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Research Paper

MANIPULATION OF PLANT GROWTH, DEVELOPMENT AND THE COMPOSITION OF ESSENTIAL OILS IN *OCIMUM SANCTUM* L. (HOLY BASIL) USING EXOGENOUS REGULATORS OF PLANT GROWTH

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The influence of exogenous regulators of plant growth and development on biomass yield, composition and amount of essential oils in holy basil, *Ocimum sanctum L.*, was investigated. Four exogenously applied plant growth regulators namely Melatran, Humiforte N6, Aminoforte, and Kadostim were used in this 3-year research by spraying them on the leaves of plants grown on a permanent experimental site. Results of the 3-year research confirm that all the exogenous regulators of plant growth used in this research supported plant growth and development. Further, all of these growth regulators especially the combinations of the growth regulators in treatments AH and AHK increased plant biomass fresh yield. In general, the growth regulators also triggered the early emergence of the flowering phase except for treatment 4 (AHK) which contained kadostim. The plants treated with the regulators, i.e., plants in treatments Mel, AH, and AHK, gave lower average number of branches in comparison to the control. The treatment Mel had plants with the least average number of branches, confirming that melatran has an inhibitory effect on branching. All of the exogenous regulators particularly the combination used in treatment 3 (AH) supported increase in yield of essential oils in the leaf and seed of *Ocimum sanctum L.*

Keywords: *Ocimum sanctum*, Essential oils, Growth regulators, Gas chromatography, Kadostim, Humiforte, Melatran, Aminoforte

INTRODUCTION

This plant physiology research was aimed at testing the influence of exogenous regulators of plant growth, i.e., synthetic plant growth regulators, on the processes of plant growth, development and formation of yields (i.e., plant physiological activities) in Holy Basil, *Ocimum sanctum L.* The work was repeated for 3 years.

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In the course of this work, the exogenous regulators of plant growth were used to influence plant hormones biosynthesis as well as their transport and uptake by *Ocimum sanctum L.* plants. The availability of these hormones and their transport to another part of the same plant where they act to maintain its growth and metabolic integrity were influenced through the use of the following substances: Melatran, Humiforte N6, Aminoforte, and Kadostim. These substances were used either separately or in combination with one another. The basis of this work was to compare the influences of the three treatments, i.e., Mel, AH, AHK, on the plant growth and development, biomass yield, and the yields of essential oils (i.e., amount and constitution) in the seeds and leaves in comparison to the control (i.e., plants not treated with any of the exogenous regulators). The agronomic design of this experiment availed the researcher the opportunity to study the effect of each treatment and to consider the difference between the factors contributing to crop yield. It also enabled the monitoring of plant reaction to the influence of specific factors such as climatic conditions, soil management practices and the effect of the used regulators of plant growth under natural conditions. To ensure the validity of the research results, the experiment was repeated on the same research site and within the same timeframe (i.e., experimental season) and while maintaining the same experimental design for 3 years.

MATERIALS AND METHODS

Experimental Materials

The plant experimental material for this 3-year research work was the seeds of Holy basil (*Ocimum sanctum L.*) which were obtained from the dried plants harvested in the previous year. The seeds were sown in garden trays in the greenhouse of the Institute of Tropical and Subtropical Agriculture at the Czech University of Life Sciences in Prague. 95% of the seeds germinated on the 14^{th} day after sowing. The seedlings were tilled after 4 weeks from seed sowing and transplanted into soil at the experimental site after eight weeks from seed sowing. The experimental plants were then maintained on the permanent site each year until harvest.

Exogenous Regulators of Plant Growth and Development

Melatran

Melatran contains the precursor of the phytohormone auxin. This substance is then metabolized into β-indoleacetic acid (IAA), an auxin, which is used for the regulation of plant growth and morphogenesis. Melatran is easily metabolized in plant roots and leaves within 24 h. Particular attention was paid to the concentration of this substance during its application on plant leaves in this agronomic research as concentrations over 2 L per ha were observed to inhibit plant growth. Melatran influences the formation of plant roots and stem, pollen fertility and the accumulation of assimilates in plant storage organs. It could be used with insecticides and organic fungicides. However, it is not recommended to be used in conjunction with copper-containing fungicides as melatran tends to increase the fungicides toxicity to the plant.

Humiforte

Humiforte is a synthetic biostimulator of plant growth, and it is characterized by its high content of biologically active amino acids, nitrogen, phosphorus, potassium, magnesium and microelements (i.e., Fe, Zn, Cu, Mn, B). It has an optimum pH range of 6.2-6.9. Humiforte reduces nutrient depletion through leaching and improves plant nutrients uptake. It increases both plant nutrients metabolism and plant adaptation to ecological factors. It is easily assimilated by plant leaves and is therefore highly recommended for quick and intensive supply of plant nutrients to plants grown in greenhouses and under sprinkler irrigation.

Aminoforte

Aminoforte supports plant growth and development, and is characterized by its quick assimilation by plants. Its effect is not dependent on chlorophyll biosynthesis. Aminoforte is readily assimilated by plant leaves and roots following its application. This substance increases the amount of chlorophyll in leaves, thereby increasing plant photosynthetic activity whilst delaying leave senescence. It supports rapid plant regeneration; it is particularly useful in the regeneration of plants after budding, grafting, etc., as well as plants affected by stress and unfavorable climatic factors such as frost.

Kadostim

Kadostim supplies plants with potassium in a complex organic form when applied on leaves. Aside from potassium, kadostim also contains: N, Cu, Fe, Zn, Mn, amino acids. Kadostim is particularly effective in supporting the accumulation of plant assimilates in bulbs, tuber and root crops, and in plants grown for seeds.

The Common Features of Melatran, Humiforte, Aminoforte, and Kadostim

The common characteristics of the abovementioned four exogenous regulators of plant growth and development are that they all contain synthetic biologically active amino acids; supply plants with exogenous source of plant nutrients; are readily assimilated by plant roots and leaves, and therefore can be used in sprinkler irrigation. Further, they can be used in conjunction with fungicides, insecticides, herbicides, and fertilizers for application on leaves. In terms of health and ecological aspects, their advantages include that they do not cause skin irritation, neither are they mutagenic nor toxic to bees.

EXPERIMENTAL METHODS

The Propagation and Treatment of the Experimental Plants

In the second week of March, the seeds were sown by covering them with a very thin layer of soil in garden trays in the greenhouse. Holy basil seeds were immersed in water for 3 h prior to being sown in soil. The seeds were not treated with any growth regulators before, during or after planting. About 80% of the seeds germinated on the 10th day after seed sowing and a total of 95% on the 14th day from the date of seed sowing. The seedlings were tilled 4 weeks following seed sowing while still in the greenhouse to a spacing of 0.20 mm $\times$ 0.20 mm. The temperatures in the greenhouse were controlled with an average temperature of 23 °C and maximum of 29 °C. The seedlings were grown under enough lighting and protected from frost and cold wind in the greenhouse. Seedlings were watered on a daily basis and plant protection measures (i.e., weeds and disease control) were ensured. The seedlings were not treated with any growth regulators before transplanting them to the permanent experimental site. The soil at the experimental site, where wheat was grown the previous year, was carefully prepared using agricultural mechanization machines. The soil pH was 6.6. The seedlings were transplanted early in the morning during the second week in May at a spacing of 0.30 m × 0.25 m. During the transplanting, special care was taken to transfer the seedlings carefully with the full integrity of the root system contained in the garden soil from the greenhouse. Plants were grown on the permanent site in summer when the temperatures were between 18 °C and 29 °C. The plants were watered daily using manual sprinklers, excepting when it rained the previous day. Removal of weed was carried out manually as needed. The experimental plots were clearly labelled in accordance with the names of the treatments (see Table 1).

After full flowering, the plants were harvested manually by cutting the stem at the height of 40 mm from its base on a sunny afternoon. The biomass was dried soon after harvest to avoid the "sweating of the leaves" and subsequent onset of leaf decay. Plants were dried in a thermostat dryer at a temperature of 25 °C.

The application of the synthetic (exogenous) regulators on the plant leaves for all the 3 treatments was carried out once in 3 weeks using portable manual sprinklers. The last application of the regulators was 3 weeks prior to plant harvest. The concentrations of the exogenous growth regulators used for the foliage application are given in the table below.

Table 1: Table of the Experimental Treatments

Treatment	Components of the Treatment	Abbrev.
Treatment 1	Control (i.e., no exogenous growth regulator used)	Ko
Treatment 2	Melatran (1 ml Melatran: 1000 ml water)	Mel
Treatment 3	0.1% Aminoforte + 0.1% Humiforte (0.1 ml Aminoforte: 100 ml water) and (0.1 ml Humiforte: 100 ml water)	AH
Treatment 4	Aminoforte 0.1%; Humiforte 0.1%; Kadostim 0.05%	AHK

Monitoring of Plant Characteristics

The morphological characteristics of *Ocimum sanctum L.* were carefully observed and systematically monitored throughout this research study. The following characteristics were observed and valid data were collected every year over the 3 years experimental period: date of seed sowing; date of seed germination; seedling transplanting date to permanent experimental site; plant height in mm (once a week); date of flowering (once a week); date of harvest; etc. The number of branches and biomass yield for each treatment were recorded during plant harvest while the dry mass yields and yields of essential oils were taken after harvest.

Determination of Essential Oils in Holy Basil (*Ocimum sanctum L.*) Seeds

Determination of essential oils in holy basil seeds was achieved by using a combination of the well-tested Gas Chromatography analysis (GC) method described by Jankovsky *et al.* (1989) and the Continuous Distillation Extraction (CDE) method after Jankovsky *et al.* (1993).

KEY RESULTS

Results on plant average height show significant differences between plants from different treatments, especially between the control and plants sprayed with the exogenous regulators of plant growth and development, ranging from 134,6 mm in control plants to 169,57 mm in plants in treatment 3 (i.e., treatment AH). Plants in treatment 4 (i.e., treatment AHK = treatment with the mixture Aminoforte + Humiforte + Kadostim) attained an average height of 169.55, and next to that was the average height of 156.42 for plants treated with Melatran. The above results clearly show that all the exogenously supplied plant growth regulators supported plant growth at the concentrations used during this research study, and that kadostim, aminoforte and humiforte acted synergistically in promoting plant growth.

The holy basil plants went into flowering phase 3 months from the date of seed sowing. The number of flowing plants for each treatment was registered every week throughout the flowering period in each year over the 3-year experimental period. The results on the emergence of the flowering phase in each treatment were consistent each year over the 3-year period. On the first day of flower emergence, treatment with melatran (Mel) had the highest average number of flowering plants (13 plants), followed by the treatment with Aminoforte and Humiforte (AH) with 9 flowering plants. The least number of flowering plants was observed in treatment (AHK) with only 5 flowering plants, while the control treatment (Ko) had 6 flowering plants. The above results clearly show that melatran most markedly supported emergence of flowers. The results suggest that while the combination of Aminoforte and Humiforte (AH) promoted early flower emergence, kadostim inhibited early flower emergence.

Results on the effect of the used exogenous regulators of plant growth and development on branching of *Ocimum sanctum L.* were recorded during harvest. Data on branching revealed that on average the basil plants had between 3 and 6 branches per plant from the height of 50 mm above soil level. The control plants (Ko) had the highest average number of branches (i.e., 6 branches). Next were treatments AH and AHK which gave an average of 5 branches. Treatment with melatran indicated a possible inhibitory effect of this substance on branching as the plants had an average of only 3 branches.

After full flowering, the plants were harvested manually by cutting the stem at the height of 40 mm from its base on a sunny afternoon. The biomass (i.e., stem, branches with leaves and flowers) yields of freshly harvested plants ranged between 14.22 kg/100 m^2 for the control (Ko) and 15.71 kg/100 m^2 for treatment AHK. The treatment with a combination of Aminoforte and Humiforte (AH) had the second highest yield of 14.98 kg/100 m^2, while the treatment with melatran (Mel) gave a yield of 14.56 kg/100 m^2. The above results clearly show that all the used exogenous regulators, especially the treatment AHK, were capable of increasing biomass (i.e., stem, branches with leaves and flowers) fresh yield in comparison to the control.

The highest dry mass yield of 3.4 kg/100 m^2 was recorded for control (Ko) and the least for treatment 3 (AH) with only 2.15 kg/100 m^2. Treatments AHK and Mel gave 2.39 kg/100 m^2 and 2.23 kg/100 m^2 respectively. The fresh mass to dry mass ratio for the basil plants ranged between 5:1 and 6:1, indicating there were no marked differences in the dry mass ratio between the control plants and the plants treated with the growth regulators. However, the control plants gave the highest dry mass yield.

Qualitative and quantitative determination of essential oils in the *Ocimum sanctum L.* seeds were carried out, using the aforementioned techniques, on seeds and leaves after drying in the thermostat dryer. Table 2 shows the detailed results obtained for each treatment. The combination of Aminoforte and Humiforte (AH) gave highest amount of essential oils in the seeds as 505 ppm, followed by treatments AHK with 443 ppm, and treatment 2 (Mel) with 380 ppm. The control yielded the least amount of essential oils, with only 360 ppm. The above data show that all the exogenously supplied regulators of plant growth and development triggered increase in the accumulation of essential oils in holy basil seeds.

Another significant and interesting finding regarding the influence of the used exogenous regulators of plant growth and development was registered on the constitution of the essential oils in holy basil seeds. The results of detailed analyses of essential oils in the seeds are given in Table 3.

Table 2: Quantitative Determination of Essential Oils in *Ocimum sanctum L.*

Treatment	Mass of Seed Sample (g)	Mass of Oil (g)	Amount of Oil (ppm)
1 = (Control)	31.00	0.0114	360
2 = Mel	31.00	0.0117	380
3 = (AH)	31.00	0.0170	505
4 = (AHK)	31.00	0.0138	443

Table 3: Determination of the Composition of Essential Oils in *Ocimum sanctum L.*

Name/Type of Essential Oil	Maximum Percentage (%) Composition of the Essential Oil Types in, Holy Basil, *Ocimum sanctum L.*, Seeds			
	Treatment 1 (Control (Ko))	Treatment 2 (Mel)	Treatment 3 (AH)	Treatment 4 (AHK)
Para cymen	4.4	16.7	0	26.1
Eugenol	40.7	49.9	50.0	30.3
Methyl cinamate	20.1	5.4	5.6	8.0
Nerolidol	11.8	0	3.5	3.0
Cineol	2.0	0	3.0	7.0 -8.0

DISCUSSION

In the course of this study, 80% of the *Ocimum sanctum L.* seeds germinated in garden trays in the greenhouse under controlled climatic conditions on the 10th day after seed sowing, and a total of 95% on the 14th day after seed sowing. The seeds were not treated with any growth regulators before, during or after planting. This result is in agreement with the study conducted by Pisarik (1959) who reported the seed germination timeframe of 10-14 days, and further supported by Atal and Kapur (1982). Furthermore, study on basil plants grown in a medium containing vermiculite and treated with 1% solution of the growth regulator ROOTS (a synthetic growth regulator containing *Ascophyllum nodosum*) only had true leaves after 14 days of treatment (Poincelot, 1993).

The results on plant average height showed significant differences between plants from different treatments, especially between the control plants and plants sprayed with the exogenous regulators of plant growth and development. Plant average height ranged from 134,6 mm in control plants to 169,57 mm in treatment with a combination of aminoforte and humiforte. The above results on plant height confirmed that all the exogenous regulators used in this research supported increase in plant height and stem elongation. This result is consistent with the work on *Ocimum gratissimum L.* where the plant growth regulator Gibberellic Acid (GA) increased plant height, length of internodes, leaf area and biomass. However the use of Cycocel had a negative effect on the above parameters (Umesha *et al.*, 1991). Further, the work on *Ocimum basilicum* L. supports the results of this study on the role of growth regulators in increasing plant height and number of leaves per plant (Shedeed *et al.*, 1990).

Data on branching which were collected during plant harvest showed that on average the basil plants had between 3 to 6 branches per plant from the height of 50 mm above soil level. The control plants had the highest number of branches followed by treatments AHK and AH. Treatment with melatran (Mel), with only 3 branches, indicated a possible inhibitory effect of this substance on branching as compared to the control.

The yields of basil plants are in a wide range, depending on the interplay of climatic and soil conditions on a specific variety. In this study, four different exogenous regulators were used to study their influences on plant growth and development, and on the yields of biomass and essential oils of holy basil in comparison to the control. The results showed that all the treatments, especially treatment AHK with a combination of three exogenous regulators, were capable of increasing biomass (i.e., stem, branches with leaves and flowers) yields in comparison to the control (Ko). The fresh mass yield per 100 m^2 ranged between 14.22 kg/100 m^2 for the control (Ko) and 15.71 kg/100 m^2 for treatment AHK. The above finding agrees with the agronomy work done by Hlava and Matejka (1988), however, the plant yield reported by the authors was of a wider range—8 to 20 kg/100 m^2.

The time of harvest is vitally important for the holy basil, *Ocimum sanctum L.*, plant as it is principally grown for its aromatic leaves and seeds. Both the leaves and seeds are rich in essential oils. The increase in the content of the aromatic substances, especially the essential oils, is progressive with the plant growing phase until full bloom. During the course of this research, the plants were harvest during full bloom when the leaves were still very fresh on the plant. This is in agreement with the studies of Pisarik (1959) and Hlava (1988). However, basil plants can also be harvested before flowering (Valicek and Dobsicek, 1993).

There are several effective methods of determination of essential oils in basil plants. The choice of a specific method or combination of methods depends on what equipment is available and the researchers' expertise. In this research, the determination of essential oils in holy basil seeds was achieved by using a combination of the well-tested gas chromatography analysis method described by Jankovsky *et al.* (1989) and the Continuous Distillation Extraction (CDE) method after Jankovsky *et al.* (1993). Another effective method of determination of essential oils in basil plant material involves the use of distilled water and 95% alcohol (Singha *et al.*, 1993). The amount and composition of essential oils can also be influenced by the cultivar of basil plant (Treutner, 1986).

In this research, the control plants yielded the least amount of essential oils with only 360 ppm. The above data show that all the exogenously supplied regulators of plant growth and development triggered increase in the accumulation of essential oils in holy basil seeds. The highest yield of oil in both leaves and seed was found in treatment AH. Further, treatment 3 (AH) contained the highest amount of eugenol in the seed.

Different cultivars of basil plants have been found to differ considerably in both the amount and composition of the essential oils. For example, the study by Singha and Gulati (1990) revealed that *Ocimum americanum* L. contained linalool, kafr, citronellal, iso-borneol, borneol and methylchavicol. In the same plant, *Ocimum americanum L.*, Sarin *et al.* (1992) found 76% citral. Differences in the amount and constituents of the essential oils in basil plants can also be attributed to the climatic conditions in the region where the basil plants are cultivated. For instance, in Northern Australia, Brophy *et al.* (1993) found that *Ocimum tenuiflorum L.* contained 87% methylchavicol and very low amount (4%) of Kafr. In *Ocimum basilicum L.*, the following essential oils were found: lemon, terpinen, geraniol, myrcen, methylchavicol, beta-pinen, alpha-pinen, eugenol, alpha-terpineol (Hodisan *et al.*, 1983). A study with *Ocimum gratissimum L.* revealed the identification of 21 different kinds of essential oils; the principal component was tymol, while the other components were p-cymen, myrcen, A3-karen and alpha-terpinen (Sainsbury and Sofowora, 1971). A plant breeding study on *Ocimum gratissimum L.* aimed at influencing the content of essential oils showed that different hybrids contained different essential oils. The content of eugenol was at least 55% (Sobti *et al.*, 1978; and Sobti *et al.*, 1980).

This study further presents the important finding that the amount and composition of essential oils in holy basil, *Ocimum sanctum L.*, was significantly influenced by the used exogenous regulators of plant growth and development as shown in Tables 2 and 3 above.

CONCLUSION

This 3-year research study on holy basil, *Ocimum sanctum L.*, was focused on the usage of exogenous regulators of plant growth and development for the manipulation of plant growth and development, the plant biomass yield as well as the composition and amount of essential oils in leaves and seeds.

The above results confirmed that all the exogenous regulators used in this research supported plant growth. The control plants grew to an average height of 134.6 mm, while the plants treated with one or a combination of the growth regulators grew to an average height between 156 mm and 169 mm. Plants in the treatment with a combination of aminoforte and humiforte (AH) had the highest average height of 169 mm.

This study further confirmed that all the growth regulators used in this research increased plant yield, especially the combination of the growth regulators in treatment AHK. This treatment gave the highest biomass yield of 15.71 kg per100 m^2. However, the control (Ko) gave the highest dry mass yield.

In general, the results confirmed that all the growth regulators triggered the early emergence of the flowering phase, except for treatment 4 (AHK). The treatment with melatran had the highest number of flowering plants (i.e., 13 plants) while the control (Ko) had only 6 flowering plants on the first day of flower emergence.

Data on plant branching showed that the control (Ko) had the most number of branches per plant, with an average of 6. The plants treated with the growth regulators (i.e., the plants in treatments Mel, AH, and AHK) had lower number of branches in comparison to the control. The treatment Mel had just 3 branches on average, confirming that melatran inhibited the branching of the basil plants.

All of the used exogenous regulators of plant growth and development enhanced the yield of oil in the leaf and seed of *Ocimum sanctum* L in comparison to the control (Ko). The highest yield of oil in both leaves and seed was found in treatment AH. In addition, treatment 3 (AH) contained the highest amount of eugenol in the seed.

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