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Mathematical models for the estimation of leaf chlorophyll content based on RGB colours of contact imaging with smartphones: A pomegranate example

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ABSTRACT

The objective of this study was to develop a mathematical model for the non-destructive, fast estimation of the leaf chlorophyll (Chl) content of pomegranate trees. For this reason, contact images of the leaf samples were firstly captured with smartphones and the RGB colours of the images were used for the estimation of the leaf Chl contents. Here, different methods were used for the contact imaging. In the present study, two closed boxes with a small hole (equal to the dimensions of a smartphone camera) on each were formed. Samples were inserted into the hole; and a red LED light and white LED light, separately, were passed through the hole and the leaf. Furthermore, a series of models were tested to best estimate the leaf chlorophyll content of the pomegranate trees by using the RGB colours of contact imaging. Results showed that the use of red LED light sources, instead of white LED light sources, during contact imaging, provides a better estimation of the leaf Chl content. Results also suggest that colour values are highly related to the total weight of the contact imaging area. According to the results obtained, the best estimation of the leaf Chl content (of a given area) is possible by using both the G and B colour values with multiple regression models. It is also found to be important to use the weight of the sampled area for the estimation of the leaf chlorophyll content in mg · g^{-1}.

Keywords: colour index, computer programs, fast estimation, multiple regression analysis, plant health, sustainable farming

INTRODUCTION

Chlorophyll ($C_{55}H_{72}O_5N_4Mg$), the pigment that gives green colour to the plants, has a vital role in photosynthesis and plant growth. It is found in the plants' chloroplasts and is responsible to convert carbon dioxide and water into carbohydrates (food) and oxygen by using sunlight. In other words, it is the basic unit of plant energy systems. During photosynthesis, sunlight is captured by chlorophyll which allows plants to do photosynthesis and grow. Chlorophyll content of plants is highly influenced by light quality, mineral nutrition and chemical metabolites produced by the plants system (Barrios et al., 2016). Therefore, environmental stress conditions and/or reduction in nutritional status may significantly reduce the chlorophyll contents of the plants, which reduces the plant growth, development and yield. Thus, the determination of the leaf chlorophyll content of the plants is important for understanding the plant health and carrying reasonable management of the crops (Yadav et al., 2010; Muñoz-Huerta et al., 2013; Iqbal and Bakht, 2019). Previous studies were reported that there is a strong correlation between the leaf chlorophyll content and nitrogen status (Tewari et al., 2013). Nitrogen is not only a major component of chlorophyll but also of amino acids and nucleic acids

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(forming DNA) and is vital for plants growth. Thus, the determination of the chlorophyll and nitrogen contents of plants gives valuable information to the farmers for the sustainable management of the crops. Managing the use of agricultural chemicals for plant nutrition not only prevents environmental pollution but also reduces the costs of production and provides better economic performance for the farmers (Sawyer et al., 2004). Most of the methods used for the determination of the leaf chlorophyll and/or nutrient contents are destructive ones, which are time-consuming and costly (Muñoz-Huerta et al., 2013). Recent studies showed that non-destructive methods (Nicolai et al., 2007) can be used to develop chlorophyll metres, that is, Konica Minolta SPAD-502®, for the determination of leaf chlorophyll content and to estimate the nitrogen status of some crops (Scharf et al., 2006; Miao et al., 2009; Vesali et al., 2015; Vesali et al., 2017). Chlorophyll metres use two wavebands, infrared light (at 930 nm) and red light (650 nm) to assess the chlorophyll content (Blackmer et al., 1994).

Mathematics is an important tool for the explanations of many natural facts. For example, the Fibonacci series and the Golden ratio appear in nature and reflect some naturally occurring patterns (Adam, 2003). These are the means of the explanation and/or estimation of nature with mathematics (Dunlap, 1997). The Fibonacci series is a sequence where each number is the sum of the two preceding ones, starting from 0 and 1. The Fibonacci series is closely related to the Golden ratio, which is a special number equalling to ~1.618. It is obtained when a line is divided into two parts, and the long part divided by the short part is also equal to the whole length divided by the long part. The Fibonacci series and the Golden ratio appear in rabbit populations, petals' arrangement around a flower, leaves arrangement round branches and seeds on pinecones and they add not only an aesthetic value but also a 'meaning'. Mario (2003) reported that one of the relationships between plants and Fibonacci is in the leaves and plant stems. The relationship in question is the phyllotaxy which is known for completing itself in five turns until two lines are aligned around the stem in which two leaves are in the system in the sequence of the leaves on the plant stem. The phyllotaxy, which is generally considered as 2/5 ratio, provides the opportunity for the sixth leaf to coincide and the two consecutive leaves to make an angle of 720/5 degrees around the stem. Mathematical studies in agriculture are mostly carried to reveal mathematical modelling and analysis of the plant species discussed (Yatat et al., 2016; Sukhova et al., 2017; Morgan and Rhodes, 2002). Considering all this, it can be assumed that mathematics is not only important in the decision-making process or the modelling needed but also in the balance of nature.

Chlorophyll content is the main pigment affecting the visual characteristics of leaves. Therefore, red, green, blue (RGB) colours of images can be used to estimate chlorophyll contents of the plant leaves without any damages on the leaves, with low cost and quick determination (Lee and Lee, 2013; Wang et al., 2013). Using standard cameras or smartphone cameras may provide usable images for the determination of RGB colours, but there is an important challenge: the lighting conditions significantly affect the RGB colours of the images. To eliminate this challenge, covers with artificial light were previously tested and found to be successful for some crops, that is, rice (Tewari et al., 2013). It was previously noted that the G colour in images can not exactly represent the visual green in the leaf, and thus the chlorophyll content. Karcher and Richardson (2003) reported that the R and B values also affect the appearance of turfgrass. Previous studies were also showed that the independent indices extracted from images (i.e. GMR 'G-R', G/R, NGI 'Normalised Green Index', Hue, DGCI 'Dark Green Colour Index') can be used to have higher estimation of chlorophyll contents (Rorie et al., 2011; Wang et al., 2013). Previously Dey et al. (2016) noted that models developed from the image-processing technique provided a strong correlation between the readings of chlorophyll metre. Moreover, Graeff et al. (2008) reported that the CIE L*a*b values have a correlation with the nitrogen content of broccoli plants. Low-cost digital cameras were previously reported to be useful for the estimation of the leaf chlorophyll and nitrogen content of field crops, that is, rice (Li et al., 2010; Saberioon et al., 2014; Rigon et al., 2016). To determine the leaf chlorophyll contents of any plants with the use of any of the above-mentioned independent indices or similar, mathematical models are required.

The world is facing an important problem, which is the increasing human population and decreasing available resources for food supply. Diversification of food resources is highly important for ensuring food safety (Ben-Simchon et al., 2019; Iqbal et al., 2019), and pomegranate fruit is highly important which is a well-known traditional crop (Korkmaz et al., 2016; Kahramanoğlu, 2019). In line with this information, the current study aimed to develop a mathematical model for the non-destructive, fast estimation of the leaf chlorophyll content of pomegranate trees by using the RGB colours of contact images taken with smartphones.

MATERIALS AND METHODS

Plant samples

Twelve different pomegranate cv. Wonderful orchards with different cultivation practices were selected for the present study. The orchards were all located within and around the Güzelyurt city in Northern Cyprus. The reason for the selection of different orchards with different cultivation practices is to have different levels of chlorophyll in the leaves. This would have made it possible to obtain different values to be able to correlate the leaf chlorophyll content with RBG colours. One tree was randomly selected from each orchard (representing the average), and three second-year branches were sampled from each selected tree on 1st of July in 2019. The branches were immediately brought to the laboratory in 1 h for further analysis.

Experimental methodology and image acquisition

First of all, two closed boxes were developed to capture the contact images of the leaf samples. The dimensions of the closed boxes were 100 mm width × 170 mm length and 100 mm height. The thickness of the material used to make the closed box was 2 mm. A hole was made onto the long side of the box with the dimensions of equalling to the camera dimensions of Samsung S6 Duos. Two different light sources were used in the present study: (1) red LED light (1 Watt/RED, 34 lm, operation voltage: 220–240 V/50–60 Hz) and (2) white LED light (1 Watt, 1,000 lm, operation voltage: 220–240 V/50–60 Hz).

After bringing the samples to the laboratory, a healthy leaf representing the branch was selected from the top-third axis of each branch sample (Figure 1). Then, the leaf samples were washed with pure water to eliminate any dust. Furthermore, the mid-point of the leaf was determined by using a ruler. Thus, the mid-point of the leaf was inserted onto the hole of the developed closed box. Hereafter, capturing the contact image with a smartphone was performed. Image capturing was performed both for red LED light source and white LED light sources separately for each 36 leaf samples. Samsung S6 Duos was used to capture images from the samples with a picture size of 187.4 cm width and 105.41 height (Pixels: 5,312 × 2,988). The picture mode was 8-bit RGB and the resolution was 72 pixels per inch. Pictures were saved as jpeg (joint photographic experts group) format and transferred to a computer by using a portable flash disc (Figure 2). Finally, the images were opened at the Adobe Photoshop 7.0 ME computer program, and the average RGB values of the images were read and noted by using the histogram function.

Chlorophyll extraction and content analysis

Chlorophyll (Chl) is vital for plants growth while it absorbs sunlight and enables its usage in photosynthesis. There is more than one type of chlorophyll with unique chemical structures each. The main types are Chl *a* and Chl *b*. Chl *a* is the primary pigment of photosynthesis and Chl *b* is an accessory pigment. Chl *a* is the primary electron donor in the electron transfer chain and it absorbs light from the orange-red and violet-blue areas of the electromagnetic spectrum. Chl *b* absorbs blue light and expands the absorption spectrum of plants. The increase of Chl *b* is a sign of adaption to shade (Roca et al., 2016).

The chlorophyll contents of the leaf samples were then determined with the method developed by Arnon (1949) as suggested by Sudhakar et al. (2016). For this reason, the same parts of the leaves (which were used to capture images) were used. The image captured parts of the leaves were cut carefully and used to determine the chlorophyll contents of the samples. The fresh weight of the plant samples was determined with a sensitive balance (±0.0001 g) and a grind with 10 mL of 80% acetone in a mortar using a pestle. The sample was then filtered through what man filter paper no. 1. The optical density of the extract is measured at 663 and 645 nm wavelengths using a spectrophotometer. Thus, the absorption coefficients were used to calculate Chl *a*, Chl *b* and total Chl contents of the samples by using the following equations (Arnon, 1949; Sudhakar et al., 2016).

$$\text{Chl } a \left(\text{mg} \cdot \text{g}^{-1}\right) = \frac{\left[12.7\left(\text{A663}\right) - 2.69\left(\text{A645}\right)\right] \times \text{V}}{1{,}000 \times \text{W}}$$

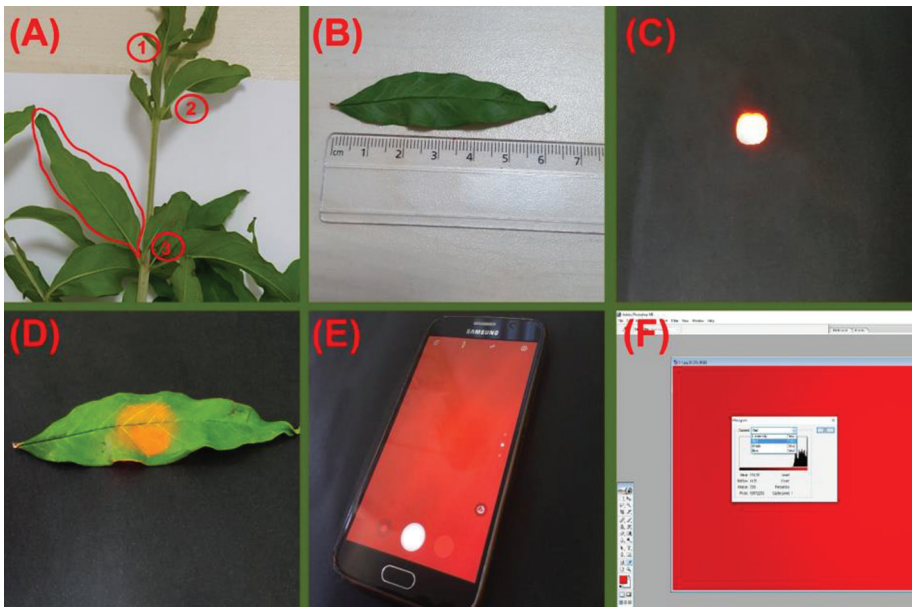

Figure 1. Experimental methodology and image acquisition: (A) selection of the leaf samples, (B): determination of the mid-point of the leaves, (C) a view of the red LED light from the closed box, (D) insertion of the leaf onto the closed box's hole, (E) capturing of the contact image with a smartphone and (F) reading the RGB values of the images by using the histogram function of the Photoshop ME 7.0.

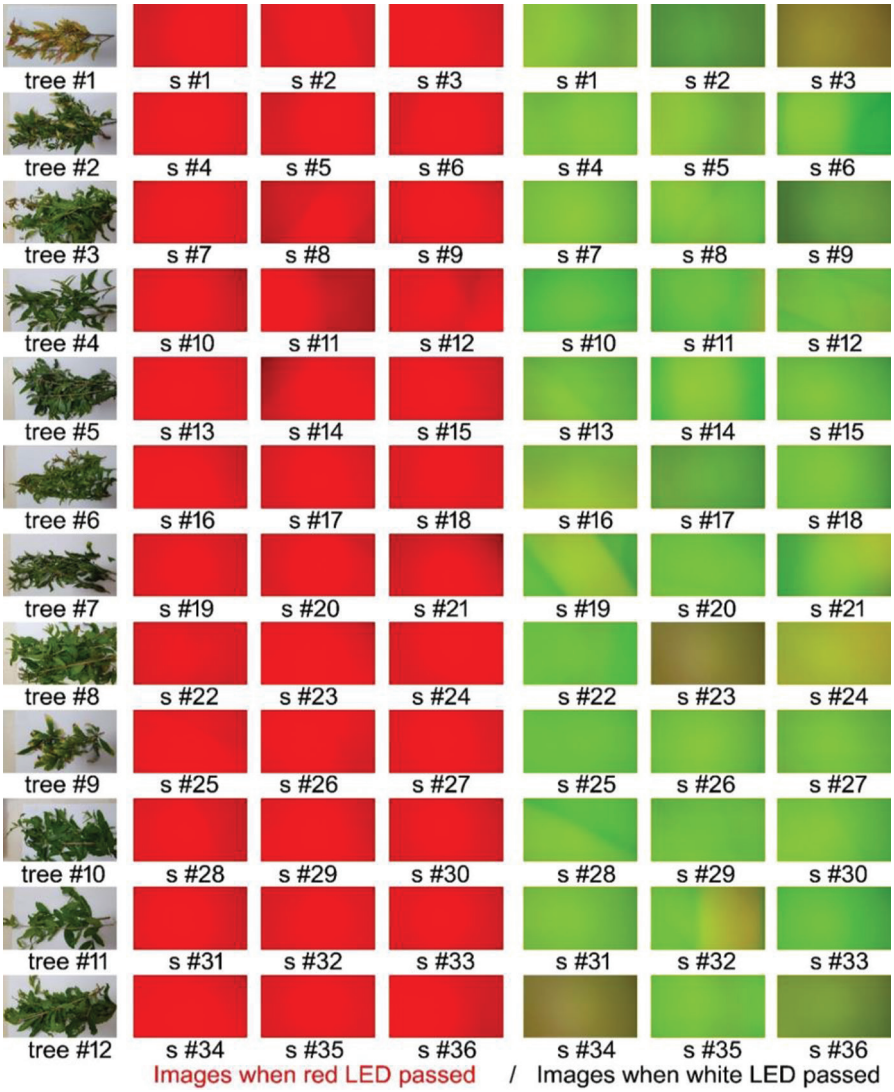

Figure 2. Captured leaf images with a smartphone when red LED passed (three red images from the left side) and when white LED passed (three green images from the right side).

$$\text{Chl } b\left(\text{mg}\cdot\text{g}^{-1}\right)=\frac{\left[22.9\left(\text{A645}\right)-4.68\left(\text{A663}\right)\right]\times\text{V}}{1{,}000\times\text{W}}$$

In the above-given formula; A = is the absorbance at specific wavelengths, V = final volume of chlorophyll extract and W = fresh weight of tissue extracted. Correlation analysis showed that there is no significant correlation among the RGB values and the leaf Chl content (mg · g^{-1}), but further analysis showed that the RGB values (which are captured from a specific area) are highly related with the Chl content of the sampled areas. Thus, the Chl content of sampled areas (in mg · MW^{-1}, MW: Measured Weight) was calculated by multiplying the Chl contents (mg · g^{-1}) with the W (fresh weight (g) of tissue extracted, which is also equal to the fresh weight of the area used for the image capturing).

Colour indices and feature extraction

After transferring the images to a computer as described earlier in the present study, the images were opened in Adobe Photoshop 7.0 ME software. Using the software histogram function, the whole image was selected and the average values of red (R), green (G) and blue (B) were obtained from each image. In addition to RGB of colour spaces, other combination indices (features) suggested by previous studies (Rorie et al., 2011; Lee and Lee, 2013; Wang et al., 2013) were calculated by using the following equations:

$$\text{NRI}\left(\text{Normalized red}\right)=\frac{\text{R}}{\text{R}+\text{G}+\text{B}}$$

$$\text{NGI}\left(\text{Normalized green}\right)=\frac{\text{G}}{\text{R}+\text{G}+\text{B}}$$

$$\text{NBI}\left(\text{Normalized blue}\right)=\frac{\text{B}}{\text{R}+\text{G}+\text{B}}$$

$$\text{GMR}\left(\text{Difference between green and red}\right)=\text{G}-\text{R}$$

$$\text{GRRI}\left(\text{Green}-\text{Red Ratio Index}\right)=\text{G}/\text{R}$$

$$\text{GRVI}\left(\text{Green}-\text{Red Vegetation Index}\right)=\frac{G-R}{G+R}$$

$$\text{DGCI}\left(\begin{matrix}\text{Dark Green}\\ \text{Colour Index}\end{matrix}\right)=\left[\begin{matrix}\left(\dfrac{\text{Hue}-60}{60}\right)+\\ \left(1-S\right)+\left(1-\text{Br}\right)\end{matrix}\right]/3$$

For the calculation of the DGCI (dark green colour index) following equations were used to obtain Hue, Saturation (S) and Br (Brightness) values as suggested by Karcher and Richardson (2003).

$$C=\max\left(R,G,B\right)-\min\left(R,G,B\right)$$

$$\text{Hue}=\begin{cases}60\times\left(\dfrac{G-B}{C}\right), & \max\left(R,G,B\right)=R\\ 60\times\left(2+\dfrac{B-R}{C}\right), & \max\left(R,G,B\right)=G\\ 60\times\left(4+\dfrac{G-B}{C}\right), & \max\left(R,G,B\right)=B\end{cases}$$

$$S=\begin{cases}0, & V=0\\ \dfrac{C}{\max\left(R,G,B\right)}, & V\neq 0\end{cases}$$

$$\text{Br}\left(\text{Brightness}\right)=\max\left(R,G,B\right)/255$$

Moreover, the following model was developed during the present study based on the uniqueness of the experimental methodology. The use of red LED light behind the leaves during contact imaging is a new method, and it was necessary to develop new models for a better correlation among the leaf chlorophyll content and extracted features.

$$\text{GMB}\left(\text{Difference between green and blue}\right)=G-B$$

Mathematical models for chlorophyll content estimation

After the calculation of the above-mentioned indices, dataset was subjected to Pearson's correlation analysis. The significant data for both red LED and white LED were then subjected to linear regression and the Student's *t*-test at 95% confidence by using IBM SPSS 22.0. The correlation and linear regression results for red LED light and white LED light were then discussed to determine any possibility to estimate leaf chlorophyll content (mg · MW^{-1}). The dataset obtained from the red LED method provided a strong correlation with the leaf chlorophyll contents (mg · MW^{-1}). Hereafter, multiple regression analysis was performed to find out the most accurate model for the estimation of the leaf chlorophyll content (mg · MW^{-1}) of pomegranate leaves by using the RGB colours of contact imaging.

RESULTS

Correlation of combination indices with leaf chlorophyll content

The analytical extraction of the pomegranate leaves resulted in an acceptable range of chlorophyll content ranging from 2.07 to 6.66 mg · g^{-1} for a good correlation and estimation of contents with some indices. The results of the present study showed that neither the RGB values from red LED light nor white LED light have a significant relationship between the leaf Chl content in mg · g^{-1} (Table 1). Furthermore, it was intended that the RGB values of the contact images might be related to the Chl content of the given areas. Thus, the fresh weight (W), in g, of tissue extracted (contact imaging area), was multiplied with the leaf Chl contents (mg · g^{-1}) to calculate the Chl content of given areas (mg · MW^{-1}, MW: Measured Weight). The leaf Chl in mg · MW^{-1} was also found to ranging from 0.033 to 0.148, which

Table 1. Correlation between the RGB colours and combination indices and leaf chlorophyll contents (for both mg · g^{-1} and mg · MW^{-1}) of a pomegranate tree

RGB colours and combination indices	Red LED passed		White LED passed	
	Chl (mg · g^{-1})	Chl (mg · MW^{-1})	Chl (mg · g^{-1})	Chl (mg · MW^{-1})
R value	−0.282	−0.552**	−0.225	−0.257
G value	0.113	0.914**	0.210	0.460**
B value	0.146	0.893**	−0.382*	−0.364*
NRI	−0.154	−0.914**	−0.195	−0.424**
NGI	0.148	0.918**	0.344*	0.476**
NBI	0.172	0.897**	−0.384*	−0.371*
GMR	0.280	0.571**	0.279	0.484**
GMB	0.000	0.756**	0.287	0.456**
GRRI	0.146	0.917**	0.233	0.450**
GRVI	0.144	0.916**	0.302	0.475**
DGCI	0.280	0.574**	−0.014	0.009

* Correlation is significant at 0.05 level and ** at the 0.01 level (2-tailed).

is useful for a good correlation and estimation of the contents with the RGB indices. The results provided a higher correlation for the RGB values and leaf Chl content in mg · MW^{-1}, and the correlation is high when red LED light passed through the leaf samples.

A negative correlation was observed between R (Red) value and leaf chlorophyll (mg · MW^{-1}) content while a positive correlation was calculated for G (Green) value and leaf Chl content, at both conditions (red LED passed and white LED passed). The negative correlation for *R*-value was moderate when red LED passed through the leaf, but very weak under white LED light source. Among the three colour values, G value was found to have the strongest correlation with leaf Chl content. The correlations for all colour values and combination indices were higher at the red LED conditions than the white LED conditions. This result suggests that the use of red LED provides a better correlation with leaf Chl content and is much useful for the estimation of leaf Chl content. The extracted indices were also found to have a higher correlation with Chl content in mg · MW^{-1}.

According to the results obtained, the B (Blue) value also found to have a very strong positive correlation with the leaf Chl content (when red LED passed). The correlation between the B value and leaf Chl content (mg · MW^{-1}) was weak and negative when white LED passed through the leaf during contact imaging. The correlation among the normalised colour values (NRI, NGI and NBI) and leaf Chl (mg · MW^{-1}) were calculated as very strong under red LED conditions. The results suggested that raw G and B values and normalised RGB values can be used for the estimation of the leaf Chl content (mg · MW^{-1}) of the pomegranate tree. GMR (Difference between Green and Red) is an extracted feature used for the estimation of the leaf Chl. Due to the nature of the present study (use of red LED), an additional feature was extracted to better estimate the leaf Chl content which is GMB (Difference between Green and Blue). The results showed that GMB has a better correlation (very strong) with leaf Chl content than GMR. The Green-Red Ratio Index (GRRI) and Green-Red Vegetation Index (GRVI) were also found to have a very strong positive correlation with the leaf Chl (mg · MW^{-1}) under the red LED conditions. Lastly, the DGCI (Dark Green Colour Index) was found to have a moderate positive correlation with the leaf Chl (mg · MW^{-1}) under red LED conditions and neutral (no) correlation under white LED conditions.

Estimation of leaf chlorophyll content with a linear model

After the correlation analysis, to determine the best model for the estimation of the leaf Chl content (mg · MW^{-1}) with different colour indices, a stepwise linear regression analysis was performed to formulate models. This classical method resulted in a close relationship for the G and B values with the leaf Chl (mg · MW^{-1}) under red LED conditions (Figure 3). In line with the obtained results from stepwise linear regression, the best indices for the estimation of the leaf Chl (mg · MW^{-1}) were found to be G value with an R^2 of 0.8355. The calculated linear formula for the leaf Chl content (mg · MW^{-1}) (y) estimation was $y = 0.1522 * G - 0.0911$.

The G value was found to have a very weak estimation for the leaf Chl content (mg · MW^{-1}) under white LED conditions with an R^2 of 0.212. According to the obtained results, the B value was also found to provide strong estimation of the leaf Chl content (mg · MW^{-1}) under red LED conditions with a R^2 of 0.797. The calculated formula for that is $y = 0.1915 * G - 0.0565$. The B values under white LED light conditions found to have some very high values which damage the regression analysis. The elimination of these extreme values was also resulted with a very weak correlation, and the values were not eliminated in the presented form. The normalised values of RGB provided a better estimation of the leaf Chl (mg · MW^{-1}) with *R*-value. The Normalised G and B (NGI and NBI) values had similar results with the G and B results. The *R*-value (under red LED) had a weak relationship with leaf Chl content (mg · MW^{-1}) ($R^2 = 0.3047$) but the NRI had a stronger relationship ($R^2 = 0.8366$). Estimation of the leaf Chl content (mg · MW^{-1}) was possible with the NRI by using the equation of $y = -0.0455 * NRI + 0.9956$. According to the results obtained, the best-fit regression model for the single colour values and also for the normalised indices was from green (G).

The GMR (Difference between Green and Red) under red LED was found to have a very weak relationship with the leaf Chl content (mg · MW^{-1}) with an R^2 of 0.3264. On the other hand, the GMB (Difference between Green and Blue) had a better relationship with leaf Chl content (mg · MW^{-1}) and the equation of $y = 1.3272 * GMB + 0.3058$ was found to have an R^2 of 0.5712. The results showed that GMB under red LED has higher correlation (very strong) with leaf Chl content (mg · MW^{-1}) than GMR. There was a good linear relationship (0.8421) between the GRRI under red LED and the leaf Chl content. The equation for the estimation of the leaf Chl content (mg · MW^{-1}) was $y = 0.0265 * GRRI - 0.0028$. The GRVI under red LED was also calculated to have a very strong positive relationship with leaf Chl content (mg · MW^{-1}) with a formula of $y = 0.0524 * GRVI + 0.9944$. Finally, the relationship between the DGCI (Dark Green Colour Index) under red LED and leaf Chl content (mg · MW^{-1}) was found to be very low (R^2 of 0.3285) and not useful for the estimation of the leaf Chl content of pomegranate trees under neither red LED nor white LED.

Model determination for chlorophyll content estimation

Results showed that, from the three colour values, the G and B values have a higher direct influence on the leaf Chl content and were selected for further model

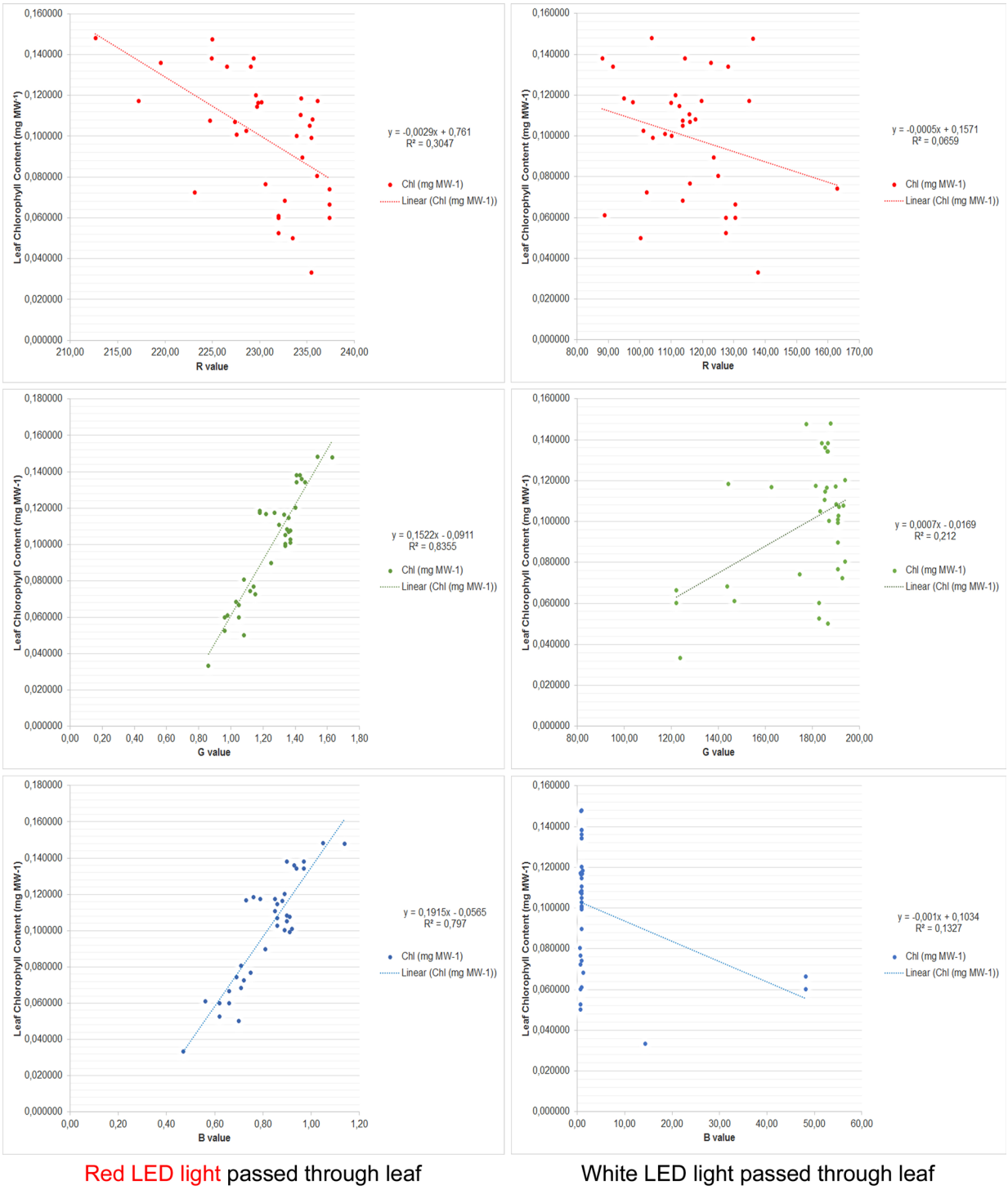

Figure 3. Stepwise linear regression analysis for leaf Chl content measured analytically in laboratory and the RGB values based on contact imaging with smartphone.

estimation. Stepwise regression, correlation analysis as suggested by previous literature (Mollazade et al., 2012; Lee and Lee, 2013; Teimouri et al., 2014) was used together for the feature selection to estimate leaf Chl content (mg · MW^{-1}). Thus, multiple regression analysis was performed to estimate the Chl content (mg · MW^{-1}) of pomegranate leaves by using the G and B values together. Results provided a similar R^2 (0.8355) with a simple regression analysis. The standard error for the model was found to be 0.0126 and the multiple R was calculated as 0.9141. The summary results of the multiple regression analysis were given in Table 2.

According to the obtained results, it was concluded that the estimation of Chl content by using both G and B values has the same efficiency with using only G factor. However, due to the reduced standard error, it is suggested to use both factors together. The calculated

Table 2. Summary results for multiple regression analysis among G value, B value and Chl (Y)

	Coefficients	Standard error	t Stat	*p*-value
Intercept	−0.0914	0.017741	−5.1518	1.18E-05
G	0.1537	0.055322	2.7782	0.008948
B	−0.0019	0.071268	−0.0271	0.978541

formula for the estimation of Chl content (mg · MW^{-1}) is: Chl = (0.1537 * G) + (−0.0019 * B) – 0.0914. Dividing the obtained result to the fresh weight of the sampled area gives the leaf Chl content of the sample is mg · g^{-1}.

DISCUSSION

The calculated leaf Chl contents of pomegranate leaf samples ranged from 2.07 to 6.66 mg · g^{-1} (and from 0.033 to 0.148 mg · MW^{-1}) and made it possible to have a good correlation and estimation of leaf Chl contents (mg · MW^{-1}) with RGB colour values and combination indices. RGB values from both red LED light and white LED light found to have a very weak correlation and non-significant relationship with the leaf Chl content in mg · g^{-1}, but very high correlation and significant relationship with the leaf Chl content in mg · MW^{-1} (Table 1). In a similar in press study by Barman and Choudhury (2020) alike method was used but constant light source (Eveready digital torch with 0.2 watt and 11 lumens) was used instead of white LED light. They did not test red LED light. The results of their study are also similar to the present results. Similarly, to our results, they reported that the pure RGB values have a weak correlation with the Chl content of the leaves. In contrast to our study, they did not calculate the Chl content as mg · MW^{-1} and tried to explain the relationship with different colour index.

Our results showed that the RGB values of the contact images are related to the Chl content of the given areas. It was then suggested that the leaf Chl content is highly influencing the RGB values of a given area and the fresh weight (W) of the area is highly important in the determination of the leaf Chl content. *R*-value found to have a weak negative correlation with leaf Chl content (mg · MW^{-1}) and G and B values provided a very strong positive correlation with leaf Chl content (mg · MW^{-1}), under red LED conditions. Previous works of literature (Yadav et al., 2010; Tewari et al., 2013) with white LED or natural light suggested that G has the highest correlation, *R* shows a good correlation with Chl content but B is poorly fitted to Chl content. In the present study, the correlations for all colour values and combination indices were higher at the red LED conditions than the white LED conditions. The main reason for this is that Chl *a*, the primary source of chlorophyll pigments absorbs orange-red and violet-blue areas of the electromagnetic spectrum (Roca et al., 2016). This is why the laboratory extraction techniques use 663 and 645 nm wavelengths at the spectrophotometer to identify the Chl contents (Sudhakar et al., 2016). According to the authors' knowledge, many studies (Karcher and Richardson, 2003; Graeff et al., 2008; Li et al., 2010; Rorie et al., 2011; Lee and Lee, 2013; Tewari et al., 2013; Wang et al., 2013; Saberioon et al., 2014; Dey et al., 2016; Rigon et al., 2016) were conducted before for the estimation of the leaf Chl contents by using image scanning techniques, but none of them tested red LED light sources. Therefore, the results of the present study provided a better correlation among the RGB values and leaf Chl content (mg · MW^{-1}) than the previous studies. For example, Vesali et al. (2015) used natural light for the contact imaging and the coefficient of determination (R^2) for linear regression among the R, G and B values with SPAD Chl value were reported as 0.56, 0.11 and 0.04. The coefficient of determination (R^2) for the same colour indices in current work (when red LED light passed through leaf) was found to be as 0.3047, 0.8355 and 0.797. The coefficient of determination (R^2) of the present study for the linear regression among the R, G and B values with leaf Chl content are also higher than the results of Dey et al. (2016), Rigon et al. (2016) and Vesali et al. (2017). When comparing the Chl *a* and Chl *b* in the present study, the estimation of the Chl *a* was higher than the Chl *b*. This is mainly a result of the absorption of blue light by Chl *b* and red LED light was used in the present study. However, this did not negatively affect the estimation of the total Chl due to the low content of Chl *b* in the leaf samples. The results also showed that the B value also has a very strong positive correlation with the leaf Chl content (when red LED passed). Furthermore, it was observed that in some cases, the determination of Chl *b* was inaccurate (Rigon et al., 2016). This resulted from the fact that the absorption peak of Chl *a* was similar to the wavelength emitted by the device, making it more difficult to separate (Neves et al. 2005). In a recent study, Yao et al. (2020) tested a different method. They did not pass the light from the leaf but reflected the light from the leaves. They developed some indexes from the photometric measurements (light reflected from leaves surfaces) and suggested that the bidirectional reflectance factor (BRF) and 1/DOLP (degree of linear polarisation) provided a 90% convenient estimation of the leaf chlorophyll content.

The normalised values of RGB provided a better estimation of the leaf Chl with *R*-value but not changed for G and B. This is due to the use of red LED during the contact imaging. Overall of the results suggested that among the raw and computed colour indices, G provides the best fit linear regression for the estimation of the leaf Chl content. This result is in accordance with the reports of Vesali et al. (2015). The GMB (Difference between Green and Blue) indices provided better fit than the GMR (Difference between Green and Red) indices due to the use of red LED for the contact imaging. As suggested by previous studies (Karcher

and Richardson, 2003; Dey et al., 2016; Rigon et al., 2016; Vesali et al., 2017) a good linear relation was observed between the GRRI and the leaf Chl content. The regression for the GRVI was also strong for the estimation of the leaf Chl content. The relationship with DGCI and leaf Chl content was found to be not useful for the estimation of the leaf Chl content on the contrary to the previous studies (Rorie et al. 2011; De Lima et al. 2012). This might be a result of the use of red LED light source, but the advantage of using red LED light is so high when compared with all results. Similarly, Vesali et al. (2015) also noted that some indices, including GRVI and DGCI, were not strongly fitted to Chl values of the SPAD estimation method under natural light contact imaging conditions. Results suggested that a better estimation of the leaf Chl content is possible with the use of multiple regression analysis. Previously some Authors (Marenco et al. 2009; Mielke et al. 2010; Rigon et al., 2016) suggested that non-linear regression models provide a better estimation of the Chl content. Using multiple regression models is not new for agricultural studies (Bramel et al., 1984; Saed-Moucheshi et al., 2013) but according to the authors' knowledge, it is first used in the present study for the estimation of the leaf Chl (mg · MW^{-1}) of pomegranate trees.

CONCLUSIONS

Overall, the results suggested that the colour index from contact imaging with smartphones, when red LED light is passed through the leaf, can be used to estimate the leaf Chl content of pomegranate trees. However, the direct estimation of the leaf Chl in mg · g^{-1} from RGB values is not possible and is highly necessary to use the fresh weight of samples for its calculation. Results suggested that using red LED light sources, instead of white LED light sources during contact imaging provides a better estimation of the leaf Chl content. It was also concluded that there is a weak negative correlation between *R*-value and leaf Chl (mg · MW^{-1}) and a strong positive correlation with leaf Chl (mg · MW^{-1}) and G and B values. Results suggested that the best estimation of the leaf Chl content (mg · MW^{-1}) of pomegranate trees is possible by using both G and B values together with the equation of Chl (mg · MW^{-1}) = (0.153695 * G) + (−0.00193 * B) – 0.0914. It is necessary to divide the result into the fresh weight of the sample to calculate the leaf Chl content as mg · g^{-1}. Further studies on different crop types are recommended to determine the effectiveness of the method and to determine multiple regression models for the estimation of leaf Chl of other crops.

AUTHOR CONTRIBUTIONS

N.Ö. and İ.K.: conceived and designed the experiments. N.Ö. and İ.K.: performed the contact imaging. İ.K.: performed the laboratory analysis. N.Ö. and İ.K.: performed data analysis and model determination and equally contributed to manuscript writing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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