Rapid and non-destructive leaf chlorophyll estimation of Fig (*Ficus carica* L.) cv. *Iraqi* grown on different root zone spatial limitation and controlled porosity level under greenhouse condition

M M Isa¹,²,³, K F Kasim¹, M F A Muttalib¹,² and M N Jaafar¹,²

¹Faculty of Chemical Engineering Technology, Universiti Malaysia Perlis, 02600, Arau, Perlis, Malaysia
²Institute of Sustainable Agrotechnology (INSAT), Sungai Chuchuh, Universiti Malaysia Perlis, 02100, Padang Besar, Perlis, Malaysia
³School of Agriculture Science and Biotechnology, Faculty Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Tembila Campus, 22200, Besut, Terengganu, Malaysia

*E-mail: muslianie@yahoo.com.my*

**Abstract.** The estimation of chlorophyll content in leaves by the chlorophyll meter (SPAD 502) is more favourable than by the extraction method for studies on photosynthesis or senescence where the total chlorophyll is estimated on the same leaf over time. However, till date there is lack of information available on leaf chlorophyll content of Fig (*Ficus carica* L.) cv. *Iraqi* grown in containers under different root zone spatial limitation and controlled porosity level. Hence, this study aim to determine the effect of both factors on leaf chlorophyll content of the crop. The experiment was design in randomized complete block design (RCBD) with four replications. The Soil-Plant Analyses Development (SPAD) chlorophyll meter (Minolta Camera Co., Ltd., Japan), has been used for instantly measuring the amount of chlorophyll present in plant leaves. Based on the results, the application of Mix 3 (50% sand: 50% clay) with high root zone spatial gave the highest chlorophyll content compared to other treatment. The application of the right root zone and porosity level may gave positive effect on leaf chlorophyll content of *F. carica* cv. *Iraqi* grown under greenhouse condition.

**1. Introduction**

Fig (*Ficus carica* L.) is the significant member of the genus Ficus. This plant is a deciduous tree belonging to the Moraceae family which also represents one of the most important crop cultivated in the worldwide [1]. It is one of the earliest cultivated fruit trees which native to western Asia and later was spread to the Mediterranean region [2]. *F. carica* is one of the only five plants stated in the holy Quran along with the grapes, olives, dates and pomegranate [3]. Besides, it is also become a symbol for both the Greek and Roman cultures [4]. This plant consists of numerous varieties with significant genetic diversity and outstanding pharmacological activities [5]. The tree grows well and produces tasteful and flavouring fruits enhanced by warm and shiny conditions during ripening. Also, the ripe fruits can be eaten either fresh, dried, or in other stored form [6] [7]. Fig trees can be grown in many parts of the world where the climate is moderate because this fruit crop species is particularly grows well to several types of soils and climates due to its tolerance certain environmental problems [8].
According to Hassaini et al. [9], fig is a common fruit worldwide due to its gaining international trade importance as consumers pursue continuously fresh and high-quality products. Figs have been reported as an important crop for both dry and fresh consumption also the fruits are considerably rich with their composition of minerals, fiber, amino acids, vitamins, carotenoids, antioxidant polyphenols, flavonoids, sugars, organic acids, and volatile compounds that provide a delightful or pleasant characteristic aroma. In addition, these substances gave a great impact on colour, flavour and sensory properties such as bitterness and astringency which contribute to good quality of the fruit as well as improved human health [10]. *F. carica* plant typically grows to about 18–20 feet. It has moderate size of stem with irregular branches also underground and extrinsic root. The aboveground part which are stem and leaves (largely green, petiolated, curly margins and hairy surface) contain milky white latex that contains a protein-degrading enzyme namely ficin. This plant also has been used for cultural, food and medicinal purposes, including recreational and sporting events [7] [11] [12].

In Malaysia, Beach Ridges Interspersed with Swales (BRIS) soil is the abundant type of soil that can be seen along the east coast of Peninsular Malaysia from Kelantan, Terengganu, Pahang and right down along the coast to the west coast of Johor. BRIS soil has a poor physical properties for instance greater percentage of sand, high drainage, low water holding capacity, low fertility level than heavier soil and this will hinder the crop performances [13] [14] [15]. Reuter [16] also Walpola and Arunakumara [17] mentioned that sandy soils have low nutrient and water retention capacity because of the greater amount of large pores and the low cation exchange capacity of sand particles. Sandy soils may also be water repellent which will result in low carbon input into sandy soil and also cause lack of nutrient uptake by the plant. Furthermore, lack of binding site for organic matter would result in high decomposition rates of organic material. Hence, sandy soils have a low C sequestration capacity or low organic matter content. Plants with smaller roots zone are facing problems as sandy soils lose moisture rapidly than other type of soil. Most of the nutrients in sandy soils are washed out quickly or prone to leaching [18] [19] [20]. BRIS soil are less fertile than other fine textured soil and this soil also become one of problematic soils because it lack in many aspects [15]. Although BRIS soil is a problematic soil, *F. carica* is suitable to be planted in this soil because it have high porosity and well aerated for deep root zone.

There are many approach have been suggested to manage sandy soils problems as mentioned above. Previous research by Blackwell et al. [21] recommended that using furrow sowing would slowly releases fertilisers in order to maximize nutrient use efficacy or amendment of organic substances to enhance water and nutrient retention. However, these management preferences improve soil fertility only temporarily due to fertilisers have to be continuously added because nutrients are quickly leached out of the root zone. Environmental problems may occurred although plant growth and yield can be increased by frequent irrigation and fertilization (especially in dry conditions). Leaching problem that arise may reduce fertiliser use efficiency and also lead to surface and ground water contamination [22] [23] [24]. Lal [25] mentioned that incorporation of organic substances such as plant residues can lead to microbial activity and organic carbon sequestration as well as supply nutrients for plants.

Therefore, strategies must be established to ensure nutrients available within the root zone and one of the approach is addition of clay soil to sandy soil because clay soils have several benefits such as existence of micropores which can hold water in the soil. Moreover, incorporating clay on or with sandy soil is a favourable method for increasing yield, improving soil moisture distribution and maximizing water use efficiency as well as enhancing water saving [19] [27]. Clays are ubiquitous materials which undergo spontaneous modification and transformation with the change of environmental conditions. This material are widely distributed in the surface of earth as fine grained rocks and soil component with the characteristics of small size. The structures and type of clay minerals critically affect the physical, chemical and biological processes of soils [28].

Clay soil is essential for the study of global sedimentary, biological and environmental processes. This soil also play significant role to such as an extent that their utilization are almost endless due to their specifically physical and chemical features [29]. Clay is a soil component which have a high capacity to hold nutrients and organic matters by binding to clay particles and within aggregates which protects organic matter from microbial degradation and stabilizes easily decomposable substrate. Furthermore, pore structure characteristics and mechanical properties such as tensile strength were
affected by the degree of packing of clay mineral particles which influences soil structure and inter-
particle bonding. Besides, clay-rich soils also have a larger microbial biomass than coarser textured soils
[18] [30].

Roots serves as a significant function in water and nutrient uptake and in balancing shoot behaviour
via phytohormones. Root form and function vary with species and genotype, texture and porosity of the
soil medium, nutrient status and location also water status and location [31]. Further, Wang et al. [32]
demonstrated that root restriction coordinate vegetative growth by reducing the root system volume
which consequently regulates the absorption of water and nutrients of greenhouse nectarines. This
reduction in root volume can be considered a stress condition. However, the advantageous of proper
root restriction volume can ensure normal plant growth, gave maximum production rates, decrease the
amount of soil need to be refill and conserve the local ecological environment. On the other hand, Brady
and Weil [33] also Zhu et al. [34] observed that extreme root restriction will lead to phenotype based
physiological variations and trigger the release of root exudates, which contains large quantities of
autotoxins and allelochemicals that can cause reductions in plant growth.

Plant leaves are the main photosynthetic organ. There are several methods for examining chlorophyll
content. Leaf chlorophyll can be extracted using acetone, dimethyl sulfoxide (DMSO), methanol,
petroleum ether and other solvents for total chlorophyll analysis. After extraction phase, the
spectrophotometric assessment of absorbance by the Chl solution and absorbance will be convert to
contents using standard published equations. This in vitro determinations are destructive, laborious, time
consuming, costly, requiring sophisticated equipment for chemical digestion and other analytical
procedures and may therefore not be applicable for all purposes. However, non-destructive, in situ
estimation of chlorophyll is desirable when gas exchange measurements are to be carried out repeatedly
on the same leaves [35] [36].

Another alternative method that can be used to measure leaf chlorophyll content is by using hand
held Soil Plant Analysis Development (SPAD) chlorophyll meter (Minolta Ltd, Osaka Japan) of series
number 502. This hand held chlorophyll meter is an ordinary used instrument which enables a rapid
(when dealing with large number of plants) and non-destructive measurement of the leaf transmittance
in two regions which are red light (650 nm) for chlorophyll absorption and sensitive to chlorophyll
concentration while near-infrared light (940 nm) which used for correction of leaf thickness and
functions as a reference to compensate leaf variables. The ratio of these two transmission values is
referred to as SPAD reading or SPAD value. A dimensionless SPAD unit ranging from 0 to 80 is
calculated by the microprocessor in the SPAD-502 chlorophyll meter based on the difference in light
attenuation. Generally, SPAD measurement is conducted on the first fully expanded leaf or on flag
leaves at many developmental stages and the measurement is obtained by inserting a leaf blade into the
head of the SPAD-502 meter [37] [38]. Markwell, Osterman and Mitchell [39] recommended that the
SPAD-502 meter is able to provide a rapid, straightforward method and reasonably accurate estimate of
leaf Chl. Till date, there is lack of information are available in terms of chlorophyll content F. carica
planted in different root zone spatial and growing media which are BRIS soil and clay soil. Therefore,
the objective of this research was to examine the effect of root zone spatial limitation and controlled
porosity level on the chlorophyll of F. carica grown under greenhouse condition.

2. Materials and methods

2.1. Plant material

This research was carried out on 48 uniform, 3 months old of F. carica cv. Iraqi. This plant was obtained
from the local nursery of research station. F. carica plant was propagated through air layering method.
This method was used because propagation through seedlings are not preferred due to characteristic
differences diverting from mother plants.

2.2. Experimental design

The study was performed at the Institute of Sustainable Agrotechnology (INSAT) Research Station of
Universiti Malaysia Perlis (UniMAP). F. carica was cultivated in polyethylene greenhouse. The
experiment was designed in Randomized Complete Block Design (RCBD) with two factors with 4
replicates which were root zone spatial and different growing media (porosity). The medium mixtures
were used by mixing Beach Ridges Interspersed with Swales (BRIS) and clay soil in different proportions (Table 1). Three cylindrical container sizes with different capacity will be used in this experiment (Table 2).

| Table 1. Container soil composition used during the experiment |
|---------------------------------------------------------------|
| **Soil composition (%)** | BRIS | Clay |
| Mix 1               | 0    | 100  |
| Mix 2               | 25   | 75   |
| Mix 3               | 50   | 50   |
| Mix 4               | 75   | 25   |

| Table 2. Root zone spatial applied during experiment |
|-----------------------------------------------------|
| **Root zone volume (L)** | Treatments |
| 15         | Low        |
| 30         | Medium     |
| 45         | High       |

2.3. Experimental set-up

Experimental site was set-up with the irrigation system encompassed of 500 gallon water tank used as water supply. The system was operated with 2 HP water pump and connected with 32 mm diameter PVC pipe. The drip line with 1.2 m dripper spacing was connected to adjustable irrigation drippers with the specification flow range of water 0-80 L/h, 360° adjustable emitter with 8 spray hole that can adjust water flow rate also for cutting off the water flow. An arrangement of 1.4 m between rows and 0.5 m between plants, which corresponds to a planting density of 0.7 plants m⁻² was used. Gypsum blocks were used to be a valuable tool to monitor soil moisture content of the growing media. Gypsum blocks were installed quickly after transplanting phase to provide time for roots to develop around the blocks. Water were applied when the reading were below the target soil water potential.

2.4. Measurement of leaf chlorophyll content

Leaves were read with a Hand-help SPAD-502 (Minolta Camera Co., Osaka, Japan) which used to estimate chlorophyll content. This portable meter determine the differential transmittance of light that come through it rapidly in order to measure the relative greenness in a leaf and the result will interpret as an index in SPAD value. The principle of the measurement of SPAD meter is the meter emits light from two diodes that producing two transmission value which were a peak wavelength near 650 nm (red, absorbed by chlorophyll) and the other a peak near 940 nm (infrared, no absorption occurs). These wavelengths are transmitted alternately through the leaf [40].

In order to maintain the stability of SPAD-502 meter, the sampling period used for leaf chlorophyll content of F. carica is at the same time used by plant breeders, physiological researchers and for penultimate leaf chlorophyll concentration which was the measurement of leaf samples between 08:00 and 10:00 h. Three SPAD measurement were performed on each side of the midrib. The six readings per leaf were averaged to produce a single observation value for each leaf. Prior to measurement, the SPAD-502 meter was calibrated by using the reading checker supplied by the manufacturer and the meter was set to zero without any sample in the sample box slot by pressing the same button that is used for data collection. Proper care was taken to ensure that SPAD meter sensor fully covered the leaf lamina and that interference from veins and midribs was avoided especially while recording SPAD readings [41][42].
2.5. Statistical analysis
An analysis of variance (two way ANOVA) was done with root zone spatial variability (low, medium, high) and different porosity level (Mix 1, Mix 2, Mix 3 and Mix 4) as factors in this experiment. When the interaction between factors was significant \( P < 0.05 \), Tukey’s HSD (honest significance difference) test was performed to detect significant difference among group least square means of the analyses.

3. Results and discussion

3.1. Effect of root zone spatial limitation and controlled porosity level on chlorophyll content of \( F. \) carica

Plants are capable of growing in a wide range of soil textures ranging from coarse sand to heavy clay. Soil texture affects the movement and availability of air and water in soil and influences root growth, water and nutrient uptake and overall plant growth [43] [44]. Growing \( F. \) carica trees in unfavourable or improper environment will lead to inadequate development, crop damage and various types of fruit loss. Based on the previous studies, plant needs appropriate growing media for gas exchange between plant roots and atmosphere, supply of nutrients and water quality, yield as well as sufficient anchorage of plant [45] [46].

| Source of variations | DF   | SPAD values |
|----------------------|------|-------------|
| Root zone            | 2    | <0.0001     |
| Mixture              | 3    | <0.0001     |
| Root zone*Mixture    | 6    | 0.0005*     |

Table 3 showed the Analysis of Variance (ANOVA) that relates to chlorophyll content in \( F. \) carica. Based on the data, the chlorophyll content was significantly affected by the different root zone spatial limitation and mixture of growing media (which involve different porosity level) and also the interaction of both factors. Post Hoc Tukey HSD method was used to find out that if there was significant effects between the treatments for leaf chlorophyll content. The results were shown in the next section.

3.2. Effect of root zone spatial limitation and controlled porosity level interaction on chlorophyll content of \( F. \) carica

The root zone spatial and porosity level of growing medium may influence the chlorophyll content of the plant. Plant growth or development will be adversely affected if both factors is limited or present in excess amount. Growing media porosity is an index for root media aeration which means when root media aeration is sufficient the nutrient elements and water is directly supplied for growth of plant [45] [47].
Figure 1. Chlorophyll content as affected by different root zone spatial and media mixture

According to Figure 1, the chlorophyll content of *F. carica* was significantly affected by root zone spatial and media mixture (different porosity level). The highest values of chlorophyll content was observed in *F. carica* grown under high root zone spatial and Mix 3 which containing 50% BRIS soil with 50% clay soil. This result is due to clay addition increases not only soil fertility, but could also protect organic matter from decomposition. In addition, the presence of clay which helps to retain water for a certain period of time and helps prevent the leaching process from happening actively [13] [18]. Application of clay-substrate to the growing media will improve water retention and reducing leaching process. This gave benefits particularly for sandy soils which rapidly dry and show higher losses of plant nutrients due to the greater seepage rate which also causes severe ground-water contamination such as by nitrogen compounds [16]. According to Sinclair et al. [48] and Turner et al. [31] coarse-textured soils with enlarged porosity have a limited capillary fringe of saturated soil which cause a low water-holding capacity and drain very rapidly also plants rapidly applied the least available water and lead to greater water deficits.

Another study done by Reuter [16] reported that utilization of clay-substrate in sandy soil significantly enhanced soil water regime specifically on the percolation processes. Another aftereffect of clay soil addition are decreasing of plant nutrient losses and eradication ground water contamination. Obst [49], Carter et al. [50], Ismail and Ozawa [19] also stated that addition of clay to the top of sandy soil has been observed to be highly practical in decreasing water repellency and maximizing g crop yield. Ismail and Ozawa [19] conducted a container experiment to study the enhancement of water productivity and crop yield of sandy soils treated with clay in cucumber and maize plant. There were three treatments applied namely control (sandy soil), overlay (4% by weight of clay) and incorporation of clay to sandy soils with four replicates. The results showed that chlorophyll content in cucumber was greatest in control treatment while there were no differences between overlay and incorporation treatments. Meanwhile, chlorophyll content in maize were increased consistently in control treatment while it decreased followed by an increase with the same rate in other two treatments (overlay and incorporation). Furthermore, overlay treatment showed small difference in comparison with incorporation treatment which maintain higher amount of water and control treatment. Utilization of
clay resulted in increased water use efficiency and water savings due to 45% till 64% of irrigation water can be saved compared to control. Nguyen and Marschner [24] comparing the effect of addition of a fine-textured soil (34% clay) to garden waste compost on nutrient availability and leaching in a sandy soil. The researchers reveal that fine textured soil can reduce N and P leaching and prolonged the positive effect of the clay as a growing media which can increase water and nutrient-holding capacity and ultimately the productivity of the crop.

In addition, Figure 1 also showed that the lowest chlorophyll content was obtained from F. carica grown under low root zone spatial with 100% clay soil composition of growing media. The SPAD value for this treatment was 22.5. According to Poorter et al. [51], detrimental effects may occur due to the application of small container for experimental study which are more associated to biological constraints. The availability of water and nutrients to the plant will be reduced due to the usage of small container (small quantity of soil or other substrate). Furthermore, the reduction of water and nutrient will also inhibit the root growth in container. This result is similar to a report on root restriction effects on growth and development of salvia (Salvia splendens) by Iersel [52] whose suggests that the small containers may cause inhibition of root growth and this would gave greater effect on shoot dry mass accumulation. Besides, the root restriction would disturb the pattern of assimilates partitioning and also reduced the root growth sharply by small containers support the root system’s capacity to act as a sink for assimilates would be much decreased. Further studies by Weyer et al. [53] found that porosity plays a significant part in the delivering of oxygen in the rooting environment. The researchers demonstrated that the oxygen concentration will be decreased to less than 20%, if the porosity reduced to less than 30% (v/v). Liu et al. [54] and Passioura [55] explained that fine-textured soils would cause compaction, drain the water slowly and impede root growth during irrigation. This soil also greater capillary fringe of saturated soil and more prone to waterlogging especially in shallow pots.

It also can be observed that F. carica grown in high root zone spatial and Mix 4 (75%BRIS soil + 25% Clay soil) gave lower value of chlorophyll content compared to plant grown in high root zone spatial with (50% BRIS soil + 50% clay soil) which was 37.7. This result can be attributed to the growing medium basically contained of greater particles will have more aeration and less water-holding capacity compared to smaller particles medium. Furthermore, plants growing in a medium composed of all large particles would dry out too rapidly while plants grown in a medium with all small particles would experience waterlogging. On the other hand, other research conducted by Zucco et al. [56] on leaf chlorophyll content of tomatoes grown on three different textural classes of soils namely sandy, loamy, and clayey with the amendment of vermicompost under greenhouse condition revealed that tomatoes grown in the sandy soil had the maximum leaf chlorophyll content in comparison with clay and loam soils while the loam soil providing the lowest. In this study, the loamy and clayey soils have become more compacted over the time which would obviously have an effect on plant growth.

4. Conclusion
The chlorophyll content of F. carica was significantly better with the application of Mix 3 (50% sand: 50% clay) and high root zone spatial than other treatments. Overall, the mixture of BRIS soil and clay in the growing media composition provided better crop performances for F. carica grown in container under greenhouse condition. Also, the use of different growth media could improve the agronomical and physiological efficiency of F. carica, producing economic and environmental benefits.

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