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Research Article

A Novel Approach to Plant Breeding and Improving Plant Yield

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Abstract

The influence of synthetic regulators of plant growth and development on biomass yield, composition and amount of essential oils in holy basil, *Ocimum sanctum* L., was investigated. Four synthetic plant growth regulators namely Melatran, Humiforte N6, Aminoforte, and Kadostim were used by spraying them on the leaves of plants grown on a permanent experimental site. Results of the 3-year research confirm that all the synthetic 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 flower induction, excepting treatment 4 (AHK) which contained kadostim. The plants treated with the regulators, i.e. plants in treatments Mel, AH, and AHK, exhibited 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 synthetic 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.

Key Words: Holy basil; essential oils; synthetic growth regulators; yield parameters; *Ocimum sanctum* L.; flower induction; etc.

Introduction

This research was focused on studying the influence of four synthetic plant growth regulators on the processes of plant growth, development and formation of yields in Holy Basil, *Ocimum sanctum L.* 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. During this work, the synthetic 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 the endogenous plant 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 Melatran, Humiforte N6, Aminoforte, and Kadostim. The synthetic growth regulators were used either separately or in combination with one another. The work was founded on the impetus 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 synthetic regulators). The research design enabled the study of the effect of each treatment and the consideration of the differences 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.

Materials and Methods

Experimental Materials

The seeds of holy basil (*Ocimum sanctum* L.) used for this research were obtained from the dried plants harvested the previous year. The plants or seeds of the previous year's planting were not treated with any plant hormones or synthetic regulators of plant growth. The seeds were sown in garden trays in the greenhouse. 95% of the seeds germinated on the 14th 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 grown on the permanent experimental site each year until the time of harvest.

The common features of the Synthetic Regulators of Plant Growth and Development - Melatran, Humiforte, Aminoforte, and Kadostim

Each of these four synthetic regulators of plant growth and development has its unique and distinguishing characteristics (Megbo, B. C. 2013). However, they also possess common characteristics in that they all contain synthetic biologically active amino acids; supply plants with synthetic source of plant nutrients; are readily assimilated by plant roots and leaves, so they can be used in sprinkler irrigation. In addition, they can be used in conjunction with fungicides, insecticides, herbicides, and fertilisers for application on leaves. With regards to their health and ecological aspects, their merits include that they do not cause skin irritation, neither are they mutagenic nor toxic to bees.

Experimental Methods

The agronomy

In the second week in 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 hours 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 0.20mm x 0.20mm. The temperatures in the greenhouse were controlled with an average temperature of 23^{o}C and maximum of 29^{o}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 0.30m x 0.25m. 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^{o}C and 29^{o}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.

After full flowering, the plants were harvested manually by cutting the stem at the height of 40mm 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^{o}C.

Treatment of the Plants in the Field

The application of the synthetic 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 synthetic growth regulators used for the leaf application are given in the table below.

Table A: Table of the experimental treatments

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

Monitoring and Measurement of Plant Characteristics

The morphological characteristics of *Ocimum sanctum* L. plants 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.

Analysis of Essential Oils in *Ocimum sanctum* L. seeds

The analysis of essential oils in holy basil seeds was achieved using a combination of the gas chromatography method by Jankovsky et al. (1989) and the continuous distillation extraction method after Jankovsky et al (1993).

Results

Results on plant average height show significant differences between plants from different treatments, especially between the control and plants sprayed with the synthetic regulators of plant growth, ranging from 134,6mm in control plants to 169,57mm 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 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 the flowering phase 3 months after the date of seed sowing. The number of flowing plants for each treatment was observed and noted 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 synthetic 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 50mm 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 40mm from its base on a sunny afternoon. The biomass (i.e. stem, branches with leaves and flowers) yields of freshly harvested plants ranged between 14.22kg/100m^2 for the control (Ko) and 15.71kg/100m^2 for treatment AHK. The treatment with a combination of aminoforte and humiforte (AH) had the second highest yield of 14.98kg/100m^2, while the treatment with melatran (Mel) gave a yield of 14.56kg/100m^2. The above results clearly show that all the used synthetic 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/100m^2 was recorded for control (Ko) and the least for treatment 3 (AH) with only 2.15kg/100m^2. Treatments AHK and Mel gave 2.39kg/100m^2 and 2.23kg/100m^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. 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 syntheticly supplied regulators of plant growth and development triggered increase in the accumulation of essential oils in holy basil seeds.

The following significant and interesting finding regarding the influence of the used synthetic 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 B.

Table B: 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

Eighty percent 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 synthetic regulators of plant growth and development. Plant average height ranged from 134,6mm in control plants to 169,57mm in treatment with a combination of aminoforte and humiforte. The above results on plant height confirmed that all the synthetic 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 were collected during plant harvest. On average the basil plants had between 3 to 6 branches per plant from the height of 50mm 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 interaction of climatic and soil conditions on a specific variety. In this study, four different synthetic 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 synthetic 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 $100m^2$ ranged between $14.22kg/100m^2$ for the control (Ko) and $15.71kg/100m^2$ 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 $20kg/100m^2$.

The holy basil, *Ocimum sanctum* L., plant as it is mainly grown for its aromatic leaves and seeds. Both the leaves and seeds are rich in essential oils. The time of harvest is of great importance as the increase in the content of the aromatic substances, especially the essential oils, is correlated with the progression of the plant growing phase until the stage of full flowering. During the course of this research, the plants were harvest at the time of full flowering when the leaves and flowers were fresh on the plant. This harvesting period is in conformity with the studies of Pisarik (1959) and Hlava (1988). Basil plants can also be harvested prior to flowering (Valicek and Dobsicek, 1993).

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 (GC) 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).

In this research, the control plants yielded the least amount of essential oils with only 360 ppm. The above data show that all the syntheticly 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.

The amount and constitution of the essential oils have been observed to significantly differ between in within the same cultivar of basil plants. For example the study by Singha and Gulati (1990) highlighted that *Ocimum americanum* L. constituted of 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 connected 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, A^3-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; 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 synthetic regulators of plant growth and development as shown in tables B and C above.

Conclusions

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

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 15.71kg per 100m^2. However, the control (Ko) gave the highest dry mass yield.

All the growth regulators triggered flower induction or 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.

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; this clearly confirms that melatran inhibited the branching of the basil plants.

All of the used synthetic 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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