Investigation of the effect of various herbicides on the development of hairy vetch (*Vicia villosa* Roth.) and soil

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Summary

Hairy vetch (*Vicia villosa* Roth.) is a legume crop that is known for high biomass yield. Weed infestation can compromise crop production. Herbicide options are less researched. Pre- and post-emergently applied herbicides were assessed through NDVI and visual phytotoxicity. Due to limited data on herbicide effects on soil microorganisms, enzyme activity tests were conducted. The pre-emergently applied flumioxazin, clomazone, and metribuzin did not cause phytotoxicity, while pendimethalin caused mild symptoms. Post-emergence application of metazachlor + quinmerac, imazamox, and bentazon caused minimal phytotoxicity. The applied herbicides significantly affected the β-glucosidase activity (BGA) of the soil. Further testing is warranted with additional measurements and seed yield results.

Keywords: herbicide, NDVI, phytotoxicity, enzyme activity, β-glucosidase

Különböző gyomirtó szerek hatásának vizsgálata a szöszös bükköny (*Vicia villosa* Roth.) fejlődésére és a talajra

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Összefoglaló

A szöszös bükköny (*Vicia villosa* Roth.) egy egynyári pillangós növény, amelyet széleskörűen termesztenek, főként magas zöldtömeghozama, valamint kiváló télállósága miatt. Ennek köszönhetően rendkívül értékes zöldtrágya, valamint takarmánynövényként egyaránt fontos szerepet tölthet be a mezőgazdasági rendszerekben. Az utóbbi években tapasztalható globális változások a mezőgazdasági művelési gyakorlatokban előrevetítik a zöldtrágya-növények és ezek vetőmagjai iránti kereslet további növekedését. Ugyanakkor a jelentős gyomfertőzöttség komolyan veszélyeztetheti a magtermesztés sikerességét és eredményességét.

A bükkönyfélék érzékenyek számos gyomirtószer-hatóanyagra, ezért a szöszös bükköny hatékony és biztonságos gyomirtása világszerte kevéssé kutatott területnek számít. Különböző hatásmechanizmusú herbicid hatóanyagok preemergens (vetés után, kelés előtt) és posztemergens (kelés utáni) kijuttatásának hatását vizsgáltuk szabadföldi, kisparcellás körülmények között a Hungvillosa fajta alkalmazásával. A növények fejlődését, illetve az alkalmazott hatóanyagok hatásait Normalizált Differenciál Vegetációs Index (NDVI) mérésével, valamint vizuális fitotoxicitási értékeléssel követtük nyomon.

A szakirodalomban igen kevés adat áll rendelkezésre a különböző gyomirtó szer hatóanyagok talaj-mikroorganizmusokra gyakorolt hatásáról. Ennek érdekében talajenzim-aktivitás méréseket végeztünk, amelyek célja az volt, hogy különböző dózisban alkalmazott herbicidek talajmikrobiológiai aktivitásra – elsősorban a β-glükozidáz enzimre – gyakorolt hatását felmérjük.

A preemergensen kijuttatott flumioxazin (0,06 és 0,08 kg/ha), klomazon (0,2 l/ha), valamint metribuzin (0,3 és 0,4 l/ha) hatóanyagok nem okoztak fitotoxicitási tüneteket a szöszös bükköny állományban, míg a pendimetalin (5,0 l/ha) csak enyhe tüneteket idézett elő; az említett hatóanyagokkal további kísérleteket érdemes végezni. A posztemergens kezelésként kijuttatott metazaklór + quinmerak (2,0 és 2,25 l/ha), imazamox (2,0 l/ha), illetve bentazon

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(3,0 l/ha) alkalmazása is minimális fitotoxicitást váltott ki, ezért ezek a szerek szintén további vizsgálatokra érdemesek.

Az alkalmazott herbicidek mindemellett szignifikánsan befolyásolták a talaj β-glükozidáz enzimaktivitását is. Az azonos hatóanyagtartalmú, de eltérő dózisú kezelések hatása azonban nem volt következetes: egyes esetekben magasabb, más esetekben alacsonyabb β-glükozidáz aktivitás mutatkozott a talajban.

Összefoglalva megállapítható, hogy az egyes hatóanyagok további vizsgálata indokolt, különösen kisparcellás kísérletek keretében, ahol a gyomborítottság, a növénymagasság, az ezermagtömeg, az esetleges termésszennyeződés, illetve a terméshozam mérésével még átfogóbb képet kaphatunk a herbicidek hatásairól és a biztonságos termesztéstechnológiai lehetőségekről.

Kulcsszavak: gyomirtó szer, NDVI, fitotoxicitás, enzim aktivitás, β-glükozidáz

Introduction

Hairy vetch (*Vicia villosa* Roth.) is a leguminous plant belonging to the Fabaceae family, originating from Europe and Western Asia (*Fuller et al. 2023*). When cultivated as a cover crop, hairy vetch can supply approximately 100–230 kg N ha^{-1} through biomass production, exhibiting superior performance compared to other vetch species in above-ground biomass production, root morphological characteristics, phosphorus and potassium uptake, and nitrogen fixation capability (*Grossman et al. 2018; Solangi et al. 2019*). Hairy vetch exhibits significant agronomic variability, making it highly promising for breeding new cultivars suitable for forage and green manure (*Mikić–Mihailović 2014*).

The vetch species exhibit effective ground coverage upon reaching a certain stage of maturity, thereby significantly reducing light penetration to the soil surface. Consequently, maintaining weed-free conditions during the initial growth phase, prior to the establishment of sufficient vegetative canopy, is crucial in vetch cultivation systems. Weeds that emerge in the autumn can potentially compete with the hairy vetch, as its initial development is slow, and consequently, the weeds can suppress the crop (*Oliva 2020*). Vetch species, including hairy vetch are sensitive to a variety of herbicide active substances (*Aarssen–Hall–Jensen 1986*). Due to the susceptibility of hairy vetch and its limited global cultivation area, weed control practices for this crop have been relatively under-researched. However, in the future, the role of green manure crops may increase at the global level, so there may also be a greater emphasis on safe seed production.

The importance of sustainable farming systems is steadily increasing; therefore, it is particularly crucial to investigate how herbicide use affects soil quality and biological activity. Soil quality is one of the basic requirements for increasing agricultural productivity across different land uses and management practices. Soil is a dynamic environment, diverse and complex due to the interaction of biotic and abiotic components in physical, chemical and biological processes (*Almeida–Naves–Mota 2015*).

Enzyme activity is a good indicator of soil quality, as it is an indicator of the microbial community, which reflects the changes in soil biochemical processes and decomposition dynamics (*Liu et al. 2021*). The enzyme β-glucosidase (BG) is widely distributed in nature and is associated with the carbon cycle and plays a role in the cleavage of cellobiose into glucose molecules (*Almeida–Naves–Mota 2015*). Soil β-glucosidase is usually adsorbed on the surface of mineral particles and organic matter in the soil (*Zhang et al. 2011*). BG plays a role in the final stage of the cellulose decomposition process by hydrolyzing the cellobiose residue (*Passos et al. 2008*). Soil β-glucosidase activity has been proposed as a soil quality indicator because it is very sensitive to environmental changes and is directly related to the quantity and quality of soil organic matter (*Zhang et al. 2011; Almeida–Naves–Mota 2015*).

Nowadays, herbicides are widely used in intensive agriculture, however, the improper or excessive use of chemical herbicides can have negative effects on the environment. In agricultural ecosystems, the use of herbicides can lead to contamination of water resources and soil, poison plants and soil microorganisms, and even pose a threat to human health (*Centner–Russell–Mays 2019; Parven et al. 2025*). The interaction between soil ecosystem components and herbicides can influence soil biogeochemical processes, which are primarily controlled by soil microorganisms. One way to determine the effects of herbicides on soil microorganisms is to assess soil enzyme activity. Based on the literature, herbicides at higher recommended doses generally have negative effects on soil β-glucosidase activity. In addition, exposure time is an important factor, similar to higher doses of herbicides, in determining negative effects on enzyme activity (*Koçak 2024*). The aim of the research was twofold. To investigate the effects of herbicides with different mechanisms of action on hairy vetch. Furthermore, the effect of herbicide active substances on the activity of enzymes in the soil.

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Material and methods

Experimental site and set up of the experiment

The experiment was set up on sandy loam soil belonging to the Nyíregyháza Research Institute of the University of Debrecen, Hungary (*Figure 1*), (47.975076, 21.696107).

The characteristic soil type of the area is *Arenosol* (*WRB 2014*). The soil type of the experimental area is sandy loam soil with alkaline pH (pH_{KCl} 7.4), with sufficient organic matter content (1.53 m/m%). The soil has a good phosphorus (231.7 mg kg^{-1}) and potassium (263.6 mg kg^{-1}) supply. The soil was analyzed by an accredited soil laboratory (*Table 1*).

The pre-crop in 2023 was corn (*Zea mays* L.) in the area. The sowing of the hairy vetch took place at the end of November 2023, but no plants emerged due to the late sowing. Consequently, the seed was resown in the spring on 4 March, using the Wintersteiger plot driller. In an open field small plot experiment, 8.5 m^{-2} (1.7 × 5.0 m) plots were used in 4 replicates and plots were randomized.

Table 1 | The main soil characteristics of the experiment field (0–25 cm soil layer)

Soil parameter	Unit	Value
pH (KCl)	-	7.4
Plasticity index by Arany	-	30
Water-soluble salt	m/m%	<0.02
Carbonated lime content	m/m%	2.42
Soil organic matter content (SOM)	m/m%	1.53
Phosphorus pentoxide (P_2O_5)	mg kg^{-1}	231.7
Potassium oxide (K_2O)	mg kg^{-1}	263.6

Source: Own work (Adapted from *Juhász et al. 2025: 4*)

Applied herbicide treatments

Various pre-emergence treatments were applied on the day of sowing, some at different doses (pendimethalin (1), flumioxazin (2, 3), clomazone (4), metribuzin (5, 6)). In the two weeks following application, more than 12 mm of rainfall fell on the area. Post-emergence treatments were applied 45 days after sowing (DAS) (flumioxazin (7, 8), chlorotoluron (9), clopyralid + picloram (10), metazachlor + quinmerac (11, 12), imazamox (13), halauxifen-methyl (14, 15), halauxifen-methyl + clopyralid (16), bentazon (17)) (*Table 2*). The active substance was applied using an SG 71 back sprayer with a water volume of 300 L ha^{-1}.

Data collection of Normalized Difference Vegetation Index (NDVI)

Crop development and herbicide-induced phytotoxicity were monitored using a Trimble GreenSeeker HCS-100 handheld sensor. NDVI values were recorded six times (51, 60, 66, 73, 79, 86 DAS).

Documentation of phytotoxicity symptoms

Phytotoxicity symptoms were evaluated through visual assessment. Phytotoxicity lesions were recorded on five occasions (53, 60, 67, 73, 79 DAS). The phytotoxicity

Figure 1 | Location of the experiment in Nyíregyháza, Hungary

Source: Google Earth 2024 (left); Source: Own work *(right)*

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Table 2 | Details of herbicide treatments applied

Codes of the treatments	Active ingredients	Application time	Active substances applied	Application rate of the herbicides	Action mechanism
1	Pendimethalin	Pre-emergence	2275 g ai ha^{-1}	5.0 L ha^{-1}	Germination and growth inhibition
2	Flumioxazin	Pre-emergence	30 g ai ha^{-1}	0.06 kg ha^{-1}	Protoporphyrinogen oxidase inhibition
3	Flumioxazin	Pre-emergence	40 g ai ha^{-1}	0.08 kg ha^{-1}	Protoporphyrinogen oxidase inhibition
4	Clomazone	Pre-emergence	96 g ai ha^{-1}	0.2 L ha^{-1}	Inhibition of carotenoid
5	Metribuzin	Pre-emergence	180 g ai ha^{-1}	0.3 L ha^{-1}	Photosynthesis inhibition in the Ps II
6	Metribuzin	Pre-emergence	240 g ai ha^{-1}	0.4 L ha^{-1}	Photosynthesis inhibition in the Ps II
7	Flumioxazin	Post-emergence	30 g ai ha^{-1}	0.06 kg ha^{-1}	Protoporphyrinogen oxidase inhibition
8	Flumioxazin	Post-emergence	40 g ai ha^{-1}	0.08 kg ha^{-1}	Protoporphyrinogen oxidase inhibition
9	Chlorotoluron	Post-emergence	1400 g ai ha^{-1}	2.8 L ha^{-1}	Photosynthesis inhibition in the Ps II
10	Clopyralid + Picloram	Post-emergence	80 + 20 g ai ha^{-1}	0.3 L ha^{-1}	Respiratory metabolism stimulant (synthetic auxin)
11	Metazachlor + Quinmerac	Post-emergence	666 + 166 g ai ha^{-1}	2.0 L ha^{-1}	Germination and growth inhibition
12	Metazachlor + Quinmerac	Post-emergence	749 + 187 g ai ha^{-1}	2.25 L ha^{-1}	Germination and growth inhibition
13	Imazamox	Post-emergence	80 g ai ha^{-1}	2.0 L ha^{-1}	Acetolactate synthase inhibitors
14	Halauxifen-methyl	Post-emergence	1.6 g ai ha^{-1}	0.5 L ha^{-1}	Auxin effect
15	Halauxifen-methyl	Post-emergence	1.9 g ai ha^{-1}	0.6 L ha^{-1}	Auxin effect
16	Halauxifen-methyl+ Clopyralid	Post-emergence	5 + 120 g ai ha^{-1}	1.0 L ha^{-1}	Auxin effect + Respiratory metabolism stimulant
17	Bentazon	Post-emergence	1440 g ai ha^{-1}	3.0 L ha^{-1}	Photosynthesis inhibition in the Ps II
18	Control	–	–	–	–

Active ingredient (ai).

Source: Own work

monitoring coincided exactly or nearly coincided with the NDVI measurements. Scale values were used to assess the severity of phytotoxicity, where a value of 1 indicates no symptoms and a value of 9 indicates total plant mortality (*Dancza 2004*).

Soil sampling, laboratory measurements

Soil samples were collected for enzyme activity tests on 02 July 2024 in four replicates. β-glucosidase activity (BGA) of the soil was measured according to *Eivazi–Tabatabai (1988)* after soil incubation with p-nitrophenyl-β-D-glucopyranoside and measurement of the p-nitrophenol absorbance at 400 nm.

Weather data

The monthly amount of precipitation and the average air temperature data were measured in 2024 by the weather station of the Research Institute of Nyíregyháza (*Table 3*).

Statistical analysis of the datasets

The analyses of the variance (ANOVA) of the data from different treatments were evaluated using the SPSS 22.0 program. For comparison of means, Tukey's and Duncan's tests were used at a significance level of $p < 0.05$. A Spearman's rho-based correlation test (2-tailed) was used, where NDVI data were correlated with phytotoxicity scores.

Table 3 | Precipitation and monthly average air temperature data in 2024 in Nyíregyháza, Hungary

	February	March	April	May	June	July
Precipitation (mm)	15	17	41	42	130	24
Monthly average temperature (°C)	8	10	14	18	22	25

Source: Own work

Results

Changes in NDVI values during the growing season

The post-emergence applied clopyralid + picloram, halauxifen-methyl, and halauxifen-methyl + picloram treatments (10, 14, 15, 16) at different doses showed similar trends with different levels of NDVI values below those of the control (18) and other treatments during the growing period. In plants treated with post-emergence flumioxazin (7, 8), NDVI values were initially lower but

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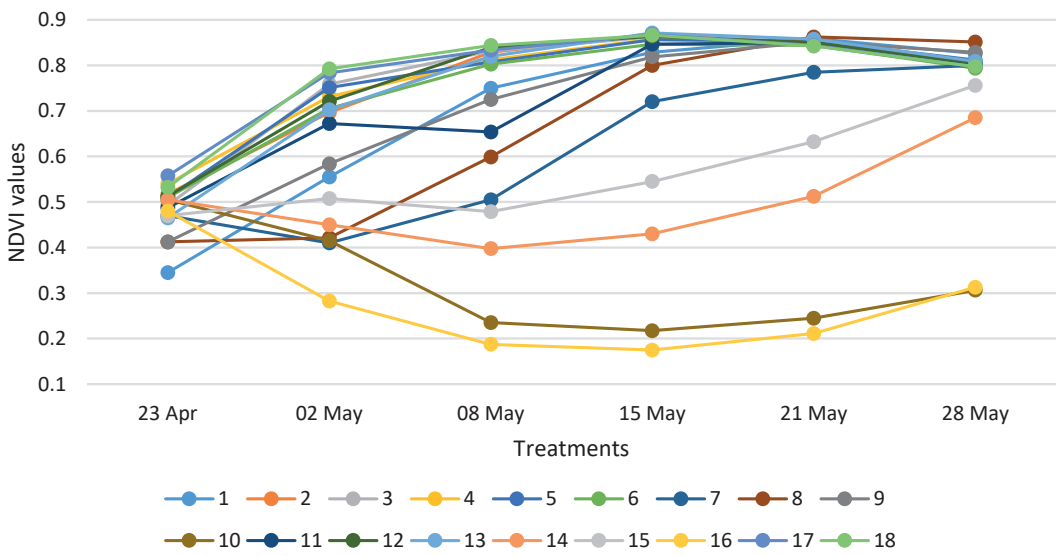

Figure 2 NDVI values over time for different herbicide treatments.

Numerals 1–18 indicate the herbicide treatments: 1 – pendimethalin (5.0 L ha^{-1}); 2 – flumioxazin (0.06 kg ha^{-1}); 3 – flumioxazin (0.08 kg ha^{-1}); 4 – clomazone (0.2 L ha^{-1}); 5 – metribuzin (0.3 L ha^{-1}); 6 – metribuzin (0.4 L ha^{-1}); 7 – flumioxazin (0.06 kg ha^{-1}); 8 – flumioxazin (0.08 kg ha^{-1}); 9 – chlorotoluron (2.8 L ha^{-1}); 10 – clopyralid + picloram (0.3 L ha^{-1}); 11 – metazachlor + quinmerac (2.0 L ha^{-1}); 12 – metazachlor + quinmerac (2.25 L ha^{-1}); 13 – imazamox (2.0 L ha^{-1}); 14 – halauxifen-methyl (0.5 L ha^{-1}); 15 – halauxifen-methyl (0.6 L ha^{-1}); 16 – halauxifen-methyl + clopyralid (1.0 L ha^{-1}); 17 – bentazon (3.0 L ha^{-1}); 18 – control. Treatments 1–6 were applied pre-emergence, whereas treatments 7–17 were applied post-emergence

Source: Own work

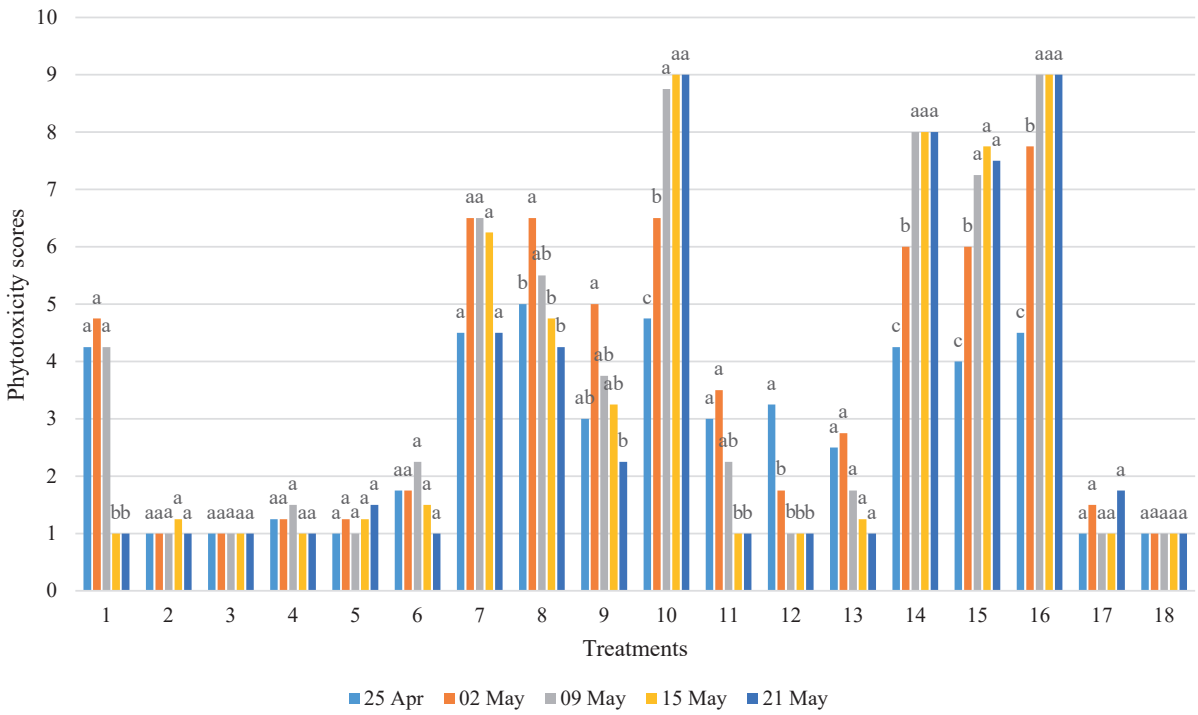

Figure 3 Changes in the phytotoxicity scores of treatments observed at different time points (25 Apr, 02 May, 09 May, 15 May, 21 May) during the growing season.

Different letters indicate significant differences between the mean values of the treatments based on Duncan ($p < 0.05$). Numerals 1–18 indicate the herbicide treatments: 1 – pendimethalin (5.0 L ha^{-1}); 2 – flumioxazin (0.06 kg ha^{-1}); 3 – flumioxazin (0.08 kg ha^{-1}); 4 – clomazone (0.2 L ha^{-1}); 5 – metribuzin (0.3 L ha^{-1}); 6 – metribuzin (0.4 L ha^{-1}); 7 – flumioxazin (0.06 kg ha^{-1}); 8 – flumioxazin (0.08 kg ha^{-1}); 9 – chlorotoluron (2.8 L ha^{-1}); 10 – clopyralid + picloram (0.3 L ha^{-1}); 11 – metazachlor + quinmerac (2.0 L ha^{-1}); 12 – metazachlor + quinmerac (2.25 L ha^{-1}); 13 – imazamox (2.0 L ha^{-1}); 14 – halauxifen-methyl (0.5 L ha^{-1}); 15 – halauxifen-methyl (0.6 L ha^{-1}); 16 – halauxifen-methyl + clopyralid (1.0 L ha^{-1}); 17 – bentazon (3.0 L ha^{-1}); 18 – control. Treatments 1–6 were applied pre-emergence, whereas treatments 7–17 were applied post-emergence

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gradually increased, reaching levels comparable to the control in the final assessments. As shown in *Figure 2*, plants treated with pre-emergence flumioxazin (1), post-emergence chlorotoluron (9), and lower doses of metazachlor + quinmerac (11) showed slightly reduced NDVI values compared to the control during the first three measurements, but values closely aligned with the control in the latter half of the observation period.

Changes in phytotoxicity scores during the growing season

The treatments clopyralid + picloram (10), halauxifen-methyl (14, 15), and halauxifen-methyl + clopyralid (16) caused progressively worsening phytotoxicity during the growing season. Post-emergence applications of flumioxazin (7, 8) caused severe phytotoxic symptoms, which showed some recovery over time but remained clearly visible at the final assessment date. Pre-emergence pendimethalin (1) and post-emergence chlorotoluron (9) treatments exhibited the most severe phytotoxic symptoms at the 2 May (60 DAS) assessment; however, both treatments showed substantial plant recovery over time. The application of metazachlor + quinmerac (11, 12) initially induced mild symptoms, followed by significant recovery. No significant phytotoxic changes during the growing season were documented for plants treated with flumioxazin (2, 3), clomazone (4), metribuzin (5, 6), imazamox (13), and bentazon (17) (*Figure 3*).

Treatment-induced changes in NDVI values and phytotoxicity scores of hairy vetch

The lowest Normalized Difference Vegetation Index (NDVI) values were observed in plots treated with the herbicide combinations clopyralid + picloram (10) and halauxifen-methyl + clopyralid (16), and these treatments also caused the most severe damage to the hairy vetch. Halauxifen-methyl treatments (14, 15) also caused severe damage, which was also reflected in the NDVI results. Post-emergence applications of flumioxazin (7, 8) induced chlorosis in the plants, and the resulting sparse plant stand contributed to elevated phytotoxicity scores, which correlated with reduced NDVI values. The NDVI values of plants treated with pendimethalin (1) and chlorotoluron (9) tended to be lower than those of control plants and caused significantly higher phytotoxicity (averaged overall results, it caused mild phytotoxicity) than many other treatments. Several pre-emergence applications of some of the active substances at different doses (flumioxazin (2, 3), clomazone (4), metribuzin (5, 6)) did not cause phytotoxicity and did not differ significantly from the control plants in the measured NDVI values. Among the post-emergence treatments, plants treated with different doses of metazachlor + quinmerac (11, 12), imazamox (13), and bentazon (17) did not differ from control plants in NDVI values and phytotoxicity scores (*Table 4*).

Pre-emergence application of flumioxazin (2; *Figure 4A*) and post-emergence application of bentazon (17; *Figure 4C*) caused no visible phytotoxicity in hairy vetch, with plant stands producing biomass comparable to the untreated control (18; *Figure 4D*) and reaching the flowering stage. In contrast, application of halauxifen-methyl (14; *Figure 4B*) initially caused chlorosis, followed by reduced plant population, absence of flowering, and minimal biomass production.

Table 4 | Normalized Difference Vegetation Index (NDVI) and phytotoxicity scores

Codes of the treatments	NDVI values	Std. error	Phytotoxicity scores	Std. error
1	.69 abc	0.015	3.1 ef	0.427
2	.76 a	0.021	1.1 g	0.050
3	.77 a	0.002	1.0 g	0.000
4	.77 a	0.004	1.2 g	0.082
5	.77 a	0.003	1.2 g	0.141
6	.75 ab	0.019	1.7 g	0.472
7	.62 bd	0.048	5.7 cd	0.330
8	.66 bcd	0.023	5.2 d	0.337
9	.70 abc	0.020	3.5 ef	0.435
10	.32 g	0.017	7.6 ab	0.141
11	.72 ab	0.016	2.2 efg	0.330
12	.77 a	0.003	1.6 g	0.082
13	.75 ab	0.011	1.9 fg	0.359
14	.50 f	0.020	6.9 abc	0.050
15	.57 de	0.025	6.5 bcd	0.238
16	.28 g	0.016	7.9 a	0.096
17	.78 a	0.003	1.3 g	0.189
18	.78 a	0.012	1.0 g	0.000

NDVI values and phytotoxicity scores are the mean of the results obtained during the growing time. Different letters indicate significant differences between the mean values of the treatments based on Tukey ($p < 0.05$). Std. error: Standard error. Numerals 1–18 indicate the herbicide treatments: 1 – pendimethalin (5.0 L ha^{-1}); 2 – flumioxazin (0.06 kg ha^{-1}); 3 – flumioxazin (0.08 kg ha^{-1}); 4 – clomazone (0.2 L ha^{-1}); 5 – metribuzin (0.3 L ha^{-1}); 6 – metribuzin (0.4 L ha^{-1}); 7 – flumioxazin (0.06 kg ha^{-1}); 8 – flumioxazin (0.08 kg ha^{-1}); 9 – chlorotoluron (2.8 L ha^{-1}); 10 – clopyralid + picloram (0.3 L ha^{-1}); 11 – metazachlor + quinmerac (2.0 L ha^{-1}); 12 – metazachlor + quinmerac (2.25 L ha^{-1}); 13 – imazamox (2.0 L ha^{-1}); 14 – halauxifen-methyl (0.5 L ha^{-1}); 15 – halauxifen-methyl (0.6 L ha^{-1}); 16 – halauxifen-methyl + clopyralid (1.0 L ha^{-1}); 17 – bentazon (3.0 L ha^{-1}); 18 – control. Treatments 1–6 were applied pre-emergence, whereas treatments 7–17 were applied post-emergence

Source: Own work

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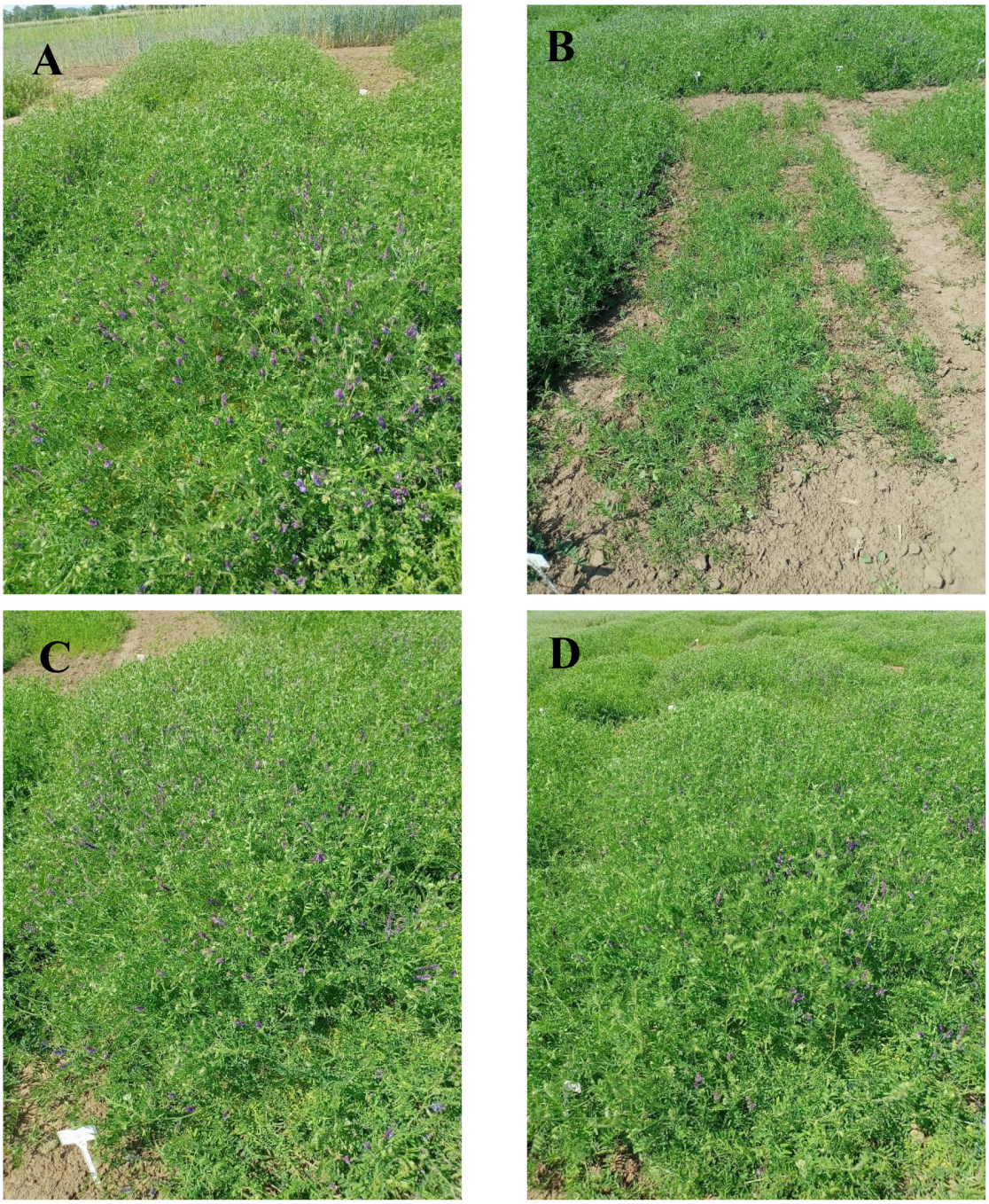

Figure 4 Photodocumentation taken 85 days after sowing shows the hairy vetch plots treated with flumioxazin (PRE; 0.06 kg ha^{-1}) (A), halauxifen-methyl (POST; 0.5 L ha^{-1}) (B), bentazon (POST; 3.0 L ha^{-1}) (C), and the untreated control plot (D)

Source: Own work

Correlation between NDVI values and phytotoxicity scores

A strong negative correlation was found between NDVI values and phytotoxicity scores based on Spearman's correlation ($r = -0.898$, $p < 0.01$). In practice, this means that as phytotoxicity increases, indicating greater herbicide-induced damage, NDVI values, which reflect the chlorophyll content and vigor of hairy vetch plants, have consistently decreased.

Impact of herbicide treatments and application rates on soil β-glucosidase activity (BGA) in hairy vetch

Significant differences in BGA can be observed in different treatments in *Figure 5*. The lowest BGA were measured in plots treated with pendimethalin (1), clopyralid+picloram (10), and imazamox (13). High BGA was found in 3 (flumioxazin, pre), 5 (metribuzin), and 7 (flumioxazin, post). The highest BGA was shown

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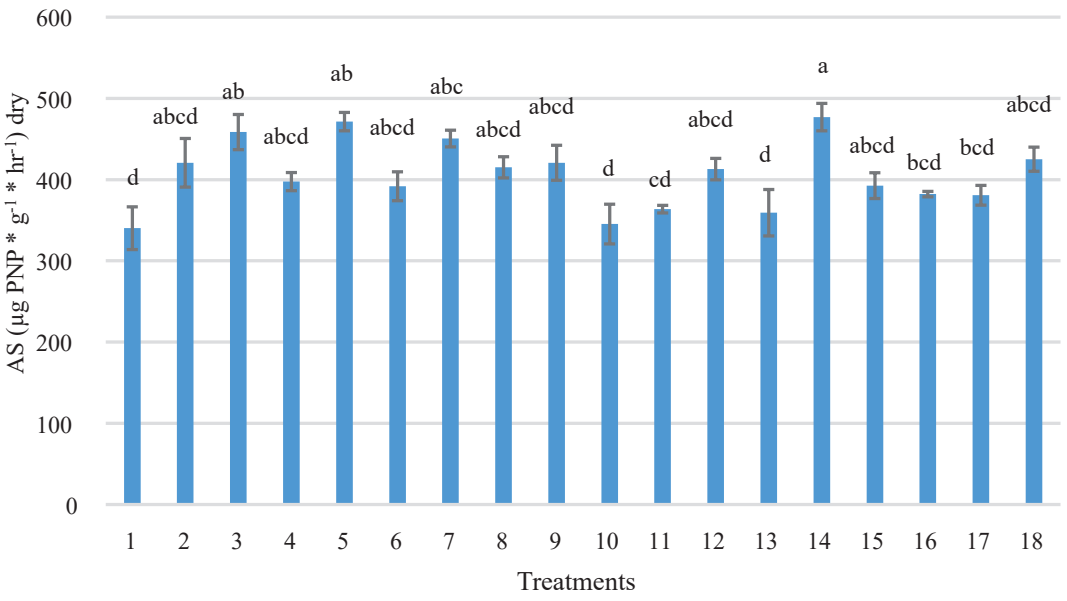

Figure 5 β-glucosidase activity (BGA) of soil in different treatments (Nyíregyháza, 02.07.2024).

a-d indexes mean different groups of means according to Tukey's test at the significance level of $p < 0.05$. Bars indicate the mean values, with error bars representing standard error in both the upward (positive) and downward (negative) directions for each dataset. Numerals 1–18 indicate the herbicide treatments: 1 – pendimethalin (5.0 L ha^{-1}); 2 – flumioxazin (0.06 kg ha^{-1}); 3 – flumioxazin (0.08 kg ha^{-1}); 4 – clomazone (0.2 L ha^{-1}); 5 – metribuzin (0.3 L ha^{-1}); 6 – metribuzin (0.4 L ha^{-1}); 7 – flumioxazin (0.06 kg ha^{-1}); 8 – flumioxazin (0.08 kg ha^{-1}); 9 – chlorotoluron (2.8 L ha^{-1}); 10 – clopyralid + picloram (0.3 L ha^{-1}); 11 – metazachlor + quinmerac (2.0 L ha^{-1}); 12 – metazachlor + quinmerac (2.25 L ha^{-1}); 13 – imazamox (2.0 L ha^{-1}); 14 – halauxifen-methyl (0.5 L ha^{-1}); 15 – halauxifen-methyl (0.6 L ha^{-1}); 16 – halauxifen-methyl + clopyralid (1.0 L ha^{-1}); 17 – bentazon (3.0 L ha^{-1}); 18 – control. Treatments 1–6 were applied pre-emergence, whereas treatments 7–17 were applied post-emergence

Source: Own work

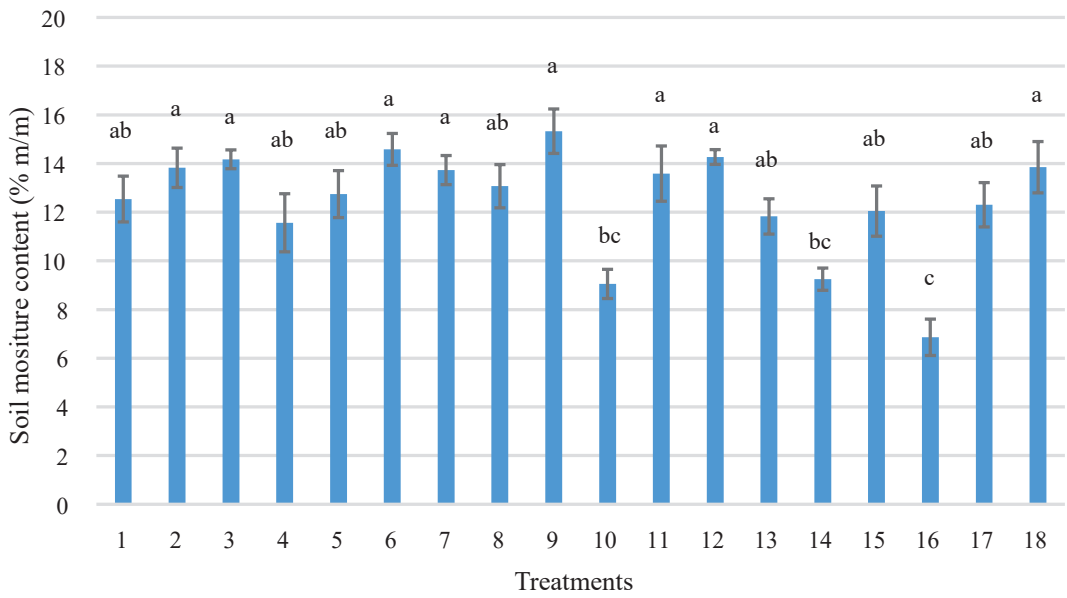

Figure 6 Changes in soil moisture content in different treatments (Nyíregyháza, 02.07.2024)

a-d indexes mean different groups of means according to Tukey's test at the significance level of $p < 0.05$. Bars indicate the mean values, with error bars representing standard error in both the upward (positive) and downward (negative) directions for each dataset. Numerals 1–18 indicate the herbicide treatments: 1 – pendimethalin (5.0 L ha^{-1}); 2 – flumioxazin (0.06 kg ha^{-1}); 3 – flumioxazin (0.08 kg ha^{-1}); 4 – clomazone (0.2 L ha^{-1}); 5 – metribuzin (0.3 L ha^{-1}); 6 – metribuzin (0.4 L ha^{-1}); 7 – flumioxazin (0.06 kg ha^{-1}); 8 – flumioxazin (0.08 kg ha^{-1}); 9 – chlorotoluron (2.8 L ha^{-1}); 10 – clopyralid + picloram (0.3 L ha^{-1}); 11 – metazachlor + quinmerac (2.0 L ha^{-1}); 12 – metazachlor + quinmerac (2.25 L ha^{-1}); 13 – imazamox (2.0 L ha^{-1}); 14 – halauxifen-methyl (0.5 L ha^{-1}); 15 – halauxifen-methyl (0.6 L ha^{-1}); 16 – halauxifen-methyl + clopyralid (1.0 L ha^{-1}); 17 – bentazon (3.0 L ha^{-1}); 18 – control. Treatments 1–6 were applied pre-emergence, whereas treatments 7–17 were applied post-emergence

Source: Own work

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by treatment 14 (halauxifen-methyl). Among the other treatments, there were no significant differences in BGA.

If the effect of herbicides with the same active ingredient content and different doses on BGA is examined, it can be concluded that in the case of flumioxazin (2–3 treatments) and metazachlor+quinmerac (11–12 treatments), higher BGA activity, while in the case of metribuzin (5–6 treatments) and halauxifen-methyl (14–15 treatments), lower BGA activity was measured.

Impact of herbicide treatments on soil moisture content

The changes in soil moisture content in different treatments can be seen in *Figure 6*. The lowest soil moisture content was measured in treatment 16 (halauxifen-methyl + clopyralid). Similarly, low moisture contents were detected in treatments 10 (clopyralid+picloram) and 14 (halauxifen-methyl). There was no significant difference in the moisture content of the other treatments.

Discussion

To the best of our knowledge, several active substances have been tested for the first time in the world in hairy vetch. The active substances metazachlor + quinmerac (11, 12) caused only mild symptoms in the hairy vetch and were also a promising active substance combination in our common vetch (*Vicia sativa* L.) small plot trials (*Juhász et al. 2025*). The plants treated with imazamox (13) recovered from the initial mild symptoms. In the experiment of *Hinds-Cook et al. (2009),* imazamox did not cause any injury in the hairy vetch, but the seed yield results of the treated plants were lower (not significantly) than those of the control. In 2023, we applied different ALS inhibitors (including the active substance imazamox) that caused both chlorosis and growth inhibition in the hairy vetch (*Juhász–Zsombik 2023*). In our experiment, bentazon did not cause any symptoms, which is in agreement with the findings of *Hinds-Cook et al. (2009)*, where bentazon was applied in combination with imazamox and did not cause any damage to the plants. *Lobos et al. (2016)* tested different herbicides on cover crops (including hairy vetch). Pendimethalin caused only very mild phytotoxicity in hairy vetch. In our experiments, the active substance pendimethalin (1) initially caused definite symptoms in hairy vetch, but the plants fully recovered.

Soil microbial enzyme activity indicates the soil's ability to decompose organic carbon and mineralize nutrients. β-glucosidase enzyme mainly participates in mineralization and cycling of carbohydrates in the soil (*Tomar-Baishya 2020*).

Soil enzyme activity varies throughout the year, depending on weather conditions. Soil moisture content is a factor regulating the biochemical processes of soil C transformation catalyzed by BG (*Zhang et al. 2011*). It was found that the catalytic ability of soil β-glucosidase was sensitive to changing soil moisture regimes (*Zhang et al. 2011*). However, we found no correlation between soil moisture content and BGA.

In general, herbicides at higher recommended doses negatively affect soil β-glucosidase activity (*Koçak 2024*).

Pinna et al. (2022) studied the effect of higher than recommended doses of imazamox on β-glucosidase activity in sandy loam soil. They found that high doses of imazamox had limited adverse effects on BGA. After 30 days, a stable effect was observed in the soil. Based on this, they concluded that imazamox weakly stimulates β-glucosidase activity.

Zhang et al. (2011) applied cloransulam-methyl herbicide at doses of 0.05, 0.5 and 2.5 mg kg^{-1} to sandy soil. They found that the applied herbicides increased β-glucosidase activity. In addition, a significant stimulating effect was observed at the 2.5 mg kg^{-1} herbicide dose compared to the control on day 14. They suggested that the 2.5 mg kg^{-1} herbicide dose may provide a rich source of carbon and organic matter that can be utilized by soil microorganisms.

Conclusion

Nowadays, the use of herbicides is widespread in modern agriculture and their global use in agricultural production has doubled in the past two decades. In our experiment, we applied herbicides with different modes of action to hairy vetch and examined their effects on both the crop and the soil. Based on our NDVI and phytotoxicity results, promising active substances include pendimethalin (pre), flumioxazin (pre), clomazone (pre), metribuzin (pre), metazachlor + quinmerac (post), imazamox (post) and bentazon (post). Herbicides are often the focus of research because of their use and efficiency, however, their effects on soil life and soil microorganisms are unknown or there is scarce information available. The interaction between soil ecosystem components and herbicides can influence soil biogeochemical processes, which are primarily regulated by soil microorganisms. Therefore, soil enzyme activity tests were performed to assess the effect of various herbicides with different doses on soil microorganisms. Soil β-glucosidase activity (BGA) is one of the most important responsible enzymes, playing a key role in the degradation of cellulose. The applied herbicides had a significant effect on β-glucosidase activity of soil. The herbicides with the same active ingredient content and different doses had variant effects on BGA, both higher and lower BGA were observed. The experiment should be repeated in an open field, small plot setting, and supplemented with additional measurements and data collection.

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