



Effect of GA₃ and Environmental Factors on Seed Germination Rates of *Hypericum perforatum* L.

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ABSTRACT

This study aimed to evaluate the effects of gibberellic acid (GA₃) on the germination of *Hypericum perforatum* (St. John's Wort) seeds. The first trial tested three GA₃ concentrations (200, 300, 400 mg·L⁻¹) and three incubation durations (24, 72, 120 hours) under dark conditions. The second trial focused on the interaction of GA₃, temperature (15, 25, 35°C), and photoperiod conditions. As a result, GA₃ significantly improves germination rates, with concentrations of 200–300 mg·L⁻¹ and treatment durations of 72–120 hours being the most effective. Temperature and light conditions were also found to be critical, with the optimal temperature for germination being 15°C. At higher temperatures, particularly 35°C, germination was either minimal or absent. Light exposure under a 16/8-hour photoperiod led to significantly better germination compared to continuous darkness. The highest germination rates (up to 96.75%) were observed under a combination of 200 mg·L⁻¹ GA₃ and light exposure at 15°C. This study highlights the synergistic effect of GA₃ and light, which together enhanced germination success.

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Introduction

The Hypericaceae family comprises approximately 500 species worldwide, with 108 species recorded in Turkey. Among these, *Hypericum perforatum* L., commonly referred to as St. John's Wort, is the most well-known. This species is native to Europe, Asia, and North Africa but has become widely distributed globally (Campbell, 1985). In Turkey, it is recognized by various vernacular names, including yara otu, kan otu, kılıç otu, kuzukıran, and mayasıl otu. Internationally, it is known as "St. John's Wort" (Özbek et al., 2019; Ersoy & Özkan, 2020).

H. perforatum has long been used in traditional medicine systems, including Chinese, Greek, and Islamic medicine, for its therapeutic properties (Nobakht et al., 2022). It is widely recognized for its antidepressant, wound-healing, and antimicrobial effects. Studies indicate that hydroalcoholic extracts of *H. perforatum* may be as effective as conventional antidepressants for mild-to-moderate depression, with fewer side effects (Mennini & Gobbi, 2004). Hyperforin, a key bioactive compound, inhibits neurotransmitter uptake and interacts with dopamine D1 and opioid receptors, contributing to its antidepressant activity (Mennini & Gobbi, 2004). Additionally, the plant demonstrates potent antimicrobial properties, particularly against Gram-positive bacteria (Saddiqe et al., 2010). Its role in wound healing is

supported by research highlighting its promotion of re-epithelialization, angiogenesis, and collagen deposition while reducing inflammation and oxidative stress (Farasati Far et al., 2024). *H. perforatum* is available as dried herbs, tablets, topical oils, creams, and ointments (Demirezer et al., 2007; Ernst, 2007).

In Turkey, *H. perforatum* naturally grows across various regions, from sea level to altitudes of 2000 meters. It is a perennial herbaceous plant that flowers between May and September, producing bright yellow blossoms and reaching a height of 30–80 cm. In addition to its flowers, the leaves and shoots are also utilized for medicinal purposes. Due to increasing demand from the pharmaceutical industry, *H. perforatum* has been cultivated commercially in Europe, America, Australia, and China. Although cultivation efforts in Turkey have only recently commenced, small-scale production has been observed. The cultivated area expanded from 6.5 decares in 2012 to 534 decares across 25 enterprises by 2019. Nevertheless, the majority of *H. perforatum* utilized in pharmaceutical production is still collected from the wild. Sustainable cultivation strategies are, therefore, critical to ensuring a stable supply of high-quality plant material for pharmaceutical applications.

Economically, *H. perforatum* is primarily propagated by seeds, although stem cuttings can also be used. The seeds are extremely small, with a 1000-seed weight of approximately 0.125 g. Due to their minute size, direct seeding is generally not preferred in cultivation, necessitating seedling production before field transplantation. One of the critical stages in the seedling production of *H. perforatum* is seed germination, which is significantly influenced by dormancy. The literature indicates that various treatments, including potassium nitrate application, temperature regulation, light exposure, and chemical treatments, can enhance germination rates (Carvalho and Nakagawa, 1988). Dormancy is a major factor limiting the germination success of *H. perforatum* seeds. Germination studies have shown that specific environmental conditions are required to achieve optimal results. For instance, Campbell (1985) reported that lower nighttime temperatures coupled with higher daytime temperatures increased germination rates in freshly harvested *H. perforatum* seeds from 44% to 70%. Additionally, light was found to be more effective than darkness in promoting germination. Similarly, Faron et al. (2004) demonstrated that a 16-hour photoperiod and a temperature of 20°C positively influenced germination. However, contrary to previous findings, they reported that potassium nitrate had no significant effect on germination. Pérez-García et al. (2006) further established that a 16/8-hour light/dark photoperiod significantly enhanced germination compared to continuous darkness, and the application of 250 ppm gibberellic acid (GA₃) further improved germination across different accessions of *H. perforatum*.

Understanding the germination characteristics of *H. perforatum* is essential for optimizing its cultivation. Various pre-sowing treatments have been explored to enhance germination success. Stratification, which involves exposing seeds to cold temperatures for a specific duration, has been reported to alleviate dormancy and improve germination efficiency (Nunes et al., 2019). Similarly, the application of GA₃ has been found to stimulate germination by breaking physiological dormancy, making it a valuable technique for commercial propagation. Temperature and light are also crucial factors influencing germination rates. Studies suggest that moderate temperatures ranging between 15°C and 25°C are optimal for *H. perforatum* seed germination (Pérez-García et al., 2006). Moreover, light exposure has been found to enhance germination, with seeds demonstrating higher germination rates under photoperiod conditions compared to complete darkness (Nunes et al., 2019).

This study investigates the impact of ecological factors and pre-treatments on enhancing the germination performance of *H. perforatum* seeds collected from the Tokat region. Various factors, including temperature, light exposure, and GA₃ applications, were evaluated to overcome dormancy and improve germination rates.

Materials and Methods

Plant Material and Trial Conditions

This study was conducted in 2020 at the Tissue Culture Laboratory of the Department of Horticulture, Faculty of Agriculture, Tokat Gaziosmanpaşa University, Turkey.

The plant material used consisted of *H. perforatum* (St. John's Wort) seeds, which were collected from the natural flora of the rural area of Ataköy, Almus District, Tokat Province (49°26' N, 36°54' E) at an altitude of 1165 meters. The seeds were stored in moisture-proof packages in dark conditions at 20°C for three months before the experiment. The 1000-seed weight was determined to be 0.111 grams.

Sterile 90 mm Petri dishes were used for seed germination. A 2 mm-thick layer of cotton was placed at the base of each dish, ensuring sufficient moisture saturation. Filter paper was then laid over the cotton layer, and seeds were evenly distributed on the filter paper. To maintain adequate moisture levels, distilled water was periodically added to the Petri dishes whenever water levels declined. The germination studies and treatments were conducted in a controlled plant growth chamber with a volume of 316 liters, equipped with a temperature control system (ranging from -10°C to +60°C), a humidity control system (20%–90%), and a three-sided illumination system with daylight and Gro-lux fluorescent lamps to provide an optimal spectrum for seed germination.

Germination Treatments

The study was conducted in two different trials. In the first trial, three concentrations of GA₃ (200, 300, and 400 mg·L⁻¹) were applied along with three incubation durations (24, 72, and 120 hours) at 15°C under dark conditions. Seeds were placed into 100 mL beakers, and 50 mL of the GA₃ solution was added to each beaker. The beakers were subjected to continuous shaking for six hours using a shaker. Following this treatment, the seeds were removed from the solution and transferred to Petri dishes. The seed bed was wetted with the GA₃ solutions. For the control group, seeds underwent the same shaking procedure, but with distilled water instead of the GA₃ solution. Afterward, the control seeds were placed in Petri dishes, and distilled water was used to maintain moisture. Following the incubation periods, all Petri dishes were transferred to a growth chamber set to a day/night temperature regime of 20/25 ± 2°C, with a photoperiod of 16 hours of light and 8 hours of darkness, and a relative humidity of 60 ± 5%. The light intensity was maintained at 10000 lux. Germination was observed and recorded on the 6th, 8th, 11th, and 14th days.

The second trial was designed based on the germination results obtained from the first trial in order to further investigate the effects of various treatments on seed germination. In this study, seeds were subjected to the same GA₃ treatments, with concentrations of 200, 300, and 400 mg·L⁻¹ (seeds were treated with GA₃ in the same manner as in the first experiment), and were incubated under two distinct photoperiod conditions. These conditions included continuous darkness for 24 hours and a 16/8-hour light/dark cycle, with the light intensity set at 10000 lux. Furthermore, the seeds were exposed to three different temperature regimes (15, 25, and 35 °C) over a period of 14 days. To monitor the progression of germination, observations were conducted on the 5th, 7th, 10th, and 14th days of the experiment. The germination status of the seeds was recorded daily at 16:00 hours, without removing the seeds from the growth chamber. This approach allowed for consistent and non-invasive monitoring of seed development throughout the duration of

the trial. In both experiments, germination was defined as the emergence of cotyledon leaves. Since no seedling mortality was observed after germination, these values were also considered indicative of the number of viable plantlets.

Trial Design and Statistical Analysis

The experiments were conducted using a split-plot design with eight replications, each consisting of 20 seeds. In the first experiment, gibberellic acid (GA₃) doses were assigned to the main plots, while application periods were assigned to the subplots. In the second experiment, dark-light treatments were assigned to the main plots, GA₃ doses to the subplots, and temperature treatments to the sub-subplots. Germination rates were calculated by determining the ratio of germinated seeds to the total number of seeds, and results were expressed as percentages. Data were analyzed using one-way analysis of variance (ANOVA) in IBM SPSS Statistics 20. Multiple comparisons of means were performed using Duncan's multiple range test, with a significance level set at P = 0.05.

Results

Effect of GA₃ and Low-Temperature Treatments on Germination Rates

The data presented in the Table 1 indicate that GA₃ treatment significantly enhances germination, with the 200 and 300 mg·L⁻¹ concentrations showing the highest effectiveness, particularly after 11 and 14 days. On the 11th

day, the highest germination percentage (67.20%) was recorded with 300 mg·L⁻¹ GA₃ and a 120-hour pretreatment, followed by 200 mg·L⁻¹ GA₃ with a 120-hour pretreatment (65.20%) and 72-hour pretreatment (64.80%). The control treatments exhibited lower germination, with a maximum of 54.00% after 24 hours of pretreatment. On the 14th day, the highest germination (88.60%) was observed with 300 mg·L⁻¹ GA₃ and a 72-hour pretreatment, followed by 200 mg·L⁻¹ GA₃ with a 120-hour pretreatment (88.00%). The control group showed significantly lower germination (69.20%). Regarding the impact of pretreatment duration (duration of exposure at 15 °C), longer times (72h and 120h) generally led to better germination outcomes. Notably, germination rates increased over time across all treatments, with the 120h pretreatment resulting in more consistent high germination rates. The control treatment, which did not receive any GA₃, showed the lowest germination rates across all observed days, highlighting the significant role of GA₃ in promoting seed germination. The statistical analysis confirmed that both GA₃ concentration and the duration of pretreatment had a significant effect on germination rates. The GA₃ concentration significantly affected germination rates (p < 0.001). The time factor had a significant impact on germination only on the 6th and 8th days (p < 0.001), while the effect became stable by the 11th and 14th days. The interaction between GA₃ concentration and time was also significant (p < 0.001), indicating that GA₃'s effect varied over time.

Table 1. Effect of GA₃ pretreatments on germination rates of *H. perforatum* seeds according to observed days

GA ₃	Pretreatment	Observation Days			
		6th day	8th day	11th day	14th day
Control	24h	9,20±1,10 ^b	34,40±3,29 ^d	54,00±3,54 ^c	69,20±4,55 ^g
	72h	3,00±1,22 ^c	34,40±3,36 ^d	47,60±2,07 ^d	60,00±4,30 ^h
	120h	1,80±0,84 ^{cd}	26,00±1,58 ^e	49,40±2,97 ^d	65,80±3,96 ^g
200 mg·l ⁻¹	24h	10,00±1,12 ^b	47,00±4,70 ^a	57,00±2,12 ^c	77,60±3,13 ^{def}
	72h	18,80±2,17 ^a	41,40±3,05 ^b	64,80±4,15 ^{ab}	75,00±3,46 ^f
	120h	0,00±0,00 ^d	38,20±2,77 ^{bcd}	65,20±2,39 ^{ab}	88,00±3,08 ^a
300 mg·l ⁻¹	24h	10,00±1,00 ^b	38,00±2,00 ^{bcd}	57,00±2,74 ^c	81,20±4,79 ^{cde}
	72h	10,00±1,22 ^b	35,80±2,59 ^{cd}	62,20±2,17 ^b	88,60±3,58 ^a
	120h	0,00±0,00 ^d	30,00±1,87 ^e	67,20±2,77 ^a	86,20±3,83 ^{ab}
400 mg·l ⁻¹	24h	6,40±1,78 ^{bc}	36,00±3,94 ^{cd}	64,00±3,32 ^{ab}	84,60±3,78 ^{abc}
	72h	9,40±1,02 ^b	39,00±2,00 ^{bc}	57,00±2,00 ^c	82,20±4,64 ^{bcd}
	120h	1,40±0,55 ^{cd}	26,00±1,58 ^e	55,60±2,30 ^c	76,60±2,97 ^{ef}
GA ₃					
Control		4,67 ^b	31,60 ^c	50,33 ^c	65,00 ^c
200 mg·l ⁻¹		9,60 ^a	42,20 ^b	62,33 ^a	80,20 ^b
300 mg·l ⁻¹		6,67 ^{ab}	34,60 ^b	62,13 ^a	85,33 ^a
400 mg·l ⁻¹		5,73 ^{ab}	33,67 ^{bc}	58,87 ^b	81,13 ^b
Pretreatments					
24h		8,90 ^b	38,85 ^a	58,000	78,15
72h		10,30 ^a	37,65 ^a	57,900	76,45
120h		0,80 ^c	30,05 ^b	59,350	79,15
Significance					
GA ₃		***	***	***	***
Pretreatment		***	***	n.s.	n.s.
GA ₃ *Pretreatment		***	*	***	***

Table 2. Effect of photoperiod, GA₃ and growth temperatures on germination rates of *H. perforatum* seeds according to observed days

Photoperiod	GA ₃	Temp.	Observation Days			
			5th day	7th day	10th day	14th day
Darkness	Control	15	0,00±0,00 ^d	0,00±0,00 ^f	0,00±0,00 ^g	0,00±0,00 ^g
		25	0,00±0,00 ^d	0,00±0,00 ^f	4,50±1,08 ^{fg}	8,50±2,52 ^{efg}
		35	0,00±0,00 ^d	0,00±0,00 ^f	0,00±0,00 ^g	0,00±0,00 ^g
	200 mg·L ⁻¹	15	0,00±0,00 ^d	0,00±0,00 ^f	2,50±0,58 ^g	9,50±2,11 ^{efg}
		25	5,25±1,06 ^{bc}	5,50±1,52 ^c	16,25±2,36 ^{de}	17,25±2,50 ^e
		35	0,00±0,00 ^d	0,00±0,00 ^f	0,00±0,00 ^g	0,00±0,00 ^g
	300 mg·L ⁻¹	15	0,00±0,00 ^d	0,00±0,00 ^f	7,00±0,81 ^{fg}	9,50±1,29 ^{efg}
		25	6,75±1,36 ^a	8,00±2,08 ^b	14,25±2,71 ^{de}	16,75±2,19 ^{ef}
		35	0,00±0,00 ^d	0,00±0,00 ^f	3,00±0,82 ^{fg}	4,50±0,99 ^{fg}
	400 mg·L ⁻¹	15	0,00±0,00 ^d	0,00±0,00 ^f	10,50±1,00 ^{ef}	16,00±1,82 ^{ef}
		25	4,50±1,00 ^c	6,25±1,89 ^{bc}	14,75±2,50 ^{de}	18,50±2,19 ^e
		35	0,00±0,00 ^d	0,00±0,00 ^f	0,00±0,00 ^g	0,00±0,00 ^g
Light	Control	15	0,00±0,00 ^d	0,00±0,00 ^f	41,50±4,11 ^c	60,50±5,38 ^c
		25	4,50±1,00 ^c	4,50±1,00 ^{cd}	21,25±2,50 ^d	45,50±3,91 ^d
		35	0,00±0,00 ^d	0,00±0,00 ^f	0,00±0,00 ^g	0,00±0,00 ^g
	200 mg·L ⁻¹	15	0,00±0,00 ^d	0,00±0,00 ^f	57,75±5,63 ^a	96,75±8,78 ^a
		25	6,25±1,50 ^{ab}	17,25±2,98 ^a	52,25±5,32 ^{ab}	79,00±6,87 ^b
		35	0,00±0,00 ^d	3,25±0,65 ^{de}	4,00±1,09 ^{fg}	4,75±1,50 ^{fg}
	300 mg·L ⁻¹	15	0,00±0,00 ^d	0,00±0,00 ^f	59,00±5,08 ^a	95,75±8,69 ^a
		25	4,00±1,15 ^c	18,50±2,89 ^a	56,25±6,85 ^a	75,25±7,54 ^b
		35	0,00±0,00 ^d	2,50±0,48 ^e	4,00±0,98 ^{fg}	4,50±1,00 ^{fg}
	400 mg·L ⁻¹	15	0,00±0,00 ^d	0,00±0,00 ^f	42,75±4,26 ^c	93,25±7,50 ^a
		25	0,00±0,00 ^d	17,25±2,50 ^a	48,25±4,50 ^{bc}	71,25±6,50 ^{bc}
		35	0,00±0,00 ^d	2,75±0,76 ^{de}	4,50±1,00 ^{fg}	4,50±1,00 ^{fg}
Photoperiod						
24h dark			1,38	1,65 ^b	6,06 ^b	8,37 ^b
16/8h day-night			1,23	5,50 ^a	32,62 ^a	52,58 ^a
GA ₃ treatments						
Control			0,75 ^b	0,75 ^b	11,21 ^d	19,08 ^b
200			1,92 ^a	4,33 ^a	22,12 ^b	34,54 ^a
300			1,79 ^a	4,83 ^a	23,92 ^a	34,38 ^a
400			0,75 ^b	4,38 ^a	20,13 ^c	33,92 ^a
Temp. Treatments						
15°C			0,00 ^b	0,00 ^c	27,63 ^a	47,66 ^a
25°C			3,91 ^a	9,66 ^a	28,47 ^a	41,50 ^b
35°C			0,00 ^b	1,06 ^b	1,94 ^b	2,28 ^c
Significance						
GA ₃			***	***	***	***
Temp.			***	***	***	***
Light			n.s.	***	***	***
GA ₃ *Temp.			***	***	***	***
GA ₃ *Light			***	***	***	***
Temp.*Light			n.s.	***	***	***
GA ₃ *Temp.*Light			***	**	***	***

Effect of Photoperiod, GA₃ and Growth Temperatures on Germination Rates

The results presented in Table 2 highlight the significant effects of photoperiod, GA₃ concentration, and temperature on the germination rates of *H. perforatum* over a 14-day observation period, with particular emphasis on the 10th and 14th days. Germination was notably higher under light conditions (16/8h day-night) than in complete darkness, particularly by the 10th and 14th days. For instance, on the 14th day, the highest germination (96.75%) was achieved under 200 mg·L⁻¹ GA₃ at 15°C in light, compared to much lower rates in the dark. Temperature was a crucial factor influencing germination

rates. At 35°C, germination was either nonexistent or minimal across all conditions, indicating that high temperatures are detrimental to *H. perforatum* seed germination. At 15°C, however, germination rates were optimal, especially when combined with light exposure. On the 10th day, the highest germination under light condition occurred with 200 mg·L⁻¹ GA₃ at 15°C, reaching 57.75%, and was even higher on the 14th day. Similar trends were observed for 300 mg·L⁻¹ GA₃, with 59.00% germination on the 10th day and 95.75% on the 14th day under light condition at 15°C. The control group showed minimal to no germination, especially at 35°C and in darkness, further confirming the necessity of GA₃

treatment, appropriate temperature, and light for maximizing seed germination. The interactions between GA₃, temperature, and light were highly significant ($p < 0.001$), particularly for the 10th and 14th days, underscoring the combined importance of these factors in optimizing germination of *H. perforatum*. The results indicate that 200 mg·L⁻¹ GA₃ at 15°C under light provides the best conditions for germination.

The interaction between GA₃ concentration and growth temperature had a significant effect on seed germination. At 15°C, higher GA₃ concentrations (200 mg·L⁻¹ and 300 mg·L⁻¹) resulted in the highest germination rates, particularly on the 14th day. At 25°C, germination was slightly reduced but still substantial. However, at 35°C, germination was minimal across all GA₃ treatments, indicating that higher temperatures inhibited seed germination. This interaction highlights that optimal germination conditions are achieved with lower temperatures combined with appropriate GA₃ concentrations, while high temperature (35°C) negatively affect germination (Figure 1).

Effects of Interactions on Seed Germination

The interaction between GA₃ concentration and photoperiod had a significant impact on seed germination. Under dark conditions, germination was generally low across all GA₃ concentrations. However, under light

conditions, the germination rates were much higher, especially with higher GA₃ concentrations. The data clearly demonstrate that light enhances the effect of GA₃ on seed germination. As the GA₃ concentration increased, seed germination improved under light, showing a positive interaction between the two factors. In contrast, under dark conditions, the effect of GA₃ was limited, with germination remaining low regardless of the concentration. This suggests that light plays a crucial role in activating the germination-promoting effects of GA₃. Therefore, for optimal seed germination, both the presence of light and an appropriate GA₃ concentration are essential, with light significantly amplifying the germination rates, particularly with higher GA₃ levels (Figure 2).

Figure 3 shows the interaction between photoperiod and growth temperature on seed germination. In the dark, the germination rates were low across all temperatures, with 15°C showing the highest germination by the 14th day, while both 25°C and 35°C had limited germination. In contrast, under a 16/8h day-night photoperiod, the germination rates were significantly higher, especially at 15°C. By the 14th day, germination reached a dramatic increase compared to the dark conditions. At 25°C, germination also improved under the day-night cycle. However, at 35°C, the germination remained low even with the light exposure.

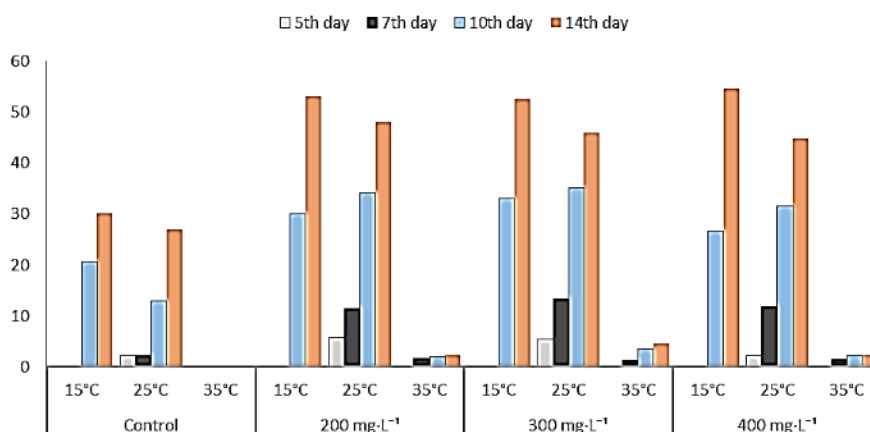


Figure 1. Effect of GA₃ and Growth Temperature Interaction on Seed Germination

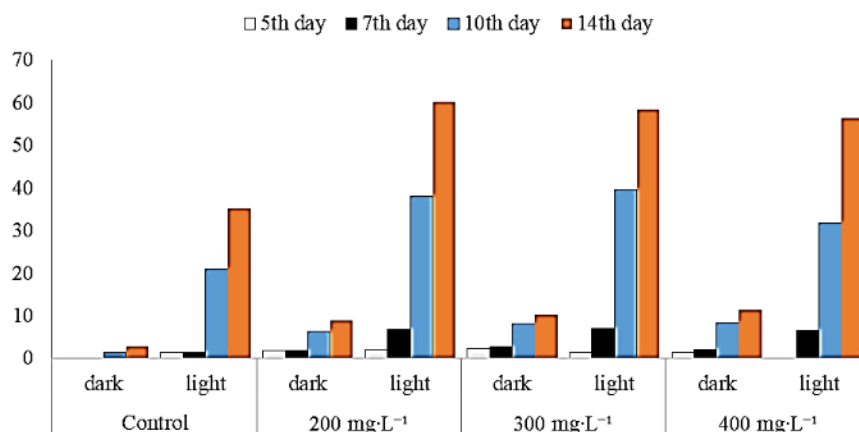


Figure 2. Effect of GA₃ and photoperiod interaction on seed germination

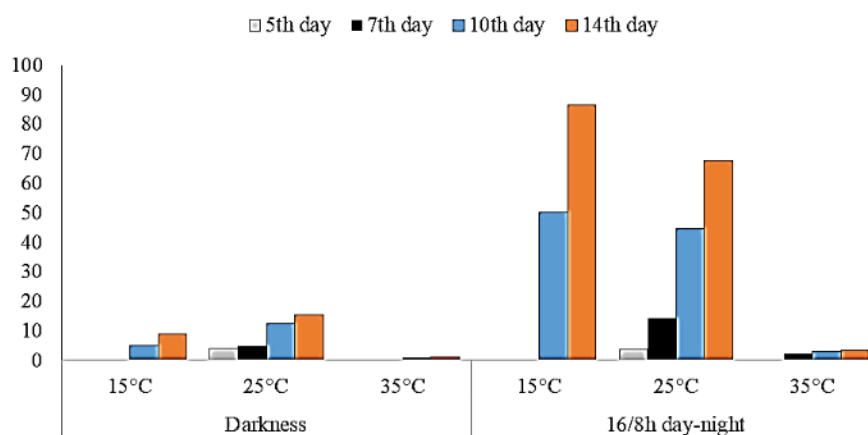


Figure 3. Effect of photoperiod and growth temperatures interaction on seed germination

This interaction indicates that the combination of light and moderate temperatures (15°C and 25°C) promotes higher seed germination. The results suggest that light plays a key role in enhancing germination rates, with moderate temperatures (15°C and 25°C) being more favorable compared to higher temperature, where germination was significantly reduced. Thus, light exposure and optimal temperatures are crucial for improving seed germination.

Discussion

This study provides valuable insights into the impact of various ecological factors, such as temperature, light, and chemical pre-treatments like gibberellic acid (GA₃), on the seed germination of *H. perforatum*, commonly known as St. John's Wort. The results contribute to optimizing germination protocols for this species, particularly in the Tokat region of Turkey. Such protocols are crucial for ensuring consistent, high-quality plant material for commercial cultivation, especially as demand for *H. perforatum* grows within the pharmaceutical industry.

One of the most compelling findings of this study is the role of GA₃ in significantly enhancing germination rates. This result is in line with prior research, which has demonstrated that gibberellins play a crucial role in overcoming dormancy in seeds exhibiting physiological dormancy (Baskin & Baskin, 2000; Pérez-García et al., 2006). GA₃ has been widely recognized for its ability to break dormancy by stimulating enzymatic activity and cellular expansion, both essential processes for successful seed germination (Bewley & Black, 2013). In the present study, the application of higher GA₃ concentrations (200–300 mg·L⁻¹) notably increased germination, particularly when the treatment was extended for 72–120 hours. This supports the hypothesis that exogenous GA₃ can effectively mitigate dormancy in *H. perforatum* seeds. Additionally, Pérez-García et al. (2006) reported a significant increase in germination when GA₃ was applied to *H. perforatum* seeds, with variations based on population and concentration, suggesting that seed origin must be considered when determining the optimal treatment for germination. The current study's observation that germination rates increased from 54% to 88.6% over time with GA₃ treatment further emphasizes the importance of both concentration and incubation period in optimizing

germination success, as also confirmed by Faron et al. (2004) and Butalo et al. (2007).

In addition to GA₃, the study highlights the critical role of temperature and light conditions in seed germination. The results indicate that the optimal temperature for *H. perforatum* germination is 15°C, with a significant decrease in germination success at higher temperatures, particularly at 35°C, where germination was minimal or nonexistent. This observation aligns with the findings of Bewley and Black (2013), who noted that high temperatures hinder *H. perforatum* seed germination. Pérez-García et al. (2006) also emphasized the importance of moderate temperatures for optimal germination, a conclusion further supported by the present study's data.

Furthermore, the study underscores the importance of light exposure in germination. Seeds exposed to a 16/8-hour light/dark photoperiod exhibited significantly better germination rates than those kept in complete darkness, a finding consistent with previous research (Pérez-García et al., 2006; Çırak et al., 2004). In this study, light exposure combined with optimal temperature conditions (15°C) resulted in germination rates as high as 96.75%, reinforcing the critical role of light in triggering the germination process in *H. perforatum*. Notably, the interaction between GA₃ concentration and light exposure was also significant, with the highest germination rates observed when both treatments were combined, especially at 200 mg·L⁻¹ GA₃. This synergistic effect suggests that GA₃ and light work together to maximize germination, a conclusion supported by Nunes et al. (2019), who found that light exposure significantly increased germination rates in *H. perforatum* seeds.

The findings of this study have significant implications for the sustainable cultivation of *H. perforatum* in Turkey. As the demand for this plant grows in the pharmaceutical industry, optimizing germination protocols will be essential to ensure a consistent and high-quality supply of plant material. The results suggest that GA₃ treatments, particularly at concentrations of 200–300 mg·L⁻¹, in combination with light exposure at moderate temperatures (15°C), can significantly enhance seed germination success. These insights are particularly relevant for the commercial cultivation of *H. perforatum*, where efficient seedling production is key to establishing high-yielding crops for pharmaceutical purposes.

Conclusion

The findings of this study underscore the significant effect of GA₃ concentration, photoperiod, and growth temperature on the germination of *H. perforatum* seeds. GA₃ treatments, particularly at 200 and 300 mg·L⁻¹, significantly improved germination rates, with the highest germination observed on the 14th day. Optimal conditions for germination were achieved at 15°C under light exposure, particularly with GA₃ concentrations of 200 and 300 mg·L⁻¹. Additionally, high temperature (35°C) negatively impacted germination, while extended photoperiods and moderate temperatures positively influenced seed development. These results highlight the importance of these factors in maximizing seed germination.

Declarations

Author Contribution Statement

E.P.: She was involved in seed collection, planning the research, conducting the experiments, evaluating the results, and writing the article.

N.G.: The execution of the study and the evaluation of the results.

Conflict of Interest

The authors declare no conflict of interest.

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