# The effect of nitrogen fertilization and stage of plant development on the mass and quality of sweet basil leaves (*Ocimum basilicum* L.)

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S u m m a r y

The effect of nitrogen fertilization in various rates and the stage of plant development (beginning and full bloom) on the mass of leaves of three sweet basil cultivars ('Dark Opal', 'Mittelgross' and 'Wala') as well as the content of total N, chloroplast pigments and essential oil in leaves were estimated. It was found that nitrogen fertilization significantly increased the mass of leaves, the chloroplast pigment content and the yield of essential oil whereas the effect of the stage of plant development on above parameters depended on the cultivar.

*Key words: sweet basil, nitrogen nutrition, development stage, mass leaves, chloroplast pigments, essential oil*

Sweet basil is one of the oldest spices originated from the Far East and presently a great, renewed interest in this plant is observed. It is also known as a medicinal plant, confirmed not only by the experience gathered over generations but also by research results of modern phytotherapy [1, 2]. This species is also used in aromatherapy, homeopathy and cosmetology [3].

Within the *Ocimum basilicum* L. species, forms and cultivars with green leaves are cultivated most frequently, although there are also forms with anthocyanin content [3, 4].

There is a close correlation between nitrogen content in leaves and pigment levels, dependent on the dose of fertilizer and date of application [5, 6].

The aim of the study was to determine the effect of nitrogen fertilization on the mass of sweet basil leaves as well as the content of nitrogen, essential oil and chloroplast pigments in them at two different stages of plant development.

## MATERIALS AND METHODS

In the years 2002-2003, three cultivars of sweet basil belonging to the group of medium-sized leaves, were grown in an unheated glasshouse, in pots of 5 $dm^3$ filled with substrate composed of mineral soil and raised peat mixed at the v:v ratio of 1:1 [7]. Cultivar 'Dark Opal' is characterized by dark anthocyanin leaves and stalks, whereas both the leaves and stalks of cv. 'Mittelgross' are green. Cv. 'Wala' distinguished by green leaves and anthocyanin stalks.

In order to obtain $pHH_2O = 6.5$, the substrate was limed on the basis of the neutralization curve, using the amount of 30 g of $CaCO_3$/plant. Nitrogen fertilizer ($NH_4NO_3$ form) was applied twice: pre-vegetatively (in 0.9, 1.2 or 1.5 g N per plant) and as top dressing after the first harvest (in 0.45, 0.6 and 0.75 g N per plant). The combination without nitrogen fertilization was a control. The other macro- and microelements constituted the background of the experiments. The following fertilizers were applied per plant: 0.4 g P as $Ca(H_2PO_4)_2 \cdot H_2O$; 0.8 g K as $K_2SO_4$; 0.5 g Mg as $MgSO_4 \cdot 7H_2O$ and 0.5 g Polichelat LS-79 (chelate polymer) of the following composition: 2.9% Mg, 2.9% Fe, 1.9% Zn, 1.4% Mn, 1.3% Cu, 0.7% B and 0.1% Mo.

In the middle of May basil seedlings at the stage of 4-5 leaves and 12-15 cm in height were planted into pots (1 plant per pot). Each combination consisted of 12 plants, 6 for each development stage.

Plants were harvested twice: at the beginning of bloom and in full bloom. Leaves were separated from stems, dried in a ventilation drier at 30°C, ground and then the mass of air-dried leaves was determined.

Plant material was subjected to wet mineralization in a mixture of sulfosalicylic acid, sodium thiosulfate and selenium mixture, followed by the total nitrogen determination using the distillation method with the use of a Parnas-Wagner apparatus [8].

For determination of chloroplast pigments leaves were pretreated with boiling water (to remove anthocyanin pigments), and next extracted with acetone. Content of chlorophylls $\alpha$ and $\beta$ and carotenoids in the extract were determined spectrophotometrically according to Arnon's method [9].

Essential oil content was determined in the air-dried leaves using the steam distillation in the Deryng's apparatus [10].

Results of experiments are presented as means from two years. The tree-way analysis of variance with the Duncan's multiple range test was performed to determine the significance of differences at $P \leq 0.05$ for the mass of basil leaves, and content of chlorophyll $\alpha$, $\beta$ and carotenoids separately.

## RESULTS

The application of nitrogen fertilizers increased the average air-dried mass of basil leaves in the investigated cultivars in comparison with the control (Table 1). However, generally, no significant differences were found in mass of leaves among the applied nitrogen doses in any of the cultivars.

**Table 1.**

Effect of nitrogen fertilization and stage of plant development on the air-dry mass of sweet basil leaves.

| cultivar | nitrogen fertilization level (g N/plant) | stage of plant development | | | | mean |
|---|---|---|---|---|---|---|
| | | beginning of bloom | | in full bloom | | |
| | | mass of leaves (g/plant) | mass increase compared with control (%) | mass of leaves (g/plant) | mass increase compared with control (%) | |
| 'Dark Opal' | 0.0 | 4.00 ab | 100.0 | 3.50 ab | 100.0 | 3.75 a |
| | 0.9* + 0.45** | 4.83 b | 120.6 | 4.83 b | 138.0 | 4.83 b |
| | 1.2* + 0.6** | 4.33 ab | 108.2 | 6.33 c | 180.9 | 5.33 b |
| | 1.5* + 0.75** | 3.16 a | 79.0 | 7.00 c | 200.0 | 5.10 b |
| | mean | 4.10 | | 5.41 | | |
| 'Mittelgross' | 0.0 | 3.83 a | 100.0 | 4.83 a | 100.0 | 4.33 a |
| | 0.9* + 0.45** | 10.16 bcd | 265.2 | 10.83 cd | 224.2 | 10.50 b |
| | 1.2* + 0.6** | 5.83 ab | 152.2 | 10.83 cd | 224.2 | 8.33 b |
| | 1.5* + 0.75** | 7.16 abc | 186.9 | 14.00 d | 289.9 | 10.58 b |
| | mean | 6.75 | | 10.13 | | |
| 'Wala' | 0.0 | 6.66 ab | 100.0 | 4.16 a | 100.0 | 5.42 a |
| | 0.9* + 0.45** | 11.66 c | 175.0 | 9.83 bc | 236.2 | 10.75 b |
| | 1.2* + 0.6** | 6.50 ab | 97.6 | 9.83 bc | 236.2 | 8.16 b |
| | 1.5* + 0.75** | 9.50 bc | 142.6 | 8.66 bc | 208.1 | 9.10 b |
| | mean | 8.58 | | 8.13 | | |

Values marked with the same letters in column do not differ significantly.
* the nitrogen rate prior to vegetation
** the nitrogen rate in top-dressing

The highest mean leaf mass was found in cv. 'Wala' and in cv. 'Mittelgross' while applying the lowest dose of nitrogen. Cultivar 'Dark Opal' showed the highest mass after fertilization at 1.2+0.60 g N/plant, but it was by approx. 40% lower in comparison with the cultivars mentioned above.

The mass of leaves from plants harvested in full bloom was higher than that obtained at the beginning of bloom – by 30% in cv. 'Dark Opal' and by 50% in cv. 'Mittelgross'. In contrast, no significant correlation was found between the mass of cv. 'Wala' leaves and the time of harvest.

At the beginning of bloom the greatest increase in leaf mass in the studied cultivars, in comparison with the control, was obtained for the lowest dose of nitrogen fertilization. On the other hand, in full bloom, leaf mass increase as compared with

the control was the highest in cv. 'Dark Opal' and cv. 'Mittelgross', with the highest nitrogen dose 1.5+0.75 g N/plant. In cv. 'Wala' the highest leaf mass increase was achieved for both combinations 0.9+0.45 g N/plant and 1.2+0.6 g N/plant.

Nitrogen content in leaves increased proportionally to the increasing nitrogen fertilization (Table 2). An exception to this trend was observed in case of the highest nitrogen contents which were found at the beginning of bloom in cv. 'Wala' and cv. 'Mittelgross' in the combination 1.2 g N/plant.

**Table 2.**

Effect of nitrogen fertilization and stage of plant development on the N-total content in leaves of sweet basil.

| cultivar | nitrogen fertilization level (g N/plant) | N - total content (% d.m.) | | |
|---|---|---|---|---|
| | | stage of plant development | | |
| | | beginning of bloom | in full bloom | mean |
| 'Dark Opal' | 0 | 1.85 | 1.01 | 1.43 |
| | 0.9* + 0.45** | 3.72 | 3.56 | 3.64 |
| | 1.2* + 0.6** | 3.89 | 3.86 | 3.87 |
| | 1.5* + 0.75** | 4.27 | 3.99 | 4.13 |
| 'Mitellgross' | 0 | 1.70 | 0.73 | 1.21 |
| | 0.9* + 0.45** | 2.23 | 1.69 | 1.96 |
| | 1.2* + 0.6** | 4.62 | 2.04 | 3.33 |
| | 1.5* + 0.75** | 4.28 | 2.54 | 3.41 |
| 'Wala' | 0 | 1.20 | 1.29 | 1.24 |
| | 0.9* + 0.45** | 3.54 | 3.14 | 3.34 |
| | 1.2* + 0.6** | 4.40 | 3.13 | 3.77 |
| | 1.5* + 0.75** | 4.03 | 3.88 | 3.96 |

* and ** as in Table 1.

Nitrogen fertilization had an effect on contents of all chloroplast pigments (Table 3). At the beginning of bloom the higher chlorophyll α contents were observed in comparison to that of full blooming stage.

**Table 3.**

Effect of nitrogen fertilization and stage of plant development on the content of chloroplast pigments in sweet basil leaves.

| stage of plant development | cultivar | nitrogen fertilization level (g N/plant) | chlorophyll *a* | chlorophyll *b* | carotenoids |
|---|---|---|---|---|---|
| | | | (mg/g d.m.) | | |
| beginning of bloom | 'Dark Opal' | 0 | 6.38 cd | 2.8 b | 1.84 b |
| | | 0.9* + 0.45** | 8.95 e | 3.61 bc | 2.15 b |
| | | 1.2* + 0.6** | 9.75 e | 3.72 c | 2.12 b |
| | | 1.5* + 0.75** | 8.92 e | 3.4 bc | 1.93 b |
| | | mean | 8.5 e | 3.38 bc | 2.01 b |
| | 'Mittelgross' | 0 | 2.85 b | 1.05 a | 0.79 a |
| | | 0.9* + 0.45** | 5.18 c | 1.7 ab | 1.08 ab |
| | | 1.2* + 0.6** | 5.88 cd | 1.81 ab | 1.6 b |
| | | 1.5* + 0.75** | 3.03 b | 1.01 a | 0.78 a |
| | | mean | 4.23 c | 1.39 ab | 1.06 ab |
| | 'Wala' | 0 | 3.15 b | 0.94 a | 0.98 a |
| | | 0.9* + 0.45** | 4.54 c | 1.66 ab | 1.42 ab |
| | | 1.2* + 0.6** | 5.73 cd | 1.86 ab | 1.47 ab |
| | | 1.5* + 0.75** | 4.44 c | 1.24 ab | 1.07 ab |
| | | mean | 5.25 c | 1.42 ab | 1.01 ab |
| in full bloom | 'Dark Opal' | 0 | 0.56 a | 0.39 a | 0.31 a |
| | | 0.9* + 0.45** | 7.42 d | 3.58 bc | 2.42 c |
| | | 1.2* + 0.6** | 7.25 d | 3.34 bc | 1.92 b |
| | | 1.5* + 0.75** | 6.31 cd | 2.54 b | 1.68 b |
| | | mean | 5.38 c | 2.46 b | 1.58 b |
| | 'Mittelgross' | 0 | 0.56 a | 0.27 a | 0.32 a |
| | | 0.9* + 0.45** | 2.37 b | 1.11 ab | 0.71 a |
| | | 1.2* + 0.6** | 3.13 b | 1.39 ab | 0.93 a |
| | | 1.5* + 0.75** | 5.06 c | 2.16 b | 1.25 ab |
| | | mean | 2.78 b | 1.23 ab | 0.8 a |
| | 'Wala' | 0 | 0.61 a | 0.1 a | 0.32 a |
| | | 0.9* + 0.45** | 2.03 ab | 0.51 a | 0.66 a |
| | | 1.2* + 0.6** | 2.48 b | 0.91 a | 0.97 a |
| | | 1.5* + 0.75** | 3.59 bc | 1.39 ab | 1.19 ab |
| | | mean | 2.31 b | 0.73 a | 0.78 a |

Explanations as in Table 1.

Maximum contents of chlorophyll α and β and carotenoids were found in all the cultivars for the dose 1.2 g N/plant at the first stage of plant development, whereas at the second one the most advantageous dose was 1.5+0.75 g N/plant for cv. 'Mittelgross' and cv. 'Wala', and dose 0.9+0.45 g N/plant for cv. 'Dark Opal'.

Chlorophyll α content in leaves was 2–3 times higher than that of chlorophyll β. Moreover, it was found that the content of chloroplast pigments in cv. 'Dark Opal' was on average twice as high in comparison with cv. 'Wala' and cv. 'Mittelgross'.

Essential oil content in the investigated basil cultivars ranged from 0.2% to 0.8% (Table 4). While analyzing this parameter of raw material quality it was found that a slightly more advantageous harvest time for cv. 'Dark Opal' and cv. 'Mittelgross' was the beginning of flowering, while for cv. 'Wala' it was full bloom phase. At the same time essential oil content in cv. 'Wala' was almost 100% higher than in the other cultivars.

**Table 4.**

Effect of nitrogen fertilization and stage of plant development on the content and productivity of essential oils in leaves sweet basil leaves.

| cultivar | nitrogen fertilization level (g N/plant) | stage of plant development | | | | | |
|---|---|---|---|---|---|---|---|
| | | beginning of bloom | | | in full bloom | | |
| | | essential oil content (% d.m.) | yield of essential oil (mg/plant) | increase in essential oil yield compared with control (%) | essential oil content (% d.m.) | yield of essential oil (mg/plant) | increase in essential oil yield compared with control (%) |
| 'Dark Opal' | 0 | 0.40 | 16.0 | 100.0 | 0.20 | 7.0 | 100.0 |
| | 0.9* + 0.45** | 0.30 | 14.0 | 87.5 | 0.40 | 19.3 | 275.7 |
| | 1.2* + 0.6** | 0.20 | 8.8 | 54.1 | 0.20 | 12.6 | 180.0 |
| | 1.5* + 0.75** | 0.40 | 1.2 | 7.9 | 0.20 | 14.0 | 200.0 |
| | mean | 0.32 | 13.0 | | 0.25 | 13.5 | |
| 'Mittelgross' | 0 | 0.40 | 15.3 | 100.0 | 0.20 | 9.7 | 100.0 |
| | 0.9* + 0.45** | 0.20 | 20.3 | 132.6 | 0.20 | 21.7 | 172.7 |
| | 1.2* + 0.6** | 0.40 | 23.3 | 152.2 | 0.40 | 43.3 | 292.8 |
| | 1.5* + 0.75** | 0.40 | 28.6 | 186.9 | 0.40 | 56.0 | 285.2 |
| | mean | 0.35 | 23.6 | | 0.30 | 30.3 | |
| 'Wala' | 0 | 0.20 | 13.3 | 100.0 | 0.60 | 24.9 | 100.0 |
| | 0.9* + 0.45** | 0.20 | 23.0 | 224.2 | 0.70 | 68.8 | 276.3 |
| | 1.2* + 0.6** | 0.60 | 39.0 | 448.4 | 0.80 | 78.6 | 315.6 |
| | 1.5* + 0.75** | 0.40 | 38.0 | 579.7 | 0.80 | 69.2 | 277.9 |
| | mean | 0.35 | 30.0 | | 0.72 | 58.5 | |

Explanations as in Table 1.

Nitrogen fertilization had an effect on the increase in essential oil yield but there was no proportional effect on essential oil content according to the increase of nitrogen doses. The highest increase in oil yield (by 480% in relation to control) was obtained for cv. 'Wala' at the beginning of flowering at the highest fertilization level of 1.5 g N/plant. An exception was cv. 'Dark Opal', where nitrogen fertilization at the beginning of flowering caused a decrease in oil yield in comparison with the control.

## DISCUSSION

Nitrogen is generally known as one of the factors determining plant biomass. Thus, it is important to find the optimum doses of fertilizers with this macroelement. In spite of various nitrogen levels, generally no differences were found in the mass of leaves of any cultivar between the applied fertilization rates. However, it was shown that for all the cultivars the lowest nitrogen dose (0.9 g N/plant) was the most advantageous for the early stage of plant development, while the highest, i.e. 1.5+0.75 g N/plant – for the later stage. This is confirmed by our results obtained previously for cv. 'Wala', which showed that the high mass of basil leaves and the contents of photosynthetically active pigments in them are dependent on both appropriate fertilization and harvest time [11, 12, 13].

Total nitrogen level found in basil leaves in our experiment reached the optimum range, which was reported by Kreij et al. [14].

Rumińska [15] and Sanda with co-workers [16] reported that the content of active substances varies depending not only on the plant species but also on the age of the plant. The amount of essential oil decreases in the course of leaf ageing, while leaf weight increases, and the yield of essential oil from leaves of various age is the result of both parameters [15, 16]. These dependencies were also confirmed in this study for cv. 'Dark Opal' and cv. 'Mittelgross' but not for cv. 'Wala'.

There is little data in literature on the effect of nitrogen fertilization on essential oil content in herbal plants. In the studies conducted on mint (*Mentha x piperita* L.) and sweet sagewort (*Artemisia annua* L.) no advantageous effect of nitrogen fertilization on essential oil contents was found [17, 18], while our earlier work showed favourable effect on basil plants [12]. Presented studies confirmed these results.

While comparing the mass of leaves and the content of chloroplast pigments of studied cultivars, it is necessary to point out that cv. 'Dark Opal' had a higher chlorophyll content, while its mass was lower by half in comparison with cv. 'Wala' or cv. 'Mittelgross'. It seems to be caused, among others, by the presence of anthocyanins in the leaves of cv. 'Dark Opal'. Anthocyanins occurring in leaf epidermis absorb some part of light energy, including that in the range of photosynthetically active radiation (PAR).

## CONCLUSIONS

1. Nitrogen fertilization increases the mass of basil leaves. The effect differs concerning cultivars and stage of plant development.
2. Leaves of the plant fertilized with nitrogen characterized increased content of chloroplast pigments. Content of chloroplast pigments of cv. 'Dark Opal' was on average twice as high in comparison with cv. 'Wala' and 'Mittelgross'.
3. Effect of nitrogen fertilization on the content of essential oil depended on nitrogen dose, cultivar and stage of plant development.

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## WPŁYW NAWOŻENIA AZOTEM I FAZY ROZWOJU ROŚLIN NA MASĘ ORAZ JAKOŚĆ LIŚCI BAZYLII WONNEJ (*OCIMUM BASILICUM* L.)

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Streszczenie

Badano wpływ zróżnicowanego nawożenia azotem oraz fazy rozwoju roślin w momencie zbioru (początek kwitnienia i pełnia kwitnienia) na masę liści oraz zawartość N- ogólnego, barwników chloroplastowych i olejku eterycznego w liściach trzech odmian bazylii wonnej ('Dark Opal', 'Mittelgross' i 'Wala'). Stwierdzono, że nawożenie azotowe zwiększało istotnie masę liści i wpływało na zawartość barwników chloroplastowych oraz znacząco wpływało na plon olejku eterycznego.

*Słowa kluczowe: bazylia wonna, nawożenie azotem, faza rozwojowa, masa liści, barwniki chloroplastowe, olejek eteryczny*