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Research Article

Effect of NPK-SRFS on the Growth, Yield and Essential Oil Composition of Basil (*Ocimum basilicum* L.)

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Abstract

Background and Objective: Basil (*Ocimum basilicum* L.), an aromatic herb, is considered one of the most important crops with essential oils as well as other bioactive compounds. Basil leaves have tremendous pharmaceutical benefits and are used for foods. Slow-release fertilizers have been developed to optimize the fertilization of crops. This work aims to discover the effect of NPK Slow-Release Fertilizer Coated by Starch (NPK-SRFS) at different rates on growth, yield and essential oil components of basil grown on the field in Northern Vietnam. **Materials and Methods:** Basil seedlings, sown from seeds, were used as plant materials. NPK-SRFS was stocked in the Faculty of Chemistry, Hanoi Pedagogical University 2. The experiments were designed in a fully randomized block model, consisting of four treatments with different rates of NPK-SRFS. Each treatment had three replicates with an area of 8 m². Duncan's Multiple Range Test was being used for statistical analysis ($p = 0.05$). **Results:** All 3 NPK-SRFS treatments significantly increased the number of buds and leaves per plant compared to the control. However, NPK-SRFS at different rates affected diversely plant height and leaf area of the basil. F5.0 and F10 treatments accelerated chlorophyll content as well as Fv/Fm value in comparison with none NPK-SRFS treatment. The application of NPK-SRFS at different rates caused slightly different changes in basil essential oil composition, especially the content of Methyl Chavicol, the most abundant oxygenated monoterpene and α -trans-Bergamotene, the most abundant sesquiterpene hydrocarbon. **Conclusion:** The present study provides further insight into the influence of NPK-SRFS on the growth, yield and essential oil components of basil.

Key words: Basil (*Ocimum basilicum*), NPK-SRFS, growth, yield, essential oil composition

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The genus *Ocimum* L. belongs to the family Lamiaceae, comprising more than 160 species and the most popular herbal shrub well-known for its immense value, medicinal properties and aroma profile^{1,2}. *Ocimum basilicum* L. (sweet basil) is an aromatic herb. This species is used as one of the most common plants in aromatherapy, perfume, cosmetics and foods³⁻⁵. Tobyn *et al.*³ suggested that *O. basilicum* contains many compounds such as phenols, alkaloids, terpenoids, aldehydes, flavonoids, steroids, glycosides, essential oils, saponins and tannins. Therefore, this species is widely used in current different industrial sectors.

Chemical fertilizers have been used in vegetable production for many years. However, unbalanced fertilization, overuse of chemical fertilizers and improper fertilization methods cause soil leaching, volatilization and low Nitrogen (N) efficiency, resulting in significant economic losses and environmental risks. Fertilizer optimization can be essential to meeting plant nutrient needs, increasing yield and quality, maintaining sustainable agricultural development and improving rural economies. As a result, Slow-Release Fertilizers (SRF) have been created to slow down or even control the rate at which nutrients are released into the soil, allowing plants to better meet their nutrient requirements⁶. When compared to Traditional Fertilizers (TFs), SRF has an advantage⁷. Biological polymers, such as starch, are being employed as encapsulating materials for boosting the performance of SRFs because of the high costs, process complexity and environmental contamination issues associated with petroleum-based polymers⁸.

The SRF application significantly promoted the leaf length of wintering Chinese chives cultivated in greenhouses, compared to TF, but had no significant effect on the plant height and stem diameter of this crop. In addition, The SRF treatments significantly improved the increase of chlorophyll content of Chinese chives leaves⁷. In another study, the application of N slow-release significantly increased all the plant growth indices such as plant height, total fresh leave weight and leaf area, as well as chlorophyll contents of *Echinacea purpurea* (L.), compared to the application of urea fertilizer⁹. Using different NPK-SRFs enhanced the vegetative growth of corn seven weeks after planting, compared to control¹⁰. Similarly, in comparison to control, two types of slow-release compound fertilizers, Matador at 30, 60 and 120 kg/Feddan and Nitrophoska at 25, 50 and 100 kg/Feddan significantly increased stem length, numbers of branches and leaves/tree of Nonpareil almond young trees¹¹. In addition, these two types of slow-release compound fertilizers induced

an increase of leaf area, leaf fresh weight and chlorophyll content at all applicated concentrations, except the Matador[®] at 30 kg/Feddan¹¹. Recently, the effect of NPK fertilizer at different rates (2.5 and 5 g/pot) on the growth, chlorophyll content and essential oils yield of sweet basil was investigated¹². Therefore, the influence of NPK-SRFS at different rates on the growth, yield, photosynthetic indices and essential oil composition is not fully clarified.

This study aims to investigate the effect of NPK-SRFS at different rates (2.5, 5.0 and 10 kg/100 m²) on the growth, photosynthetic indices and essential oil composition of basil grown on the field in Northern Vietnam.

MATERIALS AND METHODS

Study area: This work was conducted on the field at the Tien Thang village, Me Linh District, Hanoi, Vietnam from June to September, 2021.

Physio-Biochemical indices were analyzed at the Faculty of Biology and Agriculture Engineering and the Institute for Science and Application, Hanoi Pedagogical University 2, from June to September, 2021. The chemical compounds of essential oil were analyzed at the Institute of Natural Products Chemistry (INPC), Vietnam Academy of Science and Technology (VAST).

Materials: Basil seedlings were sown from seeds (made in Vietnam, distributed by Phu Dien Trading Production Joint Stock Co., Ltd.). NPK-SRFS was stocked in the Faculty of Chemistry, Hanoi Pedagogical University 2. Content of nitrogen, phosphorous and potassium reached 272, 46.760 and 70.606 mg kg⁻¹.

Research procedure

Experiments: Seeds of basil were sown in plastic cups containing sand. Seedlings with a height of 6.5 ± 0.5 cm were transferred to the field (with a density of 20×20 cm). The soil was generally added by 150 kg of compost/100 m². The experiments were arranged in a fully Randomized Block model (RCB), consisting of 4 treatments with different rates of NPK-SRFS, including control: no NPK-SRFS added, F2.5: NPK-SRFS added at 2.5 kg/100 m², F5.0: NPK-SRFS added at 5.0 kg/100 m², F10: NPK-SRFS added at 10 kg/100 m². Each treatment had three replicates with an area of 8 m².

Plant height, number of buds and number of leaves were randomly collected from 10 plants per plot. Ten plants were selected randomly from the experiments to determine the fresh weight (g) by using an analytical balance (Ohaus Pioneer

PA413, USA) with an accuracy of 0.001 g. Additionally, the third leaf (from the bud) was randomly selected from the observed plants to determine the Fv/Fm value by using OS30p+ (Opti-Sciences, Inc., USA) then collected to calculate the leaf area by using Image J v.1.8.0_172 software. Three leaves randomly taken from each experimental plot were used to determine the chlorophyll content by using Chlorophyll meter SPAD 502 (Minolta, Konica, Japan).

Hydro-distillation of essential oils: The essential oils of the basil dry leaves (500 g), were shredded and hydro-distilled for 2H₃O⁺ using a Clevenger-type apparatus and then essential oils were separated and dried with anhydrous Na₂SO₄. The obtained oils were stored at 5°C in refrigeration until analysis.

Essential oil analysis: A Hewlett-Packard (HP) 6890 gas chromatograph equipped with a Mass Spectrum Detector (MSD) Agilent Technologies 5975C and an HP-5 MS column. The column dimensions were 60×0.25 mm, film thickness 0.25 µm. The injector was established at 250°C. The temperature program was a 60°C ramp of 4°C per min up to 240°C. Helium with a flow rate of 1 mL per min was used as the carrier gas. The fractionation ratio was 100:1 and 1 mL of the extracted material was injected. The MSD scenario was derived from full scan modes at an electron-affected ionization voltage of 70 eV, an emission current of 40 mA, an acquired scan mass range of 35-450 amu. The GC-FID analysis procedure was carried out with the same GC-MS method's conditions. The EO compositions were determined by comparing their RI and MS data with those from HPCH1607, W09N08 libraries and NIST Chemistry Web-Book. The relative percentage amount of each component was estimated without any adjustment factor using the GC-FID peak area. Data are expressed as Mean±SD of three independent experiments.

Statistical analysis: Statistical analysis of all data was performed by using the ANOVA and means separated by Duncan's Multiple Range Test at the 5% level of significance (p = 0.05).

RESULTS AND DISCUSSION

Effect of NPK slow-release fertilizer coated by starch on growth, yield of basil grown in Northern Vietnam: In this study, plant height, number of buds per plant, number of leaves and leaf area of basil under influence of NPK-SRFS were investigated and shown in Fig. 1-2 and in Table 1-2.

At 30 days after treatment, the highest value of plant height was observed at the control treatment (20.77 cm). However, the plant height decreased when increasing the amount of NPK-SRFS. This value only attained 19.02, 16.50 and 14.25 cm at F2.5, F5.0, and F10 treatment, respectively. At the point of 45 days after treatment, the value of plant height was higher in F2.5 (36.96 cm) and F5.0 (32.91 cm) than in none fertilizer treatment (31.25 cm). In contrast, the plant height of basil in the F10 treatment (30.23 cm) was lower than in the control treatment (Fig. 1).

Table 1 showed that the NPK-SRFS treatments significantly increased the number of buds per plant of basil compared to the control. At 30 days after treatment, the F2.5, F5.0 and F10 treatments significantly improved the number of buds per plant of basil by 53%, 100% and 109%, respectively, compared to the control. Similarly, at 45 days after treatment, compared to the none fertilizer treatment, the F2.5, F5.0, and F10 treatments significantly raised the number of buds per plant of basil by 48%, 233% and 215%, respectively (Table 1). The highest value of the number of buds per plant was recorded in basil treated by 5.0 kg of NPK-SRFS/100 m².

Similar to the number of buds per plant, Table 2 displayed that the NPK-SRFS treatments significantly improved the number of leaves per plant of basil compared to

Table 1: Number of buds per plant of basil affected by NPK-SRFS

Treatments	0 day	30 days	% of control	45 days	% of control
Control	1.00±0.00 ^h	3.20±0.40 ^g	100	4.80±0.40 ^f	100
F2.5	1.00±0.00 ^h	4.90±0.83 ^f	153	7.10±0.70 ^{cd}	148
F5.0	1.00±0.00 ^h	6.40±0.66 ^e	200	16.00±0.77 ^a	333
F10	1.00±0.00 ^h	6.70±0.46 ^{de}	209	15.10±0.70 ^b	315

Means followed by the same letter (a, b, c, d, e, f, g, h) are not significantly different according to Duncan's Multiple Range test (p = 0.05), F2.5: NPK-SRFS treatment at 2.5 kg/100 m², F5.0: NPK-SRFS treatment at 5.0 kg/100 m², F10: NPK-SRFS treatment at 10 kg/100 m² and Mean±SD (buds per plant)

Table 2: Number of leaves per plant of basil affected by NPK-SRFS

Treatments	0 day	30 days	% of control	45 days	% of control
Control	5.40±0.49 ⁱ	48.10±2.07 ^h	100	78.50±1.57 ^d	100
F2.5	5.50±0.50 ⁱ	52.50±1.57 ^g	109	87.80±2.52 ^c	112
F5.0	5.30±0.46 ⁱ	65.10±1.22 ^e	135	209.00±3.44 ^a	266
F10	5.40±0.49 ⁱ	59.50±1.63 ^f	124	193.70±2.19 ^b	247

Means followed by the same letter (a, b, c, d, e, f, g, h, i) are not significantly different according to Duncan's Multiple Range test (p = 0.05), F2.5: NPK-SRFS treatment at 2.5 kg/100 m², F5.0: NPK-SRFS treatment at 5.0 kg/100 m², F10: NPK-SRFS treatment at 10 kg/100 m² and Mean±SD (leaves per plant)

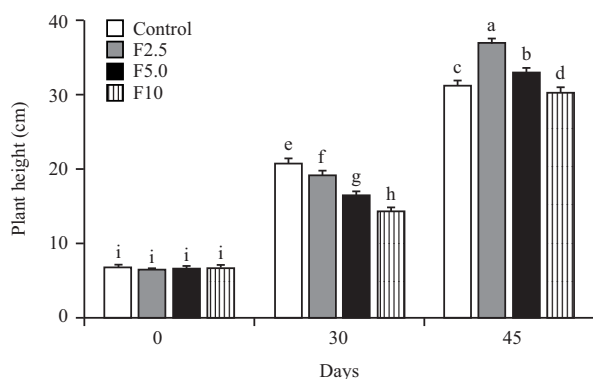


Fig. 1: Influence of NPK-SRFS on the plant height of basil
Means followed by the same letter (a, b, c, d, e, f, g, h, i) are not significantly different according to Duncan's Multiple Range test ($p = 0.05$). F2.5: NPK-SRFS treatment at 2.5 kg/100 m², F5.0: NPK-SRFS treatment at 5.0 kg/100 m² and F10: NPK-SRFS treatment at 10 kg/100 m²

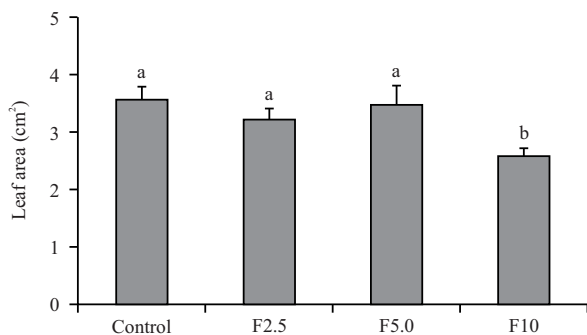


Fig. 2: Influence of NPK-SRFS on the leaves area of basil at 45 days after treatment
Means followed by the same letter (a, b) are not significantly different according to Duncan's Multiple Range test ($p = 0.05$). F2.5: NPK-SRFS treatment at 2.5 kg/100 m², F5.0: NPK-SRFS treatment at 5.0 kg/100 m² and F10: NPK-SRFS treatment at 10 kg/100 m²

the no fertilizer treatment. At 30 days after treatment, the F2.5, F5.0, and F10 treatments significantly increased the number of buds per plant of basil by 9%, 35% and 24%, respectively, compared to the control. At 45 days after treatment, compared to control, the F2.5, F5.0, and F10 treatments significantly increased the number of leaves per plant of basil by 12%, 166% and 147%, respectively (Table 2). The highest value of the number of buds per plant was recorded in basil treated by 5.0 kg of NPK-SRFS/100 m² (65.10 and 209.00 leaves per plant at 30 and 45 days after treatment, respectively).

In addition, the leaf area of basil was also calculated at 45 days of treatment (Fig. 2). The NPK-SRFS treatments had no significant effect on the leaf area of basil, except the F10 treatment. The value of leaf area in control, F2.5, F5.0, and F10 reached 3.5, 3.2, 3.5, and 2.6 cm².

Effect of NPK slow-release fertilizer coated by starch on yield of basil (*Ocimum basilicum* L.) grown in Northern Vietnam:

The yield of basil was affected by NPK-SRFS treatments (Fig. 3a-b). The application of NPK-SRFS significantly promoted the fresh weight of basil in comparison with the control. In detail, the value of air fresh weight in control, F2.5, F5.0, and F10 reached 12.01, 12.74, 15.10, and 13.82 g/plant at 30 days after treatment, respectively and gained 19.75, 21.92, 33, 63, and 26.90 g/plant at 45 days after treatment, respectively (Fig. 3a). These values corresponded to 3.00, 3.16, 3.58, and 3.40 kg/8 m² at 30 days after treatment, respectively and to 4.93, 5.53, 8.43, and 6.88 kg/8 m² at 45 days after treatment, respectively (Fig. 3b). The highest value of yield was observed in the F5.0 treatment at both 35 and 45 days after treatment.

The effect of NPK-SRFS treatments on basil growth and yield is confirmed by previous studies. Himmah *et al.*¹⁰ reported that the increase of the vegetative growth of corn seven weeks after planting has been observed under NPK-SRF applications. In detail, NPK-SRF treatments raised the corn leaf number, leaf width, stem diameter and biomass above ground compared to control. The present results were in agreement with the described study on Nonpareil almond young trees treated by two types of slow-release compound fertilizers with different rates, including Matador at 30, 60, and 120 kg/Feddan and Nitrophoska at 25, 50, and 100 kg/Feddan. These two slow-release compound fertilizers significantly increased numbers of branches and leaves/tree, leaf area and leaf fresh weight of at all applied concentrations, except the Matador[®] at 30 kg/Feddan¹¹. Similarly, SRF treatment increased the leaf length of Chinese chives cultivated greenhouses, compared to traditional fertilizer⁷. There was a slight difference between the effect of NPK-SRFS on the plant height of basil and the effect of NPK-SRF on the plant height of Chinese chives. Wang *et al.*⁷ reported that NPK-SRF treatment had no significant effect on the plant height and stem diameter of Chinese chives. On other hand, NPK-SRF applications increased the stem length of corn¹⁰ and maize¹³. Therefore, only F5.0 and F10 treatments promoted the increase of plant height of basil at 45 days after treatment in this study.

It can be seen that the effect of NPK-SRFS on plant height was significantly different between the present study and the previous ones. The reason perhaps is due to the applied doses of nitrogen fertilizer which was much lower in this study. Also, the NPK-SRFS applications enhanced the chlorophyll content in leaves. This observation was in agreement with previous findings in Chinese chives leaves treated by NPK-SRF⁷ and Nonpareil almond young treated by Matador[®] or Nitrophoska^{®11}.

Table 3: Chemical composition (%) of essential oils from leaves of basil under different rates of NPK-SRFS

EO constituents	RI	Control	F2.5	F5.0	F10
Monoterpene hydrocarbons					
α -Pinene	938	0.34	0.35	0.25	0.16
Sabinene	977	0.16	0.3	0.22	0.1
β -Pinene	983	0.3	0.62	0.42	0.21
Myrcene	990	0.73	0.38	0.36	0.28
Limonene	1034	0.63	0.36	0.35	0.31
Terpinolene	1093	0.12	-	0.11	-
β -Phellandrene	1035	0.15	0.14	0.17	0.15
E- β -Ocimene	1048	2.14	1.67	2.53	2.49
Fenchone	1096	0.4	0.35	0.43	0.55
Camphene	955	0.19	-	-	-
Sesquiterpene hydrocarbons					
cis- β -Elemene	1348	0.36	0.29	0.24	0.15
E-Caryophyllene (= β -Caryophyllene)	1438	0.42	0.4	0.24	0.13
α -Trans-Bergamotene	1447	6.27	6.45	4.76	3.45
α -Guaiene	1452	0.31	0.27	0.24	0.27
Z- β -Farnesene	1460	0.17	0.23	0.17	-
α -Humulene	1472	0.38	0.21	0.15	0.11
cis-Muurolo-4(14),5-diene	1480	0.13	0.2	0.17	-
β -Trans-Bergamotene	1498	0.38	0.42	0.3	0.22
D-Germacrene	1499	0.28	0.32	0.24	0.15
Bicyclgermacrene	1514	0.21	0.23	0.17	-
α -Bulnesene (= δ -Guaiene)	1522	0.26	0.22	0.19	0.26
g-Cadinene	1530	1	1.04	0.67	0.45
β -Sesquiphellandrene	1534	0.12	0.24	0.17	0.11
Seychellene	1465	-	-	-	0.2
Oxygenated monoterpene					
Linalool	1101	1.46	1.07	0.98	1.01
Camphor	1155	0.77	0.52	0.46	0.51
α -Terpineol	1198	0.19	0.24	0.17	0.12
Methyl Chavicol (= Estragole)	1208	76.23	77	81.04	84.41
Eucalyptol	1038	1.96	3.83	2.6	1.44
Bornyl acetate	1294	0.75	0.21	0.29	0.27
Methyl eugenol	1407	0.62	1	0.62	0.54
Fenchyl acetate	1228	0.48	-	0.11	-
Endo-Fenchol	1121	0.77	-	-	-
Borneol (= Endo-Borneol)	1175	0.43	-	-	-
Oxygenated sesquiterpenes					
Epi- α -Cadinol (= Tau-Cadinol)	1634	0.63	1.06	0.62	0.37
1,10-Di-Epi-Cubenol	1658	0.16	0.19	0.12	-
Total		99.9	99.81	99.56	98.42

Effect of NPK slow-release fertilizer coated by starch on variation of essential oil composition of basil grown in Northern Vietnam: The composition displayed by the mean percentage of total identified compounds of essential oils of basil treated by different rates of NPK-SRFS was shown in Table 3. A total of 27-35 compounds, accounting for 98.42%-99.9% of the total content, were identified in the essential oils obtained from basil leaves in different NPK-SRFS treatments, respectively. In general, the oxygenated monoterpene fraction dominated in four essential oils samples, followed by sesquiterpene hydrocarbons and monoterpene hydrocarbons (Table 3).

The amount of the total oxygenated monoterpene changed from 83.66%-88.3% in four treatments. The content

of this fraction increased when using NPK-SRFS up to 10 kg/ 100 m². The major components were Methyl Chavicol (= Estragole) which ranged from 76.23% (control)-84.41% (F10 treatment) and Eucalyptol which changed from 1.44% (F10 treatment) to 3.83% (F2.5 treatment). In contrast, using NPK-SRFS up to 10 kg/100 m² induced a significant decrease in sesquiterpene hydrocarbons (from 10.52-5.5%). The major compound of this fraction was α -trans-Bergamotene which decreased from 6.45% (F2.5 treatment) to 3.45% (F10 treatment). Content of monoterpene hydrocarbons was higher in control than in all three NPK-SRFS treatments. In detail, the concentration of monoterpene hydrocarbons was 5.16%, 4.17%, 4.84% and 4.25% in control, F2.5, F5.0, and F10 treatments. Amount of E- β -Ocimene, the

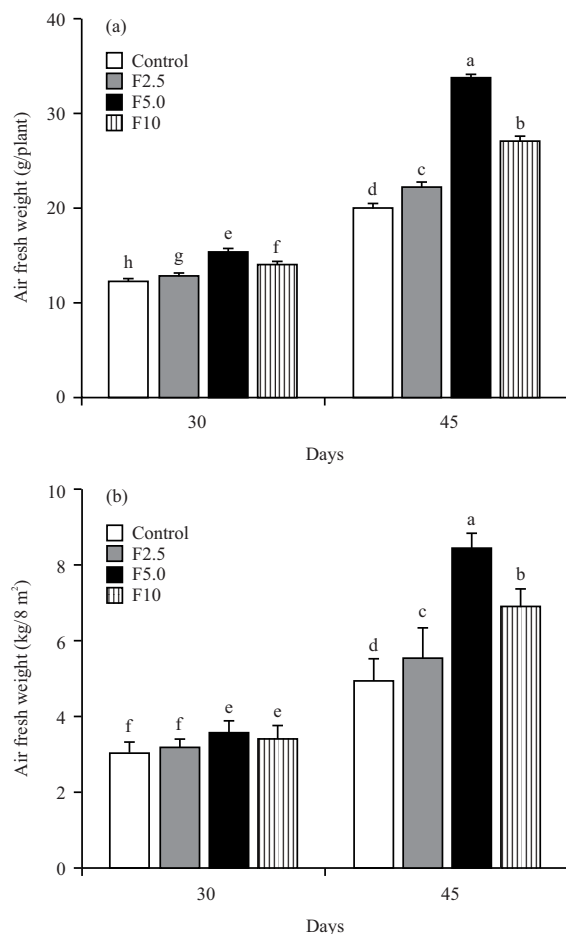


Fig. 3(a-b): Influence NPK-SRFS on the yield of basil, (a) Yield determined by g per plant and (b) Yield calculated by kg per plot area (8 m²)

Means followed by the same letter (a, b, c, d, e, f, g, h) are not significantly different according to Duncan's Multiple Range test ($p=0.05$). F2.5: NPK-SRFS treatment at 2.5 kg/100 m², F5.0: NPK-SRFS treatment at 5.0 kg/100 m² and F10: NPK-SRFS treatment at 10 kg/100 m²

most abundant monoterpene hydrocarbons, varied from 1.67%-2.53% in NPK-SRFS treatments. Indeed, the concentration of this compound was 2.14% in basil non-treated by NPK-SRFS fertilizer. Using NPK-SRFS caused a very slight or no significant change in the concentration of other components.

These results were in agreement with Hassan *et al.*¹⁴ who reported that Methyl chavicol (estragole) was increased by increasing the NPK level and linalool decreased. The variation trend of these important components in basil essential oil was caused by the increased nitrogen application¹⁵. Previously, Nurzynska-Wierdak *et al.*¹⁶ reported that the linalool concentration in oils grew up after the nitrogen application at the medium rate and subsequently slightly

decreased after the highest rate was applied. In the present work, the dominance of the Methyl Chavicol compared to Linalool in the essential oil of the basil was recorded. This observation could be explained by the very high rate of potassium per nitrogen (260-folds) in NPK-SRFS. According to the previous finding, the decrease in the proportion of linalool in the oil of the basil cultivars resulted from an increase in the rate of potassium¹⁶. However, the findings on the effect of NPK-SRF on essential oil composition, to date, were poorly described, especially in *Ocimum* genus.

CONCLUSION

This study described the growth, photosynthetic indices, yield of basil affected by different rates of NPK-SRFS. All three NPK-SRFS treatments considerably increased the number of buds and leaves per plant and the yield of basil compared to the none fertilizer treatment. NPK-SRFS at different rates caused changes on the other growth indices of the basil, including plant height and leaf area. Two treatments, F5.0 and F10, promoted a significant increase of chlorophyll content as well as Fv/Fm value in comparison to no NPK-SRFS treatment. This study also investigated the chemical composition of essential oil in basil leaves under different rates of NPK-SRFS. The application of NPK-SRFS at different rates caused slightly different changes in the composition of basil essential oils. NPK-SRFS treatment at 5 kg/100 m² was the optimal rate for basil cultivation on the field at the study area.

SIGNIFICANCE STATEMENT

The present study discovers the possible effect of NPK-SRFS treatments on cultivated basil plants. This work will help researchers to reveal the critical role of NPK-SRFS fertilization in the field that many researchers were not able to investigate. Thus, a newly-added procedure for the use of NPK-SRFS may be gained.

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