to 1093.50 nm (with reflectance at 350 to

1050 nm ranges being more consistent), were acquired [14].

2.2.1 Determination of spectral bands and vegetation indices

The reflectance values within the green, red, and near-infrared (NIR) bands were determined for each plant by averaging the reflectance values within specific wavelength ranges: 520 - 590 nm for green, 620 - 680 nm for red, and 770 - 860 nm for NIR. Subsequently, from these calculated reflectance values within the green, red, and NIR bands, several vegetation indices namely Normalized difference Vegetation indices, Simple ratio and Green Red Vegetation Indices were computed by using following formulas.

NDVI = (RNIR –RRED) / (RNIR+RRED)

Where, RRED and RNIR are spectral reflectance values in RED and NIR bands respectively. Bands [15,16,17,18]

SR = RNIR / RRED

Where, RRED and RNIR are spectral reflectance values in red and NIR bands, respectively [15,16,17].

GRVI = (RGREEN – RRED) / (RGREEN + RRED)

Where, RRED and RGREEN are spectral reflectance values in red and green bands, respectively [19].

2.3 Band Sensitivity and Vegetation Index Sensitivity Analyses

Sensitivity at a given wavelength or band was computed by using the following formula [5]

Band sensitivity = Rinf-Rctrl / Rctrl x 100

where Rctrl is the canopy reflectance of the control plants, and Rinf is the canopy reflectance of the infected plants. Similarly, the following formula was used to determine the sensitivity for the given vegetation index.

Vegetation sensitivity = VIs inf – Visctrl / VIsctrl X100

Whereas VIctrl is the vegetation index of the control plants, and VI inf is the vegetation index of the infected plants.

2.4 Corrected Sensitivity

Due to fluctuations in light intensity during observations, the sensitivity curve shifted along the y-axis. To rectify this issue, a corrected sensitivity was derived as follows: Reflectance within the wavelength range of 350-370 nm remained unaffected by pest damage, as noted in preliminary observations. Therefore, the average sensitivity values within this range were considered as zero, serving as a correction factor. This correction factor was then applied to sensitivity values at other wavelengths by either adding or subtracting it. The resulting values, termed as corrected sensitivity values, were reported as the final sensitivity values.

2.5 Statistical Analysis

The mean and standard deviation were computed for the percentage of damage caused by pink mealy bug. A randomized block design was employed to analyse the vegetative indices, namely RVI, NDVI, and GRVI, with means compared using the Least Significant Difference method. Correlation studies were conducted to examine the relationship between reflectance in different spectral bands (red, green, and NIR), vegetation indices (NDVI, GRVI, and SR), and the percentage of leaf damage caused by pink mealybug. Ten tagged plants exhibiting varying levels of pest infestation were selected, and corresponding vegetation indices were determined for each plant through spectral reflectance studies. The mean values of spectral indices from ten tagged control plants were also incorporated in the correlation coefficient calculation. Significance testing of the correlation coefficient was carried out following the method proposed by Rangswamy [20]. Furthermore, linear regression models were constructed to assess the relationship between the percentage of damage and each of the vegetation indices, considering the varied levels of pest infestation in the ten tagged plants and their corresponding vegetation indices [21].

2.6 Linear Correlation Intensity Analysis

Linear correlation intensity analysis was conducted to identify the wavelengths with the highest positive and negative correlation with pest damage. The correlation between pest damage and spectral reflectance across each of the 512 wavelength bands ranging from 350 to 1050 nm was calculated. These correlation values were then graphed against the corresponding wavelengths to generate a linear correlation intensity analysis graph, following the method proposed by [22,23]. This analysis, akin to band sensitivity analysis, aims to pinpoint wavelengths most sensitive to pest damage.

3. RESULTS AND DISCUSSION

3.1 Spectral Signature of Pink Mealy Bug Damaged Plants

When per cent reflectance value got from spectroradiometer plotted in a graph against a wavelength range from 300 nm to 1200 nm, plants affected by pink mealy bugs exhibited a decrease in NIR reflectance and an increase in green and red reflectance compared to unaffected plants as shown in (Fig.1). The percentage reflectance differed notably between the damaged and undamaged plants, particularly in the green, red, and near-infrared regions. This discrepancy in reflectance arises from the absorption of visible light by chlorophylls within the epidermal cells of leaves and the multiple reflection of NIR radiation within spongy tissues. Moreover, variations in percent reflectance were observed across different regions within both damaged and healthy plants. Plants under stress typically exhibit reduced reflectance in the NIR region (700-1300 nm), increased reflectance in the far-red region, and a notable shift in the red edge [5,24,25,26]. Wheat canopies infested with the Russian wheat aphid demonstrated a significant decrease in NIR reflectance and an increase in the visible spectrum compared to uninfected canopies [13]. Similarly, rice canopies affected by leaf folder pests showed increased reflectance in the green and red regions but a decrease in NIR reflectance throughout the observation period. The spectral signature of rice crops damaged by the brown plant hopper, both in field and greenhouse conditions, exhibited changes in reflectance. Lower at shorter wavelengths and higher at longer wavelengths, specifically in the NIR followed by the mid-infrared region. This indicated the potential for detecting damage caused by the brown plant hopper through spectral analysis [27].

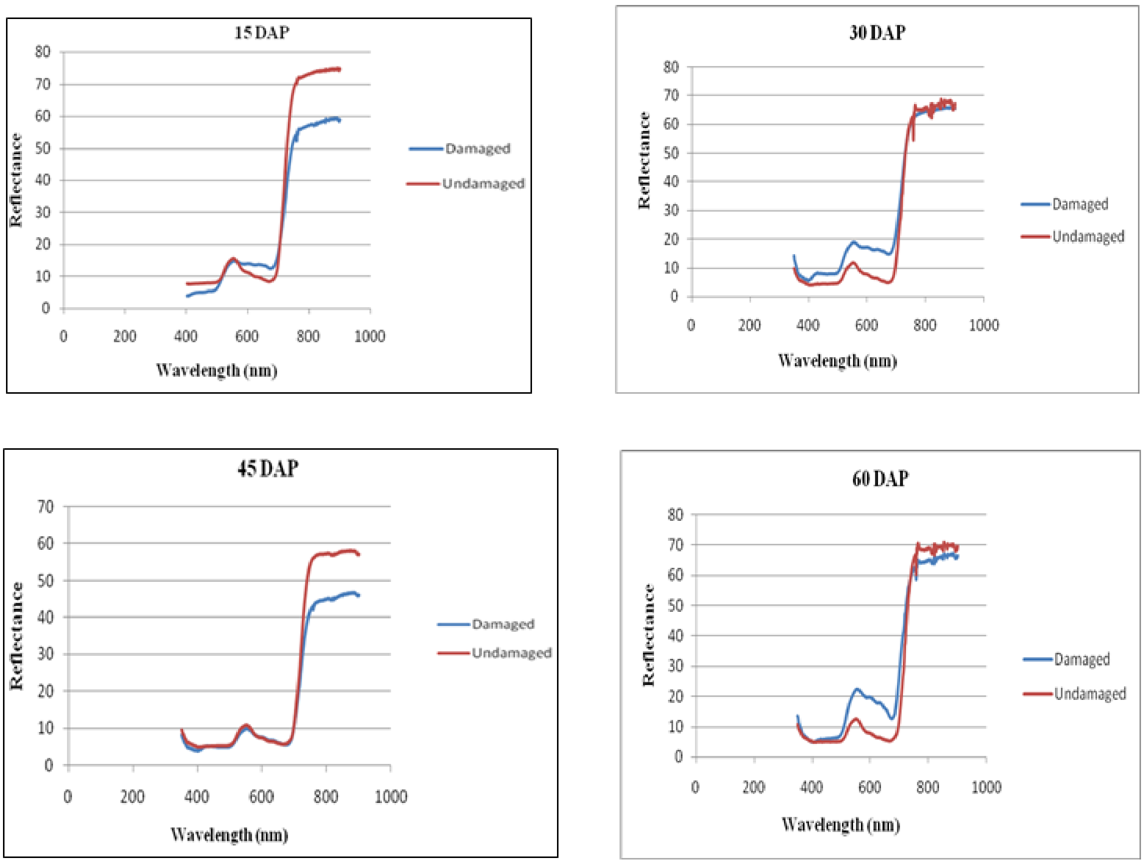


Fig. 1. Spectral signature of pink mealy bug damaged mulberry plants
**Mean of 10 damaged and 10 undamaged plants*

3.2 Vegetative Indices of Pink Mealy Bug Damaged Mulberry Plants

The Vegetative indices namely, Normalized Difference Vegetation Index (NDVI), Simple Ratio and Green Red Vegetation Indices were worked (Table 1) is often lower in damaged plants compared to healthy ones due to alterations in chlorophyll content, leaf structure, and overall plant health due to pest damage. Reason for the lower NDVI values in pink mealy bug damaged plants is the reduction in chlorophyll content caused by pest. The mealy bug infected plants showed cured leaves and stunted apical shoots. As mealy bug sucks on apical leaf tissues, alters leaf structure and canopy density, affecting the scattering and absorption of light within plant tissues. This alteration in light interactions further impacts the red and NIR reflectance components used in calculating vegetative indices, contributing to the observed decrease in values in damaged plants compared to healthy ones [28].

3.3 Sensitivity of Spectral Bands and Vegetative Indices

Comparing the sensitivity of the red, green, and NIR bands on days 15, 30, and 45 after pruning, the red bands display the highest band sensitivity value (Table 2). Red band must be helpful in identifying damage caused by pink mealy bugs, as evidenced. The red band is more sensitive to pest damage primarily because it captures changes in chlorophyll content and leaf structure that occur when plants are under stress from pest attacks. When pests damage plants, they often affect the plant's ability to photosynthesize by altering the chlorophyll content and damaging the physical structure of the leaves. Since chlorophyll strongly absorbs light in the red portion of the spectrum (around 680 nm), any reduction in chlorophyll content due to pest damage leads to an increase in red light reflectance. This change is more pronounced in the red band than in other spectral bands, making the red band particularly useful for detecting stressed or damaged vegetation [29]. Similar report has been made by [14] the sensitivity percent in YellowStemBorer infestation of red band, on 90 DAT was +113.5 percent while that of NIR and green bands were -14.5 and +33.4 which indicate that red band is more sensitive to white ear symptom caused by YSB. These were the periods when active damage was caused by YSB in rice crops. Changes in chlorophyll content significantly affect reflectance in the red band [26]. Additionally, [25] highlighted the sensitivity of the red band to variations in plant Vigor, noting that stressed plants tend to reflect more red light due to decreased chlorophyll absorption. These studies underline the reason why the red band is more sensitive to pest damage, as it directly relates to changes in the plant's primary photosynthetic pigment and overall health status. Based on the three sensitivities of reflectance indices simple ratio showed higher magnitude on all days of observation irrespective of the sign. The results corroborate the findings of Ranjitha and Srinivasan [10] who found that thrips damaged cotton plants showed Green Red Vegetation Index as most sensitive to pest damage than NDVI and GRVI.

3.4 Regression Analysis of Pink Mealy Bug Damage Based on Vegetation Indices

The regression analysis of SR, GRVI and NDVI provided possibilities of estimating pink mealy bug damage, as strong correlation existed between these parameters and pink mealy bug damage. The three indices calculated on all days of observation had significant negative correlation with pink mealy bug damage. Similarly, The R^2 values of RVI, NDVI and GRVI with pink mealy bug damage on V_1 variety of mulberry were 0.513, 0.421 and 0.330, respectively indicating the capability of all these indices to estimate pink mealy bug damage. This negative correlation between pest damage and vegetative indices suggests that as pest infestation levels rise, the biophysical properties of the leaf deteriorate, leading to a decrease in both reflectance and the values of vegetative indices. In Brinjal, Simpe ratio significantly correlated with aphid incidence [11].

The use of correlation and regression analysis to examine the relationship between pest damage and vegetative indices helps to define how these variables are interconnected. R^2 values of NDVI, RVI and GRVI were 0.42,0.51 and 0.33, (Table 3) respectively on all observations. These results indicating the capability of all the indices for estimating pink mealy bug damaged mulberry plants. And also estimating pink mealy bug damage linear equations were developed for RVI, NDVI and GRVI was 41.16-1.68 X RVI, 42.48-24.33 X NDVI and 37.05-59.82 X GRVI (Fig. 2). Regression equations were developed between percent damage and vegetative indices, thus making estimating thrips one from the other.

Per cent leaf damage by thrips = 510.0 - 604.5 x NDVI; Per cent leaf damage by thrips = 113.3 - 9.8 x RVI; Per cent leaf damage by thrips = 26.9 - 92.3 x GRVI. These equations offer insights into pest damage by using estimated vegetative index values within the formulas. They also enable the calculation of index values without the need for remote sensing data, by using the percentage of leaf damage gathered from field observations [9].

Table 1. Vegetative indices of pink mealy bug damaged mulberry plants

DAP	NDVI		SR		GRVI	
	Undamaged*	Damaged*	Undamaged*	Damaged*	Undamaged*	Damaged*
15	0.779±0.03	0.714±0.31	8.15 ± 1.07	0.550±0.22	9.32±2.64	7.51±1.65
30	0.841±0.04	0.630±0.28	11.95 ± 2.89	0.561±0.22	16.63±9.81	7.07±0.98
45	0.812±0.03	0.722±0.21	9.85 ±1.69	0.647±0.16	8.37±3.40	6.67±1.00
60	0.872±0.02	0.620±0.23	12.32 ±3.62	0.586±0.17	12.61±1.96	8.17±4.03
Mean	0.831± 0.03	0.671±0.26	10.57±2.31	0.586±0.19	11.73±4.45	7.36±1.92
Sed	0.0747		0.0422		0.8284	
CD(.05)	0.1628		0.0920		1.8050	
CD(.01)	0.2282		0.0600		2.5306	

**Calculated from mean of 10 plants in each damaged and undamaged.*
DAP-Days after Pruning.

Table 2. Sensitivity of bands and vegetative indices

DAP	Green	Red	NIR	SR	GRVI	NDVI
15	31.62	75.91	8.39	-52.49	5.34	-8.64
30	74.98	152.85	-25.90	-38.54-	26.63	-24.32
45	40.99	146.90	-37.73	-11.08	-9.10	-7.24
60	11.95	0.69	-2.59	-46.58	-30.55	-29.02

**Calculated from mean of 10 plants in each damaged and undamaged.*
**DAP-Days after Pruning.*

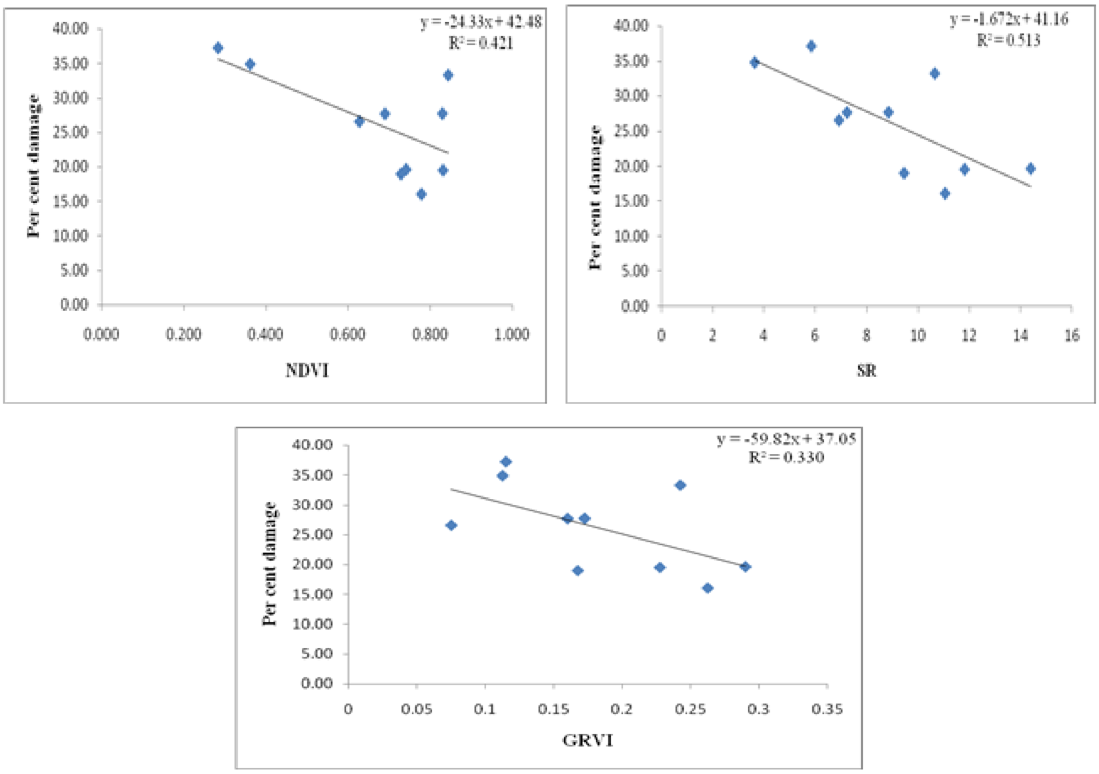


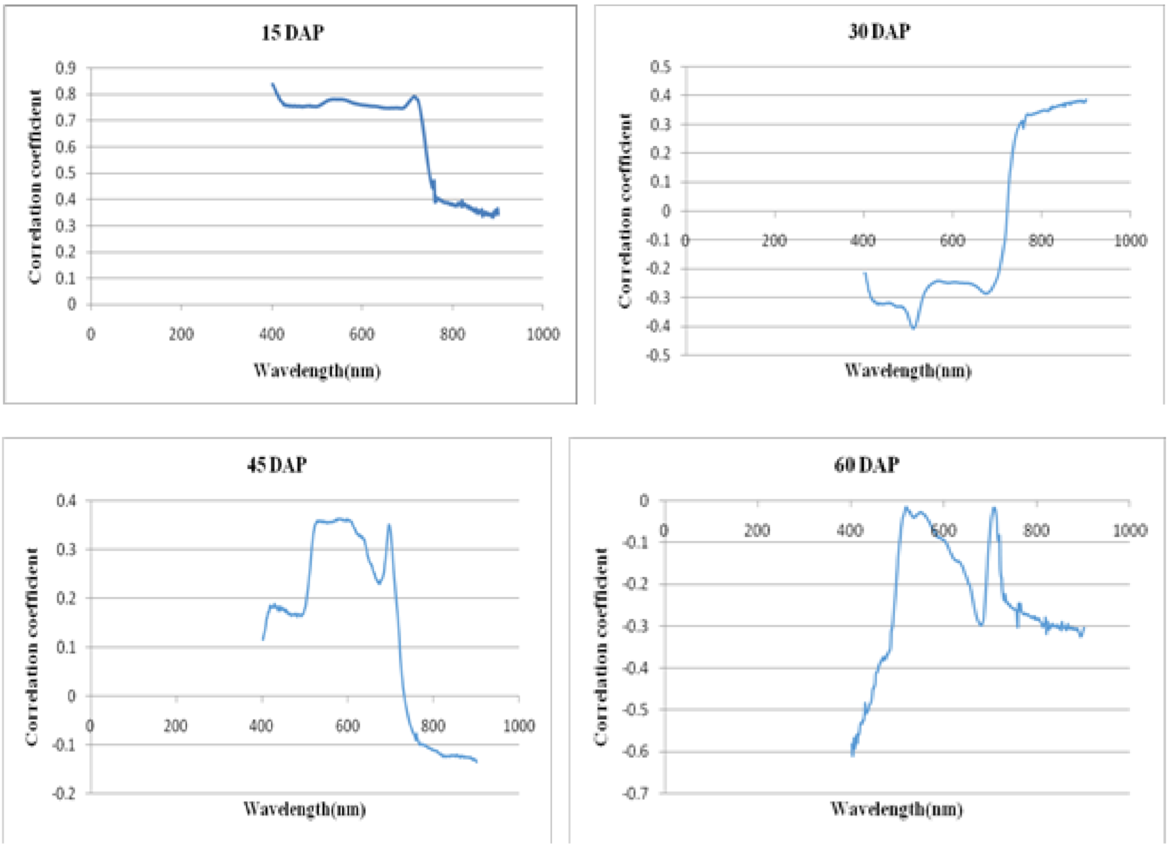
Fig. 2. Relationship* between different vegetative indices and per cent damage caused by pink mealy bug in mulberry plants

Table 3. Vegetative indices and their relationship with per cent shoot damage caused by pink mealy bug

Plant no.	Per cent damage	SR	NDVI	GRVI
1	27.67	7.23	0.690	0.16
2	26.56	6.91	0.627	0.07
3	18.98	9.45	0.729	0.16
4	16.08	11.05	0.778	0.26
5	19.64	14.39	0.741	0.29
6	33.26	10.65	0.844	0.24
7	27.70	8.84	0.830	0.17
8	19.52	11.81	0.831	0.22
9	34.84	3.64	0.361	0.11
10	37.19	5.85	0.283	0.11
R		-0.717*	-0.648*	-0.574
R2		0.514	0.421	0.33

**Significant at p=0.05*

Fig. 3. Correlation of reflectance in different wavelengths to per cent shoot damage by pink mealy bug in mulberry plants



3.5 Linear Correlation Intensity Analysis

The correlation coefficient between pest damage and spectral reflectance values were plotted against the respective wavelengths to construct linear correlation intensity curve for sucking pest of mulberry pink mealy bug. It was found that in pink mealy bug damaged mulberry plants, the maximum correlation (r = 0.77) was located in NIR region at 702.12 nm. There was a positive correlation (r = 0.34) in blue region at a wavelength of 522.23 nm and the most negative correlation (r= - 0.02) was noticed in green region at a wavelength of 515.73 nm. Hence, these wavelength ranges can be used for detecting the damage caused by pink mealy bug damage in mulberry.

Our studies are in line with the findings of [10] who found that the maximum positive correlation value between thrips damage in cotton and reflectance was located in red band between 651 to 680 nm. There was a positive correlation in blue region ranging from 420 to 490 nm which was marginally lower than red region. The most negative correlation was in NIR band with a wavelength range of 760 to 769 nm. Hence, these wavelengths ranges can be used for detecting the thrips damage in cotton plants. Plotting correlation coefficients (r) that measure the link between plant reflectance across the spectrum from 350 nm to 2500 nm at every 1-nm interval and the levels of BPH damage, revealed key sensitive wavelengths: 1986 nm (r = 0.63), 665 nm (r = 0.58), 1792 nm (r = 0.53), and 500 nm (r = 0.52) [27].

4. CONCLUSION

This study utilized hyperspectral radiometry to analyse the spectral characteristics of pink mealybug-damaged mulberry plants. Through rigorous field experiments and data analysis, we identified distinct differences in spectral reflectance profiles between healthy and infested plants. Pink mealybug damage led to an increase in red and green reflectance, along with a decrease in near-infrared (NIR) reflectance. Vegetation indices such as NDVI, Simple Ratio (SR), and Green Red Vegetation Index (GRVI) exhibited significant decreases in infested plants compared to healthy ones, indicating the potential for these indices to serve as effective indicators of pest damage. Our findings underscore the importance of specific spectral bands and vegetation indices in detecting and estimating pink mealybug damage in mulberry plants. Notably, the red band demonstrated the highest sensitivity to pest-induced stress, making it particularly valuable for pest detection. Regression analyses further confirmed the strong negative correlation between vegetation indices and pest damage, providing a basis for estimating damage levels using spectral data. Additionally, linear correlation intensity analysis identified key wavelength ranges, such as the NIR region at 702.12 nm, with the highest correlation to pink mealybug damage. These findings offer valuable insights into the potential application of hyperspectral radiometry for early detection and management of pink mealybug infestations in mulberry crops. Overall, our study contributes to the advancement of remote sensing techniques for pest monitoring in sericulture, highlighting the utility of hyperspectral radiometry in detecting subtle changes in plant health associated with pest damage. Further research in this area holds promise for improving pest management strategies and minimizing crop losses in mulberry cultivation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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