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Improvement of lucerne germination and seedling performance through a combined seed priming method

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Abstract: Seed priming is an effective seed treatment procedure and has been shown to improve the emergence of seedlings in various crops. However, there is a lack of systematic research for these techniques in lucerne (*Medicago sativa* L.), especially for combinations of priming agents. This study aimed to screen 22 biologically active compounds and then to evaluate the potential of combinations of these agents, assessing the dynamics of germination, seedling length, and performance, in a pot experiment for selected combinations. About half of the screened agents increased germination rate (on the 3rd day) or seedling length (from 8% to 75%), where chitosan and green tea improved total germination and seedling formation. The selected combination of priming agents improved only seedling growth compared to hydropriming and control, where the combination of fermented weed juice + green tea and H_2O_2 + thyme infusion seems effective (+61%). In the pot experiment, only a combination of mixed priming with the coating method led to improved lucerne root growth (+33% compared to the untreated control). These results can contribute to the adoption of easily available, cost-effective, and sustainable treatments with the potential to accelerate germination and lucerne seedling development.

Keywords: legume; alfalfa; abiotic stress; bioactive stimulants; early growth enhancement

For all crops, the time between sowing and emergence is crucial during which seeds and seedlings are vulnerable to various risks, such as cold/drought/salinity, diseases, pests and competition with other plants (Undersander et al. 1997). A shorter critical period supports the viability and development of seedlings, especially in the field environment where management of conditions is usually limited. Lucerne (*Medicago sativa* L.) is a perennial forage where seed inoculation with bacteria and nutrients could serve as an insurance for a well-established and productive crop (Tang et al. 2025). Among techniques that have the potential to support seedling development is seed priming based on controlled moistening; water at 90% to 100% of the seed weight is recommended for lucerne, where this process may take several hours or days, depending on temperature (Liu et al. 2008). Any reduction in the germination was not observed up to six months of storage after seed priming (Tu et al. 2022). This technique is generally considered an easy, sustainable, cost-effective, and attractive alternative for resource-poor farmers (Mondal and Bose 2019).

Various solutions and suspensions with variable modes of action can serve as priming agents. Some of these agents are difficult to fully define chemically

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and may also combine different mechanisms of action. Antimicrobial properties of agents can be associated with enhancement of final germination, as shown with infusions of spent tea leaves for improvement of pepper germination (Gammoudi et al. 2021) or of cinnamon essential oil for common peas (Bravo Cadena et al. 2018). There could also be various compounds that promote the growth of plants, including amino acids and hormones, the effects of which were summarised by Craigie (2011) for seaweed extracts. Hossain et al. (2024) consider the seaweed-derived carrageenans to be biostimulants and elicitors for enhancing crop emergence. The stimulating activities of thyme oil are also well documented for the seed coating method (Ben-Jabeur et al. 2023). To improve seed stress tolerance, hydrogen peroxide (H_2O_2) as a reactive oxygen agent (ROA) delivered by seed priming in the appropriate dose also promotes lucerne seed germination (Muñoz-Salinas et al. 2021) *via* mechanisms described by Wojtyla et al. (2016). Smoky compounds known as karrikins could also be mentioned, which can be delivered through wood ash, smoke treatment, smoke water, or karrikins themselves *via* seed priming (Moyo et al. 2022). In addition, priming agents can provide necessary nutrients to compensate for the seed's inherent nutrient deficiencies, thereby supporting proper germination and seedling development (Muhammad et al. 2015). Boron (borax) solution was successfully tested as a lucerne seed priming agent (Xia et al. 2019). Sulfur deficiency is believed to limit N_2 fixation by lowering ferredoxin, ATP, and leghemoglobin concentrations in lucerne plants (Scherer et al. 2008). In addition to commercial fertilisers, there are also alternative sources of nutrients where fermented plant juice has been used successfully as a seed priming agent to enhance soybean germination, vigour, and seedling length when it outperformed commercial organic fertiliser (Cejalvo and Mercado 2018). The liquid fraction of vermicompost, known as vermicompost tea, is a valuable source of nutrients with phytohormones, humic acids, and antioxidants, which help germinating seeds overcome various stresses (Benazzouk et al. 2019).

Szabó et al. (2023) summarised the potential benefits of lucerne's most frequent priming and coating methods, showing the benefits of chitosan and potassium permanganate ($KMnO_4$) as the most promising treatments. However, that review also showed that the list of potential agents is much broader, and their range remains untested as potential lucerne seed priming agents. Additionally, combinations of different action modes could be potentially effective for improving seedling development and protection. Positive response to combined priming treatments has been reported for pea yield parameters with a combination of *Bacillus thuringiensis* and carrot extract (Arafa et al. 2021). Ayoub et al. (2018) reported that the synergistic action of fungicide and peroxyacetic acid allowed for minimising the amount of applied fungicide by more than 95% in field control of *Botrytis cinerea*. There remains a lack of systematic research comparing the effects of potential synergies of seed treatment combinations. Therefore, this study aimed to screen the impact of a wide range of lucerne seed priming substances on seed germination, followed by testing selected combinations in a pot experiment associated with assessing lucerne productivity and root traits.

MATERIAL AND METHODS

A series of experiments was conducted to screen 22 seed priming agents for lucerne, to evaluate the effects of combined seed priming methods, and to test the most promising combinations in a pot experiment. In all the experiments reported here, lucerne *Medicago sativa* L. var. Frigos seeds (provided by Seed Service,Vysoké Mýto, Czech Republic), with a germination rate of 80% and WTS (weight of thousand seeds) of 1.9 g, were primed four days before the experimental setup (48 h for priming and two days for redrying). For germination tests, one hundred seeds were placed onto filter paper inside a disinfected glass Petri dish and subsequently irrigated with 10 mL of distilled water. Four replications were conducted, with up to 10 treatments within each experiment set. Petri dishes were placed at 20 °C under the 12/12 dark-light regime in a Binder KBWF 240 growth chamber (Binder GmbH, Tuttlingen, Germany). Seedlings were counted daily and, after seven days, 30 randomly selected seedlings were measured for their length, including both shoot and root parts.

Screening tests of priming agents. The screening experiment was conducted in three series, and the list of tested priming agents with applied concentrations is shown in Table 1. The priming parameters were based on Liu et al. (2008), and minimal concentrations were selected to avoid osmotic stress (O'Leary et al. 1970). Saccharose ($C_{12}H_{22}O_{11}$), salt (NaCl), green tea (Lipton), red sweet pepper (Kotányi), thyme (Kotányi), Ceylon cinnamon (Kotányi), chitosan ($C_{56}H_{103}N_9O_{39}$), commercial vermicompost tea Bopon

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Table 1. Concentrations (weight/volume) of suspensions and infusions for the screening test of priming agents

Seed priming agent	Concentration (%)
Distilled water	100
SulkaK	1
Haifa MKP	1
Urea	1
Urine	100
Potassium permanganate ($KMnO_4$)	1
Boric acid	1 and 3
Agroptim Sunset	1.5
Fermented weed juice (FWJ)	100
Algomel Push	1.5
Hydrogen peroxide (H_2O_2)	1
Cinnamon suspension	1
Saccharose	1
Pepper suspension	1
Thyme infusion	10
Green tea infusion	10
Chitosan	1
Wood ash	1
Smoke water	100 (10 min passage through)
Vermitea homemade	1
Vermitea commercial	1
Sodium chloride (NaCl)	1

Natural (Bros Sp., Poznaň, Poland), SulkaK with 3% N, 14% K_2O, 2% CaO, and 19% S (Duslo a.s., Šala, Slovak Republic), potassium permanganate ($KMnO_4$), urea (CH_4N_2O), Haifa MKP with 34% K_2O and 52% P_2O_5 (Haifa Group, Haifa, Israel), and boric acid (H_3BO_3) were purchased from commercial sources. Algomel Push (extract from *Solieria filiformis* with 7.5% Mn and 13% SO_3) and Agroptim Sunset (with 3% N, 3.5% K_2O, 0.5% MgO, 1.4% Na and 0.02% Cu) were provided by Olmix company (Olmix Group, Brehan, France). Homemade vermicompost tea (vermitea, produced by *Eisenia andrei)* and fermented weed juice (FWJ, mainly based on *Stelaria media* and *Sonchus arvensis*) were produced within homemade systems, both following standard procedure (half a year of maturation for FWJ). Wood ash was prepared by the combustion of spruce trees. Smoke water was produced by bubbling smoke from the combustion of spruce wood through distilled water for 10 min; at the same time, it was dedicated to smoking seeds, as Sparg et al. (2006) suggested. Distilled water was used in all suspensions and infusions.

Experiment combining seed priming agents. For combined seed priming methods, the selection of agents was based on the screening experiments, where the best-performing agents were divided into four groups based on the expected primary mode of action: source of nutrients, plant stimulants, ROA, and substances with antifungal properties. Combined priming agents were applied together in concentrations shown in Table 2 when soaking time, temperature, and drying time were identical to screening experiments. The description of 8 treatments based on combinations of four agents together is presented as T3–T10 (Table 2). Germination dynamics and seedling length were compared with those of the control (T1) and hydropriming (T2) treatments.

Performance of selected treatments in the pot experiment. Treatments T1, T2, T6, and T9 (Table 2)

Table 2. Concentrations (conc.) (weight/volume) of suspensions (sp.) and infusions (inf.) of priming agents in combinations used in the experiment, combining various seed priming agents based on their main mode of action

Treatment	Source of nutrients	Conc.	Stimulants	Conc.	ROA	Conc.	Antifungal	Conc.
T1	–	–	–	–	–	–	–	–
T2	Hydropriming	100	–	–	–	–	–	–
T3	Agroptim Sunset	0.25	Chitosan	0.25	$KMnO_4$	0.25	Cinnamon	0.25
T4	FWJ	99.0	Chitosan	0.33	$KMnO_4$	0.33	Cinnamon	0.33
T5	Haifa MKP	0.25	Chitosan	0.25	H_2O_2	0.25	Cinnamon	0.25
T6	Ash	0.25	Chitosan	0.25	$KMnO_4$	0.25	Cinnamon	0.25
T7	FWJ	99.0	Green tea inf.	10.0	$KMnO_4$	0.50	Cinnamon	0.50
T8	Haifa MKP	0.33	Pepper sp.	0.33	H_2O_2	0.33	Thyme inf.	10.0
T9	FWJ	99.0	Green tea inf.	10.0	H_2O_2	0.50	Thyme inf.	10.0
T10	Haifa MKP	0.25	Pepper sp.	0.25	$KMnO_4$	0.25	Cinnamon	0.25

ROA – reactive oxygen agent; FWJ – fermented weed juice; $KMnO_4$ – potassium permanganate; H_2O_2 – hydrogen peroxide

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were selected for final testing in the pot experiment based on results from the germination experiment. The treatments provided alleviated germination rates and seedling lengths. Treatment 9 (FWJ/Green tea/H_2O_2/Thyme infusion) was also additionally tested in combination with gypsum + bentonite coating (ratio 1:1) based on positive effects observed by Szabó et al. (2023). The pot experiment was established on 22nd June in four replicates. The low-fertility loamy Cambisol was used in the experiment, and the pots were placed at 20 °C, 60% air humidity, under the 12/12 dark-light regime in a Binder KBWF 240 growth chamber (Binder GmbH, Tuttlingen, Germany). Fifty seeds were sown in 15 × 15 × 25 cm containers and irrigated daily. The number of plants was counted on 25th, 27th, and 29th June. The experiment was terminated on 27th July 2024 (35 days after sowing) when the number of plants, stem length (SL, mm), root length (RL, mm), tap-root diameter (TD, mm), lateral root numbers (LRN, number of root branches above 1 mm per plant), and dry plant weight (DPW, g dry matter per pot) were assessed.

Statistical analyses. All measured data were analysed for each experiment using one-way ANOVA with a fixed effect of treatment. Values of germination rate expressed as percentage were arcsine transformed, and back-transformed – means are presented in the results. When the treatment effect was significant at $\alpha = 0.05$, the means were compared using the Tukey post-hoc test at $\alpha = 0.05$. The data were analysed using the Statistica 13.0 program (StatSoft, Tulsa, USA).

RESULTS

Screening experiments. The results of the three series of screening experiments are shown in Table 3, where back-transformed means of germination are presented. Almost all the priming agents significantly improved the germination rate on the 2nd day (16 out of 24) and seedling length on the 7th day (13 statistically significantly out of 24) compared to the untreated control. However, the higher germination rates of almost all priming agents did not persist until the end of the germination experiment, except for chitosan and green tea, which had significantly higher germination rates (+17% and 14% respectively). The treatments with a significant positive effect on seedling length ranged from chitosan (+75%) to Agroptim Sunset (+11%). Other agents showed neutral (vermicompost tea commercial or smoke water) or negative effects (Algomel Push, urine, urea, and boric acid) on germination or seedling length. SulkaK, wood ash, $KMnO_4$ and Haifa MKP positively affected the germination from 2nd to 4th days; however, they did not influence total germination and seedling length compared to the control.

Experiment combined seed priming agents. The effect of combined priming on seed germination and seedling length is shown in Table 4, where back-transformed means of germination are presented. Within this experiment, hydropriming did not show statistically significant differences from the control. Only treatment 9 consistently improved seed germination from the 2nd to the 4th day relative to the control, but not to hydropriming. Treatment 9 also reached the highest seedling length (+61%), and treatments 3, 6, 7, and 8 increased seedling length from +14% to +21% compared to the control.

The pot experiment. A comparison of five selected treatments in plant traits is presented in Table 5. A positive result was observed for FWJ/Green tea/H_2O_2/Thyme infusion + coating at root length, with the treatment exceeding control by about 33%. There was also the highest value of stem length ($P = 0.071$). Without the supplementary coating, the effect on root length with T9 was insignificant. Among other variables, there were no significant differences between treatments. Regarding the dynamics of plant numbers, no effect of treatments was observed (data not shown).

DISCUSSION

Screening experiments. Our screening of 22 potential priming agents demonstrated that about half of them were able to improve lucerne germination dynamics from the 2nd to the 3rd day, which is in line with the general advantage of primed seeds as described by Sivasubramaniam et al. (2011). Except for chitosan and green tea, this initial enhancement did not influence the total germination, in line with Szabó et al. (2023). Half of the priming agents improved seedling length, but this effect did not fully correspond with improved germination in all cases. Cinnamon, hydropriming, chitosan, green tea, Agroptim Sunset, FWJ, $KMnO_4$, Haifa MKP, thyme infusion, pepper suspension, and H_2O_2 can be generally considered as promising agents for lucerne priming. Smoke treatment did not influence germination dynamics; however, it improved seedling length by about 13%, which aligns with the findings of Hong and Kang (2011). Karrikins, naturally

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Table 3. The germination dynamics (%) from 2nd to 7th day and seedling length (mm) on the 7th day in three separate series

	Time (days)	2nd	3rd	4th	7th	Seedling length
Serie 1	Control	3.75[a]	30.9[ab]	58.1[ab]	71.1[ab]	38.6[b]
	Algomel Push	6.25[ab]	25.1[a]	47.7[a]	63.0[a]	23.7[a]
	Saccharose	11.8[abc]	45.1[bc]	59.8[b]	71.0[ab]	45.3[c]
	Cinnamon suspension	16.3[bcd]	44.8[bc]	60.1[b]	79.7[bc]	44.7[c]
	Vermitea homemade	21.0[cde]	49.1[c]	58.0[ab]	62.0[a]	52.8[de]
	Vermitea commercial	21.3[cde]	44.8[bc]	56.5[ab]	63.6[a]	37.9[b]
	Hydropriming	22.0[cde]	46.8[bc]	64.3[bc]	80.8[bc]	49.8[cd]
	Chitosan	25.5[de]	57.8[c]	71.6[c]	83.5[c]	67.6[f]
	Green tea	32.3[e]	58.6[c]	71.1[c]	81.3[c]	55.5[e]
	SEM	0.026	0.039	0.029	0.032	0.57
	P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Serie 2	Control	2.00[a]	33.6[b]	47.4[d]	79.3[c]	61.2[cd]
	Urine	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
	Boric acid 3%	0.00[a]	0.50[a]	10.5[b]	37.4[b]	23.5[b]
	Urea	6.00[a]	23.8[b]	33.5[c]	47.7[b]	26.1[b]
	Agroptim Sunset	27.6[b]	67.5[c]	70.0[e]	79.1[c]	68.1[e]
	FWJ	28.3[b]	67.7[c]	70.5[e]	81.0[c]	69.9[e]
	SulkaK	33.3[b]	65.3[c]	63.3[e]	77.8[c]	59.1[c]
	$KMnO_4$	31.5[b]	64.0[c]	64.8[e]	82.3[c]	67.2[de]
	Haifa MKP	40.0[b]	67.4[c]	72.1[e]	81.1[c]	66.1[de]
	SEM	0.029	0.036	0.032	0.039	0.92
	P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Serie 3	Control	7.50[ab]	32.6[bc]	64.9[bcde]	72.8[abc]	51.4[b]
	Boric acid 1%	2.33[a]	14.0[a]	44.7[a]	66.0[a]	43.9[a]
	Smoke treatment	4.75[a]	26.6[ab]	62.3[bc]	71.7[abc]	58.4[cd]
	NaCl	16.8[bc]	35.5[bc]	57.1[b]	66.2[a]	57.8[cd]
	Thyme infusion	22.0[c]	46.3[cde]	67.5[cdef]	75.4[abc]	62.6[d]
	Smoke water	25.0[cd]	45.0[cd]	64.6[bcd]	71.3[ab]	56.8[bcd]
	Pepper suspension	33.6[d]	64.2[f]	72.5[ef]	80.7[c]	60.3[cd]
	H_2O_2	33.8[d]	58.6[ef]	73.8[f]	79.8[bc]	61.1[cd]
	Wood ash	35.3[d]	55.5[def]	71.3[def]	78.0[bc]	56.3[bc]
	SEM	0.023	0.033	0.023	0.029	0.51
	P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

SEM – standard error of mean, presented for arcsine-transformed germination values; $n = 4$; one-way ANOVA; different letters document statistical differences for Tukey post-hoc test at $\alpha = 0.05$. FWJ – fermented weed juice; $KMnO_4$ – potassium permanganate; H_2O_2 – hydrogen peroxide; NaCl – sodium chloride

occurring also in wood ashes, have been reported as beneficial for seeds (Moyo et al. 2022), and our results showed a similar positive response of smoke treatments and wood ash in lucerne seedling growth. Observed effects of tested substances can be attributed to various mechanisms of action that have been previously described, where nutrients support plant assimilation whilst phytohormones, amino acids and antioxidants can stimuli plant growth and reduced the level of physiological stress of the plant during the initial period of growth (Craigie 2011, Soares et al. 2016, Benazzouk et al. 2019).

However, form, concentration and soaking time must continually be optimised. Even though boron

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Table 4. The germination dynamics (%) from 2nd to 7th day and seedling length (mm) on the 7th day in the experiment combining seed priming agents

	Treatment	2nd	3rd	4th	7th	Seedling length
T1	Control	17.0[a]	45.8[a]	49.8[a]	75.0[b]	41.5[ab]
T2	Hydropriming	35.3[ab]	56.6[ab]	59.9[ab]	76.0[b]	36.5[a]
T3	Agroptim Sunset/Chitosan/$KMnO_4$/Cinnamon	35.7[ab]	56.4[ab]	60.9[ab]	73.4[ab]	48.1[cde]
T4	FWJ/Chitosan/$KMnO_4$/ Cinnamon	38.5[ab]	46.8[a]	49.6[a]	59.1[a]	42.8[abc]
T5	Haifa MKP/Chitosan/H_2O_2/Cinnamon	36.3[ab]	55.1[ab]	59.3[ab]	72.5[ab]	43.3[bcd]
T6	Ash/Chitosan/$KMnO_4$/Cinnamon	39.7[ab]	59.5[ab]	67.2[ab]	72.9[ab]	50.4[e]
T7	FWJ/Green tea/$KMnO_4$/Cinnamon	39.8[ab]	58.3[ab]	63.3[ab]	69.3[ab]	49.0[de]
T8	Haifa MKP/Pepper sp./H_2O_2/Thyme infusion	39.5[ab]	57.3[ab]	64.0[ab]	73.7[ab]	47.4[cde]
T9	FWJ/Green tea/H_2O_2/Thyme infusion	55.8[b]	66.6[b]	69.7[b]	78.1[b]	66.9[f]
T10	Haifa MKP/Pepper sp./$KMnO_4$/Cinnamon	38.8[ab]	54.3[ab]	60.9[ab]	67.4[ab]	44.3[bcd]
SEM		0.051	0.045	0.045	0.041	0.47
P-value		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

SEM – standard error of mean, presented for arcsine-transformed germination values; $n = 4$; one-way ANOVA; different letters document statistical differences for Tukey post-hoc test at $\alpha = 0.05$, FWJ – fermented weed juice; $KMnO_4$ – potassium permanganate; H_2O_2 – hydrogen peroxide

was previously considered the desired nutrient for lucerne seedling development in line with concentration and time (Xia et al. 2019), there was a strong negative effect on lucerne germination observed in the present study under 1% and 3% solutions. This could be associated with the potential negative response of some lucerne cultivars to boron priming or different forms and longer duration of seed soaking in the present study (boric acid, 48 h) compared to Xia et al. (2020) with Borax, 24 h. The concentration and soaking duration should be carefully considered for further research in boric acid priming. Negative effect of urine observed in the present study can be associated with high concentration, but 1% urea seems to be an effective priming agent, like maize cultivation (Anosheh et al. 2011).

Undefined products provide variable results in seed treatment, where a 2% concentration of cinnamon powder reduced lucerne germination dynamics (Szabó et al. 2023). On the other hand, cinnamaldehyde, as the main bioactive compound of cinnamon essential oil, which was encapsulated into mesoporous silica nanoparticles, improved the germination of pea seeds (Bravo Cadena et al. 2018). In our study, prim-

Table 5. The effect of seed treatments on shoot length (SL, mm), root length (RL, mm), tap-root diameter (TD, mm), lateral root number (LRN, pcs of root branches above 1 mm per plant), and dry plant weight (DPW, g dry matter per pot) 35 days after sowing

	Treatment	SL	RL	TD	LRN	DPW
T1	Control	120	141[a]	1.13	2.46	1.20
T2	Hydropriming	150	114[a]	0.98	2.13	0.44
T6	Ash/Chitosan/$KMnO_4$/cinnamon	136	150[a]	1.27	2.67	1.47
T9	FWJ/Green tea/H_2O_2/Thyme infusion	132	156[ab]	1.22	2.93	1.03
T11	T9 + gypsum and bentonite coating	175	188[b]	1.34	2.63	1.82
SEM value		7.08	5.38	0.05	0.13	0.20
P-value		0.07	< 0.01	0.21	0.66	0.22

SEM – standard error of mean; $n = 4$; one-way ANOVA; different letters document statistical differences for Tukey post-hoc test at $\alpha = 0.05$. FWJ – fermented weed juice; $KMnO_4$ – potassium permanganate; H_2O_2 – hydrogen peroxide

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ing with a 1% concentration of cinnamon powder improved lucerne seedling growth but did not affect germination dynamics. Commercial vermicompost tea responded poorly to germination and seedling length compared to homemade vermicompost tea. This illustrates that its composition is not standard and strongly varies according to the source of organic matter and composting conditions. In rapeseed, the effect of priming with vermicompost tea on seed germination was most pronounced in older seeds and under salinity stress concerning several protective compounds (Benazzouk et al. 2019).

Experiment combined seed priming agents. Combined seed priming is rarely used in seed treatment techniques; therefore, directly comparing specific combinations is challenging. It is also essential to distinguish the effect of hydropriming from the priming effect of water in combination with other substances, where the positive impact of chitosan priming on lucerne green fodder yield was reported by Mustafa et al. (2022) when compared to hydropriming. In the present study, we observed no significant improvement in germination dynamics compared to hydropriming alone. A positive response was observed for seedling length, where 5 treatments out of 8 exceeded the control and hydropriming. The combination of nutrients with stimulants was previously reported as a beneficial solution for sugar beet seed coating compared to separate applications (Mohammadi et al. 2024). However, lucerne plant stimulation under drought stress significantly reduced forage yield (Pisarčik et al. 2022). The stimulants seemed effective at lucerne seeds, especially in combination with fertilisers containing a broader spectrum of nutrients (ash, Agroptim Sunset, FWJ). Soares et al. (2016) demonstrated that seed treatments with micronutrients improved plant assimilation and soybean growth rate, whereas amino acids and phytohormones reduced stress and increased the dry matter production. Ayoub et al. (2018) report on the strong synergic effect of ROA with fungicides. In our experiment, the antifungal cinnamon powder was effectively combined with $KMnO_4$, but not with H_2O_2 (T5). The combinations of FWJ + green tea and H_2O_2 + thyme infusion (T9) seemed to have a stable positive response in both lucerne germination and seedling growth. The FWJ had a positive synergy with green tea (T7), but not with chitosan, where treatment T4 had an even lower germination rate on the 7th day when compared to control and hydropriming. This aligns with Riseh et al. (2024), who reported that chitosan can create inappropriate combinations with other agents. In the present study, two of four treatments with chitosan improved seedling growth.

Using a priming treatment generally seeks to combine temperature, time of exposure, and amount of priming agent (Liu et al. 2008). It offers a range of possibilities that may even increase when different agents are combined. Nevertheless, all factors influencing the uptake speed of agents by seeds (e.g. seed microdamage) may result in different effects of priming compared to soaking time.

Pot experiment. The effects of seed treatments on lucerne growth in the pot experiment were minimal. Tang et al. (2025) have not shown any productivity benefit from applying micronutrients with lucerne seeds in field conditions despite their detected deficiency in the soil. Root length was the only measured response that was found to be supported by seed treatments in the pot experiment, and only T9 (Table 5), combined with coating, surpassed the control. It corresponds with the study by Szabó et al. (2023), in which the best treatments combine priming and coating. Riseh et al. (2024) reported a positive influence of chitosan seed treatment on root architecture, and chitosan priming combined with gypsum/bentonite coating increased lateral root number. The absence of effect on root branching may be associated with the short duration of the experiment, plant density and the poor soil conditions (Hakl et al. 2021). In contrast to field conditions, the size of pots can also influence root development (Poorter et al. 2012).

In conclusion, this study highlights the potential of a range of priming agents to increase the rate of lucerne seed germination and early seedling growth. Some tested treatments represent easily available, cost-effective treatments with good potential to accelerate germination and seedling development. The combination of priming agents improved only seedling growth compared to hydropriming, where combinations of FWJ + green tea and H_2O_2 + thyme infusion were found to be particularly effective. In the pot experiment, only the combination of priming and coating method improved lucerne root growth, highlighting the need to verify laboratory test seed performance results under soil conditions.

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