

Hyperspectral Imaging for Determining Reflection Variables in Chilli Leaves Infested with Green Peach Aphid

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Abstract

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Aphid infestations cause a physiological response detectable by a remote leaf reflectance sensor. Changes in the spectral signature of specific wave bands, measured with hyperspectral imagery (HI), may also relate to the absence, presence and/or level of infestation of aphids. Aphids cause significant damage to crops and yields in the field as well as in greenhouses. Green peach aphid, *Myzus persicae* (Sulzer) destroy chloroplast cells; this damage can be spectrally detected in the reflectance of the visible and near-infrared (NIR) regions. The spectral curve showed that the aphid-infested chili leaves reflectance in the NIR, decreased in time with the increase of aphid population. Although the aphid's activity occurred on the leaves underside, their damage can be spectrally detected by reflection data from the upper side. Early aphid damage was identified in limited areas of chili leaf and this damage showed to be the only harm inflicted on the plant, also proven by the HI data obtained. Surface reflectance (%) from un-infested chili was lower in the visible and higher in the NIR light 90A spectrum when compared with aphids-infested chili. The overall classification accuracies of 89% for damage detection were achieved. These results indicated that HI can be effectively used to accurately detect and quantify aphid infestation in chili for site-specific aphid management. It can be potentially applied for limited areas as well as fields as an early detection tool for aphid management. This study aimed to spectrally explore the ability to assess the level of aphids damage in a limited area grown with the chili crop.

Keywords: Hyperspectral imagery, aphids, chilli pepper, near-infrared.

Introduction

Plant stress detection has always been challenging for farmers. The commonly faced challenges across the globe include the destruction of a major part of production due to pests (Singh *et al.*, 2020). Insect infestation in crops leads to reduced yield, increase control cost, and the risk of environmental damage from insecticides (Oerke & Dehne, 2004). The presence of various pests in a plant is a major concern among farmers (Lamichhane *et al.*, 2016). Insect pests damage crop plants and deteriorate the quality of food grains and products. Plants respond to pest stress in many ways, including leaf curling, chlorosis or necrosis of photosynthetically active parts or stunted growth (Sahoo, 2022). Data from the reflected visible and NIR wavelengths of light can detect damage to leaves caused by pests (Xu *et al.*, 2007).

Hyperspectral monitoring is a technique used to monitor plant stress. It can show all the light frequencies in a single image, reducing the time and hardware needed to get all the necessary information. It is most suited for agriculture surveys; chlorophyll fluorescence imaging is a powerful tool to provide specific signatures for the diagnosis of distinct and abiotic stresses (Mahlein, 2016). Hyperspectral sensing devices appear to be the most suited for vegetation health-state monitoring (Avetisyan *et al.*, 2019). A plant has specific UV reflectance that can show signs of deterioration.

Correctly interpreting this reflectance provides a great deal about plant status (Sosa-Herrera *et al.*, 2022).

Chilli farming still faces pests attack as a limiting factor in chilli production. When infested, chilli plants suffer structural, physiological, and biochemical damage (Martínez Arias *et al.*, 2022). Pest infestations change plant physiology which alters leaf reflectance. Analysis of leaf reflectance data offers the possibility to forecast the risk of a pest infestation before it occurs (Rotondi *et al.*, 2021). The implementation of non-contact, highly efficient, and affordable methods for detecting and monitoring plant pests over vast areas could greatly facilitate pest management (Zhang *et al.*, 2019).

The aphid *Myzus persicae* (Sulzer) feeds on the underside of leaves, piercing the chloroplast-containing cells and affecting pigments as well as leaf structure (MacWilliams *et al.*, 2023). Aphids cause significant damage to crops and yields in the field as well as in greenhouses by feeding and also by transmitting viruses (Bera *et al.*, 2022; Sun *et al.*, 2020; Valenzuela, & Hoffmann, 2015). Aphids secrete sticky honeydew, which is harvested by ants, making leaves susceptible to sooty mould fungus, causing reduced photosynthesis (Lemay & Scott-Dupree, 2022). Reduced photosynthesis causes reduced food for plants, inevitably leading to stunted growth and desiccated leaves and, therefore, a change in plant leaf reflectance.

Recent developments in agriculture have led to a demand for non-destructive methods of plant stress detection

as increasingly sophisticated ground-based remote sensing systems are becoming less expensive and easier to use. The use of remote sensing techniques for the detection of crop stress due to pests is based on the assumption that stresses induced by them interfere with photosynthesis and the physical structure of the plant, affect the absorption of light energy and thus alter the reflectance spectrum of plants (Ahmad & Rasool, 2014; Sahoo *et al.*, 2015). HI technology produces precise data in real-time, distinguishing between healthy and unhealthy plants by measuring reflected electromagnetic radiation. Unusual data can indicate problems well before the damage becomes more visible and widespread. A change in leaf reflectance indicates plant stress which is caused by various factors including pests (Bjerke *et al.*, 2014). Damage caused by aphids becomes apparent in the reflected visible and NIR wavelengths. This study aimed to demonstrate that NIR radiation reflection from chilli plants changes when plants are exposed to infestation with aphids.

Materials and Methods

Preparation of raised garden beds

The study was conducted in a small plot trial in October 2021 in Bundaberg 4670, Queensland, Australia, to examine the effect of aphid infestation on the leaf reflectance characteristics of chilli plants. A randomised plot design was used with two treatments (control and aphid infested). 24 chilli cv. 'Caysan' seven weeks old seedlings were planted in two raised planting beds filled with a soil mix of pH 6.7. Each bed was further divided into six sections containing two plants per section, and 12 cages (45×75×90 cm) were placed on the beds. Each cage had 2 chilli plants. Insect-proof mesh cages were placed over each section to prevent cross-infestation with insects. The plants were grown under the recommended fertilization regime. 16 g of Aquasol was dissolved in 10 liters of water and applied to 3-week-old plants; Urea 3.6g/plant was applied 3 weeks later, and watered by automatic daily drip-irrigation (three times a day). Uniform-sized plants were selected (7-10 cm high and 6-7 weeks old), and 24 were exposed to aphids (using toothbrush to transfer the aphids). All plants were placed in insect-proof enclosures to prevent the spread of aphids to the controlled plants (weather conditions were as indicated in Table 1). Four days before the beginning of the experiment, preventive insecticide (Confidor 200 SC, Bayer) spray was applied by hand spray as per commercial practice using personal protective equipment to ensure that test plants were free from insect pests.

Green peach aphids were cultured on mature chilli plants grown in pots in a separate greenhouse. At the beginning of the experiment, a fine brush was used to collect the aphids for transfer to the chilli plants used in the trial. Between 40±5 aphids were transferred to each aphid infestation treatment plant, with the aphids transferred carefully and placed on six-single leaves (selected randomly) on each plant. All leaf reflectance measurements were made on leaves located on the aphid-infested leaves of the treated plants.

Six individual leaves from each individual plant were selected for the study. A hyperspectral camera was used to

assess leaf reflectance characteristics. Data were collected from the chilli plants six times (once before the aphids were transferred and five times after the aphids had been transferred to them, once after each two days). Data were collected on clear days between 11:00 – 14:00 when the sun was almost at its zenith. To demonstrate that radiation (infrared and visible) reflectance from chilli plants changes when plants were exposed to aphids infestation.

Hyperspectral Image (HI) data capture

HI was used in one of the experiments conducted in this project. In this experiment, images were collected using a hand-held hyperspectral camera (Specim Ltd., Oulu, Finland, Model 0604675) with integrated operating systems and controls. Camera focus needed a manual adjustment. The adjustment was needed to accurately overlay the spectral and viewfinder camera images; the image recording was started by pressing the capture button. The dark reference, representing the sensor noise without incoming light, was recorded automatically. This was done on the spectral camera home position blocked from incoming light. After the dark reference acquisition, the spectral camera was moved to the measurement starting position and the actual data acquisition started. A white reference panel was placed with each sample plant during the image capture. Each image covered a complete plant. Solar illumination provided a continuous spectrum over the wavelength range of interest. Leaves were oriented to ensure they were receiving full sun exposure for a few minutes prior to and during data capture. The captured images were analyzed using Specim IQ Studio software, which relies on the spectral angle mapper algorithm to analyze the images.

Hyperspectral capture is very sensitive to inconsistent lighting and spectral scattering. In order to avoid this problem, image capture took place as close to midday as possible, under full sunlight conditions. An adjustment was necessary to overlay the spectral and viewfinder camera images with reasonable accuracy. After these initial adjustment steps, the first image recording started by pressing the capture button. After the full image was scanned, the camera stored the reflectance changes data, which were then downloaded to produce a graph (the parameters gathered are saved into each image's metadata). Each image was radiometrically calibrated using the Spectronon tools (RESONON, Version 3.1.1 software).

Statistical design and analysis

The completely randomized design (CRD) was used in the experimental design of trials and GenStat software was used to analyze the trial data obtained.

Table 1. Temperatures and precipitation at Bundaberg region during trial period.

Month	Temperature (°C)			Precipitation (mm)
	Average	Minimum	Maximum	
September	19.4	13.8	13.8	25.1
October	21.8	16.8	16.8	26.9
November	23.7	19.1	19.1	28.4
December	25.0	20.6	20.6	29.5

Results

Data from the hyperspectral sensors, commencing one day before pests are introduced to the chilli plants, were recorded at two different angles and distances between the sensor device and the plant, with no-changes in the spectral signature of specific wave bands measured with HI (Figure 1). All plants were assessed regularly using a visual pest rating. In the data recorded one day before and two days after aphids were transferred, the average reflectance in leaves was similar whether plants were infested with aphids or not (control). This was demonstrated when later data showed increasingly less reflectance in the NIR range as the chlorophyll content of infested leaves decreased (Figure 2).

Results obtained confirmed that the changes in reflectance were due to feeding aphids on the leaves. Hyperspectral cameras reflectance of non-infested and aphid-infested leaves did not differ significantly in the light range (816.92-896.01 nm) wavelengths, one and two days before aphids transfer. Little difference was apparent after seven days, as aphid numbers started to increase, however, the differences became more apparent over the following days when aphid-infested chilli leaves exhibited clear differences in their spectral reflectance (Tables 2 and 3).

Table 2. Reflectance at 816.92 and 896.01 nm wavelengths of chilli leaves one day before aphids were transferred.

Leaf position and distance in relation to the camera	Reflectance one day before aphids transfer	
	816.92 nm wavelength	896.01 nm wavelength
45 A, 40 cm	0.63	0.65
45 A, 60 cm	0.63	0.66
90 A, 40 cm	0.65	0.68
90 A, 60cm	0.66	0.69

Hyperspectral imaging data provided canopy reflectance in large numbers of contiguous narrow bands. Low reflectance in the NIR domain is due to scattering caused by the internal leaf structure. It was found that aphids affected leaf reflectance over a narrow wavelength band within the NIR spectrum (816.92-896.01 nm). A significant difference in reflectance in the NIR region between healthy and aphid-infested chilli plants was evident in this study. Therefore, data recording of the reflectance changes in chilli leaves on which aphids are feeding is an efficient alternative to visual detection of infestation. Percentage surface reflectance from uninfested chilli was higher in the NIR

portions of the spectrum when compared with aphid-infested chilli. These results indicate that HI data can be effectively used for the accurate detection of aphid's infestation in chilli crops. These analyses resulted in 100-95% success in identifying early damage with HI data reflected from chilli leaf. HI data was analyzed and cross-validated independently by partial least squares-discriminant analysis models (Figure 2).

Sunlight from a plant canopy reflects wavelengths from 460 nm (blue) to 900 nm NIR. Chlorophyll absorbs light strongly; the absorbance efficiency of chloroplasts is reduced in unhealthy plants; thus, they reflect more light exhibiting a high reflectance coefficient (35–48%) at wavelengths above 600 nm. A significant reduction in the percentage of NIR (760 and 900 nm) reflectance becomes apparent as damage severity increases (Wu *et al.*, 2021). Remote sensing makes accurate monitoring available over a wide area. The technologies of recording and interpreting data from the handheld or aerial use of hyperspectral camera devices are constantly being improved to measure reflectance and accurately identify problems.

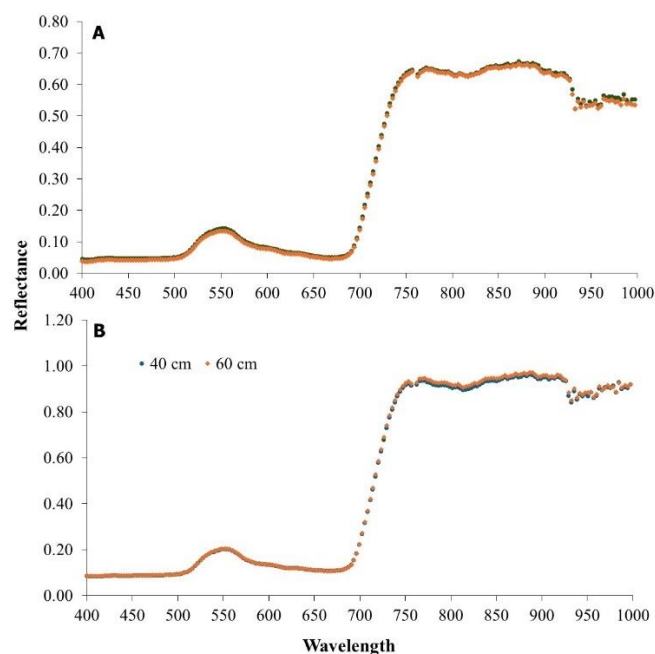


Figure 1. Non-infested chilli leaves reflectance rating at (A) 45A angel and (B) 90A angle at 60cm distance (solid square) and 40cm distance (solid circle) one day before aphid's transfer.

Table 3. Reflectance at 816.92-896.01 nm wavelengths of the chilli leaves (non-infested and aphid infested) and P-values.

Leaf condition	Reflectance X days after aphids transferring at two wavelength (nm)									
	2		4		7		9		14	
	816.92	896.01	816.92	896.01	816.92	896.01	896.01	896.01	896.01	896.01
Control	0.63	0.64	0.68	0.72	0.63	0.67	0.76	0.81	0.72	0.76
Treatment	0.63	0.66	0.70	0.73	0.60	0.63	0.62	0.67	0.52	0.54
P-value	0.52	0.64	0.82	0.98	0.53	0.52	0.00	0.00	0.00	0.00*

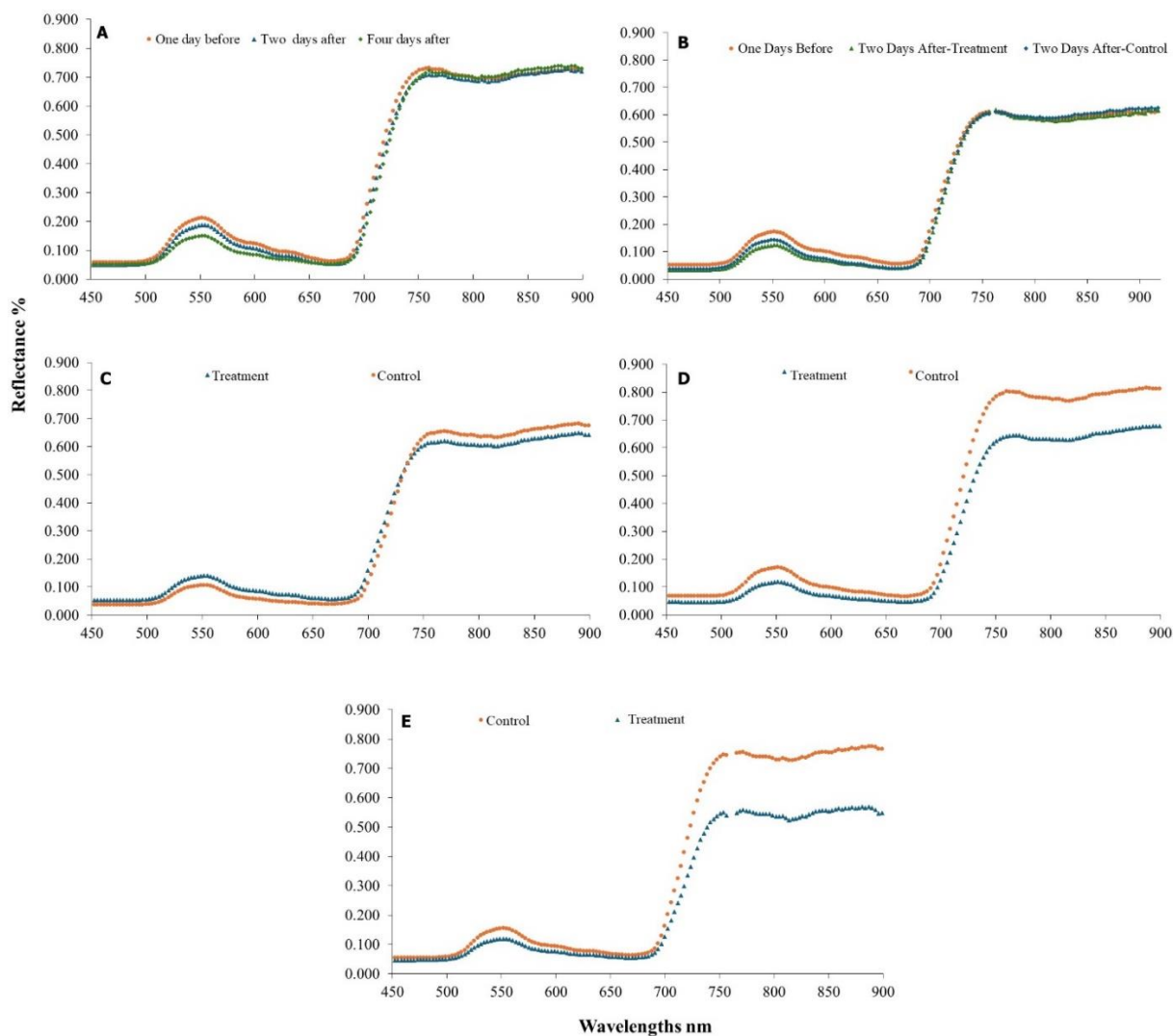


Figure 2. Describes the infested and non-infested chilli leaves reflectance at 816.92-896.01nm wavelengths. Reflectance rating for control and treatment leaves one day before aphids (A), two days after aphids transferred the infested (treatment) leaves and four days after aphids transferred (B), after seven days (C), nine days (D), fourteen days (E) from aphids transferred.

Discussion

Early detection of pests may allow farm managers to mitigate an infestation before it turns into a devastating outbreak. Aphid damage to chilli crops was studied by measuring the reflectance of leaves over a period of two weeks. Insect pests cause interference with photosynthesis and initiate physical changes to the structure of plants, affecting absorption and reflectance of light energy (Prabhakar *et al.*, 2012). Aphid-infested chilli plants had lower canopy reflectance in the NIR regions of the spectrum compared to aphid-free plants (Alves, 2017). Early aphid infestation of the chilli crop did not have uniform distribution (Atshan *et al.*, 2020). In the first recorded data, the reflectance curves in the NIR (750–900 nm) for different regions of the leaves were similar but changed steadily as the aphids population increased over the following days. Yang *et al.* (2009) noted that reflectance of plants in the NIR band (750–1300 nm) decreased under stress. The difference in the reflectance curves of two leaves

at different stages of growth can be construed to be due to the changes in absorption of NIR light by chloroplasts in epidermal cell leaves and HI reflections of NIR radiation in spongy tissues. Similar results were found by Zhou *et al.* (2018), who recorded a decrease in reflectance in the NIR region in pest-infested plants. The reflectance (%) varied in different regions of damaged and healthy plants at different growth stages. A graph recording percentage reflectance against wavelength showed a decrease in the NIR region (700-900 nm), indicating severe tissue damage to infested plants, and the NIR reflectance values decreased as the crop age increased. Nansen *et al.* (2021) indicated that the reflectance values distinguished infested Chinese cabbage from non-infested ones. Lacotte *et al.* (2022) recorded that hyperspectral imaging leads to better pest detection accuracies.

Host plants attacked by piercing/sucking (phloem feeding) aphids respond defensively, or stress interferes with the physical structure and photosynthesis of the plant tissue, in some instances releasing systemic volatile chemicals

which affect the light reflectance of leaves (Prabhakar *et al.*, 2012). The spectral reflectance of infested chilli leaves with different levels of aphid infestation was significantly lower than the reflectance of non-infested plants at NIR wavelengths (750-900 nm). However, reflectance of non-infested and infested leaves did not differ significantly in the 400-700 nm wavelength range, but a significant difference in reflectance between non-infested and infested plants in NIR wavebands was noticed, even at low infestation level (Atshan, 2021). Reflectance of both non-infested and infested plants increased with increasing wavelength up to 900 nm. Variations in plant reflectance due to aphid feeding were less at shorter wavelengths (750–900 nm) compared with non-infested plants (Figure 2).

The greatest difference in plant reflectance occurred between infested and non-infested chilli leaves in the NIR region at 816.92 nm wavelength (Table 2). These results were similar to those reported earlier (Huang *et al.*, 2012; Prabhakar *et al.*, 2012). Although there may have been variations in reflectance patterns of infested plants in the visible region, reflectance of infested plants in the NIR was previously observed to be, without exception, uniformly lower than non-infested plants (Prabhakar *et al.*, 2011). Lower reflectance of infested plants in the NIR region 816.92-896.01 nm compared with non-infested plants could be due to curling, shrinking and/or wilting of leaves due to aphid feeding, leading to light scattering instead of reflectance of incident radiation, resulting in decreased reflectance from infested plants. In previous studies, leaf damage caused by chilli aphids (Yang *et al.*, 2009), cotton leafhoppers (Prabhakar *et al.*, 2011) reduced plant

reflectance in the NIR due to photon scattering caused by leaf colour fading, cell structure damage and alteration in the air-cell spongy mesophyll. Luo *et al.* (2013) reported that the maximum reflectance from non-infested plants was due to the strongest multiple scattering and transmittance in the NIR region. This damage could be spectrally detectable in the visible and NIR spectral regions (Zibrat *et al.*, 2021). Remote sensing is one of the best available methods for detecting aphid damage in the field.

It can be concluded from this study that remote sensor data provides synoptic, spatial-temporal views, and their integration can lead to a better understanding of plant cases. However, stressors such as climate change, wind, temperature, soil moisture levels, precipitation, crop variety and soil types influence the function of remote sensing indicators of chilli crop properties. The spectral curve indicated that the infested leaves' reflectance in the NIR decreased with time, corresponding with the aphid population. These results indicate that HI is a promising method for monitoring crops. Infestation of chilli plants by aphids results in a physiological response occurring that is detectable using a remote sensor of leaf reflectance. Per cent surface reflectance from healthy chilli was higher in the NIR portions of the spectrum when compared with aphids-infested chilli. This study shows that by using sub-leaf spatial resolution, early damage by pests can be spectrally detected by HI. Early detection and remote sensors can significantly impact the agricultural industry. This information can allow a farmer to identify problems and deal with them earlier than conventional means of detection.

الملخص

عطشان، لفته عوض، هاني أحمد إبراهيم، خالد جابر الحسيناوي وكواكب عوض عطشان. 2024. استخدام صور جهاز فائق الطيفية لتحديد عوامل انعكاس الأشعة على أوراق نبات الفلفل الحار المصابة بحشرة من الدراق الأخضر. مجلة وقاية النبات العربية، 42(3): 299-305.

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تسبب حشرات المن تغيرات فيسولوجية على نبات الفلفل الحار والتي تؤدي إلى تغيرات في الأشعة المنعكسة من أجزاء النبات المختلفة والمقاسة بواسطة أجهزة الاستشعار عن بعد. تستخدم أجهزة القياس الضوئي لمعرفة التغيرات في الموجات المنعكسة من أوراق النباتات السليمة والمصابة، فهناك ثمة اختلاف في الأطوال الموجية والتي يمكن قياسها وكشفها بواسطة صور جهاز فائق الطيفية بعد تحليلها. تتسبب حشرات من الخوخ بهدم أو تدمير الصانعات الخضراء في أوراق النباتات والذي يمكن تحديده بتحليل الصور فائقة الطيفية، حيث يمكن اكتشاف هذا الضرر طيفياً في انعكاس المناطق المرئية والقريبة من الأشعة تحت الحمراء. أظهر المنحنى الطيفي أن الفلفل الحار المصاب بحشرة المن يترك انعكاساً يتناقص مع الوقت ومع زيادة أعداد حشرة المن. على الرغم من أن نشاط حشرات المن يحدث على الوجه السفلي للأوراق، إلا أنه يمكن الكشف عن أضرارها طيفياً من خلال البيانات المنعكسة من الوجه العلوي. إن الكشف المبكر عن الأضرار التي تسببها الآفات (المن) في مساحات معينة على النبات، والتي تظهر في منحنى قراءات الأجهزة فائقة الطيفية مقارنة مع المناطق السليمة، تظهر واضحة بقياسات انعكاسية الضوء غير المرئي، حيث تتخفف في أجزاء النبات المصابة مقارنة بالأجزاء السليمة، والتي زادت بشكل واضح مع تقدم الإصابة. بلغت دقة تشخيص الكشف عن الأضرار في النباتات المصابة باستخدام النسبة المئوية للانعكاس حوالي 89%. كذلك بينت النتائج أن الأجهزة فائقة الطيفية أعطت نتائجاً يمكن الاعتماد عليها في تحديد الأجزاء والمناطق المصابة من النباتات، سواء كانت ضمن مناطق محددة أو في الحقول. أشارت هذه النتائج إلى أنه يمكن استخدام أجهزة الاستشعار عن بعد للكشف عن الأضرار التي تصيب المحاصيل، وبذلك يمكن إدارة الآفة (حشرات المن) في المراحل الأولى من الإصابة قبل أن تستشري. هدفت هذه الدراسة إلى معرفة قدرة الأجهزة فائقة الطيفية على تحديد الأضرار التي تسببها حشرات المن على محصول الفلفل الحار.

كلمات مفتاحية: التصوير فائق الطيفية، حشرة المن، الفلفل الحار، الأشعة تحت الحمراء.

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