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Using Smartphone Application to Estimate the Defoliation Caused by Insect Herbivory in Various Crops

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Authors' Contribution
MIU designed the experiment. MA, SA and AA collected data and wrote the article. SK and SMAZ prepared figures. HMA analyzed the data, MA and JM. O reviewed the article.

Key words
BioLeaf, Foliar damage, ImageJ, Leaf area meter, Quantification, *Spodoptera litura*

ABSTRACT

Leaf area in plants is critical for the photosynthesis process. Thus, quantification of foliar area needed for the photosynthetic process is impartment. The recent methods used to quantify the foliar damage are relatively expensive and are mainly dependent on complex and state of art devices and instruments. Instead, smartphone application may be a good source to measure the foliar damage. In this study, foliar damage of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) on sunflower and soybean crops was measured using BioLeaf android application. The infected leaves due to citrus canker were also tested using BioLeaf for foliar analysis. Our results demonstrated that the quantification of foliar damage using BioLeaf application was easy and accurate. This method is also comparable with other techniques like digital analysis by ImageJ software and leaf area meter devices. With these results, it is concluded that the BioLeaf application has resulted in an accurate leaf area measuring tool of foliar damage with minimum time and cost and can be used in various crops for damage assessment.

INTRODUCTION

Plant leaves serve as a powerhouse for the plants. Food production in plants is dependent on photosynthesis that involves a process of converting solar energy into chemical energy using chlorophyll and in the process of evapotranspiration (Marcon *et al*., 2011). Leaf area greatly affects plant growth and reproduction (Agrawal, 2000). There are several studies reporting detrimental effects of defoliation by insect herbivory in terms of the reduced shoot and root biomass (Wirf, 2006), decreased flower, fruit or seed production (Hufbauer *et al*., 2013) and decreased seed size (Myers and Sarfraz, 2017).

Foliage quantification is important for studying the physiological features of plant-related growth, transpiration and photosynthetic processes (Filho *et al*., 2010). It is also useful in biochemical and molecular analysis of plant defense (Fescemyer *et al*., 2013; Miresmailli and Isman, 2014), plant fitness in transgenic cultivars (Letourneau and Hagen, 2012; Grinnan *et al*., 2013) and plant assaults on ecological studies (Moreira *et al*., 2014; Cronin *et al*., 2015). Therefore, quantifying the defoliation caused by insect herbivory is necessary for entomologists, experts and farmers for better decisions under integrated pest management (IPM) technique to control the insect pests including the evaluation of insecticides (Calixto *et al*., 2015).

Several conventional methods are being used to measure foliar damaged area such as visual estimation of defoliation, manual quantification based on the square-counting, determination of foliar dimensions, leaf area meter and use of electronic devices (Cristofori *et al*., 2007; Keramatlou *et al*., 2015). These methods not only demand expertise and experience but are also time-consuming and costly. Some methods also have a probability of inaccurate estimation of the foliar area (Machado *et al*., 2016).

Several latest studies also proposed automatic computational systems for estimation of the foliar area

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(Igathinathane *et al.*, 2006; Easlon and Bloom, 2014). Bradshaw *et al.* (2007), Mura *et al.* (2007) and Marcon *et al.* (2011) used scanners to quantify the foliar area of different crops. In all these studies, the area of healthy leaves was estimated only but the damage and lesions caused by insects were not quantified. Furthermore, these studies were also sensitive to noise, such as sand grains and small pieces of leaves which cause inaccuracy of leaf area quantification (Nazare-Jr *et al*., 2010).

Among the technologies invented over the last few decades, smartphones are intimately linked to the daily life of people (Xia *et al*., 2015). Smartphones have become a useful tool in agriculture because their mobility corresponds to the nature of agriculture. The cost of mobile devices is very accessible and their computational power makes it possible to create a variety of practical applications with high accuracy (Confalonieri *et al*., 2013). The farmers can easily use smartphone-based sensors and applications to increase crop production and facilitate various tasks throughout the agricultural cycle (Pongnumkul *et al*., 2015).

BioLeaf foliar analysis is the latest application which is used to estimate the foliar damage of leaves caused by insect pests. This smartphone application is a novel approach which calculates the intensity of foliar losses in relation to the total leaf area by using the image sensor (camera). This method is based on the techniques of image analysis and computational geometry which is applied to each leaf image. This application consists of different steps such as image thresholding, noise removal, and border construction using quadratic bezier curves and at last, insect herbivory quantification (Machado *et al*., 2016). The present study was conducted to correlate the accuracy of BioLeaf application with leaf area meter and image analysis technique to quantify the different type of foliar damage of soybean and sunflower leaves. The accuracy of BioLeaf was also measured for quantification of infected leaves of citrus due to citrus canker disease.

MATERIALS AND METHODS

In this experiment, leaves of soybean (*Glycine max* (L.) Merrill) and sunflower (*Helianthus annuus* L.) were collected from field located nearby University of Sargodha, Pakistan. We divided the collected leaves into two groups: natural feeding and artificial defoliation. Images of ten leaves from each group were captured using NIKON D5300, DSLR camera without flash, and positioned 25 cm from the leaves. The white portable background was used during image capture. The images were finally stored in computer hard disk in TIFF format.

Natural herbivory

For natural feeding, third instar larvae of *Spodoptera litura* were taken from the reared culture in the laboratory of Entomology, University of Sargodha. The larvae were starved for 24 hours prior to provision of leaves for natural defoliation (See Fig. 1).

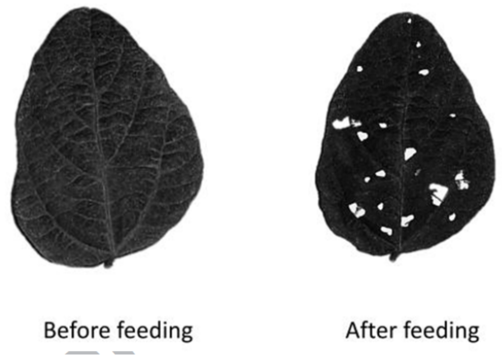

Fig. 1. Natural feeding of *S. litura* larvae.

Artificial herbivory with regular damages

The leaves were damaged manually using scissor. Firstly, 25% of biomass was removed by a single cut of a quarter portion of the leaf. Secondly, 50% of biomass removed by cutting half portion of the leaf (See Fig. 2).

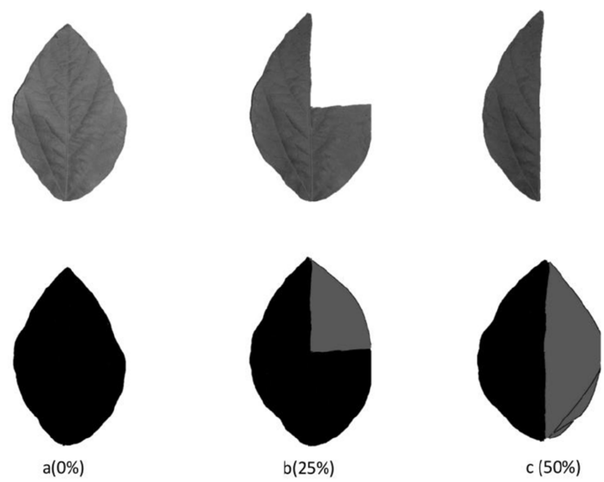

Fig. 2. Artificial regular damage of leaves (upper portion showed, a = healthy leaf, b = 25% damage, c = 50% damage and lower portion is the reconstruction of damage in BioLeaf application for damage quantification)

Artificial herbivory with irregular damages

For irregular damage, the leaves were cut by multiple circular holes of leaf blade with border damage. The location of the holes was not specific. The internal holes with border damage produced randomly. We divided irregular damage of soybean and sunflower leaves into two types. In type 1, only one internal hole with border damage was select. In type II, three internal holes with border damage were produced (See Fig. 3). The infected leaves of citrus due to citrus canker disease were also evaluated (See Fig. 4).

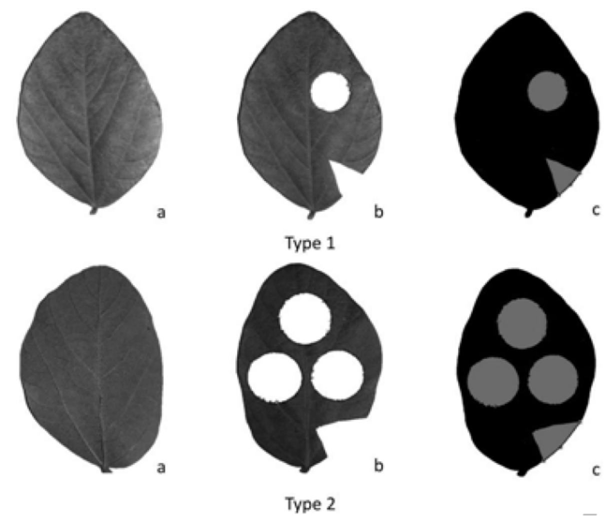

Fig. 3. Artificial irregular damage (Type 1, and II), a = healthy leaves, b = irregular damage, c = hole and border reconstruction in BioLeaf application for damage quantification.

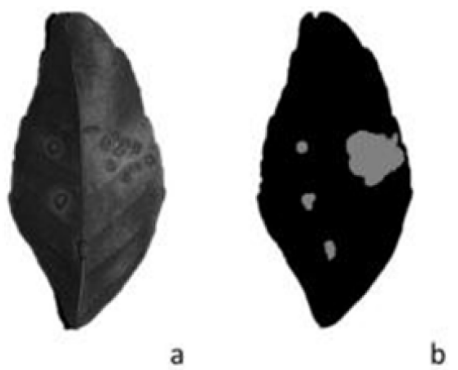

Fig. 4. Infected leaf of citrus due to citrus canker disease (a = natural damage, b = quantification of damage by BioLeaf application).

Measurement of percent defoliation

BioLeaf foliar analysis application

The quantification of leaf damage using smartphone application is a new platform which performs the task with better results. BioLeaf foliar analysis is a smartphone application to quantify the leaf damage by reducing analytical cost and time for measurement and comparable with other expensive devices (Machado *et al*., 2016). Different types of leaf damage were measured to check the accuracy of this application with leaf area meter and image analysis using ImageJ software. The application is free and can be downloaded from GooglePlay at https://play.google.com/store/apps/details?id=upvision.BioLeaf. We used the application in Samsung S5 mobile to quantify the foliar damage.

Leaf area meter

Leaf damage was also quantified with CI-202 leaf area meter (CID, Bioinc USA). Before and after natural or artificial damage, leaf area was measured by leaf area meter. The percent defoliation was calculated by dividing after value over before value and multiplied with 100.

Image analysis

An image of each selected leaf was captured by a digital camera. The leaves were placed on white background. The distance between camera lens and object (leaves) were kept constant using camera stand. Images were captured, arranged in numbers and stored in computer hard drive for further analysis. The leaf area was measured by ImageJ software. When the image was opened in software, trace mode was selected to get the desired portion of leaves and adjust the threshold to get red leaf image. Hue ranges were set from 47 to 107 and saturation from 0 to 100 (Richardson *et al*., 2001) to identify the green pixels (leaves). The leaf area measurement was obtained in pixels. So, a reference object was used to calculate the area in cm^2 instead of the pixel at a constant distance. To convert pixel values into cm^2, five rupees coin was used as a reference object. The reference object is an object with a known area (See Fig. 5).

The area of a reference object was measured as suggested by Patil and Bodhi (2011):

$$Area\ of\ coin = \pi r^2$$

$$r = \frac{d}{2}$$

r = radius, d = diameter, the diameter of coin was 1.8cm. So, the radius of the coin was 0.9cm.

$$Area\ of\ coin = 3.14\ X\ 0.9^2$$

$$Area\ of\ coin = 2.54\ cm^2$$

So, the leaf area measured in pixels was converted into cm^2 by this method.

Data analysis

To check the accuracy of BioLeaf application, we analyzed the data by linear correlation. The percent defoliation of leaves with BioLeaf was correlated with the data from CI-202 leaf area meter and ImageJ software.

Fig. 5. Reference object to convert the area from pixel to cm^2.

RESULTS

We evaluated the quantification of foliar damage using smartphone application in comparison to leaf area meter and image analysis method. First, we described the artificial defoliation with regular and irregular damage. Secondly, the natural damage by *S. litura* was quantified.

Artificial defoliation with regular damage

The results showed that there was a highly positive correlation of BioLeaf with ImageJ and CI-202 LAM. A linear correlation was observed similar in 25% artificial defoliation of soybean leaves with correlation coefficient R ≥ 99 at $P<0.001$. At 50% artificial defoliation of soybean leaves, the linear correlation of BioLeaf with CI-202 LAM was slightly lower as compared with ImageJ. The correlation coefficient was R ≥ 96.1, R ≥ 99.1 at $P<0.001$ for CI-202 LAM and ImageJ respectively (Fig. 6A). In the case of artificial defoliation of sunflower leaves, the linear correlation was similar at both 25% and 50% defoliation. The correlation coefficient was R ≥ 98.2, R ≥ 98.5 at $P<0.001$ for CI-202 LAM and ImageJ respectively, in 25% defoliation. A similar value of correlation coefficient was observed in 50% defoliation of sunflower leaves (Fig. 6B).

Artificial defoliation with irregular damage

In type I irregular damage of soybean leaves, the linear correlation was slightly higher in the case of ImageJ

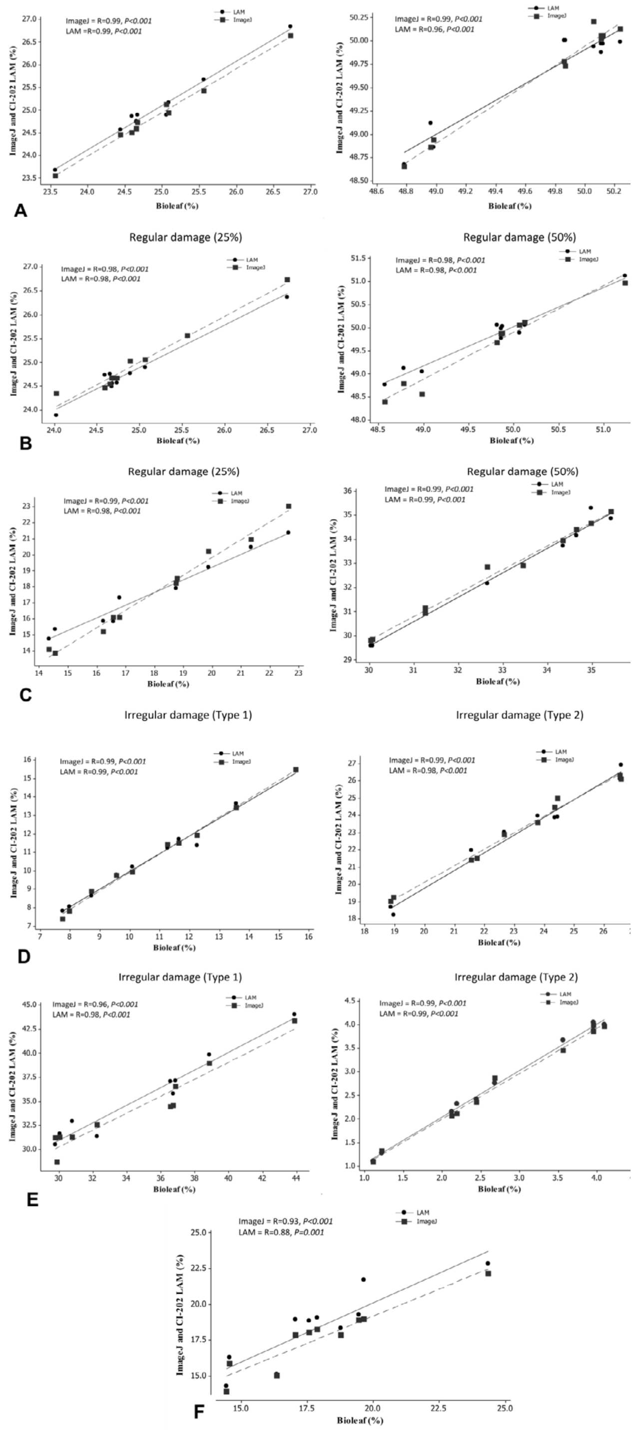

Fig. 6. Relationship of BioLeaf analysis with ImageJ and CI-202 LAM for quantification regular damage of soybean leaves (A), sunflower leaves (B), soybean leaves (C), sunflower leaves (D), and for quantification of natural damage of soybean and sunflower leaves due to *S. litura* feeding (E), and quantification of infected leaves of citrus due to citrus canker disease (F).

compared with CI-202 LAM. The correlation coefficient was R ≥ 99.4, R ≥ 98.5 at *P<0.001* for ImageJ and CI-202 LAM respectively. In type II damage of soybean leaves, the correlation of BioLeaf was observed similar with ImageJ and CI-202 LAM, which was highly positive (Fig. 6c).

In type I irregular damage of sunflower leaves, the correlation was highly positive with correlation coefficient R ≥ 99.3, R ≥ 99.7 for LAM and ImageJ respectively at *P<0.001*. But for type II damage, the correlation of BioLeaf with ImageJ was slightly higher compared with LAM. The correlation coefficient was R ≥ 99.4, R ≥ 98.9 for ImageJ and LAM respectively at *P<0.001* (Fig. 6D).

Natural feeding

The linear correlation of BioLeaf was observed higher with LAM compared to ImageJ in case of natural defoliation of soybean leaves. The correlation coefficient value was R ≥ 98.0 for LAM and R ≥ 96.0 for ImageJ at *P<0.001*. For natural defoliation of sunflower leaves, the correlation was similar for both LAM and ImageJ with correlation coefficient R ≥ 99.0 at *P<0.001* (Fig. 6E).

Quantification of citrus canker damage

The infected leaves for citrus canker disease were also quantified with the BioLeaf application and compared with LAM and ImageJ analysis. The linear correlation was higher for ImageJ with correlation coefficient R ≥ 93.7 at *P<0.001*. The correlation of BioLeaf with LAM was lower when quantified the citrus canker leaves damage. The correlation coefficient was only R ≥ 88.9 at *P = 0.001* (Fig. 6F).

DISCUSSION

The quantification of foliar damage is very important for crop production and to make strategies against this damage. Many studies were conducted on the effects of foliar herbivory, for forecasting the crop production (Strauss *et al*., 2001; Lizaso *et al*., 2003) and artificial defoliation analysis (Susko and Superfisky, 2009; Johnson, 2011; Li *et al*., 2013). Therefore, quantifying the damage caused by insect herbivory is important with respect to supporting farmers to take better decisions, such as assessments of insecticide management. There are several studies in which the foliar damage was estimated by different methodologies like visual estimates Stotz *et al*. (2000), hand tracings of injured leaves (Pfister *et al*., 2012), leaf area meter (Pandey and Singh, 2011; Ullah *et al*., 2016) and image analysis (Alchanatis *et al*., 2000; Su and Messenger, 2000). The current study was introduced the smartphone application named as BioLeaf foliar Analysis which is already described first time by Machado *et al*. (2016). The foliar damage was correlated with other methods like leaf area meter and image analysis. The results showed that BioLeaf was highly correlated with both methods either the damage was artificial or natural. In artificial defoliation either it was regular or irregular damage, the BioLeaf also showed a positive correlation. We also checked the efficacy of BioLeaf for estimation of citrus canker damage and the results were highly correlated with image analysis. The results showed that disease severity can be estimated by using BioLeaf application.

BioLeaf was proved an accurate method to quantify the foliar damage of soybean and sunflower leaves. It was easy to use, time-saving, free available and can be used to estimate foliar damage of any crop. According to Machado *et al*. (2016), the BioLeaf application is a non-destructive tool which can be used to estimate the foliar damage in the field without removing leaves from plants and can be used to contours damaged or multiple types of damages caused by insect pests. The other methods to quantify the damage are too much costly like leaf area meter and image analysis which required digital camera and desktop. In contrast, the BioLeaf application is freely available on Google play store of mobile phones which are accessible for popular prices (Machado *et al*., 2016).

The BioLeaf application proved as a highly precise method for quantification of foliar damage in comparison to other techniques such as leaf area meter and image analysis. Previously, researchers have also reported different smartphones application such as LAI Canopy (Fuentes *et al*., 2012) and Leaf IT (Schrader *et al*., 2017) to measure leaf area index (LAI). In comparison to these applications, BioLeaf is accurate application for the leaves having complex morphologies and can easily detect the margins of damaged leaves.

However, the use of LAI data using smartphone application is limited due to a lack of confidence in data recording. In addition, the user can collect data independent of each other, so the quality of the data set is often unknown. This can further lead to oversampling and incomplete data collection. Therefore, it is important to test the application systematically, and to asses the quality control mechanisms as well.

CONCLUSION

BioLeaf foliar analysis is semi-automatic, portable, multi-language and an accurate application to complete the task of foliar damage quantification. We compare this smartphone application with other traditional methodologies for damage quantification of soybean and sunflower leaves as well as infected leaves of citrus due to canker disease and found it as a highly precise application. In a future study, the field measurements should be done to quantify the LAI and have to be checked for the quality. There is a need to check the validation of the smartphone application's results and to check the accuracy for different sized leaves and for different herbivory defoliation.

Statement of conflict of interest

The authors declare there is no conflict of interest.

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