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COMPARISON OF DIFFERENT TOOLS AND METHODS IN THE MEASUREMENT OF LEAF AREA IN ALFALFA

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Abstract: Leaf area measurements in the field are very difficult to determine the yield. Use of the mobile application has come to the fore due to its practical use and ease of transportation. Therefore, in this study, the ease of use and accuracy were tested by determining area of leaf with an automatic leaf area meter and a mobile application called 'PETIOLE'. The leaf area of alfalfa plants (*Medicago sativa* L. cv Nimet) grown in the field, and measured in the field with the 'PETIOLE' mobile application and in laboratory conditions with automatically leaf area measurement device was measured. The automated leaf area meter mean for total leaf area was 61.65 ± 3.50; meanwhile the mean for the PETIOLE App assessment was 61.56 ± 3.15. As a result, the PETIOLE app measures the area of each leaf separately, more quickly and efficiently than before, particularly in the field.

Keywords: Alfalfa, PETIOLE App, LICOR, LI-3100C, Leaf area, *Medicago sativa* L cv

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1. Introduction

Plant physiological functions characterize anatomical, biochemical, physiological, or morphological properties of plant individuals or species that are significant to ecology and reveal the environmental standards a plant must cope with (Perez-Harguindeguy et al., 2013). Growing interest has been shown in researching the variance of plant properties (Díaz et al., 2016). Functional features from several species of plants, research, and locations have been compiled into sizable databases, yet there are still apparent taxonomy and geographical deficiencies (Jetz et al., 2016).

Leaf area one of the most crucial aspects of a plant's anatomy (Díaz et al., 2016). It may be viewed as a crucial attribute that affects other traits, and is used to anticipate the need for irrigation depending on evapotranspiration and performance (Perez-Harguindeguy et al., 2013). Furthermore, it is crucial for the development of the foliage economics continuity (Jetz et al., 2004), which is connected to variabilities in plant life approaches. It corresponds favorably with rate of photosynthesis, leaf nitrogen intensity, light infiltration, and comparative rate of growth and adversely with leaf durability and carbon equity (Garnier et al., 2017). Alfalfa (*Medicago sativa* L cv.) is the most widely utilized warm weather forage legume on the earth (Acharya et al., 2020). Plants are vulnerable against abiotic stress elements such as drought, salt, and high or low temperature, which results in yield loss in agricultural production (Janmohammadi et al., 2008). Alfalfa, which has %18 crude protein in its dry forage (Dale et al., 1983), is the most essential and beneficial quality roughage source in Türkiye and over the world. This plant, that has completed its growth, is still resistant to the effects of drought. It requires significantly more water, particularly in dry locations, than other farmed plants since alfalfa have a long harvest period and a rich vegetative portion (Wissuwa et al., 1997).

A variety of techniques are using to measure leaf area along with some traditional and non-traditional methods which are time consuming and laborious as well. Smart phones have an enormous opportunity for research (Welsh and France, 2012), since they are widely available, have powerful computational capability (Lane et al., 2010), and offer a variety of precise instruments such as Geolocation, cameras, and many sorts of sensing power (e.g., acceleration sensors, gyroscopes, magnetic field sensors, light sensors, barometers, thermometers, and air humidity sensors). Smart phones that use this combination of sensors might be useful tool for fieldwork (Welsh and France, 2012), especially because many of them are free. Despite the numerous precise sensors in cellphones, remarkably few software has been created as instruments for ecology and evolution, rendering them an underutilized resource (Teacher et al., 2013).

On the other hand, estimating leaf area in the field can be challenging since typical techniques involve a scanner, computer, and digital image processing using specialized and often costly software e.g. :Delta-T Devices (Cambridge, UK), LICOR (Lincoln, NE, USA), and

WinFOLIA (Regent Instruments Canada Inc.. It frequently limits leaf area analyses to labs with access to power and computers (Perez-Harguindeguy et al., 2013). As variety of techniques are available for leaf area assessment. Scientists have been exploring for further feasible and efficient ways to estimate leaf area. Therefore, the prime goal of this study is to assess the most effective leaf area measurement among traditional and mobile application methods for alfalfa crops.

2. Materials and Methods

This experiment was carried on alfalfa (*Medicago sativa* L. cv Nimet) at Niğde Omer Halisdemir University research field located in Niğde, Turkiye. As each plant have different number of leaves, so 20 random leaves samples were taken from each alfalfa plants. Leaves from three random alfalfa plants were collected to examine the comparison of two different leaf area measurement with PETIOLE mobile application and automatic leaf area meter LICOR, LI-3100C. In this research work comparison of mobile application (PETIOLE) and traditional method (automatic leaf area meter, LICOR, LI-3100C) in two different techniques: (i) individually measured (LICOR-I) and all leaves were measured at the same time (LICOR-II) as commonly applied for leaf area measurement were used for the estimation of leaf area.

Leaf area measurement directions: (1) Snap the leaf horizontally, with the leaf completely flat on the work-surface, and using a measure; (2) Launch ImageJ and open the image: File > Open; delete unnecessary things: Adjust the scoring system: image > Crop Set Scale > Analyze; (5) Configure Comparison: (6) Calculate the area: Image > Type > 8-Bit Image > Customize > Threshold; Analyze > Particle Analysis Following photography process, the leaves were measured with a leaf area meter (LICOR LI-3100C).

Leaf-IT operates on smartphone device with an Android operating systems and doesn't need a Web or data system connection. The integrated smartphone camera captures an image of leaves. Following picture acquisition, Leaf-IT performs area measurement using digital image processing in three steps: (1) border spotting of the leaf with well delineated borders, (2) pixel counts, and (3) comparison with a reference object with a known area. For optimum effects, set the leaf on a backdrop with a strong contrast to the leaf. For instance, for darker foliage, a white background works best.

Data were evaluated as means ± standard error showing $P<0.05$ were considered statistically significant. Box plots of variables were used for graphical representation.

3. Results

It is exhibited that mean of total leaf area measured by the automatic leaf area meter was 61.65 ± 3.50 cm^2 while, PETIOLE App measurement's mean was observed 61.56 ± 3.15 cm^2. According to ANOVA results, it showed non-significant difference ($P=0.106$) between the machines reading. On individual basis, no significant difference was observed in replications 1, 2, and 3. Analyses showed almost the same results with the average values of 2.89 and 2.82 cm^2 in replication 1, 3.43 and 3.36 cm^2 in replication 2, and 2.92 and 3.06 cm^2 respectively for LICOR, LI-3100C and PETIOLE app (Figure 1).

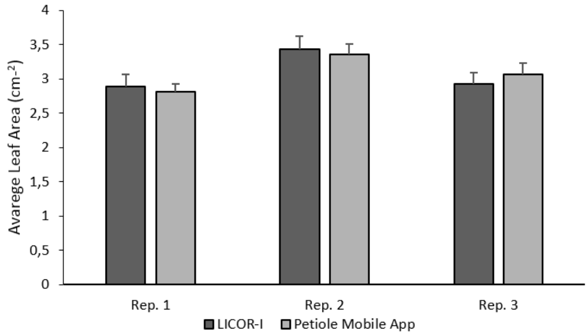

Figure 1. Average leaf area comparison of individual alfalfa leaf samples using LICOR-I and PETIOLE App (n=20).

On the other hand, when three different techniques (LICOR-I, PETIOLE App, and LICOR-II) were used to measure the leaf area, the results showed that the LICOR-II was found with the least leaf area measurement (Figure 2).

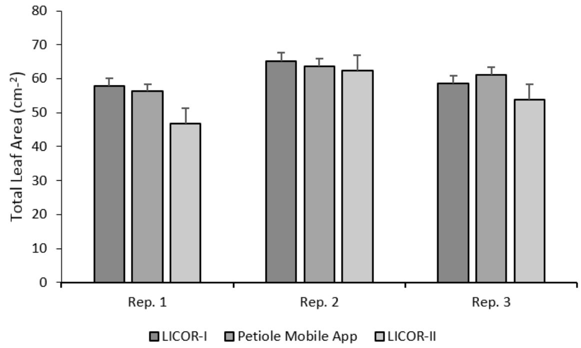

Figure 2. Total leaf area comparison of using with three different methods (LICOR-I, LICOR-II and PETIOLE App; n=20).

Whereas the LICOR-I exhibited the maximum values in replication 1 and 2 (57.8 and 65.2 cm^2) as compared to PETIOLE app values (56.3 and 63.7 cm^2) respectively. While, in replication 3 the petiole app was found with maximum leaf area 61.2 cm^2, as compared to LICOR-I 58.2 cm^2. According to the P values there is no significant difference between the two machines as shown in the box plot (Figure 3).

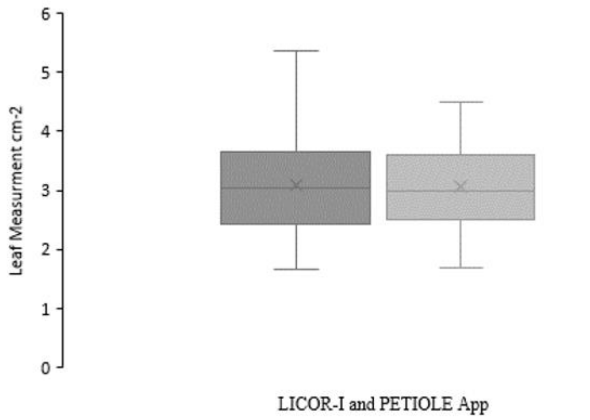

Figure 3. Box plot graph depicting comparison in individual alfalfa leaf samples measurement (LICOR-I; left) and PETIOLE App (right); n=60).

4. Discussion

This study was carried out to compare to different approaches (LICOR, LI-3100C and PETIOLE App) for leaf area measurement using alfalfa leaves as a sample. There was no significant difference was observed using both sources (LICOR, LI-3100C and PETIOLE App). A study carried out by Singh et al. (2021) estimating leaf area by using PETIOLE app also found no significant difference.

Similarly, Janmohammadi et al. (2008) found differences for height and time of year for the direct, destructive technique when comparing methods for estimating leaf area in palisade grass, and did not suggest the use of a meter for gathering LAI data for modeling purposes. Clarke and McCaig (1985) described a microcomputer-based leaf area measurement system that was capable of measuring multiple green scales, allowing the device to measure chlorotic portions of leaves. In cucumber and tomato, a time- and labor-saving alternative to calculating leaf area (using PETIOLE app) was put into place (Blanco and Folegatti, 2003). In a similar vein, Ramirez and Zullo Jr. (2010) used Quickbird satellite orbital photos to examine the leaf area parameter in a coffee crop and came to the conclusion that employing high-resolution imaging is a potential way for measuring leaf area. However, there are multiple results are available in the favor and contrast of our study.

5. Conclusion

Two different methods were used in this study for the estimation of leaf area analysis. This outcome indicates that there is a variance even in the measuring application. As a result, the PETIOLE app measures the area of each leaf separately, faster and efficiently than before, especially in the field. Individual leaf measurement with LICOR, LI-3100C is most effective than total leaf measurement with same equipment. Measurement in the filed with mobile application is not require to carry to plants until lap so it is more fast and more applicable in the field.

Author Contributions

The percentage of the author(s) contributions is present below. The author reviewed and approved final version of the manuscript.

	T.K.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

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