Improvement of germination by hormopriming technique of aleppo pine (*Pinus halepensis* Mill) seeds after a forest fire

Melhoria da germinação pela técnica de hormopriming de sementes de pinus aleppo (*Pinus halepensis* Mill) após um incêndio florestal

mejora de la germinación mediante la técnica hormopriming de semillas de pino alepo (Pinus halepensis Mill) tras un incendio forestal

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ABSTRACT

Between recurring forest fires, destructive anthropogenic practices and accentuated climatic variations, the maintenance of forests remains threatened. Fire being considered as a triggering phenomenon of secondary succession. Our work focused on the effect of the hormopriming technique on the germination of Aleppo pine seeds after a forest fire, at three sampling sites namely (sampling in the middle of a fire, between 4 and 10 of the fire and beyond 10 m from the fire).The seeds underwent immersion in a hormonal solution based on gibberillic acid (Ag3) at two concentrations 0,5% and 01% for 12h, 24h, 36h and 48h. This work on the importance of the hormopriming technique allows us to identify the most effective type(s) of treatment for improving the germination of Aleppo Pine (Pinus halepensis) seeds. The principle consists of soaking the seeds of this species in solutions of Ag3 namely: 0,5% and 01% for 12h, 24h, 36h and 48h in order to accelerate and improve the germination rate. The best germination rate is observed at sample 3, located beyond 10m from the fire. Conversely, samples 1 and 2 show low or even zero germination rates. Aleppo pine seeds collected more than 10 meters from the fire source and treated with gibberellic acid show a positive response to the hormone, but the concentration of 0,5% remains overall more effective in stimulating the germination than that of 1%.

Keywords: forest fire, aleppo pine, germination, gibberillic acid, imbibition time.

RESUMO
Entre incêndios florestais recorrentes, práticas antrópicas destrutivas e variações climáticas acentuadas, a manutenção das florestas continua ameaçada. O fogo é considerado um fenômeno desencadeador de sucessão secundária. O nosso trabalho centrou-se no efeito da técnica de hormopriming na germinação de sementes de pinheiro de Aleppo após um incêndio florestal, em três locais de amostragem nomeadamente (amostragem no meio de um incêndio, entre 4 e 10 do incêndio e além dos 10 m do incêndio). As sementes foram imersas em solução hormonal à base de ácido giberílico (Ag3) em duas concentrações 0,5% e 01% por 12h, 24h, 36h e 48h. Este trabalho sobre a importância da técnica de hormopriming permite identificar o(s) tipo(s) de tratamento mais eficaz(s) para melhorar a germinação de sementes de Pinheiro de Aleppo (Pinus halepensis).O princípio consiste em embeber as sementes desta espécie em soluções de Ag3 nomeadamente: 0,5% e 01% durante 12h, 24h, 36h e 48h de forma a acelerar e melhorar a taxa de germinação. A melhor taxa de germinação é observada na amostra 3, localizada a mais de 10m do fogo. Por outro lado, as amostras 1 e 2 apresentam taxas de germinação baixas ou até nulas. As sementes de pinheiro de Aleppo coletadas a mais de 10 metros da fonte de fogo e tratadas com ácido giberélico apresentam uma resposta positiva ao hormônio, mas a concentração de 0,5% permanece globalmente mais eficaz no estímulo da germinação do que a de 1%.

Palavras-chave: incêndio florestal, pinheiro de aleppo, germinação, ácido giberílico, tempo de embebição.

RESUMEN
Entre incendios forestales recurrentes, prácticas antropogénicas destructivas y variaciones climáticas acentuadas, el mantenimiento de los bosques sigue amenazado. Considerando al fuego como un fenómeno desencadenante de la sucesión secundaria. Nuestro trabajo se centró en el efecto de la técnica de hormopriming sobre la germinación de semillas de pino carrasco después de un incendio forestal, en tres sitios de muestreo, a saber (muestreo en medio de un incendio, entre 4 y 10 metros del incendio y más allá de 10 m del incendio). Las semillas se sumergieron en una solución hormonal a base de ácido giberílico (Ag3) en dos concentraciones 0,5% y 01% durante 12h, 24h, 36h y 48h. Este trabajo sobre la importancia de la técnica de hormopriming nos permite identificar el tipo o tipos de tratamiento más eficaces para mejorar la germinación de semillas de pino carrasco (Pinus halepensis).El principio consiste en remojar las semillas de esta especie en soluciones de Ag3 a saber: 0,5% y 01% durante 12h, 24h, 36h y 48h con el fin de acelerar y mejorar la tasa de germinación. La mejor tasa de germinación se observa en la muestra 3, ubicada a más de 10 m del fuego. Por el contrario, las muestras 1 y 2 muestran tasas de germinación bajas o incluso nulas. Las semillas de pino carrasco recogidas a más de 10 metros del foco del incendio y tratadas con ácido giberélico muestran una respuesta positiva a la hormona, pero la concentración del 0,5% sigue siendo en general más eficaz para estimular la germinación que la del 1%.

Palabras clave: incendio forestal, pino carrasco, germinación, ácido giberílico, tiempo de imbibición.

1 INTRODUCTION

The Aleppo pine is an indigenous species in the Mediterranean. In Algeria, beautiful pine forests are found at different bioclimatic stages (Kadik, 1987). Indeed, the species is present in all bioclimatic variants with a predominance in the semi-arid stage.

Aleppo pine (*Pinus halepensis*) forests are valuable ecosystems that provide numerous ecological, economic and social benefits (Tergou *and al*., 2024). However, despite its high flammability due to its resinous bark and its dense undergrowth favorable to the rapid spread of fire,

and its exposure to prolonged summer droughts, it is renowned for its pyrophilic character, where it regenerates easily after fire. Between recurring forest fires, destructive anthropogenic practices and accentuated climatic variations, the maintenance of forests remains threatened (Quezel and Médail, 2003). The reconstitution of the post-fire plant cover has been widely discussed by Mediterranean authors. Fire being considered as a triggering phenomenon of secondary succession (Lepart and Escarre, 1983).The regenerative succession of totally or partially burned plant communities, as well as their return to the initial state preceding the trauma, have been demonstrated (Trabaud, 1983; 1993; Trabaud and Galtié, 1996). The resilience of forests to fire remains variable depending on the existing species. However, despite its high flammability due to its resinous bark and its dense undergrowth favorable to the rapid spread of fire, and its exposure to prolonged summer droughts, it is renowned for its pyrophilic character, where it regenerates easily after fire.This state of affairs results in a bursting of the cones and the breaking of dormancy of the seeds projected over a hundred meters (Quezel P, 1976). An adaptation to fire frequently encountered in Mediterranean pines is the presence of serotinous cones, these cones closed by resin constitute an aerial bank of dormant seeds, subjected during a fire to much less severe temperatures and, above all, a residence time. as the soil seed bank; under the effect of heat, the resin melts and the cones open, releasing the seeds after passing the fire and thus allowing massive regeneration.The extreme heat reached on the surface of the soil, blackened by ashes, facilitates the breaking of dormancy of seeds which germinate in optimal conditions of mineral nutrition and in the face of reduced competitive pressure (Pimont et *al*., 2014).

The viability of Aleppo pine seeds, from burned forests, therefore constituted the core of our work, where germination stimulations were carried out, in order to try to understand the phenomenon of post-fire biological recovery in some pine forests. reservoirs in the Blida region.The hormone priming technique adopted in this study is also known as hormonal priming. Many studies have reported that hormopriming improves stand establishment and seedling growth of crops under optimal and suboptimal conditions (Ahmad Sher *et al*., 2019).

In this context, our study aims to analyze the effectiveness of hormopriming on improving the germination of Pinus halepensis Mill. seeds collected after a fire, with a view to better understanding the physiological mechanisms underlying natural regeneration and optimizing restoration strategies for degraded Mediterranean ecosystems.

2 MATERIAL AND METHODS

2.1 OBJECTIVES AND METHODOLOGY OF WORK

The objective of this research is to study the influence of the hormopriming technique based on Ag3 at two concentrations (0,5% and 01%) and at different seed impregnation durations (12h, 24h, 36h and 48h.) on the germination of Aleppo pine seeds collected at well-defined sampling locations, in order to optimize regeneration, and to assist the persistence of forests affected by fire, for the reforestation of this weakened forest heritage.However, few studies have focused until now on the germination behavior of seeds of this forest species under controlled conditions. The preparation of the different treatments used was carried out at the Biotechnology of Plant Production research laboratory at the University of Blida 1 (Algeria).The germination of the seeds after using the hormopriming technique was carried out in sterile Petri dishes soaked in distilled water and placed in an oven at a controlled temperature of 25 ±1°C.

2.2 PLANT MATERIAL USED:

The Aleppo Pine seeds were collected from three samples, namely in the middle of the fire, between 4 and 10m from the fire and beyond 10m from the fire respectively and this in December 2021 on trees of the commune of Chiffa wilaya of Blida (Algeria) whose latitude is 36°25'08.60"N and longitude is 2°45'07.10"E.

- 1st Sampling: altitude 410m
  N 36 25 08,6
  E 002 45 07,1
- 2nd Sampling: altitude 240m
  N36 25 20,1
  E 002 45 94,0
- 3rd Sampling: altitude 223m
  N36 25 41,5
  E 002 45 10,8

Figure 1: Geographical location of the study area (chiffa: wilaya of Blida, 2023) Source: Prepared by the authors (2025).

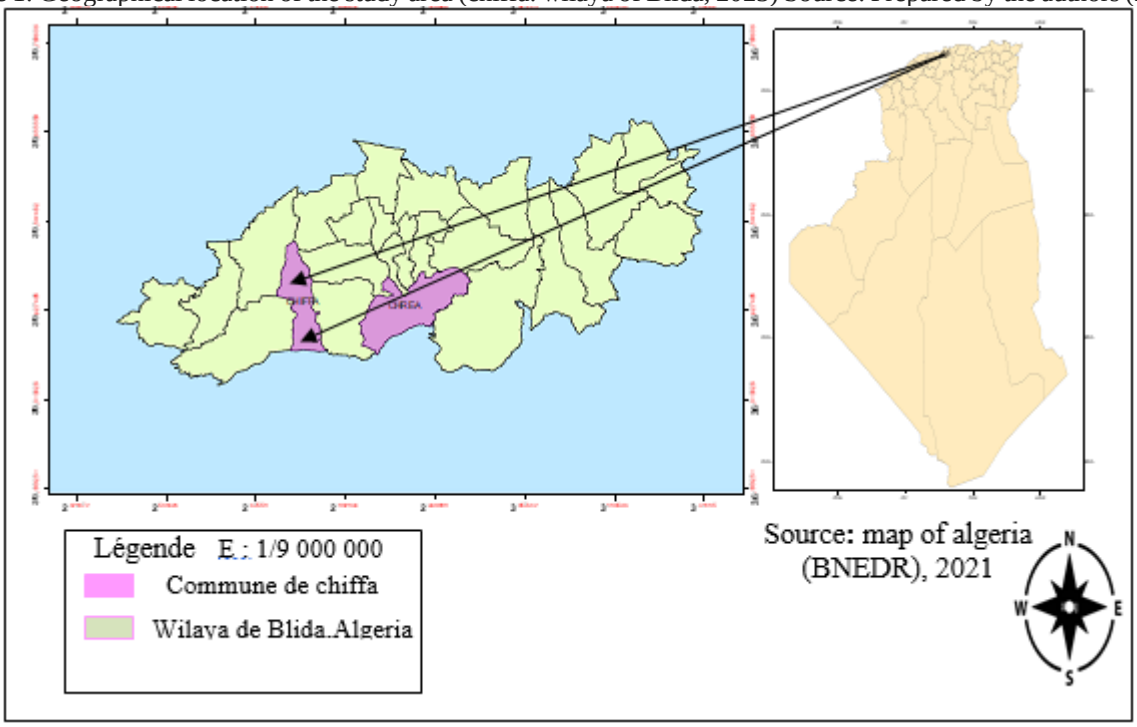


Figure 2: Leaves and Buds of Aleppo Pine (Boukli Hacene, 2021)



Figure 3: Germination phase of Aleppo Pine seeds (Boukli Hacene, 2021)



2.3 ALEPPO PINE SEED SOAKING TECHNIQUE

The seeds from the sites studied were soaked in two hormonal solutions based on Ag3 for: 12 hours, 24 hours, 36 hours and 48 hours.

It was done as follows:

- Two batches of seeds were weighed and their initial weights before soaking were noted;
- Each seed lot consisted of 10 seeds/box, with 3 replicates per treatment ;
- Each batch of seeds was soaked according to the corresponding treatment and placed in a shelter for drying until the initial dry weight was obtained before the soaking operation using the different treatments tested;

- Once this stage has been reached, the seeds from the four treatments are soaked in distilled water and placed in an oven at a temperature of 25°C;
- The number of seeds tested: is 2 treatments x 3 reps x 4 times x 3 samples of 10 seeds, i.e. 720 seeds in total;
- The seeds are soaked in two solutions, namely T1 (Ag3 at 0,5%), T2: (Ag3 at 01%).

Table 1: Distribution of treatments by collection location and duration of imbibition Source: Prepared by the authors (2025).

Area: Sebaa Chouhada (Chiffa)			
Sample	Location	Treatments used	Duration
1st sample	In the middle of a fire	Ag3 (0,5%), Ag3 (01%)	12h, 24h, 36h, 48h
2nd sample	Between 4 and 10 meters from the fire	Ag3 (0,5%), Ag3 (01%)	12h, 24h, 36h, 48h
3rd sample	More than 10 meters from the fire	Ag3 (0,5%), Ag3 (01%)	12h, 24h, 36h, 48h

3 FORMULA AND EQUATIONS

The germinated seeds are observed and counted daily. The germination criterion used in our experiment is based on the appearance of the radicle of approximately 1 mm per seed.

The measured parameters are:

3.1 FINAL GERMINATION RATE (%) AT THREE SAMPLING LEVELS

$$\textbf{Germination rate (TG\%)} = \frac{Ngg}{Npg} \times 100$$

(Npg) = expected number of seeds
(Ngg) = number of germinated seeds
(TG%) = germination rate

Based on the expected number of seeds (Npg), the number of germinated seeds (Ngg) is counted per treatment, per soaking duration and at the level of each of the three samples.

The germination rate (TG%) is calculated according to the formula described by Talhi et al., (2020).

Data analysis

The results were subjected to single-criteria analysis of variance using XLSTAT software version 2020.5.1.1059. Mean values and standard error (SE) were obtained using Excel 2013 software from 3 repetitions for the two parameters studied. The comparison of means was made with the least significant difference (or PPDS) test at 5%. A Probability (P) value of 0.05 was considered statistically significant while a value of (P) less than 0.05 was considered highly significant**.**

4 RESULTS AND DISCUSSIONS

4.1 GERMINATION RATE OF PINUS HALEPENSIS SEEDS AT SITE 1: (FULL FIRE)

The results in Table 2 show that Aleppo pine seeds collected directly from the fire did not germinate, despite treatment with gibberellic acid at concentrations of 0,5% and 1%. This suggests that the seeds suffered irreparable damage due to the intensity of the fire, and that hormonal treatments could not compensate for these effects. These results highlight the severe impact of fires on seed viability and underline the difficulty of promoting germination under such extreme conditions.

Table 2: Germination rate of Aleppo pine seeds at sampling 1 (full fire) Source: Prepared by the authors (2025).

Treatments		12h	24h	36h	48h
T1	Ag3 (0,5%)	0	0	0	0
T1	Ag3 (0,5%)	0	0	0	0
T1	Ag3 (0,5%)	0	0	0	0
T2	Ag3 (01%)	0	0	0	0
T2	Ag3 (01%)	0	0	0	0
T2	Ag3 (01%)	0	0	0	0

The data show that, despite treatment with 0,5% and 1% gibberellic acid, no seed showed any sign of germination, regardless of the imbibition time (12h, 24h, 36h, 48h). This indicates that there is a total absence of germination under the experimental conditions.

Although gibberellic acid is known to stimulate germination by breaking seed dormancy, in this case its effect was ineffective. This could suggest that, even in the presence of this hormonal inducer, the seeds were too affected by the fire to be able to respond to this stimulant (Boukli Hacene, 2024).

This lack of treatment efficacy could also suggest that gibberellic acid is not sufficient to compensate for the damage suffered by the seeds in such an extreme environment.

The fire outbreak appears to have devastating effects on the viability of Aleppo pine seeds (Boukli Hacene, 2024). Even with the application of a germination stimulator such as gibberellic acid, the seeds failed to germinate. This could indicate that the heat of the fire and other factors associated with the fire (such as smoke or chemicals) are powerful enough to destroy the seeds or render them incapable of germination, regardless of hormonal treatments.

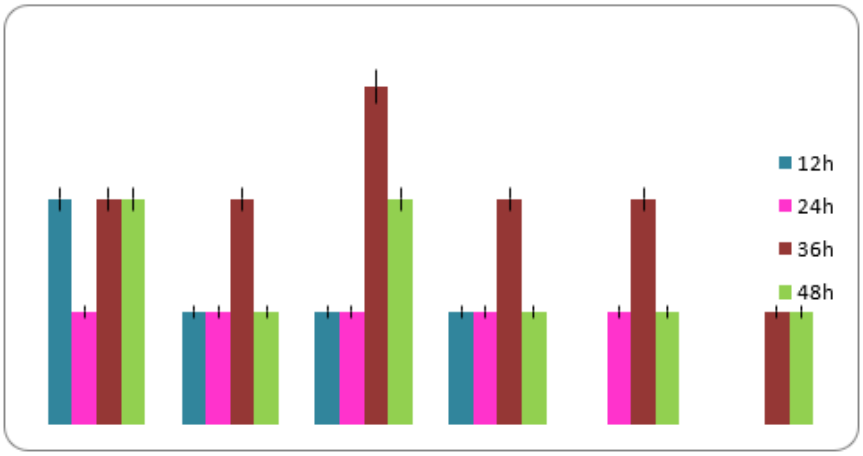
It is possible that gibberellic acid treatment, although stimulating under normal conditions, is not suitable for severely fire-damaged seeds. Gibberellic acid may be ineffective in cases where the damage is too severe to be repaired by hormone treatment, or it is possible that other necessary germination factors (such as moisture or temperature) are not optimized in this experiment to promote germination.

Mountain species, such as black pine or Scots pine, should also be classified in the resistant category. Indeed, their regeneration after fire is almost zero (generally less than 10 stems per hectare), while their survival after low-intensity fire is generally satisfactory. These species seem to be well adapted to the passage of surface fires (most often at the end of winter), as evidenced by the frequent presence of fire scars (Pimont et al., 2014).

4.2 GERMINATION RATE OF PINUS HALEPENSIS SEEDS AT SITE 2: (4 TO 10 M) FROM THE FIRE SOURCE

Figure 4 shows the results concerning the germination rate of Aleppo pine seeds collected between 4 and 10 meters from the fire source, treated with a hormonal solution based on gibberellic acid (Ag3) at two different concentrations (0,5% and 1%), after 12h, 24h, 36h and 48h of imbibition.

Figure 4: Germination rate of Aleppo pine seeds at sampling 2 (between 4 and 10 meters from the fire) Source: Prepared by the authors (2025).



Overall, seeds treated with 0,5% Ag3 show a slow but steady progression of germination. The rate remains relatively low throughout the experiment, but there is a slight improvement over time.

For seeds treated with 1% gibberellic acid solution, the trends are weaker:

Seeds treated with 1% Ag3 show less marked and less uniform germination compared to the 0,5% treatment. Germination appears to progress more slowly and less uniformly, suggesting that the higher concentration may have a limiting or inhibitory effect on germination.The 0,5% gibberellic acid treatment appears to have a slightly more favorable effect on seed germination than the 1% treatment. Indeed, although germination remains low in both cases, the 0,5% treated seeds show a more consistent progress in germination over time. In contrast, the 1% treated seeds have more varied results, with some cases where no seeds germinate after 48 h. A higher concentration (1%) could induce greater stress on the seeds or interfere with the physiological processes necessary for germination, which may explain the slower and weaker response observed.

Guirra et al.,2020 state that the use of hormopriming as a seed treatment is beneficial for seedling germination and vigor under abiotic stress conditions. Indeed, seeds that underwent pretreatment showed faster imbibition than unprimed seeds.

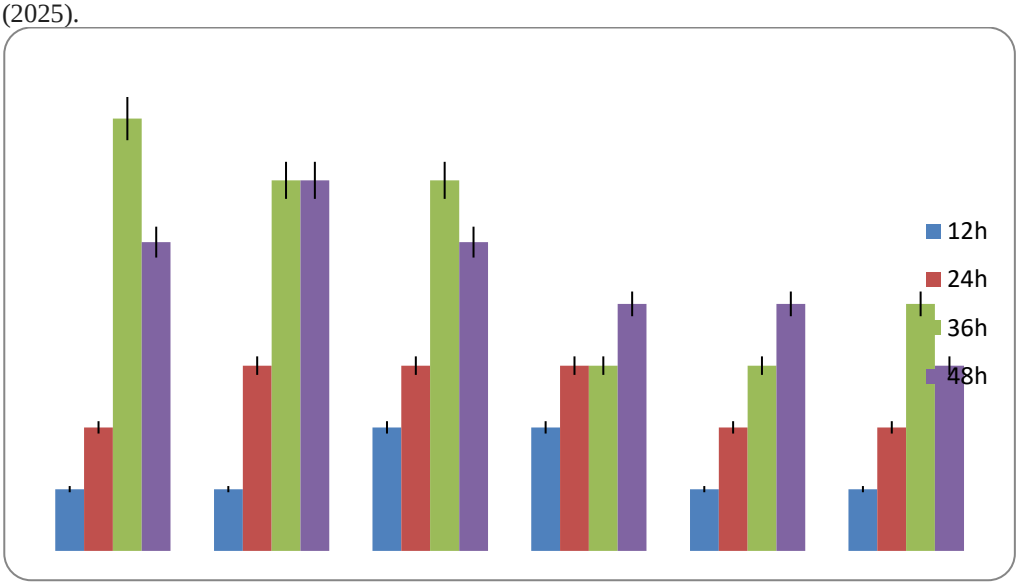
Although the results in Figure 4 do not directly specify the impact of the fire source, seeds collected between 4 and 10 meters from the fire source probably suffered thermal or chemical stress due to the proximity of the fire. This stress could have an inhibitory effect on germination, even after hormone treatment. However, gibberellic acid appears to have a stimulating effect, albeit modest, in helping seeds overcome this impact, although the effect is more pronounced at 0,5% than at 1%.

The priming technique and particularly hydropriming (distilled H2O) and hormopriming (IAA) have a positive effect on the germination rate and the quality (vigor) of seeds (Baaziz et al., 2021).

4.3 GERMINATION RATE OF PINUS HALEPENSIS SEEDS AT SITE 3: (MORE THAN 10 M) FROM THE FIRE SOURCE

Figure 5 shows the impact of gibberellic acid (Ag3) on the germination of Aleppo pine seeds collected more than 10 meters from the fire source and treated with hormonal solutions at two different concentrations (0,5% and 1%) after 12h, 24h, 36h and 48h of imbibition.

Figure 5: Germination rate of Aleppo pine seeds at sampling 3 (more than 10 meters from the fire) Source: Prepared by the authors (2025).



The values show the rate of number of seeds germinated in each replicate. However, we can make an analysis based on the trends of the results.

For seeds treated with 0,5% gibberellic acid solution, the results show a relatively stable progression of germination.

After 12 hours of imbibition, low germination was observed, with between 10 and 20% of seeds germinated per repetition. This low initial germination rate is typical, as seeds generally take some time to respond to the hormone. As for the 1% solution, the rate of germinated seeds is similar to that observed with the 0,5% concentration, with 10 to 20% of seeds germinated.

After 24 hours of moistening, the germination rate increases gradually, reaching 20 to 30% of germinated seeds. This shows that gibberellic acid begins to have a stimulating effect. The same is true for the 1% concentration where we also note that germination progresses slowly, with 20 to 30% of germinated seeds.

After 36 hours of imbibition, germination progressed further, with between 60 and 70% of seeds germinated per repetition. This suggests that the effect of gibberellic acid became more evident, with a strong increase in the number of seeds germinated. As for the 1% concentration, germination continued to progress, with between 30 and 40% of seeds germinated per repetition. However, the progression seemed less marked than with the 0,5% treatment, indicating that this concentration may have a less stimulating effect.

Finally, after 48 hours of moistening, germination remained high, with 50 to 60% of seeds germinated per repetition. Although there was a slight decrease compared to 36 hours, germination remained relatively stable. Also, with the 1% concentration of gibberellic acid, germination seemed to stabilize, between 30 and 40% of seeds germinated per repetition. There was no clear

improvement after 36 hours, suggesting that the 1% concentration might not be as effective in stimulating seed germination under these conditions.

Our results are in agreement with those of Miladinov et al., 2014 who confirmed that the priming technique namely hydropriming (H2O), osmopriming (KNO3) and hormopriming improve the germination and vigor of soybean seeds.

According to Shah et al., (2023), priming with Ag3 allows triggering pre-germination processes, treatment with 0,5% Ag3 shows a positive and progressive response over time. Germination increases continuously at each interval, suggesting that the effect of the hormone on Aleppo pine seed germination is relatively potent and is expressed more intensely after a certain imbibition time.

In parallel, treatment with 1% Ag3 shows a positive but less marked response compared to 0,5%. Although the effect is visible, it is slower and less sustained over time, indicating that lower concentrations of hormone may be more effective for some Aleppo pine seeds. Similar results were found by (Mohammed et al., 2023) who showed that pretreatment of barley seeds with AG3 clearly causes an acceleration of germination.

5 CONCLUSION

The results suggest that Aleppo pine seeds collected from the fire site may suffer irreversible damage from the fire. This highlights the importance of burnt area management for forest regeneration and ecosystem resilience after a fire. Furthermore, it shows that hormonal treatments alone are not sufficient to promote germination in extreme conditions such as those generated by a fire.

Aleppo pine seeds collected between 4 and 10 meters from the fire and treated with gibberellic acid show a positive, but modest, response to the hormone treatment. The 0,5% treatment appears to be more effective in stimulating germination than the 1% treatment, with more regular germination and continued progression over time. In contrast, the 1% treatment appears to have a less favorable effect, with slower and uneven germination. Gibberellic acid probably helps the seeds overcome the effects of fire stress, but lower concentrations of the hormone are more effective.

The 0,5% gibberellic acid treatment is generally more effective than the 1% treatment. Germination progresses more rapidly and sustainably at 0,5%, reaching higher germination rates after 36h and 48h of imbibition.

The 1% treatment shows less marked and slower germination, particularly after 36 h, suggesting that lower concentrations may be more suitable to stimulate Aleppo pine germination in this experimental environment.

In terms of germination dynamics, seeds treated with 0,5% Ag3 showed a continuous acceleration of germination over the hours of imbibition, which is typical of effective hormonal stimulation. In contrast, seeds treated with 1% showed a slower progression and a stabilization after 36h, suggesting that this concentration does not produce such a robust long-term response.

Although the effect of the fire source on germination is not explicitly mentioned in the table, it is likely that seeds collected more than 10m from the fire source were already partially exposed to the effects of heat or smoke.

However, the results indicate that gibberellic acid has a significant effect on germination even after this exposure to post-fire conditions, suggesting that hormone treatment may overcome some of the fire-induced barriers to germination.

Aleppo pine seeds collected more than 10 meters from the fire and treated with gibberellic acid showed a positive response to the hormone, but the 0,5% concentration remained overall more effective in stimulating germination than the 1% concentration. Germination progressed more quickly and more steadily with 0,5%, while with 1%, germination remained slower and stabilized earlier. This suggests that lower concentrations of gibberellic acid were more suitable for promoting germination of Aleppo pine seeds under these experimental conditions, even after a fire.

At the end of this study, the germination potential of Aleppo pine seeds from burnt forests shows remarkable differences depending on the sampling sites selected (Boukli Hacene et al., 2021). These differences between sources should guide future research, to study the physiological potential of seed germination; the modes and periods of stratification according to the hormopriming technique, which seems to significantly improve the germination rate, in view of the rehabilitation of this stand in perpetual decline due to forest fires, retained in reforestation and repopulation.

This study, while promising, remains limited by the small number of sites studied and by the evaluation of a single type of hormonel treatment at two concentrations. Furthermore, the trials conducted under controlled conditions do not fully reflect the constraints of the natural environment. For future work, it would be useful to diversify seed origins, explore other types of priming, and test the protocols in nursery conditions or directly in a post-fire forest environment.

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