

Allelopathic effects of *Poa Pratensis* cultivars on lawn grasses

H. Lipińska, M. Sosnowska*, I. Woźniak-Kostecka, A. Kocira¹ and I. Shuvar²

Department of Grassland and Landscape Forming, University of Life
Sciences in Lublin 20-950, Poland

E. Mail: malgorzata.sosnowska@up.lublin. pl; akocira@pwsz.chelm.pl

(Received in revised form: July 19, 2022)

ABSTRACT

We studied the allelopathic potential of above-ground vegetative shoots of 3-cultivars ('Ani', 'Bila', and 'Nandu') of *Poa pratensis* and their effects on the initial growth and development of 6-Acceptor plants spp (*Agrostis capillaris* cv. Niwa, *Festuca arundinacea* cv. Asterix, *Festuca ovina* cv. Espro, *Festuca rubra* cv. Areta, *Lolium perenne* cv. Stadion and *P. pratensis* cv. Bila) present in grass lawn community. Aqueous extracts from shoots of *P. pratensis* 3-cultivars were tested in Petri dishes on filter paper. Whereas, the dried chopped leaves of *P. pratensis* cultivars were incorporated in pots containing sand for assay. The phenolics and flavonoids in the plant materials were analyzed by liquid chromatography. The allelopathic potential of Donor *P. pratensis* cultivars on the acceptor plants were correlated with the leaf contents of flavonoids and phenolic acids and followed the Order: 'Ani' > 'Nandy' > 'Bila'. The allelopathic potential of Donor cultivars on the Acceptor plants in Petri dish Bioassays was higher than in Pot assays. Aqueous extracts were more inhibitory to root growth than to seed germination and shoot growth. In contrast, in Pot assay allelopathic substances released from the decomposing cut leaves were more inhibitory to sprouting seeds than shoot elongation. The *A. capillaris* was most sensitive to allelopathic effects of *P. pratensis* cultivars followed by *F. rubra* and *P. pratensis*. The plants of *F. arundinacea* and *L. perenne* were least sensitive. With this in mind, it is advised to remove the cut grass from the lawn surface.

Key words: *Agrostis capillaris*, allelopathy, decomposing leaves; *Festuca arundinacea*, *Festuca ovina*, *Festuca rubra*, lawn cultivars, *Lolium perenne*, *P. pratensis*, shoot growth, water extracts

INTRODUCTION

The phenomenon of allelopathy in agricultural and horticultural practice is known since long time. It consists of mutual influence of plants through the release of chemical compounds. These compounds are usually secondary metabolites released from living aboveground plant parts, secreted by roots or released during decomposition of dead plant residues. In the aboveground vegetative plant organs, leaves are the richest source of allelopathic substances (31).

Allelopathy has been reported in grasslands (4,8,10,34). However, the allelopathic effects of grasses are least known factors influencing the growth and development of plants, particularly lawn grasses. The allelopathic potential is defined as a capacity of a particular chemical compound to inhibit seeds germination or growth of test plant. The amount of the released compounds depends on their levels in a plant that is genetically preconditioned and which may change in relation to the developmental phase or under the influence of nutritional, thermal or water stress conditions (33). It also depends on biological and environmental factors. This potential varies not only across plant species but also across

*Correspondence author, ¹Institute of Agricultural Sciences, State School of Higher Education in Chełm, Str. Pocztowa 54, 22-100 Chełm, Poland; ²Lviv National Agrarian University, Dublany, Ukraine.

cultivars (20,31,32). Therefore, it is important to understand the allelopathic effects of selected grass cultivars on other grasses and use this knowledge in practice not only to determine their mutual compatibility in seed mixtures but also to make the right decisions to maintain the stable composition and functional values of sward (2,4,21). There is common opinion that cut grass left on the surface of lawn did not have negative impact on growth of its plant species components.

Poa pratensis L. is most valuable lawn grass specie used in lawns (23,27). It forms a part of grassland communities in all climate zones and various habitats (7,28). Its lawn cultivars shows a considerable variability in their functional features due to genetic diversity (27). However, there are few studies on the allelopathic properties of its lawn varieties (22). The knowledge of allelopathic properties of lawn cultivars (i). will enable better explanation of the reasons for changes in the composition of the lawn turf and the condition of lawn surfaces, (ii). to better manage these changes using only natural resources and (iii). to prevent harmful allelopathic effects. Only a well-maintained lawn satisfactorily meets its functions (22). Lawn varieties of *P. pratensis* are one of the most popular species used in lawns. A strong and compact root system covers the ground well and ensures good overwintering (Photo 1). These qualities make them a popular choice for almost any lawn, accompanying the lawn varieties of such species as *Agrostis capillaris*, *Festuca arundinacea*, *Festuca ovina*, *Festuca rubra* and *Lolium perenne*.



Photo 1. The donor *Poa pratensis*

The research hypothesis assumes that aboveground grass biomass (leaves and stems) contains allelopathic substances that can be leached by rain or dew from both growing and mowed decomposing grasses. Depending on the donor variety and its form (aqueous extracts or decomposing leaves), these substances can exhibit both positive and negative effects on the initial growth and development of the acceptors plants.

To verify this hypothesis, tests were conducted to explain whether these effects are due to the allelopathic substances released from (i). water extracts from leaves, (ii). the cut leaves placed on a sandy substrate and (iii). how strong are these effects? To confirm the allelopathic properties of the investigated cultivars, the contents of allelopathic phenolics compounds and flavonoids were determined in the plant material (4,6,30,36).

This study aimed (i). to assess the allelopathic potential of vegetative aboveground shoots of 3- lawn cultivars of *P. pratensis* and (ii). their influence on the initial growth and development of other lawn grasses cultivars.

MATERIAL AND METHODS

Plant material

The donor biomass was obtained from monoculture plots from the experimental field at the Didactic-Research Station, Sosnowica SE Poland (Longitude 23.0914, Latitude 51.521, mean height above sea level: 154 m, annual rainfall: 160 mm, max temp: 20.7°C, min temp. -5.9°C). When the plants were 8 cm tall, these were cut at 4 cm height. The plant material was washed under running water to remove the soil and impurities, dried (with air at 21-25°C) and finely cut to pieces (about 2 mm long). This plant material was used to prepare water extracts for the Petri dish experiment or as biomass mixed with the substrate in the pot experiment.

The allelopathic potential of the lawn cultivars of *P. pratensis* was assessed in a Petri dish and pot experiment conducted at the Department of Grassland and Landscape Forming, University of Life Sciences in Lublin (Poland), during the years 2016-2019. Three lawn cultivars, namely Ani, Bila and Nandu referred to as donors, were investigated. These cultivars are lawn components that are among the most durable ones and resistant to unfavorable conditions e.g. presence of heavy metals, salinity, frequent mowing and ground compaction, dustiness and low temperatures (21).

To prepare water extracts, 50 g plant material was soaked in 1.0 L distilled water for 24 h at ambient temperature. It was filtered through filter paper and filtrate extracts were stored at 5 °C.

Bioassays

The experimental treatments consisted of two factors : (i). Donor plant *P. pratensis* cultivars : 3 ('Ani', 'Bila', and 'Nandu') and (ii). Recipient spp. 6 (*Agrostis capillaris*, *Festuca arundinacea*, *Festuca ovina*, *Festuca rubra*, *Lolium perenne*, *P. pratensis*).

In both experiments, the following lawn cultivars frequently used in lawns were the acceptors plants: *A. capillaris* cv. Niwa, *F. arundinacea* cv. Asterix, *F. ovina* cv. Espro, *F. rubra* cv. Areta, *L. perenne* cv. Stadion and *P. pratensis* cv. Bila (Photo 2).

(i). Niwa cultivar of *A. capillaris* - small delicate leaves of vivid green color. It is resistant to drought and fungal diseases, has low soil requirements and overwinters well. It grows back intensively after mowing. Suitable for intensive and extensive use of ornamental and park lawns. Suitable for golf courses, but also for slope turfing.

(ii). Asteric cultivar of *F. arundinacea* - dark green, with broad and rough leaves. The grass produces a strong root system and overwinters well. It is resistant to drought, pollination, salinity, and the presence of heavy metals in the soil. It is used in mixtures for turfing slopes, roadsides, traffic routes, brownfields and sports grounds.

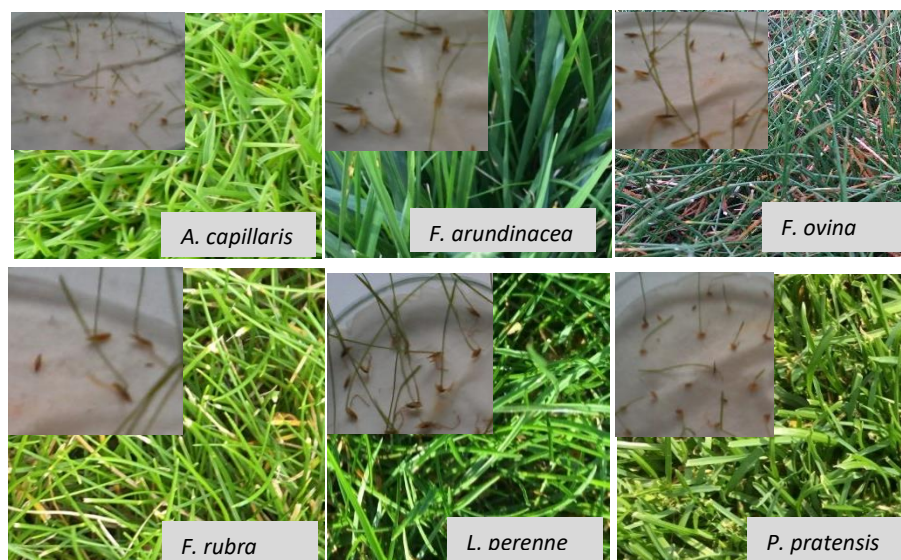


Photo 2. The test acceptor plants: *A. capillaris*, *F. arundinacea*, *F. ovina*, *F. rubra*, *L. perenne* and *P. pratensis*

(iii). **Espro cultivar of *F. ovina*** - gray-green leaves that keep their colour even in winter. The grass is used in mixtures for recreational lawns, for turfing slopes, roadsides, parking lots, and reclaimed areas.

(iv). **Areta cultivar of *F. rubra*** - abundantly foliated, dark green leaves, long underground stems. It tillers intensively and covers the ground well. After mowing, it grows back slowly. Useful for park lawns, ornamental lawns and for turfing slopes.

(v). **Stadion cultivar of *L. perenne*** - narrow leaves of vivid green color. The grass is characterized by good compositional value and turfing, as well as a slow rate of regrowth after mowing. It is recommended primarily for intensively used lawns.

Bila cultivar of *P. pratensis* - green, broad leaf blades. It is characterized by good compositional value, strong and compact root system. It covers the ground well and overwinters well. It tolerates frequent and low mowing. It is recommended for lawns used quite intensively and for sports surfaces.

(i). **Petri dish Bioassay:** The acceptor plants (20 seeds of each test cultivar) were sown equidistant in Petri dishes lined with 3-layers of Whatman No. 3001917 filter paper. Two ml of aqueous extracts (donors) were added/petridish every day. In control distilled water was used. The treatments in Petriplate bioassay were replicated 4-times in completely randomised design. Germination was defined as emergence of germ roots not shorter than seed length or its diameter and was calculated as under (17) : *A. capillaris* on 10th day, *L. perenne* and *F. arundinacea* on 14th day, *F. ovina* and *F. rubra* on 21st day, and *P. pratensis* on 28th day after sowing (9). Seven days after seeds germination, roots and shoot length were measured (mm) with a ruler.

(ii). **Pot Assay:** This experiment was done in pots (dia 18.5 cm and depth 30 cm), in a completely randomized design, with 3-replications. Each pot filled with 3-kg quartz

sand, 20 seeds of each test acceptor plants were sown (at 5-7 mm depth) and covered with 10 g of finely cut leaves of donor *P. pratensis* (equivalent to cut biomass left on lawn surface). In control, there were no leaves of donors on the substrate. The test plants were watered every day with distilled water and Hoagland's medium- 2 (21). The emergence of seedlings was evaluated, when the first leaf appeared on the ground surface. Root length and shoot growth were measured in 7-days old seedlings,

The experiments were done under 12-h (7.00 am to 7.00 pm) artificial lighting with SON-T Agro type high-pressure lamp (Mean luminance approx. 3000 lux at table level). Its special SGR 140-type socket guaranteed even distribution of light on plants (Mean luminance of approx. 3000 lux). The ambient temperature in the room ranged between 22 and 25 °C.

Chemical analysis : The flavonoids and phenolic acids content were determined in the donor plant material. The total flavonoids content was analyzed by high-performance liquid chromatography (HPLC), while the phenolic acids content was analyzed by Ultra-performance liquid chromatography (UPLC) (21). These analyses were done at the Institute of Biochemistry, Institute of Soil Science and Plant Cultivation, State Research Institute (IUNG PIB), Puławy.

UPLC-MS analysis: The grass samples were frozen, lyophilized and ground. 100 mg of the plant material was eluted with 80 % MeOH in a Dionex ASE 200 extractor (100 °C, 1500 psi, 3 static cycles). Methanol was evaporated from the extract and extract was dissolved in water, it was applied to an RP-18 microcolumn, stabilized in advance with methanol and distilled water. To remove carbohydrates, the microcolumn was washed with 1 ml H₂O, and the fraction with phenolic acids was removed from the microcolumn with 4 ml of 40 % MeOH. The phenolic fraction obtained was evaporated and quantitatively dissolved in 1 ml of 40 % MeOH to carry out the UPLC-MS analysis. Phenolic acids: Protocatechuic acid, 4-hydroxy-benzoic acid, Vanillic acid, Caffeic acid, Syringic acid, p-Coumaric acid, Ferulic acid, and Sinapinic acid, were determined with Waters ACQUITY UPLC chromatography system coupled with Waters TQD mass spectrometer in SRM mode (single reaction monitoring), negative ionization. Phenolic acids were separated on an ACQUITY UPLC HSS C18 column (1.0 x 100 mm, 1.8 µm) at 30 °C. The 0.1 % HCOOH-MeCN gradient was used.

HPLC analysis: To determine flavonoids, methanol extracts (0.5 g of the material and 20 ml of 80 % methanol to HPLC) were prepared, then the extract was evaporated, the residue was dissolved in water and applied to the preparative RP18 column (2 x 35 cm). Conditions of separation: Stationary phase RP 18 (4 x 250 mm, 10 µm), Mobile phase - linear gradient state, Eluent A - 1 % H₃PO₄, Eluent B - 40 % acetonitrile in 1 % H₃PO₄, Linear gradient 0-100 % B in 70 min, Temp. 50 °C, Flow 1 ml/min, DAD detector - detection at 228.8 nm. Based on calibration curves for routine standards, flavonoids content were calculated and expressed in mg·g⁻¹ of dry matter.

Statistical analysis

The results Data obtained in all experiments were analyzed statistically using the one-way analysis of variance, ANOVA. The normality of data distribution was assessed with the Shapiro-Wilk test and the Levene test was used to verify data heteroscedasticity. The

significance of differences between the group means was estimated with the Tukey test. Additionally, Pearson correlation was used to assess the influence of the level of flavonoids and phenolic acids on the germination and growth of plants used in the experiment. The statistical analyses were performed in SAS v.9.1 software. The significance level $\alpha = 0.05$ was assumed in all tests.

The graphs show the mean values of inhibition and the standard deviation for each group. Additionally, the homogeneous groups of means based on the Tukey test were indicated, separately for each category presented on the horizontal axis.

RESULTS

Flavonoids and phenolic compounds contents

The flavonoids and phenolic acids contents varied in the leaf blades of lawn grass cultivars and depended on the cultivar. The highest flavonoids levels were found in the leaves of Nandu cultivar, lower levels in Ani cv. and the moderate levels in Bila cv. (Table 1).

Table 1. Total flavonoid content (expressed as rutin content, $\text{mg} \cdot \text{g}^{-1}$ d.m.) in the leaves of the donor lawn cultivars of *P. pratensis*.

Donors (<i>P. pratensis</i> lawn cultivars)	Total flavonoid content expressed as rutin content [$\text{mg} \cdot \text{g}^{-1}$ d.m.]
Ani	$0.822^b \pm 0.010$
Bila	$0.669^c \pm 0.08$
Nandu	$0.912^a \pm 0.011$

* The same letters indicate the lack of significant differences between mean values in particular columns.

Table 2. Phenolic acids content ($\mu\text{g} \cdot \text{g}^{-1}$ d.m.) in the leaves of the lawn cultivars of *P. pratensis*.

Phenolic acids	Donors (lawn cultivars <i>P. pratensis</i>)		
	Ani	Bila	Nandu
	Phenolic acids [$\mu \cdot \text{g}^{-1}$ s.m.]		
Protocatechuic acid	$1.04^a \pm 0.03$	$0.32^b \pm 0.02$	$0.29^b \pm 0.02$
4-Hydroxy-benzoic acid	$1.20^a \pm 0.03$	-	-
Vanillic acid	$3.61^a \pm 0.09$	$0.80^b \pm 0.02$	$0.73^b \pm 0.03$
Caffeic acid	$4.20^a \pm 0.02$	$2.41^b \pm 0.02$	$2.35^b \pm 0.04$
Syringic acid	$2.63^a \pm 0.04$	$0.87^b \pm 0.03$	$0.92^b \pm 0.01$
<i>p</i> -Coumaric acid	$38.06^a \pm 0.06$	$15.41^b \pm 0.02$	$12.88^c \pm 0.02$
Ferulic acid	$15.02^a \pm 0.03$	$11.20^b \pm 0.18$	$10.14^c \pm 0.03$
Sinapinic acid	$0.55^a \pm 0.01$	$0.51^a \pm 0.02$	$0.52^a \pm 0.03$
Total	$66.30^a \pm 0.02$	$31.53^b \pm 0.09$	$27.82^c \pm 0.03$

Total sum of analyzed compounds in cultivars of *P. pratensis*.

* The same letters indicate non-significant differences between mean values in particular columns.

The highest levels of phenolic acids (in total) were found in the leaves of Ani cultivar. The phenolic acid levels in the other cultivars were half the level in Ani. The lowest levels of these compounds were found in the leaves of Nandu cultivar (Table 2). Furthermore, the highest content of *p*-coumaric acid was found among the identified phenolic acids in the leaves of all tested lawn varieties. The leaves of Ani cultivar had the highest levels of this

acid (Table 2). Ferulic acid showed the second-highest levels. Its highest content was found in the leaves of Ani cultivar as well.

PETRIDISH BIOASSAY

Seed germination

It was demonstrated that the seeds of Niwa cv. of *A. capillaris*, when subjected to the effect of the water extracts and decomposing leaves of all the lawn cultivars of *P. pratensis*, had significantly poorer germination than in the control treatment (Figure 1). In comparison

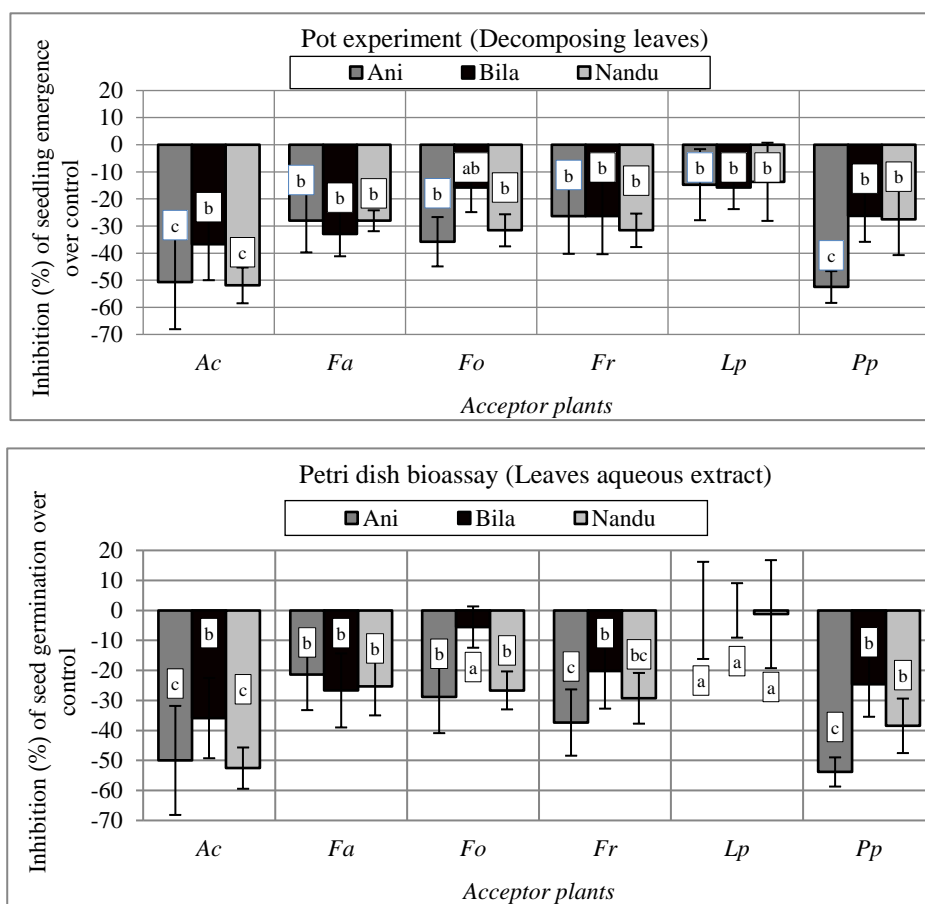


Figure 1. Inhibitory effects of leaves aqueous extract (Petridish bioassay) and decomposing leaves (Pot culture) on seeds germination/seedlings emergence (%) of *Poa pratensis* varieties 'Ani', 'Bila' and 'Nandu'. Abbreviations: Ac - *A. capillaris*, Fa - *F. arundinacea*, Fo - *F. ovina*, Fr - *F. rubra*, Lp - *L. perenne*, Pp - *P. pratensis*. The same letters indicate Non-significant differences between mean values in the particular category

to the control treatment where the paper was moistened with distilled water, significantly greater inhibition in seed germination of Asterix cv. of *F. arundinacea* was observed under the influence of allelopathic substances in the extracts from the leaves of Bila and Nandu cv. Significantly greatest inhibition of both seed germination of *F. ovina* was caused by leaves of Ani and Nandu cv. Water extracts of these donors also caused the greatest inhibition of seeds germination in *F. rubra*. The tests showed that the seed germination capacity of *L. perenne* under the influence of the water extracts from the leaves of all the studied *P. pratensis* cultivars was similar to the control conditions. The germination ability of the Bila cv. acceptor seeds. *P. pratensis* was significantly lower when moistened with water extracts from leaves of only Ani and Nandu cv.

To conclude, in comparison to the control treatments, the highest potential to inhibit seed germination was shown by Ani and Nandu cvs. Under the influence of water extracts from the leaves of these cultivars, the inhibition of germination was 30-40 %. Bila cv. caused 12 to 28 % inhibition of the parameters assessed. The smallest negative impact of the donors occurred in relation to *L. perenne*.

The above described relations confirmed the calculated coefficients of Pearson correlation. In the dish experiment, a significant positive correlation between the content of flavonoids and inhibition of seed germination was reported in case of *F. ovina* ($r=0.72$), *P. pratensis* ($r=0.50$) and *A. capillaris* ($r=0.49$). A positive correlation was proved also in case of : *F. rubra* ($r=0.37$). For the remaining acceptors (*L. perenne* and *F. arundinacea*) correlation was not observed, which is shown by the value of the coefficient of Pearson correlation which was close to zero (Table 3).

Table 3. Pearson's coefficient of correlation between the content of flavonoids and phenol acids in leaves of *P. pratensis* (Donor) and inhibition of seeds germination or seedlings germination of Acceptor plants.

Allelopathic compounds	<i>Agrostis capillaris</i>	<i>Festuca arundinacea</i>	<i>Festuca ovina</i>	<i>Festuca rubra</i>	<i>Lolium perenne</i>	<i>Poa pratensis</i>
Seed germination						
Total flavonoids	0.498	-0.062	0.724	0.375	0.002	0.504
	$p=0.036$	$p=0.807$	$p=0.001$	$p=0.125$	$p=0.992$	$p=0.033$
Total phenolic acids	0.149	-0.207	0.394	0.464	-0.024	0.698
	$p=0.554$	$p=0.409$	$p=0.106$	$p=0.052$	$p=0.924$	$p=0.001$
Seedling emergence						
Total flavonoids	0.479	-0.251	-0.095	0.169	0.162	0.646
	$p=0.044$	$p=0.316$	$p=0.708$	$p=0.503$	$p=0.520$	$p=0.004$
Total phenolic acids	0.178	-0.120	0.003	0.788	-0.125	0.444
	$p=0.481$	$p=0.634$	$p=0.992$	$p=0.000$	$p=0.622$	$p=0.065$

Taking into consideration the content of phenol acids its significant positive correlation with inhibition of germination only in case of *P. pratensis* ($r=0.69$) was confirmed. A smaller impact may be concluded also in case of *F. rubra* ($r=0.46$) and *F. ovina* ($r=0.39$). In the remaining cases (*A. capillaris*, *F. arundinacea* and *L. perenne*), no correlation of the phenol acids content on inhibition of germination was reported but in case of *F. arundinacea* one may observe a trend for occurrence of stimulation of this process.

Inhibition of acceptor plants root growth

In the tests, it was proved that water extracts from leaves of all investigated cultivars (donors) caused significant inhibition in the growth of seedlings roots *A. capillaris*. The highest inhibitions were caused by extracts from Ani cv. Leaves.

In the research it was proved that when referred to the control, water extracts from leaves of all investigated cultivars caused significant inhibitions of the growth of roots of *F. arundinacea*. Based on the tests, it was demonstrated that only water extracts from Ani leaves caused significant inhibitions in the seedlings roots length of *F. ovina*. Bila and Nandu cvs, on the other hand, had a positive influence on this parameter, as evidenced by the longer roots of this acceptor's seedlings in comparison to the control treatments. The bioassays demonstrated that the water extracts from all donors significantly inhibited the root elongation of *F. rubra* seedlings. The strongest inhibitory were leaves of Nandu cv. in the presence of which the inhibition of the root growth was as much as 74.9 %.

A significant inhibition of the roots growth under the influence of water extracts from leaves of all investigated donors *P. pratensis* was reported also in *L. perenne*, the strongest influence showed Ani cv. leaves.

The tests demonstrated that the water extracts from the leaves of all the donors studied significantly inhibited the root elongation of *P. pratensis* seedlings. The maximum inhibition was observed in the dish experiment, where tissue paper was moistened with Ani cv. leaves. The influence of this cultivar was significantly greater than that of Bila and Nandu cvs.

The allelopathic potential of donors, expressed as inhibition (%) of the acceptor roots growth, was also confirmed by calculated Pearson's correlation coefficient. In the experiment, a significant positive correlation between the content of flavonoids and inhibition of the root growth were reported in case of *F. rubra* ($r=0.73$) and *P. pratensis* ($r=0.61$), while in case of *L. perenne* this correlation is significant but negative ($r=-0.47$). For the remaining acceptors *A. capillaris*, *F. arundinacea* and *F. ovina* no significant correlation was reported. In case of the phenol acids content, significant and positive correlations with inhibition of the root growth were reported in case of *A. capillaris* ($r=0.87$), *F. ovina* ($r=0.70$) and *P. pratensis* ($r=0.61$), while in case of *L. perenne* ($r=0.44$) the correlation is close to statistically significant (Table 4).

Table 4. Pearson's coefficient of correlation between the content of flavonoids and phenol acids in leaves of *P. pratensis* (Donor) and roots growth of acceptor plants seedlings.

Allelopathic compounds	<i>Agrostis capillaris</i>	<i>Festuca arundinacea</i>	<i>Festuca ovina</i>	<i>Festuca rubra</i>	<i>Lolium perenne</i>	<i>Poa pratensis</i>
Petri dish experiment						
Total flavonoids	0.022 $p=0.930$	0.036 $p=0.886$	0.292 $p=0.239$	0.734 $p=0.001$	-0.469 $p=0.049$	0.613 $p=0.007$
Total phenolic acids	0.867 $p=0.000$	0.244 $p=0.328$	0.700 $p=0.001$	-0.208 $p=0.406$	0.442 $p=0.066$	0.643 $p=0.004$
Pot experiment						
Total flavonoids	0.922 $p=0.000$	0.379 $p=0.120$	0.840 $p=0.000$	0.091 $p=0.717$	0.221 $p=0.376$	-0.852 $p=0.000$
Total phenolic acids	-0.012 $p=0.963$	0.406 $p=0.095$	0.488 $p=0.040$	0.053 $p=0.834$	0.604 $p=0.008$	0.263 $p=0.292$

Growth inhibition of acceptor plants seedlings

The allelopathic potential of the lawn cultivars of *P. pratensis* was assessed based on their inhibitory or stimulatory effect on the seedling height of the acceptor plants (Figure 3). The leaf water extracts of the cultivars significantly inhibited the height of *A. capillaris* seedlings in comparison to the control treatment. The greatest inhibitions were found in treatments where the paper was moistened with leaf extracts of Ani cv.

The tests showed that in the conditions of the impact of water extracts from leaves of all donors and in the control conditions, the height of *F. arundinacea* seedlings did not differ significantly.

In comparison to the control object, significant inhibition of *F. ovina* seedlings growth were reported in the conditions of water extracts from leaves of all donors.

In the tests it was proved that only water extracts from leaves of Ani cv. significantly inhibited the seedlings height of *F. rubra* in the remaining objects, the height of seedlings of this acceptor was close to the one in the control object.

The *L. perenne* seedlings height in treatments with water extracts from the leaves of all studied *P. pratensis* cultivars was similar to their height in the control conditions (Figure 3A).

In the tests it was proved that the height of seedlings of the acceptor - *P. pratensis* in the conditions of the impact of water extracts from donor leaves of Ani and Bila cv. was significantly inhibited compared to the control, contrary to the conditions of impact of Nandu cv.

Table 5. Pearson's coefficient of correlation between the content of flavonoids and phenol acids in leaves of *P. pratensis* (Donor) and shoot growth of acceptor plants seedlings.

Allelopathic compounds	<i>Agrostis capillaris</i>	<i>Festuca arundinacea</i>	<i>Festuca ovina</i>	<i>Festuca rubra</i>	<i>Lolium perenne</i>	<i>Poa pratensis</i>
Petri dish experiment						
Total flavonoids	0.247 <i>p</i> =0.323	-0.053 <i>p</i> =0.836	-0.300 <i>p</i> =0.226	0.446 <i>p</i> =0.064	0.000 <i>p</i> =1,00	-0.662 <i>p</i> =0.003
Total phenolic acids	0.818 <i>p</i> =0.000	0.806 <i>p</i> =0.000	0.416 <i>p</i> =0.086	0.640 <i>p</i> =0.004	0.177 <i>p</i> =0.481	0.529 <i>p</i> =0.024
Pot experiment						
Total flavonoids	0.922 <i>p</i> =0.000	-0.005 <i>p</i> =0.983	0.517 <i>p</i> =0.028	-0.039 <i>p</i> =0.878	0.269 <i>p</i> =0.280	0.573 <i>p</i> =0.013
Total phenolic acids	0.281 <i>p</i> =0.258	0.117 <i>p</i> =0.643	0.836 <i>p</i> =0.000	0.152 <i>p</i> =0.548	0.186 <i>p</i> =0.460	0.4795 <i>p</i> =0.044

The high potential of the aqueous leaf extracts of the tested donors was also confirmed by some Pearson's correlation coefficients (Table 5). In the experiment, taking into consideration the content of phenol acids, for each acceptor, a positive correlation with inhibition of the seedlings growth was determined but statistically, significant correlation was reported in case of: *A. capillaris* ($r=0.82$), *F. arundinacea* ($r=0.81$), *F. rubra* ($r=0.64$) and *P. pratensis* ($r=0.53$). A smaller influence is also in case of *F. ovina* ($r=0.42$) and *L. perenne* ($r=0.18$). Contrarily, a significant but negative correlation between the flavonoids content and inhibition of the growth of seedlings was found in case of *P. pratensis* ($r=-0.66$), while in case of *F. rubra* the obtained value of the Pearson's coefficient of correlation ($r=0.44$) is close to a significant positive correlation between the investigated variables. No

significant correlation was observed for the remaining acceptors (*A. capillaris*, *F. arundinacea*, *F. ovina* and *L. perenne*) and the obtained negative values of the Pearson's coefficient of correlation for *F. arundinacea* and *F. ovina* may prove a trend for stimulation of the growth of their seedlings.

POT EXPERIMENT

Seed germination

It was demonstrated that the seeds of Niwa cv. of *A. capillaris*, when subjected to the effect of the decomposing leaves of all the lawn cultivars of *P. pratensis*, had significantly poorer emergence than in the control treatment (Figure 2). Significant inhibitions were caused by water extracts from Nandu and Ani leaves. In comparison to the treatment where the substrate was moistened with distilled water, seedling emergence of Asterix cv. of *F. arundinacea* in all treatments with the cut leaves of the donor plants placed on the substrate was significantly poorer than in the control treatment, especially in the conditions of influence of cut leaves of Bila cv.

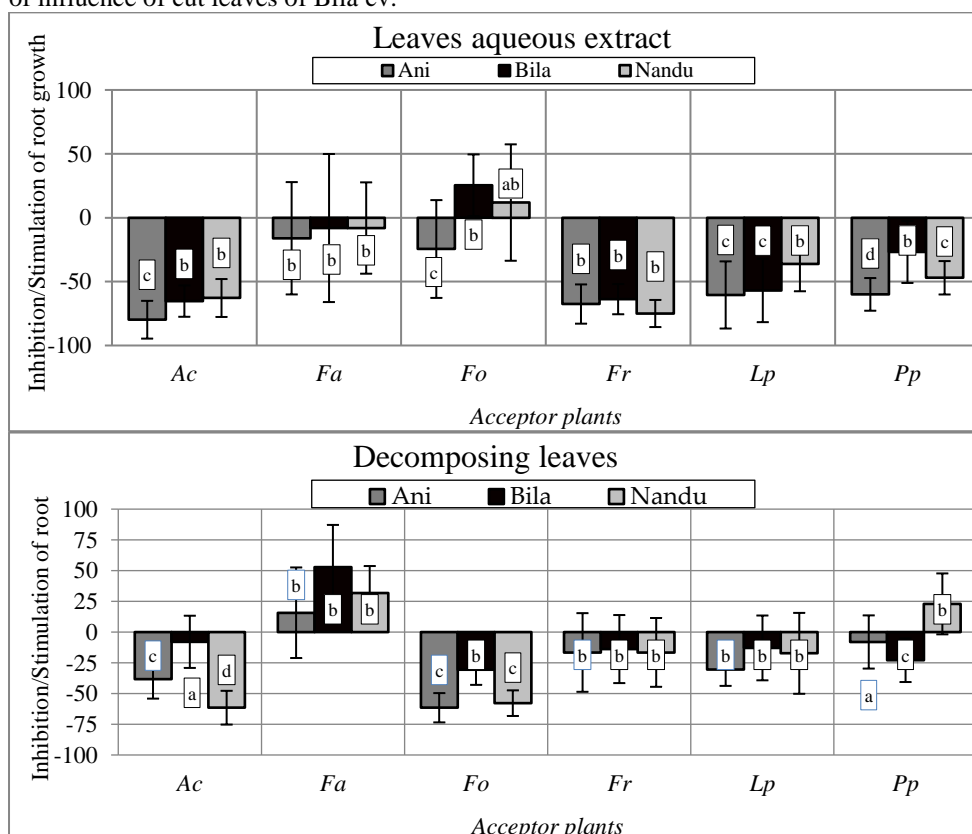


Figure 2. Inhibitory effects of leaves aqueous extract (Petridish bioassay) and decomposing leaves (Pot culture) on root growth (%) of *Poa pratensis* varieties 'Ani', 'Bila' and 'Nandu' seedlings. Abbreviations: Ac - *A. capillaris*, Fa - *F. arundinacea*, Fo - *F. ovina*, Fr - *F. rubra*, Lp - *L. perenne*, Pp - *P. pratensis*. The same letters indicate Non-significant differences between mean values in the particular category

Significantly greatest inhibition of seedlings emergence of *F. ovina* was caused by leaves of Ani and Nandu cv. In contrast, all cultivars of the donor had a significantly negative impact on seedlings germination of *F. rubra*. The tests showed that when mixed with the substrate, the cut leaves of all lawn cultivars of *P. pratensis* inhibited the emergence of the seedlings of *L. perenne*. These differences were significant in comparison with the control treatment. The substances present in the cut leaves of all the studied donors were found to have a significant inhibitory effect on the emergence of *P. pratensis* seedlings. The biggest inhibition were found in the case of Ani cv.

In the pot experiment, a significant positive correlation between the content of flavonoids and inhibition of seed germination was reported in case of *P. pratensis* ($r=0.65$) and *A. capillaris* ($r=0.48$). Contrary to the dish experiments, no correlation in case of *F. ovina* and *F. rubra*, was reported, as for the remaining acceptors (*L. perenne* and *F. arundinacea*). When analysing the impact of the content of phenol acids in the donor leaves on germination of seedlings, their significant and additional correlation with this parameter was determined only in case of *F. rubra* ($r=0.78$), while a smaller impact is also in case of *P. pratensis* ($r=0.46$). In the remaining cases (*A. capillaris*, *F. arundinacea* and *L. perenne*), no correlation of the phenol acids content with inhibition of germination of seedlings was reported, but in case of *F. arundinacea* and *L. perenne* one may observe a trend for occurrence of acceleration of germination.

Inhibition of acceptor plants root growth

In the tests it was proved that the highest inhibitions of the growth of seedlings roots *A. capillaris*. were caused by leaves of Nandu and Ani cv.

In the research it was proved that when referred to the control, cut biomass was stimulating the root growth of *F. arundinacea* seedlings because the shortest roots of this acceptor were found in the control treatment.

On the other hand, the roots of *F. ovina* seedlings in nearly all treatments were significantly shorter than in the control treatments. The leaves of Ani and Nandu caused the greatest inhibition of acceptor seedling roots elongation.

There was no significant impact of all investigated donors on the length of seedlings roots *F. rubra*. This is contrary to *L. perenne*, in which significant inhibitory impact on the root growth of the acceptor was observed under the influence of all investigated donors.

In the pot experiment the length of the seedling roots of *P. pratensis* in pots, where on the substrate, the cut leaves of Bila and Ani cvs. were placed, did not significantly differ. While, in pots with leaves of Nandu cv., a stimulating effect was reported - acceptor roots were significantly longer than in the control objects.

In the pot experiment, a significant positive correlation between the content of flavonoids and inhibition of root growth was reported in case of *A. capillaris* ($r=0.92$) and *F. ovina* ($r=0.84$), while in case of *P. pratensis* this correlation is significant but negative ($r=-0.85$). For the remaining acceptors *L. perenne*, *F. arundinacea* and *F. ovina*, no significant correlation was reported but for *F. arundinacea* there is a trend for inhibition of the root growth ($r=0.38$). In case of the phenol acids content, significant and positive correlations with inhibition of the root growth was reported in case of *F. ovina* ($r=0.49$) and *L. perenne* ($r=0.60$), while in case of *F. arundinacea* ($r=0.41$) the correlation is close to statistically significant (Table 4). In case of *A. capillaris*, *F. rubra* and *P. pratensis* correlation did not take place.

Growth inhibition of acceptor plants seedlings

The allelopathic potential of the lawn cultivars of *P. pratensis* was assessed based on their inhibitory or stimulatory effects on the seedling height of the acceptor plants. Decaying leaves of Ani and Nandu cvs significantly inhibited the height of *A. capillaris* seedlings. The leaves of Nandu cv. turned out to be the strongest inhibitor of the growth of *A. capillaris*. On the other hand, it was proved that decomposing leaves of Billa cv. in the pot assay stimulated the growth of seedlings of *A. capillaris*. While a significant stimulatory effect of decomposing leaves of the investigated donors on the height of shoots of *F. arundinacea* plants was concluded. The stimulatory effect was also observed in the pots with *F. ovina*, where cut leaves of Bila cv. were placed on the ground. The shoots of the acceptor on the pots subjected to the impact of Nandu cv. and control were similar.

Referred to the control, the obtained results proved, on the other hand, a significant positive impact of cut leaves of all investigated donors at the height of shoots of *F. rubra*. However, there was no significant effect of decomposing cut donor leaves on the height of *L. perenne* shoots. The decomposing leaves of the donors were not found to have a negative impact on the shoots height of *P. pratensis*.

The relationships described above are confirmed by correlations calculated as part of the experiment. A significant positive correlation was observed only between the content of flavonoids and inhibition of seedlings growth of *A. capillaris* ($r=0.92$), *P. pratensis* ($r=0.57$) and *F. ovina* ($r=0.52$). A positive correlation was also observed for the content of phenol acids respect to inhibition of the seedlings growth in the case of *F. ovina* ($r=0.84$) and *P. pratensis* ($r=0.58$). No significant correlations were recorded for the remaining acceptor plants.

Inhibition of assessed parameters

Summing up the degree of inhibition of the acceptor root growth, it can be concluded that the allelopathic potential of the donors was quite varied. The water extracts from the leaves caused greater inhibition of root growth than the decomposing leaves. In the case of water extracts from leaves, inhibition ranged from 25 to 80 %, while in the pot experiment from 13 to 61 %. In the case of the water extracts, longer roots were observed in *F. ovina* under the influence of Bila and Nandu cvs. In the pot experiment the leaves of all donors stimulated root growth in *F. arundinacea*, while the leaves of Nandu cv. stimulated root growth in *P. pratensis*.

Concluding the allelopathic potential of the Ani, Nandu, Bila cv. of *P. pratensis* to inhibit the seedlings height in the acceptors, it should be said that it was higher in case of water extracts than in case of the leaves placed on the ground (Figure 3A and B). Regardless of the form of donors, the greatest inhibition was found in *A. capillaris*. Ani cv. had a particularly strong inhibitory effect, inhibiting the shoot growth of this acceptor by about 60 %. This cultivar also inhibited the elongation of *F. ovina* and *P. pratensis* seedlings although this inhibition was 50 % weaker than in case of *A. capillaris*. It was particularly visible in case of the decomposing leaves of the donors that also stimulated the shoot growth of other acceptors (*Fa*, *Fo*, *Fr*, *Lp* or *Pp*).

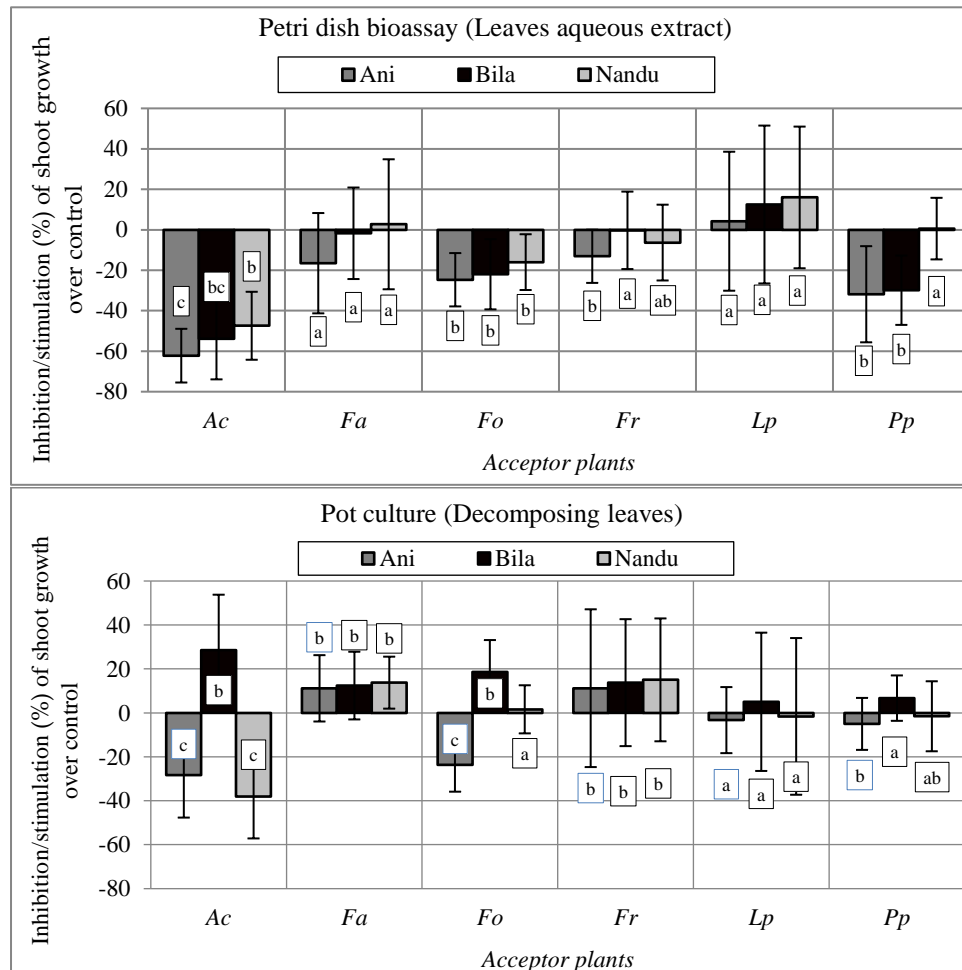


Figure 3. Inhibitory/stimulatory effects of leaves aqueous extract (Petridish bioassay) and decomposing leaves (Pot culture) on shoot growth (%) of *Poa pratensis* varieties 'Ani', 'Bila' and 'Nandu' seedlings. Abbreviations: Ac - *A. capillaris*, Fa - *F. arundinacea*, Fo - *F. ovina*, Fr - *F. rubra*, Lp - *L. perenne*, Pp - *P. pratensis*. The same letters indicate Non-significant differences between mean values in the particular category

Sensitivity of the acceptor plants indicated the highest percentage of inhibition on *A. capillaris*, followed by *P. pratensis* and *F. rubra*, regardless of the cultivar and form of the donor (Figure 4). *F. arundinacea* and *L. perenne* turned out to be the least sensitive. The acceptor plants were particularly sensitive at the stage of root elongation (except *F. arundinacea*), and the least sensitive at the stage of seedling growth.

The water leaf extracts caused the highest inhibition (%) in root elongation of the acceptor plants, while the decomposing leaves mainly impacted the emergence of the seedlings (Figure 5). The weakest impact of the leaf materials was found during the stage

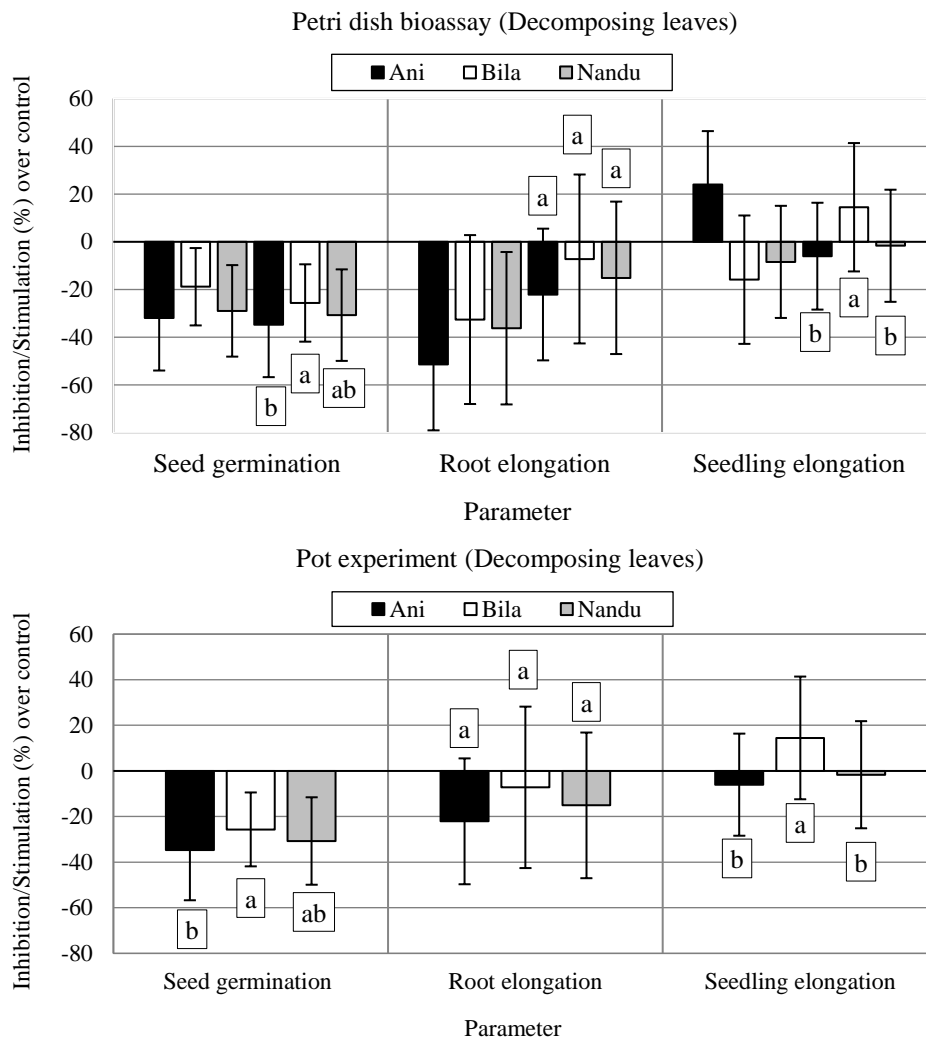


Figure 4. Inhibitory/Stimulatory effects of decomposing leaves (Pot culture) on seeds germination and root and shoot elongation (%) of *Poa pratensis* varieties 'Ani', 'Bila' and 'Nandu'. The same letters indicate Non-significant differences between mean values in the particular category

of seedling elongation, particularly when the allelopathic substances were released from the leaves in a sandy substrate. During each growth stage, Ani cv. had the highest allelopathic potential, while, Bila cv. had the lowest potential.

DISCUSSION

Petri dish and pot assays showed that the leaf materials of the *P. pratensis* cultivars had a significant impact on the growth and development of the acceptor plants. The impact was manifested as the inhibition or stimulation of seed germination or seedling emergence and elongation of the roots and seedlings of the tested acceptor plants. The negative impact of the water extracts from the aboveground vegetative shoots of this species was pointed out by other authors as well. Kovar *et al.* (17) demonstrated that water extracts from the leaves of *P. pratensis* significantly inhibited the total energy and germination capacity of the seeds, as well as the length of the roots and leaves of *Poa annua*. Kohoutek (16), showed the negative impact of water extracts from the soil from beneath the sward of *P. pratensis* on the energy and germination capacity of the seeds as well as the length of the roots and hypocotyl of *Trifolium pratense*. Other studies also confirm the allelopathic properties of the fodder cultivars of this species (4,5,19,25,3). Few studies, however, have concerned the allelopathic properties of the lawn cultivars of *P. pratensis* (20,18). Field studies by Lipińska *et al.* (20) demonstrated the negative impact of Bila cv. on the share of dicotyledons and of Nandu cv. on other grass species.

In the performed laboratory tests from among the assessed donors, Bila cv. showed the smallest negative impact on the investigated parameters of acceptors - lawns. Whereas, a considerable allelopathic potential of Nandu cv. was proved.

Contents of flavonoids and phenolic acids and calculated Pearson's coefficients confirmed the significant positive relations with the inhibition of growth of some parameters of the selected acceptors may prove the impact of allelopathic nature of lawn cultivars of *P. pratensis*. The allelopathic impact of the lawn cultivars of *P. pratensis* can be indicated by flavonoid and phenolic acid content determined in the course of the present study as well as Pearson's coefficients, confirming significant positive correlations with the increased inhibition of certain parameters of the selected acceptors. The analysis indicated that with increasing flavonoids content, the inhibition of seed germination in some acceptors was becoming greater. A significant, positive correlation was also observed between phenolic acids content and emergence (Table 3) or root growth (Table 4) and seedling growth (Table 5) in selected acceptors. The highest content of flavonoids was reported in leaves of Nandu cv., while phenol acids (total) in leaves of Ani cv. leaves. A high content of p-Coumaric acid with a high allelopathic potential proved in the literature was shown in the tests (1). High content of ferulic acid was also found in the donor leaves. This acid in considerable amounts in plants was also reported in the experiments by Zhu *et al.* (37) and Almaghrabi (1). It is considered that ferulic acid in combination with caffeic acid (which in the author's research) was on the third position concerning the amount leached from dead plant sprouts shows a significant allelopathic activity (11). A negative impact of these secondary metabolites on the plant growth is confirmed in research of other authors (30,35). Kączkowski (14) also reported the allelopathic properties of *P. pratensis* due to the presence of secondary metabolites in its tissues, such as endogenous gibberellin (A19) and abscisic acid (ABA).

The biological activity of allelopathic substances released from the donors depended on their forms (18). In the author's research, water extracts from leaves proved more inhibitory than the cut leaves placed on the sandy substrate. Additionally, a stimulatory effect of cut leaves of some cultivars (in particular Bila and Nandu) which was emphasized in a more intense growth of roots of some acceptors that develop in these conditions should

be highlighted. Together with a decomposition of donors leaves, also a stimulating effect on the seedlings height of the tested acceptors was reported most often under the influence of Bila cv. in tests (26).

This may indicate the low persistence of phytotoxins which, combined with low sensitivity of the acceptors in later stages of growth and development, can have a neutral or even stimulating effect (24). This view is also confirmed by other authors (12,15) in whose opinion the biological activity of plant residue decomposition products changes over time; it is always greater at the beginning of the decomposition period and decreases as the decomposition progresses. (They believe that allelopathic impact can be caused by simple phenolic acids characterized by short persistence, rather than compounds that degrade slowly. The authors of this study concur with the opinion and disprove a fairly common belief that decomposing aboveground shoots of grasses can enrich the soil with the necessary nutrients (21,22). These investigations did not show the impact of the biomass, including that of *P. pratensis* (22), left on the lawn surface on the soil pH and increased content of assimilating forms of phosphorus, potassium and magnesium.

The allelopathic potential determined in the course of the study also depended on the cultivar of the donor. In both experiments (dish and pot) the greatest inhibitions were caused by Ani cv., while considerably lower by Bila and Nandu cv. in the dish experiment and only Bila in the pot experiment.

The varied content of flavonoids and phenol acids among *P. pratensis* cv. in many cases translated into the level of inhibition of the assessed parameters of test species, which depended also on the donor form. These relations also confirmed calculated correlations where significantly positive correlation between the content of metabolites and a particular parameter of acceptors in the dish experiment contrary to the same parameters and acceptors in the pot experiment.

The varied responses of the acceptors to the donors demonstrated in the study should be taken into account when selecting species for seed mixtures for lawns. The highest sensitivity at each development stage under study (seed germination, seedling emergence, root elongation, seedling elongation) was shown by *A. capillaris*, followed by *F. rubra* and *P. pratensis*. These observations were also confirmed by the correlation coefficients that indicate a significant positive correlation between the levels of the metabolites under study and the parameters of these acceptors. The responses of the other acceptors were weaker and depended both on the parameter assessed and the form of the donor (extracts or cut leaves). *F. arundinacea* turned out to be the least sensitive. In the case of this species, no significant Pearson's correlation was found, and the negative values of this coefficient can even indicate the stimulation of the growth of this acceptor under the influence of *P. pratensis*. This species also showed a low sensitivity to the impact of other grass species, including those from genus *Festuca* (21) or *T. pratense* (37).

The identification of the allelopathic properties of grass species and cultivars is very important from the scientific and practical perspective because the allelopathic effects may lead to the disruption of many life processes in plants and in consequence, influence the species composition and aesthetic value of lawns (12,13). Knowledge in this field is also useful in making decisions concerning maintenance measures, including the decision of whether to leave the cut biomass on the lawn surface. From the ecological and economic perspective, *Poaceae* is a very significant family around the world. Learning about the

allelopathic mechanisms in grasses is also of key importance for understanding the natural behaviour of plants and using their allelopathic properties to combat weeds (10).

CONCLUSIONS

The substances released from water extracts and decomposing aboveground vegetative shoots of lawn cultivars of *P. pratensis* were inhibitory or stimulatory. The allelopathic potential was highest in cv. Ani, followed by cv. Nandu and Bila (lowest potential). The allelopathic inhibition of acceptor plants depended on the total contents of flavonoids and phenol acids in donor plants leaves (highest contents were in cv. Ani and lowest in cv. Bila) These relations were confirmed by the correlation coefficients between the flavonoids and phenol acids contents and their inhibitory effects on the studied parameters.

The *P. pratensis* leaves aqueous extracts were more inhibitory to roots growth than seed germination and shoot growth. The allelopathic substances released from the decomposing cut leaves strongly inhibited the seedling emergence than shoot elongation. The sensitivity of acceptors plants to allelopathic influence of donors was variable. The most sensitive was *A. capillaris* followed by *F. rubra* and *P. pratensis*. The least sensitive was *F. arundinacea* and *L. perenne*. This knowledge can be used to prepare lawn mixtures of *P. pratensis* cultivars for lawns lawn maintenance. Knowledge of the allelopathic effects of cultivars of *P. pratensis* can be useful in deciding whether to leave the mowed turf on the lawn.

DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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