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# The Effects of Different Rates of Nitrogen Fertilizer and Shade on Physiological Characteristics and Yield of Basil (*Ocimum tenuiflorum*)

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## ABSTRACT

The economic value of basil (*Ocimum tenuiflorum*) herb comes from its leaves; efforts to increase the number of leaves should be of high importance. This study was conducted to improve the growth and development of basil through investigation on the effects of applications of different rates of nitrogen (0, 100, 150 and 200 kg/ha) and percentage of shade (0, 50 and 70%). Results showed that the growth and development of basil responded to the different rates of nitrogen applications as well as the percentages of shade. Plants treated with 150 kg/ha N under 50% shade yielded the best results, reaching 48.8 cm in height, possessing the highest number of leaves (139 leaves/plant) and average leaf area (11.42 $cm^2$/leaf). It was also noticed that plants exposed to full sun light had the lowest plant height (34.55 cm), lowest number of leaves (120 leaves/plant) and lowest leaf area (5.40 $cm^2$/leaf). The application of nitrogen at 150 kg/ha and 50% shade offered better development of leaves (number, leaf area and weight) with good development of root (weight, length and surface area). As the value of basil is on its leaves, therefore, the final recommendation is made based on the growth and development of its leaves.

**Key words :** Physiology, light intensity, fertilizer, production, yield, basil

## INTRODUCTION

Basil belonging to the Lamiaceae family, is one of the most important aromatic and medicinal plants, and is widely cultivated in many countries (Pirbalout *et al.,* 2017). Under the genus of *Ocimum*, there are several types of species that are widely cultivated: *basilicum, gratissimum, citriodorum, americanum* and *tenuiflorum.* Among these, *O. basilicum* is the most popular species. Basil is an aromatic herb that grows from 20-80 cm in height (Ghasemzadeh *et al.,* 2016). More cosmetics, health related foods and pharmaceuticals have recently included basil as one of the ingredients in the products. As the part of economic importance in this herb is its leaves, increasing the number of leaves will help to increase yield production.

It is well-known that the application of nitrogen can help in enhancing the performance of the upper ground parts, particularly the leaves. It is one of the primary macronutrients that is essential to plants and is an important constituent of phospholipids, nucleic acids, chlorophyll and proteins, which support plant growth and development. Legahri *et al.* (2016) stated that nitrogen played the most vital role in various physiological processes in plants, where it promoted more leaves and produced dark-green colour in parts of plants.

Other than nitrogen, the irradiance from the sun also has multiple effects on plant physiology, growth and development. Naturally, basil can grow well under full sun light, however, they can also tolerate light shade (Tattini *et al.,* 2014). Providing shade to basil sounds potential, it is however, there were also reports which stated the adverse effects of shade (Kumar *et al.,* 2014). It is then important to determine the suitable percentage of shade to be used, as it is also dependent on the light intensity of a particular country.

To date, no study has been reported on the effects of combined applications of nitrogen

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fertilizer and shade on performance of basil. As a result, the aim of this study was to determine the effects of different rates of nitrogen fertilization and shade on the physiological characteristics of basil.

## MATERIALS AND METHODS

The experiment was conducted in an open field condition, of field 15 Universiti Putra Malaysia in Serdang Selangor. The experimental site was located at the Latitude of 2° 59′ 0.42″ N and longitude of 101°44′ 104″ E, at 30 m above the sea level. Basil seeds were first germinated in trays for 15 days fewer than 50% shade. The seeds were planted on 13 April 2019 and were transplanted to field on 28 April 2019. A commercial soil mixture (IMPRA™ Bio soil) was analyzed before transplanting of seedling to poly bags, in which elements were (6.81 pH, 0.03 N%, 0.5540 P% and 0.1837.20 $K_2O$%). Seedlings were later transplanted into the soil mixture using 14 x 14 cm poly bags. Treatments were arranged in a factorial randomized complete block design (4 x 3; nitrogen x shade) with four replications, where each replication consisted of 10 plants. Data were subjected to the analysis of variance using the Statistical Analysis System software SAS version 9.4 (SAS Institute, Cary, NC) and means were compared by least significant difference at the 5% probability level.

Transplanted seedlings were placed at different percentages of shades (0, 50 and 70%). The average temperature under the shade was between 26 to 37°C, with the relative humidity level between 75 to 95% throughout the experimental period. The average rainfall was 4.96 inch (126.0 mm) and irrigated manually two times daily. Plots were kept clean to prevent pests and diseases. However, aphids and white flies were observed at the plot. Thus, malathion [(Dimethoxyphosphorothiyl) sulfanyl] butanediote was applied twice throughout the study at concentration of 2.5 l/ha. Nitrogen in the form of urea was applied at different rates of 0, 100, 150 and 200 kg/ha on 20 DAT. The granular urea was spread evenly around the plant's canopy. No other fertilizer was added in this experiment. Non-destructive data were taken on 15 and 30 days after transplanting (DAT), focusing on plant height and number of leaves. Destructive data were taken on 75 DAT, which were number of leaves, leaf area, dry weight of leaves and roots, chlorophyll A and chlorophyll B contents, total root length, total root volume and total root surface area. During this stage, the plants were at reproductive phase and de-flowering was done.

Plant height and number of leaves were taken individually per plant basis. Plant height was measured from the soil surface until the shoot tip of the plants for each plant using a measuring tape and expressed in centimeters. As for data on leaf area, the leaves were detached from each plant and the total leaf area was taken using the leaf area meter. Data were presented in average leaf area per leaf ($cm^2$) for each treatment.

Leaves and roots were measured at 75 DAT (90 days after sowing). To obtain the dry weight, all parts were labelled according to the treatments and were placed in the oven at 60°C until a constant weight was reached.

Leaves from each treatment were cut into the size of 4 $cm^2$ and placed in a glass vial containing 20 ml of 80% acetone. Samples were kept for seven days in a dark condition at room temperature. After seven days, the samples were taken out and the absorbance of the solution was measured using spectrophotometer at 647 nm and 664 nm for chlorophyll A and B contents, respectively. The calculation for chlorophyll A and B used was as follows :

$$\text{Chlorophyll a } (\mu M) = 13.19\ A664 - 2.57\ A647/4\ cm^2$$
$$\text{Chlorophyll b } (\mu M) = 22.10\ A647 - 5.26\ A664/4\ cm^2$$

Total length, volume and surface area of root per plant were also collected at 75 days after transplanting. The freshly harvested plants were taken immediately to the laboratory. The plant parts were separated and the roots were washed thoroughly with running tap water in order to remove the soils. Five plant samples from each replicate were taken at random and were placed on digital root analyzer model Win RHIZO (Epson Expression 1680 USA) for the measurements.

## RESULTS AND DISCUSSION

Plant height was about 2 cm at 15 DAT and was twice the value at 30 DAT (Table 1). As an effect of nitrogen application and shade, a differential effect was found between the two

**Table 1.** Plant height and number of leaves as affected by the application of different rates of nitrogen and percentages of shades in basil

| Treatment | Plant height (cm) | | Number of leaves | |
|---|---|---|---|---|
| | 15 DAT | 30 DAT | 15 DAT | 30 DAT |
| **Nitrogen (kg/ha)** | | | | |
| 0 | 2.01±0.10a | 3.95±0.15a | 4.95±0.10a | 6.03±0.10a |
| 100 | 2.02±0.08a | 4.04±0.05a | 5.05±0.20a | 5.96±0.20a |
| 150 | 1.96±0.06a | 3.97±0.05a | 5.15±0.34a | 6.15±0.25a |
| 200 | 2.01±0.13a | 4.02±0.13a | 4.69±0.16a | 5.60±0.16a |
| **Shade (%)** | | | | |
| 0 | 1.99±0.13a | 4.04±0.18a | 5.03±1.46a | 6.03±1.46a |
| 50 | 2.00±0.05a | 3.94±0.12a | 4.99±0.30a | 6.05±0.36a |
| 70 | 2.01±0.18a | 4.01±0.16a | 4.85±0.34a | 5.72±0.30a |
| N x S | NS | NS | NS | NS |

All parameters reported in the manuscript were analyzed in ANOVA. Means followed by the same letter are not different using the LSD (at P<0.05). NS–Not Significant.

factors at 75 DAT (Table 2). The tallest plants were recorded from plots applied with 100 and 150 kg/ha N under 50% shades, with plant height of around 48 to 49 cm at 75 DAT. In contrast, plant height recorded from other treatment combinations was generally less than 40 cm, with the lowest being from the control (34.6 cm). There was a general trend showing that increase in nitrogen rate caused height retardation of the plants. Heavy shade of 70% also caused a decrease in plant height of the plants. It can, therefore, be inferred that both nitrogen fertilizer rates and percentages of shade play an important role in the progress and development of the physiological characteristics of basil plants. This result was in agreement with that of Nagaveni *et al.* (2018), which revealed that application of nitrogen increased plant height of basil plants. Nitrogen, being a major food for plants was an essential constituent of protein which was responsible in chemical responses, transportation of electrons and in chlorophyll (Leghari *et al.,* 2016). Besides, plant height may be since plants grown in shade resembled etiolated plants which had an unwanted tall appearance and were more apical dominant.

Number of leaves was recorded at 15, 30 and 75 DAT, and was expressed in per plant basis. The plants showed no variation in number of leaves at 15 DAT, where all plants had around five leaves per plant. It was also observed that the plants still did not show variation in the number of leaves among treatments at 30 DAT, with value of six leaves per plant. Although nitrogen was applied at 20 DAT, the effect was not clear. It was found that the plants took more than 10 days to show effects of nitrogen

**Table 2.** Effect of different levels of nitrogen and percentage of shade on plant height (cm) of basil at 75 DAT

| Nitrogen (kg/ha) | Shade (%) | | | Mean |
|---|---|---|---|---|
| | 0 | 50 | 70 | |
| 0 | 34.55c | 35.47c | 36.10bc | 35.37c |
| 100 | 35.90c | 48.35a | 36.51bc | 40.25a |
| 150 | 35.12c | 48.80a | 38.21b | 40.71a |
| 200 | 38.12b | 39.20b | 37.55b | 38.29b |
| Mean | 35.92c | 42.95a | 37.09b | |

Means with the same letter in the same column are not different at P≤0.05 based on LSD. DAT–Days after transplanting.

applications. Different percentages of shade did not influence the number of leaves produced at the earlier stages. It is also assumed that at this point the plants were still at the hardening phase or were just and were still adapting in the new environment after transplanting. According to the classification by the United States Department of Agriculture (2019), basil plant is a dicotyledon plant which may require longer hardening phase after transplanting.

After the application of nitrogen, a differential effect between nitrogen rate and shade percentage was found for number of leaves (Table 3) at 75 DAT. It was noticed that application of 150 and 200 kg/ha N under 50% shade showed greater effects compared to other treatments, with 139 and 137 leaves at 75 DAT, respectively. Plants given 0, 100 and 150 kg/ha N under 0% shade produced the lowest number of leaves, with less than 122 leaves per plant. Thus, it can be inferred that the effects of nitrogen were less effective in increasing the number of leaves under 0%

**Table 3.** Effect of different levels of nitrogen and percentage of shade on number of leaves of basil at 75 DAT

| Nitrogen (kg/ha) | Shade (%) | | | Mean |
|---|---|---|---|---|
| | 0 | 50 | 70 | |
| 0 | 121.05d | 125.30c | 124.15c | 123.5d |
| 100 | 120.85d | 125.05c | 127.25bc | 124.38c |
| 150 | 121.75d | 139.30a | 127.85bc | 129.63a |
| 200 | 125.50c | 136.50b | 123.45d | 128.48b |
| Mean | 122.28c | 131.53a | 125.67b | |

Means with the same letter in the same column are not different at P≤0.05 based on LSD. DAT–Days after transplanting.

shade in basil. Applying nitrogen at a higher rate of 200 kg/ha increased number of leaves (125.5 leaves per plant). A different trend was observed when 70% shade was given, where an increase in nitrogen rate caused a decrease in number of leaves.

However, it was also important to observe the leaf area resulting from each treatment. The effects of nitrogen rates and percentages of shade on leaf area of the plants are presented in Table 4, expressed in per leaf basis. Both factors showed differential effects between each other. The untreated plants had 6.11 $cm^2$ of leaf area, which were not different from those treated with nitrogen under full sunlight. Plants placed under heavy shade had no difference in leaf area compared to those in full sunlight, besides showing no effect from different rates of nitrogen. However, leaf area of the plants was almost doubled when applied with 150 kg/ha N under 50% shades with mean value of 11.42 $cm^2$ per leaf, making it the most effective among the treatments. The overall pattern showed that increasing the rate of nitrogen up to 200 kg/ha N reduced plant's leaf area. Springer (2015) found in *P. longum* that

**Table 4.** Effect of different levels of nitrogen and percentage of shade on number of leaf area ($cm^2$) of basil at 75 DAT

| Nitrogen (kg/ha) | Shade (%) | | | Mean |
|---|---|---|---|---|
| | 0 | 50 | 70 | |
| 0 | 6.11cd | 7.60cd | 5.83d | 6.51b |
| 100 | 5.14d | 8.48bc | 5.87d | 6.49b |
| 150 | 5.85d | 11.42a | 5.91d | 7.73a |
| 200 | 5.40d | 9.32b | 5.48d | 6.74b |
| Mean | 5.63b | 9.20a | 5.77b | |

Means with the same letter in the same column are not different at P≤0.05 based on LSD. DAT–Days after transplanting.

plants grown under shade increased number of leaves and leaf area. Similar result was also obtained by Larimi *et al.* (2014) who found that application of nitrogen fertilizers increased the leaf area of basil. Previous researchers had reported that nitrogen was involved in various metabolic processes which had direct impact on vegetative phase in plants, as it was an important constituent of nucleotides, proteins and enzymes (Zhang *et al.,* 2014), which helped in regulating the metabolism in the plant system.

Observation on chlorophyll content was taken at 75 days after transplanting. As shown in Table 5, no interaction between the factors was shown on chlorophyll A. Application of 150 kg/ha N fertilizer produced the highest chlorophyll A (0.546 mg/$cm^2$) compared to 0, 100 and 200 kg/ha N. The lowest chlorophyll A resulting from nitrogen application was from 200 kg/ha N treatment (0.544 mg/$cm^2$). With regards to percentages of shade, plants under 50% shade performed better than those under the other two percentages of shade.

**Table 5.** Chlorophyll A and leaf dry weight of basil as affected by the application of different rates of nitrogen and shades

| Treatment | Chlorophyll A (mg/$cm^2$) | Leaf dry weight (g) |
|---|---|---|
| **Nitrogen (kg/ha)** | | |
| 0 | 0.546c | 1.37b |
| 100 | 0.545b | 1.18c |
| 150 | 0.546a | 1.67a |
| 200 | 0.544d | 1.36b |
| **Shade (%)** | | |
| 0 | 0.545a | 1.17c |
| 50 | 0.546b | 1.61a |
| 70 | 0.544c | 1.41b |
| N x S | NS | NS |

All parameters reported in the manuscript were analyzed in ANOVA. Means followed by the same letter are not different using the LSD (at P<0.05). NS–Not Significant.

Meanwhile, differential effects between the two factors were found on chlorophyll B content (Table 6). Results shown in Table 7 reveal that plants placed under 0 and 70% shades had similar amount of chlorophyll B, with values of 1.5-1.6 mg/$cm^2$. Plants placed under 50% shade showed higher amount of chlorophyll B, with value of 1.81 mg/$cm^2$. When the two factors were combined, plants applied with 150 kg/ha N and 50% shade had the highest amount of chlorophyll B, with value of 2.38 mg/$cm^2$. The amount of chlorophyll B dropped to

**Table 6.** Effect of different levels of nitrogen and percentage of shade on number of chlorophyll B (mg/cm$^2$) of basil at 75 DAT

| Nitrogen (kg/ha) | Shade (%) | | | Mean |
|---|---|---|---|---|
| | 0 | 50 | 70 | |
| 0 | 1.42d | 1.59c | 1.67b | 1.56b |
| 100 | 1.47d | 1.71b | 1.42d | 1.53bc |
| 150 | 1.71b | 2.38a | 1.55c | 1.88a |
| 200 | 1.68b | 1.55c | 1.41d | 1.55b |
| Mean | 1.57b | 1.81a | 1.52b | |

Means with the same letter in the same column are not different at P≤0.05 based on LSD. DAT–Days after transplanting.

35% when the nitrogen rate was increased to 200 kg/ha N. Besides, the chlorophyll B content from plants applied with the low nitrogen rate (100 kg/ha N) was like that of the untreated plants.

It is assumed that the addition of nitrogen to plants caused an increase in the amount of chlorophyll in the leaves (chlorophyll A and chlorophyll B) and thus, improved the photosynthesis processes. Internally, higher production of photo-assimilates will occur because of the increase of cell division and cell size, as explained by Larimi *et al.* (2014). The result obtained by Ilic *et al.* (2018) showed that plants contained more chlorophyll A and chlorophyll B when planted under shade compared to those in an open area.

Furthermore, leaf dry weight was recorded at 75 DAT to determine the biomass yield of the leaves. Application of 150 kg/ha N fertilizer produced the highest leaf dry weight per plant (1.67 g) compared to rates of 0, 100 and 200 kg/ha N. The lowest leaf dry weight resulted from nitrogen application at 200 kg/ha N treatment (1.36 g). With regards to percentage of shade, plants under 50% shade performed better than those under the other two percentages of shade. Increasing shade percentage to 70% caused 12% reduction of leaf dry weight. This result was in contrast to that of Ilic *et al.* (2018) who reported that lower light intensity caused an increase in leaf dry weight. This contradiction might have been due to the difference in prevailing natural light intensities between the two studies, as these were dependent on the geographical area at which the research was conducted. Thus, in this study, it was assumed that basil can only tolerate shade of 50%, beyond which can cause a detrimental effect on its performance.

Roots being the anchor of the plants to the soil are responsible for water uptake, minerals and nutrients. Results of this study showed no effect of interaction between the two factors on root dry weight. There was also no effect among rates of nitrogen (Table 7). However, dry mass of the root was affected by the different percentages of given shades. Plants exposed to direct sunlight were found to have 1.28 g of root dry weight per plant, while 1.87 g when grown under 50% shade, because shading caused reduction in evapo-transpiration in plants. The evapo-transpiration was correlated with hydraulic conductivity in roots which altered the regulation ability of water balance in plants (Bote and Vos, 2017), which might have contributed to the dry mass of the roots as measured in this study.

**Table 7.** Root dry weight, root total length and root total volume of basil as affected by the application of different rates of nitrogen and shades

| Treatment | Root dry weight (g) | Root total length (cm) | Root total volume (cm$^3$) |
|---|---|---|---|
| **Nitrogen (kg/ha)** | | | |
| 0 | 1.2 ±0.90a | 441.29 ±27.70a | 0.18 ±0.018a |
| 100 | 1.1 ±0.42a | 443.98 ±11.95a | 0.2 ±0.027a |
| 150 | 1.4 ±0.43a | 453.65 ±47.68a | 0.18 ±0.028a |
| 200 | 1.07 ±0.51a | 458.18 ±21.56a | 0.19 ±0.028a |
| **Shade (%)** | | | |
| 0 | 1.28 ±0.53b | 422.34 ±17.72c | 0.2 ±0.027a |
| 50 | 1.87 ±0.20a | 489.72 ±73.19a | 0.19 ±0.028a |
| 70 | 0.43 ±0.46c | 435.76 ±11.95b | 0.17 ±0.007a |
| N x S | NS | NS | NS |

All parameters reported in the manuscript were analyzed in ANOVA. Means followed by the same letter are not different using the LSD (at P<0.05). NS–Not Significant.

Furthermore, the same pattern was also observed for total root length produced by basil plants. There were no differential effects of the two factors on root length (Table 7). Nevertheless, different percentages of shade affected total root length. Plants grown fewer than 50% shade had the highest total root length (489.72 cm) in comparison to the control (422.34 cm) and those grown fewer than 70% shade (435.76 cm). To date, there has been no previous report on the root length of basil plants. The finding from this study has brought a new insight on how basil grown under 50% shade maximized the expression of total root length of basil plants. It was assumed that plants under 50% shade were more efficient in absorbing water, minerals and nutrients from the soil as the roots were widely

**Table 8.** Effect of different levels of nitrogen and percentage of shade on root total surface area ($cm^2$) of basil at 75 DAT

| Nitrogen (kg/ha) | Shade (%) | | | Mean |
|---|---|---|---|---|
| | 0 | 50 | 70 | |
| 0 | 31.48b | 30.69c | 35.40b | 32.52bc |
| 100 | 29.81c | 32.25b | 36.67ab | 32.91b |
| 150 | 32.69b | 38.81a | 36.02ab | 35.84a |
| 200 | 34.07b | 30.77c | 35.37b | 33.07b |
| Mean | 32.01b | 3288b | 35.87a | |

Means with the same letter in the same column are not different at P≤0.05 based on LSD. DAT–Days after transplanting.

distributed below the soil surface. More efficient absorption of those elements would help to enhance the plant's growth.

As for the total root volume (Table 7), it was interesting to observe that there was no effect of all nitrogen rates, percentages of shade, as well as their interaction, although effects were found on other root parameters. However, the root surface area seems to be the only root characteristic that revealed differential effects between the two factors (Table 8). The increase in rate of nitrogen fertilizer led to the increase in root surface area of basil. Results showed that application of 150 kg/ha N under 50% shade percentage yielded the highest root surface area (38.81 $cm^2$), compared to other treatments applied. It was surprising to observe that the application of nitrogen fertilizer did not affect the root surface area when the plants were under 0 and 70% shade. This inferred that the effectiveness of nitrogen was much dependent on the percentage of shade, in regard to high root surface area. High root surface area in plants was function of root diameter and total root length. However, performance relating to root diameter was not investigated in this study. It can be inferred that high root surface area was beneficial to the plants, because it would contribute to availability of greater surface area for nutrient absorption for better growth and yield performance of the plants.

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