# SPAD Index in Oregano Crop: A Proposal for Interpretation Ranges

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## Abstract

The analysis of non-destructive variables in plants, such as the SPAD index, shows a growing trend of adoption in the field. However, it is necessary to determine comparative reference standards, aiming to assist in the interpretation of results obtained in the field and in making decisions about the management to be adopted. The study aimed to propose levels of interpretation of the SPAD index in oregano leaves based on the yield of the crop. The experiment was conducted in protected environment, randomized blocks design was adopted with four replications in 6 x 4 factorial scheme: six levels of water replacement (60, 70, 80, 90, 100, and 110% of the crop evapotranspiration-ETc) and four doses of bokashi (0, 100, 200, and 300 g m<sup>-2</sup>). For analysis, the data were subjected to variance analysis, multivariate analysis, regression and correlation. Productive management (water replacement and bokashi dose) influences the SPAD index response. Through mathematical analysis of the relationship between SPAD index and relative yield, the sufficiency ranges based on the SPAD index were determined in very low (<37) low (37-44), medium (44-46) and high (>46). The proposed classification of the sufficiency range for the SPAD index allows advances in the productive management of the oregano crop.

Keywords: non-destructive method, Origanum vulgare, plant analysis

## 1. Introduction

Plants are subject to different interactions with the productive environment, the consequence of which is reflected directly on the development of the plant, and by direct effect, on its productive potential (Ostadi et al., 2020; Hancioglu et al., 2021). Water management in cultivation has a direct impact on plant water potential, gas exchange, photosynthetic efficiency and mass accumulation (Wenneck et al., 2021; Andrian et al., 2022). Oregano grown under water deficit conditions presents a significant reduction in development and yield (Saath et al. 2022; Santi, 2022).

High quality soils can favor plant development, even under adverse conditions. In this sense, the application of organic compounds, such as bokashi, can contribute to physical and biological aspects, in addition to providing nutrients (Lopes Pereira et al., 2022), resulting in an increase in crop yield (Saath et al., 2022).

The measurement of morphological and/or physiological variables that correlate with productive performance, allow the evaluation of plant development and interventions in the management, aiming at productive increase (Caroll et al., 2017; Silva et al., 2021; Zhu et al., 2022).

The Soil Plant Analysis Development (SPAD), portable equipment which allows information to be obtained immediately and non-destructively, is used in various crops, the data obtained being correlated with the chlorophyll content of the leaves, the nutritional state of the plants and plant development (Dunn et al., 2018; Li et al., 2020; Benati et al., 2021). Although adopted in studies involving several species of agronomic interest, the interpretation of the results obtained is limited to local conditions. This fact is associated with limited information for some species, such as oregano (*Origanum vulgare*), and the lack of determination of ranges of values.

The definition of reference values assists in decision-making and crop management, benefiting producers, researchers and professionals in the area. Considering the limitation of information involving the SPAD index and the culture of oregano, the study aimed to propose levels of interpretation of the SPAD index in oregano leaves based on the yield of the crop.

## 2. Method

#### 2.1 Study Location

The study was based on information obtained in cultivation conducted in the Irrigation Technical Center (CTI) of the State University of Maringá (UEM), in municipality of Maringá, PR, Brazil (23°25'S, 51°57'W and 542 m altitude). The cultivation was conducted in protected environment (greenhouse), between the months of Febrary and April 2021. The characterization of temperature and relative humidity inside the protected environment during cultivation was determined with an automatic meteorological station, as described in Figure 1.





## 2.2 Experimental Design

The study was conducted using a randomized blocks design with four replications in a 6 x 4 factorial scheme: six levels of water replacement (60, 70, 80, 90, 100, and 110% of the crop evapotranspiration-ETc) and four doses of bokashi (0, 100, 200, and 300 g m<sup>-2</sup>).

#### 2.3 Crop Management

The oregano (cultivar Verdadero) was grown from seedlings in beds that were 3 m long, 0.5 m wide and 0.5 m high. Each experimental unit was composed of six plants, being considered the two central plants as a useful area. The soil in beds is classified as NITOSSOLO VERMELHO distroférrico according to the Brazilian Soil Classification System (SiBCS), which corresponds to Utissol in the US Soil Taxonomy (Santos et al., 2018). The chemical characterization of the soil is presented in Table 1. Fertilization was carried out using cured bovine manure (3 kg m<sup>-2</sup>), which was incorporated into the soil before transplanting the seedlings.

| рН                | Organic<br>matter | Р                   | Ca  | Mg   | K     | Al               | Н   | CEC  | V     |
|-------------------|-------------------|---------------------|-----|------|-------|------------------|-----|------|-------|
| CaCl <sub>2</sub> | %                 | mg dm <sup>-3</sup> |     |      | cmolc | dm <sup>-3</sup> |     |      | %     |
| 6.6               | 1.2               | 6.13                | 6.4 | 1.87 | 0.51  | 0.13             | 4.3 | 9.45 | 53.11 |

| Table 1. Soil chemica | l characterization | of the | 0-0.2 m | of drepth |
|-----------------------|--------------------|--------|---------|-----------|
|-----------------------|--------------------|--------|---------|-----------|

*Note*. P: Phosphor; Ca: Calcium; Mg: Magnesium; K: Potassium; Al: aluminum; H: hydrogen; CEC: cation exchange capacity; V: base saturation.

The determination of the crop's daily evapotranspiration (ETc) was determined with constant-level water table lysimeters present inside the protected environment, as described by Andrian et al. (2022). The oregano plants grown in the lysimeter had spacing similar to that adopted in the beds. Water replacement of the accumulated depth was carried out every two days, using self-compensating drippers, with a nominal flow of 4 L h<sup>-1</sup>, spacing of 0.3 m, and Christiansen's uniformity coefficient (CUC) of 94%.

## 2.4 Data Acquisition

The cultural management was based on the technical recommendations for the species, as reported by Santi (2022). The SPAD index was determined using SPAD-502 equipment (Minota<sup>TM</sup>), with readings taken in the morning (8 to 10 a.m.). The determination was performed on leaves in the upper third of the plant, taking three readings per plant. The evaluation was performed 60 days after transplanting-DAT. The harvest, with subsequent yield determination, was performed at 80 DAT (Saath et al., 2022).

## 2.5 Data Analysis

The SPAD index data was submitted to variance analysis and multivariate analysis, considering the primary sources of variation (water replacement and application of bokashi). The linear correlation of the variables and regression analysis was determined using the SPAD index data and the production indexes presented by Saath et al. (2022).

From the adjusted equation the estimated SPAD index was calculated and the classification by ranges was performed, considering the method adopted for determining the interpretation classes in soil analysis described by Pauletti and Motta (2019), where:

- Very low level: obtained for values where productivity is equal to or less than 40% of maximum productivity.
- Low level: obtained for values where productivity is between 40 to 70% of maximum productivity.
- Average level: obtained for values where productivity is between 70 to 90% of maximum productivity.
- High level: obtained for values where productivity is greater than 90% of maximum productivity.

SPAD index data as a function of crop management (water replacement level and bokashi doses) were submitted to analysis of variance. When determining the significant interaction of the factors (p<0.05) a multivariate regression analysis was performed. From the multivariate analysis, an adjustment equation was determined, with the respective coefficient of determination ( $R^2$ ), and a graph of the response surface.

Yield and SPAD index data were submitted to descriptive analysis, analysis of variance and regression. From the adjustment equation between performance and SPAD index, the intervals of the interpretation classes (very low, low, average, and high) were calculated.

Data analysis was performed using the softwares Statistica<sup>TM</sup> and MS Excel<sup>TM</sup>.

### 3. Results

The analysis of variance verified significant effects (p<0.05) for the interaction of the primary factors (water replacement (W) and application of bokashi (B)). Considering the sources of variation of a quantitative character, a multivariate analysis was performed and the equation of adjustment (Equation 1) was determined with a coefficient of determination (R<sup>2</sup>) equal to 0.63.

$$\hat{Y} = 12.0515 + (0.4845xW) + (0.0687xB) - (0.0023xW^2) - (4.0514E - 5xWxB) - (0.0001xB^2)$$
(1)

Were,

 $\hat{Y}$  = estimated SPAD index (dimensionless)

W= water replacement level (% of ETc)

B = dose of bokashi (mg m<sup>-2</sup>).

The data are presented in response surface graph, as shown in Figure 2.





The SPAD index data in the leaves showed a linear correlation equal to 0.94 with the dry mass yield of the aerial part of the plants (Saath et al., 2022). The descriptive analysis of the SPAD index data and yield of oregano are presented in Table 2.

| Parameter          | Yield (g plant <sup>-1</sup> ) | SPAD Index |
|--------------------|--------------------------------|------------|
| Average            | 10.30                          | 41.21      |
| Standard error     | 0.69                           | 0.80       |
| Median             | 10.31                          | 42.37      |
| Standard deviation | 3.33                           | 3.81       |
| Sample variance    | 11.10                          | 14.55      |
| Kurtosis           | 0.05                           | -1.30      |
| Asymmetry          | 0.51                           | -0.31      |
| Interval           | 12.59                          | 12.19      |
| Minimum            | 4.98                           | 34.67      |
| Maximum            | 17.56                          | 46.85      |

Table 2. Descriptive analysis of plant yield and SPAD index in oregano leaves

As the variation of the SPAD index was significant (p<0.05) with the yield of dry mass of the aerial part of the plant, regression analysis was performed as shown in Figure 3.





From the adjusted equation (Figure 3) the estimated SPAD index was calculated for yields of 40, 70 and 90% of the maximum yield. The maximum yield (17.41 g plant<sup>-1</sup>) was obtained as the average of the upper values (17.26 and 17.56). For the classification of the interpretation ranges the integer values were considered (without decimal places), and are presented in Table 3.

Table 3. Interpretation of the SPAD index in oregano leaves

| Interpretation class | SPAD Index |
|----------------------|------------|
| Very low             | < 37       |
| Low                  | 37-44      |
| Medium               | 44-46      |
| High                 | > 46       |

#### 4. Discussion

The present study proposed to define parameters for interpreting the SPAD index values obtained in oregano leaves, considering cultivation under different conditions of management. The cultivation under diverse water conditions (levels water replacement) and nutritional management (bokashi application) allowed obtaining productive data ranging from 4.98 to 17.56 g plant-1 and SPAD index ranging from 34.67 to 46.85 (Table 2). Kocabas-Oguz (2020) obtained SPAD index values (21.7 to 42.87), close to this study, when analyzing Origanum onites plants.

The SPAD index although it is a dimensionless variable, is commonly correlated to the content of chlorophylls and nitrogen content in leaves, being obtained quickly and non-destructively (Dunn et al., 2018; Vidigal et al., 2018; Mendoza-Tafolla et al., 2019; Hou et al., 2021). According to data obtained in the study, the SPAD index values obtained in field express direct effects of the development and management of the crop, especially under variable conditions of water replacement and fertilization with bokashi, according Fig 2.

According to the multivariate analysis of the SPAD index (Fig 2), under conditions of close to adequate water replacement (100% of ETc) and fertilization with organic bokashi compost, there is an increase of SPAD index obtained in the plants. Higher SPAD index values coincide with responses in morphological development and yield, as reported by Saath et al. (2022). Considering that yield potential is related to plant development, several studies have shown a relationship between the SPAD index and yield (Ostadi et al., 2020; Silva et al., 2021; Szulc et al., 2021).

Li et al. (2020) developed a model for fertilization of rice based on the values obtained with SPAD. However, there is a lack of information for most crops of agronomic interest, such as oregano, mainly related to the interpretation ranges of the index.

Benati et al. (2021) determined the range of SPAD index (39-49) in which nitrogen content in peach tree is adequate, being an indirect classification of SPAD index. Caroll et al. (2017) obtained, in maize, values of 31.9 and 37.2 respectively in nitrogen deficit and sufficiency condition. These studies demonstrate the narrow range for

classification between low and sufficient values for the SPAD index and the potential of the information obtained for the variable in the productive context. The mapping of the SPAD index is relevant for plant production, and Zhu et al. (2022) proposed the estimation and mapping of SPAD based on hyperspectral images.

In the present study, the approach of defining sufficiency ranges was adopted based on the relationship between the SPAD index obtained in the field and the respective productivity of the plants, disregarding the isolation of management factors. From the significant variation (p<0.05) between income and SPAD index, a mathematical adjustment model was obtained, demonstrating a positive trend between the variables (Fig 3).

From the mathematical model (Fig 3) and methodology used, it was determined as levels of sufficiency, according Table 3. The results allow for advances in the assessment and productive management of the crop, allowing for interventions aimed at increasing plant yields. However, further studies are necessary to compare the proposed values, the adequacy of the interpretation ranges and the definition of new ranges for the culture of oregano and other species of agronomic interest.

Limitations in the adoption of the SPAD index in the productive management of the oregano crop may still be related to problems in determining the values with the portable meter, either associated with plant age and leaf thickness (Pereyra et al., 2014) or by the location of the reading (Dunn et al., 2018), requiring standardization to obtain consistent information.

#### 5. Conclusion

When grown under different management conditions, there is variation in the SPAD index obtained from oregano leaves.

The yield of oregano and the SPAD index of the leaves show a positive relationship, making it possible to determine sufficiency ranges based on yield.

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#### **Authors contributions**

Conceptual idea: GSW; Methodology design: GSW, RS, and DST; Data collection: NOS, LLA, and VBC; Data analysis and interpretation: GSW; Writing and editing: GSW, and DST; Supervision: RS.

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The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

## Data sharing statement

No additional data are available.

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