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Research Article

Effects of Applications of Different Coloured Led Lights on Emerging and Seedling Growth of *Rhododendron luteum* Sweet Seeds**

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Abstract. This study was conducted to determine the effects of LED lights in different colours (Daylight LED, Blue LED, Red LED and Blue LED + Red LED (50%+50%)) on emerging rates of *R. luteum* seeds and seedling growth). The LED lights were applied to the seeds grown on the acidic peat in foam cups superficially in the form of 16:8 hours (light:dark). The results showed that the emerging rates in seeds subjected to Daylight, Red LED, Red + Blue LED and Blue LED light were determined as 61%, 45.66%, 43%, and 35%, respectively. The effect of different light colors on plant height was significant (p<0.01). Red + Blue LED, Red LED, Blue LED and Daylight LED subjected plants' heights were determined as 10.342 cm, 10.262 cm, 75.06 cm, 7.139 cm, respectively. Diameters of seedlings were determined in Daylight, Blue LED, Red LED, Red + Blue LED subjected seedlings as 0.147 mm, 0.104 mm, 0.085 mm, 0.077 mm, respectively (p<0.01). The number of leaves were determined as 3.547, 2.000, 1.550, 1.302 in daylight, blue LED, red + blue LED and red LED subjected plants, respectively (p<0.01). To conclude, the best emerging rate and seedling growth were obtained from daylight treatment. Although the higher seedlings were obtained from Red+Blue LED and Red LED treatments, these seedlings were found as weak with thinner stem and less leaves.

Farklı Renklerdeki Led Işıkların *Rhododendron luteum* Sweet Tohumlarının Çıkışı ve Fide Gelişimi Üzerine Etkileri

Anahtar kelimeler:
Rhododendron luteum, çıkış, fide gelişimi, yapay ışık, LED

Özet. Bu çalışma, farklı renklere sahip (Gün ışığı LED, Mavi LED, Kırmızı LED ve Mavi LED + Kırmızı LED (%50 + %50)) LED ışıklarının *R. luteum* tohumlarının çıkış oranları ve fide gelişimleri üzerine etkilerini belirlemek amacıyla yapılmıştır. LED ışıkları, köpük kaplarda asidik torf üzerine yüzeysel olarak ekilen tohumlara 16:8 saat (ışık:karanlık) şeklinde uygulanmıştır. Gün ışığı, Kırmızı LED, Kırmızı + Mavi LED ve Mavi LED ışık uygulanan tohumlarda çıkış oranları sırasıyla %61, %45.66, %43 ve %35 olarak belirlenmiştir. Çalışmada bitki boyu üzerine yapılan analizlerde, farklı ışık renklerinin bitki boyları üzerine etkisinin istatistiki anlamda çok önemli (p<0.01) düzeyde olduğu belirlenmiştir. Kırmızı + Mavi LED, Kırmızı LED, Mavi LED, Gün ışığı LED'leri ile muamele edilen bitkilerin boyları sırasıyla 10.342 cm, 10.262 cm, 75.06 cm, 7.139 cm olarak bulunmuştur. Fidelerin çap ölçümlerine ait bulgularda ise, farklı ışık renklerinin fide çapları üzerine etkisinin istatistiki anlamda çok önemli (p<0.01) düzeyde olduğu belirlenmiştir. Fide çapları Gün ışığı, Mavi LED, Kırmızı LED, Kırmızı + Mavi LED uygulanan fidelerde sırasıyla 0.147 mm, 0.104 mm, 0.085 mm, 0.077 mm olarak ölçülmüştür. Diğer parametrede ise yaprak sayıları incelenmiş ve fidelerdeki yaprak sayısına, LED ışığı uygulamasının çok önemli düzeyde (p<0.01) etkisinin olduğu tespit edilmiştir. En fazla yaprağın Gün ışığı, Mavi LED, Kırmızı + Mavi LED, Kırmızı LED'lerde sırasıyla 3.547, 2, 1.550, 1.302 adet olduğu belirlenmiştir. Sonuç olarak; en iyi çıkış oranı ve fide gelişimi gün ışığı uygulamasından el elde edilmiştir. Her ne kadar en boylu fideler Kırmızı+Mavi LED ve Kırmızı LED uygulamalarından elde edilse de bu fidelerin daha ince ve daha az sayıda yaprağa sahip cılız fideler olduğu tespit edilmiştir.

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INTRODUCTION

Plants need light to grow and develop, and even seeds, also, need light to be able to germinate. In plants, except fungi, there is an absolute need for light for the formation of pigments, especially chlorophyll, enzymes and hormones, affecting the basic physiological events such as photosynthesis, respiration, assimilation and transpiration (Ağaoğlu *et al*., 2001). Light defined as a physical form of energy affects plant growth and development by its exposure time, i.e. duration of illumination, intensity and wavelength (Kevseroglu, 2004). Considering the natural light source, these characteristics depend upon the cloudiness, direction, location of plant and length of daylight. Almost all plants give various biological responses to illumunation period. Photoperiodism is called this reaction of plants against lighting period or the length of day light (Koç *et al*., 2009). The stage in which plants grow and develop is called as photoperiod. Photoperiod affects many physiological events such as growth, development, flowering, fruit set, coloration of fruits, yellowing and shedding of leaves, and entering to dormancy period of plants (Anonymous, 2019).

The most important impact of light on plants is on photosynthesis. Like all living things, plants need physical energy in order to survive. This energy is converted into organic compounds so that they are going to be the source of chemical energy to serve the survival of plants. Photo-synthetic pigments found in chloroplasts of plants are the basic organs that convert light energy to chemical energy (Kaçar *et al*., 2010).

In greenhouse agriculture, artificial lighting is needed the seasons when the natural light source is limited or insufficient. For this reason, the new lighting technologies have been developed to be used in plant production. Initially, different types of electric lamps were used as artificial light sources. For this purpose, incandescent lamps, metal halogen lamps, fluorescent lamps, high pressure sodium vapor discharge lamps are the most commonly used lamps as artificial light sources in plant production. In parallel with the developing technology, LED lamps can be given as an example in recent years (Çakırer *et al*., 2017). However, some of these artificial light sources, for example, incandescent lamps have disadvantages such as excessive energy consumption, overheating, especially in small spaces. Considering such disadvantages, Light Emitting Diodes (LEDs) will take their place in the agricultural sector by the advantages of low energy consumption, providing light at desired wavelength, easy installation and usage.

R. luteum species are called as Zifin, Sarı Ağu and Yel in Turkey. *R. luteum* can grow up to 3–4 m, mostly 1–1.5 m in height, with densely branched, golden yellow flowers and very sharp scented flowers. Flowering period starts from the end of April to the beginning of June, depending on the altitude. The plant, which has a reddish color before the leaves fall in autumn, is an important ornamental plant that can be used in landscape planning. The leaves, nectar and pollen of *R. luteum* are poisonous due to its andromedotoxin content. In Turkey, *R. luteum* distributes naturally from the provinces of Çanakkale, Balıkesir, Kastamonu, Sinop, Amasya, Samsun, Ordu, Giresun, Trabzon, Rize and Artvin at sea level (Sinop) to Genya Mountain peak at 2000 m in Artvin. The most intensive population of this species was found in the highlands of Ordu (Stevens, 1978; Küçük, 2005; Altun *et al*., 2016). There has been a lack of study on the effects of different light colors on *R. luteum* with respect to seed germination, emerging rate and seedling growth. Therefore, the present study, was conducted to determine the effects of different coloured led lights on germination, emergence and seedling development of *R. luteum* Sweet seeds which are the only species that deciduous in winter in Turkey's natural *Rhododendron* species.

MATERIAL AND METHOD

Seed material was collected from the plants *R. luteum* Sweet at 1671 m altitude and 41° 10’424’’ N, 42° 18’943’’E geographical coordinates in Şavşat District of Artvin province, where its natural spreading location, under the concept of TUBİTAK Project called “Determining the genotype, morphological and molecular identification, duplication and ex situ conservation studies in Turkish *Rhododendron* (*Rhododendron* spp.)”. In this study, four climate cabinets (75x65x60 cm) were used for plant germination and growth purposes in the Horticulture laboratory of Agriculture Faculty of Kırşehir Ahi Evran University. A fan was used for cooling inside the cabinets. The fan in each cabinet was connected to a copper electrode thermostat and the fan operates when the temperature inside the cabinet rises above a certain degree; was automatically closed. In addition, acidic peat was used as seed germination and growth medium. Capsules bearing *Rhododendron* seeds were in the septicidal capsule group as fruit structure (Yıldız and Aktoklu, 2010; Bozcuk, 2013). These seed capsules ripen and turn brown and, then, the seeds spread out. Seed collection was done in the form of cutting off brown capsules containing seeds from the plants with the help of pruning shears. The capsules were placed on dry paper bags and moved to the trial area in this way (Altun, 2011). Seed capsules brought to the trial area were placed in large flowerpots under room conditions, and manual mixing was performed from time to time until these capsules were completely opened. When it was determined that the seed capsules were fully opened by regular controls, the capsules were shaken gently by inverting them into the container and the seeds were separated from the capsules and poured into the container. In order to remove impurities from the spilled seeds, the seeds were sieved with a sieve having a pore of 0.850 mm to be ready for their plantation. These seeds were planted on July 14, 2017 in 30x50 cm foam crates filled with sterile acidic peat with pH between 3.5-4.5. The seeds, which were counted into small papers, were made by hand in the form of superficially poured seeds. Trial was conducted with four replicates in each cabin and 75 seeds in each replicate. For lighting treatments, IP65 - 4.8W M^{-1}, silicon, three-chip 60 LED/m LED strip operating at -5 to +35 °C (AL-SMD-2835 A6, LEDON Advanced Technology, China) was used to produce different colors of light. The cabinets were grouped into four according to lighting treatments which were daylight, blue, red, and blue & red led lamps. In the last cabin, one row was illuminated with blue and another one illuminated with red lamps. Four strips of 60 cm length were cut, and each strip glued 15 cm wide to the ceiling of cabinet. The ends of the strips in each cabinet were soldered in series with cables. It was fixed to the ceiling using transparent silicone to prevent loss of adhesion. One end of the strips was connected to the adapters. The adapters were plugged into a time-adjustable outlet and 16/8 hours of light/dark application. Temperature and humidity values were recorded at the beginning of every hour by means of a data logger (MIC METTER, Air Quality Guardian) placed in cabinets. The calculated daily temperature and humidity values are given in Figure 1. After seed sowing (July 14, 2017), seedlings were counted every 7 days, and the number of seedlings departing was determined. At the end of the 5th week (August 18, 2017), when seedling numbers were fixed, the counting process was terminated. On the other saying, counting process was continued until the number of germinated seeds and seedlings fixing. Irrigation was done by hand sprayer on the sowed seeds. Emergence tests were counted regularly weekly by using a magnifying glass. The obtained germination and emergence values were recorded for each counting process. Seedling diameter measurements were conducted with a digital caliper from the midpoint of the stem between the leaves and the root collar. Seedling height measurements were determined by measuring the length between the root collar and the top of the top leaf with the help of a ruler.

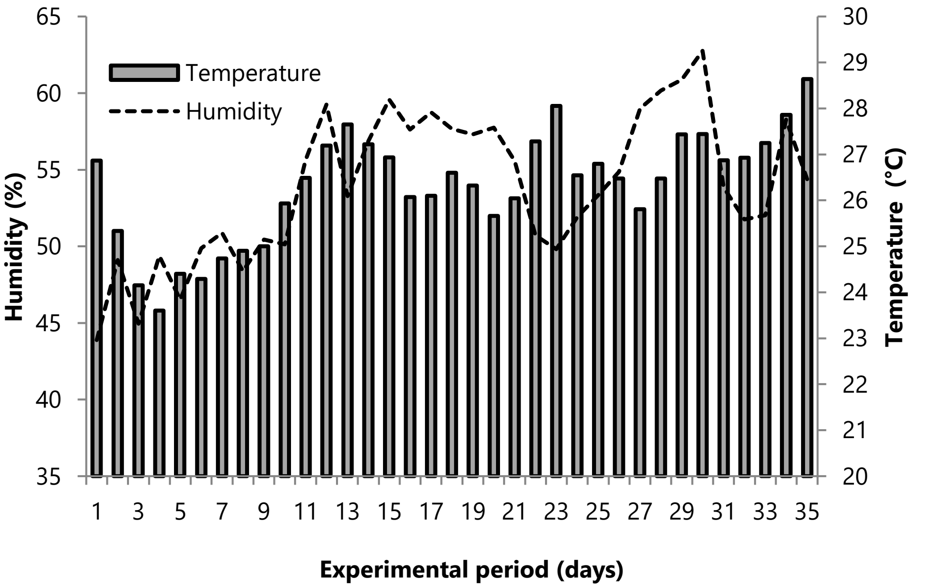

Figure 1. The daily mean climate condition of experimental cabinets.
Şekil 1. Büyüme kabinlerinin günlük ortalama sıcaklık (°C) ve nem değerleri (%).

The data was subjected to GLM procedure of SPSS statistical software (Windows version of SPSS, publication 16.00). The percentage data were subjected ArcSin transformation before statistical analysis. The means were compared by using Duncan Multiple Comparison Test in the same software.

RESULTS AND DISCUSSION

In the sown seeds, it was observed that emergences started one week after sowing. In the first count of seeds, the emerging rates were calculated as 16.33% for daylight LED application, 3.33% for blue LED light, 11.00% for red LED light and 1.66% for red+blue LED light. When emerging rates were examined on weekly basis, it was determined that daylight LED application resulted the highest emerging rate (61%) at the end of the five weeks, after that, the decreases in this rate were observed as the weeks progressed. In other light applications, it was found that seed emergences were quite high in the second week of the counting compared to the first week, but these emergences were decreased in the following weeks and fixed at the end of the 5th week. At the end of the fifth week, the highest emerging rate (61%), was observed in the cabinet where daylight was applied while the lowest emerging rate (35%) was observed in the cabin where blue light was applied. The emerging rates were as 45.66% and 43% for red LED light and Red+Blue LED treatments, respectively (Figure 2).

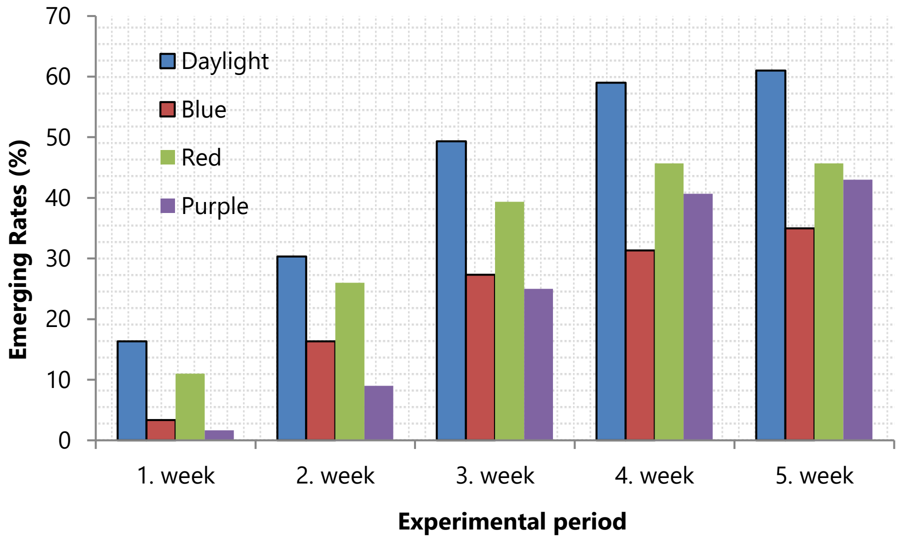

Figure 2. The effects of lighting application on emerging rates (%).
Şekil 2. Işık uygulamalarının çıkış oranları üzerine etkileri (%).

The highest plants (10.342 cm) were obtained from the combination of Red+Blue light, followed by 10.262 cm plant height in the cabinet where red light was applied alone. The lower plant heights were obtained from Blue light and daylight applications. These values were obtained as 7.506 cm and 7.139 cm in Blue light and daylight subjected cabinets, respectively, without any statistical difference (Table 1).

The thickest seedling diameter of 0.147 mm was obtained from the cabinet where the daylight was applied. The thinnest seedling diameter was obtained from the plants exposed to Red+Blue LED light (0.077 mm), followed by Red LED light (0.085 mm) treatment without any statistical significance (Table 1).

It was found out that the number of leaves in seedlings was affected by light treatments ($p<0.01$). The maximum number of leaves (3.547 leaves per seedling) was obtained in daylight application. The minimum number of leaves (1.302 leaves per seedling) was obtained from the application of Red LED light (Table 1).

Table 1. The effects of lighting application on plant growth.
Çizelge 1. Işık uygulamalarının bitki gelişimi üzerine etkileri.

Parameters	Daylight	Blue	Red	Blue+Red	P values
Length (cm)	7.139 b	7.506 b	10.262 a	0.400	0.000
Diameter (mm)	0.147 a	0.104 b	0.085 c	0.007	0.000
Leaf number (count)	3.547 a	2.000 b	1.302 d	0.227	0.000

a,b: the different letters in the same row show the statistical difference between means ($p\leq0.01$, ($p\leq0.05$)

R. luteum Sweet species is one of the members of the natural flora of Turkey. There has been not any comprehensive work on the effects of light regimen on the physiological responses on the seeds of *R. luteum* Sweet. That was why we illuminated plant growth cabinets with different colored LEDs (Daylight, Red, Blue and Blue+Red (50% 50%)) and observed the emergence and seedlings with respect to emerging rate and growth parameters.

At the fifth week of the study, the number of seedlings in the cabinets was fixed. The maximum emerging rate was 61% in cabinet subjected to daylight LED application. The emerging rate was 45.66% in the cabinet with red LED and 43% with Red+Blue LED lighting. The lowest emerging rate (35%) was observed in the cabinet illuminated with blue LED lamps. Sakharova (1993) obtained 71% germination rate in *R. luteum* seeds. Altun and Çelik (2016) conducted a germination study in *R. luteum* species. They obtained 76% germination rate on 21-d after sowing months in unheated greenhouse conditions in winter. Also, Altun *et al.* (2016) determined the highest emerging rate 67.5% with 24 h lighting period in *R. luteum* seeds. Caprar *et al*. (2013) germinated *R. luteum* seeds under greenhouse conditions 21-24 °C and found out 38%-48% emerging rates. Sakharova (1993) reported in his study that the success of germination in *Rhododendron* seeds, depended on year (weather), harvesting time, germination temperature and pH value of germination environment. Our lowest emerging rates might be attributed to the ambient temperature in cabinet as reported by Sakharova (1993). Evidently, the ambient temperature inside the cabinet was 26.38 °C with 54.25% humidity as seen in Figure 1. Shen *et al*. (2015) did a germination study on *Rhododendron protistum* var. *giganteum* species. They reported that the optimum temperature for seed germination was 15 °C and 20 °C for 45-d germination period. They determined the highest germination rate as 77% at 25 °C. They also found out that the higher temperatures affected germination negatively. Zhao (2014) obtained the highest germination rate in *R. purdomii* seeds at 20 °C and lowest rate at 30 °C heat stress. Therefore, in the current study, it can be postulated that the ambient temperature in cabinets had a negative effect on the emerging power of seeds. Even though higher ambient temperature conditions, daylight led application gave the best results, suggesting that lighting will increase the heat tolerance of seeds for germination in *R. luteum* seeds.

Current results showed that different colored led lamps affected plant height statistically ($p<0.01$). The highest seedling (10.342 cm) was obtained from the combination of Red + Blue (50%+50%) light.

When red light was applied alone, a 10.262 cm plant height was obtained as same as that of combination Red+Blue lights. The lower plant heights were obtained in blue and daylight led lights applied seeds. Akbarian *et al*. (2016) reported that the plant species reacted differently according to the light source with respect to growth characteristics. Simlat *et al*. (2016) reported that red LED light in *Stevia* plants significantly increased stem height and root length. Enache and Livadariu (2016) studied on *Artemisia dracunculus* L. seeds and found out that the higher germination and longer hypocotyl height with Red LED application compared to other applications. Liu *et al*. (2013) reported that Red light in vitro conditions gave the best results on seedling growth, leaf regeneration, stem length, leaf length, leaf width and rooting rate in *Rhododendron*. Although the species we work here were different than those of other studies, even at the genus level, our findings on seedling height was in line with the researchers' findings.

The different coloured leds affected seedling stem diameter significantly ($p<0.01$). The thickest seedling 0.147 mm was obtained from the cabinet where daylight was applied on. The thinnest seedling stem 0.077 mm was obtained from seeds subjected to Red+Blue LED light. This was statistically the same as that of Red LED light (0.085 mm) (Table 1). Akbarian *et al*. (2016) reported that red light increased stem diameter in Impatiens while blue light increased this parameter in *Petunia*. Li *et al*. (2017) studied the effects of two different LED light sources on growth of the *Arabidopsis* plant compared to white fluorescent lamp as a control. They found that red (465 nm) & blue (665 nm) combination gave better results in terms of flowering time, plant height, stem diameter, number of branches, number of capsules, fresh, and dry matter content of biomass and seed yield. The current results are not agreeing with their findings, assuming that the species difference between studies may have caused this disagreement. Evidently, Akbarian *et al*. (2016) reported that stem diameters of *Impatiens* and *Petunia* were affected differently from different colored lightings. Liu *et al*. (2013) reported that red lamps resulted thinner stem and lower rooting. Our findings are in line with their findings even though the studied species were different than ours.

Our results showed that lighting colors had a significant effect on the number of leaves in seedlings ($p<0.01$). The maximum number of leaves (3.547 leaves per seedling) was obtained by daylight led treatment while the minimum number of leaves (1.302 leaves per seedling) was obtained by Red LED lighting (Table 1). This shows that how is important daylight led lighting on vegetative growth of plants as sees in the number of leaves. This will give agriculturist or forest engineer opportunity to propagate or duplicate plants more efficiently. For nutritional point of view, the increase in the number of leaves means that more nutrients are produced in these photosynthetic areas of plants. Akbarian *et al*. (2016) reported that blue light significantly increased the number of leaves in Impatiens and *Zinnia* species. Li *et al*. (2017) used a combination of red (465 nm), blue (665 nm) and red Headlight LED (733 nm) as the light source and found out that fresh and dry weight of vegetative parts of plants were higher than that of control illuminated with white fluorescent lamp. Wongnok *et al*. (2008) conducted an in vitro study on germination of *Phalaenopsis* hybrid (cv. Cassandra Rose) seeds. They found out that the plants exposed to LED lights produced heavier biomass with higher stem and longer leaf than those of fluorescent lamps 4 months after the sowing date. An in vitro study was conducted by Liu *et al*. (2013) to investigate the effects of LED light sources with different colour on seedling growth and leaf regeneration in *Rhododendron*. Consequently, they reported that the number of shoots, and leaf numbers per shoot were increased significantly by using the combination of red+blue light (3:1) compared to fluorescent lamp, control. In the same study, it was, also, found out that these parameters examined by using red or blue light alone gave lower results than those of control. These findings are not agreeing with our results, assuming that seeds may have reacted differently in situ experimental conditions compared to in vitro.

CONCLUSION

When the emerging rate and seedling growth parameters were taken into consideration, the highest rate of emergence and the best seedling development occurred in daylight led application. Although the higher seedlings occurred in Red + Blue LED and Red LED applications, the stem diameter and number of leaves were found lower in those seedlings. To conclude, it can be suggested that daylight LED can be used as an artificial light source for generative propagation of *R. luteum* Sweet species. In further studies, different color combinations with daylight leds may be tested on the studied parameters in *R. luteum* Sweet species and other ornamental plants.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

AUTHORS' CONTRIBUTIONS

Murat Ünsal conduced this MSc study under supervision of Bahadır ALTUN. The supervisor wrote this article.

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