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The impact of fertilisers and fungicides on seed germination and the initial phase of seedling growth in black alder *Alnus glutinosa* (Gaertn.)

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Abstract. To test the influence of selected fertilisers and fungicides on the germination of black alder seeds and the initial phase of seedling growth, we conducted a laboratory experiment outlined in this paper. Six treatments were applied on petri dishes each containing 30 seeds. The substrate for germination was sterile filter paper wetted with an aqueous solution of either one of two fungicides, two organic fertilisers, a mineral fertiliser or distilled water (control). Fungicides and fertilisers were applied according to the manufacturers' recommendations. In order to keep genetic variability to a minimum, seeds originated from a single tree in a seed stand located in the Chotyłów Forest District, eastern Poland. Germination and growth took place at a temperature of 23°C ± 2°C with a 14 h/10 h day/night cycle. Seeds began to germinate as early as the second day after sowing, except for the mineral fertiliser treatment, in which the first sprouting was observed on day 3. Seedling length was measured daily from the day of germination of a given seed through to day 15. Germination was found to proceed most rapidly in the control, while the largest increments in length and dry mass occurred in the control and fertiliser treatment with the so-called N1 fertiliser (solely comprising growth stimulators in the form of humic acids, chitosan and silicon). The most limited growth was observed under the influence of the F1 fungicide (active compound Thiram) as well as the organic fertiliser N2 (a mixture of mineral components and organic growth stimulators). Roots were found to develop most rapidly in the control and in the treatment with N1 (no mineral components).

These are also the only two treatments in which the roots were longer than the stems after 15 days. Fertiliser N2 was found to have the most unfavourable influence on both, germination and the first phase of seedling development. The fact that selected fertilizers and fungicides affected black alder seeds and seedlings under laboratory conditions does not mean that they will have an impact under field conditions or on other forest tree species. Therefore, this type of rese-arch will need to be conducted individually for each forest tree species.

Keywords: organic fertilisers, fungicides, germination, dynamics of seedling growth, dry mass of seedlings, *Alnus glutinosa*

1. Introduction

The majority of forest species are highly sensitive to pathogens living in the soil environment, which include mainly fungi that massively kill seedlings from the start of seed germination until the first few weeks of plant life. This is a period when seedlings use only the reserves accumulated in seed tissues and there is no symbiosis with mycorrhizal fungi yet. In forest nurseries, for direct protection against damping off, seedlings are treated before sowing, and seedlings are sprayed with fungicides. The use of frequent spraying of pesticides protects the seedlings from damping off but has a negative effect on the soil, increasing the 'soil fatigue' effect. (Prusinkiewicz et al. 1983).

A formal barrier to the use of chemicals in forestry, including fungicides in forest nurseries, has now emerged. The natural organic substances inhibiting fungal damping off in forest nurseries include aqueous extracts from onion and garlic, which are laborious and troublesome to prepare on their own. This has forced a search for new preparations limiting the development of pathogens of forest seeds and seedlings. Based on agricultural production, various organic prepara-

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tions or other substances that are not typical pesticides are increasingly being tested and produced to stimulate growth and protect seedlings against fungal pathogens. A preparation containing, among other things, chitosan has appeared on the market, which is formed from marine crustacean chitin as a result of its partial deacetylation (Borkowski, Dyki 2004; Piek et al. 2009). Chitosan is non-toxic, biocompatible, biodegradable and has high sorption (Raafat, Sahl 2009). The influence of chitosan on the development of horticultural plants has been studied many times (Wojdyła, Orlikowski 1997; Borkowski, Kowalczyk 1999; Placek et al. 2009, Borkowski, Uliński 2012), but rarely in relation to the development of forest trees, mainly in forest nurseries (Duda et al. 2003; Stocka 2008; Aleksandrowicz-Trzcińska 2013). There are no such studies on black alder *Alnus glutinosa* (Gaertn.) conducted under laboratory conditions. The results of the analysis of the effect of chitosan indicate that it increases the resistance of plants to various foliar and soil fungal diseases (Wojdyła, Orlikowski 1997; Pięta et al. 1998; Solarska et al. 1998; Borkowski, Kowalczyk 1999; Pięta, Pastucha 2002; Ben-Shalom et al. 2003; Piek et al. 2009) and inhibits the development of fungi and bacteria (Borkowski, Dyki 2003).

In addition to chitosan, humic acids are also present in organic preparations. These are specific, amorphous substances, naturally occurring in aquatic and terrestrial environments. They are formed in the soil by the humification process, to which dead organic matter is subjected (Grandfather 2003). Humic acids have a direct or indirect effect on cultivated plants. The indirect influence is related to the improvement of the soil environment, whereas the direct influence is related to the uptake of humic substances by plants and the induction of biochemical processes (Katkat et al. 2009). The biostimulating properties of organic preparations result from the presence of humic and fulvic acids, chitosan and silicon (Gawrońska, Przybysz 2011).

This paper presents an assessment of the influence of selected chemical preparations on seed germination and the development of black alder seedlings under laboratory conditions. This tree species was selected because it is of economic importance in Poland and due to the significant threat of phytophthorosis (a disease caused by *Phytophthora* spp. fungi), which is extremely dangerous tothe seeds and seedlings of black alder (Haque, Diez 2012), but also to other forest-forming species (Oszako 2005).

2. Materials and methods

The research was conducted in 2017 in the laboratory of the Department of Silviculture, Institute of Forest Sciences at the Warsaw University of Life Sciences. The experiment used seeds collected in November 2014 from a 100-year-old commercial seed stand in the Chotyłów Forest District, in the Wólka Dobryńska Forest Unit (division 125i). After harvesting, the seeds were dried to 8–9% humidity and stored in a tightly closed container at -3°C until sowing. In order to eliminate the influence of genetic variability, the seeds came from one tree and were characterised by high germination capacity.

The experiment consisted of six variants. The seed germination substrate was sterile filter paper, which was moistened with the following aqueous solutions:

- F1 – fungicide 1 (seed treatment) with 75% Thiram as the active substance (a compound from the dithiocarbamate group) used to reduce diseases caused by fungal pathogens in soil and seed coverings;
- F2 – fungicide 2 with 41.7% thiophanate-methyl as the active substance (compound from the benzimidazole group) used for seed treatment and soil decontamination;
- N1 – organic fertiliser (growth stimulator) containing humic acids, chitosan and silicon, intended for fertilising plants and improving soil quality, while the presence of chitosan stimulates plant growth (shoot and root). Thisorganic fertiliser does not contain micro- and macro-elements;
- N2 – organic fertiliser with microelements such as: boron – 1.7%, iron – 0.4%, copper – 0.1%, zinc – 0.1%, manganese – 0.1% and cobalt – 0.022% dissolved in humic acids with chitosan, improves soil properties, stimulates plant growth and provides them with nutrients;
- N3 – multi component mineral fertiliser, containing micro- and macro-elements such as: nitrogen – 3%, potassium – 2%, iron – 400 mg/l., manganese – 170 mg/l., zinc – 150 mg/l., copper – 70 mg/l., molybdenum – 20 mg/l. and sulphur, calcium and magnesium;
- K –control, consisting of distilled water free of any organic or mineral compounds.

The aqueous solutions of the treatments were prepared according to manufacturers' recommendations. The following doses of the preparations for the individual variants were used with 0.5 litres of distilled water: F1 – 1.5 g, F2 – 1 ml, N1 – 2 ml, N2 – 2 ml, N3 – 2 ml.

Before sowing, each seed was weighed using a precision laboratory scale (Sartorius), which allowed healthy and fully developed seeds to be selected. Another indicator of seed quality was the visual assessment of seed condition using a magnifying glass. During germination, the seedlings started to shed their seed coats. These were gently separated from the seedling cotyledons and dried for 48 h at 40°C. The embryo mass of individual seeds was calculated on the basis of the dry mass of the seed coats.

The tweezers used for seedling placement and seedling removal were disinfected with analcohol-based disinfectant, while the scales and filterpaper were scalded at a high temperature before setting up the experiment. The distilled water used in the experiment came from one source, and the seeds placed on the filter paper were not disinfected.

Thirty randomly selected and weighed seeds in each variant were placed with tweezers on the filter paper, which were wrapped around Petri dishes. The seeds were placed in designated locations on the round disc at a distance of about 3 cm from the level of the designated solution. The distance between individual seeds placed on the disc was 2 cm. The seeds were arranged in such a way that the root sprouts were directed towards the solution (towards the perimeter of the dish) and the shoots grew towards the central part of the dish. This arrangement of germinating seeds made it easier to measure seedling length. Seed germination and seedling growth took place in a vegetation chamber at a temperature of 23°C (± 2°C) and with 14h of artificial lighting at an intensity of 20,000 lux and 2,000 K light colour.

Each seedling was measured for 15 consecutive days after the seed germinated on the filter paper. Each day, the total length of the seedlings was measured and the aqueous solutions of the tested preparations were filled to keep them at the level indicated on the dishes. After 15 days of growth, the seedlings were gently removed from the paper, the shoot was separated from the root and the length of the shoots and roots was measured. After drying for 48 h at 40°C, both parts of each seedling were weighed.

Due to the stability of the factors determining the growth of seedlings in the laboratory, the data analyses assumed that the repetition for each variant will be individual seeds and seedlings.

Statgraphics Plus was used to conduct the ANOVA statistical tests, the statistical significance of individual seedling parameters was checked, and homogeneous groups were determined using Duncan's post hoc test. Then, origin clusters characterised by multi-characteristic seedling similarity were created on the basis of seed germination rate and seedling parameters. For this purpose, the Ward method was applied to determine the cluster sand the squared Euclidean distance was used as a measure of similarity (Jędrzejczak 1998).

3. Results

3.1. Parameters of the seeds

The average lightest seeds (1.75 mg) and embryos (0.75 mg) were in the control variant (K), and the average heaviest seeds (1.98 mg) and embryos (0.89 mg) were in the N2 variant of the organic fertiliser with humic acids and mineral components. However, no statistical differences were found between the variants (p<0.05) (Table 1).

3.2. Survival rate of seeds

A minimal share of non-germinated seeds was found in three variants of the experiment (F2, N1 and N3), while100% of the seeds germinated in two variants (F1 and K). 100% of the seeds also germinated in variant N2, but mould appeared after a few days, causing most seedlings to die. The percentage of germinated seeds did not differ statistically between the variants (Fig.1).

3.3. Germination rate of seeds

The treatments used had a significant impact on the germination rate of black alder seeds, which already started germinating on the second day after sowing. Germination culminated on the third day, when most of the seeds exhibited visible germination. The exception was the variant with mineral fertiliser (N3), which had a time shift in this process. Its seeds began germinating on the third day, culminating on day 4. The differences between the number of seeds germinating on successive days were statistically significant (p<0.05) (Fig. 2).

3.4. Course of seedling growth

Due to the speed of the seedlings' development, three phases of the increase in seedling length were observed. The

Table 1. Mass of seeds and embryos used in the experiment

Variant	Number of seeds [pcs.]	Average weight of seeds [mg]	Number of embryos [pcs.]	Average weight of embryos [mg]
F1	30	1.96	30	0.85
F2	30	1.85	29	0.77
N1	30	1.91	29	0.84
N2	30	1.98	8	0.89
N3	30	1.97	30	0.86
K	30	1.75	26	0.75
p-value		0.0008		0.0020

first phase of increment (seed germination) lasted from the start of the experiment to the fourth day. The second, the longest growth phase lasting from the fifth to the thirteenth day of the experiment, was characterised by a large differentiation in the total length of seedlings depending on the experimental variant. In the third phase, the seedling growth stopped. The exception was variant N1 with the biostimulator containing humic acids with chitosan but lacking mineral components, in which the seedlings further increased in size (Fig. 3). Seedlings in the control variant (distilled water) were much larger than in the N2 and N3 variants having fertilisers containing mineral components, with properties stimulating plant growth.

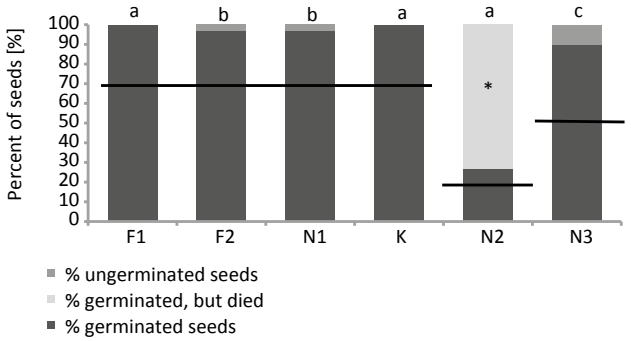


Figure 1. Germination of seeds treated with fungicides and biofertilisers the groups of homogeneous connections in which the seeds are selected are indicated by lines, and the letters indicate the groups of connections in which the seeds are not selected,

* the appearance of mold fungi

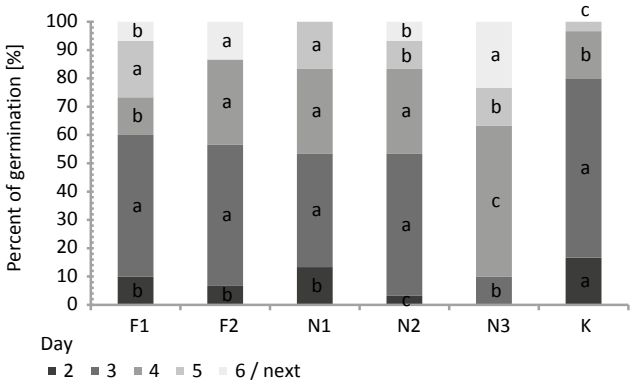


Figure 2. Share of seeds germinating after sowing on days 2, 3, 4, 5, 6 and next; letters indicate statistically homogeneous groups of variants in the following days of seed germination

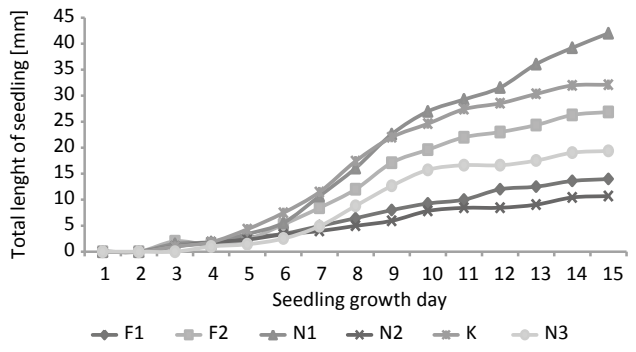


Figure 3. Course of the increase in total length of black alder seedlings

3.5. Length of seedlings

After 15 days, seedlings in the N1 variant had the longest shoots (17 mm) and the N2 variant had the shortest ones (8 mm). The length of seedling shoots in three variants (F2, N3 and K) was uniform, with lengths varying between 14 and 15 mm. The differences between average stem lengths in all variants of the experiment were statistically significant. Seedlings growing in the N2 organic fertiliser variant had the shortest roots (4 mm), while those treated with the N1 organic fertiliser had the longest roots (25 mm). Variants F1, N2 and N3 formed a homogenous group, in which the root lengths did not differ statistically. However, the differences between all average root lengths were statistically significant (p<0.001) (Fig. 4).

3.6. Dry mass of shoots

Seedlings treated with mineral fertiliser (N3) had the heaviest shoots (1.1 mg), while the lightest shoots (0.65 mg) were found in the variant with the N2 organic fertiliser. The variants with fungicides and the organic fertiliser with microelements (N2) formed a homogeneous group with no statistical difference between their mean values (Fig. 5).

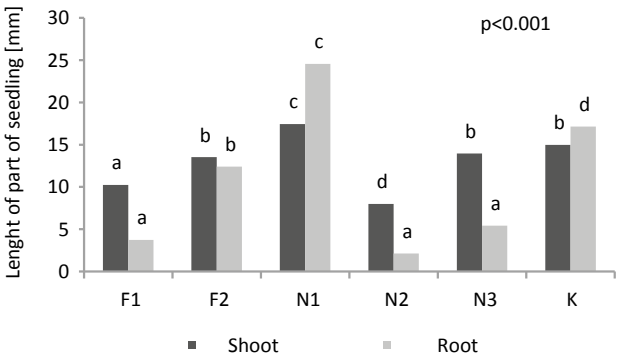


Figure 4. Length of seedlings as divided into shoot and root 15 days on from germination; letters indicate statistically homogeneous groups of variants separately for shoots and roots

Another homogeneous group was formed by the N1 organic fertiliser variant with humic acids, chitosan and silicon and distilled water variant (K). The differences between all variants were statistically significant (p<0.001). On the other hand, the heaviest roots (0.45 mg) were found for seedlings treated with the N1 organic fertiliser and the lightest (0.06 mg) in the N2 organic fertiliser variant. A homogenous group with the lightest roots was formed by seedlings from variants F1, N2 and N3. The differences between all averages were as statistically significant as in the case of shoot mass (p<0.001).

The cluster analysis performed on the basis of seed germination rate and seedling parameters allowed three groups of variants to be distinguished (Fig. 6). The first group consisted of four variants: F1 and F2 fungicides, N2 organic fertiliser and K distilled water, characterised by fast seed germination but low shoot parameters. The seedling roots in this group were of average length and weight. The N1 organic fertiliser variant differed from the others in its weaker seed germination than in Group 1, but its seedlings had longer and heavier shoots. The roots of these seedlings were of average length, but had the highest dry mass among the variants. The N3 multi component fertiliser variant had a negative effect on seed germination rate, its shoots were of average length, but the heaviest in relation to the other groups. In turn, the roots were the shortest and the lightest.

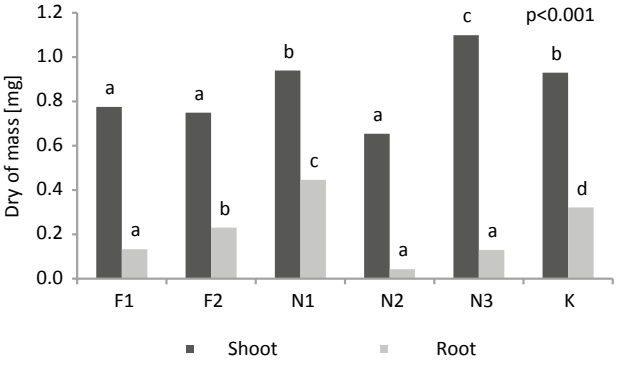


Figure 5. Dry weight of seedling shoots and roots 15 days on from germination letters indicate statistically homogeneous groups of variants separately for shoots and roots

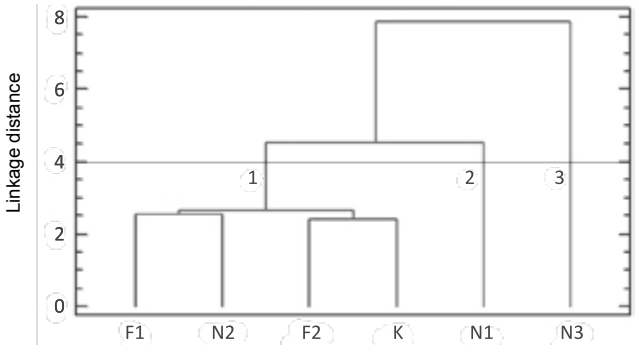


Figure 6. Clustering (1–3) of variants based on seed germination rate and seedling parameters

4. Discussion

In order to limit the influence of the genetic diversity of individual seeds on seedling growth, seeds from a single tree were chosen for testing. This is in line with the study by Bodył (2007), which demonstrated the significance of differences in energy and germination capacity of seeds from different natural and forest regions of Poland. The large differences in the properties of seeds originating from different trees and of different ages are also reported by Kaliniewicz and Trojanowski (2011).

The research of Tylkowski (2014) showed that storing black alder seeds, dried to 8–9%, for six winters did not reduce their initial germination capacity. The seeds used in this experiment were also stored under these conditions, but shorter, because only for 3 years. Thus, this factor should not negatively impact the germination process of black alder seeds. In turn, Gosling et al. (2009) noted that black alder seeds cooled at +4°C for 3 weeks showed earlier germination, more even emergence and tolerance to a wider temperature range (10–30°C) during germination. This study also noted the lack of a negative influence on seed properties after having been cooled and stored for 3 years. In most variants, over half of the seeds (even 80% in the control) germinated by the third day of the experiment. The exception was variant N3 (multi component mineral fertiliser), in which the germination process was extended by 1 day. Kaliniewicz et al. (2018) found that the germination process of black alder seeds is significantly influenced by their weight. However, it should be remembered that the average weight of the seeds used in the experiment did not differ statistically between the individual variants. In addition, the conditions during storage, seed germination and seedling growth were the same, so these factors did not affect the differentiation of the germination process.

The research showed that the fungicide and fertiliser aqueous solutions used slowed down the germination process of black alder seeds, but did not reduce germination capacity. Zamorski and Milczarek (1977) confirmed that fungicides had different effects (negative or positive) on the germination of ornamental plant seeds and showed that the fungicide with thiram as the active agent at a concentration of 75% beneficiallyaffected germination. A similar effect was observed on the germination of alder seeds, of which 100% sprouted in the F1 fungicide variant containing thiram.

The components of the N1 organic fertiliser variant used in the experiment contained humic acids, which could directly contribute to the strong growth of alder seedling roots. The stimulating effect of humic compounds on plant growth was demonstrated by Jankiewicz (1997) and Katkat et al. (2009), while Schnitzer and Poapst (1991) noted that these compounds stimulated the growth of bean roots. The influence of humic and fulvic acids on the growth of the green mass of cultivated plants is also known, at a rate of even 28% more than the control (Sarir et al. 2005). The positive effect of humic acids on plant growth (dessert grapes) is also confirmed in the work of Ferrara et al. (2007) and the Xu study (1986). Humic acids also reduce the occurrence of certain diseases caused by *Fusarium* spp. (Yigit, Dikilitas 2008).

The presented experiment found that in comparison with the control variant, treatments containing humic acids and chitosan positively affected seed germination, as well as the dimensions and dry mass of roots. On the other hand, the N2 organic fertiliser variant with a rich composition containing humic acids, chitosan and mineral components (micro- and macro-elements) showed the lowest dimensions and dry mass of both roots and shoots. The literature has information stating that although black alder is one of the less demanding native species, it reacts well to mineral fertilisation, especially phosphorus and potassium, but is sensitive to calcium deficiency (Baule, Fricker 1973). Therefore, a possible deficiency of this element may have been one of the reasons why these seedlings had the worst parameters. The nutrient-rich composition of the N2 aqueous solution variant could also stimulate the development of mould microorganisms, which weakened and killed part of the seedlings.

The N2 variant with chitosan and humic acids interacting with microelements did not exhibit a strong fungicidal effect – because the development of moulds was not limited. On the other hand, no mould fungi appeared and the percentage of dead seedlings was zero in the N1 variant, where these substances were also used. Both variants (N1 and N2) differed in the fact that the N1 organic fertiliser had no mineral components in its composition, while the N2 organic fertiliser contained microelements. The presence of mineral components should not necessarily be the main cause of the appearance of moulds, because seedlings developed properly without signs of mould in the N3 variant that had a multicomponent mineral fertiliser. It should be stressed that the presence of mould was found in only one variant of the experiment, which was conducted with the applied methods of maintaining sterility described in the materials and methods section.

Zamorski and Milczarek (1977) noticed that each plant species has a different biology and must be approached individually. The fact that selected fertilisers and fungicides affected black alder seeds and seedlings in particular ways under laboratory conditions does not mean that the impact will be similar in field conditions, where multifactorial influences of the environment and weather can further modify the results of similar experiments. Other forest tree species, such as *Pinus sylvestris* L. or *Fagus sylvatica* L. beech, may also react differently to the fungicides and organic fertilisers tested. Szołtyk and Walendzik (2003) found that forest plants have lower nutritional requirements than agricultural ones, but have a more varied individual response to particular nutrients. This confirms the need to conduct separate studies on individual forest tree species.

The results obtained in the laboratory study clearly indicate that the use of anorganic fertiliser (variant N1) containing humic acids positively affects the growth of shoots and roots of black alder in the initial growth phase. The seedling roots developed with this organic fertiliser were the longest and the heaviest, which proves that the seedlings formed quite complex root systems in a short time (only 15 days), enabling them to absorb more water and nutrients from the soil. This information is important in forest nurseries, whose task is to produce planting material of good breeding quality. A well-developed root system at the start is a basic guarantee for the proper growth of seedlings, as well as high success in forest cultivation. The chemical stimulation of growth and the proper development of seedlings is of particular importance in the production of planting material forthe covered root system of container nurseries.

5. Conclusions

1. Fungicides positively affected the germination rate of seeds, but did not positively affect seedling growth parameters.

2. The influence of organic fertilisers based on chitosan and humic acids on black alder seedlings depended on the share of mineral components. The organic fertiliser containing microelements clearly weakened the growth of seedlings, especially their roots, and stimulated the development of mould fungi.

3. Mineral fertiliser without any bio-components provided seedlings with strongly developed shoots and leaves, resulting in the highest dry mass of the shoots, but had a low mass of the roots.

4. Further research should focus on the search for effective multi component mixtures with bio stimulators, especially suitable for use in the production of seedlings in the covered root system.

Conflicts of interest

The authors declare the lack of potential conflicts of interest.

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M.B. – concept of the work, statistical analysis, preparation of figures and tables, literature review, manuscript writing, W.B. – scientific consultation, statistical analysis, correction of the work.

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