

# The Influence of Certain Types of Substrate and Biochemical Substances in Seed Germination and Plant Development of Spruce (*Picea abies*)

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## Abstract

In order to analyse seeds germination and seedlings growth of spruce (*Picea abies*) there were used five treatments with bio and chemical substances for stimulation germination: H<sub>2</sub>O, Atonik, Cropmax, CuSO<sub>4</sub>, KMnO<sub>4</sub> and five treatments with different substrates: Jiffy pots, peat + conifer humus, peat + conifer humus + perlite, peat + conifer humus + sand, peat + conifer humus + perlite + sand. Of all germination stimulants, the highest percentage of seeds germination was obtained when the seeds have been immersed in aqueous solution with Atonik. From among substrates, the highest percent of germination was obtained using peat + humus + perlite + sand, following by peat + conifer humus + perlite and Jiffy pots. The substrate containing peat and humus of coniferous can not be recommended as a properly germination substrate for spruce seeds. Results showed that using chemical substances for germination, the germination was comprised between 56.7-73.3%. Seeds treated with KMnO<sub>4</sub> had the lowest germination, and those treated with Atonik (0.75 ml active substance in one liter tap water) presented the highest germination percentage. The best growth of seedlings was assured by peat + conifer humus + perlite substrate.

**Keywords:** recoverable containers, seeds, spruce, substratum, substances

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## Introduction

The forest represents an important terrestrial ecosystem which joins different subsystems in a stable ecological equilibrium and a harmoniously integrated structure (Florescu and Nicolescu, 1996; DeFries *et al.*, 2010; Aerts and Honnay, 2011). Forests and trees contribute to sustainable agriculture stabilizing the soil and the climate, regulating the drain, creating a habitat for pollinators, and natural enemies of agricultural pests (Pauzaite *et al.*, 2017), and assure wood production, ameliorative and protective roles, but also ecological, landscape, cultural, educational, relaxation roles etc., and consequently inestimable values (Plesa *et al.*, 2017; Sestras *et al.*, 2018).

The family *Pinaceae* is the largest (more than 230 species) and most important family of conifers from an economic point of view (Price and Zandstra, 1998; Lockwood, 2013).

*Picea abies* (L.) Karst. (the Norway spruce) is of greatest importance tree species of Europe's forests (Jansson *et al.*, 2013). It widely ranges from the northern to the southern parts of Europe's forested lands, covering more than 30 million hectares, which corresponds to 38% of the continent's coniferous area (Caudullo *et al.*, 2016). In Romania, the species covers approximately 23.4% of the forested area and represents 77% of the country's conifers (Budeanu and Sofletea, 2013; Schiop *et al.*, 2015).

Norway spruce is ecologically and economically the most important coniferous tree species in Romania (Radu *et al.*, 2014). In Romanian Carpathians, Norway spruce is the dominant native forest species at elevations between 1200 and 1800 m (Feurdean *et al.*, 2011), but scattered populations can be found until 500 m above sea level (Sofletea and Curtu, 2007). Currently, Norway spruce is a widely used species in plantation forestry and plays an important role for carbon storage in the Carpathian Mountains (Dutcă *et al.*, 2010).

Spruce is a monocious, unisexual and wind-pollinated tree. It usually begins to bloom at the age of 40-50 years (Lindgren *et al.*, 1977), after which at 120-180 it begins to decrease in flowering process (Hagner, 1955). Flowering involves the differentiation of primordia into female or male flowers.

Germination begins with the uptake of water by the quiescent seed and continues with a rapid increase in metabolic activity (Bewley, 1997; Nonogaki *et al.*, 2010). Germination is often considered to be completed as the radicle emerges from the seed (Black *et al.*, 2006), while ISTA (International Seed Testing Association, 2005) rules determine the germination of conifer seeds to be complete when the radicle has grown to the length of four times the length of the seed coat (International Seed Testing Association, 2005).

The optimum temperature for spruce seed germination is 20-22°C (Bergsten, 1987; Leinonen *et al.*, 1992). According to Leinonen *et al.* (1992) 50% germination was obtained at 14°C and the germination rate was reduced to temperatures above 23°C. Along with temperature the oxygen availability affects germination. Kaila (2007) found that an oxygen content in air of 8% reduced the germination of spruce seeds in Norway compared to the level of environmental oxygen (21%).

Spruce seeds typically germinate equally well in darkness or light in optimal temperatures (Leinonen *et al.*, 1992). The optimal pH for the germination of this species is around 5-6, with decreasing germinability especially in more alkaline conditions (Rikala and Jozefek, 1990).

Germination, survival, and growth depends on "safe sites" that provide the precise environmental conditions required by a particular seedling (Harper, 1977). Site conditions like e.g. temperature, moisture, pathogenic fungi, or light are

important factors for Norway spruce (*Picea abies* (L.) Karst.) seedling development in natural forests (Ammer, 1996; Brang, 1998; Helenius *et al.*, 2005).

In forestry, the quality of planting material has a decisive role in creating some highly productive and ecological stable stands, which have an optimal structure and composition for the specific environmental conditions. To fulfil this requirement, it can be produced and planted bare-roots saplings or it can be produced and planted containerized saplings. Now, containerized sapling production is a modern and often successfully applied method. Advantages of container as compared with bareroot stock are well known, e.g., better root protection, ameliorative and nutrient effects of potting substrate, reduction of transplanting stress on planting site (Repáč *et al.*, 2014). Other advantages include more effective care for faster growth of seedlings grown individually in containers (especially under greenhouse conditions) than bareroot seedlings grown in dense spacing in a nursery bed (Nilsson and Orlander, 1995; South *et al.*, 2005; Wilson *et al.*, 2007; Repáč *et al.*, 2011a; Esen *et al.*, 2012).

It has recently been shown that the recruitment of a new spruce generation strongly depends on the quality of the seedbed (Brang, 1998; Brang *et al.*, 2003; Mori *et al.*, 2004). This applies in particular for spruce, because spruce is a species with small seeds and therefore is more substrate-restricted than other tree species (Greene *et al.*, 1998; Hérault *et al.*, 2004; Knapp and Smith, 1982).

Besides the type of spruce seedling production, the substrate plays an important role. One of the most used substrate for growing containerized plants is peat, especially *Sphagnum* peat, slightly humidified (low pH, proper balance of aeration and waterholding porosity, low inherent fertility, cation exchange capacity) and offers good growing conditions in nursery (Bernier and Gonzalez, 1995; Topić *et al.*, 2006; Vaario *et al.*, 2009; Heiskanen, 2013).

The current study aimed to identify different types of substrate with beneficial influences on germination, growth and development of spruce plantlets (1) and to identify the suitable germination stimulants in Norway spruce seeds' germination (2).

## Materials and methods

### *The biological material*

As biological material was used seeds of spruce (*Picea abies*), derived from an arboretum with age of 90 years. The arboretum is part of Provenance Region A320 Spruces, Spruce and beech forests mix with beech, O. S. Toplita, UP III Secu. This arboretum is located at a latitude N of 46° 45', longitude E of 25° 20' and an altitude of 1000 m.

The spruce cones were harvested at the end of October and were deposited for drying, at temperatures of 20-25°C. All germination experiments were accomplished in laboratory, at temperatures of 16-24°C and in conditions of natural light.

This study was divided in two parts: in the first part was analysed the germination of spruce seeds using different germination stimulants; in the second part was identified the types of substrate with beneficial influences on germination and growth of spruce seedlings.

### *The study of germination*

Seeds' germination was analysed using two types of work: a) five different bio and chemical stimulants; b) five different types of substrate (Tab. 1).

For the study, 100 seeds × 4 replicates were used per variant. In the first case, the seeds were placed for germination in Petri pots with diameter of 14 cm on two filter paper layers soaked with 5 ml bi-distillate water. A seed was considered as germinated when the radicle was at least four times the length of the seed coat (International Seed Testing Association, 2005). In the case of substrates, the seeds were sown in plastic recoverable recipients.

### *Statistical procedures*

The way in which the mean of germination was affected by different types of chemical solutions used as germination stimulants and the types of substrates used, was analysed with analysis of variance (ANOVA). When ANOVA illustrated a significant effect ( $p < 0.05$ ), post hoc comparisons between means were conducted, using Duncan's

**Table 1.** Characteristics of the two parts of the study (germination using different stimulants and germination using different types of substrate)

Nr.	Variant	Treatment	Duration treatment	Concentration
<b>The study of germination using different bio and chemical stimulants</b>				
1.	V1	Distilled water	Seeds have been soaked in distilled water for 24 hours	-
2.	V2	Atonik	Supposes soaking of seeds for 8 hours in diluted solution	In report 1:2000 (0.75 ml active substance in one liter tap water)
3.	V3	Cropmax	In case of treatment with cropmax, seeds have been soaked in watery solution for 12 hours	Used concentration being of 1 ml active substance at 1 l water
4.	V4	CuSO <sub>4</sub>	In case of treatment with CuSO <sub>4</sub> and seeds have been soaked in solution for 24 hours	A concentration of 1 g copper sulphate at 0.5 l water was used
5.	V5	KMnO <sub>4</sub>	In case of treatment with KMnO <sub>4</sub> , seeds were soaked in aqueous solution for 24 hours	Used concentration being 1.25 g potassium permanganate at 0.5 l water
<b>The study of germination using different types of substrate</b>				
1.	V1	Jiffy pills		
2.	V2	Peat <i>Sphagnum</i> (50%) + humus of coniferous (50%)		
3.	V3	Peat <i>Sphagnum</i> (33%) + humus of coniferous (33%) + perlite (33%)		
4.	V4	Peat <i>Sphagnum</i> (40%) + humus (40%) + sand (20%)		
5.	V5	Peat <i>Sphagnum</i> (40%) + humus (40%) + perlite (10%) + sand (10%)		

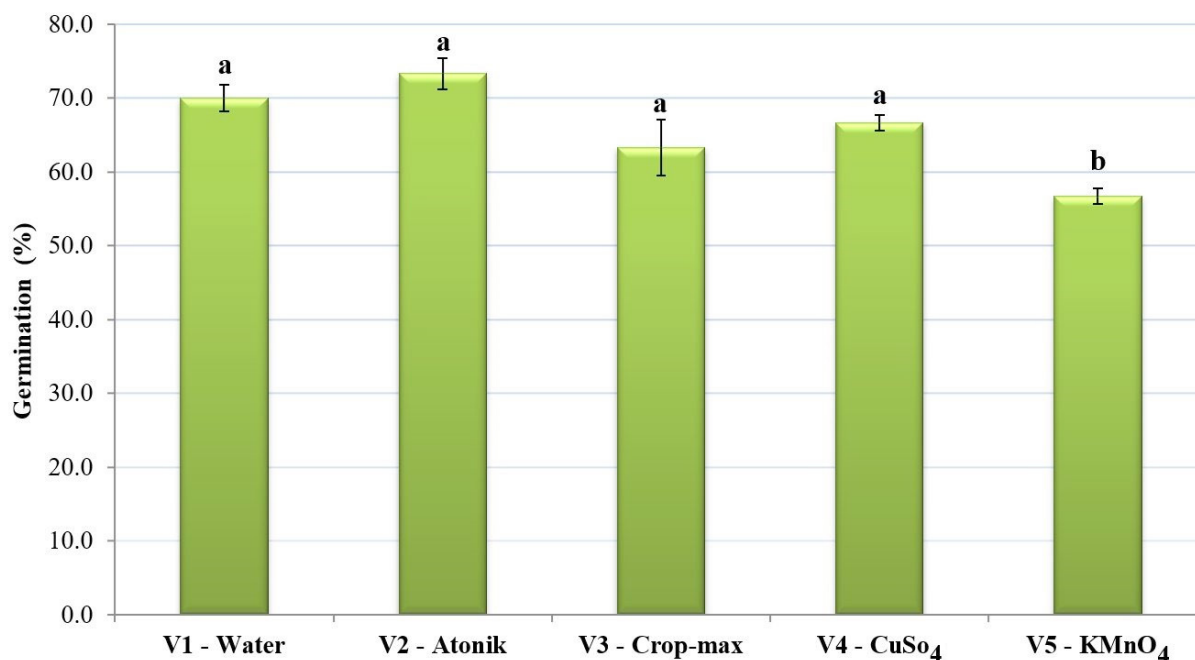


Figure 1. Germination percentage using different bio and chemical stimulants

multiple-range test. The comparison of the mean for the seeds' germination using five stimulants with mean of those germinated using five different substrates was performed using the Student test ('t' test,  $p < 0.05$ ). Data was represented using boxplot (Sestras, 2018, 2019).

## Results and discussions

### *The study of germination using different germination stimulants*

Regarding the germination of spruce seeds using different stimulants, the highest values were registered at second variant (V2 - 73.3%), followed by variant (V1) and variant (V4). Inferior values were registered at variant (V5 - 56.7%) (Figure 1).

The control of seed germination is a very sophisticated process which requires the concerted action of and interaction between diverse phytohormones (Kucera *et al.*, 2005). In seed tissues, the best investigated are the antagonistic actions of abscisic acid and gibberellins on dormancy and germination (Linkies and Leubner-Metzger, 2012).

A positive result was obtained after treating the seeds with auxin and microelements, as expected, knowing all their positive effects on a number of plant activities, including embryo development, leaf formation, fruit development,

initiation and root development (Grodnitskaya and Sorokin, 2007; Repáč *et al.*, 2014).

Treating the spruce seeds with auxin, gibberellin and copper sulphate significantly improves seed germination. These results are in agreement with Thakur and Kanwar (2008); Vilcan *et al.* (2011).

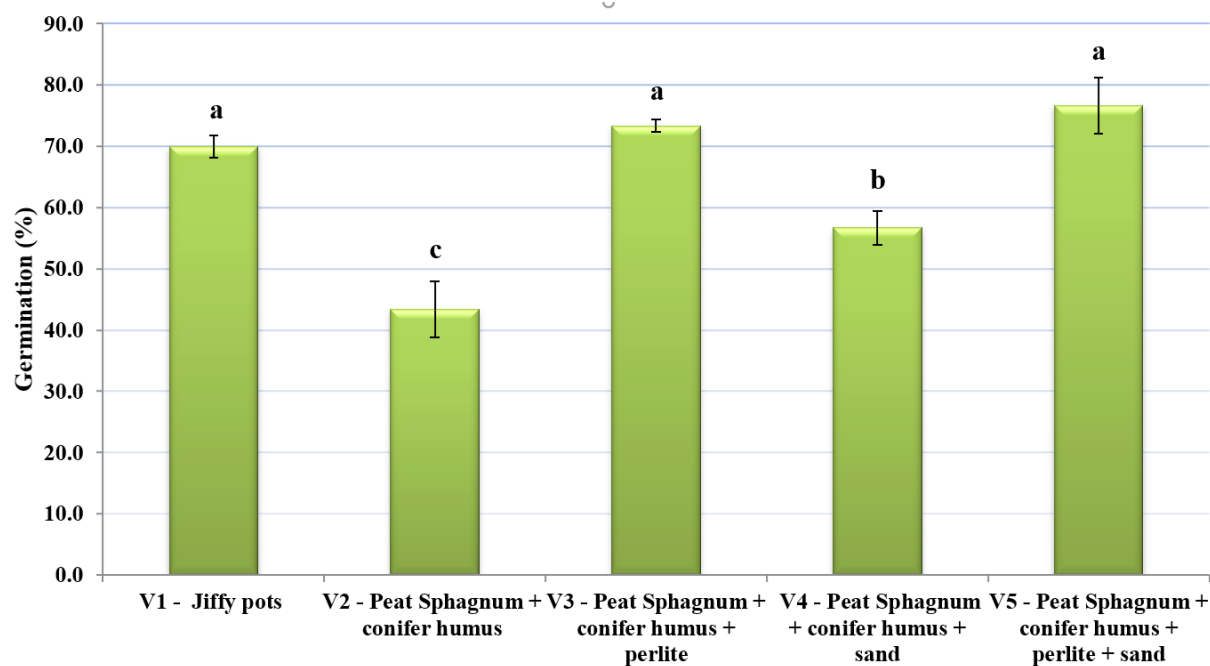
The present results indicated that seeds' treatment with an Atonik solution (0.75 ml active substance in one liter tap water) conducted to the highest germination percentages (73.3%).

### *The study of germination using different types of substrate*

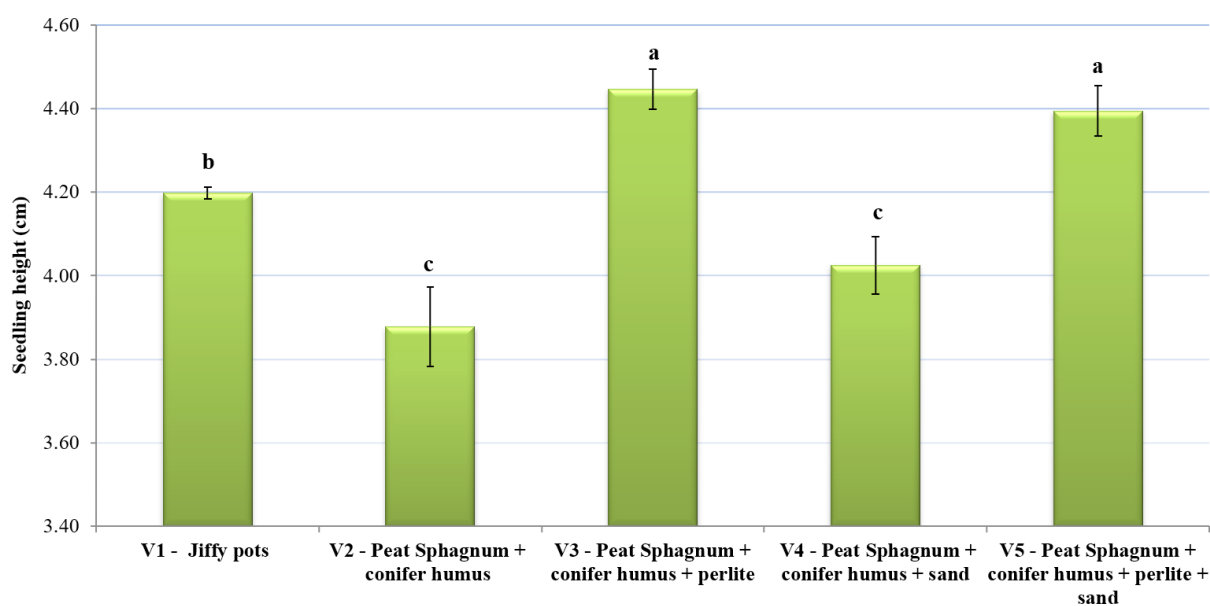
Regarding the study of germination using different types of substrate, significant differences were observed between the analysed variants (Figure 2).

One of the growth media most utilized for growing containerized plants is peat, used either alone or as a main component of peat based growing mixtures (Landis *et al.*, 1990).

Significantly superior values were registered at variants (V5 - 76.7%; V3 - 73.3%, respectively V1 - 70.0%), and significantly inferior values were registered at the second variant (V2 - 43.3%). The substrate containing peat (50%) and humus of coniferous (50%) can not be recommended as a good germination substrate for spruce seeds.



**Figure 2.** The germination percentage on different types of substrate



**Figure 3.** Seedling length on different types of substrate

Regarding the growth of seedlings, the highest height was registered at third variant (4.45 cm), respectively five (4.40 cm), variants that have in composition peat, humus, perlite, respectively sand (Figure 3).

Boxplot diagram represents a chart with the five values of a distribution. In case of seeds treated with different stimulants, the lowest germination percentage was of 33.3%, and the highest germina-

tion percentage was of 83.3%. The average of germination percentages, in this situation, was of 66.7%. In case of seeds' germination from substrate, the minimum registered percentage was of 16.7%, and the maximum value was of 76.7%. The average of germination percentages was 46.7% (Figure 4; Tab. 2).

The Student test showed that seeds' germination that were treated with five stimulant

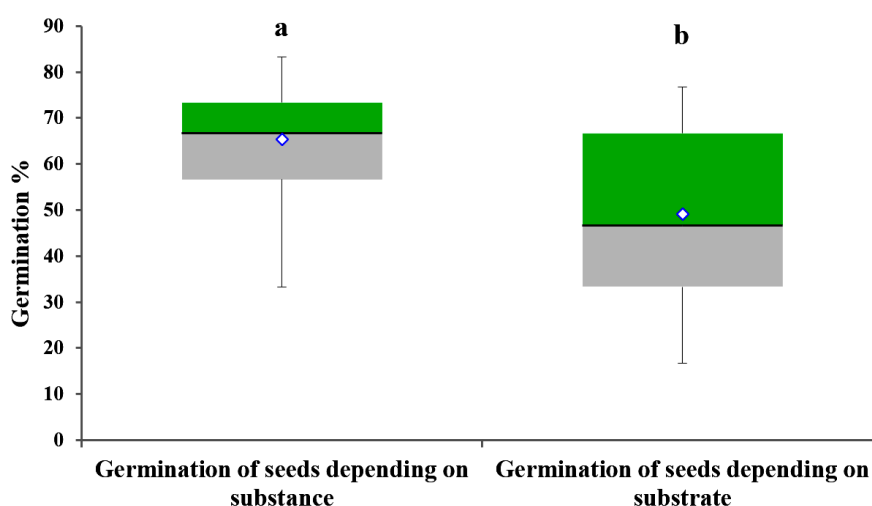


Figure 4. Boxplot diagram for germination of spruce seeds

Table 2. The main parameters of statistics for seeds' germination

Labels	Germination of seeds depending on substance	Germination of seeds depending on substrate
Min	33.3	16.7
Q <sub>1</sub>	58.3	36.7
Median	66.7	46.7
Q <sub>3</sub>	71.7	66.7
Max	83.3	76.7
IQR	13.3	30.0
Upper Outliers	0.0	0.0
Lower Outliers	1.0	0.0

substances was significantly higher compared to seeds' germination on the five types of substrates ( $p$  calculated  $0.029 < p < 0.05$ ).

### Conclusion

The study of germinative and growth parameters of spruce plantlets, showed different effects of stimulants and tested substrates. The results indicated that seeds treated with an Atonik solution might have a high germination percentage (73.3%). Regarding the substrate types and the height of seedlings the best results were obtained in case of variant peat 33% + humus 33% + perlite 33%.

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