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Methodological aspects of biotesting - comparative analysis of water uptake dynamics in seeds

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Abstract. The water absorption capacity (Ws%) and hydration potential (W%) of seeds are crucial physiological indicators for laboratory screening studies aimed at identifying allelepathic interactions between plant species. Assessing these characteristics in recipient test plants is essential, as the degree of seed imbibition determines the potential for allelochemical uptake. Temperature conditions must align with the biological requirements of the seeds, as they can influence both water absorption dynamics and the water absorption rate (R, g absorbed H_2O h^{-1}). This study investigates the water absorption capacity (Ws%) of *Lophanthus anisatus* (Nutt.) Benth. seeds and evaluates their suitability as a recipient test species in laboratory allelopathic bioassays, in comparison to the reference species *Lactuca sativa* L. Clear species-specific differences in water uptake were observed under controlled conditions. For *L. anisatus*, Ws% ranged from 16.6% at 15°C to 24.3% at 30°C, while in *L. sativa*, it remained stable at 39.2–41.6% across different temperatures. Similarly, hydration capacity (W%) for *L. anisatus* varied from 17.7% to 26.2%, whereas it remained consistent for *L. sativa* at 42.8–44.8%. The maximum water uptake rate (R, g absorbed H_2O h^{-1}) for both species occurred during the first hour of imbibition and stabilized by the fourth hour. The greater temperature sensitivity of *L. anisatus* suggests an enhanced potential for allelochemical absorption at elevated temperatures. In contrast, *L. sativa* remains a reliable reference species. Further research with different allelochemical carriers and concentrations is necessary to improve the application of *L. anisatus* in allelopathic screening.

Key words: seed water absorption, hydration capacity, imbibition, *Lophanthus anisatus*, *Lactuca sativa*, laboratory bioassays.

Introduction

Agastache foeniculum (Pursh) Kuntze, also known as *Lophanthus anisatus* (Nutt.) Benth. is a perennial herbaceous plant of the family Lamiaceae, widely recognised for its ornamental, aromatic, and medicinal value, as well as for its remarkable adaptability to diverse agroclimatic and edaphic conditions (Duda et al., 2013a; Chisnicean & Maricica, 2014; Zielińska & Matkowski, 2014; Vînătoru et al., 2015; Ivanov et al., 2019). Numerous studies (Omidbaigi & Sefidkon, 2003; Chumakova & Popova, 2011; Wesolowska, 2019) have descrybed the antimicrobial, antifungal, and antioxidant properties of *Agastache* species. According to Stefan et al. (2022), essential oils extracted from various vegetative parts of *L. anisatus* contain biologically active compounds such as estragon, limonene, eugenol, chavicol, benzaldehyde, and pentanol.

Vînătoru et al. (2015) and Kormosh et al. (2020) reported that *Lophanthus anisatus* shows high tolerance to temperature stress, withstanding both low and elevated temperatures, as well as intense light exposure. The authors note that, under thermal stress, adaptive mechanisms such as increased carotenoid content and anthocyanin accumulation are activated, resulting in a visible shift in leaf colouration toward green-purple hues.

According to Zhekova et al. (2010) and Zielińska & Matkowski (2014), the chemical composition of essential oils and the profile of biologically active compounds in *Agastache* species vary with their ecogeographical origin. Sporadic studies have also investigated the allelopathic potential of these species (Kotyuk & Rakhmetov, 2014). The authors found that aqueous extracts of *L. anisatus* exert a strong inhibitory effect on the germination and early development of *Triticum aestivum* L. and *Zea mays* L., used as test acceptor species to assess allelopathic activity.

Several studies have provided anatomical and morphological data on the genus *Agastache*, including its variability and productivity in terms of fresh and dry biomass. However, data on the water absorption capacity of the seeds and their use as test species in allelopathic screening under laboratory conditions remain limited (Duda et al., 2013b; Melnychuk & Rakhmetov, 2016; Vârban et al., 2022).

According to de Pinho et al. (2004), Bhardwaj et al. (2014), Ali & Elozeiri (2017), and Sghaier et al. (2022), the water absorption capacity of seeds is influenced by several factors - seed coat permeability, chemical composition, viability, and size fraction - which may vary depending on imbibition temperature. Although seed water absorption has been identified as a limiting factor in allelopathic screening, related studies remain scarce (Ladhari et al., 2020).

Allelochemicals present in plant residues can inhibit germination and early growth of test species (Khaliq et al., 2011; da Silva et al., 2017). Their activity often depends on the extraction method and their biochemical stability (Singh et al., 2021; Markhali & Teixeira, 2024). Therefore, seed water absorption - and consequently, the uptake of allelochemicals from aqueous extracts - should be considered a critical factor in laboratory bioassays assessing allelopathic interactions.

Lactuca sativa L. is widely used as a highly sensitive recipient species in allelopathic bioassays, suitable for detecting the presence and intensity of allelochemical effects (Sun et al., 2022; Patanè et al., 2023). Despite growing interest in the allelopathic activity of *L. anisatus* biomass (Kotyuk & Rakhmetov, 2014) and its essential oils (Haas et al., 2023), information on the water absorption capacity of its seeds and their suitability as test species for in vitro screening remains insufficient.

In this context, the present study aims to determine the water absorption capacity of *Lophanthus anisatus* (Nutt.) Benth. seeds and compare it with that of *Lactuca sativa* L., with a primary focus on evaluating the suitability of *L. anisatus* as a test species for laboratory allelopathic screening assays.

Materials and methods

Experimental conditions

The experiment was conducted in 2024 under controlled laboratory conditions at the Institute of Ornamental and Medicinal Plants in Sofia, Bulgaria. Seeds of *Lophanthus anisatus* (Nutt.) Benth. used in the study were collected in 2023 from the institute's working collection. For comparison, seeds of *Lactuca sativa* L. were used as a reference species. All seeds were stored under identical conditions before the experiment to ensure uniform moisture content and viability.

Seed water absorption experiment

Seeds of each species were manually cleaned of impurities. A total of 1000 visually undamaged seeds were selected, weighed on an analytical balance (±0.000 g), and placed between Filtrak 383 filter paper in Petri dishes (40 mm diameter). Five milliliters of pre-tempered distilled water at 15°C, 20°C, and 30°C ± 2°C were pipetted into each Petri dish. The dishes were sealed with Parafilm "M" and incubated in the dark in thermostats set at the corresponding temperatures. Each treatment was replicated four times.

After each soaking period (1, 2, 3, 4, 5, and 6 h), the remaining water in the Petri dishes was decanted, and the seeds were separated using a Büchner funnel connected to a vacuum pump. Immediately before weighing (±0.000 g), the seeds were surface-dried on triple-layer absorbent paper without applying pressure (Marinov-Serafimov et al., 2024).

Determination of water absorption parameters

The water absorption capacity (W_s%) (g absorbed H_2O g^{-1} seeds) was calculated according to Hidayati et al. (2001):

$$W_s\,\% = \frac{W_i - W_d}{W_d} \times 100$$

where W_i represents the seed mass (g) after each soaking period (1–6 h), and W_d denotes the mass of air-dry seeds (g) or the weight obtained after the previous soaking period until a constant sample weight was achieved.

The hydration degree (W%) was calculated according to Bewley et al. (2013):

$$W\% = \frac{M_2 - M_1}{M_1} \times 100$$

where M_1 is the initial mass of dry seeds, and M_2 is the seed mass after soaking for 6 h.

The seed water absorption rate (R, g absorbed H_2O h^{-1}) was measured for each soaking period following Mamonov & Kim (1978):

$$R = \frac{W_2 - W_1}{t_2 - t_1}$$

where W_1 is the initial dry seed mass (g), W_2 is the seed mass (g) at each time interval, and (t_2-t_1) represents the soaking duration (h).

Statistical analysis

Statistical analysis of the experimental data was conducted using STATGRAPHICS Plus for Windows, Version 2.1. Both one-way and two-way designs were employed in the analysis of variance (ANOVA). Fisher's least significant difference (LSD) test was performed at a 95% confidence level ($\alpha = 0.05$) to identify significant differences between means. The strength of the factor effects was assessed using the coefficient of determination (η^2), which was based on reliable factor variance, following the method outlined by Plokhinsky (1967).

Results and Discussion

Analysis of the experimental data presented in Table 1 indicates that the mass of 1000 seeds varied considerably among the studied species. In *L. anisatus*, the values ranged from 0.300 to 0.380 g, whereas in *L. sativa* the range was 1.093–1.100 g. These differences were statistically significant at $p \leq 0.05$. The experimental results obtained for the mass of 1000 seeds in *L. anisatus* differ from those reported by Dusmuratova et al. (2020) and Karpukhin et al. (2020), who found values ranging from 1.0 to 1.35 g. According to these authors, variation in seed mass depends on specific agrometeorological and edaphic conditions under which the ontogenetic development of plants occurs.

Table 1. Seed weight per 1000 seeds (g^{-1}) of the studied species.

Species	Average mass of 1000 seeds (g)	± SE
Lophanthus anisatus (Nutt.) Benth	0.340[a]	0.005
Lactuca sativa L.	1.097[b]	0.013

Legend: a, b - statistically significant differences at $p \leq 0.05$. Values indicated by the same letter are statistically insignificant; SE - standard error; MS – the mean sum of squares – ANOVA.

Analysis of the experimental results presented in Table 2 reveals that the applied temperature range (15–30°C) has a significant impact on the water absorption capacity (W_s%) and hydration level (W%) of the seeds of *L. anisatus* and *L. sativa*.

In *L. anisatus*, increasing the temperature from 15°C to 30°C causes a significant increase in W_s% from 16.6% to 24.3% and in W% from 17.7% to 26.2%, which corresponds to an approximately 45–50% relative increase in the hydration potential of the seeds. The differences are statistically significant ($p \leq 0.05$), confirming a clear temperature dependence of the water absorption capacity of the seeds in this species.

The experimental results obtained are consistent with the findings of Bradford (1990) and Bewley & Black (1994). These studies indicate that an increase in temperature during seed imbibition enhances membrane permeability and activates metabolic pathways associated with water exchange. In contrast to the observed trends in L. *anisatus*, the seeds of *L. sativa* exhibited relatively consistent values for water absorption capacity (W_s%) and hydration level (W%) across temperature ranges of 15, 20, and 30°C. Specifically, W_s% ranged from 39.2% to 41.6%, and W% ranged from 42.8% to 44.8%. The differences in these values were statistically insignificant. This consistent

water absorption capacity in *L. sativa* seeds can be attributed to effective physiological regulation and adaptive resistance to the tested temperature range.

In the comparative analysis of *L. anisatus* and *L. sativa* regarding their water absorption capacity and hydration ability, it was found that the seeds of *L. anisatus* exhibit greater sensitivity to temperature changes during seed imbibition. The observed increase in water absorption by *L. anisatus* seeds at higher temperatures in the imbibition medium is likely to enhance the uptake of allelechemicals into the seed tissues. This characteristic makes *L. anisatus* a suitable test plant for studying allelopathic interference under laboratory conditions, in contrast to *L. sativa*, which demonstrates consistent water absorption behavior and is considered a reference species. The significance of seed water absorption capacity in recipient test plants is crucial for understanding allelopathic interference under laboratory conditions. Several studies indicate that the dynamics of seed water absorption play a vital role in determining the extent to which allelochemicals are absorbed by the seed tissue. This ab-sorption, in turn, influences seed germination and the initial development of the test plants (Al Ha-run et al., 2017; Patanè et al., 2023; Li et al., 2025). The results of the two-way analysis of variance (ANOVA) conducted to evaluate the effect size of the factors (η^2) indicate that the majority of the total variation in water absorption capacity (W_s%) and hydration level (W%) of the seeds is attributed to Factor A (species), which accounts for 84.6% of the total variance (η^2). In contrast, Factor B (temperature) has a relatively minor contribution to the overall variance, accounting for only 2.1 to 2.2%. The interaction between the two factors (A×B) demonstrates a moderate but statistically significant effect, with an η^2 value of approximately 3.5 to 3.8% ($p \leq 0.05$) (Table 2).

The analysis of the experimental data presented in Table 2 shows that the seeds of *L. anisatus* are characterized by a high factorial loading, which is attributed to their increased hydration activity and sensitivity to temperature. In contrast, the seeds of *L. sativa* demonstrate low variability in response to the applied temperature range and exhibit stable hydration parameters.

These differences between the two plant species highlight the importance of determining the water absorption capacity of the seeds of the test plants prior to conducting allelopathic screening studies in the laboratory. The hydration capacity of the seeds, depending on the temperature range used, can influence the extent to which available allelochemicals diffuse into the seed tissues.

Table 2. Effect of the temperature range (15–30°C) on the water absorption capacity (W_s%) and hydration degree (W%) of *L. anisatus* and *L. sativa* seeds.

Species (Factor A)	**Temperature, °C (Factor B)**		**Water absorption capacity (W_s%)**		**Hydration level (W%)**			
L. anisatus	15		16.6[a]		17.7[a]			
	20		15.9[a]		16.8[a]			
	30		24.3[b]		26.2[b]			
L. sativa	15		41.6[a]		44.8[a]			
	20		39.6[a]		43.2[a]			
	30		39.2[a]		42.8[a]			
Effect of the factors (Analysis of Variance, ANOVA)								
Water absorption capacity (Ws%)					**Hydration level (W%)**			
Independent effects of each factor	Factor A		Factor B		Factor A		Factor B	
	a_1	18.91[a]	b_1	28.83[a]	a_1	20.21[a]	b_1	31.23a
	a_2	40.15[b]	b_2	27.56[a]	a_2	43.62[b]	b_2	30.01a
			b_3	32.21[b]			b_3	34.51[b]
Influence of interactions between factors			MS	η^2		MS		η^2
	A		4638.7	84.6	A	5636.2		84.5
	B		57.3	2.1	B	74.4		2.2
	AxB		102.9	3.8	C	116.4		3.5

Note: *W_s% – water absorption capacity; W% – hydration level; a, b – statistically significant differences between mean values at a probability level of $p \leq 0.05$; η^2 – estimate of the effect size of factors and their interactions in the factorial space Plokhinsky (1967).*

The results shown in Figs 1, 2, and 3 demonstrate a clear relationship between the applied temperature ranges (15, 20, and 30°C) and the water absorption capacity (W_s%) of the seeds of *L. anisatus* and *L. sativa*. While there is a noticeable trend indicating that water absorption capacity decreases with increasing exposure time (h), the overall amount of absorbed water continues to rise in both species. This observation aligns with findings from Doria et al. (2019), Nonogaki et al. (2010), and Li et al. (2025), who noted that the initial phase of water absorption is marked by high intensity, followed by a stabilization period where the rate of absorption decreases, yet the total water content in seed tissues continues to increase until it reaches equilibrium.

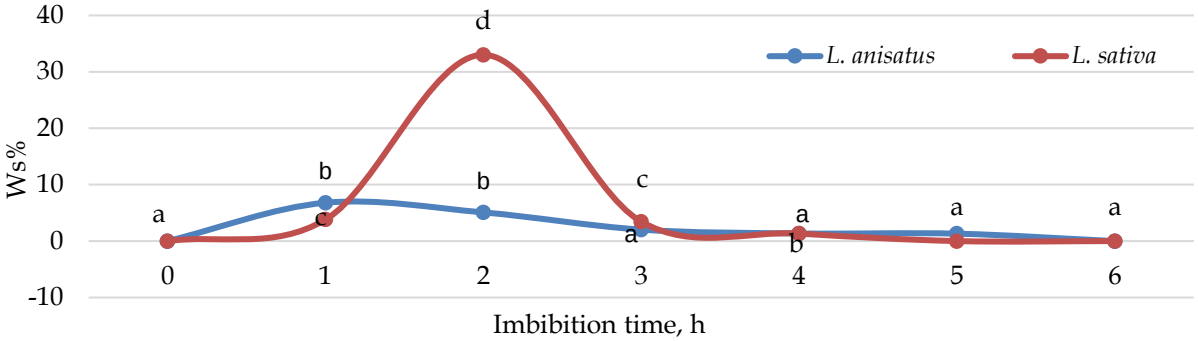

Fig. 1. Water absorption capacity of seeds from *L. anisatus* and *L. sativa* at 15°C.
Legend: a, b, c - LSD multiple range test. Treatments bearing the same letter are not significantly different at p≤0.05.

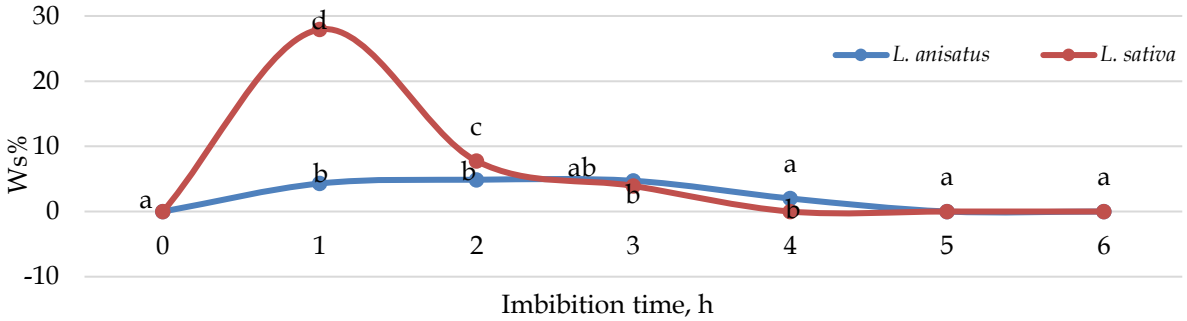

Fig. 2. Water absorption capacity of seeds from *L. anisatus* and *L. sativa* at 20°C.
Legend: a, b, c - LSD multiple range test. Treatments bearing the same letter are not significantly different at p≤0.05.

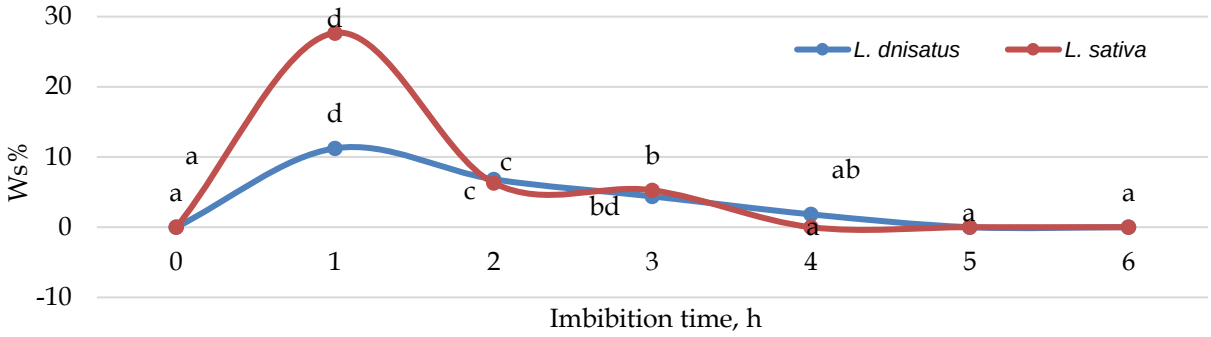

Fig. 3. Water absorption capacity of seeds from *L. anisatus* and *L. sativa* at 30°C.
Legend: a, b, c, d - LSD multiple range test. Treatments bearing the same letter are not significantly different at p≤0.05.

The analysis of the data presented in Table 3 clearly demonstrates species-specific and temperature-dependent differences in the dynamics of seed water absorption. For Factor A (species), during the initial phase of imbibition (1–2 hours), the seeds of *L. sativa* exhibited a significantly higher water absorption capacity (18.9% to 14.8%) compared to *L. anisatus*, which showed a lower absorption (8.8% to 5.8%). As the imbibition period extended to 3–5 hours, water absorption decreased sharply in both species, ultimately reaching equilibrium values. Similar trends were observed regarding Factor B (temperature), with the highest water ab-sorption recorded at 30°C (19.2%) and relatively lower absorption at 15°C (5.3%). The differences in water absorption associated with the duration of imbibition remained consistent across both species and temperatures.

The results of the two-way analysis of variance (ANOVA) for assessing the effect size of the factors (η^2) related to the hierarchical distribution of the variation in seed water absorption capacity (W_s%) at different time intervals of water absorption (1–5 h) show a clearly expressed dominance of Factor A (species), with the highest η^2 values recorded at the first (23.9%) and second (19.3%) hour. With the extension of the imbibition period to 3–4 h, the values decrease significantly (0.8–14.3%), reaching a minimum value of 0.001% at the fifth hour. Factor B (temperature) demonstrates a more pronounced influence in the factorial plane (η^2 = 2.6–51.4%), with the maximum value recorded at the fifth hour of seed imbibition (η^2 = 51.4%), while the interaction between the factors (A×B) determines a secondary but statistically proven effect (η^2 = 0.002–43.7%) at ($p \leq 0.05$) (Table 3).

The data presented in Fig. 4 demonstrates that the water absorption rate (R, g absorbed H_2O h^{-1}) of the seeds from the studied plant species is significantly influenced by temperature. For *L. anisatus*, the highest water absorption rate was recorded at 30°C, reaching 0.037 g absorbed H_2O h^{-1}. This was followed by rates of 0.022 g absorbed H_2O h^{-1} at 20°C and 0.015 g absorbed H_2O h^{-1} at 15°C, with a sharp decrease in the absorption rate occurring after the first hour of imbibition.

Table 3. Analysis of variance and degree of influence of the factors on the water absorption capacity (W_s%) of the species of *L. anisatus* and *L. sativa.*

Imbibition time, h	***1***		***2***		***3***		***4***		***5***	
Species (Factor A)										
L. anisatus	8.8[a]		5.8[a]		3.8[a]		1.6[a]		0.4[a]	
L. sativa	18.9[b]		14.8[b]		4.1[a]		0.4[a]		0.4[a]	
Temperature, °C (Factor B)										
15.0°C	5.3[a]		17.1[b]		2.6[a]		1.3a		1.3[b]	
20.0°C	16.1[b]		5.7[a]		4.4[b]		1.1a		0.0[a]	
30.0°C	19.2[c]		6.6[a]		4.8[b]		1.1a		0.0[a]	
Influence of the factors										
Factors	*MS*	η^2	*MS*	η^2	*MS*	η^2	*MS*	η^2	*MS*	η^2
A	500.3	23.9	394.9	19.3	0.4	0.8	6.5	14.3	0.0002	0.001
B	390.4	37.4	383.6	37.5	7.7	27.4	0.6	2.6	3.9	51.441
AxB	282.0	27.0	447.0	43.7	2.1	7.5	1.8	7.8	0.0002	0.002

Legend: a, b – statistically significant differences at $p \leq 0.05$. Values indicated by the same letter are statistically insignificant; MS – the mean sum of squares – ANOVA; η^2 – estimate of the effect size of factors and their interactions in the factorial space Plokhinsky (1967).

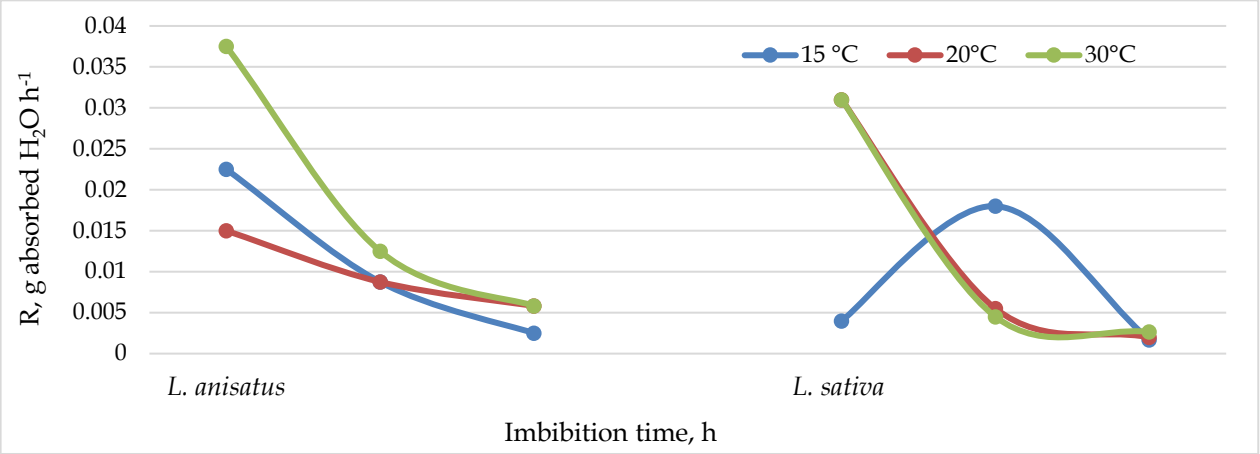

Fig. 4. Water absorption rate (R, g absorbed H_2O h^{-1}) of seeds of *L. anisatus* and *L. sativa* at different imbibition temperatures.

Legend: a, b - LSD multiple range test. Treatments bearing the same letter are not significantly different at $p \leq 0.05$.

In the case of *L. sativa*, the maximum water absorption rate was also observed at 30°C during the first hour, measuring 0.036 g absorbed H_2O h^{-1}. How-ever, at 15°C, the peak absorption rate was noted during the second hour, reaching 0.018 g absorbed H_2O h^{-1}. This suggests a slower but more sustained hydration process for the seeds at lower temperatures.

The obtained experimental results determine differences between the two species studied, likely caused by the morphophysiological characteristics of the seed coat. In *L. anisatus*, the lower permeability of the test limits the water absorption rate, while in the reference species, *L. sativa*, it is higher, suggesting more intensive water exchange. With the increase in temperature from 15°C to 30°C, a clearly expressed increase in water absorption rate (R, g absorbed H_2O h^{-1}) was recorded, rising from 0.018 g absorbed H_2O h^{-1} (15°C, 2 h) to 0.036 g absorbed H_2O h^{-1} (30°C, 1 h) in *L. sativa*, and from 0.015 to 0.037 g absorbed H_2O h^{-1} in *L. anisatus*. These results confirm the higher temperature sensitivity and faster imbibition dynamics of *L. sativa*, whereas *L. anisatus* demonstrates a more stable and resilient hydration response, characteristic of species with high ecological plasticity and stability, adapted to develop under a wide range of agroecological and edaphic conditions.

Conclusions

Species-specific differences in seed water absorption (W_s%) between *Lophanthus anisatus* (Nutt.) Benth. and *Lactuca sativa* L. were clearly observed under controlled laboratory conditions. In *L. anisatus*, the water absorption capacity (W_s%) varied from 16.6% at 15°C to 24.3% at 30°C. In contrast, *L. sativa* exhibited stable values ranging from 39.2% to 41.6%, regardless of the temperature (15–30°C). Similar trends were noted for hydration capacity (W%), which increased from 17.7% to 26.2% in *L. anisatus*, while *L. sativa* maintained values between 42.8% and 44.8%.

The water absorption rate (R, g absorbed H_2O h^{-1}) of *L. anisatus* seeds was comparable to that of *L. sativa*, with the highest uptake occurring within the first hour of imbibition and stabilizing by the fourth hour, irrespective of temperature conditions

The greater temperature sensitivity and hydration dynamics in *L. anisatus* indicate a higher potential for allelochemical uptake at elevated temperatures, while the stable hydration profile of *L. sativa* confirms its relevance as a reference species in allelopathic bioassays. Further research is needed to explore different allelochemical carriers and concentrations to enhance the use of *L. anisatus* as a recipient species in allelopathic screening studies.

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