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# Effects of Ethylimidazolium Nitrate and the Aluminum Nitrate Salt Mixtures on Germination of Three Forest Species †

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**Abstract:** Ionic liquids are synthetic compounds with melting temperatures lower than 100 °C and with high ability of modification of their physical and chemical properties from changes in their chemical structure. Although the number of applications in the last years has been continuously increasing, their effects on the different terrestrial ecosystems have been scarcely studied. In this work, the effects of the ionic liquid ethylammonium nitrate (EAN), the aluminum nitrate salt ($Al(NO_3)_3$), and the saturated mixture of both components on the germination of three forest species were studied. Different doses, from 0% to 10% weight, of the three treatments were applied at seeds of three different forest species (*Eucalyptus globulus* Labill, *Pinus radiata* D. Don, and *Pinus sylvestris* L.) and the germination of these seeds was continuously monitored for 35 days. The results showed that the addition of ionic liquid, salt, and mixture provoke the reduction of germination for all the species. Concentrations of 5% and higher incite the total inhibition of the germination of all species for all the treatments, with the EAN treatment being the most harmful.

**Keywords:** ionic liquids; toxicity; germination; *Eucalyptus*; *Pinus*

## 1. Introduction

Ionic liquids (ILs) are salts with low fusion temperature made of the combination of a cation and an anion, mostly organic. Recently, ILs have received much attention for being considered as green solvents because of their negligible vapor pressure. Besides, they present other interesting properties like high stability, and the possibility of controlling their properties by selecting the anion and the cation [1,2]. ILs are generally used as solvents, electrolytes, lubricants, as liquid crystals, supports for the immobilization of enzymes, matrices for mass spectrometry, in separation technologies, in preparation of catalytic membranes, and in the generation of high conductivity materials, among others [3,4].

The ethylammonium nitrate (EAN), the studied IL in this work, was the first discovered room-temperature IL [5]. This compound is a protic IL that is hydrophobic and ionic in character and has

the ability to exhibit hydrogen bonding. It can be used as an additive, detergent, precipitating agent or electrolyte, among other applications [6,7].

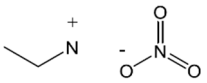
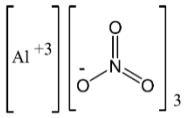
In this work, the effect of different concentrations of EAN and the aluminum nitrate salt mixtures on the germination of *Eucalyptus globulus* Labill, *Pinus radiata* D. Don, and *Pinus sylvestris* L. seeds was studied. All of these species are considered fast-growing trees, widespread around the world, and abundant in Europe [8].

## 2. Material and Methods

### *2.1. Chemicals*

The selected compounds, IL and salt, for this work, are presented in Table 1.

**Table 1.** Main characteristics of the ethylammonium nitrate (EAN) and aluminum salt.

| Name | Abbreviation CAS Number | Structure | Mw (g/mol) | Purity Provenance |
|---|---|---|---|---|
| Ethylammonium Nitrate | EAN 22113-86-6 |  | 108.096 | >0.97 Iolitec |
| Aluminum Nitrate | $Al(NO_3)_3 \cdot 9H_2O$ 7784-27-2 |  | 374.996 | >0.999 Merck |

Typical drying procedure of ionic liquids (ILs) under high vacuum was performed for EAN. Saturated solutions were obtained by mixing both components with the help of an ultrasound bath during (24 to 48 h) and by increasing molality in 0.5 mol $kg^{-1}$ intervals till saturation point, at room temperature [7].

### *2.2. Experimental*

In the germination tests, 125 seeds were used for each species in different treatments to determine the effect on the germination process. The studied doses were 0.1%, 0.5%, 1%, 2.5%, 5%, and 10%, and 0% (control) in EAN, $Al(NO_3)_3$ salt, and the saturated mixture (EAN + $Al(NO_3)_3$ 2 molal) for the three studied species. The different treatments were dissolved in distilled water and applied to seeds placed on a Petri dish with double filter paper. For each treatment and species, five replicates were used with 25 seeds. An amount of 4 mL of the corresponding dose was added initially in every Petri dish and the seeds were incubated in a Phytotron chamber with 16 h photoperiod at 24 °C in light and 8 h in dark at 16 °C [8,9].

## 3. Results and Discussion

In all cases, a decrease in germination at concentrations higher than 1% can be observed compared to the control (Figure 1). In the case of pure EAN, from 2.5%, no germination occurred for the *Pinus* species, and less than 10% of germination for *E. globulus*. However, aluminum salt did not provoke this strong effect, since a small percentage of germination can be observed, even at the 10% dose.

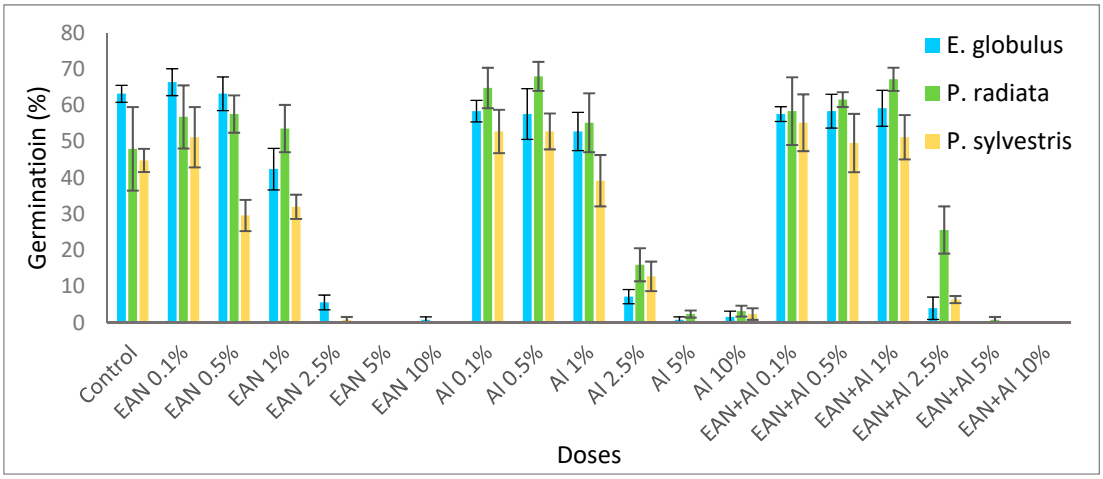



**Figure 1.** Percentages and standard error reached by each species with the studied doses of EAN, aluminum salt, and EAN + aluminum.

When the accumulated germination during these 35 days is considered, the effect of the different doses can easily be observed (Figure 2). In the case of *E. globulus*, almost all the doses present a decrease in germination values after 10 days, only 0.1% of EAN shows a small increase, and 0.5% of EAN with the same values as the control dose. In the case of the *Pinus* species, interesting effects were observed. The behavior of *P. sylvestris* is represented in Figure 2, and similar results were obtained for *P. radiata*. It is observed for EAN solutions that only a 0.1% dose induces an increase in germination in the whole studied time. Meanwhile, for the rest of the doses, a clear decrease is observed, and doses above 1% do not show germination responses on this studied period. In the case of aluminum salt, similar behavior was detected, although a 0.5% dose also showed higher germination response than the control. Finally, on the saturated mixture, interesting results have been observed, as doses of 0.1%, 0.5%, and 1% increased the germination. So, the aluminum mixture is able to alleviate EAN toxicity toward seed germination.

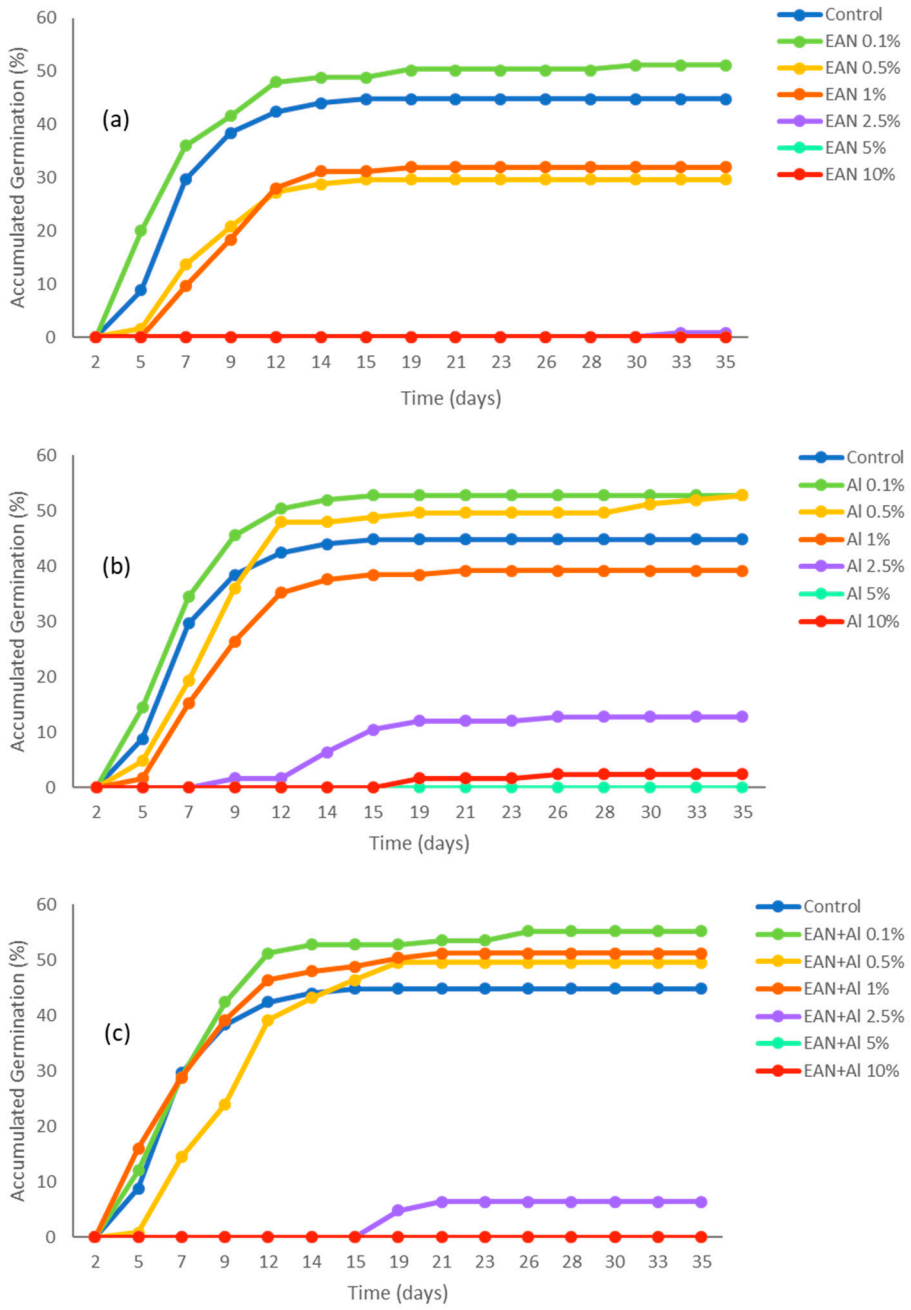



**Figure 2.** Effect of the treatments on the accumulated germination of *P. sylvestris*: (**a**) EAN; (**b**) $Al(NO_3)_3$; (**c**) EAN + $Al(NO_3)_3$ 2 molal.

These germination results are concordant and even less toxic compared with results from other authors with different ILs [8].

## 4. Conclusions

The present study reveals the effects of three compounds: EAN, $Al(NO_3)_3$, and the saturated mixture on the forest species germination, achieving their total inhibition in doses higher than 5% concentrations. It is observed that, on *P. radiata*, a higher germination percentage than the control is achieved at lower doses (up to 1%) with the three studied compounds. When comparing aluminum salt and the saturated mixture, inhibition is not as strong as the same EAN doses except for 0.1% and 0.5% in *E. globulus.*

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**Conflicts of Interest:** The authors declare no conflict of interest.

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