Caspian Journal of Environmental Sciences

Online ISSN: 1735-3866
Print ISSN: 1735-3033

# The effect of different biological products applied with different doses and exposure time on the germination of Scots pine seeds

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## ABSTRACT

The aim of the study was to determine the efficiency of methods enhancing the vitality of Scots pine, *Pinus sylvestris* L. seed by using biological products of microbial origin such as Agrarka, AgroMIX, and Epin with 10 mL $L^{-1}$, 5 mL $L^{-1}$, and 2.5 mL $L^{-1}$ doses. The exposure time of the seeds were 30 min, 1 h, 6 h, 24 h. The examined parameters included germination energy (GE) and germination capacity (GC). The results showed that all the tested methods accelerate germination of the pine seeds; using biological products is the most efficient and recommended method for Scots pine and soaking of seeds not long-term is the most efficient and recommended. The best results for the GE and GE were obtained in the pine using biological preparation at AgroMix 10 mL $L^{-1}$ under 6 h of soaking seeds.

**Keywords:** Scots pine, Soaking water, Biological preparation, Germination capacity, Germination energy.
**Article type:** Research Article.

## INTRODUCTION

The cultivation of planting material is a major component for the artificial regeneration, rehabilitation, restoration, and afforestation implements. The survival, growth and sustainability of the created forest crops depend on the quality of seeds. Often collected coniferous seeds have a low quality class for a number of reasons: adverse weather factors during flowering and ripening of cones, improper harvesting and processing of cones, violation of seed storage conditions, etc. The effect of fertilizers on Scots pine includes plant growth (Borrero 2004) and initial development after rooting. Vincent (1965) and Dušek *et al.* (1970) informed about many procedures that were tested for stimulating seed germination such as exposure of seeds to diverse forms of energy (heat, light, radiation, ultrasound) and treatment with chemicals (inorganic and organic compounds, growth stimulators). In addition to their positive influence on germination rate, growth stimulators improve morphological parameters of seedlings emerged from treated seeds (Sıvacıoğlu *et al.* 2007; Ertekin *et al.* 2009; Khurshkaynen *et al.* 2019). Regulators of plant development are the most important internal factors involved in plant growth and development. These organic substances have the property of being transported within the plant, causing growth and differentiation in the tissues and organs where they go is happening (Akman and Darıcı, 1998). Plant development regulators not only improve the seedling characters, but also increase the plant's resistance to environmental stresses (Chorievich *et al.* 2023; Gataulina *et al.* 2023; Kırdar & Allahverdiyev 2003). In addition, these regulators are also used to remove the germination barrier in some species (Quassar *et al.* 1997; Kırdar & Ertekin 2001; Kırdar 2002). Effective Microorganism (EM), i.e., effective, active, or beneficial microorganism technology has started to develop in recent years due to the harmful effects of chemical fertilizers or pesticides on human health and nature. It was established by the Japanese philosopher and naturalist Mokichi Okadain the

Caspian Journal of Environmental Sciences, Vol. 22 No. 1 pp. 23-29 Received: April 08, 2023 Revised: July 24, 2023 Accepted: Sep. 19, 2023
DOI: 10.22124/cjes.2024.7471

Publisher: University of Guilan,

1930s, considering the long-term harm of chemicals (Ayan *et al.* 2018, 2021, 2022). EM consists of mixed cultures of natural and beneficial microorganisms that are widely used in soil and is applied to plants as inoculants to increase soil quality, plant growth and yield (Higa & Parr 1994; Iwaishi 1994; Iwahori & Nakagawara 1996). EMs, which are mostly used in agriculture, have become an important part of natural agriculture. Although EM consists of numerous microbial varieties, the predominant populations are lactic acid bacteria, yeasts, actinomics, and photosynthetic bacteria (Ayan *et al.* 2018, 2021, 2022). In order to increase seed germination and accelerate the growth of seedlings, pre-sowing seed treatment is carried out. There are various methods of pre–sowing seed treatment for certain tree and shrub species - treatment with stimulants, soaking, scalding, bubbling, snowing, irradiation, etc. Seed treatments with stimulants are widely known in the scientific literature, but the offered assortment, doses and time of soaking seeds vary greatly by research regions (Cardarelli *et al.* 2022). The introduction of the Scots pine, *Pinus sylvestris* L. and its seed in Kazakhstan can indeed have significant benefits for the yield, economics, and environmental sustainability of the forestry system in the country. This review aims to focus on the bio-stimulating effect of beneficial microorganisms applied to the seed of the major crops whose cultivation cycle begins with seed sowing. The main species of forest tree whose seeds are harvested in the Akmola region, in Kazakhstan, are Scots pine *P. sylvestris* L. The seed of the Scots pine in Kazakhstan can make a substantial contribution to the yield and economics, plus the environmental sustainability of the forestry system. This study aimed to investigate the bio-stimulating effect of beneficial microorganisms when applied to the seeds, which marks the beginning of the cultivation cycle. This effect results in increased plant germination, as well as the capability to overcome seed-related issues both during and after emergence. In this regard, in the ecological conditions of Northern Kazakhstan, the influence of Agrarka, AgroMix and Epinon, on the acceleration and increase of pine seedlings when used was investigated. The aim of the research was to determine the effect of stimulants during pre-sowing seed treatment on germination and to analyse the qualities of the seeds of Scots pine.

## MATERIALS AND METHODS

The technical germination of seeds was determined on a device for germinating seeds of forest plants PLUS.441352.001 RE according to GOST 13056.6–97 at a water temperature of 24 °C. The germination of seeds (100 pcs. in 4 repetitions) was taken into account on 7 and 15 days. Weight of 1000 pcs seeds were determined by weighing 500 pcs seeds in two repetitions. The place of research was the laboratory of the RSE "Republican Forest Breeding and Seed Centre", belonging to the Committee of Forestry and Wildlife, the Ministry of Ecology and Natural Resources, the Republic of Kazakhstan, located in the Akmola region. A study to determine laboratory germination was carried out by soaking seeds in stimulants for various periods, i.e., from 30 min to 24 h. Observations were carried out according to germination energy recording on the seventh, laboratory germination – on the 15$^{th}$ day. The experiments were laid in four times repetition, with 100 seeds in each. The seeds of Scots pine for sowing were collected in test cultures of plus trees. The purity of the seeds and the weight of 1000 pieces were 99% and 11.46 g respectively. Creating an organic fertilizer based on the bird's waste products using biological products like «Agrarka» and «Agromix» is certainly feasible. Bird waste, such as poultry manure, is rich in essential nutrients like nitrogen, phosphorus, and potassium, which are beneficial for plant growth (Shumenova *et al.* 2023). Bio-fertilisers of domestic production, developed by scientists of the S. Seifullin Kazakh Agrotechnical University including "Compo-MIX", "Agrarka", "Agro-MIX", and "Trichodermin-KZ". In this study, biological products were such as Agrarka, AgroMIX, and Epin. "Agrarka" bio-fertiliser is a liquid concentrated fertilizer made on the basis of effective strains of actinomycetes, consisting of such strains as *Streptomyces xantholiticus* (pcs. 7), *S. microsporus* (pcs. 12), *S. sioyaensis* (pcs. 41), producing a complex of biologically active substances with fungicidal properties against fungal diseases and phyto-stimulating effect of agricultural crops. "Agro-MIX" bio-fertiliser was created on the basis of strains of growth-stimulating, nitrogen-fixing, anti-putrefactive microorganisms *Bacillus* spp., *Saccharomyces* spp., *Acetobacter* spp., and *Streptomyces* spp. Epin- epibrassinolide is a phytohormone of natural structure. The drug stimulates resistance to late blight, helps to reduce the content of salts of heavy metals, and nitrates. When processing seeds, it increases their germination, and enhances the protective properties to adverse environmental conditions. Composition: 24-epibrassinolide, epinbrassinolide, (24R)-brassinolide (Shumenova *et al.* 2023). Methods for determining the germination of seedlings were carried out by soaking seeds in stimulants invented in Kazakhstan. The seeds of Scots pine for sowing were collected in test cultures of plus trees. Soaking seeds was for 0.5 h, 1h, 6 h, and 24 h.

Germination capacity (GC) is defined to be equal to the number of normally germinated seeds at the end of the experiment (after 15 days) out of the total number of seeds:

$$GC (\%) = \text{total number of germinated seeds} / \text{total number of sown seeds} \times 100 \qquad \textbf{(1)}$$

Germination energy (GE) is defined to be equal to the number of normally germinated seeds determined on day 7 after setting up the germination test and specified in the percentage of germinating seeds:

$$GE (\%) = \text{Total number of germinating seeds on the seventh day/total number of sown seeds} \times 100 \qquad \textbf{(2)}$$

The data obtained for the germination characteristics were transformed into a normal distribution. Comparison of the variance factors such as stimulant, dose, and exposure time, were carried out by ANOVA with a significance level (α) of 0.05 using the STATISTICA program (Ver. 21). Multiple comparisons, i.e. the determination of differences or zero differences among the treatments, were made using the LSD test.

## RESULTS AND DISCUSSION

Results of variance analyses showed significant differences among different biological products, dose and exposure time ($p < 0.05$; Table 1). However, the germination capacity in different treatments under 30 min of soaking seeds was not significant. The seeds highest germination capacity was observed at Agrarka 2.5 mL $L^{-1}$, AgroMix 5 mL $L^{-1}$, and AgroMix 10 mL $L^{-1}$ (91.66%; 90.33% and 910% respectively), for 1 h of soaking. The lowest GC was found at Epin 5 mL $L^{-1}$, Epin 10 mL $L^{-1}$, and Control for 1-h soaking seeds. GC was significantly decreased by 6-16% at Control, Epin all doses, Agrarka 5 mL $L^{-1}$, and 10 mL $L^{-1}$ for 6 h of soaking seeds. The highest values of GC was observed at AgroMix 2.5 mL $L^{-1}$ (88.66%). It was also statistically true for Agrarka 10 mL $L^{-1}$ (85.0%) and AgroMix 10 mL $L^{-1}$ (84.66%) under 24-h soaking seeds.

**Table 1.** Germination capacity (%) of Scots pine seeds in the variants of different pre-sowing treatments under different soaking ($p < 0.05$).

| **Treatments** | **Dose** (mL $L^{-1}$) | **30 min** | **1 h** | **6 h** | **24 h** |
|---|---|---|---|---|---|
| Agrarka | 10 | $91.66 \pm 1.45^{a}$ | $87.33 \pm 2.96^{ab}$ | $90.33 \pm 0.3^{bcde}$ | $85.0 \pm 1.15^{ab}$ |
| Agrarka | 5 | $91.33 \pm 1.76^{a}$ | $85.66 \pm 1.45^{ab}$ | $89.33 \pm 1.76^{bcde}$ | $75.0 \pm 1.73^{c}$ |
| Agrarka | 2.5 | $87.0 \pm 1.0^{a}$ | $91.66 \pm 0.88^{a}$ | $93.0 \pm 1.73^{ab}$ | $80.33 \pm 0.88^{b}$ |
| AgroMIX | 10 | $91.33 \pm 1.76^{a}$ | $90.33 \pm 2.91^{a}$ | $96.33 \pm 0.88^{a}$ | $84.66 \pm 1.2^{ab}$ |
| AgroMIX | 5 | $88.0 \pm 2.5^{a}$ | $91.0 \pm 0.58^{a}$ | $92.33 \pm 1.33^{bc}$ | $81.0 \pm 1.0^{b}$ |
| AgroMIX | 2.5 | $90.33 \pm 2.84^{a}$ | $88.66 \pm 2.6^{ab}$ | $91.33 \pm 0.88^{bcd}$ | $88.66 \pm 0.66^{a}$ |
| Epin | 10 | $90.33 \pm 0.7^{a}$ | $73.0 \pm 1.73^{d}$ | $86.33 \pm 1.76^{e}$ | $57.0 \pm 1.15^{d}$ |
| Epin | 5 | $92.66 \pm 2.33^{a}$ | $78.33 \pm 1.66^{cd}$ | $88.0 \pm 1.0^{de}$ | $72.66 \pm 3.2^{c}$ |
| Epin | 2.5 | $88.33 \pm 1.2^{a}$ | $84.0 \pm 1.53^{bc}$ | $88.33 \pm 0.88^{cde}$ | $73.33 \pm 0.88^{c}$ |
| Control | | $87.0 \pm 2.0^{a}$ | $78.33 \pm 0.88^{cd}$ | $81.66 \pm 0.88^{k}$ | $83.0 \pm 0.6^{b}$ |

In the case of germination energy (%) value, statistically significant differences were recorded between the control and biological preparation treatment under 30 min of soaking seeds ($p < 0.05$; Table 2). At Agrarka 10 mL $L^{-1}$, and all doses of AgroMix under 1 hour of soaking seeds, the higher initial germination energy (above 16-24%) was still observed in comparison with the control and Epin all doses. Under 6 hours of soaking seeds the high (GE) value was noted at AgroMix (94.66%), while lowest at the control (77.66%). The highest (GE) value was also observed at Agrarka 10 mL $L^{-1}$ (79.33%) and AgroMix 2.5 mL $L^{-1}$ (77.33) under 24 h of soaking seeds.

**Table 2.** Germination energy (%) of Scots pine seeds in the variants of different pre-sowing treatments for different soaking ($p < 0.05$).

| **Treatments** | **Dose** (mL L$^{-1}$) | **30 min** | **1 h** | **6 h** | **24 h** |
|---|---|---|---|---|---|
| Agrarka | 10 | 83.66 ± 0.3$^{a}$ | 63.33 ± 1.85$^{a}$ | 89.33 ± 0.33$^{bc}$ | 79.33 ± 1.33$^{a}$ |
| Agrarka | 5 | 83.33 ± 1.45$^{a}$ | 54.0 ± 2.64$^{bc}$ | 85.0 ± 1.53$^{cd}$ | 52.0 ± 3.78$^{bc}$ |
| Agrarka | 2.5 | 77.33 ± 1.76$^{b}$ | 52.6 ± 1.45$^{bc}$ | 89.0 ± 1.0$^{bc}$ | 52.33 ± 6.48$^{bc}$ |
| AgroMIX | 10 | 83.66 ± 0.33$^{a}$ | 62.33 ± 2.84$^{a}$ | 94.66 ± 0.33$^{a}$ | 54.33 ± 4.1$^{bc}$ |
| AgroMIX | 5 | 83.66 ± 1.2$^{a}$ | 59.0 ± 2.64$^{ab}$ | 89.66 ± 1.76$^{bc}$ | 58.33 ± 0.33$^{b}$ |
| AgroMIX | 2.5 | 83.66 ± 1.2$^{a}$ | 62.66 ± 1.45$^{a}$ | 91.66 ± 0.88$^{bc}$ | 77.33 ± 0.33$^{a}$ |
| Epin | 10 | 82.0 ± 1.52$^{a}$ | 58.0 ± 2.0$^{c}$ | 82.66 ± 2.33$^{cd}$ | 24.0 ± 2.08$^{e}$ |
| Epin | 5 | 80.66 ± 0.88$^{ab}$ | 54.33 ± 2.6$^{bc}$ | 87.0 ± 2.0$^{bsd}$ | 35.33 ± 1.7$^{d}$ |
| Epin | 2.5 | 79.66 ± 0.66$^{ab}$ | 53.0 ± 1.15$^{bc}$ | 87.66 ± 1.76$^{bc}$ | 35.0 ± 1.0$^{d}$ |
| Control | | 73.66 ± 1.85$^{c}$ | 48.0 ± 2.0$^{c}$ | 77.66 ± 0.88$^{e}$ | 45.33 ± 2.9$^{c}$ |

In the case of the seeds exposed to a biological product for one day, a low percentage of germination was observed relative to the control. The percentage of germination varied from 61% to 87%. In addition, 83% of the methods of pre-sowing treatment showed an elevation in the germination energy of seeds. However, once the seeds exposed for 30 min, this figure was 100%. According to Hrabí (1990), Procházková (2004) and Hoffmann *et al.* (2005), pre-sowing treatments mentioned in the literature can be effective in enhancing the vitality of non-dormant seeds of Scots pine, particularly in the context of containerized planting stock. These treatments are aimed at promoting faster and more homogeneous germination, which is crucial for achieving high yields of seedlings. The seeds of Scots pine germinate the fastest after more 1-h stratification. Mean germination time reached the lowest values after 24-h soaking seeds and statistically significant differences were demonstrated compared with the other variants of pre-sowing treatment. Nevertheless, seed germination energy was clearly the highest after 6-h soaking seeds lots. This method of pre-sowing treatment was recommended for Scots pine also by e.g. Procházková (2004), Hoffmann *et al.* (2005) and Gordon (1992). Chang *et al*. (1991) found out that one or two dehydration-rehydration cycles stratification improved both the percentage and the rate of pine seed germination. Nonetheless, the majority of germinable seeds tested in the present study germinated at 6 h of soaking seeds within 7 days from the beginning of the germination test. Thus, the germination energy approached the germination capacity. The more 15-day soaking seeds, therefore, appears as an optimal pre-sowing treatment for Scots pine, although Hofmann *et al.* (2005) recommended its duration to be 20-40 days. It also seems obvious that more different parameters should be monitored when assessing the seed vitality (GE) in order to obtain precise information about the rate of seed germination. Germination energy increased, whereas Hrabí (1990) confirmed that in long-term stored seeds, germination energy of seeds can be increased at all times to a double by soaking in water. Similarly, Kantor (1952) reported that the yield of pine seedlings after sowing seeds soaked in water into treatment to be twice as high and sowing them into humus to be even seven times higher. After sowing the soaked seeds, the seedlings also were obtained with better morphological parameters, which was probably related to their faster emergence. The statement provided describes an experiment conducted by Dušek & Kotyza (1970), which aimed to improve the morphological parameters and emergence rate of conifer seedlings, including pine trees. According to their study, soaking the seeds in water for 24 h resulted in faster emergence of seedlings with improved morphological parameters. However, these authors noted that longer soaking periods beyond 24 h led to unfavourable outcomes. It is likely that extended soaking caused excessive water absorption or other adverse effects on the seeds, potentially hindering their germination or negatively impacting seedling development. The soaking of pine seeds in biological preparation positively affected vitality seeds; unlike in control, the positive effect was observed. A significant stimulating effect on the germination of seeds and the growth of seedlings of Chinese pine (*Pinus tabulaeformis* Carr.) was exerted by solutions obtained by diluting 0.01 and 0.02 g mL$^{-1}$ of uterine extracts (hydraulic ratio 1:10) of the root and litter of Chinese pine of various ages (Meiqiu *at al.* 2009). The positive effect of the extract on the accumulation of dry mass of 15-day-old seedlings of Scots pine was revealed when using plant materials harvested taking into account the daily dynamics of the content of growth regulators in needles (Egorova 2014, 2015).

**CONCLUSION**

All the tested pre-sowing treatment techniques for the seeds of Scots pine, i.e. soaking in water and using biological products of microbial origin, had a positive influence on the rate of seed germination. The highest values of germination energy, germination capacity at AgroMix 10 mL $L^{-1}$ under 6-h soaking seeds and the lowest values of mean germination time were recorded at Epin all doses and Control under 24-h soaking seeds. This pre-sowing treatment is therefore recommended for increasing the seed vitality. With respect to time duration, labour intensity, and costs, we recommend using biological preparation AgroMix under 6-h soaking seeds in water for enhancing their vitality.

**ACKNOWLEDGEMENTS**

We express our deep gratitude to Prof. Dr. Ainash NAUANOVA for the provided biological preparations.

**Funding acknowledgement statement**

This research is founded by the S. Seifullin Kazakh Agrotechnical University under the project: (Grant No.2VGF/23).

**Conflict of interest**

The authors declare that they have no conflict of interest.

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***Bibliographic information of this paper for citing:***

Kapar, B, Kurmangozhinov, A, Osserkhan, B, Ayan, S 2024, The effect of different biological products applied with different doses and exposure time on the germination of Scots pine seeds. Caspian Journal of Environmental Sciences, 22: 23-29.