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Effects of Different Doses of Plant Growth Regulators on Some Characteristics of Summer Snowflakes (*Leucojum aestivum* L.)[#]

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ARTICLEINFO	A B S T R A C T
[#] This study was presented as an oral presentation at the 13 th National, 1 th International Field Crops Conference (Antalya, TABKON 2019)	The aim of this study was to determine the effect of different doses and combinations of benzylaminopurine (BAP) and Putrescine elicitor on some plant characteristics of summer snowflake (<i>Leucojum aestivum</i> L.). The experiment was conducted for two years between 2017-2019 in the greenhouse of Uşak University, Faculty of Agriculture and Natural Sciences. Bulbs with
Research Article	\sim 7 cm circumference and \sim 9 g weight were used as a material of this study, and was constructed with randomized blocks design with 3 replications. The bulbs were treated for 24 hours before
Received : 25/11/2019 Accepted : 06/12/2019	planting with 5, 10 and 15 mg/l BAP; 5, 10 and 15 mg/l Putrescine elicitor in different combinations (16 different combinations). Thereafter, all bulbs were planted in crates. In the first year, only plant height, number of leaves per bulb were recorded and at the end of the second year, the plant height, number of leaves per bulb, number of leaves per daughter bulb, number of bulbs, leaf weight, root
<i>Keywords:</i> Summer snowflakes Putrescine Alkaloid Galantamine Elicitor	length and weight, bulb weight, unit bulb weight, alkaloid contents were measured after removal of bulbs from the soil. According to the results, plant height ranged 19,3-30,0 cm; the number of leaves ranged 6.5 to 12.6, and the number of bulbs ranged 0.9 to 4.6. Root weight was determined between 13.4-47.1 g/parcel, fresh leaf weight ranged 12.4-49.2 g/parcel, and one bulb weight was between 3.5-14.6 g. It was determined that the bulb plant was influenced by different treatments. The maximum growth was noted with 5 mg/l Putrescine elicitor treatment that increased plant height, number of leaves per bulb each year and number of bulbs.

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Introduction

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Summer snowflakes (Leucojum aestivum L.) is an important species of the Amaryllidaceae family. Eight (8) species exist naturally in Europe. Species of the genus Leucojum are distributed both in Europe and on the Mediterranean coasts (Jovanovic et. al., 2009). A single species in this genus Leucojum aestivum L. grows widely under wild conditions in moist areas in Turkey (Davis, 1982). It is known as Akcabardak, Kabalak and Sarıklı Kökü vernacularly among people in Turkey (Cirak et al., 2004). It has been reported and showed natural distribution in the Flora of Turkey at Istanbul, Kocaeli, Bursa, Bolu, Samsun, Konya-Beyşehir and Erzurum (Davis, 1982). Its export is prohibited or restricted except under controlled conditions with export quota of 6 million bulbs/year (Anonymous, 2019). It is grown under wild conditions or to extract galantamine for use in local industry and abroad for use as medicine. This alkaloid was first isolated from *Galanthus woronowii* (Black Sea snowdrop) in the world. Today, Galantamine is also extracted from summer snowflakes (*Leucojum* sp.) and other species of Amaryllidaceae (Takos and Rook, 2013; Klosi et al., 2016). In general, the average galantamine content in *L. aestivum* collected from natural habitats is used for industrial production and range 0.1-0.2% (dry matter) (Berkov et al. 2013; Klosi et al., 2016). Treatment of plant growth regulators and elicitor to the plants are practiced to increase the amount of alkaloids in the plants and to improve the growth and quality of the growing plants. It is established that plants grow and develop some alkaloids and flavonoids, after some chemical interaction within and between plant cells.

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Plant growth and development regulators (PGRs) are essential compounds that enable communication between cells in plants. These compounds are produced in plants. Generally, these naturally occurring compounds in plants are called phytohormones, hormones or plant growth regulators. These control growth and physiological phenomena are carried or distributed to other parts of the plant. These are effective and influence the place they are transported and can show their effects even at very low concentrations. (Okturen and Sonmez, 2005; Algul et al., 2016). Putrescine elicitor is a low molecular weight amino acid derivative which can be found in all living organisms and is one of the polyamines (PA), which is one of the substances used for preservation or improvement of quality. There are 4 types of aliphatic PAs, which are putrescine elicitor, cadaverine, spermidine and spermine, which have important functions in plants (Liu et al., 2000: Bal, 2012; Erbas, et al., 2018). There is no study using phytohormones and Putrescine elicitor under ex-vitro conditions. These chemicals are used in these combinations and style for the first time on summer snowflake bulbs. The aim of the study was to determine the effects of BAP and Putrescine elicitor in different doses and combinations on some characteristics of summer snowflake (L. aestivum L.).

Material and Method

The experiment was conducted as a two-year trial in the research and treatment greenhouse of Uşak University, Faculty of Agriculture and Natural Sciences during 2017-2019. The bulbs of summer snowflake (L. aestivum) that had an average bulb circumference of 7 cm and a weight of 9 g, were used as material. The experiment was established in 3 replications according to randomized block experimental design. The bulbs were treated for 24 hours before planting using 16 combinations of 5, 10 and 15 mg/l BAP and 5, 10 and 15 mg/l Putrescine elicitor. 5 bulbs each were planted in crates. A total of 16 treatments \times 3 replications \times 5 bulbs: 240 bulbs (240 bulbs \times 9g = 2,160 kg) were used in the experiment. Thereafter, the bulbs were treated with BAP+ putrescine elicitor concentrations and planted in plastic crates (dimension:30x50 cm) containing sieved field soil, stream sand and shaft mixture (1:1:1) in the crates. Soil analysis of this mixture was performed. This medium was slightly alkaline, loamy, medium lime, poor in organic matter and nitrogen, phosphorus and potassium in the middle range. Planting was made on 16 October 2017. Bulb harvests were carried out on 21 May 2019. Only plant height (cm/plant), number of leaves (number/plant) and their values were measured in the first year. The experiment plants were measured for plant height (cm), number of leaves per plant, fresh leaf weight per plant (g) were measured each year, number of daughter bulbs per plant, root length per plant (cm), fresh root weight per plant (g), fresh bulb weight (g /parcel), one bulb weight (g) and galantamine alkaloid content in root, bulb and leaves (%) were measured as characters in the 2nd year.

The content of galantamine alkaloid were determined at Uşak University Faculty of Agriculture and Natural Sciences Research Laboratory and Uşak University Scientific Analysis and Technological Treatment and Research Center (UBATAM) Chemistry Analysis Laboratory. The following method was used in the analysis. Specimen Preparation: 300 mg of the sample was weighed separately from dried leaves and ground plant roots, bulbs and leaves obtained from each treatment. 30 ml of 0.1 M HCl solution was then added to these samples. In the ultrasonic bath, these samples were extracted for 15 minutes. At the end of the process, the samples were filtered with filter paper and the remaining bagasse was discarded. The extract was then refrigerated until analysis.

Analysis Method

Analyzes were performed with Agilent brand 1260 HPLC device. Galantamine hydrobromide ($C_{17}H_{21}NO_3$ HBr) used as standard. First, weighing of Galantamine Hydrobromide equivalent to 100 mg Galantamine. Dissolved in deionized water, the volume was completed to 100 ml with deionized water. A standard stock solution at a concentration of 100 ppm was prepared. Prepared as standard solutions of the study in five different concentrations (20, 40, 60, 80, 100 ppm) using standard stock solution. These solutions were injected into the HPLC device. Concentration graph was generated against peak areas. (Anonymous 2008).

Chromatographic System

Detector: UV 288 nm; Column: 4.6-mm ' 15.0-cm; 5mm packing L1 Mobile Phase A: 4.0 g/L Monobasic Potassium Phosphate solution was prepared. The pH was adjusted to 6.5 with 5 N Sodium Hydroxide. (90%) Mobile Phase B: Acetonitrile (10%); Flow Rate: 1.2 mL/min; Injection Volume: 20 μ L. Galantamine calibration curve generated using the standard stock solution for the galantamine analysis in the assay was y = 10,299×-19,449; R² = 0.9996.

The variance analysis of the mean values of the characters measured in the experiment was performed by SPSS 24 statistical package program and the differences between the averages were determined by the Duncan's Multiple Range test.

Results and Discussion

Variance analysis results and the differences among the average values of the characters measured in the experiment such as plant height, number of leaves, number of sibling bulbs, root length, number of roots, leaf fresh weight, root fresh weight, bulb fresh weight, harvested bulb weight and galantamine content were found significantly different at the 0.01 level of significance in all treatments.

In the experiment, the maximum plant height value of first and 2nd years was obtained using the same treatment of 5 mg/l Putrescine elicitor for both years with plant height of 25.8 cm and 30.0 cm respectively. The minimum plant height in the first year was noted on 10 mg/l BAP + 5 mg / Putrescine elicitor and 10 mg/l BAP + 10 mg/l Putrescine elicitor (17.7 cm) treatments. In the second year, treating with 5 mg/l BAP + 5 mg /Putrescine elicitor resulted in induction of 19.3 cm long plants. It is seen that plant height is affected by all hormone+elicitor treatments, and plant length is prolonged in single treatment of hormone +elicitor, but it is seen that high doses of hormone combinations have average values lie in almost the same group as control. The maximum plant height values were obtained from10 mg/L BAP and 5 mg/L Putrescine elicitor treatments (Table 1). The maximum number of leaves was obtained from 5 mg/l Putrescine elicitor treatment (11.1

leaves per plant during first year and 12.6 leaves per plant during 2nd year). The minimum number of leaves was obtained from the treatment of 10 mg/l BAP + 10 mg/l Putrescine elicitor (6.3 leaves per plant during 1st year and 6.5 leaves per plant during 2nd year). According to the findings obtained from the experiment, it can be said that the number of leaves is affected by all treatments. The number of leaves in Putrescin single treatments was higher compared to other treatments (Table 1). In the experiment, the highest number of daughter bulbs was obtained from 5 mg/l Putrescine elicitor treatment (4.6 induced bulblets per plant) and 15 mg/l BAP + 10 mg/l Putrescine elicitor (0.9 induced bulblets per plant) was the minimum. According to the findings obtained from the experiment, it can be said that the number of induced bulblets is affected by all hormone treatments. The number of induced bulblets was higher in putrescine elicitor used singly (Table 1. Figure 1).

The maximum number of 8.47 daughter bulblets leaves were induced using 5mg/l Putrescine elicitor treatment.

The minimum number of 2.27 daughter bulblets leaves were obtained using 15 mg/L BAP + 10 mg/L Putrescine elicitor. Furthermore, the findings obtained from the experiment, showed that the number of induced daughter bulblets leaves was affected by all treatments (Table 1, Figure 1). Cirak et al (2004) observed effects of different nitrogen doses and harvest times on yield and some characteristics of bulb plants. Plant height ranged 42.63-47.57 cm; the number of leaves per plant ranged 5.52-8.42; number of daughter bulblets per bulb ranged 1-1.32. Ayan et al. (2004), treated summer snowflakes using different doses of GA₃ and NAA under shady and light conditions and found changes in bulb yield and some properties of the plants. According to the results, the plant height was 38.07 cm in light and 47.10 cm under shaded conditions. GA₃ treatments induced longer plants compared to NAA treatments. The number of leaves and the number of daughter bulblets in light and shaded environments ranged 5.28 - 6.20 and 0.63 to 1.33 respectively.

Table 1 Plant height, number of leaves per bulb, number of daughter bulb, number of leaves per daughter bulb, root length, number of root per bulb average parameters and Duncan groups

Treatments	PH		NLB		- NDB	NLDB	RL	NRB	
Treatments	1	2	1	2	- NDD	NLDD	KL	INKD	
Control	17.9 ^h	22.5 ^{def}	9.03 ^{cd}	9.5 ^{b-f}	2.9 ^{bc}	5.33 ^{bcd}	31.3 ^f	53.5 ^{ab}	
5 mg/L BAP	20.3 ^{def}	25.2 ^{a-e}	9.1 ^{cd}	9.3 ^{c-f}	2.5 ^{bcd}	4.60 ^{b-e}	30.9 ^{fg}	44.5 ^{b-f}	
5 mg/L Putrescine elicitor	25.8 ^a	30.0 ^a	11.1 ^a	12.6 ^a	4.6 ^a	8.47 ^a	28.0^{fg}	47.4 ^{a-e}	
10 mg/L BAP	25.7 ^a	29.7ª	8.0^{d-g}	8.4^{d-g}	1.8 ^{ef}	3.73 ^{ef}	26.1 ^g	57.8 ^a	
10 mg/L Putrescine elicitor	22.5°	26.8 ^{a-d}	9.13 ^{cd}	9.9 ^{b-c}	2.9 ^b	5.80 ^b	37.4 ^{cd}	43.6 ^{b-f}	
15 mg/L BAP	23.7 ^b	27.8 ^{abc}	8.40 ^{def}	8.9 ^{c-g}	2.9 ^b	4.87 ^{b-e}	28.9^{fg}	42.1 ^{c-f}	
15 mg/L Putrescine elicitor	24.3 ^b	29.1 ^{ab}	10.3 ^{ab}	11.1 ^{ab}	2.1 ^{de}	5.53 ^{bc}	42.9 ^b	52.3 ^{abc}	
5 mg/L BAP + 5 Putrescine elicitor	20.6 ^{de}	25.3 ^{a-e}	8.97 ^{cd}	9.7 ^{b-e}	2.8 ^{bc}	5.73 ^b	41.5 ^{bc}	50.4 ^{a-d}	
5 mg /L BAP + 10 Putrescine elicitor	19.4 ^{fg}	23.5 ^{c-f}	9.67 ^{bc}	10.3 ^{bc}	2.4 ^{cd}	5.33 ^{bcd}	31.9 ^{ef}	34.6 ^{fg}	
5 mg/L BAP + 15 Putrescine elicitor	18.4 ^{gh}	24.5 ^{b-e}	8.27 ^{def}	8.6 ^{c-g}	2.5 ^{bcd}	4.40 ^{cde}	27.0 ^{fg}	27.2 ^g	
10 mg/L BAP + 5 Putrescine elicitor	17.7 ^h	19.3 ^f	7.67 ^{efg}	8.0 ^{e-h}	1.9 ^{ef}	4.00 ^{ef}	36.1 ^{de}	46.9 ^{a-e}	
10 mg /L BAP + 10 Putrescine elicitor	17.7 ^h	20.3 ^{ef}	6.30 ^h	6.5 ^h	2.0 ^{de}	3.87 ^{ef}	30.8 ^{fg}	36.4 ^{efg}	
10 mg /L BAP + 15 Putrescine elicitor	19.6 ^{ef}	22.9 ^{c-f}	7.93 ^{d-g}	8.4^{d-g}	1.8 ^{ef}	3.53 ^{ef}	36.6 ^d	35.6 ^{fg}	
15 mg/L BAP + 5 Putrescine elicitor	21.0 ^d	26.0 ^{a-d}	7.4 ^{gh}	7.9 ^{fgh}	1.5 ^f	307 ^{fg}	47.7 ^a	42.1 ^{c-f}	
15 mg /L BAP + 10 Putrescine elicitor	20.7 ^{de}	27.1 ^{a-d}	6.87^{gh}	7.1 ^{fg}	0.9 ^g	2.27 ^g	41.8 ^{bc}	45.1 ^{b-f}	
15 mg /L BAP + 15 Putrescine elicitor	19.8 ^{ef}	23.2 ^{c-f}	8.67 ^{cde}	9.0 ^{c-f}	2.0 ^{de}	4.07 ^{def}	31.1 ^{fg}	40.3d ^{ef}	
General Average	20.9	25.2	8.55	9.08	2.34	4.66	34.38	43.74	

PH: Plant height (cm)**, NLB: Number of leaves per bulb**, 1: First year, 2: 'nd year, NDB: Number of daughter bulb/bulb**, NLDB: Number of leaves per daughter bulb **, RL: Root length (cm)**, NRB: Number of roots per bulb**, ** The differences between the mean values were statistically significant at the 0.01 level.

Table 2 Fresh leaf weight, fresh root weight, bulb fresh weight, single bulb weight, root, leaf and bulb alkaloid content	S
average values and duncan groups	

Treatments	FLW	FRW	FBW**	UBW	RAC	LAC	BAC
Control	16.1 ^{efg}	15.1 ^{de}	48.0 ^{d-g}	9.6 ^{d-g}	0.061 ^{cd}	0.060^{f}	0.204 ^d
5 mg/L BAP	16.7 ^{efg}	15.6 ^{cde}	51.5 ^{c-f}	10.3 ^{b-f}	0.078^{abc}	0.111°	0.236 ^{bcd}
5 mg/L Putrescine elicitor	21.1 ^{b-e}	18.7 ^{cde}	57.5 ^{bcd}	11.5 ^{bcd}	0.036 ^e	0.040^{g}	0.250 ^{a-d}
10 mg/L BAP	24.4 ^b	24.6 ^{b-e}	43.1 ^{ef}	8.6^{fg}	0.091 ^a	0.031 ^g	0.284 ^{ab}
10 mg/L Putrescine elicitor	24.2 ^b	25.6 ^{b-e}	54.5 ^{bcd}	10.9 ^{bcd}	0.032 ^e	0.158 ^a	0.253 ^{a-d}
15 mg/L BAP	12.4 ^g	13.4 ^e	38.8 ^g	7.8^{g}	0.071 ^{bc}	0.086^{de}	0.245 ^{a-c}
15 mg/L Putrescine elicitor	34.0 ^b	32.9 ^b	72.9 ^a	14.6 ^a	0.041 ^e	0.041 ^g	0.264 ^{a-c}
5 mg/L BAP + 5 Putrescine elicitor	24.5 ^b	47.1 ^a	52.2 ^{b-f}	10.4 ^{b-f}	0.047 ^{de}	0.077 ^{def}	0.210 ^d
5 mg /L BAP + 10 Putrescine elicitor	17.9 ^{def}	20.5 ^{cde}	49.4 ^{def}	9.9 ^{c-f}	0.048 ^{de}	0.096 ^{cd}	0.217 ^{cd}
5 mg /L BAP + 15 Putrescine elicitor	13.4 ^{ef}	14.3 ^e	42.8 ^{fg}	8.6^{fg}	0.068 ^c	0.075 ^{ef}	0.254 ^{a-d}
10 mg /L BAP + 5 Putrescine elicitor	49.2 ^a	22.6 ^{b-e}	17.5 ^h	3.5 ^h	0.070^{b}	0.095 ^{cde}	0.221 ^{cd}
10 mg /L BAP + 10 Putrescine elicitor	13.7 ^{ef}	18.2 ^{cde}	44.7 ^{efg}	8.9 ^{efg}	0.071 ^{bc}	0.168 ^a	0.290 ^a
10 mg /L BAP + 15 Putrescine elicitor	18.4 ^{c-f}	23.6 ^{b-e}	54.0 ^{b-e}	10.8 ^{b-e}	0.064 ^c	0.137 ^b	0.236 ^{bcd}
15 mg /L BAP + 5 Putrescine elicitor	23.4 ^{bc}	27.5 ^{bc}	61.0 ^{cb}	12.2 ^b	0.070^{bc}	0.087^{de}	0.224 ^{cd}
15 mg /L BAP + 10 Putrescine elicitor	22.1 ^{bcd}	33.7 ^b	61.4 ^b	12.3 ^b	0.063°	0.089^{de}	0.269 ^{abc}
15 mg /L BAP + 15 Putrescine elicitor	18.2 ^{c-f}	26.7 ^{bcd}	57.5 ^{bcd}	11.7 ^{bc}	0.086^{ab}	0.075 ^{ef}	0.265 ^{abc}
General Average	21.86	23.55	50.43	10.1	0.062	0.089	0.245

FLW: Fresh leaf weight (g/parcel)**, FRW: Fresh root weight (g/parcel) **, FBW: Fresh bulbs weight (g/parcel)**, UBW: Unit bulb weight (g/bulb) **, RAC: Root alkaloid contents (%)**, LAC: Leaf alkaloid contents (%)**, BAC: Bulb alkaloid contents (%) **, ** The differences between the mean values were statistically significant at the 0.01 level

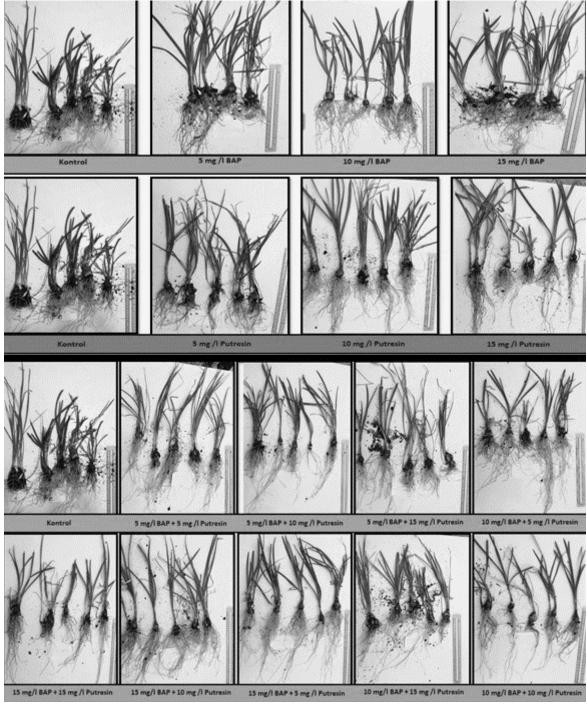


Figure 1 General conditions at time of harvest of plants to which different combinations of plant growth regulators BAP and putrescine elicitor are administered at different

The results obtained in this study showed low plant height compared to the results obtained from the researchers mentioned above. This situation was influenced by the size of the planted bulbs, under light or shady conditions. The number of bulblets obtained in this study ranged 0.9-4.6 was higher compared to the values obtained from the studies of Cırak et al. (2004) and Ayan et al. (2004). It is possible to say that plant growth regulator+elicitors are effective in inducing and improving regeneration. The maximum root length (47.7 cm) was obtained after using 15 mg/l BAP + 5 mg/l Putrescine elicitor treatment. The minimum root length was obtained using 10 mg/l BAP with root length of 26.1 cm. The findings of this experiment showed that the root length was influenced by BAP+putrescine elicitor combinations (Table 1). Additionally, the maximum number of roots was noted on 10 mg/l BAP treatment (57.8 root per plant). The concentration of 5 mg/l BAP + 15 mg/l Putrescine elicitor treatment induced minimum number of roots (27.2 roots per plant) (Table 1). Fresh leaf weight (49.2 g/parcel) was higher at 10 mg/l BAP + 5 mg/l Putrescine elicitor treatment. The minimum leaf weight (12.4 g/parcel) was noted using 15 mg/l BAP treatment (Table 2, Figure 1). The maximum fresh root weight value (47.1 g/parcel) was obtained from 5 mg/l BAP + 5 mg/l Putrescine elicitor treatment. The minimum fresh root weight (13.4 g/parcel) was noted using 15 mg/l BAP treatment (Table 2, Figure 1). The maximum fresh root weight (13.4 g/parcel) was noted using 15 mg/l BAP treatment (Table 2, Figure 1). The maximum fresh bulb weight (72.9 g/parcel) was

noted using 15 mg/l Putrescine elicitor treatment; the minimum fresh weight of bulbs (17.5 g/parcel) was noted using 10 mg/l BAP + 5 mg/l putrescin treatment (Table 2, Figure 1). If the weight of the bulbs planted in the parcels is considered as 9 g and there were 5 bulbs planted for each treatment, the total bulb weight equals around 45 g/parcel.

In most of the treatments, the bulbs were harvested well above this value and showed positive increase in their weight, while only 2 treatments were below this value. The maximum harvested bulb weight (14.6 g per bulb) values were noted at 15 mg/l Putrescine elicitor treatment; the minimum harvested bulb weight (3.5 g per bulb) was noted at 10 mg/l BAP + 5 Putrescine elicitor treatment (Table 2, Figure 1). The studies of Ayan et al. (2004), compared bulb yields in shady and light conditions, respectively and found a range between 342.78-384.4 kg/da. They noted that the light is most important factor that limits the plant growth and bulb yield. They also emphasized that growth regulators such as GA₃ and NAA are useful in obtaining large bulbs. Cirak et al. (2004) also noted that the weight of bulbs ranged 490-597.50 kg/da. They found that nitrogen treatment of 20 kg/da gives the best results and the minimum results are obtained in the control treatment.

Bulb weight and single bulb weight values obtained in this study; comparing that the weight of planted onions is 9 g at the time planting, a positive increase in their weight was noted for all treatments except 2 treatments (15 mg/l BAP and 10 mg/l BAP +5 mg/l putrescine elicitor). Here, the high amount of daughter bulblets in the treatment of 15 mg/l BAP led to a decrease in single bulb weight. When evaluated in terms of galantamine alkaloid content; The maximum alkaloids (0.091%) were noted in the roots of plants in the experiment using 10 mg/l BAP treatment. The minimum alkaloid contents (0.032%) were noted using 10 mg/l Putrescine elicitor treatment (Table 2, Figure 1). The maximum alkaloid content (0.168%) in leaf was obtained from 10 mg/l BAP +10 mg/l Putrescine elicitor treatment and the minimum alkaloid content (0.031%) in leaf was obtained at 10 mg/l BAP treatment (Table 2, Figure 1). The values of alkaloid content (0.290%) in the harvested bulbs were higher than that obtained at 10 mg/l BAP+10 mg/l Putrescine elicitor; the minimum (0.204%) was obtained from the control treatment. Some researchers in their work on L. aestivum plant populations have noted alkaloid galantamine content of 0.30%, - 0.34% (Cherkasov, 1975; Cherkasov et al., 1984; Cherkasov and Tolkachev, 2002). The amount of galantamine in dormant bulbs of summer snowflake varies between 4-99% of the total alkaloid amount (Georgieva et al., 2007; Berkov et. al., 2009). Berkov et al. (2013) found that Galantamine content of the different regions in northern Bulgarian summer snowflake bulb collected varied between 0.003-0.08%; It increased in the summer snowflakes of southern regions and reached to 0.42%. They noted that this value increased up to 0.65% in some single plant samples. They noted that there are many chemotypes according to geographical distributions. Berkov et. (2009) noted that leaves (dry matter) galantamine content varied according to geographic locations and populations from the trace amount to 0.5% (usually 0.1-0.3%). Sanguinine, N-allylnorgalantamine, N-(14methylallyl) norgalantamine, have a much higher AChE inhibitory effect than galantamine alkaloids and were isolated in much higher amounts from the bulbs

(Berkov et al., 2009). Gussev et. al. (2007), in their studies reported that galantamine content in summer snow flake bulb plants ranged 0.9-2.6 mg/g. In the daffodil plant; states that much higher levels of plant growth regulators are needed to promote the formation of adventitious shoots (Seabrook et al., 1976; Seabrook, 1990;) and bulblets (Keller, 1993) compared to other monocotyledonous plants (Codina, 2002). In a study conducted in the narcissus plant, cytokinins, such as BA or kinetin (1-3 mg/l) were used but they induced low concentrations of Galantamine accumulation in the tissues of shoot explant that was higher using BA compared to the kinetin added to the medium (Codina, 2002). Summer snow flake bulb is a perennial bulbous plant with a complex physiological cycle that also regulates the dormant period formed under the pressure of environmental temperatures and phytohormones. The above-ground parts of this plant, which die in beginning of the summer and starts regrowing beginning of the autumn. Galantamine concentration is the maximum during flowering period, but decreases rapidly during fruit formation period (Stanilova et. al., 2010). When the alkaloid contents obtained in this study were examined, alkaloid bulb galantamine in increased with hormone+putrescine elicitor treatments compared to control treatment. Galantamine content increased in bulbs but decreased in leaves. The findings for the amount of galantamine obtained in this study are consistent with the values determined by the other researchers mentioned above.

Conclusion

Hormone treatments affected yield and some yield characteristics of summer snowflake positively. In this study, the maximum amount of galantamine was detected in bulbs, followed by leaves and roots. According to these results, it can be seen that leaves can be considered as a source of galantamine. However, this plant, which has a very complex development and life cycle, needs to be investigated for different hormone+elicitor treatments by using different bulb sizes. The ratio of alkaloids should be determined in large and small bulbs. Additionally, it would be appropriate to intensify such studies to identify superior chemotypes.

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References

- Algul BE, Tekintas FE, Gunver Dalkılıc G. 2016. The Usage of Plant Growth Regulators and Hormone Biosynthesis Booster Applications (in Turkish), Journal of Adnan Menderes University Agricultural Faculty 13(2): 87-95.
- Anonymous 2008. The United States Pharmacopeial Convention. Galantamine Hydrobromide Monograph; Revision Bulletin Official Monographs / Galantamine 1 Official December 15, 1-2.
- Anonymous, 2019. Communique about 2019 export list of Natural Flower Bulbs (Communique no:2018/49); Official Gazette dated December 12, 2018 and issue: 30623.

- Ayan AK, Kurtar ES, Cırak C, Kevseroglu K. 2004. Bulb yield and some plant characters of summer snowflake (*Leucojum aestivum* L.) under shading as affected by GA₃ and NAA at different concentrations. Journal of Agronomy 3(4): 296-300.
- Bal E. 2012. Effect of Postharvest Putrescine and Salicylic Acid Treatments on Cold Storage of Sweet Cherries (in Turkish), Journal of Suleyman Demirel University Agricultural Faculty, 7 (2): 23-31.
- Berkov S, Georgieva L, Kondakova V, Atanassov A, Viladomat F, Bastida J, Codina C. 2009. Plant sources of galantamine: phytochemical and biotechnological aspects. Biotechnol. Biotechnol. Eq. 23, 1170–1176.
- Berkov S, Georgieva L, Kondakova V, Viladomat F, Bastida J, Atanassov A, Codina C. 2013. The geographic isolation of *Leucojum aestivum* populations leads to divergation of alkaloid biosynthesis. Biochemical Systematics and Ecology; 46, 152-161.
- Cherkasov OA. 1975. Leucojum aestivum L. is prospective galantamine containing species. In Resources of Wild Medicinal Plants of USSR, VILR, Minmedprom, Moscow, No. 3, pp. 238–241 (in Russian).
- Cherkasov OA, Tolkachev ON. 2002. Narcissus and other Amaryllidaceae as sources of galantamine In: (Hanks GR.) Narcissus and Daffodil the Genus Narcissus, Chapter 8: Taylor & Francis (pp:450) 242-25, London.
- Cherkasov OA, Stikhin VA, Savchuk VM. 1984. Galanthamine content in some *Amaryllidaceae* species of the flora of the Ukrainian SSR. Rastitel'nye Resursy, 20 (4), 566-568 (in Russian).
- Codina C. 2002. Production of galantamine by Narcissus tissues in vitro. In:(Hanks GR.) Narcissus and Daffodil the Genus Narcissus, Chapter 7:; Taylor & Francis, pp 215-242 London.
- Cırak C, Ayan AK, Kurtar ES, Kevseroglu K, Camas N. 2004. The Effect of Different N doses and Harvesting Times on Bulb Yield and Some Plant Characters of Summer Snowflake (*Leucojum aestivum* L.). Asian Journal of Plant Sciences 3(2):193-195.
- Davis PH. 1982. Flora of Turkey and the East Aegean Islands Edinburgh University press. vol:8 pp:364-365.
- Erbas D, Koyuncu MA, Özüsoy F, Onursal CE. 2018. Effects of pre-harvest putrescine treatment on fruit quality of sweet cherry cv. 0900 Ziraat (in Turkish), Academic Journal of Agriculture 7(2):151-156.

- Georgieva L, Berkov S, Kondakova V, Bastida J, Viladomat F, Atanassov A, Codina C. 2007. Alkaloid variability of *Leucojum aestivum* from wild populations. Zeitschrift fur Naturforschung 62c, 627–635.
- Gizba DK, Maisuradze NI, Margvelashvili NN, Gorbunova GM, Cherkasov OA.1982. Galantamine content in *Leucojum aestivum* L., growing in Abkhazia. Khimiko-Farmazevticheskii Zhurnal, 16 (2), 195–196 (in Russian).
- Gussev CH, Bosseva Y, Pandova B, Yanev S, Stanilova M. 2007. Resource assessment of *Leucojum aestivum* L. (Amaryllidaceae) populations in Bulgaria. Bocconea 21: 405-411.
- Jovanovic S, Tomovic G, Lakusic D, Niketic M, Pavlovic M, Boza P. 2009. Genus *Leucojum* L. (Amaryllidaceae) distribution and threatened status in Serbia. Botanica Serbica 33 (1): 45-50.
- Keller, E.R.J. 1993. Sucrose, cytokinin, and ethylene influence formation of *in vitro* bulblets in onion and leek. Genetic Resources and Crop Evolution, 40, 113–120.
- Klosi R, Mersinllari M, Gavani E. 2016. Galantamine content in *Leucojum aestivum* populations in northwest Albania. Albanian Journal of Pharmaceutical Sciences vol:3, No:1. 1-3.
- Liu K, Fu H, Bei Q, Luan S. 2000. Inward potassium channel in guard cells as a target for polyamine regulation of stomatal movements. Plant Physiology, 124 (3): 1315-1326.
- Okturen F, Sonmez S. 2005. The Relationship Between Plant Nutrition Elements and Some Plant Regulators (Hormones) (in Turkish) Derum, Batı Akdeniz Agricultural Research Institute 22 (2) 20:32.
- Seabrook JEA. 1990 Narcissus (daffodil). In: P.V. Ammirato, D.R. Evans, W.R. Sharp and Y.P.S. Bajaj (eds.), Handbook of Plant Cell Culture, vol. 5, Ornamental Species, McGraw-Hill Publishing Co., New York, pp. 577–597.
- Seabrook JEA, Cumming BG, Dionne LA. 1976. The *in vitro* induction of adventitious shoot and root apices on *Narcissus* (daffodil and Narcissus) cultivar tissue. Canadian Journal of Botany, 54, 814–819.
- Stanilova M, Molle ED, Yanev SG. 2010. Galantamine production by *Leucojum aestivum* culture in Vitro (in: The Alkaloids Chemistry and Biology) Chapter 5: 167-270. Elsevier Inc.
- Takos A, Rook F. 2013. Towards a molecular understanding of the biosynthesis of Amaryllidaceae alkaloids in support of their expanding medical use. International Journal of Molecular Sciences. 14 (8): 11713-11741.