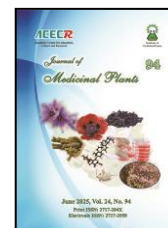




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Research Article

From seed to medicine: Photothermal effects on *Salvia spinosa* L. germination and their pharmacological relevance

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ABSTRACT

Background: *Salvia spinosa* L., a valuable medicinal species with antioxidant and anti-inflammatory properties, requires sustainable cultivation methods for pharmaceutical applications. This study investigated photothermal factors affecting seed germination indices to optimize domestication protocols. **Objectives:** The primary aims were to evaluate the effects of seed age, light conditions, and temperature on key germination indices and seedling vigor, and to establish optimal cultivation protocols for controlled growth. **Methods:** The experiment followed a completely randomized design with 10 treatments (combining seed age, temperature, and light factors) and three replicates. Measured parameters included final germination percentage, germination index, mean daily germination, germination speed, and seedling vigor indices. Data were analyzed using Duncan's multiple range test and orthogonal comparisons. **Results:** The highest germination rate (100%) was observed in aged seeds under warm temperatures and a light cycle. In contrast, the optimal treatment (No. 6: aged seeds + lab temperature [12°C night/20°C Day] + lab ambient light) achieved a balanced germination rate (85%) and robust seedling vigor. Absolute darkness was identified as the primary limiting factor for germination. **Conclusion:** Treatment No. 6 is recommended as the optimal seed germination protocol for domesticating of this plant. These findings represent a significant step toward sustainable cultivation for pharmaceutical use, reducing reliance on wild harvesting.

1. Introduction

Medicinal plants serve as a valuable source of natural products and secondary metabolites used for medicinal purposes. Excessive harvesting of these plants from natural habitats due to high demand has led to species threats,

loss of biodiversity, adulteration of herbal materials, and detrimental impacts on ecosystems. Understanding seed biology and flowering physiology alongside the development of agricultural technologies and ultimately introducing these plants to new

Abbreviations: FGP, Final germination percentage; GI, Germination Index; MGT, Mean Germination Time; SVI, Seed Vigor Index; GSP, Germination Speed; MDG, Mean Daily Germination

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habitats can improve the availability of raw medicinal plant materials and enhance their production without creating the aforementioned risks. With continuous population growth and the increasing trend of herbal medicine use, demand for medicinal plants continues to rise. Therefore, the domestication of these plants has become an urgent need to address the challenge of supplying abundant and standardized raw materials [1-3].

Salvia spinosa L. that named in Iran Thorny sage, a member of the Lamiaceae family, is an annual herbaceous plant growing 30-60 cm tall. It features heart-shaped leaves with tapering, pointed tips and a tubular corolla with a sheath-like structure, where the inner surface is scaly and hairless. This species can be identified by its broad tubular calyces with needle-like tips (during fruiting stage) and the presence of sterile lateral buds in some flowers. As an Irano-Turanian element, its distribution spans northwest, west, central, south, southwest, southeast and northeast Iran, Turkmenistan, northwest Afghanistan, Egypt, Anatolia, Palestine, Mesopotamia, Syria, Lebanon, Iraq and southeast Europe, where it typically grows in low-altitude natural habitats [4-6].

Research has demonstrated that *S. spinosa* L. possesses significant medicinal potential. This plant contains multiple bioactive compounds including flavonoids and essential oils that confer notable antioxidant, anti-inflammatory, and antimicrobial properties. Studies indicate that its extracts can effectively prevent diabetes-induced renal damage. Additionally, it plays a role in reducing oxidative stress and improving mitochondrial function. In summary, due to its valuable phytochemical composition and diverse therapeutic effects, *S. spinosa* L. has emerged as a promising candidate for treating

various diseases, particularly those associated with oxidative stress [7-9].

While the domestication of *S. spinosa* L. requires optimization of cultivation protocols, this species' pharmacological potential remains a key justification for its conservation. Recent phytochemical analyses reveal this plant produces valuable essential oils containing α -pinene (15.8%), β -caryophyllene (12.3%), and camphor (9.7%) - compounds with demonstrated anti-inflammatory and antimicrobial properties [8, 10]. Particularly noteworthy is its rosmarinic acid content (3.2 mg/g dry weight), a phenolic compound showing neuroprotective effects in murine models [11]. These bioactive constituents, while promising for drug development, exhibit concentration-dependent variability influenced by growth conditions - highlighting the importance of controlled cultivation. The species' dual significance as both a source of medicinal compounds and a model for arid-adaptation makes it an ideal candidate for integrated pharmacological-agronomic studies.

Seed germination, as a critical process in successful plant establishment, plays a decisive role in the life cycle of plants. Studies demonstrate that germination indices - including final germination percentage, germination rate, and uniformity - are influenced by the complex interplay of environmental factors such as temperature, light, and moisture [12, 13]. In *Salvia* Species, germination's physiological and biochemical mechanisms often align with the ecological conditions of their natural habitats. For instance, related research has revealed that daily temperature fluctuations (thermoperiod) and light intensity can significantly influence germination patterns [14, 15]. These findings indicate that optimizing germination conditions is crucial for rangeland restoration programs

and developing sustainable cultivation methods for these species.

Based on the previously mentioned contents, the current study aims to evaluate the domestication potential of *S. spinosa* L. under controlled conditions by documenting and examining key seed characteristics, and investigating the effects of various seed treatments (including seed age, light/dark conditions, and ambient temperature) on seed germination and early plant growth in both germinator and laboratory environments. The optimized germination protocol established in this study provides a critical foundation for future research aiming to enhance biomass production and bioactive compound yield in *S. spinosa* cultivation systems.

2. Materials and methods

2.1. Plant material

For this study, seeds of *S. spinosa* L. were collected from their natural habitat at geographical coordinates (34.309814°N, 48.832954°E) in Malayer County, Hamedan Province, Iran, in September 2019 and 2023. The plant identified by Dr. A. Shayganfar using related references [4-6] and also for taxonomic verification, a voucher specimen of the plant (Reference No. 3159) was deposited in the

Herbarium of the Plant Biology Laboratory at Malayer University following identification and cataloging.

2.2. Experimental conditions

This research was conducted as a completely randomized experimental design with three replications. Each replication consisted of 25 visually healthy seeds sterilized with 70% ethanol and 10% sodium hypochlorite and placed on sterile filter paper in sterile Petri dishes with 15 cm diameter openings (Figure 1). The seed germination test lasted 19 days, beginning with seed hydration on October 17, 2023 (25/07/1402), with daily germination data recorded for 12 consecutive days starting from October 26, 2023 (03/08/1402). A total of 10 different treatments were applied, based on standard temperature conditions for both cold-season and warm-season plants, intermediate temperatures between these two conditions, and two different light conditions (as detailed in Table 1). To apply the experimental conditions, a research germinator (GER SET model, Grouc Co., Iran) located at the Faculty of Agriculture, Malayer University, was used. This device was capable of controlling the temperature range of +5 to +50°C with a programmable accuracy of $\pm 1^\circ\text{C}$ and a light intensity of 10,000 lux.

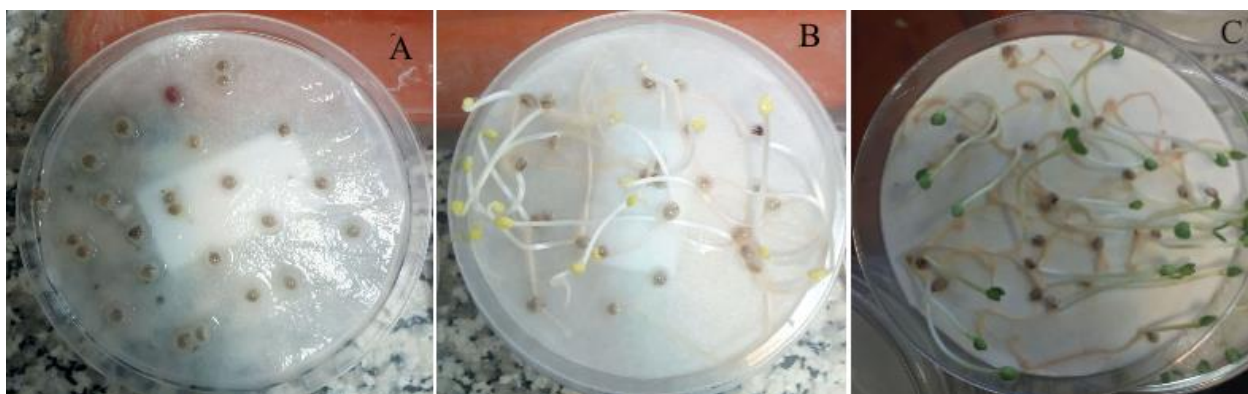


Fig. 1. Images of *S. spinosa* L. seeds under different treatments and at various stages. (A) Imbibed seed on the first day (Treatment 6). Note: The seed produces abundant mucilage during the hydrophobic phase; (B) Final day of Treatment 9; (C) Final day of Treatment 10.

Table 1. The different treatments applied for germinating *S. spinosa* L. seeds in this research.

Treatment numbers									
1	2	3	4	5	6	7	8	9	10
Fresh seed					Aged seed (4 years old)				
Lab. Temp. ^a	Cold Season Temp. ^b	Warm Season Temp. ^c			Lab. Temp.	Cold Season Temp.		Warm Season Temp.	
Lab ambient light ^d	Darkness ^e	Light cycle ^d	Darkness	Light cycle	Lab ambient light	Darkness	Light cycle	Darkness	Light cycle

a) Laboratory temperature cycle (moderate): 12°C night temperature and 20°C Day temperature.

b) Cold season temperature cycle: 7°C night temperature and 15°C Day temperature.

c) Warm season temperature cycle: 18°C night temperature and 27°C Day temperature.

d) Light cycle: 8 hours darkness and 16 hours light (this condition also applies to the control group/laboratory conditions).

e) Complete darkness: Petri dishes were covered with aluminum foil.

2.3. Evaluated Parameters

Final germination percentage (FGP): This parameter was calculated as the percentage of seeds showing either radicle protrusion or visible embryonic growth, using the formula: FGP (%) = (Number of germinated seeds/Total seeds tested) × 100 [16].

$$FGP = G = \left(\frac{\sum_{i=1}^k n_i}{N} \right) 100$$

k: Total number of observation days (e.g., Day 1 to Day *k*).

n_i : Number of seeds that germinated on the *i*-th day (e.g., n_3 = seeds germinated on Day 3).

N: Total number of seeds tested (constant for all days).

Germination Index (GI): This comprehensive seed germination parameter simultaneously integrates both germination percentage and germination rate. Higher GI values indicate superior germination performance in terms of both percentage and speed. The index was calculated using the formula: $GI = \sum (G_t/D_t)$, where G_t represents the number of seeds germinated on day t, and D_t denotes the corresponding time (in days) for G_t [17].

Mean Germination Time (MGT): This index utilizes the number of germinated seeds at specified time intervals during data collection.

Essentially, it assigns weighted value to each day based on germination counts, where lower MGT values indicate faster germination (fewer days required). The index was calculated as: $MGT = \sum (n \times d)/N$ [18], where:

n = number of seeds germinated on day d

d = days elapsed since experiment initiation

N = total germinated seeds by trial conclusion

Seed Vigor Index (SVI): Two formulas were used to calculate this index. The first formula (SVI- I) was obtained by multiplying seedling length (cm) by the final germination percentage (FGP). Seedling length was calculated as the average seedling length per seed treatment using "seedling length = radicle length + hypocotyl length". In the second formula (SVI- II), the mean fresh weight (g) of seedlings whose length was measured in the first formula was multiplied by the final germination percentage, and the resulting value was considered as the seed vigor index. Seeds showing higher seed vigor index values were considered stronger [19].

Germination Speed (GSP): This index represents the total number of seeds that germinate within a specific time interval. Higher GSP values indicate earlier and faster

germination. The index is calculated using the following formulas [18] where:

n_i = Number of seeds germinated at observation i

t_i = Time (in days) from start of experiment to observation i

k = Final germination time point

$$GSP = \left(\frac{1}{\bar{t}}\right) 100 \quad \bar{t} = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i}$$

Mean Daily Germination (MDG): This index represents the average daily number of germinated seeds during the experimental period, calculated as:

$MDG = (\text{Total germinated seeds}) / (\text{Total experimental days in days})$

Higher MDG values indicate greater germination capacity for the seed treatment [20].

2.4. Statistical analysis

The data were statistically analyzed to compare treatment effects through analysis of

variance (ANOVA) using F-tests, followed by mean comparisons with Duncan's multiple range test at the 0.01 significance level. To more clearly evaluate the effects of main treatments, orthogonal contrast analysis was performed between the treatment groups. All statistical analyses were conducted using SAS software (version 9.4).

3. Results

Analysis of variance (ANOVA) results (Table 2) revealed statistically significant differences ($P \leq 0.01$) for all evaluated indices and traits, except for the SVI II index which showed significance at $P \leq 0.05$. The table specifically presents two morphological traits: (1) final seedling length and (2) fresh seedling weight, which were used to calculate SVI I and SVI II indices respectively. All other parameters in the table represented primary germination indices derived from daily counts of germinated seeds during the observation period.

Table 2. Analysis of variance of the traits of germination of *S. spinosa* L. seed.

Source	DF	Mean Square of parameters								
		FGP	GI	MGT	GS	MDG	SL	SVI I	FW	SVI II
Model	9	94.87 *	39.21 **	4.90 **	3.67 **	0.016 **	7.36 **	5684.6	0.001 **	5.73 *
Error	20	32	1.58	0.14	0.11	0.006	0.053	642.65	0.0001	1.06
CV	-	6.36	8.48	3.47	3.55	6.36	14.83	18.8	15.44	15.21

**, *: Significant at $\alpha = 0.01$ and $\alpha = 0.05$ levels, respectively; SL: final seedling length, FW: fresh seedling weight; CV: Coefficient of Variation.

The results of the orthogonal contrast in Table 3 demonstrated statistically significant differences between warm and cold temperature treatment groups, as well as between light cycle and complete darkness treatment groups for all evaluated parameters. The GI, as the main germination parameter assessed in this research, showed significant differences in all comparisons. According to Table 4, the GI was higher in the control group compared to other treatment groups, higher in aged seeds than fresh seeds, higher under light cycle conditions

than complete darkness, and higher under warm season temperatures than cold season temperatures. Similarly, the two other important indices the MGT and GSP showed significant differences in all group comparisons, with the same patterns of significance between treatment groups as observed for GI. Specifically, the lowest MGT values (corresponding to highest GSP values) were observed in the control group, aged seeds, light cycle conditions, and warm season temperatures.

Table 3. Group comparisons of parameters evaluated in *S. spinosa* L. seed germination

Contrast	DF	Mean square								
		FGP	GI	MGT	GS	MDG	PL	SVI I	FW	SVI II
Control vs Others	1	8.53 ^{ns}	31.11**	6.09**	4.35**	0.001 ^{ns}	33.14**	283500.61**	0.001*	7.53*
Fresh vs Aged	1	0.53 ^{ns}	42.05**	7.78**	6.62**	0.0001 ^{ns}	0.39*	2013.96 ^{ns}	0.00004 ^{ns}	0.64 ^{ns}
Dark vs Photo	1	450.67**	109.68**	11.04**	7.60**	0.08**	15.45**	102736.93**	0.003**	7.33*
Cold vs Warm	1	266.67**	153.77**	18.24**	13.49**	0.05**	8.82**	56937.62**	0.01**	27.18**

**, *: Significant at $\alpha=0.01$ and $\alpha=0.05$ levels, respectively; Control (treatments no. 1 and 6); VS: Versus.

Table 4. Mean values of measured parameters in the treatment groups studied in *S. spinosa* L. seed germination

Treatment group	Mean values								
	FGP	GI	MGT	GS	MDG	PL	SVI I	FW	SVI II
Control	90.00	16.87	9.84	10.19	1.18	3.65	329.22	0.09	7.79
Others	88.67	14.33	10.97	9.24	1.17	1.03	86.19	0.07	6.53
Fresh	89.07	13.65	11.26	8.96	1.17	1.44	126.60	0.08	6.93
Aged	88.80	16.02	10.24	9.90	1.17	1.67	142.99	0.08	6.64
Dark	84.33	12.19	11.65	8.68	1.11	1.83	151.62	0.09	7.09
Light cycle	93.00	16.47	10.29	9.80	1.22	0.22	20.76	0.06	5.98
Cold	85.33	11.80	11.84	8.49	1.12	1.63	134.90	0.09	7.60
Warm	92.00	16.86	10.10	9.99	1.21	0.42	37.48	0.06	5.47

In summary, based on the results presented in Tables 3 and 4, aged seeds subjected to moderate temperatures (12°C night/20°C Day) under laboratory ambient light cycles (8 hours darkness/16 hours light) created the optimal germination conditions for *S. spinosa* L. seeds. An interesting finding from the group comparisons was that for the FGP index, light and warm temperature conditions consistently showed significantly higher values compared to darkness and cold temperatures, regardless of seed age (fresh or aged).

The summarized results of mean comparisons for the ten main treatments in this completely randomized design experiment at the 0.01 significance level can be seen in Table 5. As shown in the table, treatment No. 10 (aged seeds under warm temperature and laboratory ambient light conditions) had the highest

percentages for all germination indices but the lowest values for both seed vigor indices. Among all treatments examined, treatment No. 6 (aged seeds under laboratory conditions with laboratory ambient light cycle) showed the optimal combination of germination indices and seedling vigor. Treatment No. 2 (fresh seeds under cold temperature and complete darkness) had the poorest germination indices, although its two seed vigor indices showed favorable status. Of all treatments, when considering both germination indices and seed vigor together, treatment No. 9 (aged seeds under warm temperature and complete darkness) displayed the weakest average values for the examined indices.

Table 5. Comparisons of mean germination parameters of *S. spinosa* L. seeds under different treatments

Treatment Numbers	Mean of parameters								
	FGP	GI	MGT	GS	MDG	PL	SVII	FW	SVIII
1	92.00 ^{ab}	16.15 ^b	10.36 ^d	9.66 ^c	1.21 ^{ab}	3.52 ^a	324.08 ^{ab}	0.087 ^{ab}	7.98 ^{ab}
2	82.67 ^b	9.51 ^e	12.86 ^a	7.78 ^f	1.09 ^b	2.76 ^b	229.14 ^c	0.103 ^a	8.45 ^{ab}
3	90.67 ^{ab}	12.97 ^{cd}	11.55 ^b	8.66 ^{de}	1.19 ^{ab}	0.24 ^d	21.74 ^d	0.068 ^{bc}	6.23 ^{abcd}
4	85.33 ^{ab}	12.51 ^{cde}	11.45 ^{bc}	8.75 ^{de}	1.12 ^{ab}	0.47 ^{cd}	38.97 ^d	0.071 ^{bc}	6.01 ^{bcd}
5	94.67 ^{ab}	17.13 ^b	10.05 ^{de}	9.96 ^{bc}	1.25 ^{ab}	0.20 ^d	19.07 ^d	0.063 ^{bc}	5.98 ^{bcd}
6	88.00 ^{ab}	17.60 ^b	9.33 ^{ef}	10.73 ^{ab}	1.16 ^{ab}	3.79 ^a	334.35 ^a	0.086 ^{ab}	7.59 ^{abc}
7	81.33 ^b	10.23 ^{de}	12.35 ^{ab}	8.10 ^{ef}	1.07 ^b	3.27 ^{ab}	266.47 ^{bc}	0.108 ^a	8.76 ^a
8	86.67 ^{ab}	14.49 ^{bc}	10.61 ^{cd}	9.43 ^{cd}	1.14 ^{ab}	0.26 ^d	22.23 ^d	0.080 ^{ab}	6.95 ^{abcd}
9	88.00 ^{ab}	16.52 ^b	9.94 ^{de}	10.08 ^{bc}	1.16 ^{ab}	0.82 ^c	71.88 ^d	0.058 ^{bc}	5.12 ^{cd}
10	100.00 ^a	21.28 ^a	8.96 ^f	11.17 ^a	1.32 ^a	0.20 ^d	20.00 ^d	0.048 ^c	4.76 ^d

Different letters in each column shows statistically significant differences at P < 0.01

4. Discussion

The group comparison results regarding FGP indicate that both light and elevated temperature stimulate germination mechanisms in *S. spinosa* L., leading to increased germination rates in the corresponding treatments. While germination still occurs under dark and low-temperature conditions (8/4°C), the percentages are significantly lower. These findings align with previous studies on this species that reported enhanced germination under high light and temperature conditions (32/20°C regime), with maximum germination observed under high light intensity. The significantly reduced germination in darkness and low temperatures reflects this species' ecological adaptation to bright, warm environments [21].

Similar light and temperature effects have been documented in closely related species like *S. aegyptiaca* [22]. Like many tropical and subtropical plants, *S. spinosa* seeds respond positively to light and high temperature fluctuations as dormancy-breaking signals. This response likely involves phytochrome-mediated signaling pathways activated by white light, which trigger the germination process [12, 23].

Initial examination of the mean comparisons across all experimental treatments suggested that treatment No.10 (aged seeds under warm temperature and laboratory ambient light conditions) performed best, showing the highest values for all germination indices: FGP, GI, MGT, and GSP. This initially appeared to make it the most suitable candidate for domestication and future use. However, deeper analysis revealed an important limitation: despite its superior germination performance, this treatment resulted in the lowest values for both seed vigor indices (SVI I and SIV II), which are directly related to plant establishment and growth. This finding is consistent with similar research on *Medicago truncatula*, where high temperature stress was shown to cause imbalanced energy allocation toward germination processes at the expense of early seedling growth. In that study, despite high germination percentages at temperatures above 30°C, seedlings developed shorter roots and reduced biomass, likely due to premature activation of seed reserve-degrading enzymes like α-amylase before root development was complete [24].

In contrast, treatment No.6 (aged seeds under laboratory conditions with laboratory ambient light cycles) demonstrated the optimal balance between germination rate and seedling quality; a finding consistent with our independent comparison results. This aligns with the work of Baskin & Baskin (2000), who emphasized that laboratory ambient light cycles and moderate temperatures (20-25°C) prevent rapid depletion of seed reserves in medicinal plants and promote balanced germination and seedling growth. Previous research on *Zea mays* seeds further supports that optimal energy allocation for both germination and early growth requires intermediate temperatures (e.g., 20-25°C) that maintain equilibrium between reserve metabolism and seedling development [25]. Therefore, we recommend Treatment No.6 as the superior option for *S. spinosa* domestication.

The optimized germination conditions (Treatment 6: aged seeds, 12°C/20°C, laboratory ambient light) may indirectly enhance pharmacological potential. Studies on related *Salvia* species indicate that moderate stress during germination—particularly light and temperature regimes similar to our optimal treatment—can upregulate phenylpropanoid pathway genes, leading to increased rosmarinic acid synthesis in mature plants [26]. While this study focused on germination efficiency, the observed vigor in Treatment No.6 seedlings (evidenced by SVI values) suggests better root establishment, which could improve secondary metabolite accumulation. Notably, roots are the primary site for diterpenoid biosynthesis in *S. rhytidea* Benth [27]. Although this study did not measure phytochemicals, the established link between seedling vigor and secondary metabolism in medicinal plants [28, 29] suggests that our germination protocol could be

a critical first step in quality-controlled cultivation for pharmaceutical purposes.

The study also observed that primary germination indices (FGP, MGT, and GSP) showed significantly higher values in aged seeds. This enhancement can be attributed to: (1) reduced seed dormancy through gradual breakdown of germination inhibitors [30]; (2) improved response to light and temperature [25]; (3) balanced energy reserve utilization [24]; (4) decreased oxidative stress via better activation of oxidative enzymes like catalase and superoxide dismutase [31]; and (5) facilitated water uptake through increased permeability of the hard seed coat [32].

The most significant finding from our analysis of mean comparisons was not derived from the top-performing treatments, but rather from examining the poorest performers; specifically, treatments No.2 and No.9. These results clearly established darkness as the primary limiting factor for both seed germination and seedling vigor in *S. spinosa* L., with its inhibitory effect being more pronounced than all six other factors studied. This phenomenon likely stems from three key mechanisms: (1) suppression of phytochromes, the principal dormancy-breaking signals in seeds [23]; (2) impaired metabolic energy production due to lack of light, particularly affecting photophosphorylation processes [25]; and (3) ecological survival strategies that prevent germination when seeds are buried too deeply in soil or shaded by other vegetation [12].

5. Conclusion

This study clearly demonstrated that successful germination and establishment of *S. spinosa* L. seeds are influenced by a complex interaction of environmental factors. While Treatment No.10 (aged seeds under warm temperature with laboratory ambient light)

showed the highest germination indices, this advantage came at the significant cost of reduced seedling vigor, likely due to rapid depletion of energy reserves under temperature stress. In contrast, Treatment No.6 (aged seeds under moderate laboratory temperatures [12°C night/20°C Day] with laboratory ambient light cycles) emerged as the optimal condition, providing the best balance between germination speed and seedling quality. Importantly, complete darkness was identified as the primary limiting factor, with stronger inhibitory effects than other stress factors including low temperatures. These findings confirm this species' ecological adaptation to bright, warm environments and suggest that domestication and rangeland rehabilitation programs should prioritize both adequate light exposure and moderate temperatures (12°C night/20°C Day) when using aged seeds. The identified optimal conditions (Treatment No.6) may also enhance the plant's medicinal potential, as similar photothermal regimes have been shown to upregulate phenylpropanoid biosynthesis in related *Salvia* species. The study highlights the critical need to evaluate both germination parameters and seedling vigor simultaneously in seed quality assessments, as focusing solely on germination metrics may lead to weak seedlings

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with poor subsequent growth performance and potentially reduced secondary metabolite production.

Author contribution

ASH contributed to data collection and laboratory experiments. SFM performed data analysis and wrote the initial manuscript draft. All authors participated in research design, methodology development, and hypothesis formulation. Additionally, all authors were involved in finalizing the manuscript, including critical revisions and English language editing. Research material preparation was handled separately as part of the experimental process.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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