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Evaluation of the Performance of ImageJ, Leaf Doctor Applications, and Visual Assessments in Measuring Severity of Two Leaf Spot Diseases

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Abstract. In this research, reliability for disease severity assessment was compared for two different digital imaging quantifying techniques (ImageJ and Leaf Doctor applications) and visual assessments included ImageJ (IJ), Leaf Doctor (LD) applications, and visual assessments (VA) for the downy mildew of lettuce and Cercospora leaf spot of chard. Samples of infected chard leaves with symptoms of Cercospora leaf spot disease and lettuce leaves with symptoms of downy mildew were randomly collected from a field and a grocery in Al Anbar Governorate, Iraq. Each leaf was shot with iPhone 8 plus, provided with Dual 12 MP Camera, f1. 8, 28mm (wide), and f 2.8 (telephoto). The percentage of the diseased tissue of each shot was subjected to visual analysis and the ImageJ (IJ), Leaf Doctor (LD) applications. The measurements for them were subject to statistical analyses. Generally the results revealed a compatibility in the performance of the evaluated methods for the both diseases. There was positive and significant correlation coefficients between the measurement results of the VA and the LD for the downy mildew of lettuce ($r= 0.664$) and the Cercospora leaf spot of chard ($r = 0.8990$). Regarding the correlation between the IJ measurements and that of either VA or the LD revealed to be weak for both diseases. There were also linear regressions obtained only between the VA and the LD measurements ($R^2 = 0.441$ for downy mildew and $R^2 = 0.809$ for Cercospora leaf spot). The results of other experiment indicated positive correlation coefficients between the upper and lower disease severity measurements regardless the assessments methods for both diseases. The highest correlation coefficients and linear regression were found for the AV measurements ($r = 0.936$, $R^2 = 0.876$ and $r = 0.996$, $R^2 = 0.992$ for lettuce and chard respectively) followed by that of the LD ($r = 0.908$, $R^2 = 0.824$ and $r = 0.835$, $R^2 = 0.697$ for lettuce and chard respectively). This research indicates that the LD application is easier, faster and more reliable and accurate for disease severity measurements regardless the disease and its severity.

1. Introduction

Quantitative measurements of the plant diseases is the most difficult and important criterion in phytopathology as only on which the right decision on disease management can be taken. The right decision however, will pawn on the accuracy of the method applied for the disease severity measurement. Humans have gone through a long search for innovations in the quantitative measurements of plant diseases. Traditionally several methods of direct and indirect estimations were proposed to convert the disease injury to quantitative measurements, such as the nominal or descriptive scales, the ordinal scales, the interval or category scales, and the ratio scales [1]; [2]; [3]; [4]. In these traditional ways of the disease damage assessment specialists first need to reach the fields and measure the intensity through naked eye observation depending on a certain rating system [2]; [4]. These hand operated field assessments however, has



restrictions of being laborious, discontinuous, somewhat arbitrary, and imprecise with disease variations. Recently digital imaging quantifying techniques have been adopted using the modern information systems for automatic and semi-automatic severity measurement methods for plant diseases. The image sensing techniques have attracted the interest of many researchers and have been incorporated into plant disease study for their advantages in the analysis of automated, low-cost, non-invasive disease capabilities [5]; [6]; [7]; [8]. The application levels of such techniques ranged from unlimited field size of plant populations through the remote sensing technology to limited single plants or its single parts using professional cameras or smartphones. The digital images then treated and analyzed by the PCs, Tabs and Smart Phones provided by different software programs enable to measure disease severity in plant part such as the ImageJ and Leaf Doctor. The ImageJ is an open source java-based computer software developed by the National Institutes of Health (NIH, USA). It is one of the image processing software that has been considered in scientific researches [9]; [10]; [11]. The Leaf Doctor is a free application for quantifying plant disease severity for iPhone. The application is developed and taken into account in scientific research by Cornell University and the University of Hawaii at Manoa, College of Tropical Agriculture and Human Resources [12].

Leafy vegetable crops are affected by several leaf spot diseases as in the case of the chard and lettuce crops infected by the epidemic diseases of *Cercospora* and downy mildew respectively. The symptoms of the *Cercospora* spot disease on chard begin in form of small gray spots surrounded by a halo that started of red color and turn to brown, and when the number of spots in the leaf tissue increases a large part of the leaf tissue turns yellow. Several spots may be merged to damage significant areas, which became brown and desiccated [13]; [14]; [15]; [16]; [17]. The downy mildew on lettuce is another epidemic disease in which the symptoms of infection appear in the form of angular spots lacking chlorophyll on the upper surface, matched on the lower surface by a fluffy fungal growth. The number and size of the spots increased and a large area will have affected, appearing yellow turn brown and desiccated [18]; [19]; [20]. The most difficult thing in dealing with these diseases is measuring their severity.

Despite the fact, there are several types of research utilizing the latest applications for the PCs, Smart Phones, and iPads for disease severity assessment to the best of our knowledge, no previous research for evaluating more than one technique on a single sample of the same disease. Therefore, this is the first study to evaluate the performance of more than one image processing method (ImageJ and Leaf Doctor) along with visual assessments in assessing the severity of two different common spotting diseases.

2. Materials and Methods

Samples of infected chard leaves with symptoms of *Cercospora* leaf spot disease were randomly collected from a field in Al Ramadi city and lettuce leaves with symptoms of downy mildew were also randomly collected from a grocery in the Heet city of Al Anbar Governorate, Iraq. The leaves of each crop were classified visually into five categories in light of their disease severity rates, according to a disease severity index consisting of five categories [21]:

- 1= 1-10% Disease Damage
- 2= 11-20% Disease Damage
- 3= 21-40 % Disease Damage
- 4= 41-60% Disease Damage
- 5= > 60 %

The leaves of each crop were gathered into duplicated groups, each of five leaves represented the classes of the diseased index.

The first group was of five leaves represented the five classes of each crop. Each leaf was shot with iPhone 8 plus, provided with Dual 12 MP Camera, f1. 8, 28 mm (wide), and f 2.8 (telephoto). Each captured shot was replicated four times and given a random number of one to twenty. The second group was of four leaves represented the last four classes of the diseased index (2,3,4,5) of each crop. Each leaf shot twice, the first shot was from the upper leaf surface and the second was for the lower surface and each shot was replicated four times and given a random number of one to thirty-two. All shots background of both groups were edited by the PicsArt Editor Application for the iPhone 8 Plus. The percentage of the diseased tissue of each shot was subjected to visual analysis and two different image analysis tools. The first image analysis was the Image J 1.46 (an open-source software (National Institute of Health, USA, [http// image J](http://image.j).

NIH. Gov/I) used in PC following the same procedure described before [10]; [11] and the second tool used the Leaf Doctor Application following the instructions are provided by the Application. The results of each experiment were subject to statistical analyses, to examine their reliability, accuracy and agreement of the various disease severity estimating ways. All the statistical analyses were carried by IPM SPSS, Ver. 24.

3. Results and Discussion

The samples of the lettuce and the chard leaves were showing symptoms of the downy mildew (caused by *Bremia lactucae*) and Cercospora leaf spot (caused by *Cercospora beticola*) on lettuce and chard respectively (Fig. 1). The diseases diagnosis was confirmed by their symptom description in the literatures and their direct examination under the microscope [14]; [18]; [20]; [17].

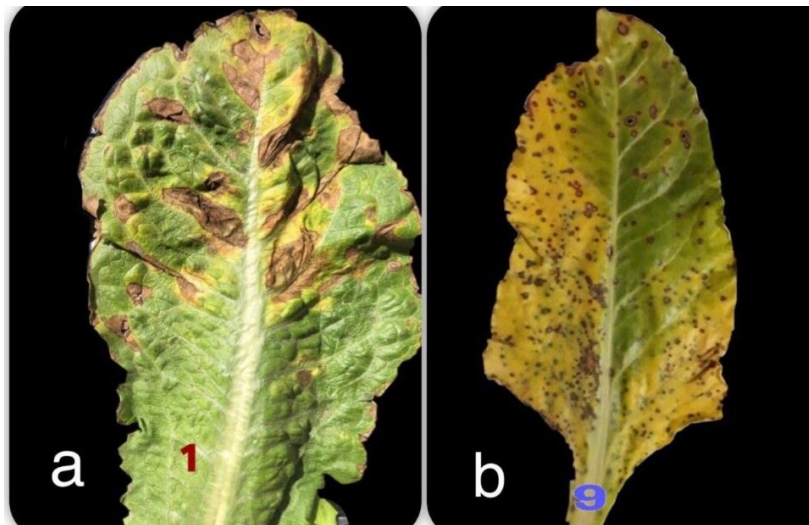


Figure 1. Disease symptoms of a: downy mildew on lettuce and b: Cercospora leaf spot on chard.

The results of evaluating the performance of measuring the disease severity for the techniques tested in this research generally showed compatibility in their performance to the varying degrees of severity of the disease and irrespective of the type of disease. The same order of the disease severity levels for both diseases was pinpointed by any of the tested methods (Figure 2).









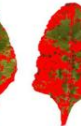
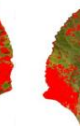














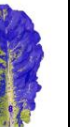

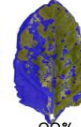
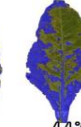
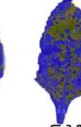
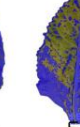
Assessment Method	Downy Mildew on Lettuce					Cercospora Leaf spot on Chard				
ImageJ Application	 8.9%	 21.5%	 30.1%	 39.5%	 50.1%	 6.6%	 30.34%	 36.81%	 58.16%	 54.16%
Visual Assessment	 10%	 20%	 40%	 50%	 60%	 10%	 30%	 40%	 50%	 60%
Leaf Doctor Application	 10%	 25%	 39%	 44%	 52%	 10%	 33%	 44%	 51%	 55%

Figure 2. The performance of the ImageJ, the Leaf Doctor and the Visual assessments for same leaves of various disease severities of downy mildew of lettuce and cercospora leaf spot of chard.

These results support the findings of several previous researches [22]; [23]; [24]). However, the measurements were not the same, statically analyses indicated that there was disagreement in their means and standard deviations of the measurements of the visual assessment (VA) and the other digital image analysis applications. The highest mean and standard deviation for both disease measurements were for the visual assessment (Table 1). The less mean and standard deviation for both diseases was for the ImageJ application (IJ). On the other hand, the statistical analysis revealed that there was a positive and significant correlation relationship only between the measurements of the VA and the Leaf Doctor application (LD). The correlation coefficient between the measurement results of the VA and the LD for the lettuce downy mildew was 0.664 and for the Cercospora leaf spot for chard was 0.899, while there was weak correlations between the VA and IJ method for both diseases. There was also a positive and significant regression coefficient between the severity assessment of the VA and LD only for the Cercospora leaf spot ($R^2 = 0.809$), but the regression between the severity assessment of the VA and LD for the downy mildew of lettuce was $R^2 = 0.441$. The essence of the disease or the host or the evaluating method may be due to the major influences of the correlation and regression coefficients between the assessments methods indicated in this research.

Table 1. Performance of various plant disease severity assessment methods and the correlation and regression between them

Disease	Assessment Method	Mean	St. Deviation	Correlation Coefficient R			Regression Coefficient R ²
				VA	IJ	LD	VA
Downy Mildew on	Visual Assessment (VA)	43	15.9	-	-	0.664*	
	Leaf Doctor (LD)	43	15.9	0.664*	0.209	0.664*	

lettuce	ImageJ (IJ)	16.	11.99	-0.209	-	-0.138	
Bremia lactucae		5					
	Leaf Doctor (LD)	33.	13.9	0.664*	-0.138	-	0.441
		5					
	Visual Assessment (VA)	58.	27.65	-	0.198	0.	
Cercospora Leaf Spot on Chard		7			8	899**	
	ImageJ (IJ)	35.	22.38	0.198	-	0.139	
Cercospora beticola		59					
	Leaf Doctor (LD)	42.	19.46	0.899**	0.139	-	0.809**
		8			9		

* Significant (p<0.05).

** Highly significant (p<0.01).

There were also significant correlations between the measurements of the disease severity on the leaves regardless of the leaf surface or the disease or its severity. The disease severity estimated by either VA or the LD methods showed highly correlation coefficient between the disease severity measurements of the upper or the lower leaf surfaces. The disease severity estimates of both methods were correlated positively regardless of the type of the disease or the crop. The correlation coefficient between the estimated severity of the upper and lower surface of the downy mildew on lettuce was 0.936 by the VA and was 0.908 by the LD and for the Cercospora on chard were 0.996 and 0.835 for both methods respectively. The results also indicated there were positive regressions of highly significant coefficients between the readings of the two surfaces for the lettuce with downy mildew (0.876 and 0.8244) and the chard with Cercospora (0.992 and 0.697) for the VA and LD respectively. On the contrary the results of assessing the disease severity on leaf surfaces by IJ application was of weak positive correlations for both diseases and non-significant regression coefficient (Table 2).

Table 2. The correlation and regression between the assessed disease severity on the upper and lower leaf surfaces of the various assessment methods

Disease	Assessment Method	Upper Leaf Surface	
		Correlation Coefficient R	Regression Coefficient R ²
Downy Mildew on lettuce <i>Bremia lactucae</i>	Visual Assessment	0.936**	0.876**
	Image J	0.581	0.337
	Leaf Doctor	0.908**	0.824**

	Lower Leaf Surface			
Cercospora Leaf Spot on Chard Cercospora beticola		Visual Assessment	0.996**	0.992**
		Image J	0.581	0.338
		Leaf Doctor	0.835**	0.697*

* Significant (p<0.05).

** Highly significant (p<0.01).

On the other hand, the results also revealed that the performance of LD application was highly efficient in determining the severity of the disease regardless of the disease type, its severity, or the crop. It was found that the frequent assessment on the same leaf of different severity levels for three times successive assessment for the downy mildew disease on lettuce and the Cercospora leaf spot on chard of no big differences between the readings. The differences were not exceeded 3% on of both diseases or crops in any of the severity levels examined (Figure 3).

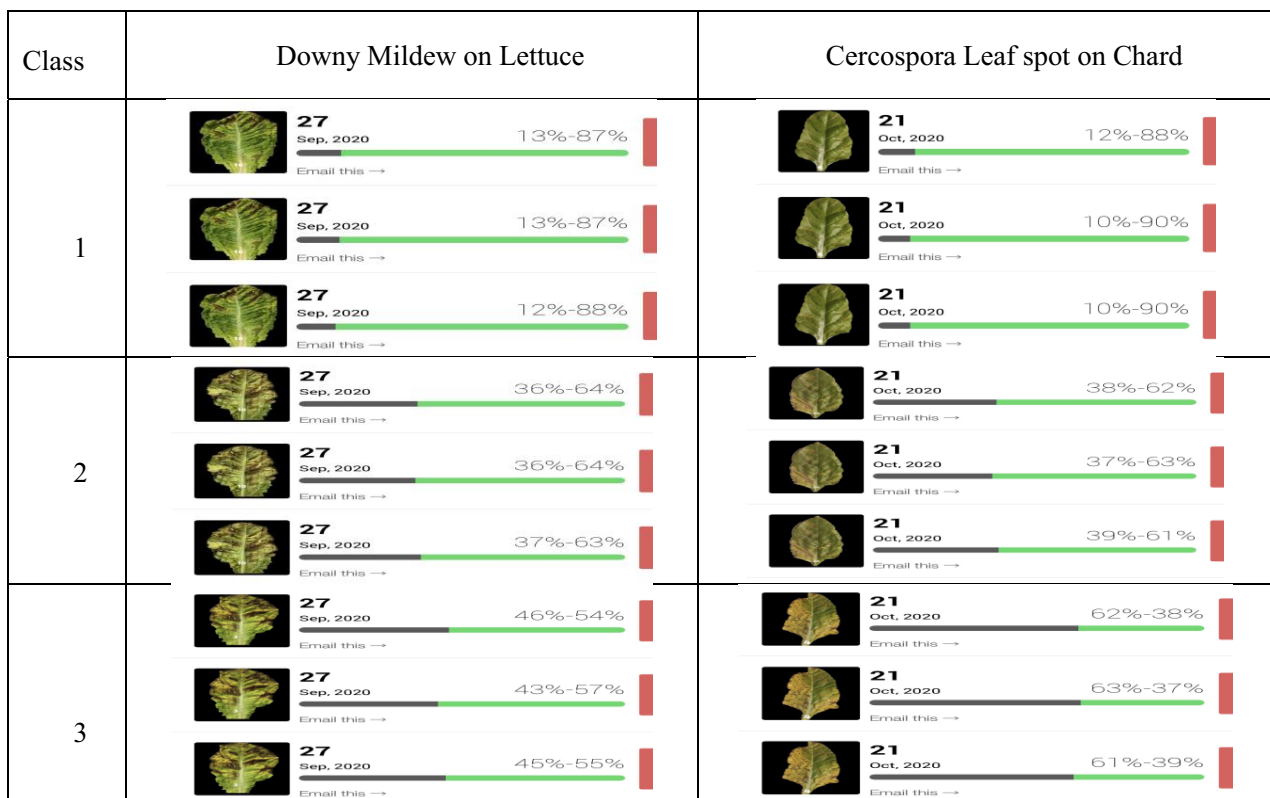


Figure 3. The performance of the Leaf Doctor application for disease severity assessments of different leaf spot diseases on different hosts for three successive assessments.

4. Conclusion

For a sustainable agriculture production, rapid and accurate measurement of plant disease severity are needed for several purpose, including predicting yield loss, monitoring and predicting epidemics, assessing host resistance and studying host-pathogen interaction. Traditionally several methods of visually estimations were adopted for measuring the plant disease severity and recently digital imaging quantifying techniques have been introduced. The personal skill and proficiency however are still played an important role in the performance accuracy of the image analysis. The results of this research relatively revealed that the LD application is easier, faster, and more reliable than the IJ application, but a further updating of the application for the disease severity still needed to overcome the personality interferences.

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