

The effect of an aqueous extract of seeds of *Lupinus polyphyllus* Lindl., naturalized on the Kola peninsula, on *Allium cepa*

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Abstract. *Lupinus polyphyllus* Lindl is a perennial herbaceous plant widely distributed in the Northeastern states of the USA, Canada, Europe, Argentina and New Zealand. This plant is distributed on the Kola Peninsula. *L. polyphyllus* contains alkaloids, the main of which are lupanine, lupinine, and sparteine. The aim of the study was to determine the cytotoxicity of aqueous extracts of seeds of the studied plant and their effect on biomass growth by the Allium-test method. It was found that aqueous solutions of *L. polyphyllus* seeds extract in concentrations 5 – 0.1325 %v/v doesn't have an inhibitory effect on the mitosis of *A. cepa* root meristem cells but have a statistically significant effect of stimulating the growth of biomass *A. cepa* in a concentration of 5% v/v.

1 Introduction

Active scientific and economic development of the territory of the Kola Peninsula in the first half of the XX century led to the expansion of biodiversity due to introduced or accidentally introduced plant species. Some species have naturalized and moved beyond the territory of controlled cultivation. One of these species is *Lupinus polyphyllus* Lindl., a family of legumes. The natural distribution area of the species is North America: the Northeastern states of the USA, Canada. *L. polyphyllus* came to Europe at the beginning of the XIX century as an ornamental plant, and by the end of the century, wild populations were found in Sweden, Germany, Finland and Great Britain. Currently, in many European countries, as well as in Argentina and New Zealand, this plant is classified as an invasive species [1]. Experimental work on the introduction of new plant species, carried out in the 30s of the last century at the experimental sites of the Polar-Alpine Botanical Garden-Institute, caused the spread of *L. polyphyllus* in the cities of Apatity and Kirovsk and the surrounding area [2]. The distribution of *L. polyphyllus* on the Kola Peninsula is insignificant in area and, to date, does not pose a threat to the ecological and economic security of the region. At the same time, the ability of *L. polyphyllus* to symbiotic nitrogen fixation, seed and vegetative reproduction, resistance to

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pathogens and other biological features ensure its ecological plasticity in a wide range of agro-climatic conditions.

The unique biochemical composition of this plant deserves special attention, and the high biological activity of its metabolites attracts attention to their comprehensive study. *L. polyphyllus*, like other members of the genus, are capable of synthesizing specific alkaloids, the main of which are lupanine, lupinine, and sparteine [3,4]. The toxicity of these substances causes their fungicidal, antibacterial, insecticidal effect [5]. In addition, lupine alkaloids have antiarrhythmic and hypoglycemic activity and are included in the composition of medicines [6]. There are known experiments to increase the yield of agricultural crops using an aqueous extract of seeds of lupine with high alkaloid content [7].

However, there is still insufficient data on the cyto- and genotoxicity of substances contained in *L. polyphyllus* seeds. The onion (*Allium cepa* L.) due to the high proportion of cells in the process of mitosis, large, well-stained chromosomes, easy of setting up an experiment, lack of ethical problems and sensitivity [8]. Grant [9] suggested using the Allium-test as a standard test for determining and evaluating chromosome damage. Such a test takes into account a number of parameters that allow us to assess the picture of cyto- and genotoxicity.

The aim of the study was to determine the cytotoxicity of aqueous extracts of seeds of the studied plant and their effect on biomass growth by the Allium-test method.

2 Materials and methods

2.1 Plant material and extraction procedure

Fully ripened *L. polyphyllus* seeds were used as plant material, collected at the Polar-Alpine Botanical Garden-Institute experimental site (Apatity) in August 2021. The selection of ripened beans was carried out from an area of 10 m² within a separate self-propagating population. The seeds were powdered in an agate mortar and sifted through a 0.63 mm sieve. For the extraction 1.5 g of seeds, 15 ml of water was poured and kept in an ultrasonic bath at 45 °C for 60 minutes. The resulting mixture was centrifuged at 4k rpm. Then five aqueous solutions of the extract were obtained in concentrations: 5, 2.5, 1.25, 0.625 and 0.1325% v/v.

2.2 Allium-test

In our study, the mitotic index was used as an indicator of cell division, the percentage of cells in each phase of mitosis and the registration of chromosomal aberrations. The germination of bulbs was also noticed after 96 hours of being on experimental solutions, so it was decided to apply the indicators of biomass and feather length as the effects of substances from solutions.

Onion bulbs (*Allium cepa* L., 2n = 16) of the Stuttgarten Riesen variety were kept in a dark cool (+4, +5 °C) place for 14 days before the experiment to equalize the vital processes of all bulbs and synchronize mitosis, cleaned of dry scales and examined. For each extract concentration and control, 5 bulbs were taken with preliminary germination for 24 hours in distilled water and the selection of bulbs with roots of 2-3 mm [10]. Distilled water was used as a control. In total, the experiment lasted 96 hours in a darkened place. Then the roots were cut and fixed in acetic alcohol for 2 hours, followed by washing and storage in 80% alcohol. To prepare the preparations, the roots were subjected to hydrolysis and simultaneous staining in acetoorcein heated to a minimum boiling in ceramic crucibles. After cooling, the crucibles were left at a temperature of 4°C for 24-48 hours in a dye [11]. Five roots were taken from each bulb for analysis. About 1000 cells were counted in each preparation with registration

of mitosis phases and chromosomal aberrations at magnification $\times 400$ on the microscope "Micromed-1, var.1-20" ("Micromed", Russia). The mitotic index was calculated as the ratio of the number of all dividing cells to the total number of counted cells in the preparation, expressed as a percentage. Also, phase indices were calculated.

2.3 Statistical analysis

Data processing was carried out using freely distributed software.

Normal distribution according to the Shapiro–Wilk criterion. Significant differences in mitotic and phase parameters between the experimental and control groups were determined using variance analysis. In addition, multiple pairwise comparisons of values were carried out using the Tukey's criterion of significant difference. The significance level was assumed at $p < 0.05$. To analyze the characteristics of the green mass, we used the nonparametric Kruskal–Wallis criterion and the a posteriori Dunn criterion.

3 Results and discussion

No statistically significant differences in the values of the mitotic index were observed in the control and in the experimental groups (Table 1). It was obtained no an inhibitory effect of aqueous solutions of lupine seed extract on the mitosis of onion root meristem cells.

There was a statistically significant increase in the prophase index in the cells of the onion root meristem in the control group and in the experimental groups corresponding to the extract concentrations of 0.625% and 0.3125% in compare with concentrations of 5% and 1.25%. At a concentration of 0.625%, there was a statistically significant decrease in the metaphase index compared to 1.25% and 2.5%. At a concentration of 0.3125%, a decrease in the metaphase index was noted compared to all experimental solutions and controls. The anaphase index significantly decreased at concentrations of 0.625% and 0.3125% compared to 5% solution. The telophase index decreased in the control compared to 2.5% and 5%. It can be assumed that an aqueous solution of lupine seed extract does not have a mitodepressive effect and there was an adaptation of cellular processes to the action of substances from solutions.

An increase in the green mass of *A. cepa* was noted, which may indicate the stimulating effect of aqueous solutions of *L. polyphyllus* seeds. The weight and length of the grown biomass were analyzed (Figure 1). In the group corresponding to the concentration of an aqueous solution of 5%, there was a significant stimulation of the growth of onion feathers in length and weight.

Table 1. Mitotic and phase indices for the meristematic cells of the *A. cepa* roots treated with aqueous solution of *L. polyphyllus* seed extract ($x \pm SD$, $n=5$).

Concentration, %	Mitotic index, %	Prophases, %	Metaphases, %	Anaphases, %	Telophases, %
C, distilled water	17.1 \pm 1.8	58 \pm 5.2 ^a	21.9 \pm 7.4	10.8 \pm 3.7	9.4 \pm 2.7 ^a
5	14.6 \pm 2.3	44.3 \pm 5.4 ^b	21.6 \pm 5.1	15.4 \pm 1.6 ^b	18.6 \pm 4.7 ^b
2.5	17.7 \pm 4.6	38.9 \pm 4.5 ^b	25.4 \pm 2.9	15.9 \pm 1.9 ^b	19.7 \pm 3.8 ^b
1.25	15.3 \pm 2.1	42.8 \pm 8.4 ^b	25.3 \pm 3.5	14.8 \pm 4.2	17.1 \pm 2.3
0.625	14.9 \pm 1.7	63.8 \pm 9.6 ^a	15.3 \pm 4.5	9.3 \pm 2 ^a	11.5 \pm 6.4
0.3125	14.3 \pm 0.6	57.1 \pm 7.2 ^a	12.8 \pm 1.8	10.3 \pm 2.5 ^a	19.8 \pm 4.7

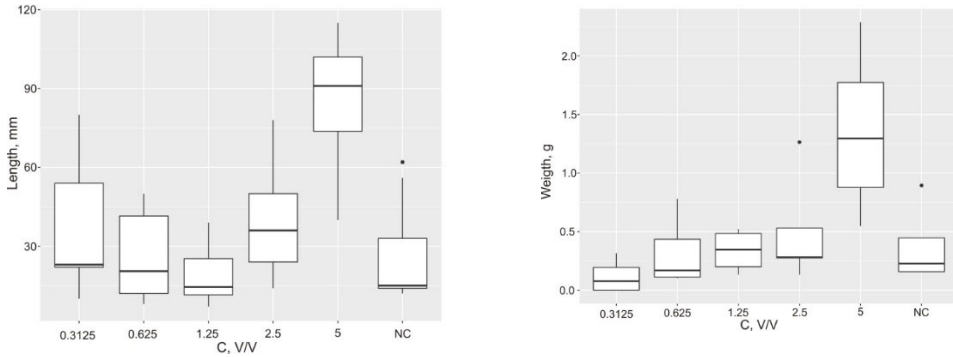


Fig. 1. The weight (a) and the length (b) of the grown biomass of *A. cepa* after treatment with aqueous solutions of *L. polyphyllus* seed extract, g.

Note: a, b – significant differences in phase indices in the experimental variants $P < 0.05$.

4 Conclusions

In the present work the effect of an aqueous extract of *L. polyphyllus* seeds on the mitosis of root meristem cells and the accumulation of *A. cepa* biomass was studied. The effect of the diluted aqueous extract in concentrations of 5, 2.5, 1.25, 0.625 and 0.1325% v/v was evaluated. It was found that an aqueous solution of *L. polyphyllus* seed extract in these concentrations did not have an inhibitory effect on the mitosis of *A. cepa* root meristem cells. However, the genotoxic effect of the extract should be studied in more detail. It should be noted that there is a statistically significant effect of stimulating the growth of biomass *A. cepa* obtained extract in a concentration of 5% v/v.

Further areas of work may be related to the study of the action of *L. polyphyllus* seed extracts, to confirm its stimulating and other effects both on plant objects and on other test systems with the selection of concentrations and the clarification of detailed chemical composition.

The obtained data can be useful for further study of economically valuable properties of plant extracts based on *Lupinus polyphyllus* and the possibilities of their application in various fields.

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