



RESEARCH ARTICLE

Optimising germination of *Verbascum speciosum* Schrad. seeds: Effects of temperature, salinity and pH for sustainable cultivation

Mohsen Zafaranih*

Department of Agriculture, Velayat University, Iranshahr, Iran

*Correspondence email - Mohsen.Zafaranih245@gmail.com

Received: 03 November 2026; Accepted: 19 June 2026; Available online: Version 1.0: 08 July 2026

Cite this article: Mohsen Z. Optimising germination of *Verbascum speciosum* Schrad. seeds: Effects of temperature, salinity and pH for sustainable cultivation. Plant Science Today (Early Access). <https://doi.org/10.14719/pst.12575>

Abstract

Defining the germination niche of *Verbascum speciosum* Schrad., a medicinal pioneer species for semi-arid rangeland restoration, is crucial for sustainable propagation. This study evaluated the effects of temperature (10–25 °C), salinity (0–10 g L⁻¹ NaCl) and pH (2–9) on seed germination using seeds from two Iranian populations, Zarand and Kohbanan, in Kerman Province. Seeds were incubated in darkness using sequential single factor experiments with 3 replicates and 90 seeds per treatment. Final germination percentage was analysed by one-way analysis of variance (ANOVA followed by Tukey's honestly significant difference (HSD) test at $p < 0.05$. Germination showed a bell-shaped thermal response, peaking at 15 °C, with $93.6 \pm 2.1\%$ in Zarand and $83.0 \pm 3.5\%$ in Kohbanan, while germination was completely inhibited at 25 °C. Increasing salinity significantly reduced germination and delayed seed germination, with near-complete inhibition at NaCl concentrations of 7.5 g L⁻¹ or higher. Germination occurred across a pH range of 3.5 to 9, with the highest germination at neutral pH 7, whereas extremely acidic conditions (pH 2) almost completely inhibited germination. These results indicate a narrow thermal optimum, moderate salinity sensitivity and broad pH tolerance in *V. speciosum*, supporting cool-season establishment in autumn microsites and providing practical guidance for restoration and cultivation in semi-arid rangelands.

Keywords: germination niche; medicinal plant; pH; salinity stress; seed germination; temperature; *Verbascum speciosum*

Introduction

Verbascum speciosum Schrad. is a biennial herbaceous species of the family Scrophulariaceae, native to Eastern Europe and Western Asia and it commonly occurs in open, dry and disturbed habitats such as roadsides, fields and semi-arid rangelands (1, 2). The species is characterised by large basal leaves, erect flowering stems, yellow flowers and a dry capsule containing numerous small seeds (2) (Fig. 1). Owing to its drought tolerance, ability to establish disturbed habitats and medicinal value, *V. speciosum* has potential as a candidate species for sustainable cultivation and restoration-oriented propagation in semi-arid landscapes (2, 3).

Successful establishment of this species depends on germination success, because the germination niche determines the range of environmental conditions that permit seed germination and early seedling establishment (4). In dryland restoration and medicinal plant production, seed-based propagation is often the most practical and cost-effective approach, but its success is highly dependent on abiotic conditions during germination (5, 6). Even small reductions in germination percentage or speed may prevent seedling establishment under stressful field microsites, thereby limiting restoration success and nursery production efficiency (7, 8). Therefore, identifying the environmental thresholds that regulate

germination is essential for designing propagation protocols that are both ecologically reliable and operationally feasible.

Although temperature, salinity and pH are recognised as major filters of seed germination in dryland species, the germination responses of *V. speciosum* to these factors remain poorly understood (9–11). This gap limits our ability to define the species' germination niche and to recommend suitable conditions for cultivation and restoration in semi-arid Iranian rangelands. It is still unclear which temperature maximises germination, how strongly salinity suppresses germination and whether the species tolerates a broad or narrow pH range under controlled conditions.

To address this gap, the present study tested the following hypotheses: (i) germination of *V. speciosum* is maximised at moderate cool temperatures rather than at high temperatures; (ii) increasing salinity progressively reduces germination percentage and delays germination; (iii) seeds germinate best under near-neutral pH, whereas strongly acidic or alkaline conditions inhibit germination. By defining these thresholds, this study provides a basis for improving nursery propagation, supporting sustainable cultivation and guiding restoration of *V. speciosum* in Iranian semi-arid rangelands.



Fig. 1. Morphological characteristics of *Verbascum speciosum*. A: habit; B: first-year mature leaf rosette (bar = 10 cm); C: partial inflorescence (bar = 1 cm); D: capsules (bar = 5 mm); E: seeds (bar = 2 mm).

Materials and Methods

Seeds collection and identification

Mature seeds of *V. speciosum* (Fig. 1) were collected in August 2023 from 20 mother plants per population at two sites in Kerman Province, Iran: Zarand (30°48'45.7" N, 56°33'50.4" E) and Kohbanan (31°24'36" N, 56°17'12" E). Collection was carried out with landowner's permission and in accordance with local regulations. The study sites represent typical semi-arid rangelands characterised by hot summers, mild winters and mean annual precipitation of approximately 250 mm yr⁻¹. After collection, seeds were manually cleaned to remove immature and damaged seeds, air-dried to approximately 8 % moisture content and stored in sealed glass vials at 20–25 °C and about 30 % relative humidity for 6–8 months before experimentation. Seed viability was determined by tetrazolium (TTC) staining, which indicated viability of 94 % in Zarand (n = 50) and 82 % in Kohbanan (n = 50).

Germination experiments

Germination thresholds for temperature, salinity and pH were assessed using a sequential single-factor experimental design following established protocols for medicinal plants (12). This design was selected to identify the main abiotic filters governing the germination niche while conserving limited seed material from natural populations. No dormancy-breaking treatment was applied, because preliminary observations indicated that seeds germinated readily under favourable conditions, suggesting that dormancy was not a major limitation under the tested regime (13, 14).

Before incubation, seeds were surface-sterilised using 1 % sodium hypochlorite (NaOCl) for 1 min, then rinsed three times with distilled water and air-dried. Seeds were placed in 9 cm Petri dishes containing two layers of Whatman No. 1 filter paper moistened with

1.5 mL of test solution. Dishes were sealed with Parafilm to prevent moisture loss and replenishment of solution during the 30-day incubation period. All dishes were randomised weekly across three wire shelves in Percival Scientific growth chambers (USA; 120 L; ± 0.5 °C precision; 60–80 % RH) and chamber conditions were monitored using HOBO sensors. Germination was defined as radicle protrusion of at least 1 mm and recorded every 30 days. Three spatial replicates of 30 seeds were used per treatment, for a total of 90 seeds per treatment (13).

Temperature treatments consisted of constant temperatures of 10, 15, 20 and 25 °C and were used to identify the thermal optimum. Salinity treatments included NaCl concentrations of 0, 1, 2.5, 5, 7 and 10 g L⁻¹, corresponding to approximately 0–17.1 dS m⁻¹ and were tested at the identified thermal optimum of 15 °C. These concentrations were selected to represent the salinity gradient typically encountered in Kerman rangelands (approximately 0.5–12 dS m⁻¹). The pH treatments consisted of distilled water adjusted to pH 2, 3.5, 5, 7 and 9 using 0.1 M HCl for acidic solutions and NaOH for alkaline solutions immediately before use (Merck, Germany) and were conducted at 15 °C. No buffer was added and pH was not monitored continuously during incubation; however, the dishes were sealed and the incubation period was relatively short, so pH drift was expected to be minimal (14, 15). A supplementary photoperiod test was conducted at 15 °C under continuous darkness and 12 hr light/12 h dark conditions using cool white fluorescent light (50 μmol m⁻² s⁻¹; n = 3 replicates per treatment) (13). The main germination experiments were conducted in continuous darkness. In addition, a supplementary photoperiod test was performed at 15 °C under continuous darkness and 12 hr light/12 hr dark conditions using cool white fluorescent light (50 μmol m⁻² s⁻¹). Seeds were stored for 6–8 months before experimentation under sealed conditions at 20–25 °C and approximately 30 % relative humidity.

Germination indices included final germination percentage (% germinated / total seeds $\times$ 100), viable seed germination (% germinated / TTC-viable seeds), mean germination time (MGT; Σ (Dayi $\times$ ni) / Σ ni) and median germination time (T50; days to 50 % viable seed germination) (16).

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post hoc test ($p < 0.05$) in SPSS v.21. Effect sizes were quantified using partial eta squared (η^2), representing the proportion of variance explained by each factor (8). Germination percentages met ANOVA assumptions based on Shapiro-Wilk normality and Levene's homogeneity tests ($p > 0.05$), so no transformation was applied. Population $\times$ treatment interactions were tested where applicable. Pearson correlation analysis was used to assess the relationship between salinity and germination and significance was evaluated using r values and 95 % confidence intervals based on Fisher's z-transformation ($df = n - 2$). One-way ANOVA was also used to compare MGT and T50 across temperatures and populations. Spatial replication ($n = 3$) provided robust precision, whereas temporal replication was not possible because of seed limitation (15, 17).

Results and Discussion

The germination responses of *V. speciosum* across temperature, salinity and pH gradients defined a clear physiological niche for this species in semi-arid rangeland ecosystems. Germination was highest at 15 °C, under low salinity and neutral pH, indicating that successful establishment is most likely under cool-season microsite

conditions rather than under the hotter and more stressful conditions typical of exposed summer environments (1, 2, 4, 6, 7). This pattern supports the view that *V. speciosum* is adapted to autumn or early spring recruitment, when soil moisture is still available and temperatures are more favourable for seedling emergence (18).

Temperature had a highly significant effect on germination performance. After 30 days, the highest germination occurred at 15 °C, reaching 88 % in Zarand and 68 % in Kohbanan, whereas germination declined markedly at higher temperatures and was strongly reduced at 25 °C (Fig. 2 and 3). The bell-shaped thermal response observed here is typical of temperate pioneer species and reflects the role of temperature in regulating metabolic activation, membrane function and radicle protrusion (2, 18). The similar response pattern in both populations suggests that thermal requirements are largely conserved, although Zarand seeds consistently performed better than Kohbanan seeds.

Salinity was the most restrictive abiotic factor in this study. Increasing NaCl concentration progressively delayed germination and reduced final germination percentage, with almost complete inhibition above 7 g L⁻¹ NaCl in both populations (Fig. 4 and 5). In the absence of salt stress, germination was higher in Zarand seeds (72 %) than in Kohbanan seeds (57 %) at 15 °C (Fig. 4 and 5). The strong negative correlation between germination and salinity confirms that *V. speciosum* seeds are moderately salt-sensitive during germination (Fig. 6). This response is likely caused by osmotic stress, which reduces water uptake during imbibition and ion toxicity, which disrupts enzyme activity and embryo metabolism (3, 6, 7). The longer lag phase under salt stress further indicates that

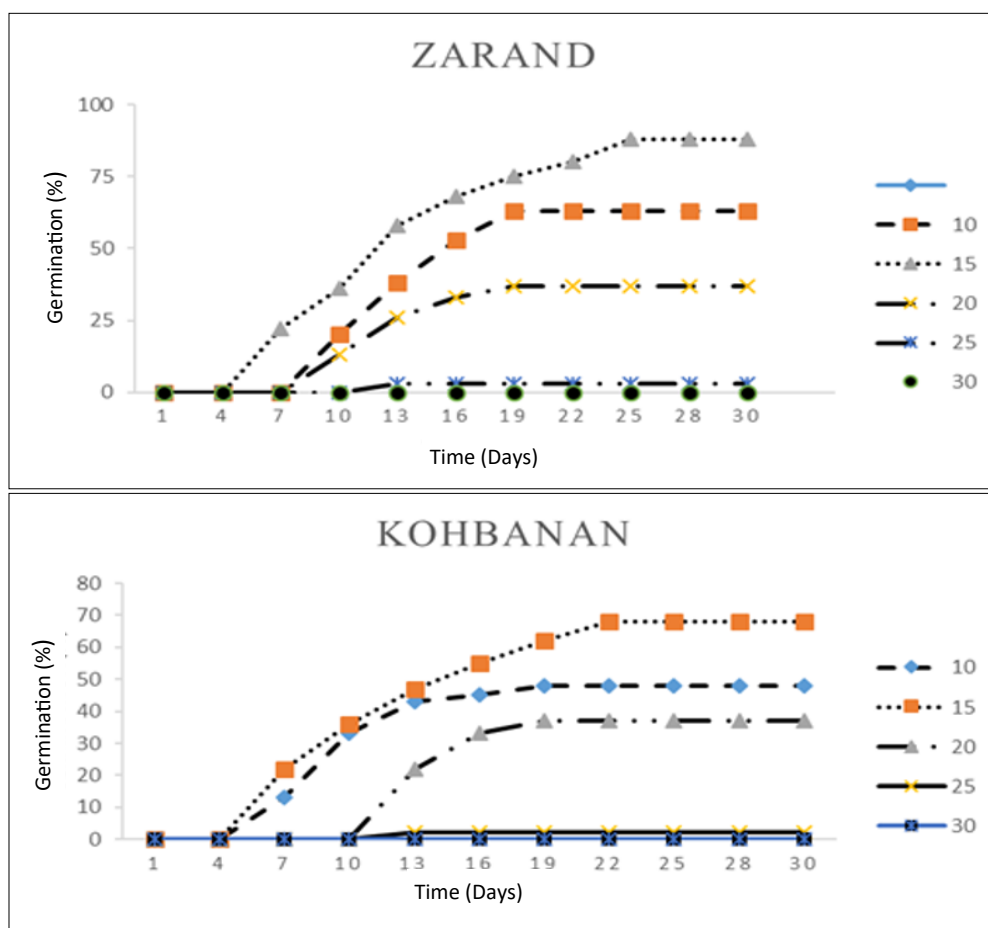


Fig. 2. Cumulative germination (%) of *Verbascum speciosum* seeds over 30 days under different temperature regimes (10, 15, 20 and 25).

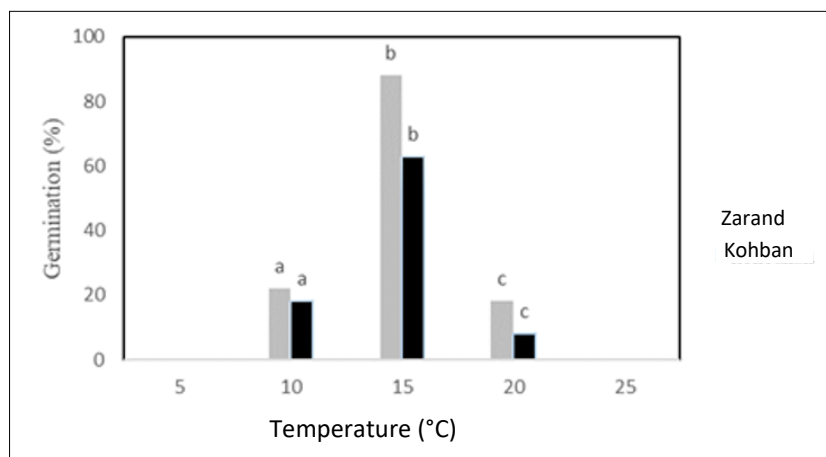


Fig. 3. Final seed germination of *Verbascum speciosum* seeds in aqueous medium at different temperatures. Data are presented as means $\pm$ SD. Different letters indicate significant differences among treatments ($p < 0.05$).

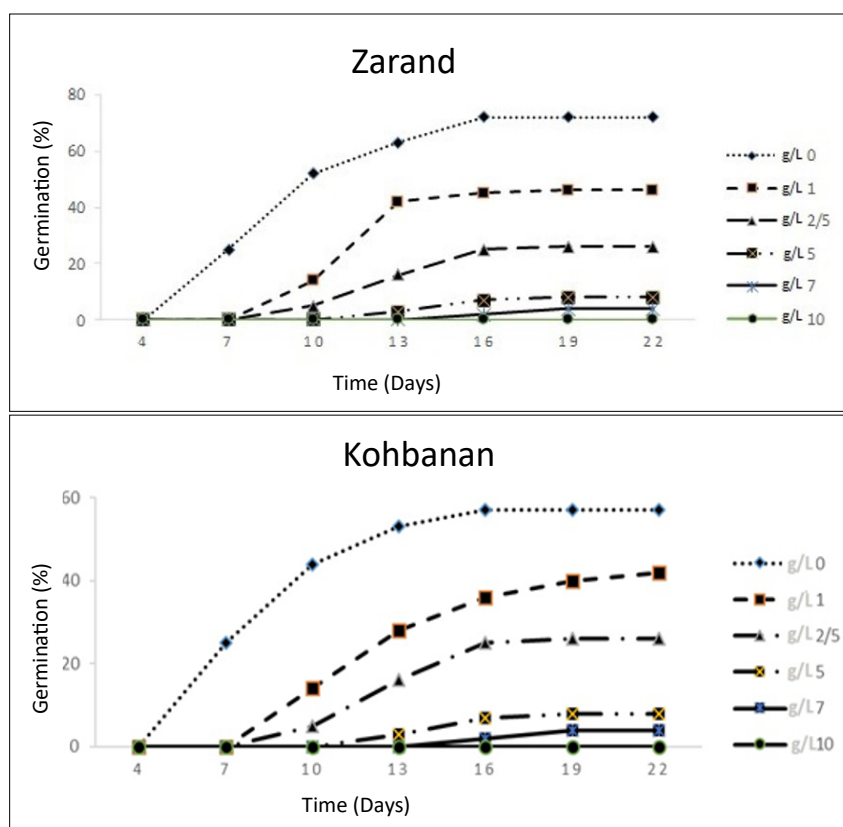


Fig. 4. Cumulative germination percentage of *Verbascum speciosum* seeds over 22 days under different NaCl concentrations (0, 1, 2.5, 5, 7 and 10 g L⁻¹).

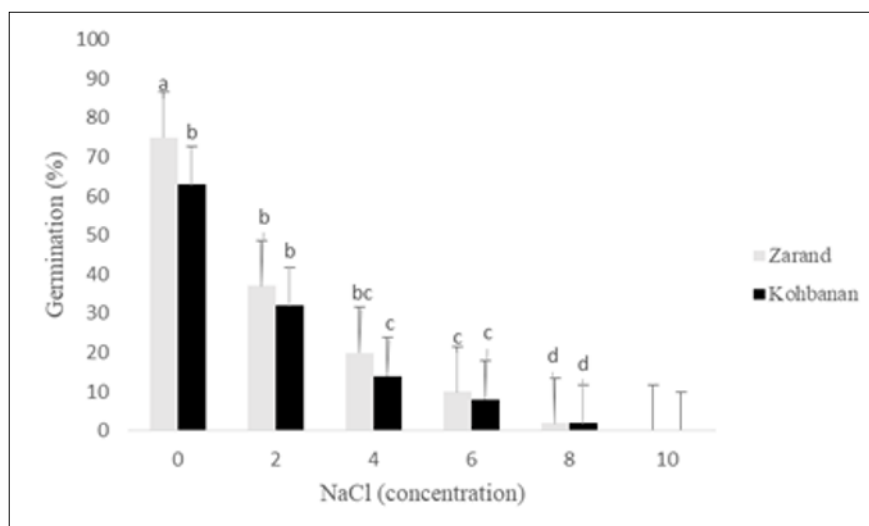


Fig. 5. Final seed germination of *Verbascum speciosum* seeds in aqueous medium with different NaCl concentrations. Data are presented as means $\pm$ SD. Different letters indicate significant differences among treatments ($p < 0.05$).

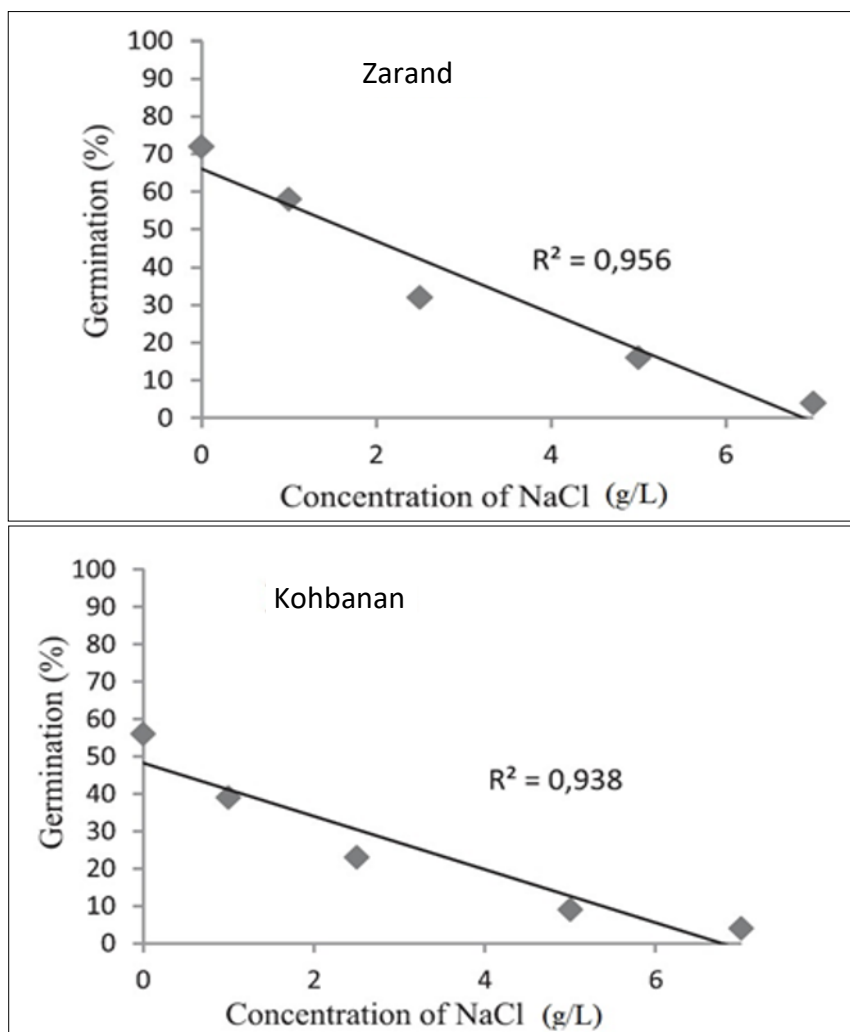


Fig. 6. Correlation between seed germination percentage and NaCl concentration in *Verbascum speciosum*.

germination was delayed rather than simply suppressed, especially at intermediate salinity levels.

Population-level differences were also evident under salinity stress. Zarand seeds maintained 28 % germination at 5 g L⁻¹ NaCl, compared with 15 % in Kohbanan, suggesting greater tolerance in the Zarand population (Fig. 5). This may reflect local adaptation or ecotypic differentiation across the geographic gradient separating the two populations (19). From a practical perspective, Zarand seeds may have a broader capacity for establishment in slightly saline microsites, whereas Kohbanan seeds are more restricted to low-salinity habitats.

The pH also influenced germination, but its effect was less restrictive than salinity. Both populations showed maximum germination at pH 7, with 71 % in Zarand and 61 % in Kohbanan, whereas germination was strongly inhibited at pH 2 and pH 9 (Fig. 7 and 8). This bell-shaped response suggests that seed metabolic processes, including enzyme activity and reserve mobilisation, are optimised near neutral pH (20, 21). The slightly broader response of Zarand seeds again suggests greater physiological flexibility, particularly under moderately acidic conditions.

Taken together, the results show that temperature sets the basic seasonal window for germination, salinity imposes the strongest environmental constraint and pH further limits establishment at the extremes. The combined abiotic thresholds define the germination niche of *V. speciosum* and indicate that this species is best suited to cool, low-salinity, near-neutral microsites. The consistently higher performance of Zarand seeds across

treatments suggests that this population may be more adaptable to environmental variation than Kohbanan. These findings have implications for restoration, seed sourcing and conservation planning in semi-arid rangelands, where matching seed provenance to local stress conditions may improve establishment success.

Conclusion

Based on the findings of the present study, it can be concluded that seed germination of *Verbascum speciosum* is significantly influenced by abiotic factors such as temperature, salinity and pH. The optimal temperature range for effective germination was found to be between 10 °C and 15 °C, providing favourable conditions for the metabolic and enzymatic processes necessary for germination. High salinity levels, as indicated by increasing NaCl concentrations, negatively affected germination percentage, showing an inverse relationship. Moreover, seed germination occurred within a pH range of 3.5 to 9, with neutral pH 7 identified as optimal. Extreme acidic or alkaline conditions reduced germination, likely due to enzyme inhibition and other physiological constraints. These results highlight the critical role of these environmental factors in enhancing germination success, which is essential for sustainable propagation and conservation of *V. speciosum*, a medicinally valuable and often overused species. Understanding and applying these optimal conditions can improve cultivation strategies and ensure maximal seedling establishment and survival. The germination of *V. speciosum* was strongly shaped by temperature, salinity and pH, with the best performance at 15 °C, low salinity and near-neutral pH.

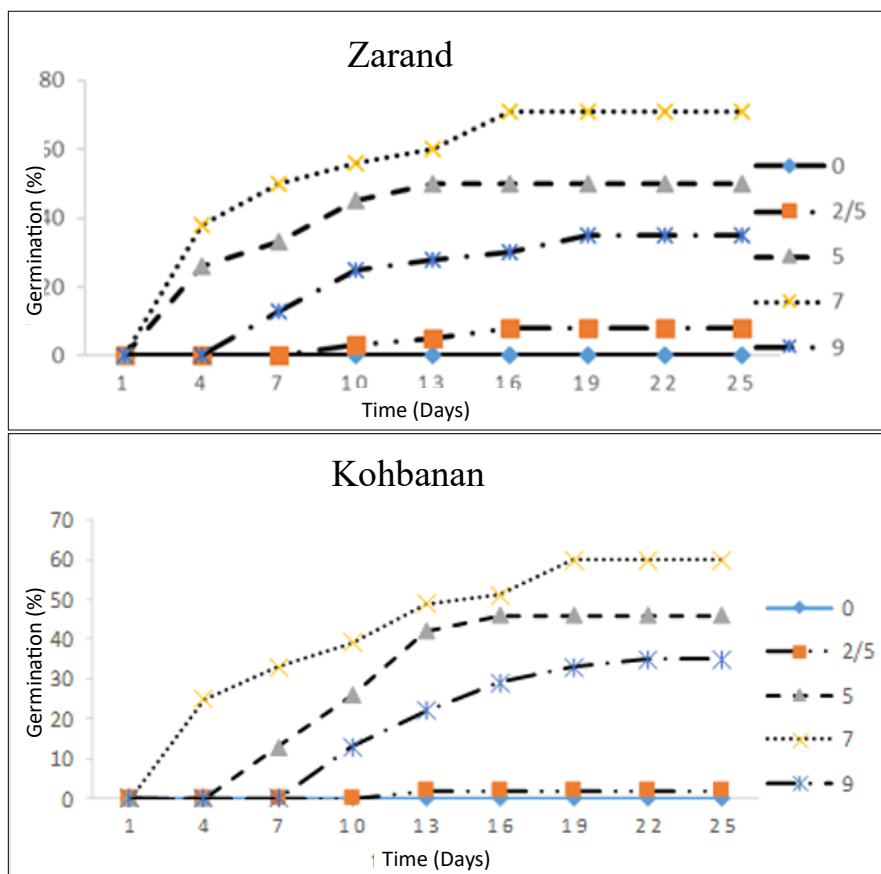


Fig. 7. Cumulative germination percentage of *Verbascum speciosum* seeds over 25 days at different pH values.

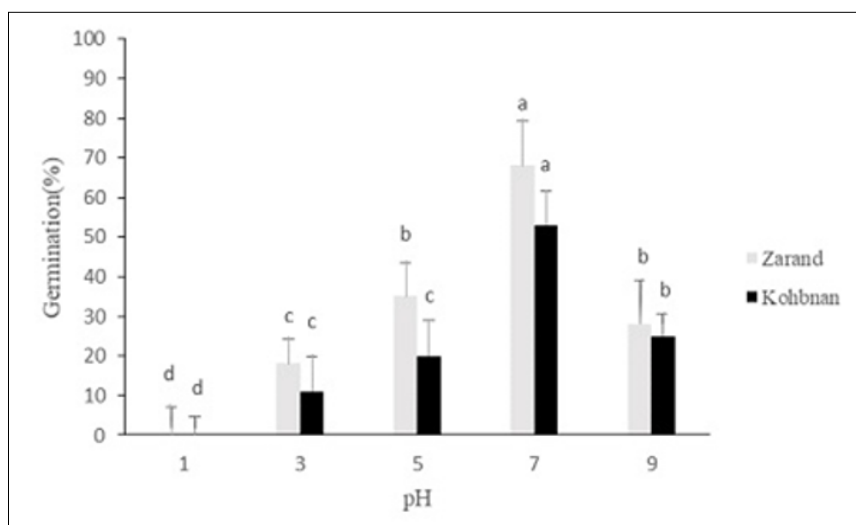


Fig. 8. Final seed germination of *Verbascum speciosum* seeds growing in aqueous medium with different pH. Data are represented as mean $\pm$ SD. values having different letters are significantly different ($p < 0.05$).

These findings suggest that sowing should be targeted to cool, moist periods on non-saline, near-neutral soils to improve establishment success. Zarand seeds showed greater tolerance to salinity than Kohbanan seeds, indicating that provenance choice matters for restoration on marginal sites. Future work should test soil-based emergence, factorial interactions among abiotic factors and field performance under natural rangeland conditions.

Technical and Engineering, Velayat University, for providing laboratory facilities and support.

Compliance with ethical standards

Conflict of interest: Author do not have any conflicts of interest to declare.

Ethical issues: None

Acknowledgements

The author gratefully acknowledges all individuals who provided technical assistance and valuable suggestions during this study. Special thanks are extended to the Department of Agriculture,

References

- Kumar V, Sharma S, Sharma RK, Kumar V, Sharma SS. Influence of seed pre-treatment and storage on germination and physiological characteristics of seeds of common mullein (*Verbascum thapsus* L.). *J Appl Res Med Aromat Plants*. 2024;43:100573. <https://doi.org/10.1016/j.jarmap.2024.100573>
- Kayır S, Demirci Y, Demirci S, Ertürk E, Ayaz E, Doğan A et al. The *in vivo* effects of *Verbascum speciosum* on wound healing. *S Afr J Bot*. 2018;119:226–9. <https://doi.org/10.1016/j.sajb.2018.09.013>
- Al-Jibouri AMJ, Abed AS, Ali AJA, Majeed DM. Improvement of phenols production by amino acids in callus cultures of *Verbascum thapsus* L. *Am J Plant Sci*. 2016;7(1):84–91. <https://doi.org/10.4236/ajps.2016.71010>
- Motamedi J, Ahmadzadeh N, Alijanpoor A. Ecological and morphological characteristics of *Verbascum speciosum* Schrader in the mountainous rangelands of Sahand. *Rangeland*. 2019;13(1):76–89.
- Hasnain A, Naqvi SAH, Ayesha SI, Khalid F, Ellahi M, Iqbal S, et al. Plants *in vitro* propagation with its applications in food, pharmaceuticals and cosmetic industries: current scenario and future approaches. *Front Plant Sci*. 2022;13:1009395. <https://doi.org/10.3389/fpls.2022.1009395>
- Irik HA, Bikmaz G. Effect of different salinity on seed germination, growth parameters and biochemical contents of pumpkin (*Cucurbita pepo* L.) seed cultivars. *Sci Rep*. 2024;14(1):6929. <https://doi.org/10.1038/s41598-024-55325-w>
- Haj Sghaier A, Tarnawa Á, Khaeim H, Kovács GP, Gyuricza C, Kende Z. The effects of temperature and water on the seed germination and seedling development of rapeseed (*Brassica napus* L.). *Plants*. 2022;11(21):2819. <https://doi.org/10.3390/plants11212819>
- Laghmouchi Y, Belmehdi O, Bouyahya A, Senhaji NS, Abrini J. Effect of temperature, salt stress and pH on seed germination of medicinal plant *Origanum compactum*. *Biocat Agric Biotechnol*. 2017;10:156–60. <https://doi.org/10.1016/j.bcab.2017.03.002>
- Bewley JD, Black M. Seed: physiology of development and germination. New York: Plenum Press; 1994. <https://doi.org/10.1007/978-1-4899-1002-8>
- Ndour P, Danthu P. Effet des contraintes hydriques et salines sur la germination de quelques acacias africains. In: Campa C, Grignon C, Gueye M, Hamon S, editors. *L'acacia au Sénégal*. Orstom; 1998. p. 105–22.
- Catara S, Cristaudo A, Gualtieri A, Galesi R, Impelluso C, Onofri A. Threshold temperatures for seed germination in nine species of *Verbascum* (Scrophulariaceae). *Seed Sci Res*. 2016;26(1):30–46. <https://doi.org/10.1017/S0960258515000343>
- Erken K. Investigation of vegetative properties and generative production of the potential ornamental and narrow endemic species *Verbascum yurtkuranianum* (Scrophulariaceae) for ex situ conservation. *BioResources*. 2021;16(4):7530–47. <https://doi.org/10.15376/biores.16.4.7530-7549>
- Ben Miled D, Boussaid M, Cherif A. Tolérance au sel d'espèces annuelles du genre *Medicago* au cours de la germination. In: *Colloque sur les végétaux en milieu aride*; 1986. p. 586–93.
- Basra SMA, Ashraf M, Iqbal N, Khaliq A, Ahmad R. Physiological and biochemical aspects of pre-sowing heat stress on cottonseed. *Seed Sci Technol*. 2004;32(3):765–74. <https://doi.org/10.15258/sst.2004.32.3.12>
- Sun W, Shahrajabian MH. Therapeutic potential of phenolic compounds in medicinal plants: natural health products for human health. *Molecules*. 2023;28(4):1845. <https://doi.org/10.3390/molecules28041845>
- Zhan-Wu G, Yong-Guang M, Jian-Jun D, Ke-Jia DI, Jia-Tong LI, Xin-Ning LI, et al. The effects of salinity stress on *Amorpha fruticosa* Linn. seed germination, physiological and biochemical mechanisms. *Not Bot Horti Agrobi*. 2024;52(1):13552. <https://doi.org/10.15835/nbha52113552>
- Singh J, Sastry ED, Singh V. Effect of salinity on tomato (*Lycopersicon esculentum* Mill.) during seed germination stage. *Physiol Mol Biol Plants*. 2012;18(1):45–50. <https://doi.org/10.1007/s12298-011-0097-z>
- Nasri N, Saïdi I, Kaddour R, Lachaâl M. Effect of salinity on germination, seedling growth and acid phosphatase activity in lettuce. *Am J Plant Sci*. 2015;6(1):57–63. <https://doi.org/10.4236/ajps.2015.61007>
- Awasthi P, Karki H, Bargali KI, Bargali SS. Germination and seedling growth of pulse crop (*Vigna* spp.) as affected by soil salt stress. *Curr Agric Res J*. 2016;4(2):159–70. <https://doi.org/10.12944/CARJ.4.2.05>
- Adnan M, Shahzad B, Collins M, Bajwa AA. Seeds of success: seed biology and germination response of *Gazania* weed in Australia. *Front Agronomy*. 2025;7:1596787. <https://doi.org/10.3389/fagro.2025.1596787>
- Bewley JD, Bradford K, Hilhorst H. Seeds: physiology of development, germination and dormancy. New York: Springer; 2012. <https://doi.org/10.1007/978-1-4614-4693-4>

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonpublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAAS, UGC Care, etc
See https://horizonpublishing.com/journals/index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (<https://creativecommons.org/licenses/by/4.0/>)

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.