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POTENTIAL FOR EARLY DETECTION OF POWDERY MILDEW IN OKRA UNDER FIELD CONDITIONS USING THERMAL IMAGING

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Abstract

In recent years, apprehensions surrounding the pervasive employment of chemical control methods in global agricultural production have intensified, primarily due to their detrimental effects on non-target organisms. This situation accentuates the importance of technology-driven alternatives for managing plant diseases in agriculture. One such technological innovation, thermal imaging technology, has emerged as a promising tool for the early detection of plant diseases. Infections often induce stress in plants, leading to either elevated or reduced temperatures at the point of infection. It is postulated that thermal imaging may effectively identify such temperature deviations in plant tissues afflicted by disease during the initial stages. The study investigated temperature differences in leaves infected by Erysiphe cichoracearum, with disparities up to 1.6 °C. Over three weeks, the surface temperatures of numerous leaves were analysed at 30-minute intervals. In three weeks period, it was shown that infected leaf surfaces had significantly lower average daily temperatures than ambient and healthy leaf temperatures. Furthermore, healthy leaf temperatures remained consistently lower than ambient temperatures throughout the study.

Key words: *thermal imaging, okra, powdery mildew*

INTRODUCTION

Globally, plant diseases have been responsible for substantial yield losses in agricultural regions where cultivation occurs [18]. For years, pesticides have served as the primary means of mitigating these diseases [29]. However, recent policy decisions by the European Union have led to restrictions on the usage of chemical pesticides that negatively affect non-target organisms [45]; [5]; [38]; [12]. Consequently, these developments have spurred interest in alternative control methods that do not rely on chemical applications. The employment of agricultural technologies, such as Precision Agriculture and Remote Sensing, has emerged as a vital approach for managing diseases within this context [39]; [33]; [14]. Early detection is one of the critical components of Precision Agriculture [9]. By utilizing sensors and other technologies such as thermal imaging, farmers can monitor their crops in real-time and detect signs of disease before they become visible to the naked eye. This allows farmers to take proactive measures, such as applying targeted treatments, adjusting irrigation and fertilization, and implementing other management practices, to mitigate the impact of diseases on crop yields. In summary, early detection of plant diseases is an essential component of Precision Agriculture, which can help to optimize crop production and improve sustainability in agriculture [9]; [6]; [8].

Okra (*Abelmoschus esculentus*) is a mallow family semi-fibrous plant. It is grown as a single in warm climates and as a perennial in hot climates. Okra is a vegetable grown for its fruit, but it is also significant for the economic functions of its leaves, seeds, flower parts, and stems. *Abelmoschus* is thought to have evolved in South and Southeast Asia. It is also a popular vegetable grown in tropical, subtropical, and temperate climates across the world. Commercial cultivation occurs in Turkey, India, Pakistan, Bangladesh, Afghanistan, Iran, West Africa, Burma, Yugoslavia, Japan, Malaysia, Brazil, Ghana, Ethiopia, Cyprus, and the southern United States [22]. In 2018, 9,872,826 tons of okra were produced in the world [32]. Plant diseases are the leading factors that cause yield loss and a decrease in product quality in agricultural areas where okra is grown in the world. Powdery mildew caused by *Erysiphe cichoracearum* can be an important disease of okra, as it can cause significant yield losses and affect the quality of the crop. Although the disease affects the plants' above-ground sections, the leaves are the most impacted [1]. *E. cichoracearum* becomes visible as the infection progresses. Initially, small white spots or patches can be seen on the upper surface of the plant leaves [17]; [30]. These spots may appear as small, raised bumps or as a thin layer of white dust. As the infection progresses, these spots can grow larger and merge with each other, forming a continuous coating over the leaf surface. Eventually, the entire leaf can become covered with a white or greyish powdery coating. It may be difficult to see powdery mildew infection in its early stages, as the initial spots can be quite small and difficult to distinguish from other leaf discolourations. It is important to regularly inspect plants for signs of powdery mildew infection, as early detection and treatment can help prevent the spread of the disease [9]; [7]. As the disease progresses, many conidiospores form on the leaf and can easily spread throughout the plant, causing a secondary infection. The disease factor also spreads intracellularly and intercellularly in the plant tissue, weakening the plant [43]; [23]. Infections caused by diseases in plants reveal temperature differences that can be detected using thermal imaging methods before visible symptoms occur in the regions where infections occur on plants. The use of thermal imaging to examine temperature changes resulting from stressful situations allows for the early detection of pests before their symptoms are noticeable [37]. As opposed to human observation, technology-based automatic detection techniques, such as thermal imaging, can help us gather data more quickly and accurately. Contrasted with human observation, technology-based automated detection methods, such as thermal imaging, facilitate the expeditious and precise acquisition of data [24]. This approach also holds the potential to reduce the costs associated with early detection [25].In a study conducted by Pineda et al. (2020) [37], a localized accumulation of salicylic acid (SA) was observed in areas where the plant exhibited a hypersensitive response (HR) to tobacco mosaic virus (TMV) infection, accompanied by a temperature increase. Likewise, Williamson et al. (2007) [46], demonstrated that *Botrytis* infection led to a moderate elevation in bean leaf surface temperature. Temperature changes caused by fungal diseases in plant parts can potentially be a clue for early diagnosis. Fungal diseases can alter the temperature of the infected plant tissue, and these temperature changes can sometimes be detected with thermal imaging or infrared cameras. This research aims to describe the potential for early detection of *E. cichoracearum* on the okra leaves using thermal imaging. Early detection of this infection may indicate that it is potentially possible to control the infection before it has spread to all other healthy plants. Preventing the disease before it spreads widely could reduce yield loss and damage to product quality as a result.

MATERIALS AND METHODS

This study was carried out in the fields of okra cultivation at Bursa Uludağ University (Figure 1). It was investigated that the possible stress caused by *E. cichoracearum* on okra could potentially be detected by thermal imaging methods, considering the temperature differences in the plant.

Fig. 1. A: The cultivation area. B: The location of the okra cultivation area. Coordinates: lat 40° 13' 36.10" N, long 28° 51' 53.80 "E, alt 50 m asl
Source: Image taken by the authors from field.

Collection of Temperature Data and Disease Diagnosis

The Near-Infrared (NIR), Mid-Wavelength Infrared (MWIR), and Long-Wavelength Infrared (LWIR) are distinct areas of the infrared spectrum, a subset of the electromagnetic spectrum. In this study, LWIR cameras are deemed more suitable for detecting plant disease for several reasons. In the LWIR range (8-14 m), most items, including plants, have a high emissivity, meaning they emit infrared radiation efficiently. This strong emissivity results in more precise temperature measurements and enhanced thermal imaging. Compared to other infrared bands, LWIR radiation is less absorbed by atmospheric gases such as water vapour and carbon dioxide. This results in less interference and improved image quality while imaging plants and their environment [19]. The study employed a portable LWIR camera with a detector resolution of 464 x 348 pixels and a thermal sensitivity of fewer than 40 millikelvins (mK) (Figure 2).

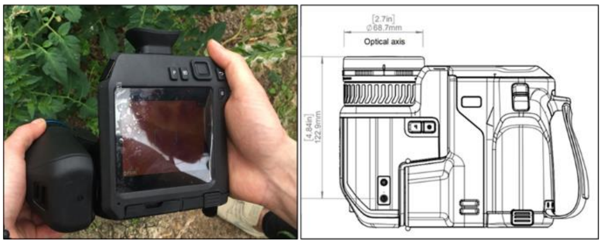

Fig. 2. A portable thermal camera with a detector resolution of 464 x 348 pixels and a thermal sensitivity of fewer than 40 millikelvins (mK).
Source: Image taken by the authors from field.

To obtain more precise results, the emissivity was tuned near to one and a lens with a spatial resolution of 0.90 m/rad pixels was utilized.

In the 200 m^2 okra field, a total of 100 young leaves on 50 plants with homogenous distribution were randomly selected and labelled. Throughout the study, all thermal measurements were made manually on the labelled leaves with a portable camera. The average temperatures of leaf surfaces infected by *E. cichoracearum* and healthy leaf surfaces were monitored and recorded simultaneously with the mean temperature of the environment. The FLIR Thermal Studio software was used to compute the mean temperatures of the leaf surfaces in the thermal images. As a control, leaves that were not infected were used. Powdery mildew infects young leaves more frequently than mature or aged leaves. Young leaves are especially sensitive due to their delicate and fast-expanding tissues, which create optimal conditions for the spores to germinate and invade the plant cells. In addition, immature leaves typically have a thinner cuticle, which makes it simpler for the fungus to start and thrive [13]. For these reasons, in the present study, all measurements were made on the young leaves of okra plants.

Light microscopy can be used to diagnose the powdery mildew disease caused by the fungus *E. cichoracearum* in okra. Typically, field samples of infected leaves are collected and examined under a light microscope to identify the fungus' distinctive features, including mycelium, conidiophores, and conidia. *E. cichoracearum* was identified using the similar methods of Newcombe et al. (2004) [34], using a light microscope. Infected leaves other than *E. cichoracearum* were excluded from the study. Compared to healthy leaves, those exhibiting abnormal temperature differences were monitored, and the presence of the disease was subsequently diagnosed over time using the microscopic examination. Leaves displaying infections caused by agents other than *E. cichoracearum* were excluded from the study, with attention solely given to the target disease and healthy leaves under consideration.

Measurement time

Within the field where the study took place, 100 young okra leaves were randomly marked in the region where the disease began to spread. At least one healthy young leaf and one infected young leaf were tagged for each plant to determine the mean temperature of the surface. The research was conducted on young leaves of okra. The temperature measurements on the leaves may be affected by environmental factors such as the sun's intensity [40]; [15]. Therefore, thermal images of all labelled leaves were taken every 30 minutes between 05:30 and 17:30 for three weeks. A handheld thermometer was used to record the ambient temperature at the same time. Due to the distance between plants, it took about 15 minutes to photograph every 30 minutes with a portable thermal camera. All thermal imaging was performed manually.

Measuring distance

Thermal imaging decreases the margin of error in temperature readings by using high-resolution cameras and close-range observations. To increase the sensitivity of the temperature measurement in this investigation, measurements were taken with a thermal camera at 0.4 m from the leaf surface [33]; [26]. All temperature measurements on the leaves were carried out at approximately the same angle (90°) and distance (0.4 m). The "FLIR Thermal Studio" tool was used to compute the temperature averages of infected and healthy leaf surfaces. The JMP®16 software was used to analyse the mean temperature of the ambient air and the leaves.

Statistical analysis

Statistical analysis was performed with the daily averages of the surface temperatures of the leaves infected by *E. cichoracearum* and the leaves without any infection and the ambient temperature measured simultaneously. The statistical significance of the temperature differences was established using the JMP®16 software's analysis of variance (ANOVA) methodology. To examine the difference between the means, the Tukey HSD post hoc test was performed (0.05).

RESULTS AND DISCUSSIONS

In certain leaves infected by *Erysiphe cichoracearum*, while the ambient temperature averaged 16 °C, significant temperature differences were observed between the infected and healthy regions of the leaf. The temperature disparity between healthy and infected areas can be as high as 1.6 °C, as illustrated in Figure 3.

This serves as an example of the localized temperature variations that can occur on the leaf surface.

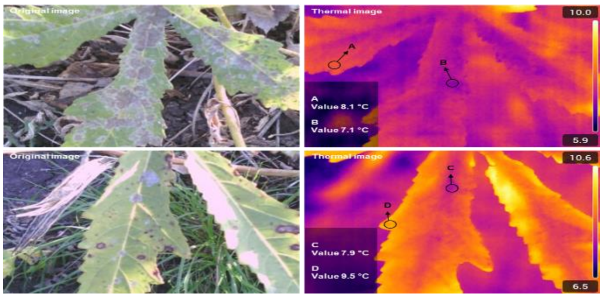

Fig. 3. The region where the mildew disease is intense (B and C) and the region where the infection is not (A and D). The ambient temperature was 16 °C.

Source: Image taken by the authors from field.

Table 1. The average surface temperature of leaves without any infection was measured every 30 min between 5:30 - 17:30 for 3 weeks. (A: Ambient Temperature, B: Healty Leaves Temperature, C: Infected Leaves Temperature, W: Weeks)

Time	A			B			C		
	1W	2W	3W	1W	2W	3W	1W	2W	3W
05:30	9.0	9.7	9.9	7.4	7.9	8.1	6.8	7.3	7.5
06:00	9.5	10.2	10.2	7.5	8.0	8.2	6.8	7.3	7.6
06:30	10.1	10.5	8.4	7.6	8.1	8.3	6.8	7.3	7.6
07:00	11.1	11.6	11.5	7.7	8.2	8.4	6.8	7.3	7.6
07:30	12.1	12.7	12.9	7.8	8.3	8.5	6.9	7.4	7.6
08:00	12.4	12.6	13.1	7.9	8.4	8.6	6.9	7.4	7.6
08:30	13.8	14.3	14.5	8.6	9.1	9.4	7.6	8.1	8.4
09:00	14.7	15.2	15.6	9.3	9.8	10.1	8.3	8.8	9.1
09:30	16.2	16.7	16.9	10.8	11.3	11.6	9.8	10.3	10.6
10:00	18.9	19.5	19.6	11.5	12.0	12.3	10.6	11.1	11.3
10:30	19.6	20.2	20.5	12.2	12.7	13.0	11.3	11.8	12.0
11:00	21.5	22.1	22.0	12.9	13.5	13.7	12.0	12.5	12.7
11:30	22.7	23.2	23.2	14.5	15.1	15.3	12.7	13.2	13.5
12:00	24.2	24.6	25.2	15.2	15.8	16.0	13.5	13.9	14.2
12:30	25.7	26.3	26.2	15.9	16.5	16.7	14.2	14.7	14.9
13:00	26.5	26.9	27.6	16.7	17.2	17.4	14.9	15.4	15.6
13:30	27.7	28.0	28.7	16.8	17.3	17.6	15.0	15.5	15.7
14:00	28.9	29.4	29.6	17.5	18.0	18.3	15.7	16.2	16.5
14:30	29.4	29.8	30.3	18.2	18.7	19.0	16.4	16.9	17.2
15:00	31.1	31.5	31.5	18.9	19.3	19.7	17.2	17.6	17.9
15:30	28.0	28.2	28.8	18.8	19.4	19.5	17.3	17.7	17.8
16:00	25.5	26.0	26.5	17.8	18.2	18.8	16.5	16.9	17.2
16:30	23.2	23.7	24.2	16.9	17.4	17.7	15.7	16.2	16.5
17:00	20.8	21.2	21.6	15.7	16.5	16.5	15.0	15.5	15.8
17:30	19.0	19.4	20.1	14.9	15.4	15.6	14.3	14.8	15.0

Source: The data was obtained by the authors as a result of fieldwork.

However, to obtain more precise data, the average surface temperature of a larger number of leaves was analysed between 5:30 and 17:30 hours at 30-minute intervals for 3 weeks.

The weekly averages of surface temperatures for *E. cichoracearum*-infected leaves and healthy leaves, along with the simultaneously measured ambient temperature data between the hours of 5:30 and 17:30, are presented in Table 1.

Drawing upon the data presented in Table 1, the average daily surface temperatures of both infected and healthy leaves, as well as the average daily ambient temperature, were subjected to statistical analysis.

Throughout the three-week thermal imaging period, the infected leaf surfaces' average daily temperatures were found to be statistically lower compared to those of the ambient and healthy leaf temperatures. Moreover, healthy leaf temperatures were consistently and significantly lower than ambient temperatures across all weeks (Figure 4).

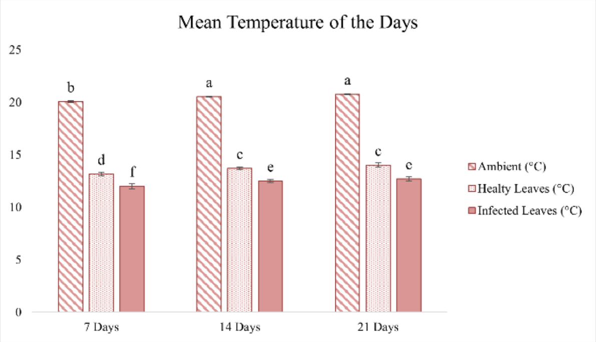

Fig. 4. The daily mean surface temperatures of infected and healthy leaves and the daily mean value of ambient temperature were statistically evaluated over a 3-week period (df: 8;54, F: 612.1962, P < 0001).

Source: The author's JMP®16 program calculations based on field data.

Fungal diseases can cause stress in plants, affecting various physiological processes such as photosynthesis, respiration, and transpiration. These factors can lead to changes in the temperature of the leaf surface [10]; [11]; [16]; [31]. Thermal imaging can be used to detect an increase or decrease in temperature in infected areas compared to healthy areas [44]; [28]; [47]. Therefore, in this study, the thermal imaging method was used for the potential early detection of the powdery mildew disease agents in okra.

Thermal imaging has previously been shown to be capable of detecting certain viruses and fungal infections. In the study by Pineda et al. (2020) [37], localized accumulation of salicylic acid (SA) was observed in the regions of the plant with a hypersensitive response (HR) to Tobacco mosaic virus (TMV) infection and a temperature increase occurred. Like fungal diseases, viruses can negatively affect transpiration, respiration, and photosynthesis in plants through various mechanisms. Thus, the temperature may increase on leaf surfaces that are unable to transpire properly [4]; [20]; [35]. However, in the present study, it was found that the average daily temperatures were lower in okra leaves infected by *E. cichoracearum* compared to healthy leaves. In a manner consistent with our study, Oerke et al. (2006) [36], used thermal imaging to investigate the effects of downy mildew (caused by the fungus *Pseudoperonospora cubensis*) and environmental conditions on cucumber leaves. The study shows that the disease agent can lower leaf temperature. In this study, the temperature decreases on leaf surfaces due to *E. cichoracearum* may result from fungal evaporation. The observed effect may be attributed to the rapid reduction of surface temperature resulting from fungal dehydration through evaporation. Unlike plants, fungi do not possess stomata, a vascular system, or the capacity for transpiration. Although fungi can lose water through evaporation from their hyphae, this process is passive and cannot be equated to plant transpiration in terms of regulation and velocity [42]; [3]; [2].

In contrast, Stoll et al. (2008) [41] report that in studies on wheat canopies, higher temperatures were observed for *Fusarium*-infected ears (containing grains). The increase or decrease in temperature on infected leaves compared to healthy ones may be dependent on the location of the phytopathogenic fungi on or within plant tissues, their developmental stage, or fungal species [36]; [27]. There are other plant pathogenic fungi, like *E. cichoracearum* used in this study, that produce mycelium on the plant leaf or fruit surface. For example, the fungus *Botrytis cinerea* causes gray mold disease in a wide range of plant species, including fruits, vegetables, and ornamental plants. This fungus forms hyphae on the plant surface, leading to the decay of plant tissues [46]. On the other hand, some plant pathogenic fungi, such as *Sclerotinia sclerotiorum*, *Phomopsis spp*., *Venturia inaequalis*, and *Fusarium*, can overwinter inside plant leaves, seeds, or fruits, either as spores, mycelium, or fruiting bodies [1]. In future studies, when investigating temperature differences on the leaf surface, it may be necessary to consider factors such as the type and developmental stage of the phytopathogenic agent, the plant's photosynthesis [9], and some environmental factors that could influence thermal imaging [21].

In summary, although it was found that *E. cichoracearum* caused a temperature decrease in okra leaves, it is evident that there may be many factors affecting the temperature fluctuation.

CONCLUSIONS

Depending on the type of disease agent, environmental temperature, time, or plant, an increase or decrease in temperature on the leaf surface may occur. These temperature differences, which are not detectable by the human eye but can be detected by thermal imaging, may hold considerable potential for the early detection of diseases. Activities that allow early control of diseases in the field before they spread to large areas can greatly prevent economic loss of crops and reduce the use of environmentally harmful chemicals in agricultural control. It is thought that further studies in this field will contribute to sustainable agriculture.

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