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Seed germination of exotic species under the influence of biostimulants

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Abstract
The aim of this study is to investigate the effect of different growth stimulants on seed germination of exotic species such as: Korean fir (*Abies koreana*), cypress (*Cupressus sempervirens*), California cypress (*Chamaecyparis lawsoniana*), Mediterranean pine (*Pinus pinea*), Macedonian pine (*Pinus peuce*). For this research, growth stimulants such as Atonik, Cropmax and Sprintene were used. The results obtained indicate that the germination capacity of the studied exotic species is significantly influenced by the application of these biostimulants. They are recognized for their unique content of organic compounds that have demonstrated the ability to enhance germination as well as seedling development. Among the three types of biostimulants used, Cropmax and Sprintene had a notable impact on the germination rate of all the analyzed species, compared to the control variant. The findings of this study highlight the importance of using growth stimulants to enhance seed germination of exotic species. These results will be useful in optimizing cultivation and care practices, thereby contributing to the conservation and diversification of plant biodiversity.

Keywords: development, provenance, seeds, stimulants, treatment

Introduction

A species is considered native to a certain region when it has evolved naturally in that area or has arrived there through natural dispersal processes [15]. In contrast, an exotic species is defined as one that has been introduced into a specific area solely through human intervention. Exotic forest species native from a particular region and brought to other parts of the world are used for various purposes, such as forestry or landscaping. These species have the potential to provide significant economic and ecological benefits, however they may also present major challenges to the balance of local ecosystems. Furthermore, exotic species often face difficulties related to seed germination.

Germination is an important stage in the life cycle of a plant, serving as the essential starting point for establishing a new generation [22]. This initial phase marks the beginning of the development and regeneration process, playing a fundamental role in the perpetuation and evolution of the species. Due to the extreme fragility of the seedling, germination constitutes a period of critical vulnerability, representing a moment of maximum risk in the development of the seedling [17]. During this early phase, any adverse factor can permanently compromise the viability of the new generation, highlighting the importance of optimal conditions for the success of the process. Thus, the germination duration exhibits an adaptive character [4,3], reflecting the seed's ability to synchronize the onset of development with favorable environmental conditions. The use of biostimulants in the germination process may be necessary to support seedling development, helping to reduce the negative effects of environmental stress and improve or maintaining seedling productivity [10]. Conifers represent a diverse group among other species and are widely used in landscape design. These plants include species such as *Abies*, *Pinus*, *Cedrus*, *Picea*, *Juniperus*, *Chamaecyparis*, *Cupressus*, etc. [11]. Several of these species are also present in Romania, where they are used in landscaping projects, urban forests, and botanical gardens.

Abies koreana is an endemic species in Korea [14] and it is included in the National Red List as an endangered species by the Ministry of Environment and it is recognized as a Biological Indicator Species Sensitive to Climate Change (CBIS) [19,14].

Pine species constitute an integral part of European forests, having an significant importance in the current context of global climate change [26]. *Pinus pinea* is a valuable species, appreciated for its aesthetic, ecological and commercial uses [26], as well as for its seeds, known as pine nuts or pinoli, which are recognized for their high nutritional content [24,27] and are classified among the most expensive globally [27]. They play an important role in the Mediterranean diet due to their refined flavor [18] and are mainly found in countries such as Spain, Italy, Portugal, Tunisia and Morocco [6,16,31]. *Pinus peuce*, commonly known as the Macedonian pine, was first identified in 1939 by the German botanist August Grisebach, on Mount Baba in Macedonia. This marked its recognition as a new species of five-needle pine that had not been previously reported in the Balkan Peninsula. [9]. This ornamental tree, admired for its impressive height of 35-40 meters, is often cultivated in parks and gardens, especially in Europe [23,12]. Its durable wood is highly valued, and its resin has valuable applications in the chemical, optical, and pharmaceutical industries, and local communities use this resin to treat wounds, stomach ailments and various other health issues [2,28].

Another exotic species is *Cupressus sempervirens* also known as the Mediterranean cypress, which is of European origin and belongs to the *Cupressaceae* family. This species is particularly valued for its therapeutic properties, as well as for its potential pharmacological interest [25]. *Chamaecyparis lawsoniana*, known as the California cypress, is a highly valued ornamental species that belongs to the *Cupressaceae* family [20,8]. It has various uses, including its use in construction and the production of railway sleepers [13]. Additionally, it has a long history of traditional use in treating ailments such as abdominal pain, tumors, and lipomas [21].

The current study aims to analyze the effects of various growth stimulants on the germination process of seeds from exotic species, including Korean fir (*Abies koreana*), cypress (*Cupressus sempervirens*), California cypress (*Chamaecyparis lawsoniana*), Mediterranean pine (*Pinus pinea*) and Macedonian pine (*Pinus peuce*).

Material and Method

The biological material used in this research was represented by the seeds of the four exotic species, harvest from two countries: Italy - Saturnia (*Pinus pinea*, *Cupressus sempervirens*), Romania - Cluj-Napoca - Campus USAMV (*Chamaecyparis lawsoniana*, *Pinus peuce*) and Baia Mare (*Abies koreana*).

As biostimulants, products such as: Atonik, Cropmax, Sprintene were used. In order to test the germination before this process, the seeds were soaked for 12 hours in solution containing each of the three biostimulators. The treatments were applied for 30 days. For seed germination, specialized Linhardt vessels were utilized, featuring a unique paper substrate composed of vegetable cellulose. Subsequently, after the seedlings emerged, they were transferred to alveoli with peat substrate, thus ensuring optimal conditions for their development. This method facilitated proper drainage and aeration, supporting healthy root growth and overall.

(a) *(b)* *(c)* *(d)*

Figure 1. (a) Seeds location; (b) preparation of biostimulants; (c) Linhardt vessels; (d) seedlings

Atonik (mono-nitroguaic sodium) is an organic growth regulator that is non-phytotoxic and harmless to both humans and the environment. It does not act as a growth hormone and is not considered a pesticide

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[30]. The active substances in Atonik include 0.2% ortho-nitrophenolate sodium(O-NP), 0.3% para-nitrodenolate of sodium (P-NP) and 0.1% nitroguaiacolate of sodium (NB) [5].

Cropmax is a growth stimulant, whose activity is based on a combination of trace elements, amino acids, vitamins and polysaccharides [29]. This combination works synergistically to enhance plant growth and development, promoting better nutrient uptake and overall vitality.

Sprintene is an efficient growth stimulant for seeds, that enhances germination rates and promote the rapid development of roots, activating biological processes and accelerating cellular metabolism for faster and more uniform germination.

By utilizing these biostimulants, the research aimed to explore their potential to optimize the germination process of the selected exotic species, contributing to more effective cultivation and management practices.The obtained results were statistically processed using analysis of variance (ANOVA), and if the null hypothesis was rejected, the differences were subsequently evaluated through Duncan's Multiple Range Test (Duncan's MRT, $p < 0.05$).

Results and Discussion

Seed germination is the process through which the embryo transitions into a seedling under favorable conditions. This stage of plant development occurs when environmental factors such as moisture, temperature, and light are optimal, allowing the seed to activate metabolic processes and begin growth [3]. This process involves a sequence of physiological, biochemical and morphological transformations, which, taken together, lead to the germination of the seed [3].

In this study, the most promising results regarding the germination capacity of the selected exotic species seed were noticed using Cropmax and Sprintene. The germination percentages ranged between 40-80%, highlighting the effectiveness of these treatments in facilitating the germination process. These values reflect not only a significant improvement compared to the control sample, but also the positive impact that biostimulants can have on the physiological and biochemical processes that promote seed germination. This suggests that the use of biostimulants can enhance the overall germination efficiency and support healthier seedling development.

In the control variant, where no biostimulants were applied, the germination percentage varied significantly, ranging from 13.33% for *Pinus pinea* to 23.33% for *Chamaecyparis lawsoniana*, this interval suggests a considerable difference in germination capacity.

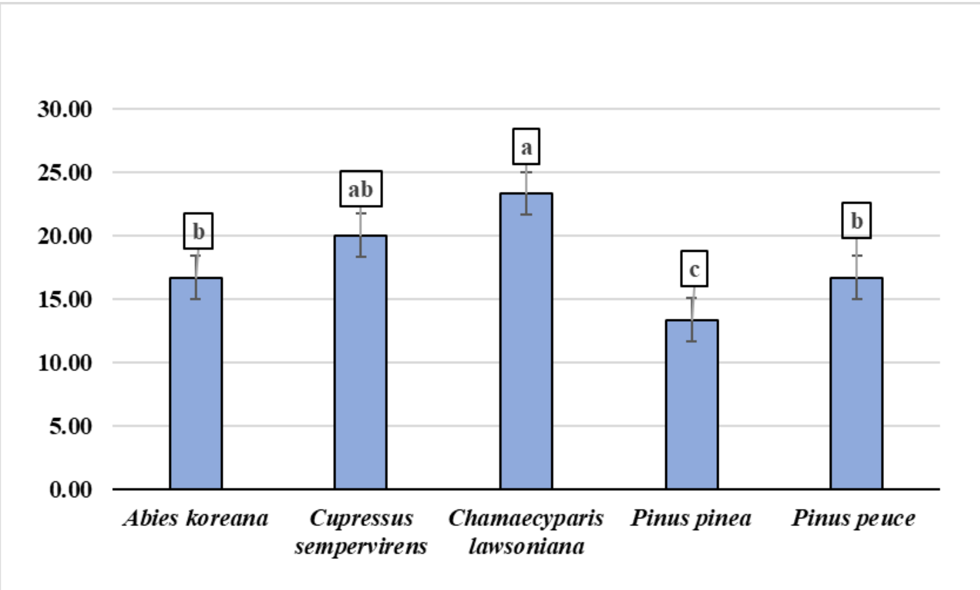

Figure 2. Germination capacity – Control variant. Different letters within means indicate statistically significant differences for treatments, at a significant level of p < 0.05 (Duncan test).

In the context of the study on the germination of selected species seeds and the application of the biostimulant Atonik, the obtained results demonstrated a significant improvement in the germination percentage compared to the Control variant. This trend was especially observed in the species *Cupressus sempervirens*, which recorded a germination percentage of 30%. This result suggests that Atonik can positively influence embryo development by stimulating the physiological processes necessary for germination.

On the other hand, the species *Pinus pinea* exhibited the lowest germination rate among all the investigated species, with a percentage of 20%. This aspect may indicate not only the influence of external variables, such as environmental conditions and seeds characteristics, but also the biological specificity of each species. Notably, significant results were also recorded for the seeds of sorghum soaked in the nutrient solution of Atonik, which led to a considerable increase in the average germination characteristic [1]. This underscores the importance of both the biostimulant and the inherent traits of the species in determining germination success.

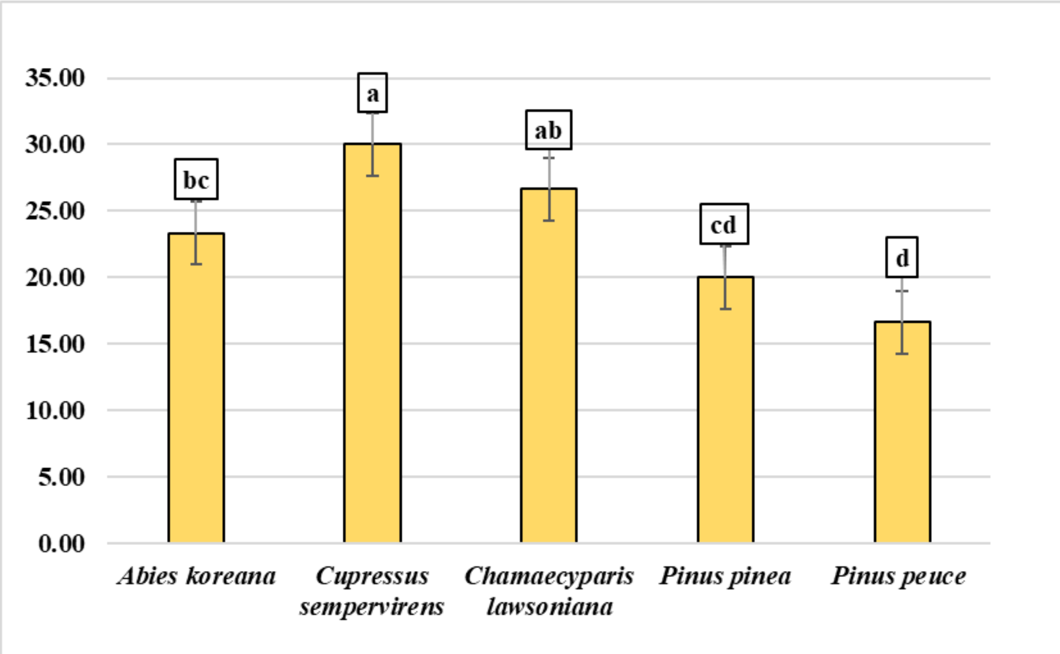

Figure 3. Germination capacity – *Atonik* variant. Different letters within means indicate statistically significant differences for treatments, at a significant level of $p < 0.05$ (Duncan test)

For the variant in which the Cropmax biostimulant was used, the germination percentages were significantly higher, demonstrating its effectiveness in stimulating the germination process. In this situation, the germination percentage varied between 33.33% for *Pinus pinea* and 83.33% for *Cupressus sempervirens*.

The increased germination rates observed with Cropmax can be attributed to its unique formulation, which includes essential trace elements, amino acids, vitamins, and polysaccharides. These components work together to stimulate metabolic processes within the seeds, improving their ability to absorb water and nutrients, which are critical for successful germination.

Additionally, the higher germination percentage for *Cupressus sempervirens* indicates that this species may be particularly responsive to the biostimulant, potentially due to its biological and physiological characteristics

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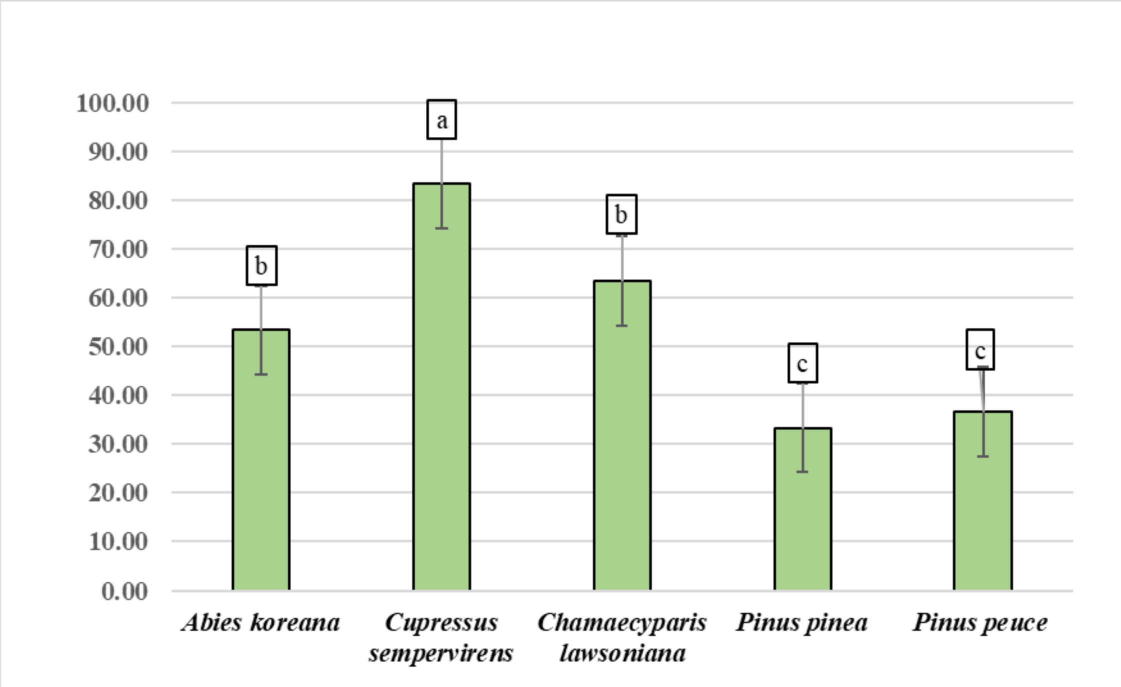

Figure 4. Germination capacity – *Cropmax* variant. Different letters within means indicate statistically significant differences for treatments, at a significant level of $p < 0.05$ (Duncan test)

In the variant where the biostimulant Sprintene was applied, the germination percentages showed a notable improvement. The species that obtained the highest percentage of germination was *Abies koreana*, with a remarkable rate of 83.33%. This performance suggests a favorable reaction of the seeds of this species to the stimuli provided by Sprintene, allowing for optimal embryo development.

On the other hand, *Pinus peuce* exhibited the lowest germination rate among the investigated species, with a percentage of 40%. This suggests that, despite the application of the biostimulant, the seeds of *Pinus peuce* had a more limited germination capacity.

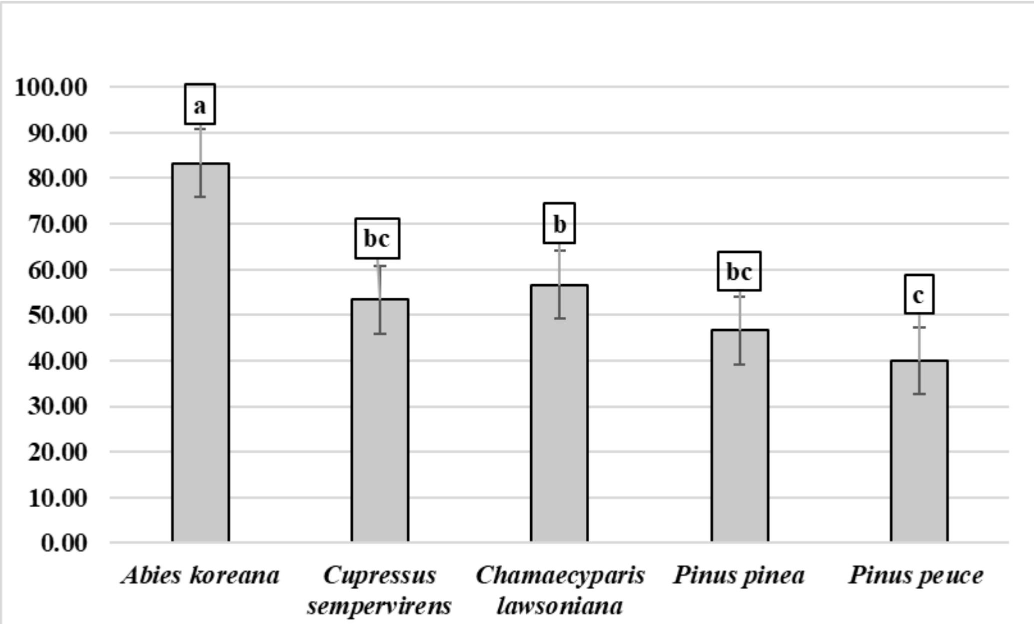

Figure 5. Germination capacity – *Sprintene* variant. Different letters within means indicate statistically significant differences for treatments, at a significant level of $p < 0.05$ (Duncan test)

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In the analysis of seed germination for the species *Abies koreana*, the data presented in Figure 6, indicate a clear upward trend in germination percentage following the application of the four experimental treatments. Among these, the treatment with Sprintene stood out an impressive germination rate of 83%. This value suggests not only the effectiveness of the biostimulant Sprintene in activating essential physiological processes, but also the ability of *Abies koreana* seeds to respond favorably to the conditions created by this stimulant.

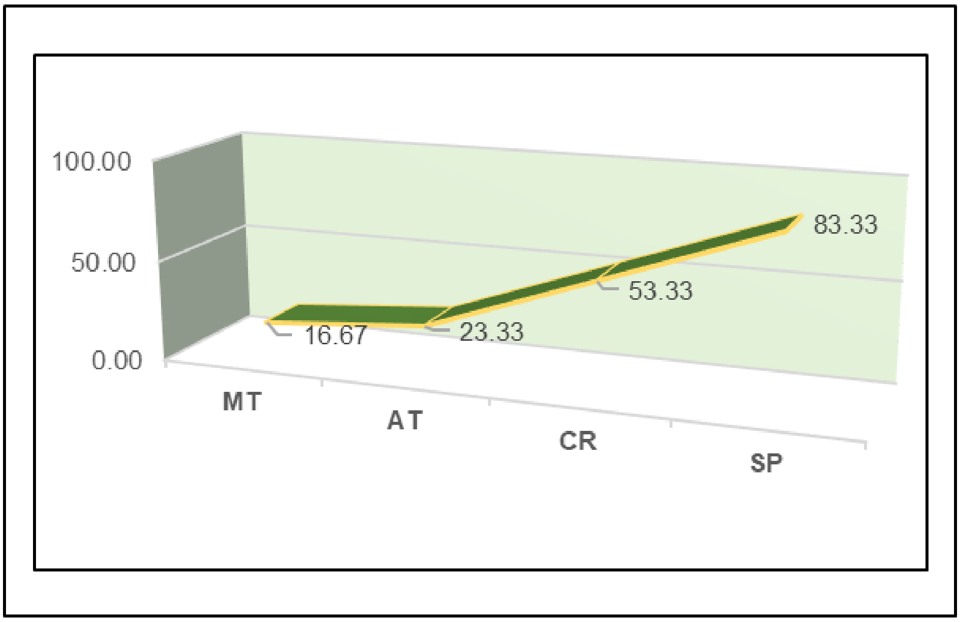

Figure 6. Germination of *Abies koreana* species for the four treatments

In contrast, the analysis of *Cupressus sempervirens* seeds, shown in Figure 7, reveals that the treatment with the biostimulant Cropmax generated the highest germination rate, also reaching 83%. This result underscores the importance of the biostimulant Cropmax in supporting the germination of the seeds of this species, also highlighting its effectiveness in stimulating processes. The high germination percentage achieved by *Cupressus sempervirens* indicates an excellent adaptability of the seeds to the conditions favored by Cropmax, which may lead to healthy and robust seedling development during subsequent growth stages.

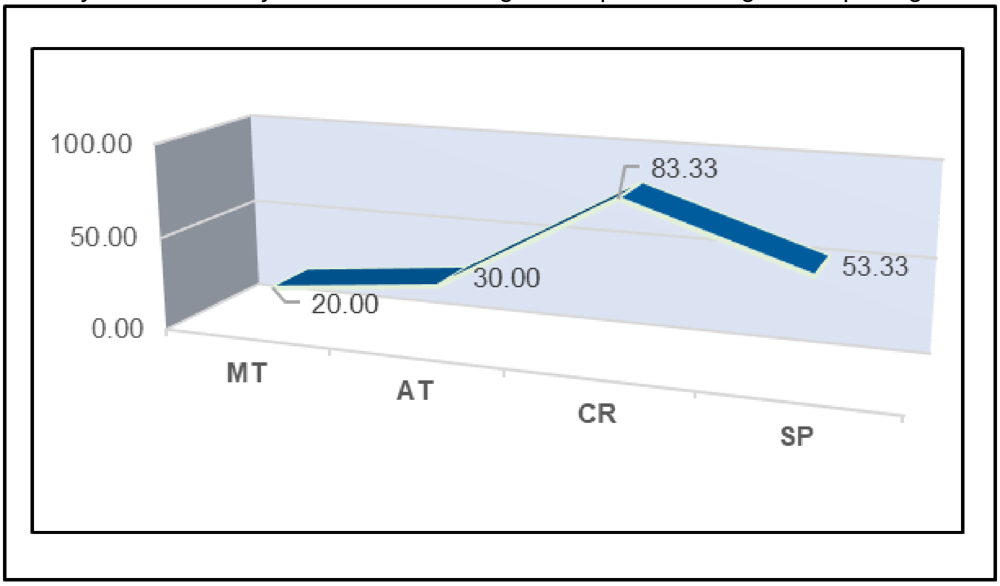

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Figure 7. Germination of *Cupressus sempervirens* species for the four treatments

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The results obtained for *Chamaecyparis lawsoniana* seeds were promising, highlighting the effectiveness of treatments with biostimulants, especially Cropmax and Sprintene. In the case of the Cropmax treatment, the germination percentage of 63% indicates a favorable reaction of the seeds, which benefited from the metabolic stimulation provided by this biostimulator. Additionally, the result obtained with Sprintene, showing a germination rate of 56%, confirms the efficacy of this biostimulant. Although slightly lower than that of Cropmax, the 56% rate is still significant and suggests that Sprintene is capable of activating the necessary processes for successful germination, albeit in different ways compared to Cropmax.

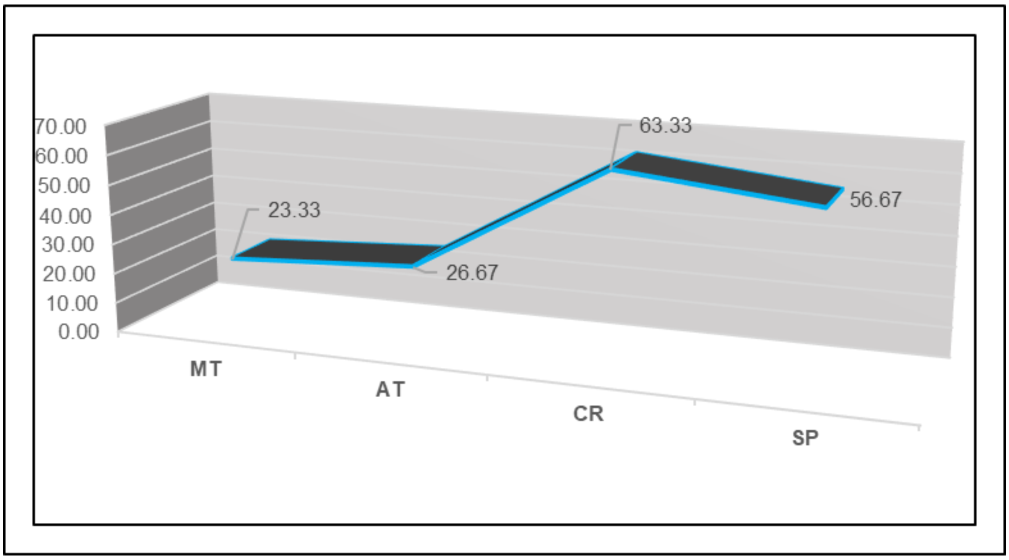

Figure 8. Germination of *Chamaecyparis lawsoniana* species for the four treatments

Regarding the seeds of the species *Pinus pinea*, the germination percentages were noticeably lower compared to the other species analyzed in the study. The germination percentage of only 13% in the Control variant indicates that *Pinus pinea* seeds were unable to realize their biological potential in the absence of external stimulants, which can be attributed to the characteristics of the seeds themselves. On the other hand, the application of the biostimulant Sprintene had a significant impact, resulting in a germination percentage of 46%.

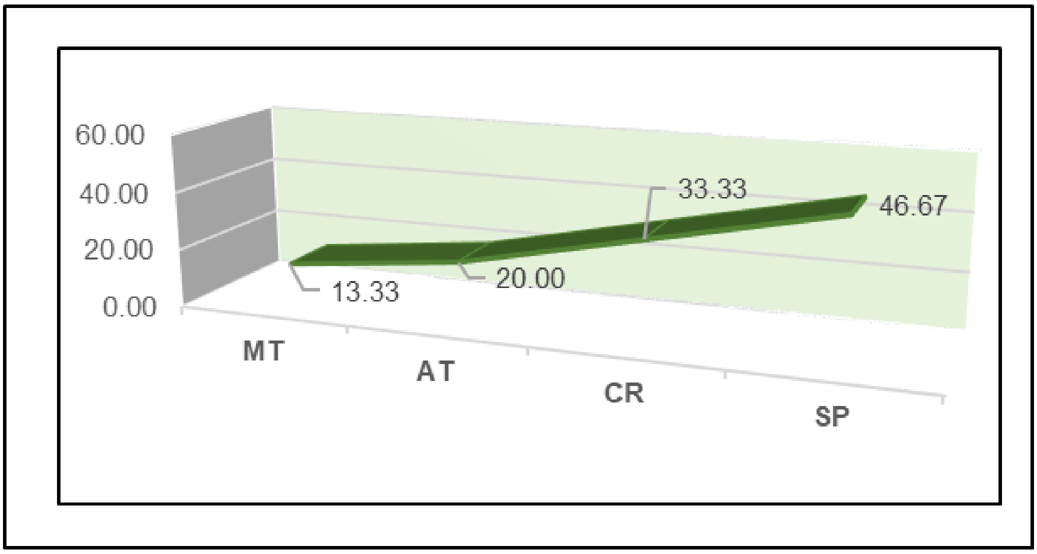

Figure 9. Germination of *Pinus pinea* species for the four treatments

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Regarding the seeds of *Pinus peuce*, the observed trend was similar to that of *Pinus pinea*, showing relatively low germination rates. As in other previous specialized studies, it can be observed that seed germination is a highly intricate process that involves various biological factors influencing the embryo within the seed [7]. However, the application of biostimulants led to significant improvements in this process, with the best results recorded in the variants treated with Cropmax, which achieved a germination percentage of 36% and Sprintene, which had a percentage of 40%.

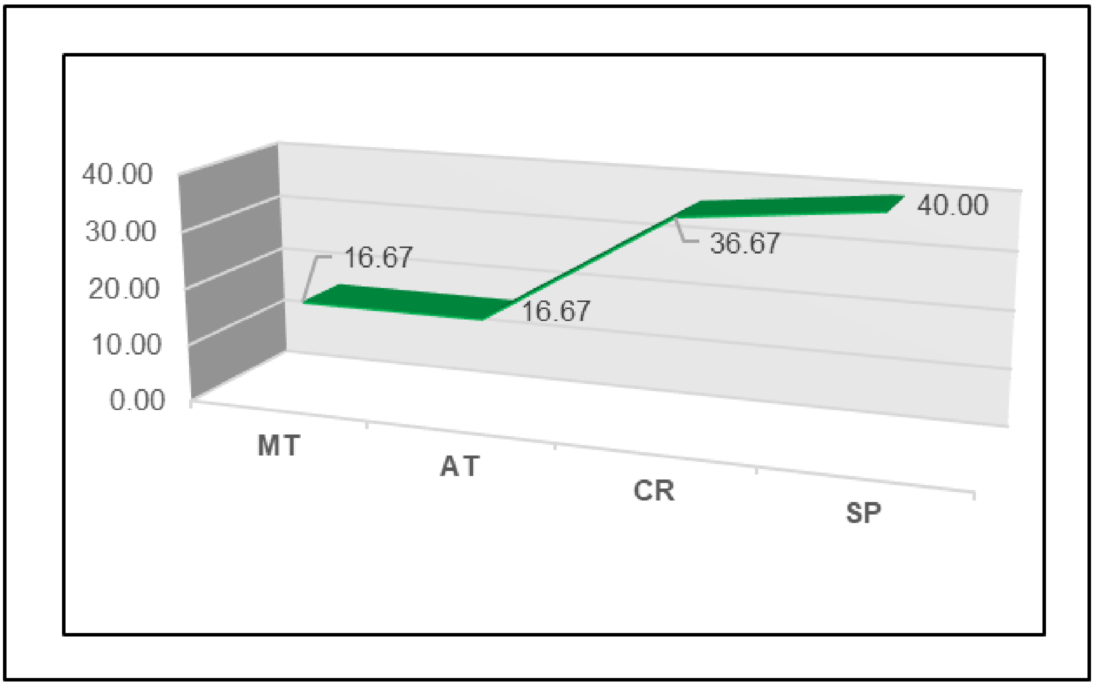

Figure 10. Germination of *Pinus peuce* species for the four treatments

Conclusions

In conclusion, this study highlights the significant influence of treatments with biostimulators on the germination capacity. As a result of the conducted experiment, it was highlighted that the germination of seeds is significantly influenced by the use of biostimulants, which contribute to the improvement of seedlings productivity. These biostimulants, through their mechanisms of action, can stimulate the biological processes of seeds, promoting the development of roots and the aerial part of plants, thereby increasing their viability and vitality. At the same time, It is essential to note that the efficiency of germination does not depend exclusively on the application of biostimulants, but also on the specific characteristics of each species. Each species has its own ecological and biological requirements, which influence how it responds to external stimuli. The results of the experiment suggest that an optimal combination of biostimulant use and appropriate species selection can lead to a significant improvement in the germination rate and, consequently, the overall productivity of plants.

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