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Nanoparticles and magnetic field as novel elicitors improve seed germination and early growth of Mediterranean cypress

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Abstract

This study aimed to investigate the possibility of increasing the germination traits and early growth characteristics of Mediterranean cypress by applying nanopriming and magnetic field. To perform nanopriming, seeds were primed with nanochitin, nanochitosan and nanocellulose at a concentration of 1% for 2 h. For magnetopriming, the seeds were exposed to a magnetic field at three levels (20, 30 and 40 mT) for 1, 10 and 20 min and then sown. In combined treatment, the seeds were exposed to the magnetic field and then primed in nanoparticle solutions at the same treatments as mentioned earlier, and were planted. Non-exposed seeds were used as a control. The selected seed germination parameters (the percentage and speed of germination, stem and radicle length, collar root diameter, fresh and dry weights of the radicle and stem, number of leaves and leaf area) of young seedlings were examined. The interaction of nano × magnetic resulted in significant differences in the number of leaves, leaf area, length of the radicle, length of the stem, fresh weight of the radicle, fresh weight of the stem and stem dry weight traits. The results showed that among all the applied treatments, 10 min magnetism to 20 mT and then priming with 1% solution of nanocellulose was the best. It increased the germination percentage (1.31 times), germination rate (1.46 times), number of leaves (1.57 times), leaf area (1.36 times) and radicle length (1.74 times) compared to the control (no treatment). A 20-min treatment under a 30-mT magnetic field (without nanoprime) is also recommended.

Key words

Mediterranean cypress, nanochitin, nanocellulose, nanochitosan, magnetic treatment

Introduction

Cupressus sempervirens L., a member of the Cupressaceae family, is commonly known as the Mediterranean cypress. This species is also found on the southern shores of the Caspian Sea in Iran (Orhan and Tumen 2015). However, with the increasing trend of deforestation, it is important to explore methods to improve the success rate of producing seedlings from multi-purpose and suitable species in recent years. Among these meth-

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ods, enhancing seed germination has proven to be an effective strategy for increasing the quality and quantity of seedlings (Ranal and Santana 2006). The initial growth traits of seedlings in the nursery significantly impact their success in later stages (Mirsaleh Gilani et al. 2020).

Unfortunately, cypress seed germination is weak (Kostopoulou et al. 2010). So, the average rate of germination in different studies is about 30% (Ahmadloo et al. 2009; Darikvand and Zolfaghari 2014). Also, germination speed is slow and requires a significant amount of time to develop, which directly affects the seed quality and performance (Darikvand and Zolfaghari 2014). Considering that one should always try to provide the best possible conditions for the growth of seedlings in the nursery (Mirsaleh Gilani et al. 2020), improving germination traits is one of the most appropriate strategies to increase the quantity and quality of seedlings (Ranal and Santana 2006). Although Mediterranean species are resistant to harsh environmental conditions, the seed germination stage and the presence of water and nutrients are very effective on the quality and quantity of seed germination and seedling growth. Furthermore, successful seedling production highly depends on seed origins and seed sowing time (Mirsaleh Gilani et al. 2020). To improve seed germination and the growth characteristics of produced seedlings, applying new technologies has become crucial for successful seedling production and forest restoration. Seed priming is a valuable technique in seed technology that aims to enhance the percentage and speed of seed germination (Feizi et al. 2013). A recent innovative approach in seed priming is the use of nanomaterials, known as nanopriming. The seeds may or may not absorb the nanomaterials, which can either remain as a coating on the seed surface or be absorbed into the seeds themselves (Khan et al. 2023). The nanomaterials used for seed priming can be classified into different types, including metal-based nanoparticles (such as silver, gold, copper, iron, iron disulphide, titanium dioxide, zinc and zinc oxide nanoparticles), carbon-based nanoparticles (e.g. fullerene and carbon nanotubes) or polymer nanoparticles (Yavari et al. 2022). Seed nanopriming has emerged as a successful method not only to enhance plant seed germination and seedling establishment, but also the growth of the plant (Imtiaz et al. 2023). Sobze et al. (2022) showed that carbon nanoparticle treatments improved seed germination in alder. Using multi-walled carbon nanotubes functionalised with carboxylic acids can effectively break seed dormancy in forest species like *Shepherdia canadensis* L. and *Alnus viridis* L. by modulating lipid metabolism in the cell membrane (Ali et al. 2020). Due to the unique properties of nanomaterials (e.g. high surface–volume ratio and extremely small size), the effects and toxicity of nanomaterials on the environment with respect to their interactions with biological material are still relatively poorly defined (Kabir et al. 2018). The destiny of nanomaterials in the environment is managed by the combined effects of their physicochemical properties and their interactions with other pollutants (Maiti et al. 2016). Meanwhile, using bio-based nanomaterials can minimise probable problems.

The magnetic field is known as a new elicitor; it has been shown to interact with biological systems and has the potential to increase plant germination, growth and productivity. Although it is known as a low-cost and promising approach, the mechanism that increases growth is not fully understood yet (Ercan et al. 2022). Researches show that in addition to germination, it has affected the yield and quality of crops (Sarraf et al. 2021). Plants germinated from magnetoprimed seeds often exhibit increased plant height, leaf area, fresh weight, chlorophyll and carotenoid contents, stomatal conductance, enzyme activity and overall yield (Sarraf et al. 2021). Successful examples of applying a magnetic field to seeds include birch (Pordel et al. 2022), maple (Ayan et al. 2018) and loblolly pine (Yao and Shen 2015). Also, magnetising oil palm seeds leads to rapid germination with a high success rate (Sudsiri et al. 2023). It has been confirmed that with the increase of magnetic field forces, the content of macroelements gradually decreases and, on the contrary, the content of microelements in the root increases (Ercan et al. 2022). It is worth noting that the duration of seed exposure to the magnetic field intensity can yield different results (Payamnoor et al. 2020). In a study by Pordel et al. (2022), a 1-min magnetic treatment at 30 mT was combined with a 2-h prime using nanocellulose on birch seeds. The results demonstrated significant improvements in various growth parameters, including a 1.58 times increase in greening percentage, 1.9 times increase in greening speed, 1.2 times increase in leaf number, 1.84 times increase in leaf area and

1.4 times increase in root length (Pordel et al. 2022). This study aims to investigate the potential for enhancing germination traits and growth of 3-month-old Mediterranean cypress seedlings through the application of nanopriming (bio-based nanomaterials: chitin nanofiber, chitosan nanofiber and cellulose nanofiber) and a magnetic field, both individually and in combination. It should be mentioned that this experiment was conducted in the weak magnetic field range; also, the nanomaterials are ultra-renewable, biodegradable and costly, and they are polysaccharides that have a protective role in plants and animals.

MATERIAL AND METHODS

Mediterranean cypress seeds originating from Gorgan (Golestan province, Iran) were obtained from the Caspian Forest Tree Seed Center, Amol, Mazandaran province, Iran. The seeds were sterilised in a 1% benomyl solution for 24 h. The seeds were then treated. For nanoprime treatment, the sterilised seeds were immersed in a 1% nanochitin, nanochitosan and nanocellulose solution for 2 h before planting. In the control treatment (Ctr), the seeds were not primed. In the magnetisation treatment, the seeds were exposed to a magnetic field at three levels (20, 30 and 40 mT) for different durations (1, 10 and 20 min) and then sown. For the combined treatment of nanopriming and magnetic field, the seeds were first exposed to the magnetic field at the same three levels and durations as mentioned earlier. Subsequently, they were primed with 1% solutions of nanochitin, nanochitosan and nanocellulose before being sown. Figure 1 shows the scanning electron microscopic structure of the tested nanomaterials from the manufacturing company.

During the experiment, irrigation was conducted twice daily (morning and evening) until the germination stage and then once a day. After 30 days, the percentage and rate of germination were determined, along with other desirable traits such as stem and radicle length (using a ruler with centimetre accuracy), collar diameter (using calipers with millimetre accuracy), fresh weight (with a scale accurate to one hundredth) and dry weight of the radicle and stem (weighed after placing the seedlings at 70°C for 48 h), number of leaves and leaf area. These measurements were taken 3 months after sowing. Germination percentage was obtained using the following formula: (the number of germinated seeds each day/the total number of seeds sown) × 100). The germination rate was obtained using the following formula: sum (the number of germinated seeds each day/number of days) (Ahani et al. 2015). The normality of data was evaluated using the Kolmogorov–Smirnov test. The experiment was conducted in three replications, in a completely random factorial design, considering the three levels of nanomaterials, three levels of magnetic field intensity and three different durations. Duncan's multiple range test was employed to compare the means.

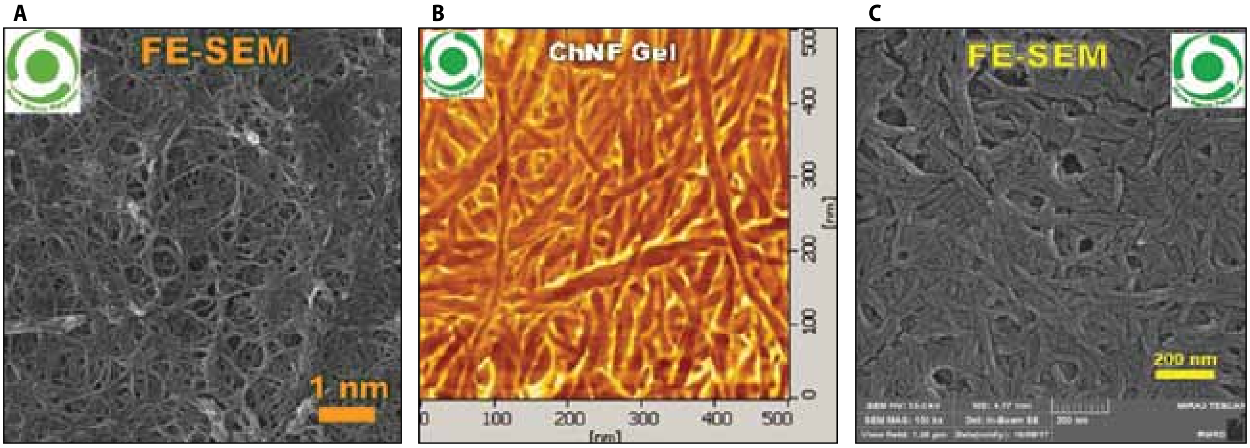

Figure 1. The used nanoparticles' images: A – nanochitosan, B – nanochitin and C – nanocellulose (photos by the manufacturer, Nano Novin polymer company, http://nanonovin.com). The average diameters at in at particular applied voltages are 35 ± 10, 24 ± 9 and 32 ± 8 respectively. (SEM: scanning electron microscopic)

RESULTS

The results of the analysis of variance, examining the impact of nano treatment, magnetic field, time and their combined effects on the germination traits and initial characteristics of Mediterranean cypress seedlings, are presented in Table 1. Nanoprime treatment showed a significant difference in all traits, except germination percentage. The magnetic field application also significantly impacted all traits, except germination rate and collar diameter. The duration of treatment alone did not yield significant differences in the measured traits, except for the number of leaves ($p > 0.05$). The interaction of these factors resulted in significant differences in certain traits. Nano × magnetic interaction caused significant differences in the traits of leaf number, leaf area, radicle length, stem length, fresh weight of the radicle and stem, and dry weight of the stem. The interaction of magnetisation × time caused a significant difference in the trait of leaf number. The interaction effect of nano × magnetic exposure time resulted in significant differences in radicle fresh weight. Also, the nano × magnetic × time interaction caused a significant difference in characteristics of radicle length, radicle fresh weight and stem dry weight traits (Tab. 1).

The mean comparison revealed that nanocellulose exhibited the most favourable outcomes compared to other treatments, improving all traits, except stem length, compared to the control group. Nanochitin also showed an increase in germination percentage, germination speed, radicle length, stem length, collar root diameter and stem dry weight, compared to the control. The results indicated that nanocellulose, nanochitosan and nanochitin positively influenced the increase in stem and radicle length. Nanocellulose enhanced the percentage and speed of germination compared to the control treatment, while nanochitosan and nanocellulose improved stem fresh weight compared to the control. Treatment under nanoprime yielded significant results for germination percentage and rate, length of radicle, length of the stem and fresh weight of the stem (Fig. 2).

Using a magnetic field treatment at 30 mT resulted in an increased number of leaves and at 20, 30 and 40 mT increased the leaf area and germination percentage compared to the control (Fig. 3). In addition,

Table 1. Analysis of variance for the effect of nano treatments, magnetic field and time on the germination traits and characteristics of Mediterranean cypress seeds and seedlings

Source of variation	Degree of freedom	Germination percentage	Germination speed	Number of leaves	Leaf area	Length of the radicle	Length of the stem
Nano	3	0.889^{ns}	8.6**	46.6**	33**	49.2**	55.6**
Magnetic	2	3.18*	1.54^{ns}	22.3**	6.4**	0.03^{ns}	8.7**
Time	2	1.39^{ns}	0.78^{ns}	3.6*	2.96^{ns}	0.05^{ns}	1.7^{ns}
Nano × magnetic	6	1.8^{ns}	1.07^{ns}	20*	6.09**	3.9*	5.3**
Nano + magnetic × time	6	0.58^{ns}	1.38^{ns}	0.97^{ns}	0.94^{ns}	1.83^{ns}	0.35^{ns}
Magnetic × time	4	2.46^{ns}	2.34^{ns}	3.2*	1.7^{ns}	1.53^{ns}	1.02^{ns}
Nano × magnetic × time	12	1.26^{ns}	1.37^{ns}	1.35^{ns}	0.7^{ns}	4.08**	0.41^{ns}
Source of variation	collar diameter	fresh weight of radicle	fresh weight of the stem	radicle dry weight	stem dry weight		
Nano	5.76**	130.07**	85.3**	20.7**	118.4**		
Magnetic	2.14^{ns}	7.64**	5.2**	3.6*	10.5**		
Time	0.127^{ns}	0.17ns	0.45ns	0.7ns	1.01ns		
Nano × magnetic	2.28ns	11.05**	5.7**	1.56ns	8.9**		
Nano + magnetic × time	0.15ns	2.34*	0.32ns	0.61ns	0.96ns		
Magnetic × time	0.95^{ns}	1.46ns	1.6ns	0.94^{ns}	2.3^{ns}		
Nano × magnetic × time	0.18ns	5.01**	0.65ns	0.92ns	2.48*		

ns, * and ** indicate the non-significance of the difference, the difference at the level of 0.05 and the difference at the level of 0.01, respectively.

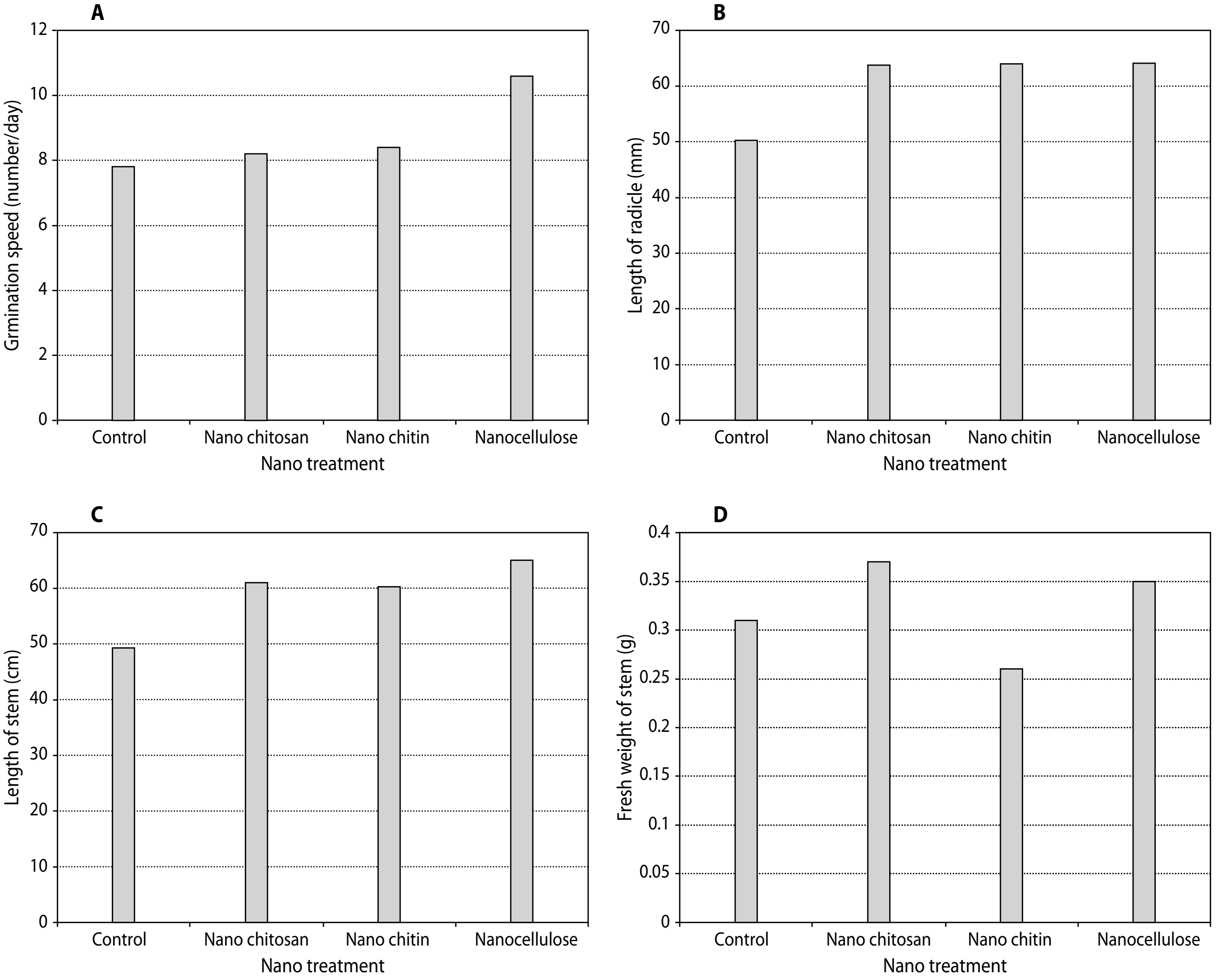

Figure 2. The effect of nanopriming on (a) germination percentage, (b) germination rate, (c) length of radicle, (d) length of the stem and (e) fresh weight of the stem. Different letters above the bars indicate statistically significant difference.

40 mT treatment improved the germination percentage compared to the control treatment. Regarding the other investigated traits, apart from leaf area, the treatments used did not significantly differ from the control treatment, or even showed a decrease in some cases. Treatment at 30 mT for 20 min yielded satisfactory results.

The best results were obtained from magnetisation for 10 min in a 20 mT field, followed by seed priming with a 1% solution of nanocellulose. Similar results were achieved through the combined treatment of nanocellulose on magnetised seeds for 20 min under a 20-mT field, leading to an increase in germination percentage, germination speed, number of leaves, leaf area and radicle length. Treatment for 20 min under a 30-mT magnetic field (without nanopriming) also yielded significant results (Tab. 2, 3).

Table 2. Average comparison of nano treatments, magnetic field and time on Mediterranean cypress seed germination characteristics

Source of variation	Germination percentage	Germination rate
1	2	3
Cellul-N	53.3^{abc}	10.6^{abcdef}
CTS-N	45.3^{abcdef}	8.2^{cdefg}
CT-N	46.6^{abcdef}	8.4^{bcdefg}
20 mT + 1 min	50.6^{abcd}	8.1^{cdefg}

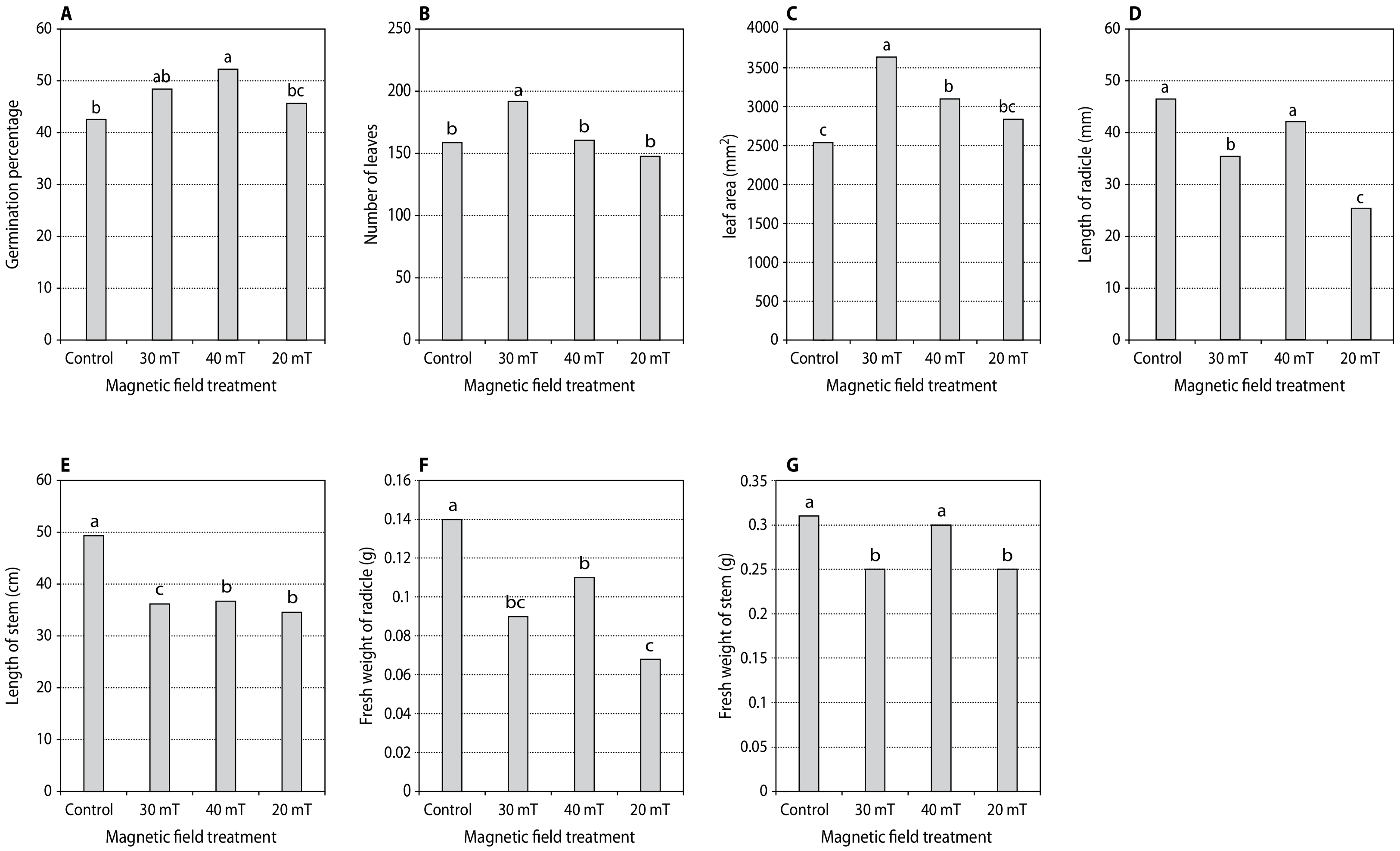

Figure 3. The effects of magnetopriming on (a) germination percentage, (b) the number of leaves, (c) leaf area, (d) length of the radicle, (e) length of the stem, (f) fresh weight of the radicle and (g) fresh weight of the stem. Different letters above the bars indicate statistically significant difference.

1	2	3
20 mT + 10 min	41.3^{bcdef}	7^{g}
20 mT + 20 min	45.3^{abcdef}	7.78^{efg}
30 mT + 1 min	44^{bcdef}	8.3^{bcdefg}
30 mT + 10 min	48^{abcde}	8.32^{bcdefg}
30 mT + 20 min	53.3^{abc}	11.2^{abcd}
40 mT + 1 min	49.3^{abcd}	8^{defg}
40 mT + 10 min	47.6^{abcde}	$8.9^{abcdefg}$
40 mT + 20 min	60^{a}	10.93^{abcdef}
CT-N + 20 mT + 1 min	46.6^{abcdef}	$9.94^{abcdefg}$
CT-N + 20 mT + 10 min	56^{ab}	$9.84^{abcdefg}$
CT-N + 20 mT + 20 min	52^{abc}	$9.58^{abcdefg}$
CT-N + 30 mT + 1 min	42.6^{bcdef}	12^{a}
CT-N + 30 mT + 10 min	33.3^{de}	$10.1^{abcdefg}$
CT-N + 30 mT + 20 min	33.3^{de}	8.3^{bcdefg}
CT-N + 40 mT + 1 min	44^{bcdef}	$9.8^{abcdefg}$
CT-N + 30 mT + 10 min	48^{abcde}	10.6^{abcdef}
CT-N + 30 mT + 20 min	44^{bcdef}	8.65^{bcdefg}
Cellul-N + 20 mT + 1 min	32^{f}	7.16^{g}
Cellul-N + 20 mT + 10 min	56^{ab}	11.4^{abc}
Cellul-N + 20 mT + 20 min	49.3^{abcd}	11.25^{abcd}
Cellul-N + 30 mT + 1 min	40^{cdef}	$9.46^{abcdefg}$
Cellul-N + 30 mT + 10 min	50.6^{abcd}	$9.86^{abcdefg}$
Cellul-N + 30 mT + 20 min	38.6^{cdef}	8.69^{bcdefg}
Cellul-N + 40 mT + 1 min	50.6^{abcd}	10.9^{abcdef}
Cellul-N + 40 mT + 10 min	38.6^{cdef}	8.49^{bcdefg}
Cellul-N + 40 mT + 20 min	44^{bcdef}	8.64^{bcdefg}
CTS-N + 20 mT + 1 min	42.6^{bcdef}	$8.93^{abcdefg}$
CTS-N + 20 mT + 10 min	45.3^{abcdef}	11.54^{ab}
CTS-N + 20 mT + 20 min	48^{abcde}	11^{abcde}
CTS-N + 30 mT + 1 min	49.3^{abcd}	8.08^{bcdef}
CTS-N + 30 mT + 10 min	48^{abcde}	7.65^{def}
CTS-N + 30 mT + 20 min	36^{def}	$9.3^{abcdefg}$
CTS-N + 40 mT + 1 min	50.6^{abcd}	$8.4^{abcdefg}$
CTS-N + 40 mT + 10 min	52^{abc}	$9.76^{abcdefg}$
CTS-N + 40 mT + 20 min	45.3^{abcdef}	8.7^{bcdefg}
Ctr	42.6^{bcdef}	7.8^{efg}

Table 3. Average comparison of nano treatments, magnetic field and time on the vegetative characteristics of Mediterranean cypress seedlings

Source of variation 1	Length of the stem 2	Collar diameter 3	Fresh weight of the radicle 4	Fresh weight of the stem 5	Radicle dry weight 6	Stem dry weight 7	Number of leaves 8	Leaf area 9	Length of the radicle 10
Cellul-N	65^{a}	3.56^{abc}	0.166^{efg}	0.353^{cdefgh}	0.025^{ef}	0.069^{fghi}	166.6^{ghijkl}	$2783.3^{fghijkl}$	64.15^{cdef}
CTS-N	61^{a}	3.83^{ab}	0.186^{def}	0.37^{cdefg}	0.033^{ef}	0.063^{hijk}	168^{ghijk}	$2912^{efghijkl}$	63.8^{cdef}
CT-N	60.3^{a}	3.41^{abc}	0.103^{fgh}	0.256^{hi}	0.026^{ef}	0.065^{ghij}	157.3^{ghijkl}	2193.3^{ijkl}	64^{cdef}
20 mT + 1 min	34.5^{fghij}	3.65^{abc}	0.053^{h}	0.236^{i}	0.011^{f}	0.035^{mn}	127.3^{jklm}	2037.3^{jkl}	24^{kl}
20 mT + 10 min	34.16^{hij}	3.84^{ab}	0.076^{gh}	0.246^{hi}	0.007^{f}	0.036^{lmn}	119.6^{lm}	2057.3^{jkl}	28.16^{kl}
20 mT + 20 min	35.3^{efghij}	4.08^{a}	0.073^{gh}	0.276^{fghi}	0.014^{f}	0.043^{klmn}	196^{cdefgh}	$3822^{abcdefgh}$	30.3^{ghijkl}
30 mT + 1 min	36.1^{efghij}	3.97^{ab}	0.076^{gh}	0.25^{hi}	0.0327^{ef}	0.041^{klmn}	172.6^{ghijk}	$3005.3^{defghijkl}$	29.3^{kl}
30 mT + 10 min	34.3^{ghij}	3.56^{abc}	0.073^{gh}	0.236^{i}	0.009^{f}	0.047^{jklmn}	188^{efghi}	$3276^{cdefghijk}$	28.5^{kl}
30 mT + 20 min	$38.3^{cdefghi}$	3.67^{abc}	0.12^{fgh}	0.28^{fghi}	0.034^{ef}	0.06^{hijk}	215.6^{bcdefg}	4628^{abc}	57.5^{efgh}
40 mT + 1 min	35.3^{efghij}	3.71^{abc}	0.12^{fgh}	0.306^{defghi}	0.022^{ef}	0.055^{ijklm}	143^{ijklm}	$2763.3^{fghijkl}$	43.16^{ghijkl}
40 mT + 10 min	36.16^{efghij}	3.43^{abc}	0.123^{fgh}	0.306^{defghi}	0.025^{ef}	0.057^{hijkl}	163.6^{ghijkl}	$3384^{bcdefghij}$	50.5^{fghi}
40 mT + 20 min	$38.6^{cdefghi}$	3.26^{abc}	0.093^{fgh}	0.296^{efghi}	0.020^{ef}	0.056^{ijklm}	175^{fghij}	$3146.6^{defghijkl}$	43.3^{ghijkl}
CT-N + 20 mT + 1 min	39.5^{cdefgh}	3.86^{ab}	0.166^{efg}	0.4^{cde}	0.042^{cdef}	0.074^{fghi}	156.3^{ghijkl}	$2810.6^{fghijkl}$	40^{hijkl}
CT-N + 20 mT + 10 min	40.3^{cdefgh}	3.56^{abc}	0.12^{fh}	0.38^{cdef}	0.022^{ef}	0.07^{fghi}	148.6^{ghijkl}	2875.3^{efghi}	35.8^{igkjll}
CT-N + 20 mT + 20 min	41^{cdefgh}	3.6^{abc}	0.163^{efg}	0.393^{cde}	0.034^{ef}	0.077^{fghi}	175^{fghij}	$3500^{abcdefghi}$	43^{ghijkl}
CT-N + 30 mT + 1 min	30.5^{ij}	2.96^{c}	0.11^{fgh}	0.263^{ghi}	0.027^{ef}	0.031^{n}	100.3^{m}	1962^{kl}	28.8^{kl}

1	2	3	4	5	6	7	8	9	10
CT-N + 30 mT + 10 min	35.5^{efghij}	3.29^{abc}	0.11^{fgh}	0.316^{defghi}	0.032^{ef}	0.062^{hijk}	126.6^{jklm}	1849.3^{l}	39.8^{hijkl}
CT-N + 30 mT + 20 min	35.5^{efghij}	2.95^{c}	0.17^{efg}	0.373^{cdefg}	0.035^{def}	0.069^{fghi}	173.6^{ghij}	$3332^{bcdefghijk}$	50.6^{fghi}
CT-N + 40 mT + 1 min	40.6^{cdefgh}	3.34^{abc}	0.16^{efg}	0.416^{cde}	0.035^{def}	0.079^{fghi}	224^{abcde}	$3730^{abcdefgh}$	49.8^{fghij}
CT-N + 30 mT + 10 min	41.1^{bcdefg}	3.51^{abc}	0.183^{def}	0.416^{de}	0.03^{ef}	0.069^{fghi}	235.3^{abcde}	$4073.3^{abcdefg}$	50.6^{fghi}
CT-N + 30 mT + 20 min	42.8^{bcdefg}	3.71^{abc}	0.16^{efg}	0.41^{cde}	0.026^{ef}	0.072^{fghi}	222^{abcde}	$3523.3^{abcdefghi}$	42.5^{hijkl}
Cellul-N + 20 mT + 1 min	45^{bcd}	3.72^{abc}	0.366^{c}	0.6^{a}	0.063^{abcde}	0.113^{bc}	223^{abcde}	4138.6^{abcdef}	62.3^{cdefg}
Cellul-N + 20 mT + 10 min	43bcdef	3.52^{abc}	0.58^{a}	0.596^{a}	0.102^{a}	0.131^{ab}	249.3^{ab}	4800.6^{a}	87.6^{ab}
Cellul-N + 20 mT + 20 min	46.5^{bc}	3.9^{ab}	0.463^{a}	0.633^{a}	0.082^{abc}	0.134^{a}	265^{a}	4610^{abc}	76.3^{abcd}
Cellul-N + 30 mT + 1 min	43.8^{bcde}	3.85^{ab}	0.443^{bc}	0.55^{ab}	0.077^{abcd}	0.126^{ab}	237.6^{abcd}	4127.3^{abcdef}	91.8^{a}
Cellul-N + 30 mT + 10 min	45.8^{bcd}	3.71^{ab}	0.386^{bc}	0.616^{a}	0.085^{ab}	0.117^{abc}	242.6^{abc}	4544^{abc}	80.1^{abcd}
Cellul-N + 30 mT + 20 min	43^{bcdef}	3.83^{ab}	0.266^{d}	0.556^{ab}	0.062^{abcde}	0.102^{cd}	241^{abcd}	4680.6^{ab}	52^{efghi}
Cellul-N + 40 mT + 1 min	40.3^{cdefgh}	3.4^{abc}	0.373^{bc}	0.543^{ab}	0.064^{abcde}	0.099^{cde}	225.6^{abcde}	4356.6^{abcd}	71.6^{bcde}
Cellul-N + 40 mT + 10 min	$41.8^{bcdefgh}$	3.65^{abc}	0.19^{def}	0.456^{bc}	0.04^{def}	0.086^{defg}	193.6^{defg}	$3649^{abcdefgh}$	47.1^{fghijk}
Cellul-N + 40 mT + 20 min	$41.8^{bcdefgh}$	3.91^{ab}	0.233^{de}	0.466^{bc}	0.0513^{bcdef}	0.089^{def}	189^{efghi}	$3532.6^{abcdefgh}$	59^{defgh}
CTS-N + 20 mT + 1 min	39.5^{cdefgh}	3.86^{ab}	0.166^{efg}	0.4^{cde}	0.042^{cdef}	0.074^{fghi}	155^{ghijkl}	$2784^{fghijkl}$	41.1^{hijkl}
CTS-N + 20 mT + 10 min	37.6^{defghi}	3.35^{abc}	0.12^{fh}	0.38^{cdef}	0.082^{abc}	0.07^{fghi}	138.6^{jklm}	2675.3^{ghijkl}	35^{ijkl}
CTS-N + 20 mT + 20 min	$38.6^{cdefghi}$	3.5^{abc}	0.156^{efg}	0.393^{cde}	0.033^{ef}	0.076^{fghi}	164^{ghijkl}	$3280^{cdefghijk}$	40.5^{kl}
CTS-N + 30 mT + 1 min	29^{j}	2.96^{c}	0.079^{gh}	0.263^{ghi}	0.014^{f}	0.043^{klmn}	101.3^{m}	1982^{kl}	30.3^{ghijkl}
CTS-N + 30 mT + 10 min	35.8^{efghij}	3.29^{abc}	0.11^{fgh}	0.316^{defghi}	0.032^{ef}	0.062^{hijk}	125^{klm}	2428^{hijkl}	41.1^{hijkl}
CTS-N + 30 mT + 20 min	35.5^{efghij}	2.95^{c}	0.17^{efg}	0.373^{cdeefg}	0.035^{def}	0.069^{fghi}	173.6^{ghij}	$3332^{bcdefghijk}$	51.6^{fghi}
CTS-N + 40 mT + 1 min	40.6^{cdefgh}	3.39^{abc}	0.16^{efg}	0.416^{cde}	0.035^{def}	0.076^{fghi}	224^{abcde}	$3730^{abcdefgh}$	50.8^{fghi}
CTS-N + 40 mT + 10 min	41^{cdefgh}	3.51^{abc}	0.183^{def}	0.416^{cde}	0.03^{ef}	0.069^{fghi}	235.3^{abcde}	$4073.3^{abcdefg}$	47.1^{fghijk}
CTS-N + 40 mT + 20 min	43^{bcdef}	3.71^{abc}	0.16^{efg}	0.41^{e}	0.026^{ef}	0.072^{fghi}	220.6^{abcef}	4246.6^{abcde}	41.6^{hijkl}
Ctr	49.3^{b}	3.14^{bc}	0.146^{efg}	0.31^{defghi}	0.027^{ef}	0.058^{hijk}	158.6^{ghijkl}	2538.6^{hijkl}	50.3^{fghi}

CT-N – n anochitin, CTS-N – nano-chitosan, Cellul-N – nanocellulose

Discussion and conclusions

Due to habitat destruction and climate changes over the years, Mediterranean cypress seeds have shown low germination rates and high porosity, posing a challenge to reviving this endangered species in Iran (Kostopoulou et al. 2010; Darikvand and Zolfaghari 2014). To address this issue, researchers have turned their attention to the potential of nanomaterials in enhancing seed performance. Studies on forest species are limited, but the positive effects of nanomaterials on seed efficiency have been reported in various studies. Notable examples include the use of carbon nanoparticles with carboxylic acids to improve alder seed germination (Ali et al. 2020), the application of titanium dioxide nanoparticles on mahlab seeds (Goodarzi et al. 2017) and the use of carbon nanotubes on *Alnus subcordata* seeds (Rahimi et al. 2018) and *Brassica napus* seeds (Lin and Xing 2007).

Among the various nanoprimes tested, nanocellulose proved to be the most effective. This type of nanomaterial, derived from biological sources, is biocompatible and renewable, making it highly suitable for use. Nanocellulose can promote high water absorption capacity and slow release of nutrients (Dutta et al. 2022). The superior absorption efficiency and specific leaf area of nanoparticles compared to conventional particles justify their enhanced effectiveness. Another approach explored in this research was using magnetic field treatment to improve the seed quantity and quality. Magnetic fields at doses of 20, 30 and 40 mT were applied for durations of 1, 10 and 20 min, respectively. Notably, treatment with a magnetic field for 20 min at 30 mT (without nanopriming) yielded significant results, increasing the germination percentage (1.25 times), germination speed (1.43 times), number of leaves (1.35 times), leaf area (1.36 times) and radicle length (1.14 times). Magnetic field treatment promotes plant growth rate, protein production and radicle development. Sudsiri et al. (2023) demonstrated that magnetic treatment of oil palm (*Elaeis guineensis*) seeds resulted in rapid and highly successful seed germination, and a 4-h dosage was required to stimulate germination. The results of another study indicated that magnetic treatment applied to wheat seeds before sowing enhanced the growth and improved their fruit yield due to increase in plant nutrients, and the intensity of the magnetic field caused positive effects on seed germination. At the same time, the exposure time did not show significant differences with the control conditions (Hussain et al., 2020). A study revealed that magnetic field exposure regulates the expression of different enzymes, which play a vital role in stimulating the rapid germination of soybean seeds (Radhakrishnan, 2019).

The activity of ions and polarisation of dipole molecules in living cells are affected by magnetic treatment (Dhawi et al. 2009). Research has shown that additional magnetism influences ion activity and the activity of hydrolysing enzymes such as alpha-amylase, dehydrogenase and protease, leading to faster germination, improved seed structure and better radicle characteristics in treated seeds (Vashisth and Nagarajan 2010). Different plant species exhibit varying responses to the intensity of magnetic fields. Some plants may experience enhanced performance with a specific intensity, while others may see a decline. A weak magnetic field inhibited primary radicle growth by disrupting cell division and mitochondrial size (Belyavskaya 2001). Conversely, higher-intensity magnetic fields did not affect germination percentage, but increased the fresh weight of radicles and stems (Fischer et al. 2004). An excitability effect was observed in the early stages of wheat growth when exposed to magnetic fields of 125 and 250 mT (Martinez et al. 2002). Therefore, the optimal magnetisation intensity should be evaluated for each plant species. In a study on seeds, a 15-min treatment of magnetism at an intensity of 10 mT was deemed the most effective, and a combined pretreatment of magnetism and osmopriming with 25 mM humic acid was recommended to improve germination and growth traits (Payamnoor et al. 2020). Investigating the effects of magnetic fields on *Pinus pinea* seed germination and seedling growth revealed that exposure to a magnetic field of 9.42 mT for 30 and 45 min increased the germination energy (43%) and germination percentage (55%), while the control group without treatment exhibited the lowest germination energy (6%) and germination percentage (32%). The magnetic field also increased stem length, radicle neck diameter and radicle length in almond pine seedlings (Kırdar et al. 2016). In a separate study, loblolly pine seeds underwent various magnetic treatments, and the optimal outcome was observed in seeds treated with 150 mT of magnetism for 60 min (Yao and Shen 2015). In the current study, the combined treatment of nanocellulose on magnetised seeds for 10 min under a 20-mT field yielded the best results, increasing the germination percentage (1.31 times), germination rate (1.46 times), number of leaves (1.57 times), leaf area (1.36 times) and radicle length (1.74 times). It has been shown that on 1-min magnetic treatment of birch seeds at 30 mT, followed by a 2-h priming with 1% nanocellulose, the best results were obtained (Pordel et al. 2022). In recent years, there have been significant advances in technologies aimed at increasing plant reproduction, particularly through the use of nanomaterials and magnetic fields. These innovative approaches have shown promising results in improving plants' germination capacity and overall performance. Every year, a large number of Mediterranean cypress seedlings are produced and planted in Iran and other semi-arid countries of the world. Considering the low germination percentage observed in Mediterranean cypress seeds, nanocellulose and magnetic field hold great potential for enhancing germination and, consequently, improving various seedling traits. These superior treatments can be recommended in tree planting and forestry projects with this valuable species. In future experiments, it is recommended to investigate the efficacy of other nanomaterials and emerging technologies, such as cold plasma, to further enhance plant propagation results.

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