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Methodological approaches to sample preparation for xenobiotic content analysis

Abstract. The aim of the article was to improve sample preparation protocols and chromatographic analysis conditions for the determination of selected xenobiotics in agricultural products. To achieve this goal, a method of artificial fortification of homogenised samples of sunflower seeds, corn kernels, lettuce leaves, and apples with model xenobiotics of different classes was used, followed by extraction with organic solvents using a modified QuEChERS approach and quantitative analysis. For the effective extraction of acetochlor and prometryn from lettuce leaves and apples, which are characterised by a high water content, an optimal raw material-to-extractant ratio of 1:3-1:5 was found. In the case of corn kernels, which have a structure containing a negligible amount of lipids, the extraction was performed with a ratio of 1:10. The greatest difficulties were observed during the processing of sunflower seeds, where a three-phase system formed due to the high fat content, complicating the mass transfer of analytes. Nevertheless, the use of acetonitrile as an extractant in a 1:17-1:20 ratio allowed for high extraction rates of the target xenobiotics to be achieved. For the extraction of benomyl and cyprodinil, a mixture of acetonitrile and methanol in a 4:1 ratio was used, which ensured effective transfer of the specified analytes in all studied matrices. Specifically, in lettuce and apple samples, the extraction rates for both substances exceeded 96%, which indicated good solubility in the mixture used and a low matrix effect. For diquat, a bipyridylium compound with a pronounced ionic nature, the best results were obtained using a solution of trifluoroacetic acid in methanol in a 9.5:0.5 ratio. This composition ensured stable extraction from all types of plant matrices, with maximum values in lettuce samples and minimum values in sunflower

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seeds (86%), which still meets modern requirements for analytical accuracy. In the case of corn kernel samples, the extraction level for all substances was 88-92%, which indicated satisfactory extractability in cereal matrices. The lowest extraction values were recorded for sunflower seeds; however, even under these conditions, the extraction remained within the 80-86% range, which is acceptable according to international standards for pesticide analysis quality control. The results of the chromatographic analysis confirmed the high reproducibility and sensitivity of the method

Keywords: high-performance liquid chromatography; gas chromatography with mass-selective detectors; extracts; pesticides; plant extracts

INTRODUCTION

The contamination of plant products with pesticides and other xenobiotics is a serious problem, as agrochemical residues in plant-based foods pose a potential threat to food safety, consumer health, and the natural environment. The detection of residual amounts of these substances requires high-precision analytics, particularly chromatographic methods, the effectiveness of which is highly dependent on correct sample preparation. Pesticides, which are widely used in modern agriculture to increase crop yields, can remain in food products in the form of xenobiotics that require strict control (Salnikova & Salnikov, 2021; Garud *et al.*, 2024). In response to the need for effective monitoring, international and national regulations, including DSanPiN 8.8.1.2.3.4-000-2001 (2001) and Regulation (EC) No 396/2005 (2005), regulate the maximum permissible levels of such substances in food products.

One of the key areas in this field is the improvement of analytical control methods. As noted in the work of K. Psczolińska *et al.* (2024), modern analytics is based on multi-residue approaches using gas chromatography in combination with mass spectrometry, which allows for the identification of dozens of pesticides in a plant matrix. The study by S. Kang *et al.* (2023) emphasises the importance of standardising methods for analysing pesticide residues in plants, taking into account their unique chemical composition. The work of J. Veiga-del-Baño *et al.* (2024) demonstrates the effectiveness of the QuEChERS method as a universal approach for the extraction and purification of plant-based samples, which significantly reduces preparation time and improves the quality of analytical results. The prospects for improving these

approaches are also confirmed by the results of S. Perumal *et al.* (2024), who found the possibility of optimising salts and sorbents in the QuEChERS method to increase the recovery of target analytes in vegetable samples.

In the context of food safety, it is relevant to study the selectivity, effectiveness, and simplicity of the practical application of various sample preparation protocols for multi-residue analysis. The QuEChERS method, which combines the stages of homogenisation, extraction, purification, and analysis, is one of the leading technologies in the field of pesticide control in plant-based products. The aim of the presented study was to provide an experimental basis for the conditions of sample preparation and analysis of plant raw materials with varying water and lipid content to ensure the reliable quantitative determination of pesticide residues by chromatography. The practical value of the study lies in establishing a complex relationship between the properties of the matrix, the type of xenobiotic, and the extraction conditions, which allows the control method to be adapted to a wide range of plant products. The data obtained can be implemented in laboratory practice to improve the control of food product safety in accordance with modern requirements.

LITERATURE REVIEW

In the works of N. Casado *et al.* (2022) and N. Patel *et al.* (2024), the concept of the QuEChERS method and its evolution through various modifications that have expanded its scope of application were analysed. Particular attention has been paid to the use of QuEChERS for the determination of xenobiotics in food products, specifically pesticide residues in fruits, the control

of which is an important task for complying with regulatory requirements and ensuring their safety. For this purpose, QuEChERS is combined with liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), which is characterised by high sensitivity and specificity. The study by N. Patel *et al.* (2024) describes in detail the use of LC-MS/MS for the extraction, purification, and quantitative analysis of pesticide residues in fruit samples. The QuEChERS method involves the initial homogenisation of samples, the use of acetonitrile and salting out for effective separation, as well as subsequent dispersive solid-phase extraction (d-SPE) to remove matrix interferences. After purification, the extract is analysed using LC-MS/MS: liquid chromatography ensures the separation of pesticides, and tandem mass spectrometry provides their ionic fragmentation and identification. This approach allows for the simultaneous detection of trace amounts of numerous pesticides with high accuracy and reliability, which confirms the universality and practical significance of the QuEChERS method in controlling food product safety.

For the simultaneous determination of several pesticides using high-performance liquid chromatography (HPLC), a careful optimisation of analytical conditions for each active substance is necessary (Liu *et al.*, 2023; Dong *et al.*, 2023). One of the key approaches is the use of gradient elution, which facilitates the effective separation of compounds in combination with detection at a variable wavelength. This method is rarely used in procedures focused on the determination of individual pesticides. Most often, the effective separation of both individual and multiple pesticides is performed using: 1) reversed-phase chromatography, where a non-polar C18 adsorbent is used as the stationary phase, and the mobile phase is formed on the basis of water, acetonitrile and/or methanol with the possible addition of modifiers, particularly orthophosphoric acid; 2) detection in the ultraviolet range, and in some cases, by the fluorescent method (Korshun, 2024).

In the study by K. Pszczolińska *et al.* (2022), the proposed method was applied to the analysis of residual pesticides in vegetable samples. Pendimethalin was found to be the most common

compound, with concentrations ranging from 0.007-0.065 mg/kg, while for difenoconazole, this value was 0.013-0.025 mg/kg. An important step of the method is the preparation of calibration curves specific to each product type, which complicates the conduct of a multi-component analysis of residual pesticides. However, the method demonstrates high effectiveness and involves the purification of plant extracts (6 ml) using QuEChERS, with 900 mg of anhydrous MgSO₄, 300 mg of C18, and 300 mg of ChloroFiltr at the d-SPE stage. Pesticides are then analysed by gas chromatography with tandem mass spectrometry, which allows for the detection of up to 164 compounds in vegetables with varying chlorophyll content. This approach allows calibration curves built for one matrix to be applied to the analysis of pesticides in products with different levels of chlorophyll, increasing the accuracy and reproducibility of the results.

The methodological aspects of multi-residue pesticide analysis are aimed at developing environmentally safe, effective, and reliable approaches to guarantee the quality, safety, and traceability of products. As noted in the study by A. Santana-Mayor *et al.* (2023), the QuEChERS method is characterised by high versatility, which allows for obtaining accurate results for a wide range of matrices and analytes. Due to the ability to vary the solvents, salts, salting-out buffers, and sorbents for purification, this approach provides significant flexibility. In addition, QuEChERS is an environmentally safe alternative to traditional sample preparation methods, corresponding to modern trends in sustainable development in analytical chemistry.

Apples are one of the most common crops grown by European producers. Before they enter the market, it is necessary to check the content of residual pesticides used to protect the harvest from pests, in order to guarantee the safety of the products for consumers. For this purpose, a new simple method for the simultaneous determination of captan, folpet, difenoconazole, and chlorpyrifos in apple samples was developed and verified (Velkoska-Markovska *et al.*, 2024). The method includes the extraction of residual pesticides with acetone, as well as liquid-liquid (LLE) and solid-phase (SPE) extraction. Reversed-phase high-performance liquid

chromatography (RP-HPLC) with a diode-array detector (UV-DAD) was used for the separation and quantitative analysis of the analytes. Optimal results were obtained using a LiChrospher 60 RP-select B analytical column (250 mm × 4 mm, 5 µm) with isocratic elution, where the mobile phase was a mixture of acetonitrile and 0.1% acetic acid in water (70:30, V/V). The eluent flow was 1 ml/min, and detection was performed at wavelengths of 220 and 230 nm. The linearity of the method was confirmed in the concentration ranges of 1.50–3.60 mg/kg for captan and folpet, and 0.35–0.60 mg/kg for difenoconazole and chlorpyrifos. Recovery varied from 94.94% to 114.63%, and the relative standard deviation (RSD) was 0.09–9.25%. The developed method was successfully applied to the analysis of apple samples for residual pesticide content, which confirms its effectiveness and reliability.

The study by D. Yun *et al.* (2024) was aimed at developing a fast and accurate method for the simultaneous analysis of multi-residue pesticides, as well as monitoring their content in agricultural products. Within its framework, representative samples of brown rice, soybeans, potatoes, tangerines, and green peppers were selected. Gas chromatography with tandem mass spectrometry (GC-MS/MS) was used for the analysis of 272 pesticide residues. Sample extraction was performed using the QuEChERS-EN method with subsequent dispersive solid-phase extraction (d-SPE) using anhydrous MgSO₄ and primary-secondary amine (PSA) sorbents. The proposed approach demonstrated high effectiveness and accuracy, which made it possible to ensure reliable monitoring of residual pesticides in various types of agricultural products.

MATERIALS AND METHODS

The research was carried out during the spring and autumn of 2024 at the Ukrainian Laboratory for the Quality and Safety of Agricultural Products of the National University of Life and Environmental Sciences of Ukraine (NULES). The study used samples of plant products, including sunflower seeds (LG50479 SX hybrid from Limagrain), corn kernels (Rist SV hybrid), lettuce leaves (Iceberg), and apples (Golden Delicious). These were collected from a variety of sources: private farms in the Kyiv region that

use integrated pest management or practice organic farming with minimal use of chemical agents, as well as the NULES research farms in Kyiv. The collection of seeds, kernels, leaves, and fruits was done when they reached full biological maturity, in line with the sampling procedure approved by the current Order of the Ministry of Agrarian Policy of Ukraine No. 289 (2018). All samples were transported to the laboratory within 12 hours of collection at a temperature of 4–6 °C, which meets the requirements for transporting food and biological materials. To prevent changes in their chemical composition and the breakdown of active biological substances, the samples were stored in sterile, light-protected containers made of an inert material. Before the analytical procedures began, all material was kept in refrigerators at +4 °C, except for samples intended for the study of volatile components or pesticides, which were stored at -18 °C according to the methodological guidelines of the European Food Safety Authority (EFSA, n.d.).

For each experiment, four parallel laboratory samples of 100 g each were prepared. Preparing the samples for analysis began with homogenisation, a standard step for different food groups. Homogenisation was done by grinding the samples in a glass laboratory grinder-homogeniser (LHM-1) at varying temperatures (from +4 °C to +25 °C) and a speed of 10,000 rpm. Due to the different chemical compositions of the raw materials, the resulting homogenised samples had different physical states: fine-grained, pasty, or liquid. Using the coning and quartering method, 5 g was taken from each homogenised sample for analysis, and the remaining 95 g was hermetically sealed and stored in a freezer at -18 °C until the end of the experiment.

To simulate contamination, three of the four samples were artificially fortified with solutions of analytical standards of the target xenobiotics produced by Sigma-Aldrich (with a purity of ≥ 98%). The range of concentrations of added xenobiotics was developed individually for each substance and corresponded to a level of 0.5 to 5 times the maximum permissible concentration (MPC). The control sample was not contaminated and was used to assess the background content of analytes and to validate the chromatographic method, specifically to evaluate matrix noise, detect and

quantify the background content of xenobiotics, and determine the extraction efficiency of the added analytical standards (recovery rate, %).

Organic solvents of “pure for analysis” (“p.f.a.”) grade were used to obtain plant extracts. Organic solvents were used as individual extractants or in mixtures, in particular with deionised water. Buffering of the homogenised sample solution and purification of the plant extract were carried out using magnesium sulphate, sodium chloride, sodium citrate, calcium chloride and aluminium oxide (“p.f.a.”). Extraction was performed in polytetrafluoroethylene plastic tubes, dark glass flasks, and polymethylpentene plastic containers, which were all protected from light with opaque covers. Mass transfer during analyte extraction was intensified by changing the ratio of raw material to extractant, regulating temperature, mechanical mixing, and using ultrasonic waves (37 kHz, Advantage Lab generator). The plant extract was purified using dispersive or liquid-liquid extraction methods with organic solvents and cartridges containing amine sorbents (Supelco). The purified extract was concentrated using an IKA rotary evaporator. Xenobiotics were analysed using High-Performance Liquid Chromatography with mass-spectrometric detectors (HPLC/MS/MS) and Gas Chromatography/Mass Spectrometry (GC/MS) on Dionex and Agilent instruments. Spectral data was processed using Cromeleon 6.0 software and the NIST 0.5 mass spectral library. The chromatographic analysis was performed with a Dionex Summit MSD-3200Q Trap instrument with a ChromSpher C18 column (3 µm, 4.6×5 mm). The mobile phase was a methanol/acetic acid gradient, transitioning from 10:90 to 90:10 over 0-5 minutes, with a reverse transition back to 10:90 over 12-15 minutes. Analytes were identified using a programmed screening method.

Based on data from M. Arnold *et al.* (2023) and Z. Li *et al.* (2024) regarding the nutritional value and chemical composition of the tested samples, the mass fractions (W, %) of the main chemical components of the matrix were calculated using formula (1).

$$w = \frac{m_{\text{component}}}{m_{\text{homogenised sample}}} \times 100\%. \quad (1)$$

Homogenised sunflower seeds were classified as a group of samples with a high lipid content (30-52%), indicating their belonging to a fatty raw material. Corn kernels contain a small percentage of lipids (6%). In contrast, homogenised samples of “Iceberg” lettuce leaves and apples (with skin) are characterised by a predominant water content of over 95% and 85% respectively. This composition places these samples in the category of high-moisture plant products, where the proportion of lipids, proteins, and other components is negligible. As the choice of extractants is determined by the physico-chemical properties of the matrix and the target xenobiotics, which influence their solubility and extraction, the study used polar (acetonitrile, methanol, deionised water) and non-polar (chloroform), protic (methanol, water, acetic, trifluoroacetic acid) and aprotic solvents (acetonitrile, chloroform), as well as their mixtures (acetonitrile + methanol, methanol + trifluoroacetic acid), which are common in laboratory practice. Additionally, organic and mineral acids were used to shift the equilibrium of the dissociation process of ionisable xenobiotics towards the formation of molecular forms, which facilitated their effective extraction from the homogenised raw material.

In conducting this study, the authors relied on data from the scientists S. Petrović *et al.* (2023) and V. Goel *et al.* (2025), whose work provides an in-depth analysis of the relationship between the physico-chemical properties of target analytes (including xenobiotics) and the characteristics of plant matrices, which directly affects the efficiency of the extraction process. For example, the article by S. Petrović *et al.* (2023) details the influence of polarity, acidity, hydrophobicity, and the degree of ionisation of substances on their extraction from different types of matrices (moist, fatty, fibrous, etc.). The authors also highlight the importance of selecting acids and salt components that allow for the optimisation of the medium’s pH to achieve the maximum conversion of target compounds into their molecular form, which is critical for extraction. The work by V. Goel *et al.* (2025) presents a comprehensive comparative analysis of polar and non-polar solvents, their mixtures, and the role of organic acids in improving the extraction of ionised pesticides and other xenobiotics from matrices containing

high levels of moisture or lipids. These works are practically oriented, and their results were directly applied in this study during the development of the extraction protocol. In addition, the work by C. Poole (2023) provided a scientifically substantiated basis for the selection of solvents and medium modifiers in the analytical procedure used in the current study.

To establish optimal sample preparation conditions, the method of artificial fortification was applied to both homogenised and non-homogenised blank samples of the raw material with the xenobiotics acetochlor (a marker for chloroacetamide herbicides), prometryn (a marker for triazine herbicides), benomyl (a marker for benzimidazole fungicides), cyprodinil (a marker for pyrimidine fungicides), diquat (a marker for bipyridylium herbicides), 2,4-dichlorophenoxyacetic acid (2,4-D), and 4-chloro-2-methylphenoxyacetic acid (MCPA) (markers for phenoxy herbicides). Compounds belonging to different chemical groups of xenobiotics were used as markers. The optimal ratio of extractant to homogenised sample was determined on the basis of a visual analysis used to observe the hydrodynamic regime of the extraction process, as well as on the results of the quantitative analysis of the xenobiotic extracts. The optimal ratio of sample-to-extractant components was chosen experimentally by a stepwise increase in the amount of extractant in a ratio range from 1:1 to 1:20. Under optimal sample-to-extractant conditions, there is no formation of compaction zones in the material, and the material freely contacts the extractant, allowing for the most complete isolation of the xenobiotic within the extract. The chromatographic control of the analyte content in the plant extracts allowed for the evaluation of xenobiotic extraction efficiency under various hydrodynamic conditions.

Statistical processing of the experimental data was performed using the Microsoft Excel software package. The measurement error was calculated using the standard deviation (S_r , %), and the degree of extraction of xenobiotics from the artificially fortified laboratory samples was estimated in percentages (r , %). For quantitative analysis, calibration was performed and the following were established: the linearity of the method (coefficient of determination $R^2 > 0.99$),

the limit of detection (LOD), and the limit of quantification (LOQ) for each of the xenobiotics.

RESULTS AND DISCUSSION

As shown in Figures 1 (a) and 1 (b), the level of extraction of xenobiotics such as the herbicide acetochlor (a marker for chloroacetamides) and promethrin (a marker for triazines) depended on the amount of extractant added to 1 gram of homogenised sample. The studies showed that sunflower seeds and corn kernels required the most extractant in experiments with crop products – about 20 ml per 1 g of ground sample. Homogenised samples of oilseed crops were studied using different extractants. The most effective extraction of these xenobiotics was observed when using acetonitrile. The method of extracting pesticides from sunflower seeds and corn kernels using acetonitrile allowed the removal of up to 98% of artificially added pesticides at a sample-extractant ratio of 1:20 (Fig. 1 (a, b), curve 4).

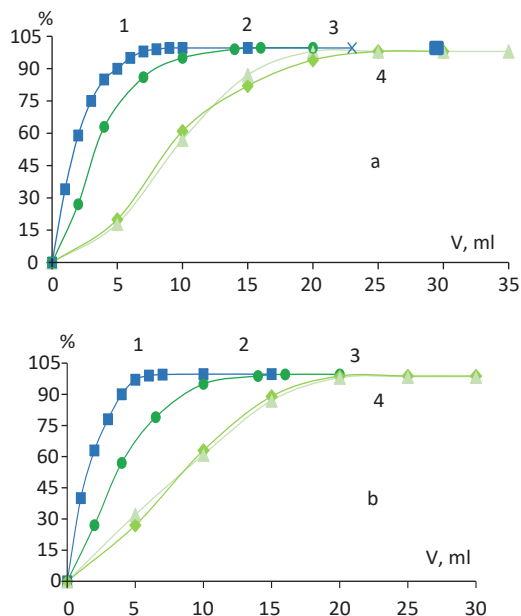


Figure 1. Percentage of extraction of acetochlor (a) and promethrin (b) from 1 g of artificially enriched homogenised samples into plant extract

Note: 1 – lettuce leaves; 2 – apples; 3 – sunflower seeds; 4 – corn kernels. Extraction agent – acetonitrile. Extraction duration – 5 min

Source: developed by the authors

For another group of xenobiotics, it was necessary to optimise the composition of the extractant. Taking into account the hydrophobic properties of xenobiotics, the peculiarities of their chemical structure, and based on the

results of chromatographic analysis (Figs. 2, 3), extractant systems were identified that promote the effective transfer of analytes from the plant matrix to the organic phase of the extraction medium.

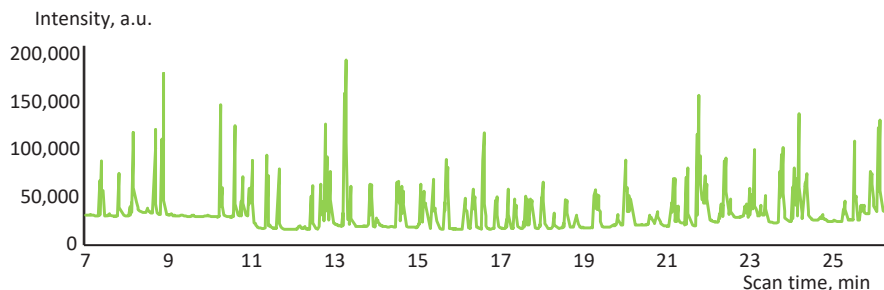


Figure 1. Gas chromatography-mass spectrometry (GC-MS) chromatogram of an extract from apples enriched with xenobiotics (Benomyl, Iprodione, Diquat)

Note: Chromatographic analysis of xenobiotic residues was performed for all studied matrices, including sunflower seeds, corn kernels, and lettuce leaves. All samples underwent procedures for artificial enrichment with the target compounds, extraction, and subsequent chromatographic determination. The chromatogram obtained from the homogenised apples was included in the main text of the work as a representative example because it has the most representative signal structure, clear peak separation, and reflects the effectiveness of the analytical method used

Source: developed by the authors

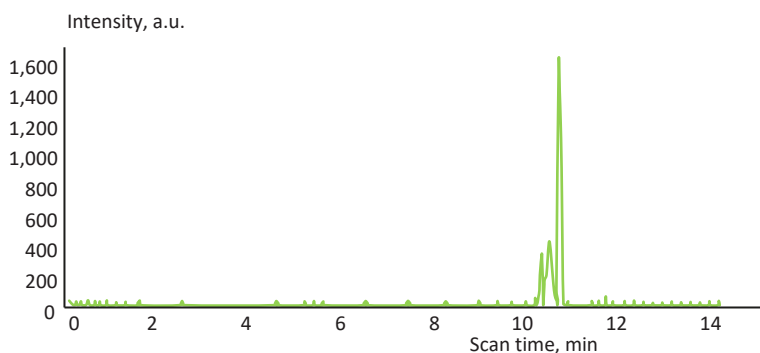


Figure 2. Liquid chromatography chromatogram with mass spectrometric detection (LC-MS) of plant extract from apple enriched with xenobiotics (2,4-D and MCPA)

Note: Chromatographic analysis of xenobiotic residues was performed for all matrices studied, including sunflower seeds, corn kernels, and lettuce leaves. All samples underwent artificial enrichment with target compounds, extraction, and subsequent chromatographic determination. The main text of the study included a chromatogram obtained from homogenised apples as an illustrative example, as it has the most representative signal structure, clear peak separation and reflects the effectiveness of the analytical method used

Source: developed by the authors

The peaks recorded on the chromatograms (Figs. 2, 3) were identified using a mass spectrum library and by comparing the retention

times of the analytes and characteristic ions of the xenobiotics under study with the corresponding parameters of the analytical standards.

The chromatogram of the extract from homogenised apples, obtained by gas chromatography with mass spectrometry (GC/MS), recorded about 70 analytical signals (Fig. 2). From the total number of peaks, using standardised solutions of target analytes, peaks corresponding to the presence of the xenobiotics Benomyl, Cyprodinil and Diquat were identified. It should be noted that the use of liquid chromatography with mass spectrometric detection (LC-MS) allows obtaining chromatograms with minimal noise from matrix components (Fig. 3). The visual and analytical characteristics of such chromatograms differ

significantly from chromatograms obtained using gas chromatography with mass spectrometry (GC-MS) (Fig. 2). Thus, Figure 3 shows a chromatogram of a plant extract obtained after alkaline hydrolysis of a sample in a 0.1% sulphuric acid solution, followed by extraction with a mixture of acetonitrile and methanol in a 4:1 ratio.

The results of the identification of target compounds are presented in Table 1. The analysis shows that the highest extraction rates of xenobiotics are observed in samples of plant products with a low content of lipid components in the matrix.

Table 1. Quantitative indicators of xenobiotic content in extracts from samples artificially enriched with target compounds

Xenobiotic	Benomyl	Cyprodinil	Diquat
Extractant	mixture of acetonitrile with methanol		solution of trifluoroacetic acid in methanol
	Sunflower seeds		
Added, µg/kg	0.50 ± 0.01	0.50 ± 0.01	0.50 ± 0.01
Determined, µg/kg	0.41 ± 0.03	0.40 ± 0.03	0.43 ± 0.03
Extracted, %	82.0 ± 6	80.0 ± 6	86.0 ± 6
	Lettuce leaves		
Added, µg/kg	0.50 ± 0.01	0.50 ± 0.01	0.50 ± 0.01
Determined, µg/kg	0.49 ± 0.01	0.49 ± 0.02	0.51 ± 0.03
Extracted, %	98.0 ± 2	98.0 ± 4	102.0 ± 6
	Apples		
Added, µg/kg	0.50 ± 0.01	0.50 ± 0.01	0.50 ± 0.01
Determined, µg/kg	0.48 ± 0.01	0.49 ± 0.02	0.47 ± 0.02
Extracted, %	96.0 ± 2	99.0 ± 4	94.0 ± 4
	Corn kernels		
Added, µg/kg	0.50 ± 0.01	0.50 ± 0.01	0.50 ± 0.01
Determined, µg/kg	0.45 ± 0.02	0.46 ± 0.01	0.44 ± 0.02
Extracted, %	90.0 ± 1	92.0 ± 3	88.0 ± 2

Source: developed by the authors

Based on the results of experimental studies that covered the process of extracting xenobiotics using different extractants and the subsequent quantitative analysis of the content of target compounds using chromatographic methods, optimal conditions for the extraction of compounds of different chemical natures were established. For the extraction of acetochlor and prometryn from samples with a water content of 90-96%, the optimal ratio of homogenised sample to extractant was 1:3-1:5 (Fig. 1 (a, b), curve 1). For samples with a water content of 75-85%, extraction was performed at a raw material-to-solvent ratio of 1:10 (Fig. 1

(a, b), curve 2). Sunflower seeds, which contain a large percentage of lipids, required a raw material-to-extractant ratio in the range of 1:17-1:20 (Fig. 1 (a, b), curve 3).

Pesticides belonging to the benzimidazole group are characterised by high lipophilicity and good solubility in organic solvents. In the presence of a buffer medium in the aqueous phase of the extraction system, benzimidazole-series compounds that do not have ionisable fragments are effectively extracted into the organic phase, for example, into acetonitrile. Many benzimidazole derivatives, such as benomyl, contain several functional groups,

which necessitates improving the composition of the extractant. To enhance the extraction of such compounds, especially those with a carboxyl group that can dissociate with the release of a proton, weak organic acids were added to the extraction mixture. A small amount of it, present in the acetonitrile extract, does not interfere with the chromatographic analysis and does not affect the operation of the mass spectrometric detectors. For the effective extraction of benzimidazole (Benomyl) and anilinopyrimidine (Cyprodinil) derivatives, a mixture of acetonitrile and methanol in a volume ratio of 4:1 is recommended. In turn, bipyridylium derivatives (Diquat) are best extracted using a methanolic solution of trifluoroacetic acid in a ratio of 9.5:0.5. As can be seen from Table 1, the highest indicators of xenobiotic extraction efficiency are observed in samples of lettuce leaves and apples, whose matrix is characterised by a low lipid content. In particular, the extraction from sunflower seeds presents the greatest difficulty in terms of sample preparation for analysis and, accordingly, the level of extraction of the target compounds. Despite the fact that the percentage of xenobiotics extracted from sunflower seeds is lower compared to the results obtained for samples of lettuce leaves and apples, the sample preparation process in this case is assessed as satisfactory. This is due to the fact that the average level of extraction of the added xenobiotic exceeds 80%, which corresponds to acceptable analytical criteria (Guidance document..., 2023; DSTU EN 15662:2023, 2024).

The most complete extraction of xenobiotics for sunflower seeds was achieved under the following conditions: 1 g of homogenised sample; 10 ml of acetonitrile; 9 ml of deionised water; 1 ml of acetic acid; 4 g of Na₂SO₄; 1 g of NaCl. Shaking was carried out for 3 minutes, and centrifugation of the extraction system took place at 7,000 rpm for 5 minutes. In addition, an important factor affecting the effectiveness of xenobiotic extraction from samples artificially fortified with analytes is the temperature regime and the duration of contact with the extractant. The most optimal extraction conditions were established in the temperature range from 4 °C to 25 °C with a duration of exposure to the extraction medium for 5-25 minutes.

In contrast to many previous works, this study was focused not only on optimising the pesticide extraction method, but also on a systematic analysis of the relationship between the physico-chemical characteristics of the plant matrix and the effectiveness of xenobiotic extraction. Specifically, K. Tong *et al.* (2022) applied a combination of the QuEChERS method with high-resolution gas and liquid chromatography for screening 237 pesticides in cottonseed husks. Similar to the approaches implemented in the current study, the authors noted the high effectiveness of acetonitrile as a universal extractant. At the same time, it is in this study that the dependence of extraction efficiency on the lipid composition of the matrix is emphasised, which was not considered in the work of K. Tong *et al.* (2022). X. Theurillat *et al.* (2021) also studied fatty food matrices and emphasised that high lipid content is a factor that complicates the extraction process. The results obtained in this work confirm these conclusions: sunflower seeds, which are characterised by high fat content, demonstrated a lower level of extraction (80-86%) even under optimised extraction conditions. A common feature of both studies is the need to modify the extraction protocol – in particular, by using acetonitrile with pH regulators or buffer components. P. Aralimarad *et al.* (2025) highlighted the importance of careful sample preparation for fatty matrices (e.g., peanut oil), taking into account the assessment of analytical uncertainty and risks. These principles were also implemented in the current study, which used standardised sample fortification and performed a comparative analysis of extraction for different plant matrices with different polarities. F. Lan *et al.* (2024) demonstrated the advantages of liquid chromatography with mass spectrometry (LC-MS) in detecting fungicide residues in fruits. Similar results were obtained in this study, where LC-MS provided high sensitivity, clear peak separation, and minimal background noise – especially for matrices with a high water content. In comparison with other modern studies, this study is more comprehensive: it covers the analysis of matrix effects, the optimisation of solvents depending on the type of raw material, the influence of the acid-base nature of xenobiotics, and also the hydrodynamic conditions of the extraction.

CONCLUSIONS

As a result of the completed research, optimal conditions were established for the preparation of plant product samples for the qualitative and quantitative determination of xenobiotics, specifically pesticide residues. The study confirmed the feasibility of applying the QuEChERS method in combination with High-Performance Liquid (HPLC) and Gas Chromatography (GC), especially for samples with high moisture and low lipid content, such as leafy vegetables and fruits. For such samples, the highest extraction rates were achieved, which indicates the effectiveness of the extraction procedure. The study established that the effectiveness of xenobiotic extraction is significantly dependent on the physico-chemical properties of the plant matrix. Samples with high water content, such as lettuce leaves (>95%) and apples (>85%), required a significantly smaller volume of extractant (a ratio of 1:3-1:5) compared to fatty sunflower seeds (up to 52% lipids), where a ratio of 1:17-1:20 was used to achieve high extraction efficiency.

For the extraction of benomyl and cyprodinil, a mixture of acetonitrile with methanol (4:1) proved effective, ensuring a high level of extraction in all matrix types, particularly over 96% in lettuce and apples, which points to good solubility and a low matrix effect. For diquat, the best results were obtained using a solution of trifluoroacetic acid in methanol (9.5:0.5), with maximum

values in lettuce and acceptable values (86%) in sunflower seeds. In corn kernels, the extraction level was 88-92% for all compounds, which confirms the effectiveness of the applied methodology across different plant matrices. It was also established that optimal extraction conditions – a temperature within 4-25 °C and an extraction duration of 5-25 minutes – ensure stable mass transfer and minimise matrix interferences. The chosen method, which includes a modified QuEChERS system, demonstrated high accuracy and analytical reproducibility, underscoring its suitability for the routine control of pesticide residues in various types of plant products. The obtained results have practical significance for improving laboratory control of the safety of plant products, which is particularly relevant in the context of increased demands for food and environmental safety. Prospects for further research include expanding the methodology to new classes of xenobiotics, automating sample preparation, and studying the stability of analytes during storage.

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CONFLICT OF INTEREST

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Методологічні підходи до підготовки проб для аналізу вмісту ксенобіотиків

Анотація. Метою статті було вдосконалення протоколів підготовки зразків та умов хроматографічного аналізу для визначення вибраних ксенобіотиків у сільськогосподарській продукції. Для досягнення мети використовували методіку штучного збагачення гомогенізованих зразків насіння соняшнику, зерна кукурудзи, листя салату та плодів яблук модельними ксенобіотиками різних класів, подальше екстрагування органічними розчинниками з використанням модифікованого підходу Q₁EChERS та кількісний аналіз. Для ефективного вилучення ацетохлору та прометрину з листя салату та яблук, які характеризуються високим вмістом води, оптимальним виявилось співвідношення сировини до екстрагенту на рівні 1:3-1:5. У разі зерна кукурудзи, структура якого містить незначну кількість ліпідів, екстракцію здійснювали зі співвідношенням 1:10. Найбільші труднощі спостерігалися під час обробки насіння соняшнику, де через високу жирність формувалася трифазна система, що ускладнювала масоперенос аналітів. Незважаючи на це, застосування ацетонітрилу як екстрагенту у співвідношенні 1:17-1:20 дозволило досягти високих показників вилучення цільових ксенобіотиків. Для екстракції беномілу та ципродинілу застосовувалася суміш ацетонітрилу та метанолу у співвідношенні 4:1, яка забезпечила ефективне перенесення зазначених аналітів у всіх досліджених матрицях. Зокрема, у зразках листя салату та яблук показники вилучення для обох речовин перевищували 96 %, що свідчило про добру розчинність у застосованій суміші та низький матричний вплив. Для диквату, який є представником біпіридилієвих сполук із вираженою іонною природою, найкращі результати були отримані при використанні розчину трифтороцтової кислоти в метанолі у співвідношенні 9,5:0,5. Такий склад забезпечував стабільне вилучення з усіх типів рослинних матриць, із максимальними значеннями у зразках салату і мінімальними – у соняшнику (86 %), що все одно відповідає сучасним вимогам до аналітичної точності. У випадку зразків зерна кукурудзи рівень вилучення для всіх речовин становив 88-92 %, що свідчило про задовільну екстрагованість у зернових матрицях. Найнижчі значення вилучення зафіксовано для насіння соняшнику, тим не менш, навіть у цих умовах вилучення перебувало в межах 80-86 %, що є прийнятним відповідно до міжнародних стандартів контролю якості пестицидного аналізу. Результати хроматографічного аналізу підтвердили високу відтворюваність та чутливість методу

Ключові слова: високоефективна рідинна хроматографія; газова хроматографія з мас-селективними детекторами; екстракти; пестициди; рослинні витяжки

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Ozonation as a means of increasing the ecological safety of agrosystems: Purification of soils and irrigation water from toluene and its derivatives

Abstract. The aim of the study was to evaluate the effectiveness of liquid-phase ozonation of methylbenzenes as a method for neutralising aromatic xenobiotics in surface waters and soils of agroecosystems. Spectrophotometric, iodometric, chromatographic, and elemental analysis methods were used to study the dynamics of aromatic ring destruction, the formation of intermediate and final products, and to determine the reactivity of arene derivatives. It was established that the main pathway for the conversion of methylbenzenes is the ozonolytic cleavage of the benzene ring, with the formation of aliphatic ozonides. The yield of benzoic acid did not exceed 5.8%, which indicates insignificant stabilisation of the aromatic fragment. For p-xylene, the degree of mineralisation reached over 70% after 30 minutes of ozonation. The presence of donor substituents in the benzene ring ($-\text{CH}_3$, $-\text{OH}$, $-\text{NH}_2$) contributed to deep core destruction, while acceptor substituents ($-\text{NO}_2$, $-\text{COOH}$) reduced its reactivity, changing the direction of the reaction to the side alkyl chain. Derivatives with functional groups capable of nucleophilic attack ($-\text{OH}$, $-\text{NH}_2$, $-\text{SH}$) were characterised by a significantly higher reaction rate (up to 25 times) compared to toluene. The acylation of such groups with acetic anhydride lowered the reaction rate and increased the selectivity for the formation of stable products. Particular attention was paid to the composition and structure of the reaction products. Peroxides formed as a result of the destruction of the aromatic ring have different structures, ranging from monomeric to oligomeric forms. They were highly soluble in acetic acid but were easily precipitated or removed from the solution, which was a key advantage of the process in water and soil purification applications. The data obtained demonstrated the potential of liquid-phase ozonation as an effective tool for the selective and controlled neutralisation of aromatic compounds in soils and waters for agricultural use. The results obtained can be used by ecologists, agrochemists, and water purification specialists to increase the ecological safety of agricultural areas and irrigation systems contaminated with aromatic compounds

Keywords: ozone; methylbenzenes; destruction; peroxides; water purification; kinetics

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INTRODUCTION

Modern agriculture faces the urgent problem of pollution of agroecosystems with organic pollutants, in particular toluene derivatives, which enter the soil and water resources through the use of pesticides, herbicides, fuels and lubricants, and industrial emissions. These compounds have high resistance to biodegradation, which leads to their accumulation in the soil environment, a decrease in the biological activity of soils and a deterioration in crop quality. Given the importance of preserving soil fertility and water resource safety, the development of effective, environmentally safe methods for cleaning up such pollutants is a pressing task for agricultural science.

The authors S. Lim *et al.* (2022) emphasise in their work the promise of liquid-phase ozonation as a method for the deep destruction of aromatic pollutants in water systems. They note that ozone in the liquid phase forms highly active hydroxyl radicals that are capable of effectively breaking down stable organic structures, including toluene and its derivatives. B. Liao *et al.* (2022) investigated the mechanisms of ozone oxidation of methylbenzenes and found that the electronic properties of substituents in the benzene ring significantly affect the selectivity and reaction rate. The work of H. Luo *et al.* (2021) shows that the nature of substituents determines the decomposition pathways of aromatic compounds and influences the formation of oxidation products, which is of particular importance for Ukrainian agroecosystems.

Research by A. Salnikova & S. Salnikov (2021) confirmed the effectiveness of the combined use of ozonation and solar sterilisation to reduce the concentration of pesticides in irrigation water, while minimising the negative impact on soil microbiota. O. Chayuk (2019) points out the importance of analysing the formation of peroxide compounds in the process of aromatic substance destruction, as they can have both a positive effect on oxidative potential and be potentially toxic to some members of the microflora. M. Tahamolkonan *et al.* (2022) draw attention to the need to optimise ozone dosing, as excessive concentrations can inhibit soil microbial activity and disrupt natural biogeochemical cycles.

The work of S. Lim *et al.* (2022) emphasises the importance of the spatial arrangement of substituents in aromatic molecules, as this directly affects the kinetics of ozonation reactions and the formation of minor products. A recent study by X. Li *et al.* (2021) demonstrated that enhanced ozonation with the addition of mechanical stirring provides over 90% degradation of benzo[a]pyrene in soil in just 90 minutes, while improving the bioavailability of residual products for further microbial degradation. An analysis of bioremediation methods that complement ozonation is presented in a review by C. Rusănescu *et al.* (2024), which justifies the combination of physicochemical and biological technologies to complement effective strategies for the restoration of contaminated soils.

Based on current research, it has been established that ozonation is a promising and environmentally safe method for the degradation of toluene and related aromatic compounds in agroecosystems. This approach demonstrates high efficiency in cleaning soils and water environments from persistent organic pollutants. At the same time, for practical implementation, it is necessary to study in depth the mechanisms of ozonolysis under different soil conditions, in particular its impact on soil fertility, enzymatic activity and microbiota structure. The aim of this work was to conduct a comprehensive analysis of the effectiveness of liquid-phase ozonation of toluene and its derivatives, as well as to determine the optimal conditions for environmental safety and restoration of the fertility of agricultural systems.

LITERATURE REVIEW

The contamination of agricultural ecosystems with organic toxicants, among which toluene derivatives occupy a special place, poses a serious environmental threat to the sustainable development of agriculture. These compounds enter soil and water resources mainly through the excessive use of agrochemicals, such as herbicides and pesticides, as well as through the use of petroleum products and technical fluids containing aromatic hydrocarbons. Sources of pollution also include untreated industrial wastewater. Due to their high chemical stability and low

susceptibility to natural decomposition, these substances accumulate in the soil environment. This, in turn, leads to a decrease in the enzymatic activity of soil biota, disruption of the microbial balance and a general deterioration in fertility, which negatively affects the quality and safety of agricultural products (Aidoo *et al.*, 2023).

Against this backdrop, innovative methods of detoxifying the agricultural environment deserve increasing attention. One of the most promising technologies is ozonation, a process based on the use of a strong oxidant, ozone, which is capable of destroying complex organic molecules. Ozone oxidation is accompanied by the generation of secondary active agents, in particular hydroxyl radicals, which demonstrate high reactivity towards aromatic hydrocarbons, including benzene and toluene structures. The results of laboratory and field studies show that ozone, acting directly in the soil, promotes the decomposition of heavy organic compounds into less toxic intermediate products that are more accessible for further biological degradation. This significantly enhances the ability of soil microorganisms to mineralise hydrocarbon residues (Yu *et al.*, 2024).

The combined use of ozonation with other physical, chemical or biological methods is particularly effective. For example, the combination with soil solarisation demonstrates a synergistic effect on the decomposition of persistent pesticides, while maintaining the overall microbial viability and bioactivity of the soil at an acceptable level (Díaz-López *et al.*, 2021). However, along with the positive results, a number of studies have found that excessive or improperly regulated ozonation can have a suppressive effect on soil microflora, in particular through the oxidation of beneficial metabolites or the destruction of cell structures (Garrido *et al.*, 2023). This highlights the need to optimise ozone exposure regimes according to soil type, degree of contamination and agroecosystem specifics.

In a study by E. Potapenko *et al.* (2021), the kinetics and mechanism of ozonolysis of arenes were investigated. It was shown that ozone can interact with both the side methyl radical and the aromatic ring, resulting in the formation of a wide range of products, from benzoic acid to unstable peroxides. The authors proved that

the solvent significantly influences the direction of the reaction. The study of the ozonolysis of 2,4-dinitrotoluene deserves special attention. It was found that in the presence of electron-accepting substituents ($-\text{NO}_2$), ozone attacks both the benzene ring and the side chain. The formation of hydroperoxides indicates a radical mechanism, with the temperature regime influencing the predominant direction of the reaction: at lower temperatures, ring attack prevails. The work of A. Galstyan *et al.* (2018) considers the reactions of ozone with aminotoluenes. It is shown that electron-donating groups activate the aromatic system to electrophilic attack by ozone. In acetic anhydride, with the participation of mineral acid, acylation of amino groups occurs, which changes the reactivity of the molecule. Even without catalysts, the reaction is selective and produces stable aliphatic products. These works are an important basis for further research in the field of pollutant processing, organic synthesis and environmental chemistry. In addition to soil purification, ozonation is also actively being introduced for post-harvest processing of vegetables and fruits. In particular, the use of ozonated water for washing products significantly reduces the residual concentrations of agrochemicals, unlike conventional water washing, which has limited effectiveness in removing hydrophobic compounds (Singh *et al.*, 2022). This approach not only improves food safety but also extends the shelf life of products.

Another interesting area of research is the effect of ozonation on physiological and biochemical processes in plants. For example, ozone treatment of seeds causes changes in the profile of volatile organic compounds, which can serve as biomarkers of germination or stress response (Khanashyam *et al.*, 2021; Dong *et al.*, 2022). Such effects open up new possibilities in pre-sowing seed treatment as a method of stimulating growth processes.

Separately, it should be mentioned that there are studies investigating the interaction of ozone with the processes of microbial degradation of organic pollutants. For example, hybrid systems combining ozonation with biological treatment have shown high efficiency in destroying polycyclic aromatic hydrocarbons in soil, even under conditions of increased

pollution (Olak-Kucharczyk *et al.*, 2023, Malek & Choudhary, 2023). Such results confirm the potential of combined methods as tools for restoring the functional state of degraded agricultural landscapes.

Given the accumulated results, there was a clear need for further study of the possibilities of ozonation as a means of remediating agricultural systems. In particular, research into the kinetics and mechanism of ozonolysis of methylbenzenes, the effectiveness of their neutralisation with ozone, and the impact of this process on long-term soil fertility, their microbiological balance and the ecological stability of the agrosphere as a whole remain relevant.

MATERIALS AND METHODS

Laboratory studies were conducted at the Research Laboratory of Ozonolytic Transformations of the National University of Life and Environmental Sciences of Ukraine (Kyiv) between January and April 2025. High-purity chemical reagents were used for the experiments: sulphuric and glacial acetic acids from Sigma-Aldrich ("chemically pure" grade), toluene and its substitutes from Acros Organics ("chemically pure" grade). The water for the solutions was demineralised.

Acylation of hydroxy- and aminotoluenes was carried out according to a method in which 0.4 mol·l⁻¹ of aminotoluene and 30 ml of 80% acetic acid were loaded into a 50 ml round-bottom flask. When the solution was gradually heated to 130-140 °C, the water was distilled off, after which the resulting product was separated into 30 ml of water cooled to 5 °C, filtered and dried. Ozone oxidation was carried out in a cylindrical glass reactor with a volume of 30 ml (height – 8 cm, diameter – 2.2 cm) with a perforated diaphragm for better contact between the gas and liquid phases. 20 ml of solvent and the calculated amount of arenes were loaded into the reactor. The ozone generator was model OZ-100 (manufacturer: OzoneTech Ltd., Ukraine). Ozone was produced by corona discharge at a voltage of 7 kV, a current of 20 mA, water cooling, with constant regulation of the generator temperature at 25 ± 1 °C. The ozone concentration in the gas mixture was controlled using a built-in spectrophotometric analyser with an

accuracy of ± 0.02% by volume and was automatically stabilised by a feedback system.

The kinetics of the reactions were studied as follows. The ozone-air mixture was fed into the reactor at a rate of 20 l/h, with an ozone concentration of 0.8-1.2% by volume. Ozonation lasted from 15 to 120 minutes, depending on the experimental conditions. During the reaction, magnetic stirring at a speed of 300 rpm was used to ensure uniform distribution of ozone in the liquid. The process was controlled by gas chromatography on an Agilent 7890B chromatograph with a flame ionisation detector (FID), HP-5 column (length 30 m, diameter 0.32 mm, film thickness 0.25 µm) (Potapenko *et al.*, 2021). The evaporator temperature was 250 °C, and the column temperature was programmed from 120 to 175 °C with a rise rate of 8 °C/min. The carrier gas is nitrogen, flow rate: 2.0 l/min, hydrogen – 1.8 l/min, air – 18 l/min.

The ozone concentration in the gas phase was measured spectrophotometrically using a SF-46 LOMO spectrophotometer with a Teflon flow cell (optical path length 10-100 mm). The optical density of the gas flow was recorded automatically by a potentiometer with an error of ≤ 5%. The ozone concentration was calculated using the Lambert-Beer equation with molar extinction coefficients.

Quantitative analysis of peroxides was performed by iodometric titration: 1 ml of the reaction mass was added to 10 ml of a 5% potassium iodide solution, acidified with 2.5 ml of 0.1 M sulphuric acid, kept for 1 and 24 hours at 20 ± 2 °C, and then titrated with 0.05 M sodium thiosulphate solution. The concentration of peroxides was calculated using the formula (1):

$$[oz] = \frac{V_T M_T}{n V_p}, \text{ mol} \cdot \text{l}^{-1}, \quad (1)$$

where V_T is the volume of sodium thiosulphate for titrating the iodine solution, ml; V_p is the volume of the reaction mass, ml; M_T is the molar concentration of thiosulphate, mol · l⁻¹; n is the number of transferred electrons.

The relative measurement error was ±8%.

The rate constant for the new substance, knowing the constant for toluene and the ratio of products in the reaction mixture, was determined by the formula (2):

$$K_{\text{sub}}/k_{\text{st}} = [P_{\text{sub}}]/[P_{\text{st}}], \quad (2)$$

where k_{sub} – the desired value of the constant; k_{st} – the known constant for the standard substance; $[P_{\text{sub}}]$, $[P_{\text{st}}]$ – the concentrations of the corresponding reaction products.

Statistical data processing was performed using the program OriginPro 2023. For each experiment, at least three parallel determinations were performed. The results are presented as the mean arithmetic value \pm the standard deviation. Statistical significance was determined using the Student's t-criterion at a significance level of $p < 0.05$.

Table 1. Oxidation of methylbenzenes with ozone in acetic acid at 293 K.
 $[O_3]_0 = 4.7 \cdot 10^{-4}$; $[ArCH_3]_0 = 0.4 \text{ mol} \cdot \text{l}^{-1}$; $V_p = 0.011$

Compounds	Selectivity, %	
	oxidation via the side chain	oxidation via the aromatic ring
Toluene	5.9	93.6
1,4-Dimethylbenzene	5.1	92.3
1,3-Dimethylbenzene	4.6	92.5
1,2-Dimethylbenzene	0.0	99.5
1,3,5-Trimethylbenzene	0.0	100.0
2-Nitrotoluene	6.2	89.0
3-Nitrotoluene	12.4	82.0
4-Nitrotoluene	14.2	81.5
3-Chlorotoluene	13.1	84.2
4-Toluenesulfonyl chloride	26.3	72.1

Source: developed by the author

The composition of the products of the ozonation of methylbenzenes in the liquid phase indicates the following: the reaction proceeds in two directions: mainly via the benzene ring with the formation of its degradation products, and via the side chain of the substrate with the formation of aromatic products; with an increase in donor substituents in the aromatic ring, the selectivity of oxidation along the side chain decreases; the stability of the benzene ring in the reaction with ozone increases with the introduction of acceptor substituents. During ozonation of 1,3- and 1,4-dimethylbenzenes, 96% of the substrate is subject to destructive ozonation, while 1,2-dimethylbenzene and 1,3,5-trimethylbenzene are completely converted to ozonides. The stability of the aromatic ring increases with the introduction of acceptor

RESULTS AND DISCUSSION

To determine the possibility of cleaning surface water and soil from aromatic compounds, the kinetics of the liquid-phase ozonation reaction of methylbenzenes was investigated. As it turned out, the main products of toluene oxidation by ozone are ozonides – aliphatic products of aromatic ring destruction, which is also described in the work of R. Samsami *et al.* (2023). In trace amounts, benzyl alcohol and benzaldehyde, CO_2 accumulate as intermediate products, while benzoic acid is the final product of the aromatic reaction, the yield of which does not exceed 6% (Table 1).

substituents. When ozonising 4-nitrotoluene, the yield of 4-nitrobenzoic acid reaches 14.2%. Similar results were obtained during the oxidation of 3-nitrotoluene. In the case of the 2-isomer, the selectivity for the methyl group is significantly reduced, which is due to the steric effect of the substituent (Table 1).

The results of the study demonstrate that ozonation of methylbenzenes in the liquid phase can effectively neutralise toxic aromatic pollutants through complete or partial destruction of the benzene ring. Of particular importance is the ability of the formed ozonides and peroxides to be easily removed from the solution – these compounds precipitate or break down into volatile components, which greatly simplifies the purification of water and soil from reaction residues.

The values of the ozone absorption rate constants in reactions with methylbenzenes given in Table 2 indicate a satisfactory correlation between the reactivity of substituted toluenes (with the exception of hydroxy-,

amino- and mercapto-toluene) and the substituent constants (σ). The calculated ρ for this series is -2.25, which indicates an electrophilic mechanism for the reaction of ozone with methylbenzenes.

Table 1. Rate constants for the reaction of ozone with methylbenzenes at 293 K

Compound	$[O_3]_0 \cdot 10^4, \text{ mol} \cdot \text{l}^{-1}$	$[ArH]_0 \cdot 10^2, \text{ mol} \cdot \text{l}^{-1}$	$k_{\text{eff}}, \text{ l} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$	
			AcOH	Ac ₂ O
Toluene	0.28 ÷ 0.57	7.7 ÷ 28.3	0.80 ± 0.08	0.88 ± 0.08
1,2-Dimethylbenzene	0.18 ÷ 0.33	5.4 ÷ 7.3	0.88 ± 0.08	3.08 ± 0.30
1,3-Dimethylbenzene	0.18 ÷ 0.33	5.4 ÷ 7.3	4.15 ± 0.40	4.56 ± 0.50
1,4-Dimethylbenzene	0.18 ÷ 0.33	5.4 ÷ 7.3	5.00 ± 0.50	5.61 ± 0.50
1,3,4-Trimethylbenzene	0.18 ÷ 0.33	2.4 ÷ 5.3	29.60 ± 3.00	32.54 ± 3.00
1,3,5-Trimethylbenzene	0.18 ÷ 0.33	2.4 ÷ 5.3	45.10 ± 0.45	50.56 ± 5.00
4-Hydroxytoluene	0.24 ÷ 0.51	0.2 ÷ 0.6	2.22 · 10 ³	2.43 · 10 ³
4-Aminotoluene	0.14 ÷ 0.41	0.2 ÷ 0.6	2.42 · 10 ³	2.66 · 10 ³
4-Acetamidotoluene	0.18 ÷ 0.45	10.1 ÷ 25.9	0.81 ± 0.07	0.85 ± 0.05
4-Acetoxytoluene	0.28 ÷ 0.57	15.1 ÷ 35.9	0.59 ± 0.05	0.66 ± 0.06
4-Nitrotoluene	0.38 ÷ 0.55	25.5 ÷ 48.0	0.035 ± 0.004	0.083 ± 0.006
3,4-Dinitrotoluene	0.38 ÷ 0.55	25.5 ÷ 48.0	0.020 ± 0.002	0.022 ± 0.002
2,4-Dinitrotoluene	0.38 ÷ 0.55	25.5 ÷ 48.0	0.016 ± 0.002	0.018 ± 0.002

Source: developed by the author

A different kinetic picture is observed upon ozonation of methylbenzenes that have substituents with lone electron pairs (-OH, -NH₂, -SH). Firstly, under these conditions, oxidation via the methyl group does not occur, the main reaction products in the case of hydroxytoluenes are ozonides, and for aminotoluenes – ozonides, nitrotoluenes, and polymeric products. Secondly, their reactivity is three orders of magnitude higher ($k_{\text{eff}} = 2.22 \cdot 10^3 \text{ l} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$) than the reactivity of methylbenzenes ($k_{\text{eff}} = 0.8 \text{ l} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$). All of this is explained by the fact that ozone attacks not the methyl group or the aromatic ring, but primarily the lone pair of electrons of the heteroatom (Galstyan *et al.*, 2009). A change in the direction of ozone attack and its partial

redirection to the methyl group is achieved under the conditions of prior acylation of the hydroxy- and amino groups with acetic anhydride. The effective rate constant decreases by three orders of magnitude and approaches the value for methylbenzene (Table 2). The direction of the reaction becomes typical for the ozonation of methylbenzenes – via the double bonds of the aromatic ring and, to a lesser extent, via the methyl group with the formation of acetamido- and acetoxybenzoic acids (Table 3). Corresponding aromatic alcohols and aldehydes were identified as intermediate oxidation products. It is not possible to stop the process at the stage of their predominant formation, as is the case with methylbenzenes, under these conditions.

Table 3. Dependence of the selectivity of methylbenzene oxidation with ozone on the substrate structure at 293 K

Compound	Selectivity, %		
	Via the methyl group	Via the aromatic ring	Unidentified products
Toluene	5.9	80.3	3.7
2-Acetamidotoluene	5.1	92.9	2.0
3-Acetamidotoluene	14.2	84.7	1.1

Table 3, Continued

Compound	Selectivity, %		
	Via the methyl group	Via the aromatic ring	Unidentified products
4-Acetamidotoluene	15.5	83.5	1.0
2-Acetoxytoluene	7.5	90.5	2.0
3-Acetoxytoluene	17.3	80.5	2.2
4-Acetoxytoluene	19.8	78.6	1.6

Note: $[O_3]_0 = 4.7 \cdot 10^{-4}$; $[ArCH_3]_0 = 0.4 \text{ mol} \cdot \text{l}^{-1}$; $V_p = 0.01 \text{ l}$

Source: developed by the author

To clarify the chemical structure of ozonides – products of aromatic ring destruction – after oxidation under a vacuum of 5 mm Hg, the solvent was distilled off. The isolated compounds were oily, viscous, light yellow liquids, highly soluble in acetic acid and its anhydride, but poorly soluble in dichloroethane. They reacted with potassium iodide, but in different ways. Toluene, di- and trimethylbenzene peroxides interact with potassium iodide in two stages: the first being rapid, during which molecular iodine is released in an amount equivalent to one peroxide group, and the second being slow (≈ 24 hours) with the release of iodine in an amount equivalent to one or two more peroxide groups. These facts, as well as the stoichiometric coefficient for ozone ($n \approx 2-3$), indicate that the specified peroxides are formed through ozonides and are oligomers of linear structure (Galstyan *et al.*, 2009).

Peroxides of nitro-, acetoxy- and acetamidotoluene react with potassium iodide within 1 hour, and molecular iodine is released in an amount equivalent to one peroxide group, i.e. they have a monomeric structure (scheme). These data are consistent with the literature, according to which it is the hydroperoxide groups that easily react with potassium iodide, and the reduction of dialkyl peroxide groups is completed in $\approx 20-24$ hours (Potapenko *et al.*, 2021). Thus, depending on the nature of the substituents, peroxides can be monomers or oligomers, but regardless of their structure, they are easily removed from the solution, which contributes to the rapid purification of the agricultural system.

The results confirm the effectiveness of liquid-phase ozonation as a method for deep purification of natural waters and soils from alkyl aromatic pollutants. It has been established

that the selection of conditions (environment, temperature, type of substituents) allows the selectivity of the process to be regulated, directing it either towards the preservation of the aromatic ring (for synthetic purposes) or towards its destruction (for ecological neutralisation). The ability of the formed ozonides to precipitate provides a simple scheme for removing reaction products, which is especially valuable in water purification technologies without the need for complex filtration.

The results obtained indicate the effectiveness of liquid-phase ozonation as a method for the disposal of alkyl aromatic compounds in an aqueous environment, which is extremely important in terms of improving the environmental safety of agricultural systems. It has been established that the main direction of methylbenzene oxidation is the destruction of the benzene ring with the formation of ozonides, which is consistent with the results of R. Samsami *et al.* (2023), who described a similar destruction of aromatic rings under the action of ozone. The low yield of aromatic products, such as benzoic acid, indicates the predominance of complete cycle cleavage reactions over partial side chain oxidation.

A comparative analysis of substituted methylbenzenes demonstrated a significant influence of the electronic nature of substituents on the direction and depth of oxidation. The presence of acceptor groups, such as $-NO_2$ and $-SO_2Cl$, contributes to an increase in the stability of the aromatic ring and, accordingly, a decrease in side chain selectivity. This confirms the conclusions made earlier in the work of A. Galstyan *et al.* (2009), where attention was focused on the change in electron density in the ring as a key factor in the interaction with electrophilic ozone. The determined value of $\rho = -2.25$ further

confirms the electrophilic nature of the mechanism of interaction of ozone with methylbenzenes, which correlates well with the Hammett parameters of the substituents.

The observation of a change in the reaction mechanism in the presence of substituents with a free electron pair ($-OH$, $-NH_2$, $-SH$) deserves special attention. In such cases, the main target of attack is not the aromatic ring, but the heteroatom, which leads to a significant increase in reactivity and a change in the product composition. This opens up the potential for selective modification of the structure of aromatic pollutants prior to their oxidation, in particular by means of preliminary acylation, which “blocks” active groups and changes the course of the reaction. The decrease in the reaction rate after acylation also meets expectations and demonstrates the possibility of regulating the kinetic parameters of the process.

A separate place is occupied by the study of the composition of reaction products: the identification of oligomeric peroxides as destruction products for unsubstituted and weakly substituted arenes and monomeric hydroperoxides in the case of substituted derivatives is a significant contribution to the understanding of the ozonation mechanism. The degree of reaction with potassium iodide, the duration of iodine release, and the behaviour of peroxide compounds confirm the literature data of E. Potapenko & I. Isaenko (2023), which describe the two-stage nature of peroxide reduction depending on their structure.

Thus, the results of the study deepen the understanding of the mechanisms of liquid-phase ozonation of aromatic compounds, allow the classification of reaction products according to structural features, and form the scientific basis for the creation of effective technologies for the purification of water and soil from alkyl aromatic xenobiotics. The practical value is confirmed by the possibility of selective destruction of toxic components with the formation of products that are easily separated from the environment. Since the literature, such as the work of H. Einaga & X. Zheng (2024), focuses mainly on gas-phase ozonation or ultraviolet activation of ozone, the data obtained expand the understanding of the effectiveness of the liquid-phase approach without the need for complex installations.

CONCLUSIONS

Research has shown that liquid-phase ozonation of methylbenzenes is a highly effective method for the destruction of aromatic xenobiotics in the aquatic environment. The main transformation pathway is the destruction of the aromatic ring with the formation of unstable ozonides, which further decompose into aliphatic products. Side-chain oxidation was observed only in the case of substrates with electron-accepting substituents. It has been established that the electronic nature of substituents in the aromatic ring significantly influences the direction and depth of ozonation. Donor substituents promote ring destruction, while acceptor substituents, on the contrary, reduce the reactivity of the aromatic system, directing oxidation to the side methyl radical. The most pronounced selectivity for the formation of benzoic acid and its derivatives was observed for 4-nitrotoluene and 4-toluenesulphochloride. The rate constants of ozone absorption by substrates show a clear dependence on the Hammett parameter σ , with a correlation coefficient $\rho = -2.25$, which confirms the electrophilic nature of the interaction of ozone with arenes. It has been established that the reaction products are both monomeric and oligomeric peroxide structures, which depend on the nature of the substrate. Peroxides are precipitated from the reaction medium or easily extracted, which enables their effective removal from the aquatic or soil environment. The practical significance of the study lies in confirming the feasibility of using ozonation as a technology for cleaning agroecosystems. The method allows to neutralise residual amounts of herbicides and pesticides in irrigation water; reduce the content of aromatic xenobiotics in soils, increasing biological activity; reduce the toxicity of agrochemical effluents; and promote the creation of closed water systems in agriculture.

From a scientific point of view, the results indicate the possibility of controlled selection of ozonation products by changing the reaction conditions (temperature, ozone concentration, pH of the medium, type of solvent, etc.), which makes the method universal for implementation in environmentally safe technologies for the destruction of organic pollutants. Prospects

for further research include the development of scalable ozonation technologies for the treatment of agricultural wastewater; assessment of the impact of ozonation products on soil microbiota; investigation of combined destruction methods (ozonation + bioremediation/photolysis); and study of the toxicological profile of intermediate reaction products.

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Озонування як засіб підвищення екологічної безпеки агросистем: очищення ґрунтів і поливної води від толуену та його похідних

Анотація. Метою дослідження було оцінити ефективність рідиннофазного озонування метилбензенів як способу знешкодження ароматичних ксенобіотиків у поверхневих водах та ґрунтах агроєкосистем. У роботі використано спектрофотометричні, йодометричні, хроматографічні методи аналізу та елементний аналіз для вивчення динаміки руйнування ароматичного ядра, утворення проміжних та кінцевих продуктів, а також визначення реакційної здатності похідних аренів. Встановлено, що основним шляхом перетворення метилбензенів є озонолітичне розщеплення бензенового ядра з утворенням аліфатичних озонідів. Вихід бензойної кислоти не перевищував 5,8 %, що свідчить про незначну стабілізацію ароматичного фрагмента. Для *p*-ксилолу ступінь мінералізації сягав понад 70 % за 30 хв озонування. Наявність донорних замісників у бензеновому кільці ($-\text{CH}_3$, $-\text{OH}$, $-\text{NH}_2$) сприяло глибокій деструкції ядра, тоді як акцепторні ($-\text{NO}_2$, $-\text{COOH}$) зменшували його реакційну здатність, змінюючи напрямок реакції на боковий алкільний ланцюг. Похідні з функціональними групами, здатними до нуклеофільної атаки ($-\text{OH}$, $-\text{NH}_2$, $-\text{SH}$), характеризувались значно вищою швидкістю реакції (до 25 разів), порівняно з толуеном. Ацилювання таких груп оцтовим ангідридом знизило швидкість реакції та підвищило селективність утворення стабільних продуктів. Окрему увагу приділено складу та структурі продуктів реакції. Пероксиди, що утворились в результаті деструкції ароматичного ядра, виявили різну будову – від мономерних до олігомерних форм. Вони добре розчинились в оцтовій кислоті, однак легко осадились або видалились з розчину, що було ключовою перевагою процесу в застосуванні до очищення води та ґрунтів. Отримані дані засвідчили потенціал рідиннофазного озонування як ефективного інструменту для селективного та контрольованого знешкодження ароматичних сполук у ґрунтах та водах сільськогосподарського призначення. Отримані результати можуть бути використані екологами, агрохіміками та спеціалістами з очищення води для підвищення екологічної безпеки аграрних територій і зрошувальних систем, забруднених ароматичними сполуками

Ключові слова: озон; метилбензени; деструкція; пероксиди; очищення води; кінетика

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Biochemical composition and nutritional value of promising purple-leaved hazelnut (*Corylus avellana* L.) genotypes developed in Ukraine

Abstract. This study presented the biochemical composition and nutritional value of three promising hazelnut (*Corylus avellana* L.) genotypes with anthocyanin colouration: 'Profesorskyi', 'Aspirantskyi', and 'Akademichnyi', developed at the National University of Life and Environmental Sciences of Ukraine (NULES). This study aimed to compare the content of key nutrients, such as protein, sugars, and fats, as well as the fatty acid profile, in these genotypes. Nut samples from the 2023-2024 harvests, collected from the orchard of the Educational and Production Laboratory of Genetic Resources, Introduction and Breeding of Non-Traditional Fruit and Ornamental Plants at NULES, were analysed using standard methods (Kjeldahl for protein, Bertrand for sugars, Rushkovskiy for fatty acids, and FAME-GC for fatty acid profiling). All three genotypes exhibited high oil content (66.2%-68.2%) and a predominance of oleic acid (79.8%-80.8%). A high content of monounsaturated fatty acids (averaging 81.1%) and a low level of saturated fatty acids (averaging 9.2%), together with the presence of essential fatty acids, indicate a favourable lipid profile similar to that of traditional high-quality hazelnut cultivars. The protein content ranged from 11.7% to 12.8%, while total sugars varied between 1.6% and 1.9%, reflecting good nutritional balance.

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The homogeneity of the genotypes in terms of sugar, protein, and oil content can be attributed to their common genetic origin. The results highlighted the genetic potential of purple-leaved hazelnuts for food and nutraceutical applications. These genotypes may serve as a basis for further breeding programmes targeting both aesthetic and nutritional traits

Keywords: hazelnut oil; oleic acid; essential fatty acids; forest-steppe of Ukraine; nut crops

INTRODUCTION

The hazelnut (*Corylus avellana* L.) is one of the world's most important nut crops. It is highly valued for its nutritional profile and its versatility in food and industrial applications. Despite being native to Ukraine and providing significant ecological benefits, domestic hazelnut production falls short of national demand. This has led to growing interest in industrial hazelnut cultivation and the development of new, high-quality varieties. While traditional breeding has focused on green-leaved genotypes, recent research is exploring forms with anthocyanin colouration, which offer aesthetic appeal and promising nutritional characteristics. Understanding the biochemical composition of these purple-leaved hazelnuts is crucial for advancing breeding programmes, expanding their use in the food and nutraceutical industries, and ultimately enhancing Ukraine's domestic supply.

The hazelnut (*Corylus avellana* L.), native to the forest zone of Ukraine, grows naturally in the understorey of oak-birch and oak-hornbeam forests (Moroz & Terletsyky, 2023). According to V. Sliusarchuk (2006), *Corylus avellana* performs protective functions in steppe forestry; in phytomelioration plantations, it protects the soil from water erosion and improves the hydrological regime and soil fertility. As a pioneer species, it is valuable for forest restoration and succession.

In horticulture, hazelnuts are among the five most important nut crops, alongside cashews, walnuts, almonds, and chestnuts. Hazelnuts are highly nutritious: they are consumed fresh, dried, or roasted and are widely used in cooking and the confectionery industry. The oil extracted from the kernels is important for both nutrition and technology and is also used in painting and perfumery (Goluch *et al.*, 2019; Motti *et al.*, 2022). B. Olas (2024) notes that nut consumption plays an important role in

preventing cardiovascular disease, one of the leading health challenges facing modern society. A. Allegrini *et al.* (2022) regard hazelnuts as a multipurpose crop that enhances the sustainability of agro-ecosystems. However, Ukraine's domestic supply does not meet national demand, with consumption per person per year reaching only 21.5% of the optimal nutritional standard (Weisfeld *et al.*, 2018). This situation could be improved by increasing domestic production. In recent years, there has been a significant increase in interest in industrial hazelnut cultivation in Ukraine (Mezhenskyj, 2022). Researchers have pointed out considerable variations in the economic traits and biochemical composition of hazelnuts, depending on the geographical region in which they are grown and their varietal composition. The industrial hazelnut varieties cultivated internationally are largely based on typical green-leaved genotypes. However, in Europe, genotypes with anthocyanin colouration of organs – particularly leaves and fruit husks – have been identified, giving the plants a distinctive decorative effect (Holstein *et al.*, 2018). These characteristics have led to the incorporation of redpigmented mutants in the development of new varieties with exceptional ornamental qualities in Europe and North America (Johnson & Moore, 2023). These varieties have gained considerable popularity, yet the use of purple-leaved hazelnuts for nut production has remained limited. More recently, they have been introduced into breeding programmes aimed at improving hazelnuts as a nut crop (Kosenko *et al.*, 2023). In 2021–2022, the State Register of Varieties of Ukraine included two purpleleaved hazelnut varieties for the first time (Ministry..., n.d.). Similar forms promising for nut production have also been developed at the National University of Life and Environmental Sciences of Ukraine (NULES). This study

aimed to analyse the biochemical composition of nuts from the most promising purple-leaved hazelnut genotypes developed at NULES.

MATERIALS AND METHODS

Orchard disposition and plant materials. The experimental study was conducted in the collection orchard of the Educational, Research and Productive Laboratory of Genetic Resources, Introduction and Breeding of Rare Fruits and Ornamental Plants of Prof. V.L. Symyrenko Department of Horticulture of NULES, located at the Agronomic Research Station in Pshenychny Village, Bila Tserkva District, Kyiv Region. The region is part of the forest-steppe natural zone. According to the Köppen climate classification scheme, the area is characterised by a typical warm-summer humid continental climate. The soil is meadow chernozem with artificial irrigation. The three best selections of *Corylus avellana* L., named 'Profesorskyi', 'Aspirantskyi', and 'Akademichnyi', were used. The adult, multistemmed shrubs were grown at a planting distance of 5×4 m, and standard cultural practices were applied, including inter-row cultivation and mechanical weed control, with the exception of irrigation. The study was conducted in accordance with the ethical standards of the Convention on Biological Diversity (1992), which ensures the conservation and sustainable use of biological diversity, as well as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1973). The ripe nuts were harvested in mid-August, once they had begun to separate easily from the husk.

Determination of protein content. Determination of nitrogen content was carried out by digesting organic matter with sulphuric acid in the presence of a catalyst, according to the Kjeldahl method. All the nitrogen released in this process is converted into ammonium sulphate (NH₄)₂SO₄. In an alkaline medium, this compound releases ammonia, which is distilled into a receiving flask containing boric acid via steam distillation. The final measurement involves titration with a standard acid solution, which changes colour at the end point. The nitrogen content is determined by the amount of ammonia produced. The crude protein content is calculated by multiplying the measured nitrogen value by the corresponding coefficient

(K=1.754). The determination was carried out using an automatic distillation unit, Kjeltec 8200 TM Foss (Methodology..., 2023).

Determination of sugar content. The Bertrand method for the quantitative determination of sugar content is based on the ability of reducing sugars – those with a free aldehyde or ketone group – to reduce copper(II) sulphate to copper(II) oxide in an alkaline medium. The resulting copper oxide precipitate is then dissolved in iron sulphate in the presence of sulphuric acid. Copper oxide is oxidised by iron, reducing it to copper oxide, which is subsequently oxidised by potassium permanganate solution. This method can also be used to determine the content of disaccharides, but only after hydrolysis with diluted mineral acids. However, the amount of sugar in the solution is not directly proportional to the mass of copper oxide precipitate formed, as some decomposition of Fehling's solution occurs upon heating (with the formation of copper oxide), and some monosaccharides decompose due to the alkalinity of Fehling's solution. Therefore, Bertrand's tables, which show the relationship between the mass of precipitate, copper oxide, and glucose concentration in the solution, are compiled empirically. Deviations from the recommended methodological conditions (temperature, heating duration, solution concentration, degree of alkalinity, etc.) may lead to inaccuracies in the results (Methodology..., 2023).

Determination of fatty acid content. The Rushkovskyi method for assessing kernel oil content is based on measuring the oil remaining in the defatted residue. Samples of dried, ground nuts were placed in filter paper bags and extracted with diethyl ether in a Soxhlet apparatus until complete defatting was achieved. The extracted fat content was calculated by subtracting the mass of the defatted residue from the initial sample mass (Methodology..., 2023).

Determination of the fatty acid composition of oil. The Fatty Acid Methyl Ester (FAME) GC method involves converting fatty acid triglycerides into methyl esters, followed by gas chromatographic analysis using a Nexis GC-2030 Shimadzu gas chromatograph (DSTU ISO 5509-2002, 2003).

Statistical analyses. Experimental data were expressed as mean ± standard deviation. The effects of genotype, harvest year, and their

interaction on fatty acid composition were analysed using ANOVA. When a significant main effect of genotype or a significant interaction effect was detected ($p < 0.05$), Tukey's HSD post-hoc test was performed to identify specific differences between means. All statistical analyses were conducted using SPSS software (Version 17.0). The non-parametric Kruskal-Wallis test was also applied to determine the statistical significance of differences in fatty acid content between hazelnut varieties. This method was chosen due to the small sample size (two years for each variety) and the inability to reliably verify the assumptions of normal distribution and homogeneity of variance, which are prerequisites for parametric tests.

RESULTS AND DISCUSSION

The studied genotypes were characterised by large nuts with a high kernel content, comparable to that of registered purple-leaved hazelnut

cultivars. The nut weights of 'Profesorskyi', 'Aspirantskyi', and 'Akademichnyi' were 3.0-3.4 g, 2.7-2.8 g, and 2.3-2.6 g, respectively. Their corresponding kernel percentages were 46.7%-49.3%, 49.4%-52.6%, and 51.3%-52.2% (Mezhenskij *et al.*, 2025). The first purple-leaved varieties, 'Barbakan BSI' and 'Bahrianyi', listed in the State Register of Varieties of Ukraine, had nut weights of 4.6 g and 2.4 g and kernel percentages of 35.0% and 47.0%, respectively (Vishtak, 2021; Vishtak, 2022). The seeds of fruit crops differ significantly in biochemical composition from the pulp, containing high levels of protein and oil; therefore, they are particularly valuable in human nutrition. The nuts of the studied purple-leaved hazelnut genotypes contained substantial amounts of essential nutrients that determine their nutraceutical value: sugars (1.6%-1.9%), protein (11.7%-12.8%), and oil (66.2%-68.2%) (Table 1).

Table 1. Oil, protein, and carbohydrate content of nuts, average 2023-2024 ($x \pm SD$)

Content	'Profesorskyi'	'Aspirantskyi'	'Akademichnyi'
Oil, %	68.15 \pm 0.919	66.15 \pm 1.626	67.75 \pm 0.636
Crude protein, %	12.25 \pm 0.778	11.70 \pm 0.566	12.85 \pm 1.202
Total sugar, %	1.82 \pm 0.240	1.86 \pm 0.287	1.64 \pm 0.190

Source: compiled by the authors

These results are consistent with previous studies indicating the limits of variation in protein, carbohydrate, and kernel oil content (Rondanelli *et al.*, 2003; Bignami *et al.*, 2005; Król & Gantner, 2020). However, the biochemical composition is also influenced by varietal characteristics and growing conditions. While the protein content of the studied genotypes (11.7%-12.8%) falls within the average range reported for *Corylus avellana* cultivars globally (7.0%-24.6% according to K. Król & Gantner, 2020), it is slightly lower than that of the recently registered purple-leaved Ukrainian cultivars 'Barbakan BSI' (17.0%) and 'Bahrianyi' (14.8%) (Vishtak, 2021; Vishtak, 2022).

This difference is offset by the exceptionally high oil content observed in 'Profesorskyi', 'Aspirantskyi', and 'Akademichnyi', which averaged 66.2%-68.2%. This range not only aligns with but often surpasses the upper limits reported for many commercial hazelnut varieties cultivated

across Europe, Asia, and North America (Król & Gantner, 2020), which typically contain 56.3%-67.5% oil. Notably, these genotypes exhibit oil levels comparable to, or even exceeding, those of highly prized Italian cultivars such as 'Nocchione', 'Tonda Gentile Langhe', 'Tonda Gentile Romana', and 'Tonda di Giffoni', which accumulate 66.3%-67.2% oil under Italian conditions (Bignami *et al.*, 2005). At the same time, according to A. Müller *et al.* (2020), 'Tonda di Giffoni' contains 62.7% oil under German conditions. The superior oil content of these genotypes, especially when compared with other purple-leaved varieties, underscores their significant potential for industrial processing and use in high-value products. The registered purple-leaved cultivars 'Barbakan BSI' and 'Bahrianyi' contain lower amounts of oil: 63.0% and 54.0%, respectively (Vishtak, 2021; Vishtak, 2022). The European purple-leaved 'Red Lambert' contains 64.8% oil (Müller *et al.*, 2020).

The sugar content of the kernels of the studied genotypes is approximately half that of the Italian varieties mentioned above, which accumulate 3.9%-4.8% under Italian conditions (Bignami *et al.*, 2005). The lower sugar content of the studied genotypes may be related not only to varietal characteristics but also to differences in soil and climatic conditions, as well as to the methodology applied: in this case, the Bertrand method. In contrast, Italian researchers determined sugar content using the more accurate gasliquid chromatography method. On the other hand, lower sugar content is advantageous for individuals with diabetes. The genotypes studied are relatively homogeneous in terms of their nutraceutical content, which can be explained by their shared genetic origin (Mezhenskyj *et al.*, 2025). Nevertheless, due to small sample sizes and limited replication in biochemical analyses, the study has certain constraints. These

factors may reduce the statistical power of the tests and affect the interpretation of the results. When analysing mean data over two years, no significant differences in sugar, protein, or oil content were observed. However, a significant interaction was observed between genotypes and years. In 2023, 'Aspirantskyi' had a statistically higher sugar content than 'Akademichnyi' and 'Profesorskyi', while 'Akademichnyi' and 'Profesorskyi' did not differ significantly from each other. In 2024, however, 'Profesorskyi' had a statistically higher sugar content than 'Akademichnyi' and 'Aspirantskyi', whereas 'Akademichnyi' and 'Aspirantskyi' again did not differ significantly. Thus, the interaction between genotype and year is statistically significant, meaning that the effect of genotype on sugar content varies depending on the year, and vice versa. The qualitative composition of hazelnut oil in the studied genotypes is shown in Table 2.

Table 2. Fatty acid composition of hazelnut oil (%), average 2023-2024 ($\bar{x} \pm SD$)

Acid		'Profesorskyi'	'Aspirantskyi'	'Akademichnyi'
Myristic	C14:0	0.033 ± 0.001	0.020 ± 0.002	0.0275 ± 0.005
Palmitic	C16:0	6.592 ± 0.226	6.072 ± 0.486	5.350 ± 0.086
Palmitoleic	C16:1	0.402 ± 0.008	0.347 ± 0.080	0.262 ± 0.001
Stearic	C18:0	2.735 ± 0.086	3.190 ± 0.165	2.836 ± 0.057
Oleic	C18:1	79.816 ± 0.358	80.759 ± 1.281	80.413 ± 0.404
Linoleic	C18:2	9.784 ± 0.035	8.038 ± 0.816	10.230 ± 0.660
Linolenic	C18:3	0.152 ± 0.007	0.263 ± 0.201	0.278 ± 0.110
Arachidic	C20:0	0.133 ± 0.016	0.154 ± 0.012	0.145 ± 0.040
Gondoic	C20:1	0.145 ± 0.014	0.281 ± 0.236	0.189 ± 0.025
Eicosadienoic	C20:2	0.001 ± 0.0b	0.006 ± 0.001a	0.002 ± 0.001ab
Behenic	C22:0	0.087 ± 0.010b	0.145 ± 0.020a	0.111 ± 0.005ab
Erucic	C22:1	0.004 ± 0.006	0.571 ± 0.805	0.001 ± 0.001
Docosadienoic	C22:2	0.001 ± 0.001	0.001 ± 0.000	0.001 ± 0.001
Nervonic	C24:1	0.005 ± 0.004	0.004 ± 0.005	0.001 ± 0.001

Note: different letters indicate values that are significantly different within a row, according to the results of Tukey's test ($P \leq 0.05$)

Source: compiled by the authors

The main fatty acids present in the oil of the studied genotypes are oleic acid (79.8%-80.8%), linoleic acid (8.0%-10.2%), palmitic acid (5.4%-6.6%), and stearic acid (2.7%-3.2%), while other fatty acids occur only in small amounts. When the genotypes are compared with one another and with the average values across two years, no statistically significant differences are observed.

This similarity is attributable to their common genetic origin. The results are consistent with the typical order of fatty acid contribution in hazelnuts: oleic acid > linoleic acid > palmitic acid > stearic acid > linolenic acid. As reported in previous studies, oleic acid remains the predominant fatty acid (Król & Gantner, 2020; Gardeli *et al.*, 2025).

Over the two-year period, the average proportions of oleic acid in the kernels of 'Professorskiy', 'Aspirantskiy', and 'Akademichnyi' were 79.8%, 80.8%, and 80.4%, respectively. These levels are similar to those of popular Italian varieties, whose oil contains between 80.7% and 82.1% oleic acid under Italian conditions (Big-nami *et al.*, 2005). Among the 15 European varieties studied, the Italian cultivar 'Tonda di Giffoni' had the highest content (81.7%) under German conditions, while the purple-leaved 'Red Lambert' had a moderate content (80.3%) (Müller *et al.*, 2020). P. Benitez-Sanchez *et al.* (2003) and C. Gardeli *et al.* (2025) point out the similarity between hazelnut oil and olive oil, both of which are characterised by a high oleic acid content when different vegetable oils are compared. Its high similarity means that hazelnut oil can even be used to adulterate extra virgin olive oil (Ordoudi *et al.*, 2022; Pereira *et al.*, 2023).

A high oleic acid concentration is known to lead to a longer shelf life and greater oxidative stability (Król & Gantner, 2020; Sun *et al.*, 2022). On average, the proportion of monounsaturated fatty acids (MUFAs), including oleic acid, in the oil of the studied genotypes is 81.1%; polyunsaturated fatty acids (PUFAs) account for 9.6%, and saturated fatty acids for 9.2%. The low level of saturated fatty acids in hazelnut kernels compared with other food sources is advantageous (Sun *et al.*, 2022; Rondanelli *et al.*, 2023). According to E. Whitney & S. Rolfes (2007), only two fatty acids are essential for humans: α -linolenic acid (ω -3) and linoleic acid (ω -6). The content of these polyunsaturated fatty acids in the kernel oil of the studied genotypes is 0.15%-0.28% and 8.0%-10.2%, respectively. Linoleic acid ranks second after oleic acid, which highlights the valuable composition of the oil from these genotypes.

ANOVA of the kernel oil composition showed that, for the majority of fatty acids, there were no statistically significant differences between the genotypes or harvest years. This suggests a high degree of homogeneity in the fatty acid profiles of the studied genotypes, as well as stability across the two growing seasons for most components. This is likely due to their common genetic origin. However, statistically significant findings were observed for eicosadienoic acid and behenic acid. Both the main effect of genotype and the

interaction effect between genotype and year were statistically significant. This interaction indicates that differences in acid content between genotypes varied depending on the harvest year. 'Aspirantskiy' had a significantly higher average content of both acids compared with 'Professorskiy'. 'Akademichnyi' did not differ significantly from the other two genotypes. It is also noteworthy that, although the main genotype effects were not statistically significant ($p > 0.05$), gondoic acid and erucic acid exhibited highly significant interaction effects between genotype and year. This implies that, although these genotypes may not differ significantly in their overall average content of these fatty acids across both years, their performance or accumulation patterns for these acids were strongly influenced by the environmental conditions of the individual harvest year. Further in-depth analysis would be beneficial to fully elucidate these genotype responses dependent on the harvest year. In summary, the statistical analysis confirms the overall genetic uniformity of the developed hazelnut genotypes with regard to their major fatty acid profiles; however, specific fatty acid compositions can be modulated by environmental conditions. This is consistent with the findings of R. Gianferri *et al.* (2023), who demonstrated that the chemical composition of hazelnuts is significantly influenced by the pedoclimatic conditions of the growing site and the variety.

In the Kruskal-Wallis test, no statistically significant differences were found between the genotypes at the p -value threshold of < 0.05 for any of the fatty acids. Despite the absence of statistically significant differences confirmed by the Kruskal-Wallis test and Dunn's post-hoc comparison for most fatty acids in the limited dataset, some genotypes demonstrated notable compositional trends that may be biologically relevant. For instance, oleic acid was consistently the predominant fatty acid across all three cultivars, contributing over 79.8% to the total oil content. The lack of significant differences underscores its stability and suggests that oleic acid content is a robust varietal trait. Linoleic acid varied among genotypes, with 'Akademichnyi' exhibiting the highest average content ($10.230\% \pm 0.660\%$) and 'Aspirantskiy' the lowest ($8.038\% \pm 0.816\%$). Although the difference was

not statistically significant, it may influence oxidative stability and nutritional value. Linolenic acid, an omega-3 fatty acid, showed higher average levels in 'Akademichniy' ($0.278\% \pm 0.110\%$) and 'Aspirantskiy' ($0.263\% \pm 0.201\%$), which may enhance their nutritional profile. Despite the lack of statistical significance, these differences may be biologically or technologically important, suggesting the need for further studies with larger sample sizes.

CONCLUSIONS

The hazelnut genotypes 'Profesorskiy', 'Aspirantskiy', and 'Akademichniy', developed at the NULES, were found to have a high content of essential nutrients, indicating their high nutritional value and suitability for dietary supplementation. The protein content ranged from 11.3% to 13.7%, the sugar content from 1.5% to 2.1%, and the oil content from 65.0% to 68.8%. Notably, the oil content in these genotypes is particularly high, with oleic acid (79.6%-81.7%) being the predominant fatty acid. This is a characteristic feature of high-quality vegetable oils, such as olive oil. The high content of monounsaturated fatty acids (averaging 81.1%) and the low level of saturated fatty acids (averaging 9.2%) highlight the health benefits of these nuts, particularly in the prevention of cardiovascular disease. The presence

of linoleic and α -linolenic acids, the two main essential fatty acids, indicates the oil's valuable composition. Despite some fluctuations in biochemical composition over the years of cultivation, the studied genotypes show relative homogeneity, which is explained by their common genetic origin. A comparison with existing registered hazelnut varieties (in particular the registered red-leaved cultivars 'Barbacan BSI' and 'Bahrianyi' and Italian cultivars used in industrial plantations in Ukraine shows that the NULES of Ukraine genotypes have competitive or even superior performance in terms of key biochemical parameters, especially oil content. This suggests significant potential for increasing the industrial production of hazelnuts in Ukraine, which could reduce dependence on imports and boost nut consumption among the population. Further research could focus on other bioactive compounds in these genotypes to better assess their value.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Біохімічний склад і харчова цінність перспективних пурпуроволисткових генотипів фундука (*Coryllus avellana* L.) дібраних в Україні

Анотація. Дослідження представило біохімічний склад та харчову цінність трьох перспективних генотипів фундука (*Coryllus avellana* L.) з антоціановим забарвленням: 'Професорський', 'Аспірантський' та 'Академічний', створених у Національному університеті біоресурсів і природокористування України (НУБіП України). Дослідження мало на меті порівняти вміст ключових поживних речовин, таких як білки, цукри та жири, а також профіль жирних кислот у цих генотипів. Зразки горіхів урожаю 2023-2024 років, зібрані з саду Навчально-виробничої лабораторії генетичних ресурсів, інтродукції та селекції нетрадиційних плодкових та декоративних рослин НУБіП України, аналізували стандартними методами (К'ельдала для білків, Бертрана для цукрів, Рушковського для жирних кислот та FAME-GC для профілювання жирних кислот). Усі три генотипи показали високий вміст олії (65,0-68,8 %) та переважання олеїнової кислоти (від 79,8 % до 80,8 %). Високий вміст мононенасичених жирних кислот (в середньому 81,1 %) і низький рівень насичених жирних кислот (в середньому 9,2 %) та наявність незамінних жирних кислот свідчить про сприятливий ліпідний профіль, подібний до профілю традиційних високоякісних сортів ліщини. Вміст білка коливався від 11,7 % до 12,8 %, тоді як загальний вміст цукрів становив від 1,6 % до 1,9 %, демонструючи хороший харчовий баланс. Однорідність генотипів за вмістом цукру, білка та олії пояснюється їхнім спільним генетичним походженням. Результати підкреслили генетичний потенціал пурпурнолисткових ліщин для харчових та нутрицевтичних застосувань. Ці генотипи можуть слугувати основою для подальших селекційних програм, спрямованих як на естетичні, так і на харчові ознаки

Ключові слова: фундукова олія; олеїнова кислота; незамінні жирні кислоти; лісостеп України; горіхоплідні культури

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Nitrogen-fixing and phosphate-mobilising bacteria improve photosynthetic activity and productivity of buckwheat (*Fagopyrum esculentum* Moench)

Abstract. One effective way to increase the yield of agricultural crops is pre-sowing seed treatment with nitrogen-fixing and phosphate-mobilising bacteria. The specific features of productivity formation in buckwheat restrict the planned yield and the realisation of varietal potential, leading to an increase in vegetative growth indicators at the early stages of growth and development. The research was conducted on three buckwheat cultivars during 2022-2023 under the conditions of the forest-steppe zone of Ukraine. This study aimed to determine the effect of seed treatment on the content of photosynthetic pigments, biomass accumulation, and leaf area index in buckwheat during the generative period of development. It was found that the influence on biomass growth from mid-flowering to fruit formation was limited, though different trends in the formation of photosynthetic pigments in leaves were observed. The biopreparations significantly increased the content of photosynthetic pigments at the budding stage (BBCH 51), mainly due to chlorophyll a, while at the flowering stage (BBCH 65), phosphate-mobilising complexes were most effective. It was established that seed treatment with nitrogen-fixing bacteria enabled buckwheat to form a leaf area index of 5.27-5.48 at the fruit formation stage, which was significantly higher compared with the control (4.19). At the same time, biomass accumulation during the generative period changed insignificantly across the seed treatment options, but morphological changes were noted that contributed to higher yields and, consequently, greater productivity. It was established that pre-sowing seed treatment has a significant effect on biometric indicators and yield, though the varietal factor often manifests itself, indicating the prospects for further research into evaluating the formation of the buckwheat generative apparatus under the influence of microbiological preparations

Keywords: chlorophyll content; dry matter; leaf area index; net photosynthetic productivity; yield

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INTRODUCTION

Global food security has long been under the scrutiny of many governments and international organisations (Kuchechuk, 2022). This is driven by population growth, which requires ever-increasing production of agricultural products – products that must meet the standards of environmental safety and the quality of food and feed (Shuvar *et al.*, 2019). One approach to addressing this issue is the development of technologies for growing crops on an organic basis. Organic production is gradually becoming more popular in Ukraine (Kotykova & Ten, 2018). Even those farms that previously engaged exclusively in non-organic crop production and traditionally used mineral fertilisers and pesticides are now showing interest in organic crop production (Kunsook *et al.*, 2024; Hudzovskyi *et al.*, 2024). The basis of organic production is weed control (in which the observance of crop rotations is of great importance, because herbicides are not used), plant nutrition, and storage of finished products.

Since the use of chemicals is prohibited in the organic cultivation of crops, the introduction of biological preparations based on beneficial microorganisms with different mechanisms of action into crop-growing technologies is gaining popularity, including in buckwheat production (Almashova & Skok, 2022). As noted by X. Wei *et al.* (2024), their use in crop production technologies helps reduce the application rates of mineral fertilisers, increase plant productivity, and improve product quality. The optimal combination of plant life factors contributes to the formation of ecological equilibrium in agrobiocenoses and affects both the yield and quality of agricultural products. Therefore, it is important to use measures aimed at increasing the number and activity of agronomically valuable microorganisms in the rhizosphere of plant root systems.

Ukraine grows approximately half of all organic buckwheat in the world. Growing interest in the production of organic buckwheat from both agricultural producers and the state makes this area highly promising (Vieites-Álvarez *et al.*, 2024b). As buckwheat is cultivated organically, the use of readily available synthetic fertilisers is prohibited. According to U. Karbivska *et al.* (2024), to increase the yield of organic

buckwheat, it is advisable to apply fertilisers of organic origin. Therefore, the study of the use of organic preparations in buckwheat seed cultivation technologies where mineral fertilisers are not used is relevant. The use of such preparations in buckwheat cultivation has been shown to improve the phytosanitary condition of crops. Seed treatment and spraying of buckwheat crops contributed to enhanced plant growth, greater increased branching, and greater vegetative mass (Honchar *et al.*, 2020). As established by Y. Vieites-Álvarez *et al.* (2024a), the competitiveness of buckwheat plants against weeds throughout the growing season also increased.

The value of buckwheat is determined by its exceptional characteristics, both as an agricultural crop – including its role as a precursor, soil improver, and component of organic cultivation technology – and as a source of raw materials obtained from cultivation: grain (for processing into cereals), biologically active substances, honey, and waste-free processing technology (Breslauer *et al.*, 2023). Despite these positive qualities, buckwheat remains a niche crop. Producer interest is also positively influenced by new varieties with yields exceeding 2.5-3.0 t/ha, capable of realising this potential in diverse environmental conditions. One of the main priorities in introducing buckwheat to modern agrocenoses is the waste-free nature of its production technology and its versatile use, which must be taken into account when calculating the economic efficiency of buckwheat production.

A key issue in modern buckwheat cultivation is increasing its grain productivity. It is known that the biomass potential it synthesises is sufficient to form a yield of 4-7 t/ha of seed if it were cultivated as a grain crop. However, the biological characteristics of buckwheat limit its productivity, so the conversion of photosynthetic activity into seed biomass faces several constraints. According to research results, yield increases often occur extensively – through biomass growth. Therefore, studying the production processes of crops during the generative period of development will enable the prediction and selection of an effective cultivation strategy. Previous research has shown that bio-preparations containing PGPM influence stress adaptation in

buckwheat and induce plant modifications in other crops by improving nutrient availability and synthesising phytohormones in the rhizosphere. A comprehensive assessment of plant growth processes and photosynthetic activity under the influence of rhizosphere modification factors using bio-preparations is the core of the present research.

This research aimed to improve certain elements of buckwheat cultivation technology under the conditions of the forest-steppe zone and to determine the influence of the growing season's weather conditions, the content of photosynthetic pigments, biomass accumulation, and the leaf area index on achieving the highest crop productivity. The objectives of the study included determining the growth and development characteristics of buckwheat plants depending on the variety and pre-sowing treatment with biological preparations, and investigating the dynamics of external architectural development of buckwheat plants at different growth stages (according to the BBCH scale) under the influence of the studied factors. For the first time, the peculiarities of yield formation in buckwheat varieties under the influence of growing-season weather conditions in the Forest-Steppe zone were identified. The influence of biological preparations

on the photosynthetic activity and productivity of buckwheat plants was established, and the technology of buckwheat cultivation in the forest-steppe zone was optimised.

MATERIALS AND METHODS

The field experiment was conducted in 2022-2023 in the Educational-Scientific Laboratory Demonstration Collection Field of Crops of the Department of Plant Science (Kyiv, Ukraine; 50° 22' N, 30° 30' E). The investigation was performed under field conditions. The soil of the experimental field was grey forest light loam, with 2.3%-3.0% humus content in the arable layer (0-20 cm), pH_{KOH} 5.8-6.1, hydrolysed nitrogen content 73 ± 6 mg per kg of soil, phosphorus 103 ± 17 mg per kg of soil, and potassium 78 ± 12 mg per kg of soil. The study complied with the principles of the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1973).

Climate conditions. The weather conditions during the research years and the buckwheat growing season were contrasting, allowing for a comprehensive assessment of the effects of the preparations. The primary difference between the years was precipitation, which is crucial for the activity of microbiota in the rhizosphere and for biomass growth (Table 1).

Table 1. Weather conditions during field trials in 2022-2023

Parameter	Year	Month					Average per vegetation
		April	May	June	July	August	
Air temperature, °C	2022	8.1	14.6	21.7	20.8	22.3	17.5
	2023	9.6	16.0	19.6	21.5	23.8	18.1
	MA*	10.0	15.8	19.5	21.3	20.4	17.4
Monthly precipitation, mm	2022	26	20	46	34	80	206
	2023	102	1	87	136	19	345
	MA*	42	65	74	68	56	305

Note: MA* – multi-annual parameter based on data from 1990 to 2015

Source: compiled by the authors from Meteopost (n.d.)

The average daily temperature during the buckwheat growing season in 2022 was 17.5°C (longterm average: 17.4°C), whereas 2023 was hotter, with an average daily temperature of 18.1°C. The air temperature at the time of sowing in May 2022 was 15.1°C, while in 2023 it was 16.4°C. May 2023 had a variable temperature regime: at the

beginning of the month, the temperature was optimal for buckwheat sowing, fluctuating between 15°C-19°C, but on 6 May there was a sharp drop to 5.6°C. Later, the temperature increased, exceeding 25°C by 18 May, and there was no precipitation throughout the month. The year 2023 was marked by the most variable temperature indicators and precipitation

sums, with frequent sharp fluctuations between day and night, affecting buckwheat plants. Precipitation was also uneven, with both absence (in May) and excess (in July), which hindered the ripening of buckwheat fruits.

Field trial design and cultivation conditions. Cultivar sensitivity to pre-sowing treatment with free-living bacteria was established

in a two-factor field experiment. The first factor comprised three buckwheat (*Fagopyrum esculentum* Moench) cultivars: 'Volodar', 'Podilska', and 'Kamyanchanka'. The second factor was the treatment of buckwheat seeds before sowing with preparations based on microorganisms – a diazotroph complex and a phosphate-mobilising bacteria complex (Table 2).

Table 2. Experimental scheme

Cultivar	Seed treatment before sowing
C1. 'Volodar' C2. 'Podilska' C3. 'Kamyanchanka'	B1. Without treatment B2. Diazotroph complex, 1 L/t of seed B3. Phosphate-mobilising complex, 1 L/t of seed

Source: compiled by the authors

The diazotroph complex was based on free-living nitrogen-fixing bacteria: *Azotobacter chroococcum*, *Azotobacter vinelandii*, and associative nitrogen-fixing bacteria *Azospirillum brasilense*, *Azospirillum lipoferum* (1 mL contained 1×10^9 colony-forming units (CFU)). The phosphate-mobilising complex consisted of spores of *Bacillus megaterium*, *Bacillus amyloliquefaciens*, and micromycetes *Trichoderma harzianum* (1 mL contained 1×10^9 CFU).

The experiment was conducted in four replications. The size of the elementary plots was 36 m² (24 m² for harvesting). The tillage system included disking after the preceding crop (winter wheat) and autumn ploughing to a depth of 18–20 cm. Disking was carried out in early spring, followed by presowing cultivation to a depth of 3–4 cm before sowing. The fertilisation regime included the application of Ecosoil organic fertiliser at a rate of 250 kg/ha (P₂O₅ – 5.0%, K₂O – 20.0%, S – 3.5%, Ca – 8%, Mg – 5.5%, Fe – 1340 mg/kg, Zn – 300 mg/kg, Cu – 240 mg/kg, Mn – 200 mg/kg, Mo – 1.5 mg/kg, Co – 0.37 mg/kg), applied before autumn ploughing. Buckwheat was sown with 15 cm interrow spacing at a rate of 300 seeds/m². Sowing time (first ten-day period of May) depended on soil temperature (optimum 10°C–12°C). No pesticides were applied during the growing season.

Plant sampling procedure. Plant samples were collected at five stages: BBCH 51 (inflorescence emergence), BBCH 65 (50% of inflorescences flowering), BBCH 67 (70% of

inflorescences flowering), and BBCH 71 (beginning of fruit filling). Samples were taken from an area of 0.5 m², and biometric parameters were analysed on ten plants (n=10) from each variant. The chlorophyll content was determined in the upper, fully developed leaf at the BBCH 51 and BBCH 65 stages. Fresh samples were collected and extracted in 96% ethanol. Optical density was measured using a UNICO 1201 spectrophotometer to determine the content of chlorophyll *a* and *b* in the solution (wavelengths 665 nm and 649 nm). These values were then recalculated per unit dry matter of leaf tissue. Chlorophyll concentration was determined using the following equations:

$$Cl_a = 13.7 D_{665} - 5.76 D_{649}, \quad (1)$$

$$Cl_b = 25.8 D_{649} - 7.6 D_{665}, \quad (2)$$

where Cl_a – chlorophyll *a* content, mg/L; Cl_b – chlorophyll *b* content, mg/L; D_{665} – optical density at 665 nm; D_{649} – optical density at 649 nm.

The leaf area index (LAI) was determined by scanning all leaf blades from ten plants and converting the results to 1 m² based on actual plant density. Dry matter accumulation (g/m²) was assessed by evaluating the increase in dry biomass at each stage compared with the previous stage. Biomass was dried at 105°C to a constant moisture content.

Statistics. Statistical analysis was performed using Statistica version 13.0 (Tibco, USA). ANOVA was applied to each studied parameter and included the influence of weather

conditions. Tukey's HSD test at a 95% confidence level was used to assess differences between variants. Bars in the figures represent the standard error (\pm SE).

RESULTS AND DISCUSSION

The content of photosynthetic pigments in buckwheat leaves varied significantly depending on the developmental stage (Table 3). Notably, seed treatment was a determining factor in pigment variation at the BBCH 51 stage, indicating qualitative modifications to the photosynthetic apparatus under the influence of these factors.

The chlorophyll *a* content varied significantly across all factors, but the influence of the year and variety was significantly less than that of seed treatment. The chlorophyll *b* content also varied depending on seed treatment and variety, but was not directly sensitive to annual conditions. The variation in the interaction between seed treatment and variety or annual conditions was greater than the influence of the variety itself. The total content of photosynthetic pigments in the BBCH 51 stage varied due to the direct effect of the factors and their interaction, except for the "year \leftrightarrow treatment" interaction.

Table 3. ANOVA for photosynthetic pigments in leaves at BBCH 51 and BBCH 65

Effect	MS					
	Cl _a ₅₁	Cl _b ₅₁	Cl _{a+b} ₅₁	Cl _a ₆₅	Cl _b ₆₅	Cl _{a+b} ₆₅
Cultivar (C)	1.56**	0.1435**	2.48*	45.73**	7.2176**	75.36**
Treatment (T)	105.53**	2.7426**	83.32**	31.20**	2.8293**	48.00**
Year (Y)	11.05**	0.0022ns	11.36**	6.91**	0.0013ns	7.09**
C×T	8.41**	0.4360**	9.17**	2.05**	0.9335**	3.61**
C×Y	2.50**	0.0193ns	2.10*	1.48*	0.0029ns	1.35*
T×Y	1.89*	0.6793**	0.98ns	5.77**	0.6329**	10.18**
C×T×Y	1.84**	0.0326*	2.05*	1.86**	0.1483**	2.70**
Error	0.32	0.0089	0.42	0.19	0.0065	0.26

Note: ns – not significant ($p > 0.05$); ** – $p < 0.001$; * – $p < 0.05$; Codes: Cl_a – chlorophyll *a*, Cl_b – chlorophyll *b*, "51" – BBCH 51 stage (inflorescence emergence), "65" – BBCH 65 stage (50% of inflorescences flowering)

Source: compiled by the authors

The situation with photosynthetic pigments changed substantially in the flowering stage (BBCH 65). During this phase, the formation of new leaves ceased, so varietal characteristics became the primary factor. The cultivar accounted for the greatest variation in the content of chlorophyll *a*, chlorophyll *b*, and their total. The second most significant factor was seed treatment, while the influence of annual conditions was lower and, in the case of chlorophyll *b*, insignificant.

The dynamics of the leaf area index also varied significantly depending on the developmental stage (Table 4). It is noteworthy that the direct factors (cultivar, treatment, weather conditions/year) always had a significant impact, but their interactions were only significant at certain developmental stages. Seed treatment and weather conditions produced greater LAI variation in the BBCH 51 stage than in the cultivar. All factor interactions, except for "cultivar \leftrightarrow treatment", were insignificant.

Table 4. ANOVA for leaf area index (LAI) and dry matter accumulation (DMA) during the generative period of buckwheat development

Effect	MS						
	LAI ₅₁	LAI ₆₅	LAI ₆₇	LAI ₇₁	DMA ₅₁₋₆₅	DMA ₆₅₋₆₇	DMA ₆₇₋₇₁
Cultivar (C)	0.5884**	5.8742**	7.719**	3.342**	4,877.1**	2,491.8**	2,491.8**
Treatment (T)	2.3207**	10.3486**	6.032**	11.572**	950.4**	284.8ns	284.8ns
Year (Y)	1.6928**	5.6224**	12.997**	16.589**	26,075.7**	35,161.1**	35,161.1**
C×T	0.2287**	0.9888**	0.930**	0.156*	261.6**	373.7ns	373.7ns
C×Y	0.0030ns	0.0713*	0.053ns	0.007ns	2,106.8**	1,065.6*	1,065.6*

Table 4, Continued

Effect	MS						
	LAI ₅₁	LAI ₆₅	LAI ₆₇	LAI ₇₁	DMA ₅₁₋₆₅	DMA ₆₅₋₆₇	DMA ₆₇₋₇₁
T×Y	0.0075ns	0.0501*	0.017ns	0.018ns	313.5**	586.9*	586.9*
C×T×Y	0.0070ns	0.0116ns	0.090*	0.076ns	331.5**	295.3ns	295.3ns
Error	0.0036	0.0106	0.033	0.033	37.0	181.6	181.6

Note: ns – not significant ($p > 0.05$); ** – $p < 0.001$; * – $p < 0.05$; Codes: LAI – leaf area index, DMA – dry matter accumulation, “51” – BBCH 51 stage (inflorescence emergence), “65” – BBCH 65 stage (50% of inflorescences flowering), “67” – BBCH 67 stage (70% of inflorescences flowering)

Source: compiled by the authors

LAI variation during the BBCH 65 stage was determined by the main factors and their pairwise interactions (with the exception of the interaction of all three factors simultaneously). In the subsequent developmental stages (BBCH 67), weather conditions became the primary factor, and the variation attributable to seed treatment in relation to the cultivar increased up to and including the BBCH 71 stage. The formation of dry biomass during interphase periods differed significantly from that of the leaf apparatus. The fertilisation factor affected the variation in dry mass growth only between BBCH 51 and BBCH 65, while in subsequent periods it was insignificant. This pattern was also observed for the “cultivar ↔ seed treatment” interaction. Year-specific conditions caused the greatest variation in dry matter accumulation during the interphase periods. Although cultivar and interactions were significant in most cases, they were less influential compared with year-specific conditions.

The content of photosynthetic pigments in leaves during the BBCH 51 phase (Fig. 1a) varied significantly according to seed treatment, allowing identification of the best options based on the total chlorophyll content. ‘Kamyanchanka’ produced significantly fewer photosynthetic pigments (14.8 mg/g DM) without seed treatment with microbial preparations, compared with other cultivars (16.1-17.2 mg/g DM). However, the application of microbial preparations increased the content to 19.519.6 mg/g DM, with no significant difference between the preparations. A similar situation was observed in ‘Podilska’, while the application of a phosphate-mobilising complex (PMC) in ‘Volodar’ showed a significant difference from the control treatment. It is worth noting that ‘Volodar’ had a high pigment content in the control treatment, so the effect of the preparations was lower, indicating a maximum content limit for these indicators.

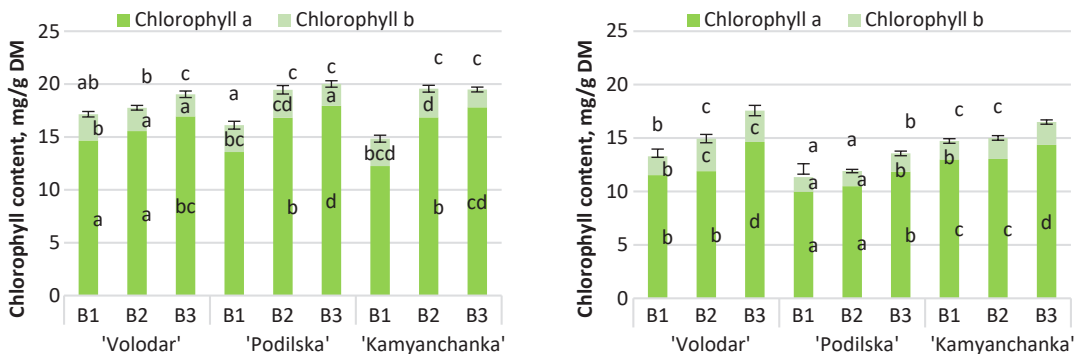


Figure 1. The content of photosynthetic pigments in buckwheat leaves, mg/g dry matter at BBCH 51 (left) and BBCH 65 (right)

Note: same letters indicate no difference between variants at a given stage according to Tukey’s HSD post-hoc test; bars indicate standard error (\pm SE)

Source: compiled by the authors

The situation with pigment content changed during the BBCH 65 phase, as the highest concentration was observed in variants treated with the phosphate-mobilising complex (17.6 mg/g DM). In contrast, treatment with a diazotroph complex showed a significant difference from the control only in 'Volodar'. This indicates the importance of improving phosphate nutrition during the generative period and the reduced role of nitrogen. The content of chlorophyll *a* increased markedly with the application of PMC, while the content of chlorophyll *b* rose significantly with the application of both preparations. The increase in chlorophyll *b* content with seed treatment was greatest in the 'Volodar'.

The positive effect of improved trophic conditions is manifested in two ways – through increasing the linear dimensions of individual organs and increasing the intensity of green colouration due to the higher concentration of photosynthetic pigments (Honchar *et al.*, 2021). The results of the current study indicate that the content of chlorophyll *a* and *b* increases significantly both in the early generative period and in the middle of flowering. Previous studies, such as that by V. Karpenko *et al.* (2021), have noted that a significant increase often occurs with the combined application of seed treatment with biostimulants, which primarily leads to an increase in pigments in fresh mass, while the increase in dry mass can be more modest. In the studies by the authors of this article, it seldom exceeds 20% for chlorophyll *a*, but it can nearly double for chlorophyll *b*. According to M. Chen (2014), this phenomenon is related to the specifics of chlorophyll *b* synthesis, which

can compensate for this factor when crops are grown under stressful conditions. Despite the substantial existing knowledge base about the impact of PGPM on agricultural crops, there remains a significant gap in the family *Polygonaceae*. For example, a meta-analysis of research on the relationship between nitrogen nutrition and microbiota in 'Tartary' buckwheat indicates that microbiota activity in the rhizosphere does not significantly change in the presence of fertilisers. Seed treatment of buckwheat with certain bacterial strains can improve indicators that affect NDVI, such as leaf area, chlorophyll concentration, and colour intensity (Witkiewicz *et al.*, 2021). However, a plausible theory suggests an increase in soil microorganisms due to crop rotation and the introduction of a qualitatively new microbiota (Zhang *et al.*, 2021).

Buckwheat is a plant with limited potential for leaf surface growth. The maximum leaf area index (LAI) depends on the cultivar characteristics and type of variety. Indeterminate cultivars are more flexible in this regard, but there is a biological limitation that makes buckwheat less productive than cereal crops. It has been established that seed treatment positively affects the LAI of buckwheat (Table 5). In the control variant during the BBCH 51 phase, the cultivars did not significantly differ from each other, whereas with treatment, there was a significant increase in LAI. 'Kamyanchanka' formed the maximum LAI of 2.05 ± 0.06 , with no significant difference between the treatments, while the other cultivars had significantly lower values. For the 'Volodar' and 'Podilska', the phosphate-mobilising complex was more effective than the diazotroph one.

Table 5. Leaf area index of buckwheat at BBCH 51, 65, 67, 71

Cultivar	Treatment			Average
	B1	B2	B3	
BBCH 51				
'Volodar'	1.31 ± 0.06 a	1.59 ± 0.06	1.78 ± 0.07 b	1.56 ± 0.05
'Podilska'	1.22 ± 0.04 a	1.45 ± 0.05	1.70 ± 0.07 b	1.46 ± 0.05
'Kamyanchanka'	1.21 ± 0.07 a	2.05 ± 0.06 c	2.04 ± 0.07 c	1.77 ± 0.09
Average	1.25 ± 0.03	1.70 ± 0.06	1.84 ± 0.05	1.59 ± 0.04
BBCH 65				
'Volodar'	2.33 ± 0.10 ab	3.83 ± 0.13 cd	3.98 ± 0.16 d	3.38 ± 0.17
'Podilska'	2.02 ± 0.07	2.76 ± 0.11	2.47 ± 0.10 b	2.41 ± 0.08
'Kamyanchanka'	2.27 ± 0.09 a	3.24 ± 0.11	3.76 ± 0.11 c	3.09 ± 0.14
Average	2.21 ± 0.06	3.27 ± 0.11	3.40 ± 0.16	2.96 ± 0.09

Table 5, Continued

Cultivar	Treatment			Average
	B1	B2	B3	
BBCH 67				
'Volodar'	3.89 ± 0.19	4.60 ± 0.17 a	4.61 ± 0.20 a	4.37 ± 0.12
'Podilska'	3.11 ± 0.10	3.59 ± 0.14	4.73 ± 0.20 a	3.81 ± 0.17
'Kamyanchanka'	4.54 ± 0.21 a	5.08 ± 0.18 b	5.20 ± 0.16 b	4.94 ± 0.12
Average	3.85 ± 0.16	4.42 ± 0.16	4.85 ± 0.12	4.37 ± 0.10
BBCH 71				
'Volodar'	4.11 ± 0.20 a	5.00 ± 0.17 b	5.10 ± 0.21 bc	4.74 ± 0.14
'Podilska'	3.94 ± 0.13 a	5.05 ± 0.19 b	5.37 ± 0.23 c	4.79 ± 0.17
'Kamyanchanka'	4.50 ± 0.20	5.76 ± 0.20 d	5.97 ± 0.16 d	5.41 ± 0.17
Average	4.19 ± 0.11	5.27 ± 0.13	5.48 ± 0.14	4.98 ± 0.10

Note: B1 – without treatment, B2 – diazotroph complex, B3 – phosphate-mobilising complex. Same letters indicate no difference between variants at a given stage according to Tukey's HSD post-hoc test

Source: compiled by the authors

LAI in the BBCH 65 phase differed notably from the previous observation. The maximum value of 3.98 ± 0.16 was observed for the 'Volodar', when treated with the phosphate-mobilising complex, but there was no significant difference compared with the diazotroph complex. The LAI in the 'Podilska' increased markedly less than in the other cultivars following seed treatment, with the maximum value observed when treated with the diazotroph complex. LAI in the 'Kamyanchanka' reached a maximum value when treated with the phosphate-mobilising complex (3.76 ± 0.11), which was also significantly higher than that recorded under treatment with diazotrophs (3.24 ± 0.11).

In the next phase (BBCH 67), the difference between the control and treated variants decreased. The maximum LAI value was recorded for the 'Kamyanchanka' (5.20 ± 0.16) when treated with phosphate-mobilising bacteria, with no significant difference from seed treatment with diazotrophs. A similar pattern was observed in 'Volodar', although with a lower LAI (4.61 ± 0.20). For

'Podilska', seed treatment with phosphate-mobilising bacteria resulted in a significantly higher LAI (4.73 ± 0.20) than with other treatments and the control. In the last accounting period (BBCH 71), the maximum LAI was again recorded for the 'Kamyanchanka' (5.97 ± 0.16). In the control variant, the LAI in this cultivar remained almost unchanged compared with the previous period, while it increased in the other cultivars. LAI increased to 5.00-5.37 in both cultivars in the treated variants, but phosphate-mobilising bacteria were more effective in the 'Podilska'.

A significant increase in the leaf apparatus in cereal crops enhances assimilation processes, but in the case of buckwheat, which has indeterminate growth, these processes occur differently. The average biomass increase from BBCH 51 to BBCH 71 was $220 \pm 12 \text{ g/m}^2$, but phase-by-phase distribution indicates that from the beginning of budding to the middle of flowering, more dry mass was accumulated than during the end of flowering and final fruit formation (Table 6).

Table 6. Dry matter accumulation (g/m^2) of buckwheat during BBCH 51-65, 65-67, 67-71

Cultivar	Treatment	Period			BBCH 51-71
		BBCH 51-65	BBCH 65-67	BBCH 67-71	
'Volodar'	B1	101.8 ± 10.8 c	63.6 ± 9.1 bc	68.7 ± 10.9 ab	234 ± 29 cde
	B2	111.9 ± 7.9	68.1 ± 13.5 c	63.3 ± 7.2 a	243 ± 25 de
	B3	123.5 ± 15.7	52.6 ± 9.7 abc	71.3 ± 7.9 ab	247 ± 29 e
'Podilska'	B1	89.0 ± 7.2 ab	35.5 ± 4.8 a	67.7 ± 10.3 ab	192 ± 18
	B2	98.6 ± 4.6 bc	46.7 ± 8.2 abc	80.5 ± 7.7 ab	226 ± 17 abc
	B3	94.7 ± 4.6 bc	44.2 ± 8.2 ab	91.9 ± 12.4 b	231 ± 22 bcd

Table 6, Continued

Cultivar	Treatment	Period			BBCH 51-71
		BBCH 51-65	BBCH 65-67	BBCH 67-71	
'Kamyanchanka'	B1	81.5 ± 6.5 a	52.3 ± 11.4 abc	77.6 ± 6.7 ab	211 ± 22 a
	B2	79.6 ± 5.1 a	57.1 ± 12.5 abc	81.3 ± 7.7 ab	218 ± 22 ab
	B3	91.8 ± 4.9 b	63.2 ± 9.1 bc	76.9 ± 7.2 ab	232 ± 18 bcd
Average		96.9 ± 3.0	53.7 ± 3.3	75.5 ± 2.9	220 ± 12

Note: B1 – without treatment, B2 – diazotroph complex, B3 – phosphate-mobilising complex. Same letters indicate no difference between variants in the column according to Tukey's HSD post-hoc test

Source: compiled by the authors

It should be noted that 'Volodar' produced a significantly greater amount of dry matter (101.8-123.5 g/m²) during the period BBCH 51-65 compared with other cultivars. Seed treatment with the phosphate-mobilising complex resulted in a significantly higher increase than with the diazotroph complex and the control variant. The difference between the variants was insignificant for the 'Podilska', while the phosphate-mobilising complex showed significant effectiveness in the 'Kamyanchanka'. As mentioned earlier in the ANOVA section, the seed treatment factor did not have a significant impact on the accumulation of dry biomass during BBCH 65-67 and BBCH 67-71, so individual variation was observed only among cultivars. 'Volodar' accumulated dry matter more intensively during BBCH 65-67 than the others, but the situation reversed during BBCH 67-71.

The results of this research confirm the well-known thesis regarding the effectiveness of the combined application of diazotrophs from different genera, manifested in increased plant biomass, yield, and individual productivity (Singh *et al.*, 2015; Mazurenko *et al.*, 2020). It should be noted that diazotrophs respond differently to weather conditions; therefore, the effect of their application may depend on available moisture, temperature, nutrient reserves, and varietal sensitivity. As noted by Y. Tao *et al.* (2004), diazotrophs can also participate in the decomposition of organic matter, thereby improving trophic interactions in the buckwheat rhizosphere. In their pot experiment, S. Tummaramatti *et al.* (2016) found that seed treatment complexes containing *Azotobacter chroococcum*, *Azospirillum brasilense*, *Trichoderma harzianum*, and mycorrhiza fully realise the potential of plants in biomass accumulation and the formation of maximum leaf parameters. In the present study, a similar complex proved highly effective, resulting in growth increases of several tens of per cent compared with the control.

It should be noted that buckwheat productivity is limited; thus, biopreparations act as stimulants, with the productivity potential constrained by the nutrients available in the soil (Oljača *et al.*, 2012; Dykyi *et al.*, 2022). Photosynthetic activity in the post-anthesis period shows a significantly higher correlation with yield, as biomass is transferred to the fruits. For this reason, larger biomass usually leads to higher yield levels (Ehdaie *et al.*, 2008; Tang *et al.*, 2017). The obtained results for leaf area index and dry matter accumulation are also consistent with existing knowledge and confirm the importance of the post-anthesis period, when optimal conditions for plant productivity must be ensured (Guglielmini *et al.*, 2019). This underscores that the biostimulating effect of plant growth-promoting microorganisms is as important as supplying plants with nutrients.

CONCLUSIONS

In this study, the influence and interrelationship between the content of photosynthetic pigments and the leaf area index (LAI) of buckwheat plants under pre-sowing seed treatment were identified. Seed treatment with phosphate-mobilising and diazotrophic complexes enhanced vegetative indices and buckwheat productivity. The pigment content in buckwheat leaves largely depended on and varied under the influence of the pre-sowing seed treatment compared with the weather factor at the BBCH 51 stage. At the BBCH 65 stage, the varietal factor assumed significant importance in determining chlorophyll concentration relative to other studied factors. A variable trend in the formation of photosynthetic pigments in leaves was observed. Both preparations significantly increased the content of photosynthetic pigments during the budding phase (BBCH 51), mainly due to chlorophyll *a*, but only phosphatmobilising complexes were generally effective during the flowering phase (BBCH 65).

The leaf area index depended on the developmental stage of buckwheat and the studied factors. Seed treatment and weather conditions produced greater variation in LAI at the BBCH 51 stage than the varietal factor. The pre-sowing seed treatment factor had no significant effect on the leaf area index overall. The increase in indices does not have universal species-level significance, as the varietal factor influences all parameters. The application of biopreparations significantly increased the leaf area index and dry biomass accumulation until mid-flowering. However, the impact on biomass growth from midflowering to fruit formation was not statistically significant.

Despite previous studies on the use of biopreparations in buckwheat cultivation technology, no published results have examined their effect on chlorophyll content, leaf area index, and dry biomass accumulation at different growth and development stages. The present findings confirm the positive influence of pre-sowing

buckwheat seed treatment on chlorophyll concentration, leaf area index, and dry biomass accumulation. These conclusions are particularly relevant for improving buckwheat cultivation technology under organic production systems using biopreparations. Further research should assess the impact of microbiological preparations on the generative development of buckwheat, including the formation of floral organs and subsequent seed yield components.

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Фотосинтетична активність та продуктивність гречки (*Fagopyrum esculentum* Moench) за дії азотфіксуючих та фосфатмобілізуючих бактерій

Анотація. Одним з ефективних способів підвищення урожайності сільськогосподарських культур є передпосівна обробка насіння азотфіксуючими та фосфатмобілізуючими бактеріями. Специфічні властивості формування продуктивності гречки обмежують планову врожайність та реалізацію сортового потенціалу, обумовлюючи зростання вегетативних показників на початкових етапах росту та розвитку. Дослідження проводилися на трьох сортах гречки протягом 2022-2023 рр. в умовах лісостепу України. Метою досліджень було встановити вплив обробки насіння гречки на вміст фотосинтетичних пігментів, накопичення біомаси, індекс листової площі в генеративний період розвитку та врожайність. Встановлено вплив на ріст біомаси від середини цвітіння до формування плодів, який був незначним, але відзначено різну тенденцію формування фотосинтетичних пігментів у листі. Біопрепарати значно підвищили вміст фотосинтетичних пігментів у фазі бутонізації (ВВСН 51), головним чином за рахунок хлорофілу *a*, але у фазу цвітіння (ВВСН 65) у більшості випадків ефективні були тільки фосфатмобілізуючі комплекси. Встановлено, що обробка насіння азотфіксуючими бактеріями дала змогу сформувати у гречки у фазу утворення плодів індекс листової площі 5,27-5,48, що достовірно вище порівняно з контролем (4,19). При цьому накопичення біомаси в генеративний період змінювалось несуттєво за варіантів обробки насіння, але виникали морфологічні зміни, що сприяли формуванню більш високого показника врожаю, а отже, і врожайності. Встановлено, що передпосівна обробка насіння суттєво впливає на біометричні показники та врожайність, але нерідко проявляється сортовий чинник, що свідчить про перспективу подальших досліджень у напрямі оцінки формування генеративного апарату гречки під впливом мікробіологічних препаратів

Ключові слова: вміст хлорофілу; суха маса; листовий індекс; чиста продуктивність фотосинтезу; урожайність

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Effects of phosphorus rich organic manure (PROM) on growth and yield of soybean in calcareous soil

Abstract. The phosphorus fixation and its availability are a major challenge not only in alkaline or calcareous soil but also in acidic soil. The stress of nutrient availability from soil is a major challenge for more phosphorus demanding leguminous crops like soybean. Using organic manure and rock phosphate in presence of phosphorus solubilising bacteria (PSB) plays a vital role not only by enhancing phosphorus availability but also by reducing stress of other nutrients too. The purpose of this study was to evaluate the impact of various phosphorus sources, including organic and inorganic inputs, on the growth parameters and productivity of soybean cultivated in calcareous soil conditions. The study found that applying 75% or 100% P_2O_5 through phosphorus rich organic manure or 100% via vermicompost significantly improved soybean growth, with larger leaves and plant height at days 28 and 56 after sowing. Phosphorus rich organic manure amendment for 100% P_2O_5 also resulted in the largest leaf area per plant (1,657.26 cm^2), leaf area index (7.37), and total chlorophyll content (1.23 $mg\ g^{-1}$). Additionally, 100% P_2O_5 through phosphorus rich organic manure led to significantly more pods per plant (46.33), seeds per pod (3.00), and seeds per plant (150.27). Subsequently, significantly higher grain (25.94 $q\ ha^{-1}$) and straw (35.16 $q\ ha^{-1}$) yield of soybean was achieved with the application of 100% P_2O_5 via phosphorus rich organic manure and which was followed by incorporation of 75% P_2O_5 via phosphorus rich organic manure or 100% P_2O_5 through vermicompost. Hence this could be a sustainable way of producing P-rich organic manure to

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improve the growth and yield attributes of soybean. The findings of this study can be beneficial to soil scientists, agronomist, farmers and agricultural enterprises working involved in legume production and other phosphorus demanding crops

Keywords: phosphorus organic sources; phosphorus inorganic sources; diammonium phosphate; single super phosphate; vermicompost

INTRODUCTION

Phosphorus is a major nutrient involved in energy translocation, root development, and flower initiation. It is taken up by plants in two forms – HPO_4^{2-} and H_2PO_4^- . Phosphorus affects plant metabolic processes like photosynthesis, translocation, transpiration, and storage of energy in the form of adenosine triphosphate and adenosine diphosphate.

Phosphorus (P) availability in Indian soils is limited, particularly in alkaline and acidic soils, where P fixation occurs. Only 15-20% of applied P is used by crops in the first year, with the rest contributing to the residual P pool (Nakeertha et al., 2023). Different forms of aluminium, iron, magnesium, and calcium bind the available P, which makes it unavailable to plants. P deficiency is most prevalent in soil types that are characterised by high levels of withering, calcareousness, and alkalinity. The economic and ecological viability of attempts at reclamation through the utilisation of phosphatic fertilisers is questionable, considering that the efficiency of these fertilisers when incorporated is remarkably poor. In highly calcareous soils, phosphorus availability is further restricted due to the development of insoluble calcium phosphate compounds, which reduce the nutrient's uptake by plants. The process of phosphate solubilisation, which is accompanied by the production of organic acids and acid phosphatases, is of paramount importance in the mineralisation of organic phosphorus in soil. The utilisation of rock phosphate, in conjunction with manure and phosphate-solubilising bacteria, has been demonstrated to ensure consistent performance. The application of these bacteria has been shown to enhance plant growth-promoting rhizobacteria (PGPR) activities, thereby strengthening resistance against disease pathogens. Phosphate solubilising bacteria like *Bacillus subtilis* and *Bacillus megaterium* play a crucial role in solubilising

the insoluble forms of phosphorus into soluble or plant available form (Khan et al., 2021). Recent studies by K. Sadvakasov & Ye. Islamov (2023) also highlighted the potential of *Bacillus subtilis* in microbial biotechnology, particularly its effective growth on alternative microbial substrates, which supports its application in sustainable agriculture.

Phosphorus-rich organic manure (PROM) offers a sustainable solution for enhancing the P use efficiency (PUE) in calcareous soils. PROM is created by enriching compost or farmyard manure (FYM) with rock phosphate and inoculating it with phosphate-solubilising microorganisms. The gradual mineralisation of organic P provides a slow-release, plant-available source of phosphorus. The Ministry of Agriculture (India) approved the use of PROM, recognising its potential to improve soil fertility and nutrient uptake in challenging soil conditions (Measures..., 2024). Recent studies suggest that applying PROM can markedly increase soybean growth and yield. Application of NPKM (30% nitrogen, phosphorus, and potassium + 70% manure) resulted in greater rice yields, PUE, P uptake, and overall balance (Hayatu et al., 2023). M. Ahmad et al. (2022) concluded that addition of 90 kg of P_2O_5 as single super phosphate or 100% acidulated rock phosphate was found superior for wheat yield and phosphorus nutrition compared to other mineral sources and control.

PROM is also gaining attention due to its ability to address P lockout issues in both alkaline and calcareous soils, making it a promising approach for improving crop productivity and soil health. Overall, the use of PROM, integrated with other nutrient management practices, can markedly improve phosphorus availability and soybean yields in highly calcareous soils. An investigation was performed to assess the growth and yield of soybean grown in highly calcareous

with application various sources of phosphorus. K. Noor *et al.* (2020) also concluded that the application of integrated use of organic manures resulted in a significant increase in yield components and maize yield, attributable to the incorporation of inorganic phosphorus sources, such as rock phosphate. The release of phosphorus from rock phosphate was found to be greater in combination with manure.

Therefore, the purpose of the present study was to explore the comparative effectiveness of PROM, vermicompost, and inorganic phosphorus sources in improving phosphorus availability, enhancing plant physiological traits, and increasing the productivity of soybean under the constraints of highly calcareous soil conditions.

MATERIALS AND METHODS

The experiment was conducted during kharif-2023 on alkaline (pH 8.18) and highly calcareous soil (12.47% CaCO₃) at the Post Graduate Research Farm, College of Agriculture, Pune. The soil, classified as Inceptisol (*Vertic Haplustepts* family), was medium-deep black, 70-80 cm deep, and dominated by smectite clay. Initial soil testing revealed a low organic carbon content (0.62%), with available nitrogen, phosphorus, and

potassium levels at 113.50 kg ha⁻¹, 10.79 kg ha⁻¹, and 536 kg ha⁻¹, respectively. The diethylene tri-amine penta acetic acid (DTPA)-extractable micronutrients were also measured, showing 1.18 mg kg⁻¹ Fe, 3.08 mg kg⁻¹ Mn, 2.28 mg kg⁻¹ Zn, and 2.24 mg kg⁻¹ Cu. PROM was prepared by using partially decomposed compost of soybean waste at 1,000 kg in high density polyethylene bags. Subsequently, phosphorus solubilising bacteria in liquid form at 2 lit t⁻¹ of partially decomposed compost was thoroughly mixed and allowed to incubate for 72 hours (Plate-1). Rock phosphate in powder form (size < 0.5mm) at 250 kg per tonne of partially decomposed compost of soybean waste was thoroughly mixed. Total 5-6 turnings at an interval of 15 days were carried out and moisture level was maintained at 40-45%. After 75-80 days, phosphorus rich organic manure was found ready and representative sampling was performed accordingly to assess nutrient status. Vermicompost was also prepared by using partially decomposed compost of soybean straw along with *Eisenia fetida* earthworm species in high-density polyethylene polybags. The composite sample of vermicompost and FYM were analysed for nutrient status (Table 1).

Table 1. Proximate analysis of PROM, FYM, and vermicompost

Seq. No.	Parameter	Unit	PROM	FYM	Vermicompost
1	pH (1: 10)	–	7.18	7.49	6.91
2	EC	(dSm ⁻¹)	1.74	1.66	2.12
3	Moisture	(%)	24.02	23.5	25.45
4	Organic Carbon	(%)	21.06	22.26	30.79
5	Total N	(%)	0.78	0.69	1.48
6	Total P	(%)	14.57	0.39	0.79
7	Total K	(%)	0.37	0.38	0.81
8	Total Fe	(ppm)	10,550	186	388
9	Total Mn	(ppm)	600	35.7	65.7
10	Total Zn	(ppm)	125	14.8	18.3
11	Total Cu	(ppm)	30	4.39	15.2
12	Total Ca	%	13.63	–	–
13	Total Mg	%	0.52	–	–
14	Total B	(ppm)	30	–	–
15	C:N ratio	–	24:1	39:1	20:1
16	C:P ratio	–	1.44:1	57.07:1	38.97:1

Source: compiled by the authors

The soybean variety 'KDS-726' (*Phule Sangam*) was used as the investigated crop for the experiment. PROM was formulated at the Vermicompost Yard, Division of Soil Science, College of Agriculture, Pune. The recommended fertiliser dose (50:75:45 kg ha⁻¹ of N, P₂O₅, and K₂O) was applied, except for the control plots. Phosphorus was provided from various sources, including PROM and vermicompost as organic sources, and Diammonium Phosphate (DAP) and Single Super Phosphate (SSP) as inorganic sources. Nitrogen and potassium were supplied through urea and muriate of potash, respectively. PROM, FYM, and vermicompost were applied at the time of sowing, and their proximate analysis was conducted before the experiment (Table 1).

P-rich organic manure and vermicompost were analysed for total nitrogen (N) by H₂SO₄ along with digestion mixture (K₂SO₄:CuSO₄ in 10:1 ratio) using macro-Kjeldhal method (Herlich, 1990), while phosphorus (P), potassium and other micronutrients were estimated by digesting 1 g dry manure sample with 10 mL tri-acid mixture (9:3:1 HNO₃:HClO₄: H₂SO₄) at 180-200°C. The P content in the acid digest was determined by means of the vanadomolybdate-phosphate yellow colour method, utilising a spectrophotometer. Meanwhile, the K content was determined by means of a flame photometer. A randomised block design (RBD) with analysis of variance (ANOVA) was employed to assess treatment effects on all studied characteristics (Panse & Sukhatme, 1985). All experimental procedures involving plants were conducted following institutional, national, and international ethical standards. No endangered or protected plant species were used in the study (Statement..., 2025).

RESULTS AND DISCUSSIONS

Effects of various sources and levels of phosphorus on growth of soybean in calcareous soil

The findings showed that periodical number of leaves and plant height (28 and 56 days after sowing (DAS)) of soybean grown in calcareous soil were significantly influenced by phosphorus application through PROM, vermicompost, and phosphatic fertilisers (Table 2, Figs. 1-2). The count of leaves on soybean plants at 28 and 56 DAS, as influenced by phosphorus application via PROM, vermicompost, and phosphatic fertilisers, ranged within 4.81-5.80 and 13.58-19.80, respectively. However, the plant height ranged within 14.40-19.80 cm on 28 DAS and 45.87-53.20 cm on 56 DAS. Application of 100% P₂O₅ through PROM (5.80), 75% P₂O₅ through PROM (5.80), 100% P₂O₅ through vermicompost (5.60), 100% P₂O₅ through DAP (5.47), and 50% P₂O₅ through PROM (5.47) were found significantly superior over 100% P₂O₅ through SSP (5.20), RDF (5.13), and control (4.81) on 28 DAS. Furthermore, these superior treatments were found to be statistically on par with each other. Significantly greater number of leaves (19.80) on 56 DAS was reported with application of 100% P₂O₅ through PROM, which was found to be statistically on par with rest of treatments except absolute control (13.58). Addition of 100% P₂O₅ through PROM yielded significantly greater (19.80 cm) plant height, which was found to be statistically on par with all the treatments under study except RDF and control. However, remaining treatments comprising phosphorus application either at 50% or 75% or yet 100% through either PROM, DAP, SSP, or vermicompost yielded statistically on-par results in terms of plant height of soybean. Analogous findings for plant height were reported on 56 DAS for soybean.

Table 2. Effects of phosphorus through PROM and phosphatic fertilisers on morphological features of soybean in calcareous soil

Seq. No.	Treatment	Count of leaves		Plant height (cm)	
		28 DAS	56 DAS	28 DAS	56 DAS
1	Absolute Control	4.81	13.58	14.40	45.87
2	RDF (NPK)	5.13	16.20	18.07	46.00
3	50% P ₂ O ₅ : PROM	5.47	18.33	18.87	47.20
4	75% P ₂ O ₅ : PROM	5.80	19.47	19.60	51.00
5	100% P ₂ O ₅ : PROM	5.80	19.80	19.80	53.20

Table 2, Continued

Seq. No.	Treatment	Count of leaves		Plant height (cm)	
		28 DAS	56 DAS	28 DAS	56 DAS
6	100% P ₂ O ₅ : DAP	5.47	18.60	19.40	47.93
7	100% P ₂ O ₅ : SSP	5.20	17.33	18.40	46.07
8	100% P ₂ O ₅ : Vermicompost	5.60	18.93	19.53	48.87
	SE+	0.15	1.01	0.22	1.95
	CD at 5%	0.44	3.05	0.67	5.91

Note: SE: Standard error, CD at 5% : Critical difference at 5% significance level, Recommended dose of fertilisers (RDF), (N – Nitrogen, P – Phosphorus, and K – Potassium), P-rich organic manure (PROM), Diammonium phosphate (DAP), Single super phosphate (SSP), Days after sowing (DAS)

Source: compiled by the authors

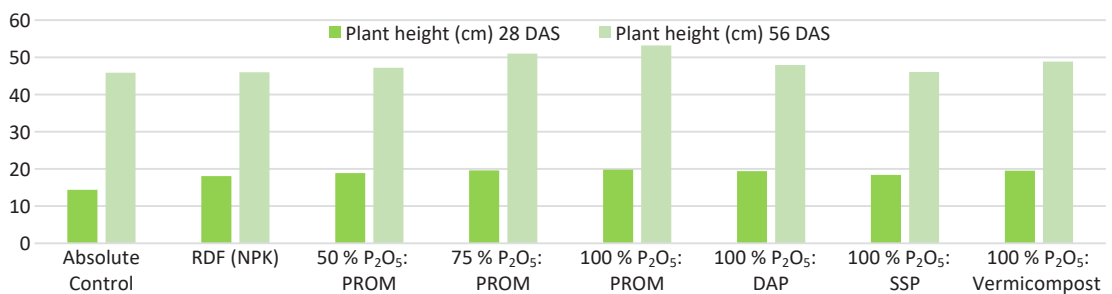


Figure 1. Plant height

Note: RDF: Recommended dose of fertilisers (N – Nitrogen, P – Phosphorus, and K – Potassium), P-rich organic manure (PROM), Diammonium phosphate (DAP), Single super phosphate (SSP), days after sowing (DAS)

Source: compiled by the authors

As for the interactive effects of phosphorus application through PROM and phosphatic fertilisers, data revealed that application of phosphorus at 75% or 100% through PROM and vermicompost or DAP yielded prominently greater number of leaves and plant height in calcareous soil. This might be explained by the application of PROM which might have greater content of available P₂O₅. The PSB present in PROM might have solubilised the unavailable forms of calcium-fixed phosphate by releasing organic acid like gluconic, fumaric, succinic, lactic, and propionic acids, leading to lowering the pH of the rhizosphere, which directly solubilised to available form. R. Boje *et al.* (2024) revealed that the number of pods plant⁻¹, filled pods plant⁻¹, nodulation, protein, and oil content in soybean were greatly improved when 78 kg P₂O₅ ha⁻¹ was applied through SSP: 100 kg ha⁻¹ + PROM: 600 kg ha⁻¹. Same treatment also produced maximum seed yield (22.35 q ha⁻¹),

stover yield (30.52 q ha⁻¹), nutrient content, and uptake in seed and stover by the crop.

Application of phosphorus through SSP + PROM and seed inoculation with *Aspergillus awamori* + *Pseudomonas striata* is a rewarding approach in maximising crop productivity (Yadav *et al.*, 2017). R. Singh *et al.* (2018) also reported that plant height, number of primary and secondary branches per plant, and number of leaves per plant increased significantly with increasing level of phosphorus up to 80 kg ha⁻¹ + PSB. Co-application of residual reactive phosphate rock with *Rhizobium* and soluble P-bacteria reported significantly greater plant height of soybean on 30 and 90 DAS. U. Ade *et al.* (2018) also reported analogous findings. In addition, the provision of phosphorus by PROM is accompanied by the provision of a substantial quantity of nutrients such as N, K, Ca, Mg, and some metallic micronutrients. This results in increased vegetative growth,

as evidenced by an increase in the number of leaves and plant height. It could be summarised that application of phosphorus at the rate

of either 75% or 100% through P-rich organic manure was found superior for the plant height and number of leaves.

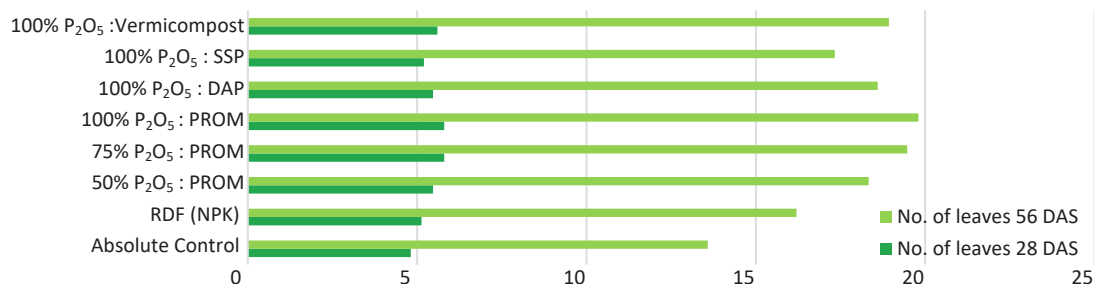


Figure 2. Periodical number of leaves

Note: RDF: Recommended dose of fertilizers (N – Nitrogen, P – Phosphorus and K – Potassium), P-rich organic manure (PROM), Diammonium phosphate (DAP), Single super phosphate (SSP)

Source: compiled by the authors

Leaf area plant⁻¹, leaf area index and total chlorophyll

Leaf area plant⁻¹, leaf area index and total chlorophyll content in soybean grown in calcareous soil was influenced significantly by the application of P₂O₅ through PROM, vermicompost, and phosphatic fertilisers. Application of PROM, vermicompost, and phosphatic fertilisers to soybean in calcareous soil produced varied leaf area plant⁻¹, leaf area index, and chlorophyll, which ranged within 1,040.64-1,657.26 cm², 4.63-7.37, and 0.98-1.23 mg g⁻¹ of fresh tissue, respectively (Table 3; Figs. 3-4). Significantly greater leaf area plant⁻¹ (1,657.26 cm²), leaf area index (7.37), and total chlorophyll (1.23 mg g⁻¹ of fresh tissue) were reported with the addition of 100% P₂O₅: PROM which was followed by 75% P₂O₅: PROM. In case of vermicompost application for the substitution of 100% P₂O₅ reported greater leaf area plant⁻¹ (1,497.36 cm²), leaf area index (6.65), and total chlorophyll (1.12 mg g⁻¹ of fresh tissue) than RDF (1,260.52 cm², 5.60, and 0.99 mg g⁻¹ of fresh tissue). Further application of 100% P₂O₅: DAP

yielded greater leaf area plant⁻¹ (1,461.96 cm²), leaf area index (6.50), and chlorophyll (1.12 mg g⁻¹ of fresh tissue) than 100% P₂O₅ through SSP.

Maximum leaf area, leaf area index, and chlorophyll content were reported with P application through PROM which might be explained by the fact that P-rich manure not only supplies P but also a considerable amount of other nutrients involved in chlorophyll synthesis. K. Boubekri *et al.* (2023) reported analogous findings. The study examined the plant growth-promoting role of phosphorus-solubilising bacteria and associated microbes, hypothesising that these might have synthesised gibberellin, resulting in superior leaf area and leaf area index. Greater photosynthetic rates in PROM phosphorus-supplied treatments are attributed to higher nutrient availability from calcareous soil, which leads to increased leaf area. P. Lokhande *et al.* (2023) also concluded that application of a liquid organic formation like panchagavya increased chlorophyll content due to greater population of photosynthetic bacteria.

Table 3. Effect of PROM and phosphatic fertilisers on leaf area, Leaf area index (LAI), and total chlorophyll of soybean in calcareous soil

Seq. No.	Treatment	Leaf area plant ⁻¹ (cm ²)	Leaf area index	Total Chlorophyll (mg g ⁻¹ of fresh tissue)
1	Absolute Control	1,040.64	4.63	0.92
2	RDF (NPK)	1,260.52	5.60	0.99
3	50% P ₂ O ₅ : PROM	1,437.26	6.39	1.08
4	75% P ₂ O ₅ : PROM	1,624.38	7.22	1.13

Table 3, Continued

Seq. No.	Treatment	Leaf area plant ⁻¹ (cm ²)	Leaf area index	Total Chlorophyll (mg g ⁻¹ of fresh tissue)
5	100% P ₂ O ₅ : PROM	1,657.26	7.37	1.23
6	100% P ₂ O ₅ : DAP	1,461.96	6.50	1.12
7	100% P ₂ O ₅ : SSP	1,358.85	6.04	1.01
8	100% P ₂ O ₅ : Vermicompost	1,497.36	6.65	1.12
	SE+	40.76	0.18	0.09
	CD at 5%	123.63	0.55	0.27

Note: SE : Standard error, CD at 5% : Critical difference at 5% significance level, RDF: Recommended dose of fertilisers (N – Nitrogen, P – Phosphorus, and K – Potassium), P-rich organic manure (PROM), Diammonium phosphate (DAP), Single super phosphate (SSP)

Source: compiled by the authors

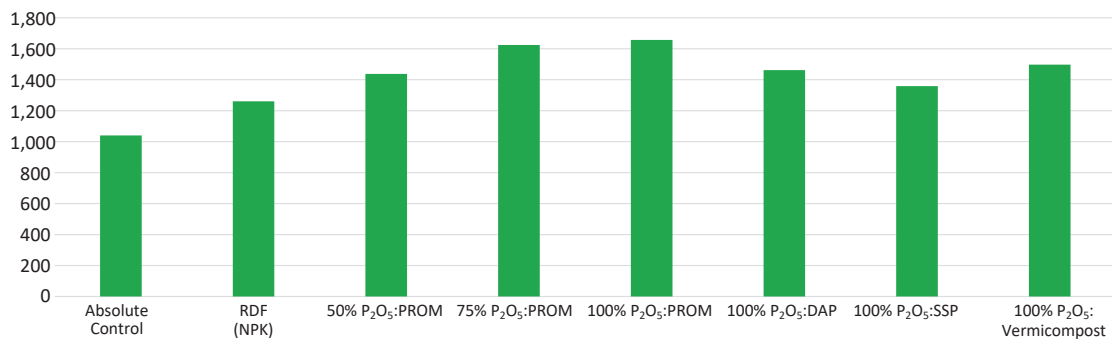


Figure 3. Leaf area per plant (cm²)

Note: RDF: Recommended dose of fertilisers (N – Nitrogen, P – Phosphorus, and K – Potassium), P-rich organic manure (PROM), Diammonium phosphate (DAP), Single super phosphate (SSP)

Source: compiled by the authors

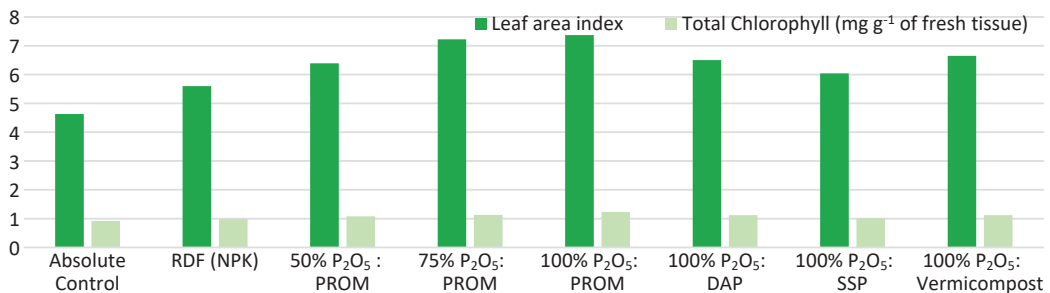


Figure 4. Leaf area index and total chlorophyll

Note: RDF: Recommended dose of fertilisers (N – Nitrogen, P – Phosphorus, and K –Potassium), P-rich organic manure (PROM), Diammonium phosphate (DAP), Single super phosphate (SSP)

Source: compiled by the authors

The organic acids diffused in soil at the time of decomposition of organic matter present in PROM might have reduced P exchange sites through chelation and released more

plant available phosphorus in calcareous soil there by higher root exploration in soil resulted into greater nutrient uptake, as well as greater leaf area, leaf area index, and chlorophyll.

Plant height, chlorophyll, and 100 grain weight of soybean were significantly impacted by different levels of P_2O_5 at 0, 30, 60, and 120 kg ha⁻¹ with broadcast and band application (Moreira et al., 2020). These findings suggest that optimising phosphorus sources and application methods can substantially enhance physiological traits and nutrient uptake efficiency in soybean cultivated under challenging soil conditions.

Effect of various sources and levels of phosphorus on yield characteristics of soybean in calcareous soil

Data presented in Table 4 and Figure 5 present significant variation in the number of pods

plant⁻¹, seeds pod⁻¹, seeds plant⁻¹ of soybean in calcareous soil as influenced by P_2O_5 application through PROM, vermicompost, and phosphatic fertilisers. Significantly greater number of pods plant⁻¹ (46.33), seeds pod⁻¹ (3.00), and seed plant⁻¹ (150.27) were noticed with application of 100% P_2O_5 : PROM which was followed and statistically on par with 75% P_2O_5 : PROM, 100% P_2O_5 vermicompost, and 50% P_2O_5 : PROM. Lower number of pod plant⁻¹, seeds pod⁻¹ and seeds plant⁻¹ were observed in control treatment where no fertilisers were added. Yield characteristics of soybean, particularly in calcareous soil, depends on nutrient availability and soil pH.

Table 4. Effects of PROM and phosphatic fertilisers on yield characteristics of soybean in calcareous soil

Seq. No.	Treatment	Pods per plant	Seeds per pod	Seeds per plant
1	Absolute Control	27.20	2.80	92.33
2	RDF (NPK)	39.80	2.93	132.40
3	50% P_2O_5 : PROM	41.47	2.93	136.27
4	75% P_2O_5 : PROM	45.87	3.00	146.73
5	100% P_2O_5 : PROM	46.33	3.00	150.27
6	100% P_2O_5 : DAP	44.20	2.93	144.27
7	100% P_2O_5 : SSP	40.87	2.93	134.73
8	100% P_2O_5 : Vermicompost	45.67	2.93	145.60
	SE+	1.92	0.03	5.66
	CD at 5%	5.82	0.11	17.17

Note: SE: Standard error, CD at 5%: Critical difference at 5% significance level, RDF: Recommended dose of fertilisers (N – Nitrogen, P – Phosphorus, and K – Potassium), P-rich organic manure (PROM), Diammonium phosphate (DAP), Single super phosphate (SSP)

Source: compiled by the authors

S. Arintonang & L. Sidauruk (2020) reported greater number of branches, pods, and weight of seeds per plant in soybean with vermicompost P application treatment. The researchers further discussed the increased number of pods in soybeans following P-substituted vermicompost treatment. This phenomenon could be attributed to the higher and more available form of nutrients supplied by organic manure. It can be hypothesised that the presence of further organic manure may have resulted in the release of low-molecular-weight organic acids. This, in turn, may have led to an enhancement of N fixation and an increase in N absorption by the leaves. This may have positively influenced Mg absorption from the soil. The addition of further

P-solubilising bacteria to soil through PROM resulted in the enhancement of bacterial population. This is likely to have led to a greater release of plant growth-promoting substances, such as auxins, gibberellins, and cytokines, which may have subsequently resulted in a greater number of pods per plant. These findings are in agreement with the results reported by P. Lokhande et al. (2023). The researchers also reported enhanced leaf area, chlorophyll, and number of pods with the application of vermicompost and organic formulations. Further application of PROM and vermicompost for P substitution stimulates release of microbial origin organic acids dominantly gluconic acid resulted into reduction of soil reaction in rhizosphere which

leads to more availability of P and other nutrients too resulted into more growth and yield contributing characters. A. Bamagoos *et al.* (2021) also reported inoculation of calcareous soil with P-SB (a 1:1 mixture of two *Pseudomonas sp.*; *Ps. mallei* and *Ps. cepaceae*) significantly exceeded leguminous compost, humic acid, or even leguminous compost + humic acid for the positive results obtained. P-SB facilitated nutrient solubility (e.g., N, K, Fe, and Mn), including conversion of insoluble phosphorous into a form available in the tested soil due to increased soil enzymatic activities (e.g., phosphatases and phytases). This mechanism, combined with a decrease in soil calcium carbonate content and an increase in cation exchange capacity (CEC) and organic matter (OM) content, increased the availability of various nutrients to plants, including P, in the soil, which contributed to the increased plant output in case of *Phaseolus vulgaris*.

Yield characteristics of soybean such as the number of pods⁻¹, number of seeds pod⁻¹, and number of seeds plant⁻¹ were on par for P₂O₅ application either with SSP or DAP. However, a

greater count of pods plant⁻¹, seeds pod⁻¹, and seeds plant⁻¹ were observed with P₂O₅ application through DAP. This could be attributed to a better fixation of P applied through SSP as compared to DAP. These findings agree with those reported by A. Jamal *et al.* (2023). The researchers reported that most of the applied P fertiliser was found to be fixed to the surfaces of calcite, thereby becoming unavailable in the rhizosphere. Further abundance of CaCO₃ along with alkaline pH lowers solubility and availability of P in rhizosphere (Aswitha *et al.*, 2024). Neutralisation of CaCO₃ content due to organic acid release resulted into lowering the pH causes release of available nutrients in rhizosphere. M. Khan *et al.* (2021) concluded that combined application of rock phosphate, poultry manure, and *Bacillus Spp.* MN-54 showed most effective for increasing shoot and root length to the tune of 37.5% and 42.6% in chickpea. The researchers explained that incorporation of manure along with *Bacillus Spp.* MN-54 helped to improve nutritional status and availability of nutrients by supplying macro and micronutrients leads to greater number of pods in chickpea.

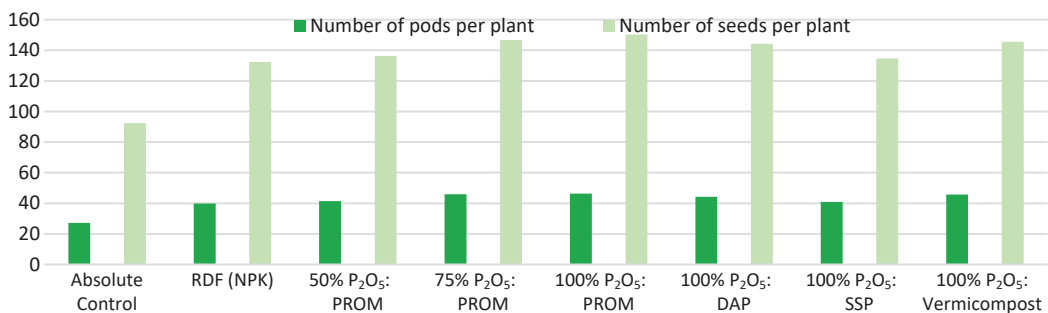


Figure 5. Soybean yield characteristics

Note: RDF: Recommended dose of fertilisers (N – Nitrogen, P – Phosphorus, and K – Potassium), P-rich organic manure (PROM), Diammonium phosphate (DAP), Single super phosphate (SSP)

Source: compiled by the authors

Effect of various sources and levels of phosphorus on grain and straw yield of soybean in calcareous soil

Grain and straw yield of soybean grown on highly calcareous soil, as influenced by levels of P₂O₅ incorporation through PROM, vermicompost, SSP, and DAP, were noted at the harvest stage of soybean. Application of P₂O₅ through various organic and inorganic sources significantly

influenced grain and straw yield of soybean (Table 5; Fig. 6). Data indicated that grain yield of soybean ranged within 19.38-25.94 q ha⁻¹ while straw yield – within 26.33-35.16 q ha⁻¹ in various treatments of phosphorus application. Significantly greater grain (25.94 q ha⁻¹) and straw (35.16 q ha⁻¹) yield of soybean was observed with 100% P₂O₅ application through PROM and this treatment was followed by and statistically on

par with 75% P₂O₅ (25.00 and 32.66 q ha⁻¹), 100% P₂O₅ vermicompost (24.69 and 32.00 q ha⁻¹) and 100% P₂O₅ through DAP (24.38 and 31.66 q ha⁻¹). Use of either 75% or 100% P₂O₅ through PROM

and 100% P₂O₅ through vermicompost resulted in almost 27-33% increase over control while 17% over RDF for grain yield of soybean grown in highly calcareous soil.

Table 5. Effect of PROM and phosphatic fertilisers on grain and straw yield of soybean in calcareous soil

Sr. No.	Treatment	Yield (q ha ⁻¹)	
		Grain	Straw
1	Absolute Control	19.38	26.33
2	RDF (NPK)	22.13	26.91
3	50% P ₂ O ₅ ; PROM	23.75	28.25
4	75% P ₂ O ₅ ; PROM	25.00	32.66
5	100% P ₂ O ₅ ; PROM	25.94	35.16
6	100% P ₂ O ₅ ; DAP	24.38	31.66
7	100% P ₂ O ₅ ; SSP	22.44	27.83
8	100% P ₂ O ₅ ; Vermicompost	24.69	32.00
	SE+	0.69	1.06
	CD at 5%	2.01	3.22

Note: SE: Standard error, CD at 5%: Critical difference at 5% significance level, RDF: Recommended dose of fertilisers (N – Nitrogen, P – Phosphorus, and K – Potassium), P-rich organic manure (PROM), Diammonium phosphate (DAP), Single super phosphate (SSP)

Source: compiled by the authors

The presence of calcium carbonate (CaCO₃) in soil, when the pH is alkaline, can restrict the availability of phosphorus, iron, zinc, and potassium. Subsequently, the added phosphatic fertiliser is rapidly transformed into an unavailable form, which has the effect of reducing the plant's usable form of P in the rhizosphere. Phosphorus-enriched compost incorporated for soybean in highly calcareous soil produced higher yield which might be attributed to a greater availability of P and other nutrients in soil. Among inorganic fertilisers, 100% P₂O₅ through SSP application produced lower yield (22.44 q ha⁻¹) than that of DAP (24.38 q ha⁻¹). Single super phosphate is a calcium phosphate that is applied in soil with a high calcium carbonate content and an alkaline pH. This may result in the restriction of solubilisation of phosphorus, which is rapidly converted into insoluble complexes of tricalcium phosphate.

The organic acids like fumaric, succinic, formic, propionic, gluconic, etc., released under the application of PROM via phosphate solubilising bacteria and decomposition of organic manure, resulted in lowering the soil pH near rhizosphere, leading to the conversion of insoluble phosphorus into soluble form. In addition,

these phenomena have been demonstrated to accelerate mineralisation rates, not only for P, but also for micronutrients. The further plant growth-promoting effect of PSB (*Bacillus megaterium* and *Bacillus thuringiensis*) and other microorganisms present in the PROM might have played a role in higher microbial-oriented nutrient availability. This, in turn, could have resulted in increased nutrient uptake, higher photosynthetic rate and higher chlorophyll synthesis, which could have led to an increase in yield. P. Bagade *et al.* (2025) also reported analogous findings. The utilisation of organic manure, which is abundant in phosphorus, has been demonstrated to have a substantial impact on the soil's biological condition, owing to its role as a nutrient source for microbes. This increase in microbial population consequently stimulates heightened levels of biological activity within the rhizosphere. In conjunction with organic components such as fluvic and humic acid, these microbes may have retained nutrients through a process of chelation, thereby enhancing nutrient availability to plants. This approach contrasts with the conventional methods of leaching, fixation, and precipitation of nutrients.

Application of either 75% or 100% P_2O_5 through PROM and 100% P_2O_5 via vermicompost improved leaf area, chlorophyll, yield, and yield characteristics, which might be attributed to greater solubility of calcium-fixed phosphorus by PSB. Subsequent addition of manure might have enhanced release of organic acids during decomposition, lowering the rhizosphere pH and increasing solubilisation of fixed and precipitated P in calcareous soil. The experiment yielded results that lend further credence to the hypothesis that the suppression of pH changes can exert a substantial influence on the physical, chemical, and biological characteristics of soil, thereby engendering conditions conducive to optimal root growth and enhanced access to the soil. This can engender an increase in the yield of soybeans.

P applied via SSP may not have been available to plants due to its alkaline calcareous nature, resulting in smaller yield compared to PROM and vermicompost. P application through SSP might have increased the Ca content in calcareous soil that might have enhanced $CaCO_3$ and alkanisation, resulting in less microbial activity, subsequently leading to poor nutrient availability. Furthermore, PROM and vermicompost not only supplied P, but also provides significant amount of other essential nutrients contributing to improvement in growth and yield features resulted into higher yield. PSB and beneficial microflora added via PROM and vermicompost might have secreted plant growth promoting constituents leads to positive growth over SSP and DAP application.

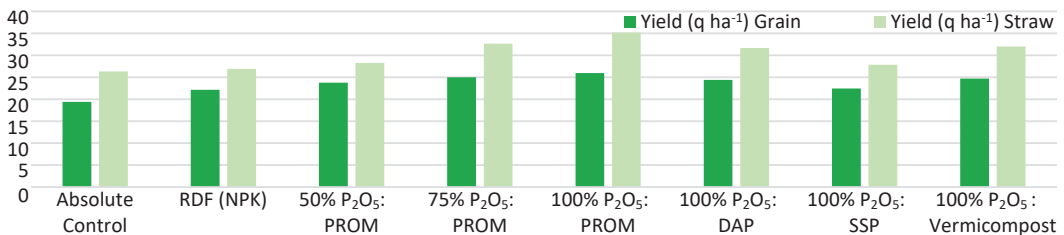


Figure 6. Grain and straw yield of soybean

Note: RDF: Recommended dose of fertilisers (N – Nitrogen, P – Phosphorus, and K – Potassium), P-rich organic manure (PROM), Diammonium phosphate (DAP), Single super phosphate (SSP)

Source: compiled by the authors

It was hypothesised that the application of P derived from organic matter, has enhanced the quality of the soil. This improvement has been demonstrated by an increase in soil porosity and water-holding capacity, alongside a reduction in soil pH. The impact of this enhanced soil quality has been shown to result in a decrease in nutrient fixation, an increase in microbial population and activity, and an enhancement in enzyme synthesis. These changes have collectively led to a higher availability of nutrients. The outcome of this experiment is in agreement with the findings reported by K. Khan *et al.* (2023). Results of the present study are also uniform with K. Aswitha *et al.* (2024). The researchers found that among many P-activators like FYM, SSP and DAP, vermicompost and P rich organic manure were found to be the best P-activators in calcareous soil. Husnain *et al.* (2021) also concluded that the residual effect of reactive rock phosphate in combination

with Rhizobium and P solubilising bacteria resulted in a reported increase in soybean yield of 40.9% in the absence of reactive rock phosphate and microbes. T. Dev *et al.* (2020) reported that the efficiency of rock phosphate as a source of phosphorus for crop production is enhanced by increased solubility due to the application of farmyard manure (FYM) and phosphate-solubilising bacteria (PSB). The researchers further concluded that the combined application of phosphate-solubilising bacteria (PSB) and farmyard manure (FYM) enhanced the mineralisation of organic phosphorus, thereby increasing soil phosphorus availability, promoting plant growth, and improving the dry matter yield of soybean. P. Lokhande *et al.* (2023) also concluded that addition of 30:60:30 kg ha⁻¹ N, P_2O_5 , and K_2O to soybean in combination with Beejamruth seed treatment, Jeevaamrut (soil application at 500 L ha⁻¹ on 30 and 45 DAS) and two foliar sprays

of panchyagavya at 3% reported significantly greater leaf area, chlorophyll, yield characteristics, and yield amount itself. The combined application of rhizobia inoculant, P-fertiliser, and organic manure increased grain yield fourfold, whereas the combined application of rhizobia inoculant, P-fertiliser, and cattle manure increased grain yield threefold. Harvest index, phosphorus agronomic efficiency, and rainwater use efficiency were relatively greater with the manure (prepared from poultry manure, rice husks, and urban waste) treatment combinations than with solely cattle-based manure combinations and the control treatment. The application of rhizobia inoculant, P-fertiliser in combination with manure (poultry manure, urban waste, and rice husks) was profitable with value-cost ratio of 2 as compared to the combination of cattle manure which had a value cost ratio of 0.40. The findings revealed that manure offers a better option of improving soybean response to rhizobia inoculant and P-fertiliser and can enhance rainwater use efficiency. However, the long-term benefit must be quantified (Ulzen *et al.*, 2020). In summary, numerous studies confirmed that the combined application of organic manure, phosphorus fertilisers, and microorganisms such as phosphate-solubilising bacteria and inoculants markedly enhances phosphorus availability in calcareous soils, improves soil physicochemical properties, boosts plant productivity, and increases soybean yield. This integrated approach shows promise for sustainable agriculture and improving resource use efficiency under challenging agroecological conditions.

CONCLUSIONS

In conclusion, the study showed that phosphorus application through PROM, vermicompost, and phosphatic fertilisers significantly influenced soybean growth in calcareous soil. Application of 100% P_2O_5 through PROM and vermicompost, as well as 75% P_2O_5 through PROM, resulted in superior plant height, chlorophyll and leaf count. The presence of PSB in PROM may have contributed to solubilising unavailable forms of calcium-fixed phosphate, influencing plant growth. These findings highlighted the potential of phosphorus application to improve soybean growth through PROM to improve

soybean growth and yield. Furthermore, application of P_2O_5 through PROM, vermicompost, and phosphatic fertilisers significantly influenced leaf area $plant^{-1}$, leaf area index, and total chlorophyll content in soybean. The highest values of leaf area $plant^{-1}$, leaf area index, and total chlorophyll were reported with the addition of 100% P_2O_5 through PROM. Notably, the application of 100% P_2O_5 through phosphorus-rich organic manure resulted in a significantly greater number of pods $plant^{-1}$ (46.33), seeds pod^{-1} (3.00), and seed $plant^{-1}$ (150.27), followed by 75% P_2O_5 via PROM, 100% P_2O_5 vermicompost, and 50% P_2O_5 : PROM treatments. Furthermore, the study supported the hypothesis that the application of PROM and vermicompost creates favourable conditions for root growth, leading to a greater yield of soybean in alkaline calcareous soil, as evidenced by the improved grain and straw yield, better availability of nutrients, improved soil pH, and enhanced microbial population and activity. The study demonstrated that phosphorus application through PROM and vermicompost significantly improved the grain and straw yield of soybean in highly calcareous soil. The use of organic matter-based P application resulted in greater availability of nutrients, improved soil pH, and enhanced microbial population and activity, and positive growth over inorganic fertiliser application. This rock phosphate-based organic manure further needs to be assessed in various types of soils and crops to maintain and improve soil health and quality. The findings of the presented study can be used to produce P-rich organic base manure which will maintain soil sustainability. This approach warrants further investigation across diverse soil types, climatic conditions, and crop species to validate its broader applicability and long-term impact on soil health and productivity.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Вплив фосфоровмісного органічного добрива (PROM) на ріст і врожайність сої на карбонатних ґрунтах

Анотація. Фіксація фосфору та його доступність є серйозною проблемою на лужних і карбонатних ґрунтах. Обмежена доступність поживних речовин у ґрунті є значним викликом для фосфорозалежних бобових культур, таких як соя. Проте використання органічного добрива та фосфориту за участі фосфат-розчинювальних бактерій (PSB) відіграє важливу роль не лише у підвищенні вмісту фосфору, а й у зниженні стресу. Метою дослідження було оцінити вплив різних джерел фосфору, включаючи органічні та неорганічні добрива, на параметри росту та продуктивність сої, що вирощується в умовах вапняного ґрунту. Дослідження показало, що застосування 75 або 100 % P_2O_5 через PROM або 100 % через вермикомпост значно покращувало ріст сої, зокрема збільшувалася кількість листків і висота рослин на 28-й та 56-й день після сівби. Використання PROM з 100 % P_2O_5 дало найвищі показники листової поверхні ($1657,26 \text{ cm}^2$), індексу листової поверхні (7,37) та загального вмісту хлорофілу ($1,23 \text{ mg g}^{-1}$). Крім того, застосування 100 % P_2O_5 через PROM призвело до значного збільшення кількості стручків на рослині (46,33), насінин у стручку (3,00) та насінин на рослині (150,27). Також значно вищу врожайність зерна (25,94 ц/га) та соломи (35,16 ц/га) сої забезпечило внесення 100 % P_2O_5 через PROM, за яким ішло внесення 75 % через PROM або 100 % через вермикомпост. Отже, виробництво фосфоровмісного органічного добрива може бути сталим підходом для підвищення росту та врожайності сої. Результати дослідження можуть бути використані агрономами, фахівцями з ґрунтознавства та агрохімії, а також фермерами і сільськогосподарськими підприємствами, які працюють із карбонатними (кальцієвими) ґрунтами для підвищення ефективності використання фосфорних добрив і покращення врожайності сої

Ключові слова: органічні джерела фосфору; неорганічні джерела фосфору; діамонійфосфат; простий суперфосфат; біогумус

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The impact of primary tillage and soil herbicides on sunflower productivity in the right-bank forest-steppe zone of Ukraine

Abstract. Sunflower is one of Ukraine's most important oil crops, which is of great economic importance due to the export of sunflower oil. The growth in sunflower sowing areas is due to high profitability and market demand. At the same time, sunflower cultivation technology is complex, and production efficiency depends on many factors, including primary tillage and herbicide protection systems. The aim of the study was to investigate the effect of different options for primary soil cultivation and herbicide protection on the yield and quality of sunflower seeds in the conditions of the right-bank forest-steppe of Ukraine. The research was conducted in 2022-2024 on typical low-humus chernozem at the private enterprise "Shans" (Cherkasy region). Ploughing and non-ploughing soil cultivation were used, as well as various herbicide protection options, including manual weeding. The main indicators were yield, basket diameter, weight of 1,000 seeds and seed quality. It was found that the use of effective herbicide schemes and mouldboard tillage significantly increased sunflower yield (up to 4.1 t/ha) compared to the control. Weeds significantly reduced crop yield (by 0.4-1.1 t/ha), reducing yield parameters and seed quality. Manual weeding provided a high level of control, but was labor-intensive and economically inefficient. The best results were obtained with the use of Primextra TZ Gold (4.5 l/ha) and Challenge (5.0 l/ha) herbicides in combination with mouldboard tillage. It was found that combining mouldboard tillage with an optimally selected herbicide protection scheme resulted in better indicators of basket diameter, seed weight per basket, and weight of 1,000 seeds. The results demonstrated the importance of integrating soil cultivation and chemical protection techniques to increase sunflower crop productivity. The results of the study can be used to optimise sunflower cultivation technologies in the region and increase production efficiency

Keywords: crop structure; weeds; yield; mouldboard tillage; non-mouldboard tillage; Challenge; Primextra TZ Gold

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INTRODUCTION

Sunflower is one of the strategically important crops for agricultural production in Ukraine, as it provides the majority of oil production for domestic and export markets. With growing global demand for oilseed products, particularly vegetable oil, it is important not only to expand sunflower cultivation areas, but also to increase its yield and stability of productivity in modern climatic conditions. To achieve these goals, it is necessary to improve cultivation technologies, in particular those elements that most significantly affect the growth, development and formation of the crop structure. In particular, it is especially important to optimise the system of primary soil cultivation in combination with effective herbicide protection of crops, which allows for more efficient use of moisture, reduces weed load and ensures favourable conditions for the development of cultivated plants. In this regard, there is a need for scientific justification of adaptive elements of sunflower cultivation technology that would meet modern requirements for resource conservation and environmental safety.

In recent years, scientific works have paid considerable attention to studying the impact of basic soil cultivation measures on the agro-physical properties, soil fertility and yield formation of field crops. Authors H. Pinkovskiy & S. Tanchyk (2020) studied the impact of depth and method of cultivation on sunflower productivity and noted that under forest-steppe conditions, reducing the depth of cultivation with the correct selection of aggregates does not reduce crop yield but contributes to improving the water regime of the soil. In turn, Y. Gadzalo *et al.* (2022) emphasised that sunflower yield depends to a large extent not only on the hybrid and weather conditions, but also on the effectiveness of each technological element – tillage, sowing, weed control and nutrition. V. Gurtovenko & O. Tsyuk (2023) found in their research that the choice of the main soil cultivation system significantly affects the density, moisture capacity and temperature regime of the arable layer, which is directly related to the initial growth rates of sunflowers and overall crop formation. At the same time, authors E. Achankeng & W. Cornelis (2023) drew attention to the potential risks of excessive intensification:

the unjustified use of deep tillage or its complete avoidance (no-till) can worsen germination conditions and increase weed infestation of crops.

The problem of weeds remains relevant in modern intensive agriculture. Y. Joshan *et al.* (2019) noted that the presence of weeds in sunflower crops significantly reduces yield – during the active growing season, they compete with the crop for moisture, nutrients and light, and also complicate mechanised harvesting. That is why herbicide protection, especially under conditions of simplified or minimal tillage, must be adaptive and differentiated. In the modern conditions of climate change, when there is an increase in the average annual temperature and a decrease in the amount of effective precipitation during the growing season, adaptive farming systems are of particular importance. According to research by A. Molla *et al.* (2022), the use of minimum tillage in combination with agronomic measures to conserve moisture contributes to the increased efficiency of sunflower cultivation in crop rotation systems. They found that even under conditions of reduced water supply, conservation agriculture ensures stable crop formation. Similar conclusions were reached by A. Sher *et al.* (2021), who pointed out that preserving soil structure when using surface or zero tillage reduces evaporation and improves conditions for the action of soil herbicides. Thus, studying the interaction between the main tillage system and herbicide protection in sunflower cultivation allows to justify effective adaptive technologies for the conditions of the Right-Bank Forest-Steppe of Ukraine. Scientific research shows that sunflower yield is influenced by a complex of technological factors, among which the main tillage system, agrochemical support and herbicide protection are of particular importance.

In the course of modern field studies in Ukraine, considerable attention is paid to the impact of basic soil cultivation methods on moisture retention and sunflower productivity. A study by Z. Pacanoski & A. Mehmeti (2021) found that different tillage methods significantly determine crop yield and energy efficiency. In particular, minimum and partial tillage help reduce energy and water consumption without

a noticeable decrease in yield compared to traditional ploughing. Thus, after maize, the yield of sunflower with traditional tillage was 2.78 t/ha, while with shallow tillage it was 2.65 t/ha. A similar pattern was observed after soybeans, although this predecessor had a generally negative effect on the yield. Thus, with mouldboard tillage, the yield reached 2.56 t/ha, and with shallow tillage, only 2.41 t/ha. In addition, the tillage method also affected the quality characteristics of the seeds: with shallow tillage, the oil content decreased by 2.4-3.6% compared to plowing.

Research by Y. Havrylyuk & N. Matsai (2019) showed that both the quantity and species composition of weeds affect sunflower yield. In the complete absence of weeds, the sunflower yield was 2.5 t/ha, and in the presence of 25 weeds/m², the yield decreased to 2.19 t/ha. With 50 weeds per m², the yield was even lower – 1.74 tonnes. The work of Z. Degtyareva & S. Kudrya (2023) analysed the agroecological aspects of herbicide protection of sunflowers depending on weather conditions in the forest-steppe zone of Ukraine. The authors note that the effectiveness of soil herbicides largely depended on the amount of precipitation in the post-emergence period: under drought conditions, the effectiveness decreased to 40-60%, while in the presence of

moisture, it reached 85-90%. In addition, the use of combined protection schemes with the use of insurance herbicides ensured a stable reduction in weed infestation and an increase in yield regardless of weather conditions.

The aim of the study was to assess the impact of different systems of primary soil tillage and herbicide protection on the elements of yield structure and sunflower productivity in the conditions of the forest-steppe zone of Ukraine.

MATERIALS AND METHODS

The research was carried out during 2022-2024 in the right-bank forest-steppe zone at the private enterprise “Shans” in Cherkasy region (Ukraine). The soil cover of the experimental site was typical low-humus chernozem. According to soil analysis, the 0-30 cm layer contained: humus – 2.1-2.3%; easily hydrolysable nitrogen – 10.3-11.5 mg/kg; available phosphorus – 153.1-160.3 mg/kg; available potassium – 130-140 mg/kg; pH 6.7-6.8. The preceding crop was maize for grain. The hybrid grown in the NK Kondi studies is characterised by traditional cultivation technology. It should be noted that only soil herbicides were used in this chemical protection experiment; no other treatments were carried out. The experiment design is shown in Table 1.

Table 1. Field experiment scheme

Variant No.	Primary soil tillage, cm (Factor A)	Scheme of soil-applied weed control (Factor B)
1	Mouldboard tillage at 25-27 cm	Without herbicides (control)
2		Manual weeding
3		Primextra TZ Gold (312.5 g/l s-metolachlor, 187.5 g/l terbuthylazine) – 4.5 l/ha (Etalon)
4		Racer (fluorochloridone, 250 g/l) – 3.0 l/ha
5		Prometrex (prometryn, 500 g/l) – 3.0 l/ha
6		Challenge (aclonifen, 600 g/l) – 5.0 l/ha
7		Prometrex 1.5 l/ha + Racer 1.5 l/ha
8		Challenge 2.5 l/ha + Prometrex 1.5 l/ha
9		Challenge 2.5 l/ha + Racer 1.5 l/ha
10		Challenge 2.5 l/ha + Aspect Pro (terbuthylazine, 333 g/l + diflufenican, 200 g/l) – 2.0 l/ha
11		Prometrex 1.5 l/ha + Aspect Pro – 2.0 l/ha
12		Racer 1.5 l/ha + Aspect Pro – 2.0 l/ha

Table 1, Continued

Variant No.	Primary soil tillage, cm (Factor A)	Scheme of soil-applied weed control (Factor B)
1	Non-mouldboard tillage at 25-27 cm	Without herbicides (control)
2		Manual weeding
3		Primextra TZ Gold (312.5 g/l s-metolachlor, 187.5 g/l terbutylazine) – 4.5 l/ha (Etalon)
4		Racer (fluorochloridone, 250 g/l) – 3.0 l/ha
5		Prometrex (prometryn, 500 g/l) – 3.0 l/ha
6		Challenge (aclonifen, 600 g/l) – 5.0 l/ha
7		Prometrex 1.5 l/ha + Racer 1.5 l/ha
8		Challenge 2.5 l/ha + Prometrex 1.5 l/ha
9		Challenge 2.5 l/ha + Racer 1.5 l/ha
10		Challenge 2.5 l/ha + Aspect Pro (terbutylazine, 333 g/l + diflufenican, 200 g/l) – 2.0 l/ha
11		Prometrex 1.5 l/ha + Aspect Pro – 2.0 l/ha
12		Racer 1.5 l/ha + Aspect Pro – 2.0 l/ha

Source: developed by the author

The following materials and methods were used during the research: field experiment, laboratory analysis, data processing and summarisation. The total area of the experiment, including the protective strip, was 0.18 hectares. The area of the sowing plot was 18 m² (3m*6m), and the accounting plot was 10 m² (2m*5m) with four repetitions. The placement of variants was randomised. Under the gradation of factor A, the influence of basic soil cultivation on sunflower yield was located. Mouldboard tillage was performed using a PLN-3-35 unit (Ukraine), which was characterised by ploughing at a depth of 25-27 cm. Non-mouldboard tillage was performed using an AGR-17 chisel unit (Ukraine) at a depth of 25-27 cm. Under gradation B, there are various options for soil herbicides, as well as a control (without the use of any weed control) and options with manual weeding. The study included a comprehensive agronomic analysis, which included measuring sunflower yield, 1,000-seed weight, and yield structure. The weight of 1,000 seeds was determined by weighing replicated seed samples selected from each treatment. To assess the yield structure, the ratio of the main components of the yield was analysed: seeds, husks and waste.

Statistical data processing was performed using analysis of variance (ANOVA) with the calculation of the least significant difference (LSD) at a significance level of 0.05, which allowed determining the reliability of differences between the mean values of the indicators. During the

years of research, uneven precipitation distribution and significantly extreme air temperature increases were observed, which had a negative impact on sunflower yield. At the same time, weather conditions in the research years made it possible to study their impact on the effectiveness of the factors under study. During the years of research, the start of the growing season varied, which affected the timing of sunflower sowing. Thus, in 2022, it took place on 1 May, in 2023 – on 11 May, and in 2024 – on 11 April. During the 30 days after sowing sunflowers in 2022, 27 mm of precipitation fell, and in 2023, 17 mm. The spring of 2024 was wet with heavy rains: 55 mm of precipitation fell during the 30 days after sowing.

The study was conducted in accordance with the ethical standards of the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1976).

RESULTS AND DISCUSSION

Yield indicators and seed quality characteristics are important when studying the effectiveness of herbicide protection of crops, since both quantitative and qualitative parameters of the harvest largely depend on the level of weed control. It should be noted that mechanical methods of weed control do not always ensure their complete destruction, which reduces the overall effectiveness of this approach. Despite its relative effectiveness, manual weeding is

labour-intensive, which makes it economically unfeasible in modern agricultural production (Mazur & Matusevych, 2023). Sunflower yields

varied throughout the years studied, which can be explained by different climatic weather conditions (Fig. 1).

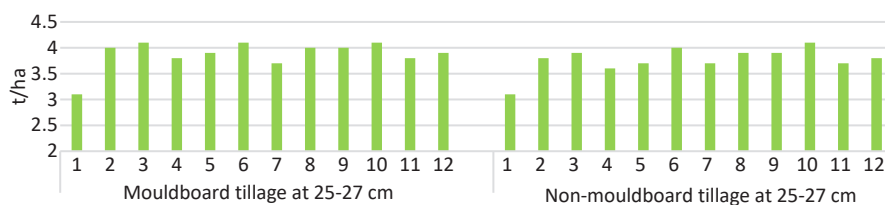


Figure 1. Sunflower yield depending on primary tillage options and weed protection, average for 2022-2024, t/ha, PE “Shans”

Source: developed by the author

In the experiment, the resource provision for sunflower seed yield was calculated at 3.5 t/ha. The manual weeding option provided a yield of 4.0 t/ha with mouldboard tillage and 3.8 t/ha without mouldboard tillage. The yield increase compared to the control variant was 0.9 t/ha. At the same time, despite its high efficiency, this method is too labour-intensive for use on large areas. The maximum average yield within the experiment reached 4.1 t/ha. This result was obtained both with the use of individual preparations and in tank mixture variants. Among individual herbicides, the highest yield was achieved with Primextra TZ Gold at a rate of 4.5 l/ha and Challenge at a dose of 5 l/ha. Among tank mixtures, the best result was observed with the combined use of Challenge (2.5 l/ha) and Racer (1.5 l/ha). Other products demonstrated yields within the range of 3.0-3.9 t/ha, which is statistically significantly lower than the reference option ($LSD_{0.5} = 0.41$ t/ha). Most tank mixtures with yields of 3.5-4.0 t/ha were also inferior in terms of yield and weed control efficacy compared to the most effective treatment schemes. Similar dependencies were confirmed by the results of other studies. L. Peleh & O. Onufriichuk (2024) found that the effectiveness of soil herbicides is largely determined by the type of primary tillage, the moisture content of the seedbed, and the uniformity of application; in case of precipitation deficit after application, the effectiveness decreased by 30-40% regardless of the dose, especially in no-till systems. Similarly, D. Chuyko *et al.* (2021) noted that under conditions of minimum tillage, it is advisable to

combine mechanical and chemical methods to increase the effectiveness of weed control.

Similar results were obtained by M. Husain *et al.* (2018), who emphasised that the use of herbicides in combination with optimal agronomic measures reduces competitive pressure from weeds and increases sunflower yield by 15-20%. In studies, the increase in yield when using the most effective herbicides exceeded this figure, which indicates the high efficiency of the applied preparations and the compliance of agricultural technology with modern requirements. Authors A. Babenko (2017) and N. Meena *et al.* (2019) focused on the impact of weather conditions on the effectiveness of herbicide protection, in particular the role of precipitation during the treatment period. The data confirm this dependence and also show that the use of tank mixtures of herbicides contributes to a more stable action of the preparations under different climatic conditions. V. Borysenko (2024) and V. Kozechko & O. Ivanchenko (2025) noted the limitations of mechanical methods of weed control due to their labour intensity and incomplete effectiveness, which is consistent with the conclusions of the study. The combination of mechanical and chemical measures increases the overall effectiveness of the protection system.

It has been established that the use of different options for primary soil cultivation and herbicide protection systems significantly influenced the formation of sunflower yield components. This dependence is explained by a reduction in competitive pressure from weeds as a result of the effective action of soil herbicides,

which contributed to improved conditions for the growth and development of cultivated plants and increased their productivity. Thus, the diameter of the basket in the control variant was 14 cm, which is 18-34% less than in other variants

of the experiment. In the variants with the use of the reference herbicide and manual weeding, the largest baskets were formed, with a diameter of 18 cm, which is a significant increase compared to the control indicators (Fig. 2).

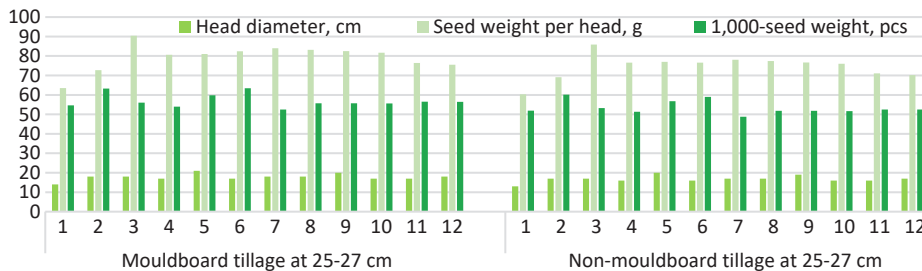


Figure 2. Sunflower yield components depending on the herbicide protection system for the crops, average for 2022-2024, at PE “Shans”

Source: developed by the author

A similar relationship can be observed with regard to the weight of seeds from a single basket: the larger the diameter of the basket, the higher the weight of the seeds, which indicates the direct impact of herbicide protection on the structural elements of the crop. The experimental variants significantly exceeded the control indicator in terms of the weight of 1,000 seeds: manual weeding provided an increase of 13.7%, and treatment with the herbicide Challenge at a rate of 5.0 l/ha provided an increase of 13.8%. Other variants showed a tendency to exceed the control by 1-3%.

It is important to note that the structure of the yield – basket diameter, weight of seeds from one basket, weight of 1,000 seeds – are informative indicators of yield quality that closely correlate with the level of weed control. Similar conclusions were made by V. Kaminskyi *et al.* (2022) and I. Mostoviak *et al.* (2024), who also noted the positive impact of effective herbicide protection on these indicators in their work. The study by S. Shcherban *et al.* (2023) also confirmed the importance of integrated agricultural technologies in achieving high crop quality, in particular emphasising the relationship between herbicide load and morphological characteristics. Thus, the comparative analysis shows that the results of the study are closely related to current scientific data, while supplementing them with new information

about the impact of herbicide tank mixtures and the role of tillage systems. This highlights the relevance and practical value of the proposed agrotechnological measures for increasing sunflower productivity in a changing climate.

CONCLUSIONS

It has been established that the presence of weed vegetation has a significant negative impact on sunflower productivity. Weeds caused a significant reduction in crop yields ranging from 0.4 to 1.1 t/ha, depending on the degree of weed infestation. The highest sunflower seed yields in the field trial – 4.1 t/ha – were recorded when using the reference herbicide Primextra TZ Gold (4.5 l/ha) and when using the product Challenge (5.0 l/ha) in combination with mouldboard tillage. Analysis of all herbicide protection options showed that even less effective products contributed to higher yields compared to the control, providing results in the range of 3.0-3.9 t/ha. This indicates the general feasibility of using herbicides as an important component of the sunflower crop protection system, particularly in conditions of intensive farming.

A comparison of the main soil cultivation systems revealed the advantages of the mouldboard tillage method, which provided higher yields compared to the non-mouldboard tillage method. Mouldboard tillage in combination

with herbicide protection improved conditions for sunflower growth, reduced the competitive influence of weeds and created a more favourable soil water and air regime, confirming the need to adapt agricultural techniques to the specific soil and climatic conditions of the right-bank forest-steppe of Ukraine. The use of tank mixtures of herbicides has demonstrated the potential to improve weed control under various weather conditions, which is an important advantage in a changing climate. At the same time, the labour intensity of manual weeding limits its use on large areas, although it contributes to improved yield performance. Therefore, the comprehensive use of effective herbicides in combination with mouldboard tillage is a feasible and effective agronomic measure to increase yields and improve the quality characteristics of sunflowers in the right-bank forest-steppe zone of Ukraine.

Promising areas for further research include optimising herbicide application rates and timing in different primary tillage systems, studying the impact of herbicides on soil biocenoses, and developing the agroecological sustainability of crops. It is also advisable to study combined protection systems that combine chemical, mechanical and biological methods of weed control in order to increase the efficiency, environmental safety and economic feasibility of sunflower cultivation in the region.

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CONFLICT OF INTEREST

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<https://orcid.org/0000-0002-9719-6374>**Вплив основного обробітку ґрунту та ґрунтових гербіцидів на продуктивність соняшнику в умовах правобережного лісостепу України**

Анотація. Соняшник є однією з найважливіших олійних культур України, яка має велике економічне значення через експорт соняшникової олії. Зростання посівних площ соняшника обумовлене високою рентабельністю та попитом на ринку. Водночас технологія вирощування соняшника є складною, а ефективність виробництва залежить від багатьох факторів, зокрема основного обробітку ґрунту та системи гербіцидного захисту. Метою дослідження було вивчення впливу різних варіантів основного обробітку ґрунту та гербіцидного захисту на врожайність і якість насіння соняшника в умовах правобережного Лісостепу України. Дослідження проводились у 2022-2024 роках на чорноземі типовому малогумусному в приватному підприємстві «Шанс» (Черкаська область). Використовувалися полицевий та безполіцевий обробіток ґрунту, а також різні варіанти гербіцидного захисту, включно з ручним прополюванням. Основними показниками були врожайність, діаметр кошика, маса 1000 насінин та якість насіння. Встановлено, що застосування ефективних гербіцидних схем і полицевого обробітку значно підвищувало врожайність соняшника (до 4,1 т/га), порівняно з контролем. Бур'яни істотно знизили врожайність культури (на 0,4-1,1 т/га), зменшуючи параметри врожаю та якість насіння. Ручне прополювання забезпечило високий рівень контролю, проте було трудомістким і економічно неефективним. Найкращі результати отримано за застосування гербіцидів Примекстра TZ Голд (4,5 л/га) та Челендж (5,0 л/га) у поєднанні з полицевим обробітком. Встановлено, що при поєднанні полицевого обробітку з оптимально підбраною схемою гербіцидного захисту формуються кращі показники діаметра кошика, маси насіння з кошика та маси 1000 насінин. Результати засвідчили важливість інтеграції технологічних прийомів ґрунтообробітку та хімічного захисту для підвищення продуктивності посівів соняшника. Результати дослідження можуть бути використані для оптимізації технологій вирощування соняшника в регіоні та підвищення ефективності виробництва

Ключові слова: структура врожаю; бур'яни; врожайність; полицевий обробіток; безполіцевий обробіток; Челендж; Примекстра TZ Голд

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