

RESEARCH ARTICLE

The Effect of Hormone Treatments on Germination and Seedling Characters of Sage (*Salvia officinalis* L.) Seeds

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ABSTRACT

Medicinal Sage is consumed as tea in sore throat and kidney diseases caused by cold and flu. It also has sedative, diuretic, antiperspirant and disinfectant effects. Thujone, which is found in the essential oil of *Salvia officinalis* species, is an essential oil component with very strong antiseptic and antibiotic effects. Sage (*Salvia officinalis*), which is a medicinal and an aromatic plant and has a wide area of usage, is cultivated due to these properties. However, the most critical cost item in the production of sage is the weeding done in the first years. The understory weeding done without using herbicides continues until the sage seedlings shield the soil and prevent the development of other herbs. The aim of this research was to determine the effects of hormone treatment on germination success and seedling morphological characters in sage seeds. Within the scope of this research, sage seeds were planted by being exposed to IAA, IBA, GA3 and NAA hormones at 1000, 2500 and 5000 ppm concentrations for 3 to 5 seconds and at 50, 100 and 200 ppm doses for 24 hours, and thus 26 applications were performed together with the control groups. The seeds were planted in sterile peat medium after the hormone treatments, and the effect of hormone treatments on the germination percentage and some seedling characters was tried to be found after 30 days of germination. As a result of the research, it was found that the hormone treatments positively affected most of seedling characters.

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Introduction

Sage, which is officially recognized for medicinal use in Europe, is the *Salvia officinalis* L. plant. *Salvia officinalis* is a typical Mediterranean plant that contains essential oil, and belongs to the Labiatae family. It has subshrub and hairy roots with lengths varying between 60 and 100 cm. Its leaves are hairy and vary in colour from whitish grey to silver (Ceylan, 1996). Sage leaves and the essential oil obtained from those leaves are used in the pharmaceutical industry (Baytop, 1963). Sage is consumed as tea for sore throat and kidney diseases caused by cold and flu.

Since its oil is externally antiseptic and has fungicidal effect, it is used in throat and respiratory tract inflammation (Zeybek and Zeybek, 2002). It also has some sedative, gastric, diuretic, antiperspirant and disinfectant effects (Baytop, 1963). Thujone, which is contained in the essential oil of *Salvia officinalis* species, is an essential oil component with strong antiseptic and antibiotic effects. For this reason, especially the thujone-rich essential oils are used as the additives of drugs made for throat infections, gum inflammations and cankers (Baydar, 2005).

Sage, which is a medicinal and aromatic plant with a wide area of use, is cultivated because of its above-mentioned

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properties. However, the most significant cost item in the production of sage is the weed control carried out in the first years. The understory control carried out without using herbicides continues to a level where sage seedlings can shield the soil and prevent the development of other weeds. For this reason, the rapid growth of sage seedlings is of great importance for shortening this process and reducing costs. This study aims to determine the effect of hormone treatments in sage seeds on germination success and morphological characteristics of seedlings.

Materials and Methods

This study was conducted on sage seeds. Hormone treatment in seeds was carried out in two groups: concentrated hormone treatment and dilute hormone treatment.

Concentrated Hormone Treatment

In the first application under the scope of the study, IAA (Indole acetic acid), IBA (Indole butyric acid), GA3 (Gibberellic acid) and NAA (Naphthalene acetic acid) hormones were applied at the doses of 1000 ppm, 2500 ppm and 5000 ppm. Thus, a total of 13 treatments were conducted in the first application including 12 applications (4 hormones x 3 concentrations) and a control group. At this stage, the seeds were dipped into the prepared hormone concentrations for 3 to 4 seconds, and then placed in the rooting medium.

Dilute Hormone Treatment

In the second application, the IAA, IBA, GA3 and NAA hormones were applied at the doses of 50 ppm, 100 ppm and 200 ppm. Thus, also in the second application, a total of 13

treatments were conducted including 12 applications (4 hormones x 3 concentrations) and a control group. At this stage, the seeds were kept inside the hormone concentrations (distilled water in the control group) for 24 hours, and then were placed in the rooting medium.

Thus, a total of 26 applications were conducted in the study, and each application was carried out in 3 replicates with 5 seeds in each replicate. After the hormone treatment, the seeds were planted in germination medium formed with sterile peat, and then left in rooting medium for 30 days with regular irrigation. During this period, the seeds were regularly checked in order to avoid the occurrence of fungal contamination, insect damage etc. At the end of the specified period, their germination conditions were examined, and germinated ones were counted. After 30 days, the seedlings were uprooted and were subjected to measurement.

Determination of Seedling Characters

Within the scope of the study, first, the germinated seeds were counted, and non-germinated seeds were cut and checked to see if they were damaged. Germination percentage (GP) was determined by proportioning the number of germinated seeds to total number of undamaged seeds. Afterwards, the seedlings were carefully uprooted, and as a result of the measurements made, the following characters were determined: Root Length (RL), Number of Roots (RN), Root Collar Diameter (RCD), Stem Diameter (SD), Unbranched Stem Length (USL), Total Length (TL), Number of Leaves (LN), Number of Layers (LAN), Length of the Largest Leaf (LLL), Width of the Largest Leaf (LLW) and Leaf Stem Length (LSL). Length measurements were performed using a digital micro-compass with a precision of 0.01 mm. The measurement points are shown in Figure 1.

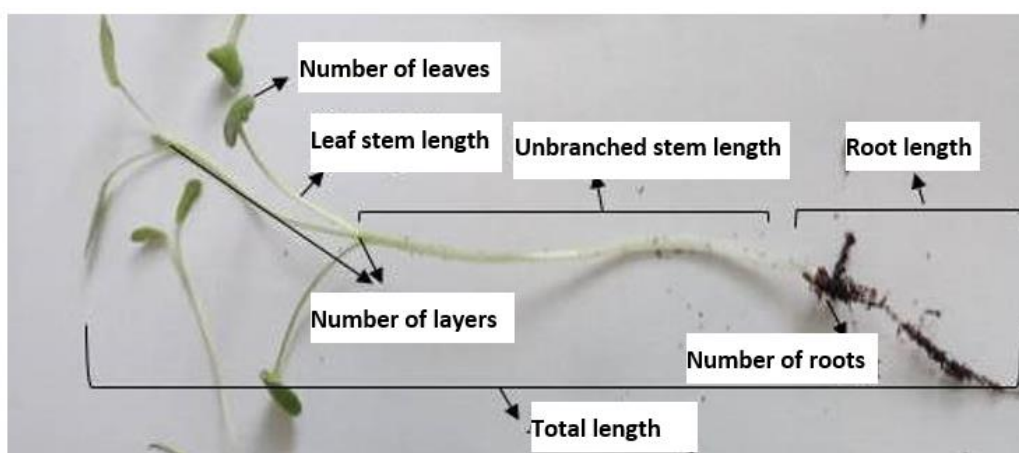


Figure 1. Morphological characters measured on the seedling

The data obtained were assessed with the help of SPSS package program. Variance analysis was applied to the data, and F value, error rate, and thus the difference between the factors were determined to be at 95% confidence level. Duncan test was applied to the factors which were found to have statistically significant differences at minimum of 95% confidence level. The results obtained were interpreted after being simplified and tabulated.

Results

26 applications were made within the scope of the study. The F value, error rate and mean values obtained as a result of the variance test conducted in order to determine the effect of hormone treatments on GP, RL, RN, RCD, USL and SD characters, and the groupings formed as a result of the Duncan test are given in Table 1.

Table 1. Effect of treatments on the seedling characters

TREATMENTS	DOSAGE	HORMONES	GP	RL	RN	RCD	USL	SD
CONCENTRATED	5000	NAA	52.00 cde	32.52 a	2.40 d	1.02 abcd	80.68 fghi	0.94 abcd
		IAA	84.00 g	44.69 abc	1.70 abc	0.98 abc	79.84 fghi	0.94 abcd
		GA3	78.18 g	44.13 abc	1.09 ab	1.15 bcdefg	86.83 i	1.03 cdefg
		IBA	33.33 ab	66.66 d	1.33 abc	1.04 abcde	82.25 hi	1.12 fg
	2500	NAA	36.00 abc	39.07 ab	1.80 bcd	1.24 efgh	73.59 defgh	1.00 abcdef
		IAA	55.00 de	44.38 abc	1.38 abc	1.08 abcdefg	78.90 fghi	1.00 abcdef
		GA3	60.00 ef	38.92 ab	1.29 abc	1.04 abcde	87.36 i	1.03 cdefg
		IBA	82.86 g	46.53 abc	1.43 abc	0.96 ab	74.28 defgh	0.91 abc
	1000	NAA	76.00 g	41.56 ab	1.60 abc	1.06 abcdef	70.29 defgh	1.02 cdefg
		IAA	46.67 cde	49.83 abc	2.00 cd	1.06 abcdef	81.57 hi	0.96 abcde
		GA3	85.00 g	44.84 abc	1.50 abc	0.93 a	81.39 ghi	0.97 abcde
		IBA	52.00 cde	48.57 abc	1.20 ab	1.26 fgh	63.06 cd	1.01 bcdefg
	Control			71.43 fg	43.14 abc	1.43 abc	1.00 abc	73.00 defgh
DILLUTE	200	NAA	20.00 a	42.96 abc	1.00 a	1.23 defgh	74.01 defgh	1.09 efg
		IAA	46.67 bcde	44.57 abc	1.00 a	1.15 bcdefg	68.92 cdefg	1.00 abcdef
		GA3	20.00 a	36.67 ab	2.00 cd	1.05 abcde	44.82 a	1.15 g
		IBA	20.00 a	46.72 abc	1.50 abc	1.15 bcdefg	76.74 efghi	1.06 defg
	100	NAA	40.00 bcd	39.66 ab	1.50 abc	0.95 ab	50.31 ab	0.87 ab
		IAA	40.00 bcd	48.32 abc	1.25 abc	1.08 abcdefg	86.95 i	0.91 abc
		GA3	44.00 bcde	51.75 bc	1.40 abc	1.18 cdefg	65.94 cde	0.96 abcde
		IBA	30.00 ab	37.13 ab	1.50 abc	1.06 abcdefg	79.82 fghi	0.89 abc
	50	NAA	60.00 ef	48.40 abc	1.50 abc	1.10 abcdefg	77.08 efghi	0.86 a
		IAA	33.33 ab	45.94 abc	1.33 abc	1.05 abcde	71.64 defgh	0.89 abc
		GA3	36.00 abc	60.05 cd	1.20 ab	1.11 abcdefg	68.57 cdef	0.99 abcdef
		IBA	40.00 bcd	73.90 de	1.50 abc	1.27 gh	58.24 bc	0.95 abcd
	Distilled Water			30.00 ab	82.05 e	1.00 a	1.39 h	43.09 a
F Value			21.559	3.904	2.735	3.714	8.853	2.821
Sig.			0.000	0.000	0.000	0.000	0.000	0.000

When the table results are examined, it is seen that the applications are statistically significant and effective at 99.9% confidence level on all characters. When the groupings formed as a result of Duncan test and the mean values are examined, it is seen that in terms of germination percentage, the values obtained in dilute hormone treatments are generally low; whereas the values obtained in concentrated hormone treatments are quite high, and that the germination percentage reaches up to 85% in 1000 ppm GA3 application. It is also noteworthy that while there were not many differences between the treatments made in the RL application, the highest value was obtained in the control application.

While the highest values in terms of RN are generally obtained in NAA application, it is seen that RCD and SD

characters have the least difference between the applications, and that the RCD values vary between 0.95 mm and 1.36 mm, while SD values vary between 0.86 mm and 1.15 mm. It is seen that the highest value in terms of RCD is obtained in the control application. In terms of USL, the highest values were generally obtained in concentrated hormone treatments, while the lowest values were obtained in control application. Especially the values obtained from concentrated GA3 application are quite high.

The F value, error rate and mean values obtained as a result of the variance test conducted in order to determine the effect of hormone treatments on TL, LN, LAN, LLL, LLW and LSL characters, and the groupings formed as a result of the Duncan test are given in Table 2.

Table 2. Effect of treatments on the seedling characters

TREATME NTS	DOSAGE	HORMONES	TL	LN	LAN	LLL	LLW	LSL	
CONCENTRATED	5000	NAA	129.59 abc	3.00 abcd	1.60 bcd	6.02 a	4.78 ef	12.14 abcd	
		IAA	170.97 gh	3.80 cd	1.90 cd	8.65 ef	4.28 bcdef	18.72 fgh	
		GA3	169.90 fgh	3.45 bcd	1.73 bcd	7.17 abcde	4.45 def	17.49 efgh	
		IBA	194.97 h	3.33 bcd	1.67 bcd	11.49 g	6.26 g	17.07 defgh	
	2500	NAA	138.12 abcd	3.60 cd	1.80 cd	6.56 abc	4.46 def	17.11 defgh	
		IAA	151.49 cdefg	3.25 bcd	1.63 bcd	7.44 abcde	4.57 def	15.29 bcdef	
		GA3	147.76 bcdefg	2.86 abc	1.43 abc	5.84 a	4.56 def	15.61 bcdef	
		IBA	142.72 abcdefg	3.00 abcd	1.57 bcd	6.17 ab	3.75 abcde	15.25 bcdef	
	1000	NAA	138.92 abcde	3.10 bcd	1.50 abcd	6.29 ab	5.19 f	16.94 defgh	
		IAA	168.49 fg	3.33 bcd	1.67 bcd	5.78 a	4.28 bcdef	19.74 fgh	
		GA3	152.73 cdefg	3.00 abcd	1.50 abcd	6.38 ab	3.91 abcde	17.97 fgh	
		IBA	152.77 cdefg	3.60 cd	1.60 bcd	7.38 abcde	3.54 abcd	17.18 defgh	
		Control	150.99cdefg	3.71 cd	1.86 cd	7.51 abcde	4.42 cdef	17.05 defgh	
	DILLUTE	200	NAA	137.90 abcd	2.00 a	1.00 a	7.01 abcde	3.26 ab	20.91 ghi
			IAA	158.46 defg	3.33 bcd	1.67 bcd	8.38 def	4.25 bcdef	21.77 hi
			GA3	119.72 a	4.00 d	2.00 d	9.31 f	3.25 ab	10.58 ab
IBA			139.65 abcde	2.00 a	1.00 a	6.33 ab	5.15 f	12.27 abcde	
100		NAA	121.03 ab	3.00 abcd	1.50 abcd	7.94 bcdef	3.06 a	11.51 abc	
		IAA	162.43 defg	2.50 ab	1.25 ab	6.43 ab	2.99 a	11.40 abc	
		GA3	156.37 cdefg	3.20 bcd	1.60 bcd	6.14 ab	3.12 a	17.98 fgh	
		IBA	138.54 abcde	2.50 ab	1.25 ab	6.00 a	3.34 abc	14.60 bcdef	
50		NAA	156.09 cdefg	3.00 abcd	1.50 abcd	6.60 abcd	3.09 a	16.08 cdefg	
		IAA	141.94 abcdef	2.00 a	1.00 a	6.19 ab	3.26 ab	16.80 defgh	
		GA3	155.96 cdefg	2.80 abc	1.40 abc	6.86 abcde	3.48 abcd	17.97 fgh	
		IBA	169.97 fgh	3.00 abcd	1.50 abcd	8.33 cdef	3.63 abcd	25.29 i	
		Distilled Water	166.60 efg	3.00 abcd	1.50 abcd	11.21 g	4.59 def	8.97 a	
F Value			4.253	3.123	2.984	7.359	6.598	4.549	
Sig.			0.000	0.000	0.000	0.000	0.000	0.000	

When the table values are examined, it is seen that the application-based changes in all characters are statistically significant at 99.9% confidence level. In terms of TL, it is seen that the values range between 119.72 mm and 194.97 mm, and that the lowest value is obtained from 200 ppm NAA application, while the highest value is obtained from 2500 ppm IBA application. In terms of LN and LAN, the values obtained from concentrated hormone treatments are generally higher than those obtained from dilute hormone treatments, but the highest values are obtained from 200 ppm GA3 application.

While the highest value is obtained from 2500 ppm IBA application (11.49 mm) for LLL, the following highest value is obtained from the control group (11.21 mm). In terms of LLW, the highest values are obtained from 2500 ppm IBA (6.26 mm),

1000 ppm NAA (5.19 mm) and 100 ppm IBA (5.15 mm) applications. Whereas for LSL, the lowest value is obtained from the control group (8.97 mm), while the highest value is obtained from 50 ppm IBA (25.29 mm) application.

The results of the correlation analysis conducted to determine the relationship levels of the characters with each other are given in Table 3.

Table 3. Correlation analysis results

	RL	RN	RCD	USL	SD	TL	LN	LAN	LLL	LLW	LSL
GP	-0.053	-0.037	-0.16	0.101	-0.034	0.04	0.033	0.038	0.003	0.068	0.075
RL	1	0.088	.292**	-0.11	0.124	.619**	0.109	0.088	.222**	-0.161	0.031
RN	0.088	1	-0.004	-0.08	0.056	0.037	0.075	0.097	-0.008	-0.119	-0.102
RCD	.292**	-0.004	1	-0.092	0.128	0.112	0.012	0.011	0.127	0.059	-0.103
USL	-0.11	-0.08	-0.092	1	0.048	.449**	.214*	.205*	-0.066	0.049	0.148
SD	0.124	0.056	0.128	0.048	1	.241**	.247**	.207*	.209*	-0.046	.318**
TL	.619**	0.037	0.112	.449**	.241**	1	.495**	.475**	.418**	-0.046	.291**
LN	0.109	0.075	0.012	.214*	.247**	.495**	1	.977**	.272**	0.04	.324**
KATS	0.088	0.097	0.011	.205*	.207*	.475**	.977**	1	.279**	0.066	.302**
LLL	.222**	-0.008	0.127	-0.066	.209*	.418**	.272**	.279**	1	.296**	0.082
LLW	-0.161	-0.119	0.059	0.049	-0.046	-0.046	0.04	0.066	.296**	1	-0.001

According to the correlation analysis results, the relationships between RL and RCD; TL and LLL; USL, TL, LN and LAN; and SD, TL, LN, LAN, LLL and LSL were found to be statistically significant at minimum of 95% confidence level. All relationships, which were found to be statistically significant at minimum of 95% confidence level, were positively related. The strongest relationships were determined between LAN and LN (0.977); KOCB and TL (0.619); and TL and LN (0.495). Interestingly, no significant relationship was found between GP and any other character.

Discussions

As a result of the study, the highest values were generally obtained in concentrated hormone treatments. While the highest values in the characters were mostly obtained at 5000 ppm hormone dose, in some characters the highest values were obtained in the control group. It was observed that GP, one of the most important characters assessed in the study, decreased to 20% in dilute hormone treatments, while it increased up to 85% in 1000 ppm GA3 application and up to 84% in 5000 ppm IAA application. Similar results were obtained in studies conducted on different species as well. Guney et al., (2016a) reported that *Lilium artvinense* seeds' germination percentage, which was 40% in the control group, increased with increasing GA3 dosage, and that it reached up to 72% at 1000 ppm, 80% at 3000 ppm and to 100% at 5000 ppm. Guney et al., (2016b) stated that hormone treatment significantly increased the rooting percentage in *Lilium martagon* seeds, that the rooting percentage, which was 28.4% in the control group, could be increased through IAA, IBA, NAA and GA3 hormone treatments, and that the highest rooting percentage was obtained from 5000 ppm IAA application (86.6%).

Within the scope of the study, the highest germination percentage value was obtained in 1000 ppm GA3 application. GA3, unlike the other three hormones used in the study, belongs to the gibberellins group. Gibberellins are the third most widely used natural plant growth regulators with the utilization rate of 17%. Gibberellins are growth-promoting hormones (Turhan, 2015). The effect of GA3 on rooting has also been the subject of many studies. The effectiveness of GA3 was investigated by Hepaksoy (2004) in *Prunus avium* and *Prunus mahaleb* seeds, by Aygun and Dumanoglu (2006) in *Cydonia oblonga* seeds, by Cosge et al., (2005) in *Capparis ovata* seeds, by Selby et al., (1992) in *Picea sitchensis* seeds,

by Sevik et al., (2015) in *Schefflera arboricola* seeds, and by Guney et al., (2016a) in *Lilium artvinense* seeds. Although no significant effect of GA3 is found on rooting in many species, there are some studies available showing that GA3 significantly increases the rooting percentage (Cosge et al. 2005; Guney et al., 2017).

It was found that hormone treatments significantly affected the rooting percentage in cuttings as well as the germination percentage. In many species such as *Robinia pseudoacacia* (Swamy et al., 2002), *Pseudotsuga menziesii* (Stefancic, et al., 2005), *Oryza sativa* (Chhun et al., 2003) and *Schefflera arboricola* (Sevik et al., 2015), it was determined that the rooting percentage could be increased with the use of hormones. It was stated that IBA significantly increased rooting in sage cuttings subjected to this study, and that the rooting percentage, which was 16.25% in the control group, could be increased by 78.75% with 100 ppm of IBA application (Ayanoglu and Ozkan, 2000).

The treatments made during the study were found to have significantly affected the seedling characters, but each application had a different effect on the characters. For example, while TL and LSL values obtained as a result of GA3 application were in the first homogeneous groups, LN, LAN and LLL values were among the highest values obtained in the same application.

In a study conducted by Sevik and Guney (2013) on *Melissa officinalis* cuttings, the lowest root length value was obtained from the control group in concentrated hormone treatment, while the value obtained from the IBA application was about 5 times higher than the value obtained in the control application. While Sevik and Cetin (2016) obtained one of the lowest values in concentrated hormone treatments from the 1000 ppm NAA application in their study on *Lilium artvinense* bulbs, the value obtained in GA3 application was one of the highest values. The value obtained in 3000 ppm GA3 application was approximately 5 times higher than the value obtained in NAA application.

In a study carried out by Pulatkan et al., (2018) on *Berberis thunbergii* cuttings, the lowest value in concentrated hormone treatments was obtained in 1000 ppm NAA application, while the highest value was obtained in 3000 ppm NAA application.

In this study, it was intended to identify the effects of different hormone treatments on the development of sage seedlings. Nowadays, plant growth regulators, meaning hormone treatments, are used in many stages of plant

production. This is because, the increasing population brings along many problems with it such as environmental pollution and a decrease in agricultural areas. Alongside these problems, food problem is also growing, and the growing food problem is tried to be solved in the most practical way by increasing the amount of product taken from unit area (Ozel, 2019; Turkyilmaz et al., 2020).

There exist many studies aiming to determine the effect of hormone treatments on plant growth and development. However, most of the studies are intended for use in vegetative production (Babu et al., 2019; Amini et al., 2019; Shao et al., 2018). Whereas, the number of studies on hormone treatments on seeds is quite limited (Guney et al., 2016a, b). When the studies on the subject are examined, it is seen that hormone treatments in general increase plant growth and development in various ways, but this increase varies depending on the plant type as well as the hormone type and dosage. This result was also obtained at the end of this study. In fact, when the study results are examined, it is seen that different hormones affect different characters at different levels, and similar results are obtained in numerous studies conducted (Guney et al., 2016a, b; Sevik et al., 2015).

The growth performance of plants, meaning the phenotypic characteristics, is the result of the mutual interaction of genetic structure and environmental conditions (Yigit et al., 2016; Hrivnák et al., 2017), and it is known that each genetic structure can give different reactions to the same environmental conditions (Yucedag et al., 2019; Sevik et al., 2019a,b; Yigit et al., 2018). Thereby, the components of these factors may affect the growth performance of plants, i.e. phenotypic characteristics. For instance, the subtype, form, variety and origin of the same plant can also be expected to give different reactions to the same hormones. Likewise, the studies conducted show that many phenological, morphological and anatomical characters are significantly affected by these factors (Yigit et al., 2016; Cetin et al., 2018a,b; Ozkazanc et al., 2019).

The responses given by plants to hormone treatments are closely related to plant metabolism (Guney et al., 2017; Sevik et al., 2015). Therefore, many factors, which significantly affect plant metabolism such as plant stress (Sevik and Cetin, 2015; Turkyilmaz et al., 2018a,b; Ozel et al., 2021; Varol et al., 2021), and genetic structure (Hrivnak et al., 2017), are likely to affect the level of response to be given by plants to hormone treatments.

Conclusions

As a result of the study, the effects of the hormones applied on the sage seeds on germination percentage and some seedling characters were determined. The data obtained from the study show that the hormones applied affect the study characters at different levels. This result is of critical importance for practice. Using the results of the study, the hormone treatment, which affects the desired character, can be selected in practice. For example, if the seedling height is desired to be high, it is preferable to apply 5000 ppm, and 200 ppm GA₃, when the stem diameter is desired to be high.

As a result of the study, it was observed that concentrated and dilute hormone treatments gave similar values in some of the characters. In practice, the most expedient treatment can be selected from concentrated and dilute hormone treatments by evaluating these results. For instance, dilute hormone treatments can be preferred, if less cost is a required; and concentrated hormone treatment, if less labour is required.

In this study, the effects of hormone treatments on sage seeds were examined. However, in the literature reviews conducted, it was seen that the responses of different species to different hormones were at different levels. For this reason, similar studies should be carried out for each species separately, and the hormone types and doses affecting the desired character should be determined for each species, separately.

Within the scope of the study, only 4 hormones were evaluated at different doses. However, in order to obtain the best result, it may be suggested to carry out similar studies by increasing and diversifying, and to use hormone mixtures in addition to different hormones and doses.

Conflict of Interest

The authors declare no conflict of interest. The none of the authors have any competing interests in the manuscript.

Author Contributions

All authors equally contribute on this research.

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