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Effects of ethylimidazolium nitrate and the aluminium 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 ºC and with high ability of modification of their physical and chemical properties from changes on 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 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 were 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, being EAN treatment 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 are getting much attention for being considered as green solvents because of their negligible vapor pressure. Besides, they present otherinteresting 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-temperatureIL [5].This compound is a protic IL, that has hydrophobic and ionic character and the ability to hydrogen bonding. It can be used as an additive, detergent, precipitating agent or electrolyte, among other applications. [6,7]

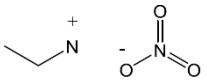
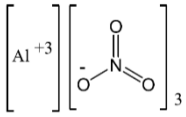
In this work, the effect of different concentrations of EAN and the aluminum nitrate salt mixtures on the germination of *Eucalyptus globulus* Labill, *Pinus radiata* D. Don and *Pinus sylvestris* L. seeds was studied. All of these species are considered fast-growing trees, widespread around the world 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 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
Aluminium Nitrate	$Al(NO_3)_3 \cdot 9H_2O$ 7784-27-2		374.996	>0.999 Merck

Typical drying procedure of 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

On the germination tests 125 seeds were used for each specie, 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 + $Al(NO_3)_3$ 2m), 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 specie, five replicates were used with 25 seeds. 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 light and 8 h dark at 16 °C. [8,9]

3.Results and discussion

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

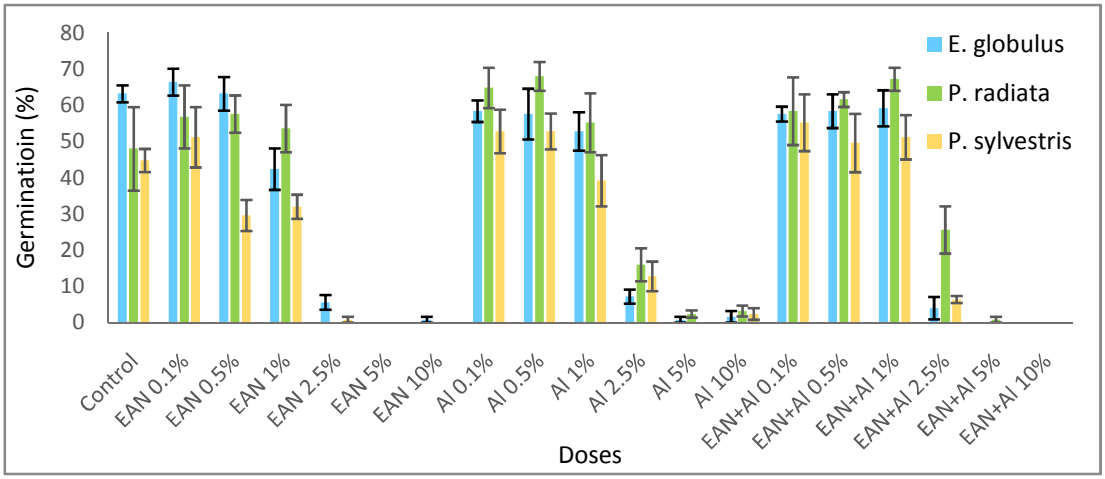


Figure 1. Germination percentages and standard error reached by each species with the studied doses of EAN, aluminium salt and EAN+aluminium.

When the accumulated germination during these 35 days is considered, it can easily be observed the effect of the different doses (Figure 2). In the case of *E. globulus* almost all the doses present a decrease on germination values after 10 days, only 0.1% of EAN shows a small increase, and 0.5% of EAN the same values of control dose. In the case of *Pinus* species interesting effects were observed, *P. Sylvestris* behaviour is represented on Figure 2, and similar results were obtained for *P. radiate*. It is observed for EAN solutions that only 0.1% dose induces an increase on 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 response on this studied period. In the case of aluminium salt similar behaviour was detected, although 0.5% dose also showed higher germination response than control. Finally, on the saturated mixture, interesting results have been observed, as doses of 0.1%, 0.5% and 1% increased the germination. So, aluminium mixture is able to alleviate EAN toxicity towards seed germination.

These germination results are concordant, even less toxic, with results from other authors with different Ils [8].

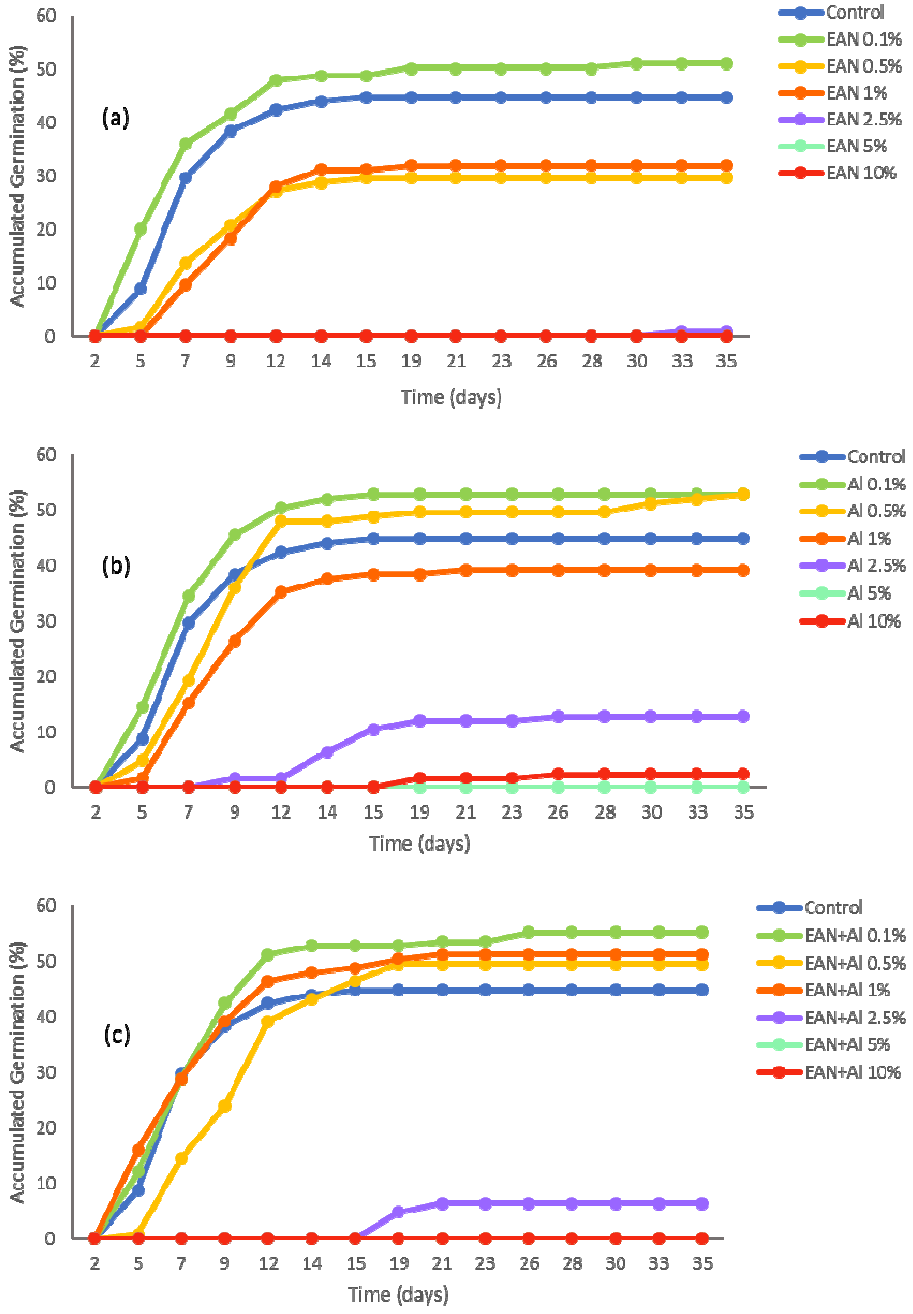


Figure 2: Effect of the treatments on the accumulated germination of *P. Sylvestris*: (a) EAN, (b) $Al(NO_3)_3$, (c) EAN + $Al(NO_3)_3$ 2m.

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*, higher germination percentage than control is achieved at lower doses (up to 1%) with the three studied compounds. When comparing aluminum salt and 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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