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Optimisation strategies for sustainable molecular farming

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Abstract. Molecular farming is a rapidly developing field of modern plant biotechnology that can provide industrial-scale support of biologically active therapeutics for medicine and veterinary. The review aimed to identify the main strategies for improvement of the efficiency of therapeutic protein production in plants. To achieve the aim, analyses of Scopus and PubMed databases, generalisation, and critical evaluation of data regarding to strategies enhancing the efficiency of therapeutic protein production in plants were conducted. Key criteria that should be covered in selection of a vector for transformation were identified. Super-binary or tertiary vectors are recommended to optimise the genetic transformation of recalcitrant species. For transient transformation, magnICON systems and geminivirus-based vectors were proposed. The ways to overcome plant recalcitrance to the *in vitro* culture and to the genetic transformation were reviewed. Methods such as overexpression of morphogenic genes (WOX, BBM) that increase the efficiency of regeneration were mentioned. The newest gene delivery systems for precise and accurate gene insertion were reviewed. Tissue-specific, inducible, or active at certain stages of plant development, promoters are recommended to increase the expression of target proteins. The study determined that protein stability can be increased by its targeting to cell compartments or co-expression with protease inhibitors. The study also established that correction of protein post-translational modifications can improve its biological activity. Reduction in toxic secondary metabolites of the host is also among the goals. The conclusions can be used to develop new strategies for optimisation of plant transformation, increasing the yield of recombinant protein, and improving its therapeutic efficacy

Keywords: plant biotechnology; genetic engineering; plant-based expression systems; pharmaceutical proteins

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INTRODUCTION

Molecular farming is a modern technology for obtaining pharmaceutically valuable proteins in transgenic plants for medical and veterinary purposes. In contrast to other systems for expressing recombinant proteins (e.g., bacteria, yeasts, animal and insect cell cultures) plants are characterised by their ability to express physiologically active heterologous proteins, accumulate large amounts of biomass in a relatively short period of time, without the risk of contamination with pathogens to which humans and animals are susceptible. This significantly reduces the safety concerns associated with such products and the cost of their purification. However, the commercialisation of plant-derived therapeutic proteins is limited by low efficiency of existing genetic transformation protocols for an economically relevant plants, low protein yield and glycosylation pattern differences that influence the efficacy of the plant-derived protein *in vivo*.

The review aimed to improve the yield and quality of recombinant therapeutic proteins by highlighting several strategies that can be applied at different stages of the technology development. J.K. Bharathi *et al*. (2024) summarised these strategies, including optimised gene-delivery methods, appropriate vector design, use of codon-optimised sequences of the genes of interest, and the peculiarities of plant cell culture approaches. Several specific methods used in experimental studies to increase the efficiency of transformation were also investigated. The gene integration efficiency can be increased by using transposon systems, as was reported by V. Kumar *et al.* (2024). The overview by S. Kambampati *et al*. (2025) stated that plant transformation using gene editing systems (e.g., CRISPR-Cas9, zinc fingers) delivered via nanoparticles could also achieve higher gene integration accuracy. The following studies highlighted strategies aimed to increase transgene expression. M.O. Pydiura & Ya.B. Blume (2023) reported that transposons enhance the expression of transgene. The expression also increased with integration of genome elements of plant viruses into vectors (e.g., polymerases). This was demonstrated in research conducted by Ya.R. Sindarovska & M.V. Kuchuk (2024) for transient expression in *Nicotiana benthamiana*.

The improvement of transformation protocols for the recalcitrant species is also noteworthy. M. Mirzaee *et al*. (2022) discussed the strategies applied for efficient transformation of monocots, which are considered as one of the most recalcitrant species. The study mentioned that the selection of suitable promoters, codon optimisation, incorporation of 5` and 3` untranslated regions (UTRs) into mRNA to increase its stability, and subcellular targeting of the recombinant protein expression are the main strategies that increase the efficiency of the transformation. Methods for improved regeneration were also developed for recalcitrant species. Some more strategies to overcome the recalcitrance of these species were discussed. M.B. Belaffif *et al*. (2025) discussed that introduction of morphogenic regulator genes such as Babyboom (BBM), Wuschel-related homeobox (WUS), and other growth-regulating factors enhanced regeneration rates in several recalcitrant plants (e.g., apple – *Malus domestica*, rice – *Oryza sativa*). X. Han *et al.* (2025) demonstrated that various in-planta methods can be applied for recalcitrant species.

The review aimed to explore the key strategies explored to improve the efficiency of therapeutic protein production in plants. For this review, the studies published in journals indexed in Scopus were used. The systematic literature search by keywords was performed in Scopus and PubMed databases. The search was filtered by year (2000-2025). Strategies used by authors among selected articles to increase the expression or stability of recombinant proteins and improve their biological availability were analysed. The data obtained were critically evaluated, compared, and grouped into semantic sections.

VECTOR DESIGN

The transfer of the target gene into the plant genome (stable nuclear and chloroplast transformation) or into the cell nucleus for its transient expression is conducted using vectors that are transferred into the plant cell by various metods (Zhi *et al.*, 2022). For plant genetic transformation and transient expression, vectors based on *Agrobacterium tumefaciens* Ti-plasmids, *Agrobacterium rhizogenes* Ri-plasmids, and plant viruses of the genera *Bunyavirus, Potexvirus, Tobamovirus, Mastrevirus, Tobravirus, Hordeivirus, Cytorhabdovirus,* and *Nucleorhabdovirus* are used (Mahmood *et*

al., 2023). The vector choice is fundamental in the efficiency of plant transformation and target protein yield. For stable nuclear transformation, Ti- or Ri-plasmids are used; specific binary and ternary vectors were created on their basis. A binary vector was obtained by removing the genes responsible for crown gall formation found in the wild-type *A. tumefaciens* plasmid. This resulted in a system consisting of a T-DNA binary vector with the gene of interest and a helper Ti-plasmid, containing virulence genes (*vir*) necessary for the insertion of T-DNA into the nuclear genome. Such binary vectors as pBIN19, pBI121, and pPZP are widely used, as reported by T. Komori *et al.* (2007). However, the use of binary vectors demonstrates rather low efficiency for species that are difficult to transform (e.g., monocots). This led to the emergence of updated vectors with increased number of *vir* genes. In particular, these are super-binary systems, in which the T-DNA binary vector contains additional *vir* genes, and ternary vector systems containing an additional third plasmid with a *vir* region. J. Jeong *et al*. (2025) demonstrated that the use of ternary vectors for the transformation of corn, sorghum, and soybeans increases the efficiency of transformation by 1.5-21.5 times. Thus, the choice of vector for stable transformation is determined by the sensitivity of the producer to *Agrobacterium*-mediated transformation.

Chloroplast transformation and transient expression result in relatively higher protein yield compared to stable nuclear genetic transformation, but the chloroplast transformation is rather complicated, while transient expression may cause some ecological concerns. To perform chloroplast stable transformation, vectors containing flanking sequences homologous to plastid DNA regions are developed. M. Narra *et al*. (2025) demonstrated that addition of flanking sequences enabled the insertion of the target fragment to the selected site via homologous recombination. In addition, the developed vectors usually contain regulatory elements, binding sites for proteins that link RNA with expression regulators (PPR – pentatricopeptide repeat), and a selective marker (usually, spectinomycin/streptomycin resistance gene). Thus, a substantial aspect in the vector design for plastid transformation is the selection of the insertion site and the isolation of the necessary sequence for the synthesis of flanking regions.

Transient protein expression is primarily achieved by using viral vectors. The main biological limitation of virus-based systems is the sensitivity of the selected host to the virus on which the vector is based. Q. Chen *et al*. (2013) reported that an effective strategy for transient expression is the use of vectors based on deconstructed plant RNA viruses, such as the magnICON system. As reported by Y. Gleba *et al*. (2013), there are many modifications to the magnICON system, and they are based on tobacco mosaic virus (TMV), potato virus X (PVX), cowpea mosaic virus (CPMV), and bean yellow dwarf virus (BYDV) DNA. With magnICON system, several monoclonal antibodies (mAb), antigens, and other therapeutics proteins have been expressed transiently. J. Hurtado *et al*. (2020) highlighted expression of anti-Chikungunya virus mAb with TMV-based vectors (pICH21595 and pICH11599), K.T. Hamorsky *et al*. (2013) reported about anti-HIV mAb expression with single-component vector based on TMV/TVCV – pICH38099. H. Karauzum *et al*. (2012) reported that the TMV- and PVX-based vectors were used to express staphylococcal enterotoxin B, meanwhile in H. Nausch *et al*. (2012) used TMV-based pICH29912 vector and PVX-based pICH27727, pICH31160 vectors for interleukin-6 expression.

Q. Chen *et al*. (2013) noted that the use of vectors based on geminiviruses (in particular, BYDV) can be more preferable, in comparison to the magnICON system, due to a wider range of susceptible species and a shorter replication period. Furthermore, N. Pisuttinusart *et al*. (2024) used the pBYR2eK2Md (pBYR2e) vector, which demonstrated high efficiency in the expression of a anti-respiratory syncytial virus mAb. The pBYR2fp vector used by J.-H. Ha *et al*. (2017) enabled efficient expression of human fibroblast growth factor. Additionally, the effectiveness of the geminivirus-based vectors can be increased when the gene of interest is co-deliverd with a Rep/RepA-supplying vector, coding Rep and RepA proteins necessary for replication. The successful super-transformation of *N. benthamiana* plants stably producing Rep/RepA proteins under control of an ethanol-inducible promoter with a geminivirus-based vector, encoding Rep/RepA, p19 suppressor, and vitronectin under control of the same promoter (INPACT platform) was also reported by B. Dugdale *et al*. (2018).

The use of other vectors has also been reported. In particular, D.A. Morris *et al*. (2021) determined that agroinfiltration of *N. benthamiana* with a TMV-based vector (GENEWARE system) yielded significantly higher amounts of cholera toxin subunit B compared to previous studies. Similar observations were made by A. Varsani *et al*. (2006) in the case of human papillomavirus (type 16) L1 protein expression. The PVX-based vectors are used for the efficient expression of human fibroblast growth factor (vector pgR107) in J. Liu *et al*. (2007) research, human granulocyte-macrophage colony-stimulating factor (vector pPVXG1) in F. Zhou *et al*. (2006), and keratinocyte growth factor 1 (pgR107) in Z.-G. Feng *et al*. (2014) researches. Thus, the choice of vector type for plant transformation should be based on the selected transformation method, the plant's sensitivity to *A. tumefaciens* (in the case of stable nuclear transformation) and viruses (in the case of virus-based transient expression). For chloroplast transformation, it is necessary to determine correctly the site of gene integration to use a suitable flanking sequence.

TRANSFORMATION METHOD OPTIMISATION

The choice of delivery method for various genetic constructs determines the overall effectiveness of plant transformation. Physical, chemical, and biological methods of genetic information transfer are utilised depending on the type of transformation (stable, transient) and the characteristics of the host. Stable transformation is applied to obtain producers that can inherit and transmit the target gene for the protein of interest to subsequent generations. Such expression results in comparatively low protein yield. Therefore, this method is used for plants with high biomass production. In contrast, transient expression demonstrates higher protein yield during a short period of time, but there is a need for repetitive rounds of plant treatment to obtain the target product.

Optimisation of transformation methods can relate to both the selection of optimal conditions for DNA transfer and to the improvement of plant regeneration during stable transformation. For example, callus cultivation in pneumatic bioreactors has been proposed by S.S. Barretto *et al*. (2017) to increase the regeneration frequency of tobacco callus expressing the C subunit of tetanus toxin. Another strategy for improvement of plant regeneration is the induction of overexpression of transcription factors. The WUSCHEL-related homeobox (WOX) transcription factors are essential plant growth and development regulators. In a study on *N. tabacum* overexpressing the *MaWOX* gene isolated from mulberry, conducted by H. Zhou *et al*. (2025), an increase in regeneration rates and the *Agrobacterium*-mediated transformation efficiency was demonstrated. J.A. Teixeira da Silva (2005) reported that *Agrobacterium*-mediated treatment with sonication promotes shoot regeneration from transverse thin cell layers as explants. J. Zhang *et al*. (2025) reported that the regeneration rate increased while overexpressing BBM morphogenic genes under constitutive and somatic embryogenesis-specific promoters. For genetic transformation, the expression of BBM morphogenic genes under inducible promoters is preferential, as their constitutive expression results in developmental abnormalities and regenerant mortality was highighted by S.D. Bakulin *et al*. (2025). The study by D.U. Santos-Ballardo *et al*. (2019) on cell pre-treatment with 0.5 M sorbitol prior to particle bombardment demonstrated optimised transformation efficiency rate at 60%. Improved transformation rate in chloroplast transformation with biolistic could be achieved through increasing the size of plastids. Research with overexpression of the *FtsZ1* gene (regulating chloroplast division) in transgenic *Solanum tuberosum* increased organelle size (generating macro-chloroplasts), as was previously reported by A. Occhialini *et al*. (2020). Such modified plants can be a suitable hosts that produce pharmaceutical proteins.

However, new methods of DNA delivery into plastids have been proposed. For instance, A. Matsuoka & P. Maliga (2021) highlighted an approach to edit the *VirD2* and *VirE2* genes of *A. tumefaciens* to use female gametocytes as explants in the chloroplast transformation with the floral dip protocol. Genetically engineered *A. tumefaciens* integrates T-DNA into the plastid because of the removal of nuclear localisation signals, and fusion of the C-terminus of the target protein with VirF Type IV secretion system signal. Another method proposed by C. Thagun *et al*. (2019) is based on the self-assembled complex, containing DNA, cell penetrating peptide (CPP), and chloroplast targeting peptide (CTP), delivered via syringe/vacuum

infiltration, microinjection into leaves, roots, fruit parts, and tubers. One of the methods for the genetic transformation of recalcitrant plants developed by A. Jakubiec *et al*. (2021) is the creation of "minichromosomes". They are based on the beet curly top geminivirus (BCTV) and contain the Rep protein required for vector replication, which recognises specific VOR sequences (viral origin of replication), flanking the transgene. For chloroplast introduction, cognate VORs flanking the chloroplast cassette were used. This vector was delivered biolistically and amplified independently of the chloroplast genes. The study also reported the case of episomal plasmid usage, which can be used for recombinant protein expression in chloroplasts without integration into plastid DNA proposed by A. Occhialini *et al*. (2024). Similarly to classic chloroplast transformation, this approach can be used for stable expression of multiple genes. Previous research noted that carbon nanotubes (CNT) could be used as novel carriers for recombinant DNA for plant transformation. As discussed by O.M. Burlaka *et al*. (2015), CNTs have higher cell-penetrating properties, enabling the delivery process tracking and non-covalent loading of the DNA. Thus, the biological properties of recombinant DNA provide no changes.

The various strategies can be used to increase the efficiency of transient expression. J.F. Buyel & R. Fischer (2012) demonstrated that protein yield depends on concentration of *Agrobacterium* cells, temperature, plant, leaf age and its position. The optimal cell density for *N. tabacum* transformation is 0.13-2 at OD_{600}, plant age – 6-7 weeks after seeding. This was supported by Y.V. Sheludko *et al*. (2007). The study determined that for *Nicotiana* species optimal leaf age for treatment is 1-6 days, and the plant age – 8-10-week-old. To increase the rate of transient expression, many more approaches can be applied. K. Norkunas *et al*. (2018) summarised that addition of the virus-derived elements into the vector (e.g., CMV 2b, TBSV p19) decreased post-translational gene silencing effects; addition of molecules that enhance transformation rate (acetosyringone, vanillin, cinnamic acid), reduce of oxidation stress (e.g., PVP, lipoic, ascorbic acid) and also addition of surfactants (e.g., Silwet-L77, Pluronic F-68, Tween 20) into a co-cultivation or infiltration medium, and heat shock treatment of the plants after transformation enhanced transient expression rate. Thus, treatment of 6-week-old leaf plants with Silwet L-77 (optimal concentration – 0.001%, as higher concentration caused leaf damages), resulted in 60-80% transient expression ratio as has reported by Y. Zhang *et al*. (2020). H. Zhao *et al*. (2017) additionally mentioned that agents which reduce DNA methylation (e.g., 5-azacytidine), also increased the efficiency of expression.

Several optimised transient expression systems such as AGROBEST (*Agrobacterium*-mediated enhanced seedling transformation), FAST (Fast Agro-mediated Seedling Transformation), and magnICON were proposed by Y. Gleba *et al*. (2005). The AGROBEST method is based on the use of a disarmed *Agrobacterium* strain with pre-induced vir-genes and an optimised culture medium, resulting in a 100% transformation rate of seedlings. It is necessary to maintain a stable pH of 5.5, as it suppresses calcium ion uptake, thus inhibiting immunity against *Agrobacterium* infection. The FAST method is based on the co-cultivation of seedlings in the medium with surfactants. The absence of specialised equipment renders this method simple and low-cost, and it can also be applied to some plants recalcitrant to *Agrobacterium* infection, such as rice. The magnifection technology (magnICON) is based on *Agrobacterium*-mediated delivery of viral DNA vector encoding RNA viral replicons, which are later spread cell-to-cell within the plant. This method is fast and does not require specialised equipment, while providing high transformation efficiency and expression level (Gleba *et al*., 2005).

Therefore, the choice of transformation method is one of the steps that determines the efficiency of obtaining a producer with the desired characteristics. Optimisation of stable transformation methods mainly includes strategies that improve gene integration into the genome and improve regeneration frequency. Optimisation of transient expression involves creation of conditions that increase the permeability of the explant to *Agrobacterium*, thereby increasing the entry of vectors into the plant.

STRATEGIES FOR OPTIMISATION OF GENE EXPRESSION AND PROTEIN TRANSLATION

Translation is a subtantial step for the expression of a recombinant protein. M. Beihaghi *et al*. (2018)

in with a study on transient expression of CCL21 protein highlighted the Kozak sequence and ER retention signal (SEKDEL) to the upstream region of codon-optimised target gene sequence. The strategy enhanced translational efficiency and protein accumulation in plants. M.O. Pydiura & Ya.B. Blume (2023) reported that the inclusion of introns is central in the enhancing of the target protein expression. The study suggested that introns may contribute to the stabilisation of mRNA or improve its processing, thereby increasing the overall efficiency of protein synthesis.

In some cases, the use of constitutive promoters in transplastomic plants negatively affects the expression of the protein of interest and the host's physiology. Such observations were made by J.I. Arevalo-Villalobos *et al*. (2020) when expressing vaccine candidates against multiple sclerosis (BV5S2, BV6S5, and BV13S1) under the constitutive promoter. The constitutive expression of these proteins was toxic for host plants, and successful expression was achieved by the Algevir expression system (geminivirus-based vector with inducible AlcA promoter from *Aspergillus nidulans*). The last one is based on the geminivirus elements with an ethanol-inducible promoter, which leads to regeneration and stable expression of the peptides. K. Saba *et al*. (2019) demonstrated that tuberculosis antigen ESAT-6 expressed under T7 ethanol-inducible promoter in plastids caused no pleiotropic effects. Therefore, the use of inducible promoters is recommended for the expression of pharmaceutical proteins in transplastomic hosts.

The choice of promoters can influence the success of transient expression. Strong promoters accelerate efficient protein synthesis, like those isolated from cotton GhEF1A8 with a 5'UTR region. B. Sun *et al*. (2016) demonstrated that GhEF1A8 provides higher expression rates of the target gene compared to the CaMV 35S promoter. For edible plants (e.g., tomato – *Solanum lycopersicum*), an effective solution is the use of promoters involved at certain stages of plant development. N. Kurokawa *et al*. (2013) proposed to use the E8 promoter, which becomes active during fruit ripening and results in higher protein accumulation. The study showed that protein yield in fruits was at least 4-times higher with E8 promoter, compared to CaMV 35S promoter. This can be explained by the fact that CaMV 35S promoter reduces its activity during fruit ripening, thus resulting in lower yields that was demonstrated by S.H. Park *et al*. (2022) in with a study on colorectal antigen expression in tomato fruits. The analysis of expression revealed that protein expression was higher in leaves, compared to fruits, therefore the researches also suggest using promoters with high activity when fruit ripening. Another approach to increase the efficiency of the biosynthesis is the editing of regulatory elements. J. Limkul *et al*. (2015) reported that usage of 5`UTR from *Arabidopsis* alcohol dehydrogenase, along with HSP (heat shock protein) terminator increased the yield of β-glucocerebrosidase up to 2 times. S. Kanagarajan *et al*. (2021) demonstrated that addition of the Kozak sequence into the 5`UTR region and 12-amino acid linker could improve expression of heterodimeric proteins (e.g., hemoglobin), providing additionally correct folding and stability of the protein. E. Frangedakis *et al*. (2021) reported that gene expression could be greatly increased due to the presence of an mRNA site for pentatricopeptide repeat (PPR), which stabilises mRNA. Such stabilisation occurred due to protection against exoribonuclease degradation, which can increase protein yield up to 15% of the TSP.

There are several studies in which increased efficiency of translation was achieved when plant viral elements and RNA silencing suppressors were inserted into the vector. P. Habibi *et al*. (2018) demonstrated that insertion to the vector of the p19, p1, p0 genes, which prevent gene silencing, resulted in higher levels of griffithsin (used as a potential HIV entry inhibitor) expression. S. Kanagarajan *et al*. (2021) demonstrated that p19 suppressor was effective for transient expression of human fetal hemoglobin with the p19 gene solely. M.-C. Jiang *et al*. (2019) highlighted the efficiency of p19 suppressor and ER retention signal co-expression with human interferon gamma. Transient co-expression of human iduronate-2-sulfatase with p38 suppressor and folder enhancer (human caltreticulin fused with GB1 – streptococcal protein G, domain B1), also demonstrated high protein yield production in *N. benthamiana*. K. Matsuo & T. Matsumura (2017) proved that transgenic *N. benthamiana* plants with repressed DCL2 and DCL4 genes, which mediate foreign RNA cleavage, produce larger amounts of acidic human fibroblast growth factor transiently, compared to wild-type

plants. Targeting protein expression to cellular compartments is also an effective strategy to increase yield. A. Sorrentino *et al*. (2005) noted that for human tissue transglutaminase, higher yields (up to 2.5 times) were observed when the protein was fused with the apoplast targeting signal. Similar observations were made by S. Singhabahu *et al*. (2015) for adenosine deaminase expressed in tobacco BY-2 suspension cells. In this case, the protein yield increased up to 336 times compared to the cytosol-directed proteins.

Apoplast targeting in S. Wirth *et al*. (2004) was noted to provide a fourfold increase in expression for the epidermal growth factor in *N. benthamiana*. However, for some proteins, the apoplast targeting was inefficient. As reported by N.R. Nair *et al*. (2014) for human granulocyte colony stimulating factor stably expressed in BY-2 cells and targeted to ER, on the contrary, increased protein yields up to 4 times compared to apoplast targeting. Moreover, in the case of β-glucuronidase, addition to the E7 protein and targeting of expression into thylakoid lumen, M. Morgenfeld *et al*. (2014) reported up to 30-40 times higher protein expression. As was previosly reported by J. Patel *et al*. (2007), fusion of elastin-like polypeptides (ELP) with interleukin-10 not only enhanced the purification efficiency but also increased transient or stable expression of the target proteins in tobacco leaves.

Thus, the efficiency of expression and translation can be improved by various methods. To improve translation, it is recommended to optimise the codons of the target gene and use a co-expression strategy with genes that regulate mRNA degradation. Expression can be improved by selecting a promoter that considers the characteristics of the transformation method (for stable transformation, inducible promoters are recommended), the site of protein accumulation, and the stage of plant development at which synthesis will occur. Protein expression can also be improved by fusion with various proteins that direct protein expression to the desired cellular compartment.

IMPROVEMENT OF PROTEIN STABILITY AND ENHANCING THE BIOAVAILABILITY OF RECOMBINANT PROTEINS

Final protein yield is greatly influenced by stability in plants. Increased protein stability is frequently achieved by co-expression of protease inhibitors or auxiliary proteins, which stabilise the target protein and protect it from proteolysis. It is known that if the protein is expressed in the cytosol, it can be degraded by proteases. M. Niemer *et al*. (2014) showed that co-expression of the protein with protease inhibitors (e.g., tomato cystatin 9, tomato cathepsin D), or, in the case of transient expression, by using young leaves, which tend to have lower protease content, the protein yield could be increased up to 40%. Fusion of interleukin-2 with different protease inhibitor proteins (e.g., CMTI – *Cucurbita maxima* trypsin inhibitor 1) resulted in a protective effect against trypsin activity. Protease level decreased when suspension culture of tobacco cells was immobilised in the alginate, resulting in the rise of protein yield up to 33-fold. This was demonstrated by K. Nakashima *et al*. (2013) on NT1 cells expressing human alkaline phosphatase.

Another strategy to protect proteins from proteolysis is directing protein translation into the endoplasmic reticulum (ER). Hence, the addition of ER retention signal at the C-terminus (C-KDEL signal) could enhance protein expression in *N. tabacum*. This was shown in A. Bidarigh Fard *et al*. (2019) research with etanercept (a recombinant fusion protein of TNFR2 receptor with the Fc portion of human IgG1 used for autoimmune diseases treatment) expression. Similar observations were made by N. Gutierrez-Valdes *et al*. (2024) for anti-HIV Fc fusion proteins, and by K. Deepa *et al*. (2013) for human platelet-derived growth factor BB. B. Dugdale *et al*. (2018) in the study on vitronectin transient expression in *N. benthamiana* also showed that ER localisation of the protein enhances its accumulation. Similar observations were made by M. Mazalovska & J.C. Kouokam (2020). The study demonstrated that addition of ER retention signal to lectin III, enhances protein expression up to 3 times, when transiently expressed in *N. benthamiana*. However, some recombinant proteins may be unstable in ER due to folding defects in this compartment (e.g., Nef protein of the HIV). M. de Virgilio *et al*. (2008) proposed fusing of the target protein (Nef) with zeolin that forms protein bodies in which protein is more stable. Some proteins when their expression is targeted in ER, may lose their biological activity. This was reported by A.P. Soni *et al*. (2022)

when expressing tumor growth factor β1 and fusing ER retention signal with C-terminus. Thus, they suggest that the optimal strategy for such proteins is chloroplast transformation.

Another substantial factors in molecular farming are bioavailability and therapeutic efficacy of the recombinant plant-derived proteins. Proteins derived from plants may have reduced efficacy due to differences in post-translational processes, especially – glycosylation. To overcome this problem I. Andrasevic *et al*. (2025) proposed the strategy of recombinant protein expression in CRISPR-Cas9 edited *N. benthamiana* plants with knock-down genes of β-1.2-xylosyltransferase and α-1.3-fucosyltransferase, responsible for plant-specific protein N- glycosylation pattern. In case of Chikungunya virus antigen expression, J. Hurtado *et al*. (2020) reported that glycoengineered plants showed slightly higher productivity compared to wild-type plants. Another research in *N. tabacum* BY-2 suspension cells, performed by K. Fujiyama *et al*. (2007) addressed co-expression with β-1.4-galactosyltransferase gene, which resulted in "humanised" N-glycosylation pattern monoclonal antibody. Similar results were obtained by C. Navarre *et al*. (2017) for IgG antibody, "humanised" protein. The researchers expressed erythropoietin in tobacco plants.

For proteins that require an O-glycosylation pattern, the strategy of co-expression with genes responsible for *N*-acetylgalactosamine synthesis can be used, as was previously reported by I.A. Ramírez-Alanis *et al*. (2018). J. Jez *et al*. (2013) showed the successful case of sialylated human erythropoietin expression in plants. This was achieved by the addition of six mammalian genes responsible for sialylation into transformation vectors. To achieve improved efficiency of the plant-derived therapeutic proteins, the latter can be fused with specific peptides or other receptor-binding ligands, which increase their affinity to the target receptor and permeabilises the blood-brain barrier for the therapeutic drug delivery. This was demonstrated via fusion of myelin basic protein with cholera toxin B subunit (CTB) for Alzheimer's disease treatment, performed by N. Kohli *et al*. (2014). P.S. Lakshmi *et al*. (2013) noted that chloroplast-delivered *Mycobacterium tuberculosis* antigens (ESAT-6, Mtb72F) fused with CTB and cell wall protein LipY demonstrated that such fusion resulted in higher immunogenicity and the possibility of the development of low-cost oral-delivery vaccines.

The production of oral therapeutic agents in some plant species is limited due to the presence of toxic secondary metabolites. This is characteristic of the Solanaceae family species, which are often used for genetic transformation. Therefore, new strategies are being developed to reduce the expression levels of certain toxic secondary metabolites. The study by J. Jeong *et al*. (2024) highlighted the possibility ofnicotine reduction by induction of mutation in BBL and A622 genes by using the CRISPR-Cas9 gene editing system. K.D. DeBoer *et al*. (2011) reported that lowered nicotine content can be achieved via RNA interference method. D. Li *et al*. (2006) highlighted the expression of B-chain of insulin fused to CTB in tobacco plants with reduced nicotine content. Several metabolic engineering strategies were also applied to *S. tuberosum*. L.V.T. Shepherd *et al*. (2015) noted the engineered *S. tuberosum* plants with reduced content of glycoalkaloids, which was achieved via down-regulation of glycosyl transferases.

Increasie of protein stability in plants is a promising area of research, as the final product yield is highly dependent on this parameter. Even with the right choice of producer, transformation method, vector, and additional strategies that promote effective protein expression, protein degradation in the cell will significantly reduce the efficiency of the entire biosynthesis process. The study discussed that protein stability can be increased by targeting the endoplasmic reticulum and chloroplasts through the addition of signal sequences. Co-expression of target proteins with protease inhibitors is also possible. Bioavailability is also a critical parameter in the development of therapeutic protein biosynthesis technology. The main areas of work in this field are the "humanisation" of post-translational modification patterns and the minimisation of the expression of toxic plant secondary metabolites.

CONCLUSIONS

Molecular farming is a promising area of modern plant biotechnology. However, active development is limited by the recalcitrance of many plant

species to existing genetic transformation protocols. Therefore, the analysis of strategies that can increase the efficiency of transformation and the yield of the target protein from the producer is a substantial and relevant research area. The efficiency of therapeutic protein expression in transgenic plants depends on several factors: vector selection and its design, transformation method, translation rate, mRNA and protein stability in the host cell, and the presence of correct post-translational modifications. For the correct selection of the vector, it is necessary to choose the proper transformation method for the biological characteristics and demands of the selected host. It is recommended to use super-binary or ternary vectors with Ti-plasmid-based ones for species recalcitrant to *Agrobacterium*-genetic transformation. In other cases (for example, transformation of dicotyledonous plants), effective transformation can be achieved using binary vectors. The choice of viral vector should be based on the sensitivity of the selected host to the applied virus.

The choice of transgene delivery method also depends on the biological characteristics of the host. As a result, plants with high biomass yield are rationally used for stable transformation, while plants with a short life cycle, relatively low biomass yield, and high sensitivity to plant viruses are used for transient transformation. The choice of optimisation strategy is aimed at improving regeneration, reducing the negative effects of possible interference between target protein synthesis and basic metabolism (stable transformation), and creating favorable conditions for the penetration of a sufficient number of vectors into the plant cell (transient transformation). Optimisation of transcription, translation, and post-translational modification levels should be considered at the vector design stage. To increase the translation rate, a strategy of codon optimisation, co-expression of the target protein with RNA silencing suppressors, and editing of gene regulatory elements is used. At the stage of vector design, it is possible to increase future protein stability by adding signal molecules directing protein expression to the proper cellular compartments. It is necessary to consider the post-translational modifications of the target protein that are essential for its activity *in vivo*.

For proteins with activity determined by certain patterns of glycosylation, sialylation, and other modifications, the need for additional genes or silencing of certain plant genes is considered at the vector development stage. The immunogenicity of therapeutic proteins can be enhanced by fusion of the target protein with peptides that have adjuvant properties or increase the affinity of the protein to the receptor. In addition, the bioavailability and safety of the protein can be increased by altering the secondary metabolism of the plant hosts to reduce the expression of toxic secondary compounds. Maybe soon specially edited plants amenable to genetic transformation producing high yields of correctly glycosylated pharmaceutical proteins without the necessity of complicated protein purification will appear.

Further development of more effective protocols for the genetic transformation and gene editing approaches of cereals, legumes, etc, is required. The stability of recombinant protein storage due to their low moisture and protease content and high protein yield in seeds of these hosts can increase the recombinant protein production. Therefore, molecular strategies to increase plant host regeneration or attempts to increase the bioavailability of obtained proteins are relevant areas of future research. The search for new hosts without harmful secondary metabolites for transient expression of therapeutic proteins and novel vector design is relevant.

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CONFLICT OF INTEREST

None.

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Стратегії оптимізації сталого молекулярного фермерства

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Анотація. Молекулярне фермерство – це галузь сучасної рослинної біотехнології, яка швидко розвивається та дозволяє експресувати біологічно активні терапевтичні засоби для медицини та ветеринарії у промислових масштабах. Метою цього огляду був пошук основних стратегій, що застосовуються для підвищення ефективності виробництва терапевтичних білків в рослинах. Для досягнення мети було застосовано аналіз баз даних Scopus та PubMed, узагальнення та критичну оцінку даних щодо стратегій, що підвищують ефективність виробництва терапевтичного білка в рослинах. Було визначено ключові критерії, які слід враховувати під час вибору вектора для трансформації. Для оптимізації генетичної трансформації стійких видів рекомендуються суперподвійні або третинні вектори. Для транзієнтної трансформації запропоновано системи magnICON та вектори на основі гемінівірусів. Розглянуто способи подолання стійкості рослин до культури *in vitro* та генетичної трансформації. Згадано такі методи, як надекспресія морфогенних генів (WOX, BBM), що підвищують ефективність регенерації. Розглянуто новітні системи доставки генів для точного та правильного введення генів. Для збільшення експресії цільових білків рекомендуються тканинно-специфічні, індуцибельні або активні на певних стадіях розвитку рослин промотори. Визначено, що стабільність білка можна підвищити шляхом його таргетування в клітинні компартменти або коекспресії з інгібіторами протеаз. Також в ході дослідження визначено, що корекція посттрансляційних модифікацій білка може покращити його біологічну активність; зменшення токсичних вторинних метаболітів хазяїна також є однією з цілей. Висновки можуть бути використані для розробки нових стратегій оптимізації трансформації рослин та збільшення виходу рекомбінантного білка

Ключові слова: біотехнологія рослин; генетична інженерія; рослинні системи експресії; фармацевтичні білки

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Innovative methods for diagnosing RNA-containing plant viruses: New approaches to control and prevention

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Abstract. This study aimed to conduct a comparative analysis of traditional methods (enzyme-linked immunosorbent assay, polymerase chain reaction) and innovative techniques for diagnosing ribonucleic acid-containing viruses in plants, in order to assess their effectiveness in detecting pathogens at early stages of infection. Experimental trials were carried out under laboratory conditions using wheat, tomato, and cucumber samples infected with typical viral agents. The detection limit, specificity, analysis duration, cost, and scalability of each method were examined. It was found that modern molecular approaches – particularly targeted cleavage of genetic material using clustered regularly interspaced short palindromic repeats with associated proteins, as well as next-generation sequencing – significantly outperformed traditional methods in terms of sensitivity, accuracy, and the ability to detect viruses in latent form. In particular, the detection limit for the sequencing method was 0.01 copies per microlitre, while for the targeted cleavage method it was 0.1 copies per microlitre. The targeted cleavage technology offered an effective balance of speed (1-2 hours), cost (approximately 20 USD per sample), and throughput (up to 200 samples per day), whereas next-generation sequencing, despite its highest accuracy, required considerable financial and technical resources. The practical significance of the results lies in providing a scientific basis for integrating innovative diagnostic platforms into the phytosanitary control system. The proposed approaches enhance the efficiency of viral pathogen detection, reduce economic losses in crop production, and support the development of modern biosecurity strategies in agriculture

Keywords: pathogen diagnostics; detection sensitivity; latent infections; agricultural biosecurity; molecular analytical methods; phytovirus monitoring

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INTRODUCTION

Ribonucleic acid (RNA) plant viruses represent one of the major threats to the stability of agricultural production, causing significant yield losses and deterioration in product quality. Timely detection of viral pathogens is essential for limiting the spread of infections; however, traditional diagnostic methods often fail to achieve the required accuracy or speed. This underscores the need for implementing innovative approaches capable of improving the effectiveness of phytosanitary monitoring. The relevance of the topic is determined by several factors. Climate change and the intensification of anthropogenic pressure on agroecosystems have led to an increase in the number of new and recombinant viral strains, complicating diagnosis. At the same time, the growing demands of food security require precise and rapid methods for early pathogen detection under field conditions. Traditional methods, such as enzyme-linked immunosorbent assay and polymerase chain reaction, remain widely used but are frequently outperformed by modern molecular technologies in terms of sensitivity and specificity.

In the scientific literature, the issue of diagnosing plant viral diseases has been examined from multiple perspectives. In the study by A.O. Potrokhov (2024), it was demonstrated that assessing the virus resistance of transgenic plants of the Solanaceae family requires the application of highly precise molecular diagnostic methods capable of detecting infection at early stages of development. The author emphasised the importance of integrating biotechnological tools into the phytosanitary control system. The study of V.Yu. Mashika & L.Yu. Pushkash (2024), devoted to pathogenic human viruses, summarised knowledge on immune response mechanisms in viral infections, which is relevant for understanding virus–plant interactions at the molecular level. In the study by T.P. Shevchenko *et al.* (2019), attention was drawn to the role of specific molecular markers in diagnosing infectious agents. The authors highlighted that the effectiveness of such methods in crop production largely depends on the quality of biomaterial preparation and the validation of diagnostic protocols. It was particularly noted that test sensitivity directly depends on the quality of RNA extraction, confirming the advisability of conducting analyses under laboratory conditions to ensure the reliability of results.

A review of modern approaches to managing viral diseases of agricultural crops was presented in the article by S. Tatineni & G.L. Hein (2023). The researchers pointed out the insufficient sensitivity of traditional methods for detecting viruses under field conditions and emphasised the potential of using advanced molecular technologies. In the study by B. Devi *et al.* (2024), a comprehensive analysis of strategies for diagnosing viral infections in plants was carried out. The authors established that classical methods, including enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR), have limitations in specificity and sensitivity, particularly when dealing with asymptomatic infections or emerging viral strains. Special attention was given to the advantages of innovative platforms based on next-generation sequencing (NGS) and CRISPR/Cas12a (clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins), which enable the detection of viral infections before the appearance of clinically evident symptoms.

In the monograph by S. Kumar & S. Maurya (2021), particular emphasis was placed on innovative diagnostic tools that provide exceptionally high testing sensitivity and minimise the likelihood of false results, even in cases of mixed infections. A comprehensive analysis of current and prospective diagnostic methods was presented in the research of A. Singh *et al.* (2025), which described the trend towards a shift from traditional approaches to molecular technologies with greater sensitivity and speed. The integration of RNA interference (RNAi) strategies for protecting plants against viral infections was examined by I.T. Bocos-Asenjo *et al.* (2022). The authors highlighted the potential of RNAi mechanisms not only as a tool for precise virus detection but also as an effective means of controlling pathogens at the level of genomic regulation. Specifically, RNAi technologies suppress the expression of viral genes by introducing double-stranded RNA molecules that activate the plant's cellular defence mechanisms. This approach ensures high specificity of action, minimises damage to non-target organisms, and can be adapted for the development of transgenic varieties with enhanced virus resistance.

In the context of advances in molecular diagnostics of plant viruses, particular attention is drawn to high-throughput methods that enable the simultaneous identification of a wide range of pathogens, including viruses, viroids, and phytoplasmas. Such approaches are discussed in the study of L. Rithesh *et al.* (2025), which substantiates the advantages of next-generation molecular platforms for the rapid and accurate detection of pathogens in complex biological samples. It is noted that integrating high-throughput sequencing with bioinformatic tools opens new opportunities for comprehensive monitoring of viral infections in agriculture. D. Trippa *et al.* (2024) emphasised that next-generation sequencing methods provide the highest sensitivity for early diagnosis of diseases in cultivated plants, yet require substantial resources for implementation in agricultural production. A review of the literature indicates that, despite the rapid development of innovative diagnostic technologies, the practical comparison of these with traditional approaches under real production conditions remains insufficiently explored, particularly in relation to criteria such as sensitivity, specificity, analysis time, cost-effectiveness, and scalability.

Based on the identified gaps, the aim of the present study was formulated: to determine the effectiveness of traditional and innovative methods for diagnosing RNA-containing viruses in plants by assessing their technical characteristics, economic feasibility, and potential for practical application under laboratory control conditions. To achieve this aim, the following objectives were set: to determine the sensitivity, specificity, and detection limits of traditional and innovative diagnostic methods; to assess their effectiveness under laboratory conditions; and to identify the limitations and prospects for applying innovative technologies in the rapid monitoring of viral infections in crop production.

MATERIALS AND METHODS

The research was conducted between March and December 2024 at the Laboratory of Environmental Plant Genetics and Biotechnology of the State Institution "Institute of Plant Protection of the National Academy of Agrarian Sciences of Ukraine" (Kyiv), which specialises in the application of molecular genetic methods for the identification of phytopathogens and genes conferring resistance to diseases and pests in agricultural crops. Plant samples were collected from farms belonging to three agricultural enterprises: Agro-Centre Limited Liability Company (LLC) (Kyiv region), Zoria Agricultural Farm (AF) (Odesa region), and Ovochevyi Rai Private Enterprise (PE) (Odesa region). These enterprises were selected for their active involvement in the cultivation of wheat, tomatoes, and cucumbers, as well as for the presence of reported cases of viral diseases in these crops. The study utilised plant samples of wheat (*Triticum aestivum* L.), tomato (*Solanum lycopersicum* L.), and cucumber (*Cucumis sativus* L.). The total sample size comprised 500 plant specimens, determined through a preliminary statistical power analysis ($\alpha = 0.05$; $\beta = 0.2$; medium effect size), which allowed for the detection of differences of up to 10% between the diagnostic methods. Samples were collected randomly from three sources: open field plots, greenhouses, and experimental plantations. The quantitative distribution across crops was as follows: 200 cucumber samples, 150 tomato samples, and 150 wheat samples. For each crop, appropriate diagnostic methods were applied: all four methods (ELISA, RT-qPCR, CRISPR/Cas12a, NGS) for cucumbers (50 samples per method); three methods (ELISA, RT-qPCR, CRISPR/Cas12a) for tomatoes (50 samples per method); and two methods (RTqPCR, NGS) for wheat (75 samples per method). Subsamples were formed randomly, taking into account the inclusion criterion of visible symptoms or suspected latent infection. Exclusion criteria included mechanical damage or confirmed bacterial aetiology of the lesion.

Within the study, each of the six viruses was associated with its corresponding crop, enabling a comparative evaluation of diagnostic methods within specific phytopathosystems. Cucumber samples (*Cucumis sativus* L.) were tested for Cucumber mosaic virus, a typical pathogen of this crop. This group was subjected to all four diagnostic methods – ELISA, RT-qPCR, CRISPR/Cas12a, and next-generation sequencing (NGS) – allowing for a comprehensive comparison of approaches. Tomato samples (*Solanum lycopersicum* L.) were examined for Tomato spotted wilt virus and Squash yellow mosaic virus, both of which can also infect tomatoes under mixed infection conditions. For these pathogens, three diagnostic methods were applied: ELISA, RT-qPCR,

and CRISPR/Cas12a. The NGS method was not used for these viruses due to funding constraints and the extended time required for analysis.

In the wheat group (*Triticum aestivum* L.), the target pathogen was Grapevine leafrollassociated virus, detected in laboratory samples as a potential model of a latent infectious process. Although this virus is not a typical wheat pathogen, it was selected as a model of latent infection owing to its stable inoculation under laboratory conditions, well-characterised genomic structure, and absence of visible symptoms during the first 72 hours after infection – criteria suitable for testing detection limits. For this crop, only RT-qPCR and NGS were employed. Additionally, wheat samples included control targets – Tobacco mosaic virus and Barley yellow dwarf virus – to verify the specificity of the diagnostic protocols. These were not primary viruses for the main analysis but were used to assess false-positive signals and cross-reactivity. Thus, not all viruses were tested across all crops. The complete set of diagnostic methods was applied only to Cucumber mosaic virus, providing a representative basis for comparing the performance of diagnostic platforms. Other viruses were analysed partially, following their biological characteristics and the methodological constraints in place. This should be taken into account when interpreting the aggregated results.

To analyse the detection dynamics at the early stages of infection, separate subsamples (n = 50) were formed for each crop and method, which were examined at time points of 12, 24, 48, and 72 hours post-infection. In particular, for the CRISPR/Cas12a method, such dynamics were assessed in tomato samples (Tomato spotted wilt virus) and cucumber samples (Cucumber mosaic virus). To model economic efficiency, two parallel subsamples of 50 artificially infected tomato (*Solanum lycopersicum* L.) samples with Tomato spotted wilt virus were formed, in which the virus was detected using either CRISPR/Cas12a or ELISA. Both groups were analysed for detection time and associated yield losses within a laboratory scenario, with plant isolation immediately after infection confirmation. Yield losses were calculated by comparing the biomass of infected plants that remained in the population until the appearance of symptoms with that of a control group (n = 10), where infected plants were removed immediately after laboratory confirmation.

ELISA diagnostics were performed using commercial kits from Agritest (Italy), RT-qPCR was carried out on the CFX96 Real-Time PCR Detection System platform (Bio-Rad, USA), CRISPR/Cas12a assays were implemented using kits from Sherlock Biosciences (USA), and nextgeneration sequencing was conducted on the MiSeq platform (Illumina, USA). All analyses were performed in parallel under laboratory conditions. Both negative controls (no RNA) and positive controls (known virus concentration) were used to ensure testing accuracy. To determine the limit of detection (LOD), serial dilutions of viral RNA were prepared. The specificity of the methods was assessed by testing for cross-reactivity with phytoplasma, Tobacco mosaic virus, and Barley yellow dwarf virus. Additional cross-reactivity testing was performed exclusively on wheat (*Triticum aestivum* L.) samples, which were selected as a model system for specificity assessment due to the absence of the target pathogen at the time of sampling. Other crops were not included in the crossreactivity analysis. The ability to detect latent infections was evaluated by sampling plant material at 12, 24, 48, and 72 hours after artificial infection through mechanical inoculation. This involved rubbing the leaf surface with an inoculation suspension containing concentrated viral RNA and an abrasive (carborundum, 600 mesh), under the standard laboratory infection protocol.

Data processing was carried out in the R software environment, version 4.3.1, using the ggplot2 and dplyr packages. Statistical comparison of method sensitivity employed one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, as well as Student's t-test for independent samples and the Mann-Whitney U test for non-parametric data. The reliability of the results was evaluated using the bootstrap method (1,000 iterations) with the construction of 95% confidence intervals. The critical significance level was set at $\alpha = 0.05$. The comprehensive evaluation of method performance encompassed sensitivity, specificity, limit of detection, analysis duration, test cost, cross-reactivity frequency, and scalability potential in laboratory practice. Experimental research on plants, including the collection of plant material, complied with institutional, national, or international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

The comparative evaluation of diagnostic methods for RNA-containing plant viruses revealed distinct differences between traditional and innovative approaches. Enzyme-linked immunosorbent assay (ELISA) and real-time reverse transcription polymerase chain reaction (RT-qPCR) exhibited lower sensitivity when detecting low concentrations of viral RNA. In contrast, CRISPR/Cas12a systems and next-generation sequencing (NGS) provided higher accuracy and enabled the identification of viruses at early stages of infection. Table 1 below summarises the comparative characteristics of these methods according to key performance criteria: sensitivity, specificity, turnaround time, analysis cost, requirement for specialised equipment, and suitability for use outside laboratory settings. All methods were tested under laboratory conditions.

Table 1. Comparative characteristics of diagnostic methods for RNA-containing viruses in cucumber, tomato, and wheat samples, according to actual laboratory

Method	Sensitivity (copies/μL)	Specificity	Analysis time	Cost (USD/sample)	Equipment requirements
ELISA	10	85%	4-6 hours	5	Medium
RT-qPCR	1	95%	2-3 hours	15	High
CRISPR/Cas12a	0.1	99%	1-2 hours	20	High
NGS	0.01	100%	24-48 hours	100	Very high

Note: data for NGS refer only to cucumber (Cucumber mosaic virus) and wheat (Grapevine leafroll-associated virus); the method was not applied to tomato (Tomato spotted wilt virus) due to constraints. ELISA was not applied to wheat samples
Source: developed by the authors based on original data

As shown in the Table 1, the NGS method achieved the highest sensitivity (down to 0.01 copies of viral RNA per microlitre) and specificity; however, it was also the most resource-intensive in both time and cost. The CRISPR/Cas12a method combined high accuracy with a relatively short analysis time (up to two hours) but also required specialised laboratory equipment, making its use outside a laboratory setting impractical. RT-qPCR remained the most balanced option in terms of reproducibility, moderate cost, and adequate sensitivity. ELISA proved to be the least effective, both in terms of sensitivity and its ability to detect infection at early stages. To illustrate the results, Figure 1 presents the average detection efficiency for each method applied to cucumber, tomato, and wheat samples within the scope of their actual use. Data are provided only for crop–virus–method combinations used in the study: cucumbers (*Cucumis sativus* L.) – Cucumber mosaic virus: all four methods (ELISA, RT-qPCR, CRISPR/Cas12a, NGS); tomatoes (*Solanum lycopersicum* L.) – Tomato spotted wilt virus: three methods (ELISA, RT-qPCR, CRISPR/Cas12a); wheat (*Triticum aestivum* L.) – Grapevine leafroll-associated virus: two methods (RT-qPCR, NGS). NGS was not applied to tomatoes, and ELISA was not applied to wheat (Fig. 1).

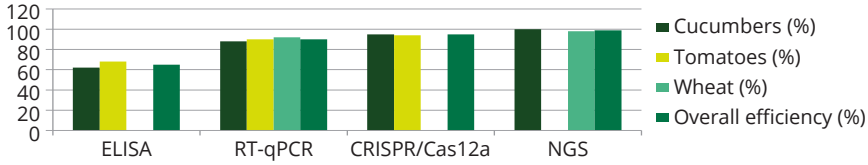

Figure 1. Average detection efficiency of RNA-containing viruses in cucumber, tomato, and wheat samples depending on the diagnostic method applied (n = 500)
Note: n = 500 represents the total number of plant samples examined for the comparative assessment of methods: 200 cucumber samples (50 per method – ELISA, RT-qPCR, CRISPR/Cas12a, NGS), 150 tomato samples (50 per method – ELISA, RT-qPCR, CRISPR/Cas12a), and 150 wheat samples (75 per method – RT-qPCR, NGS). Data are grouped by crop according to the diagnostic methods applied
Source: developed by the authors based on original data

The analysis (Fig. 1) confirmed that NGS and CRISPR/Cas12a methods achieved substantially higher detection efficiency compared with ELISA and RT-qPCR, particularly when applied to samples at early stages of infection. However, their laboratory complexity and resource requirements must be carefully considered when planning their use in phytosanitary monitoring systems. To statistically assess differences in method sensitivity, p-value analysis was conducted using test results from wheat, tomato, and cucumber samples under laboratory conditions. The comparison between CRISPR/Cas12a and real-time polymerase chain reaction (RT-qPCR) showed a statistically significant difference ($p < 0.05$), indicating the higher sensitivity of CRISPR/Cas12a. In contrast, the difference between CRISPR/Cas12a and next-generation sequencing (NGS) was not statistically significant ($p = 0.15$), suggesting a comparable level of effectiveness between these two methods in detecting RNA-containing viruses in the tested crops.

The choice of molecular diagnostic method should take into account the laboratory environment, technical capabilities, urgency of analysis, and resource availability. In cases requiring large-scale testing within limited budgets, ELISA and RT-qPCR remain suitable for laboratory use due to their relative simplicity, low cost, and sufficient accuracy. Conversely, for applications where achieving maximum sensitivity, specificity, and the ability to detect viruses at early or latent stages is critical, CRISPR/Cas12a and NGS remain the most effective options. These methods proved to be the most informative for detailed laboratory analysis of viral infections in agricultural crops.

A comparative analysis of traditional and innovative diagnostic methods, conducted under laboratory conditions, confirmed the limitations of classical approaches, particularly ELISA and RTqPCR, in detecting viral pathogens at early stages of infection and when viral RNA concentrations are low. In the study by Y.M. Wang *et al*. (2022), the advantages of modern molecular platforms in diagnosing latent infections were examined in detail. Similar conclusions were reached in the research of N. Yadav & S.P. Khurana (2016), which highlighted the insufficient sensitivity of traditional serological methods for the early detection of plant viral infections and emphasised the need to introduce molecular tools for identifying latent disease forms. The publication by A. Mandal *et al*. (2025) focused on the advantages of innovative approaches, particularly CRISPR systems, for the highly specific identification of novel and mutant viral strains, aligning with findings from the present study that demonstrated the high efficiency of CRISPR/Cas12a in detecting mutations. Likewise, the study of A. Olmos *et al*. (2007) noted the superiority of molecular methods, such as RT-PCR, in accurately diagnosing complex pathogens, a point supported in this study by the absence of cross-reactivity when using RT-qPCR and CRISPR/Cas12a.

This was made possible by the ability of next-generation sequencing to perform whole-genome analysis without the need for prior knowledge of the virus's nucleotide sequence. The obtained results were consistent with the findings of M.M. López *et al.* (2003), which demonstrated that nextgeneration sequencing enables the detection of previously unknown viral pathogens in complex plant samples without the need for prior identification. In the study by C. Delmiglio *et al.* (2023), the effectiveness of next-generation sequencing as a tool for strengthening national biosecurity through the early diagnosis of novel pathogens was emphasised – an application also demonstrated in the present research in the context of detecting viruses at low concentrations. The experience described by G. Tarquini *et al.* (2023) highlighted the practical significance of sequencing for monitoring phytoviruses in agricultural crops within intensive farming zones – conditions comparable to those replicated in the laboratory modelling conducted in this experiment.

A separate branch of the experiment focused on assessing the capability of the CRISPR/Cas12a system to detect early mutations in the known RNA-containing Tomato spotted wilt virus, a typical pathogen of *Solanum lycopersicum* L. (tomato). The experiment was carried out on laboratoryinfected tomato samples ($n = 50$), which formed part of the subsample designated for testing via the CRISPR/Cas12a method. This method was selected for its high specificity to point mutations and sensitivity to nucleotide sequence alterations at the early stages of infection. As a result, within 12 hours of controlled inoculation, CRISPR/Cas12a successfully detected

65% of characteristic point mutations. After 24 hours, this figure reached 90%, and after 48 hours – 95% (Fig. 2). The data obtained demonstrate the high sensitivity of CRISPR/Cas12a to molecular changes in the viral genome, even at the early stages of pathogenesis. The method proved particularly effective in cases where RT-qPCR failed to provide a stable signal due to insufficient target RNA quantity. This capacity for early mutation detection is promising for monitoring viral evolution and controlling the emergence of new strains (Fig. 2).

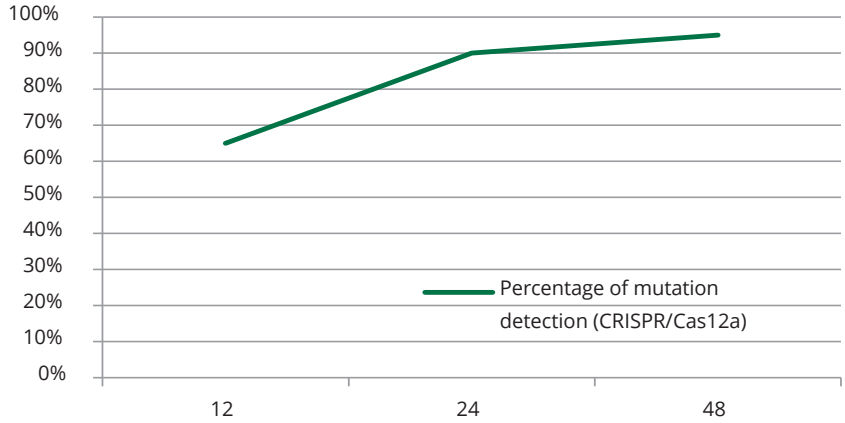

Figure 2. Dynamics of detecting point mutations of the Tomato spotted wilt virus in *Solanum lycopersicum* L. (tomato) samples using the CRISPR/Cas12a system under laboratory conditions (n = 50)
Source: developed by the authors based on original data

The graph (Fig. 2) illustrates the increase in mutation detection efficiency over time following inoculation. Unlike conventional methods, which require the accumulation of substantial amounts of viral material, the CRISPR/Cas12a system enabled the detection of molecular changes within the first 24 hours. A separate example of the laboratory application of CRISPR/Cas12a demonstrated its ability to diagnose Cucumber mosaic virus in *Cucumis sativus* L. (cucumber) samples collected during the study that showed no visible symptoms at the time of testing. A positive result was recorded 10 days before the first external signs of infection appeared. This made it possible to simulate a rapid response scenario aimed at isolating conditionally infected plant samples, thereby confirming the method's potential for the preventive detection of pathogens at the latent stage. This approach has gained particular significance in the context of active viral evolution and the high susceptibility of agroecosystems to the spread of infections. The combined use of next-generation sequencing (NGS) for the identification of novel viruses and the CRISPR/Cas12a system for tracking genomic mutations in established pathogens has formed the conceptual basis of a new model for laboratory-based plant biosecurity. It has been established that under conditions of increasing anthropogenic pressure and climate change, which favour the emergence of new pathogens, such a combination of methods ensures the timely detection of infectious agents and the monitoring of their evolutionary dynamics – an aspect of critical importance in the context of food security.

The results obtained were compared with findings from other scientific studies. In the research of M. Raza *et al.* (2025), it was demonstrated that optimising guide RNAs in CRISPR systems can substantially reduce the risks of cross-reactivity when diagnosing viruses with high sequence homology, which aligns with the present findings on the high specificity of CRISPR/Cas12a in detecting Cucumber mosaic virus. The study by A. Soni *et al.* (2024) confirmed the effectiveness of the CRISPR/Cas13a system in detecting mutations in Tomato spotted wilt virus with a sensitivity exceeding 95%, consistent with the detection rates established in this research. In the study by

B. Krenz *et al.* (2024a), emphasis was placed on the ability of the CRISPR/Cas12a system to identify point mutations within 24 hours of infection, which corresponds with the results obtained from the temporal dynamics analysis.

The analysis of sensitivity and specificity was of critical importance for assessing the effectiveness of diagnostic methods, particularly under conditions of low viral RNA concentration or the presence of genetically related pathogens. Innovative approaches, notably CRISPR/Cas12a systems and next-generation sequencing (NGS), demonstrated substantial advantages over traditional technologies; however, they required separate evaluation in the context of the limit of detection (LOD) and the risk of cross-reactivity, which can distort diagnostic results in cases of mixed infections. The comparison was conducted on laboratory samples as follows: for cucumber (*Cucumis sativus* L.), Cucumber mosaic virus was diagnosed using four methods – ELISA, RT-qPCR, CRISPR/Cas12a, and NGS; for tomato (*Solanum lycopersicum* L.), Tomato spotted wilt virus was diagnosed using three methods – ELISA, RT-qPCR, and CRISPR/Cas12a (NGS was not applied); for wheat (*Triticum aestivum* L.), Grapevine leafroll-associated virus was diagnosed using two methods – RT-qPCR and NGS (ELISA and CRISPR/Cas12a were not applied). In total, 500 samples were analysed: 200 cucumber samples (50 for each of the four methods), 150 tomato samples (50 for each of the three methods), and 150 wheat samples (75 for each of the two methods) (Table 2).

Table 2. Comparison of sensitivity, specificity, and cross-reactivity of diagnostic methods for RNA-containing viruses in cucumber, tomato, and wheat samples under laboratory conditions

Method	Limit of detection (LOD)	Specificity	Cross-reactivity (number of tested pathogens)
ELISA	10 copies/μL	85%	15% (phytoplasma, bacteria)
RT-qPCR	1 copy/μL	95%	5% (viral strains with similar sequences)
CRISPR/Cas12a	0.1 copies/μL	99%	0% (10 related RNA-containing viruses)
NGS	0.01 copies/μL	100%	0% (metagenomic selectivity)

Note: the distribution of subsamples corresponded to the actual application of methods for each crop without overlap between groups
Source: developed by the authors based on original data

As shown in Table 2, the highest sensitivity was recorded for NGS (0.01 copies/μL), enabling pathogen detection even at minimal viral loads. The CRISPR/Cas12a system demonstrated a detection threshold of 0.1 copies/μL, surpassing RT-qPCR by a factor of ten. The ELISA method had the highest limit of detection (10 copies/μL) and the lowest specificity among all tested technologies. In contrast, CRISPR/Cas12a and NGS exhibited no cross-reactivity in any of the tests, confirming their advantage in diagnosing mixed infections. By comparison, RT-qPCR produced false-positive signals in 5% of cases when genetically related viruses were present, while ELISA produced such signals in 15% of cases in the presence of phytoplasma and bacterial agents. These findings confirm the suitability of highly sensitive molecular platforms for the diagnosis of latent infections, particularly in situations where traditional methods do not provide the required accuracy.

During cross-reactivity testing, RT-qPCR and NGS, applied to wheat (*Triticum aestivum* L.) samples, demonstrated high specificity. Verification was carried out by additional testing for the presence of related RNA-containing pathogens – Tobacco mosaic virus and Barley yellow dwarf virus – which served as control organisms. Neither method produced false-positive results, indicating their high specificity and absence of cross-reactivity. The NGS method enabled precise reading of unique nucleotide sequences, eliminating the risk of misidentification in complex samples. Although RT-qPCR exhibited high sensitivity, it produced occasional false-positive signals in the presence of viruses with close homology; however, such cases accounted for less than 5%. The CRISPR/Cas12a and ELISA methods were not applied to wheat and were therefore excluded from the specificity analysis at this stage of the study.

Innovative approaches, particularly CRISPR/Cas12a and NGS, not only provided lower limits of detection but also demonstrated enhanced specificity, thereby minimising the risk of diagnostic errors. This combination of parameters is crucial when monitoring emerging or mutant virus variants, where accurate viral identification is essential for rapid response and containment. It was established that under conditions of potential multi-component infections or in the early stages of disease development, CRISPR/Cas12a and NGS maintained consistent performance, whereas traditional methods exhibited significant limitations. In addition to the technical characteristics of the methods themselves, the accuracy and reproducibility of results were strongly influenced by the quality of plant sample preparation. In a study by N.V. Verma *et al.* (2022), the importance of standardising ribonucleic acid extraction procedures was emphasised as a key factor in improving the reliability of molecular diagnostics. The findings of the present experiment confirmed this relationship: samples processed according to high-purity RNA extraction protocols exhibited more consistent signals in RT-qPCR and CRISPR/Cas12a, as well as reduced variability in repeated measurements.

The practical effectiveness of diagnostic methods is determined not only by their technical characteristics but also by temporal, financial, and logistical factors. Even with high sensitivity, innovative techniques may be less suitable for large-scale implementation owing to high costs, limited availability of specialised equipment, or the requirement for highly trained personnel. For a comprehensive evaluation, the diagnostic methods were compared according to the criteria of average analysis duration, cost per test, scalability, and availability of laboratory equipment (Table 3). The ELISA method was applied to cucumber samples (Cucumber mosaic virus) and tomato samples (Tomato spotted wilt virus). The RT-qPCR method was used for all three crops: cucumbers (Cucumber mosaic virus), tomatoes (Tomato spotted wilt virus), and wheat (Grapevine leafrollassociated virus). The CRISPR/Cas12a method was applied to cucumbers and tomatoes, while the NGS method was used only for cucumbers and wheat, owing to funding constraints and the extended analysis cycle. The total number of samples analysed was 500: 200 cucumbers, 150 tomatoes, and 150 wheat samples.

As shown in Table 3, the CRISPR/Cas12a method provided the shortest analysis time (on average 1.5 hours) and a high potential for scalability, offering the capacity to process a substantial number of samples under laboratory conditions. However, its application remained limited by the low availability of specialised equipment and the need to optimise the reaction system for each target virus. The RT-qPCR method, although slower, offered a balanced compromise between cost, processing time, and equipment requirements, which underpins its use as the baseline standard for diagnostics.

Table 3. Comparison of analysis time, cost per test, scalability, and availability of laboratory equipment for diagnostic methods applied to the detection of RNA-containing viruses in cucumber, tomato, and wheat samples

Method	Average analysis time	Cost per sample (USD)	Scalability	Equipment availability
ELISA	4-6 hours	5	Low	High
RT-qPCR	2-3 hours	15	Medium	Medium
CRISPR/Cas12a	1-2 hours	20	High	Low
NGS	24-48 hours	100	Very low	Very low

Note: all methods were used exclusively under laboratory conditions
Source: developed by the authors based on original data

Next-generation sequencing (NGS), despite its superior analytical accuracy, required 24 to 48 hours to process a single sample and was the least accessible in terms of both cost (100 USD per sample) and technical demands. The ELISA method, owing to its low cost, is suitable for routine screening; however, its limited sensitivity restricts its use for the early detection of infections. Therefore, when selecting a diagnostic method, it is essential to consider not only analytical efficiency but also the practical feasibility of implementation, depending on available

resources, the nature of the tasks, and the number of samples to be analysed.

Speed and efficiency emerged as key criteria for comparing the methods, particularly in the context of detecting RNA-containing viruses at the latent stage. Under laboratory conditions, CRISPR/Cas12a systems enabled the processing of up to 200 samples per day through an optimised parallel reaction protocol, although they required specialised equipment. The next-generation sequencing (NGS) method, despite its high analytical potential, was limited to an average throughput of 10 samples per day, owing to the time required for library preparation and the computational complexity of data analysis.

The ELISA and RT-qPCR methods remained suitable for use in settings with limited funding, particularly for large-scale routine screening. The results obtained were consistent with the findings of M. Sakthivel *et al.* (2024), which reported the effectiveness of these methods for the rapid diagnosis of infections in silkworms within sericulture farms. The authors emphasised the speed, simplicity, and cost-effectiveness of such technologies, confirming their value as fundamental tools for the initial detection of pathogens where access to advanced equipment is limited. However, in cases of low pathogen concentration or absence of clinical symptoms, their effectiveness was considerably reduced. The choice of diagnostic method depended on specific requirements: CRISPR/Cas12a was considered the most suitable for rapid detection and mutation screening; NGS for identifying novel or mixed infections; and RT-qPCR and ELISA for baseline monitoring under stable viral conditions. This justified the need for a combined approach, adapted to the target objectives and resource constraints. The data were obtained under laboratory conditions using subsamples of 50 specimens per method for cucumber and tomato cultures, and 75 specimens per method for wheat, in accordance with the actual application of the methods. Within each subsample, the dynamics of latent infection detection and the associated yield losses were assessed. Cucumber (*Cucumis sativus* L.) – Cucumber mosaic virus: all methods (ELISA, RT-qPCR, CRISPR/Cas12a, and NGS) were tested. Tomato (*Solanum lycopersicum* L.) – Tomato spotted wilt virus: ELISA, RT-qPCR, and CRISPR/Cas12a were used (NGS was not applied). Wheat (*Triticum aestivum* L.) – Grapevine leafroll-associated virus: RT-qPCR and NGS were employed (ELISA was excluded due to low specificity; CRISPR/Cas12a was not used owing to the model-specific nature of the virus). The values presented in Table 4 were averaged from results obtained only for those crops to which the respective method was actually applied.

Table 4. Comparison of diagnostic methods by time of virus detection at the latent stage of infection, using cucumber, tomato, and wheat as examples

Method	Time to detection after infection	Detection efficiency	Impact on yield*
ELISA	72 hours	35%	15-20% loss
RT-qPCR	48 hours	60%	10-15% loss
CRISPR/Cas12a	24 hours	80%	5-7% loss
NGS	24 hours	95%	3-5% loss

Note: * – yield losses were estimated under a model scenario in which infected plants were removed immediately after diagnosis, compared with groups where removal occurred after the onset of symptoms
Source: developed by the authors based on original data

As shown in Table 4, CRISPR/Cas12a and NGS demonstrated the capacity to detect infection as early as 24 hours after inoculation, achieving 80-95% detection efficiency and enabling the modelling of minimal yield losses. By comparison, RT-qPCR detected viruses with up to 60% efficiency at 48 hours post-infection, while ELISA detected them in only 35% of cases, with a delay of up to 72 hours. This confirmed the advisability of using highly sensitive methods in laboratory monitoring systems for early diagnosis and minimisation of economic risks caused by viral infections (Fig. 3).

Figure 3 illustrates changes in the efficiency of Tomato spotted wilt virus detection at the latent infection stage depending on the diagnostic method used. In a laboratory experiment on 50 tomato

(*Solanum lycopersicum* L.) samples infected with Tomato spotted wilt virus, the CRISPR/Cas12a system provided positive detection in 80% of samples as early as 24 hours after artificial inoculation. By comparison, ELISA detected only 10% of infected samples within the same time frame. By 72 hours, CRISPR/Cas12a efficiency increased to 95%, whereas ELISA reached only 35% positive results.

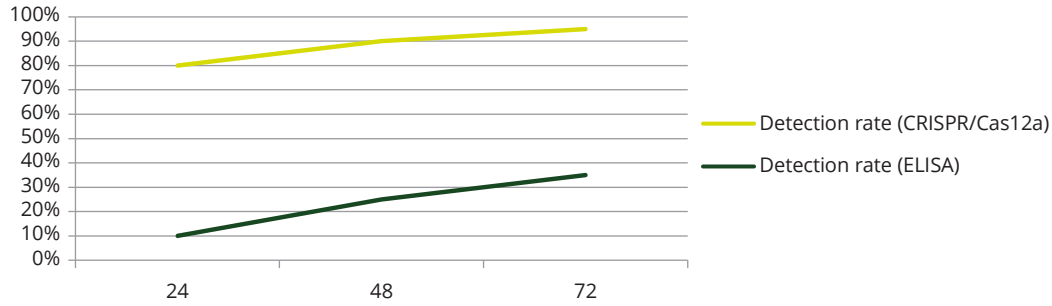

Figure 3. Dynamics of Tomato spotted wilt virus detection at the latent stage in tomato (*Solanum lycopersicum* L.) samples using ELISA, RT-qPCR, and CRISPR/Cas12a methods under laboratory conditions (n = 50 per method)

Note: data for RT-qPCR were obtained but not included in the figure due to high variability of signals during the first 24 hours, which impeded accurate representation of comparative dynamics. The comparison is based on the occurrence of positive diagnoses at each time point within the examined latent infection period

Source: developed by the authors based on original data

Economic impact modelling based on the obtained data demonstrated that the use of CRISPR/Cas12a reduced tomato yield losses by 50% compared with the control subsample of 50 specimens, in which Tomato spotted wilt virus was detected using ELISA. Statistical analysis confirmed significant differences between the methods (Student's t-test, $p < 0.001$), indicating the advantage of highly sensitive molecular tools for detecting viruses at the early stages of infection development. The study also established that in groups where diagnosis was performed using CRISPR/Cas12a, Tomato spotted wilt virus was detected prior to the appearance of visible symptoms, including necrotic spots. Removal of infected plants immediately after laboratory confirmation, followed by targeted treatment, reduced virus transmission within the population by almost 50%. In contrast, in the groups where ELISA was applied, intervention was carried out only on the third day after infection, resulting in yield losses increasing to 30%. The findings were compared with the conclusions of F. Tenllado *et al.* (2004), who emphasised the importance of molecular diagnostics at preclinical stages of infection for effectively limiting virus spread. Similarly, the research of I. Chakrabartty *et al.* (2022) confirmed that timely detection of infections in tomatoes can substantially reduce yield losses, even without the use of intensive chemical measures. These results were consistent with the outcomes of the laboratory study, demonstrating the advisability of incorporating CRISPR/Cas12a into preventive phytosanitary monitoring systems.

Despite their high sensitivity and specificity, innovative diagnostic methods such as CRISPR/Cas12a and next-generation sequencing (NGS) were subject to a number of limitations that constrained their widespread adoption in laboratory practice. The main barriers related to the need for sophisticated equipment, lengthy optimisation procedures, and high costs, which restricted the accessibility of these technologies to laboratories with limited resources (Table 5). The assessment was based on the results of experimental application of ELISA, RT-qPCR, CRISPR/Cas12a, and NGS to a total sample set of 500 plant specimens: 200 cucumber samples (50 for each method), 150 tomato samples (50 per method), and 150 wheat samples (75 per method). All methods were applied exclusively under laboratory testing conditions. In the study by I. Buja *et al.* (2021), the potential of molecular platforms for rapid viral diagnostics was noted, but difficulties in scaling

up in the absence of adequate infrastructure were emphasised. Similar conclusions were drawn by K. Kalimuthu *et al.* (2022), who highlighted the importance of increasing the adaptability of innovative technologies to local conditions, particularly by reducing costs and simplifying logistics.

Table 5. Main technical, economic, and logistical limitations of diagnostic methods for RNAcontaining viruses, identified under laboratory conditions during the study of cucumber mosaic virus (cucumber), tomato spotted wilt virus (tomato), and grapevine leafroll-associated virus (wheat)

Method	Technical limitations	Economic barriers*	Logistical challenges
CRISPR/Cas12a	Need for gRNA optimisation (2-7 days)	High equipment cost	Limited availability in developing countries
NGS	Complexity of metagenomic data processing	100-150 USD per sample	Lack of trained personnel
RT-qPCR	Dependence on primer quality	15 USD per sample	Requirement for a stable power supply
ELISA	Low sensitivity to novel strains	5 USD per sample	Risk of cross-reactivity

Note: * – barriers were assessed with consideration of reagent costs, technical requirements for equipment, need for skilled personnel, and pathogen-specific adaptation constraints
Source: developed by the authors based on original data

CRISPR/Cas12a and NGS, despite their high accuracy, exhibited the most significant limitations. For example, optimising gRNA for CRISPR/Cas12a could take up to seven days for newly identified viruses, while the cost of NGS rendered it inaccessible for small-scale farms. To illustrate the impact of these factors on practical application, a chart of limitations was constructed. The evaluation was based on the experimental application of the methods to a total sample of 500 plant specimens, collected without overlap: 200 cucumber samples (Cucumber mosaic virus, 50 per each of the four methods), 150 tomato samples (Tomato spotted wilt virus, 50 per method), and 150 wheat samples (Grapevine leafroll-associated virus, 75 per method). CRISPR/Cas12a was applied to cucumbers and tomatoes; NGS was used for cucumbers and wheat; RT-qPCR was applied to all three crops; and ELISA was used only for cucumbers and tomatoes (Fig. 4).

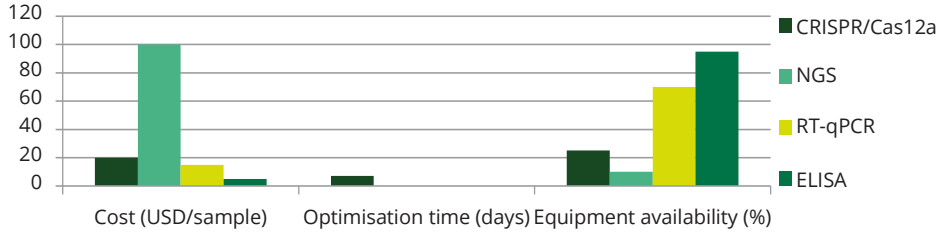

Figure 4. Comparison of key limitations of RNA virus diagnostic methods (CRISPR/Cas12a, NGS, RT-qPCR, ELISA) according to technical and logistical parameters under laboratory testing conditions for cucumber and tomato samples
Source: developed by the authors based on original data

As shown in Table 5 and Figure 4, CRISPR/Cas12a and NGS required the most advanced equipment and resources, which restricted their use in laboratories with limited technical capacity. For example, optimising guide RNAs (gRNAs) for CRISPR/Cas12a required between two and seven days when detecting newly identified viruses, whereas processing metagenomic data with NGS could take up to 48 hours and required qualified bioinformatics support. In addition, the cost of NGS testing (100-150 USD per sample) remained prohibitively high for small research facilities and agricultural producers. The RT-qPCR method proved to be more technically accessible, although

it depended on primer quality and required a stable power supply for the equipment. ELISA remained the cheapest and easiest method to implement, but it showed low efficiency in detecting new or weakly expressed strains, reducing its reliability in diagnosing latent infections.

Prospects for addressing these limitations included several key directions. In particular, the development of universal guide RNAs (gRNAs) for CRISPR/Cas12a systems was proposed, which would shorten the optimisation phase when working with new viruses. For next-generation sequencing (NGS), the use of cloud-based computing platforms with automated metagenomic data analysis was considered promising, as it could significantly reduce bioinformatics costs and simplify procedures for users without specialised training. Another potential solution was the creation of mobile laboratory units for the basic implementation of molecular methods, including CRISPR/Cas12a, in remote regions. These initiatives would require the integration of biotechnological advances with infrastructural changes in the agricultural sector, necessitating an interdisciplinary approach at the intersection of virology, informatics, agro-engineering, and logistics. Despite existing technical and economic barriers, innovative methods remained the most promising tools for high-precision laboratory diagnosis of viral infections, ensuring effectiveness in situations where traditional approaches lacked sufficient sensitivity or adaptability to emerging pathogenic threats.

The findings were correlated with current scientific developments. In the study by B. Krenz *et al*. (2024b), trends in plant virology were analysed, particularly the application of highly sensitive diagnostic platforms for early pathogen detection. The demonstrated effectiveness of CRISPR/Cas12a and NGS in laboratory testing was fully consistent with the authors' conceptual conclusions. In the study of S.K. Sharma *et al.* (2021), emphasis was placed on the potential of CRISPR/Cas12a to enable diagnosis prior to the appearance of clinical symptoms, a finding confirmed by experiments in which viruses were detected as early as 24 hours after infection. Similarly, the study by A.E. Voloudakis *et al.* (2022) examined the prospects for RNA-based technologies in strategies to combat viral infections, particularly at the stages of detection and immunisation. The present data aligned with this approach, as molecular methods based on viral RNA detection proved effective in diagnosing latent infections. In the study of C. Padmanabhan & F.J. Jan (2025), the importance of combining molecular platforms within a single diagnostic system was highlighted as a means of enhancing the resilience of agroecosystems. The results of the study confirmed the value of this approach: the combination of NGS for detecting new pathogens and CRISPR/Cas12a for monitoring mutations provided the highest effectiveness in the assessment of infected plant samples under laboratory conditions.

The study established that CRISPR/Cas12a demonstrated the highest efficiency for laboratory diagnosis of RNA-containing viruses in tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.) samples, delivering high sensitivity and specificity at the early stages of infection. Nextgeneration sequencing (NGS) proved most effective for wheat (*Triticum aestivum* L.L.) and cucumber samples, particularly in the context of comprehensive metagenomic analysis and confirmation of the absence of cross-reactivity. RT-qPCR showed stable performance across all three crops, although some variability in results was observed during the early phase of the latent period. ELISA remained suitable for primary screening but was limited in sensitivity to new strains and specificity in cases of mixed infections.

CONCLUSIONS

The study enabled a comprehensive evaluation of the effectiveness of both traditional (ELISA, RTqPCR) and innovative (CRISPR/Cas12a, NGS) diagnostic methods for RNA-containing viruses in laboratory conditions using samples of wheat, tomato, and cucumber. Each method was applied following the characteristics of the viruses typical for the respective crops, providing representative data that reflected the specificity of the phytopathosystems. It was established that CRISPR/Cas12a, as a highly sensitive diagnostic system, allowed effective detection of Tomato spotted wilt virus in tomatoes and Cucumber mosaic virus in cucumbers at the latent stage, as early as 24 hours postinfection. Next-generation sequencing (NGS), applied to wheat and cucumber samples, achieved maximal sensitivity (LOD = 0.01 copies/μL) and was able to detect even genetically variable or

previously unknown virus strains. Neither NGS nor CRISPR/Cas12a exhibited cross-reactivity, indicating high specificity for both methods. RT-qPCR, applied across all three crops, confirmed its reliability with a moderate limit of detection (1 copy/µL) and a low level of false-positive results. ELISA, used for tomatoes and cucumbers, remained suitable for basic screening but demonstrated the lowest sensitivity (10 copies/µL) and a higher likelihood of cross-reactivity (up to 15%).

Practical results indicate that CRISPR/Cas12a provided an optimal combination of high accuracy, rapid analysis time (1-2 hours), scalability (up to 200 samples/day), and relatively moderate cost (20 USD/sample), making it a promising baseline tool for laboratory-based phytosanitary diagnostics. Although NGS remained the most precise method, its routine application was constrained by high costs (100 USD/sample), the need for sophisticated technical equipment, and specialised personnel. The limitations identified in these innovative methods – specifically the requirement for gRNA optimisation in CRISPR/Cas12a and the complexity of metagenomic data processing in NGS – highlight the need for further research. Future directions include the development of universal gRNA sets, automated analytical platforms, and integrated diagnostic strategies. Both CRISPR/Cas12a and NGS have the potential to substantially enhance agri-biosafety by enabling early detection of infections and reducing crop losses, which is critically important in the context of climate and epidemiological challenges.

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CONFLICT OF INTEREST

None.

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Інноваційні методи діагностики РНК-вмісних вірусів у рослинах: нові підходи до контролю та профілактики

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Анотація. Метою дослідження було здійснення порівняльного аналізу традиційних (імуноферментний аналіз, полімеразна ланцюгова реакція) та інноваційних методів діагностики вірусів, що містять рибонуклеїнову кислоту, у рослинах для оцінки їхньої ефективності у виявленні патогенів на ранніх стадіях інфекційного процесу. Експериментальні випробування проводилися в лабораторних умовах із використанням зразків пшениці, томатів і огірків, інфікованих типовими вірусними збудниками. Було досліджено межу детекції, специфічність, тривалість аналізу, вартість дослідження та можливості масштабного застосування кожного з методів. Установлено, що сучасні молекулярні підходи, зокрема технологія спрямованого розщеплення генетичного матеріалу за допомогою кластерних повторів коротких паліндромних послідовностей зі сполученими білками, а також секвенування нового покоління, значно перевищували традиційні методи за чутливістю, точністю та здатністю до виявлення вірусів у латентній формі. Зокрема, межа детекції для методу секвенування становила 0,01 копії на мікролітр, а для методу спрямованого розщеплення – 0,1 копії на мікролітр. Встановлено, що технологія спрямованого розщеплення забезпечувала ефективне поєднання швидкості (1-2 години), вартості (близько 20 доларів за зразок) та пропускної здатності (до 200 зразків на добу), тоді як секвенування нового покоління, попри найвищу точність, потребувало значних фінансових і технічних ресурсів. Практична значущість результатів полягає у створенні наукового підґрунтя для інтеграції інноваційних діагностичних платформ у систему фітосанітарного контролю. Запропоновані підходи сприяють підвищенню ефективності виявлення вірусних патогенів, зниженню економічних втрат у рослинництві та формуванню сучасних стратегій біобезпеки у сільському господарстві

Ключові слова: діагностика патогенів; чутливість виявлення; латентні інфекції; біобезпека агросектору; молекулярні методи аналізу; моніторинг фітовірусів

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Unauthorised landfills as a source of soil contamination with heavy metals: Phytotoxic aspect

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Abstract. The article examined the issue of soil biotesting as an effective indicator approach to assessing the ecological state of land subjected to anthropogenic pressure from local sources of pollution. The objects of the study were soil samples taken near potentially hazardous man-made sites, namely landfills. Toxicity was assessed using phytotests of oil radish (*Raphanus sativus d*. var. *oleifera* Metzg.), which proved to be a sensitive indicator of the presence of harmful substances in the soil environment. At the same time, an analytical determination of the content of heavy metals (Cd, Pb, Zn, Cu) in the soil was carried out. The results of phytotesting showed a decrease in seed germination compared to control samples, which is evidence of the toxic effect of the soil environment. A decrease in germination energy and suppression of primary biomass growth were also observed. A close correlation was found between high metal content and suppression of growth parameters of oil radish, indicating an interdependence between chemical indicators of pollution and the biological response of organisms. In particular, a decrease in the length of seedlings was observed, indicating the biological activity of heavy metals. In some samples, 30% germination was recorded, indicating a critical level of toxicity. Biotesting combined with chemical analysis made it possible to identify areas with the most critical environmental conditions. The proposed approach can be useful for environmental services, local authorities and land management institutions for operational monitoring of soil condition and identification of pollution hotspots. The methodology can be adapted for monitoring industrial areas, sanitary surveillance areas, and areas near transport routes. The results of the study confirm the feasibility of using integrated approaches to monitoring, including both analytical methods and

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bioindication tests. This approach provides a more objective assessment of environmental risks and can be used to plan measures for the rehabilitation of contaminated areas, soil fertility conservation, and sustainable land use

Keywords: ecotoxicology; germination index; biomonitoring; agroecosystem risks; test plants; toxicological sensitivity

INTRODUCTION

Unauthorised landfills, especially those located near agricultural land, pose a significant environmental threat due to the release of heavy metals into the soil. Heavy metals are highly persistent, accumulate easily and have a toxic effect on plants, soil microflora and food chains. Phytotoxicity – reduced germination, growth inhibition and reduced plant biomass – is often an early indicator of soil contamination. Analysis of the phytotoxicity of soils contaminated with heavy metals due to unauthorised landfills is crucial for assessing environmental risk and protecting food security.

International studies focus on the analysis of heavy metals in soils near illegal landfills and related phytotoxicity. In their article, authors C. Du & Z. Li (2023) investigated soils near a historic landfill in northern China. As part of the study, 376 soil samples were collected to assess the content of nine heavy metals: As, Hg, Sb, Cu, Pb, Cd, Ni, Zn and Tl. The results showed that in many places, metal concentrations exceed the established limits for agricultural land. In addition, based on the calculation of the health risk index, a high potential for carcinogenic effects on the population living near the specified territory was established.

In the article by M. Sabir *et al.* (2022) investigated the level of heavy metal contamination (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn) in soils near open landfills in Pakistan and assessed the risks to human health and phytotoxicity due to the bioaccumulation of metals in plants. The authors analysed soil and plant samples and found that heavy metal content often exceeded safe limits, especially near landfill boundaries. They also showed that plants growing in these conditions could be a source of toxic elements entering the food chain, highlighting the need for monitoring and remediation of such areas. Research by M. Bozym (2022) indicates that leachates from landfill waste are highly toxic to *Lepidium sativum*, leading to a significant reduction in the germination index (GI < 50%), especially in the case of blast furnace dust waste. This indicates the significant phytotoxic potential of waste that enters the soil near landfills.

Among Ukrainian studies, there was a publication by V. Melnychenko (2024) that examined the phytoremediation of soils contaminated by military and anthropogenic influences, with an emphasis on the accumulation of heavy metals and the possibility of using phytotests to assess phytotoxicity. In a study conducted by O. Datsko *et al*. (2024) from Sumy National Agrarian University, an ecological assessment of the heavy metal content in soils in the Sumy and Chernihiv regions contaminated by explosions and anthropogenic influences was carried out, with an analysis of the consequences for agrocenoses. Another study by L. Kucher *et al*. (2023) revealed soil damage near Donbas coal dumps, where exceedances in the concentrations of Pb, Co, Cr, Cu, and Zn led to changes in the composition of plant cover and a decrease in plant biomass.

Other international studies also emphasise the relevance of using phytotesting for early detection of toxicity. For example, S. Morales-Hernández *et al*. (2022) described the use of various plants for phytoremediation of soils from urban landfills in Mexico, where sensitive plants capable of accumulating Pb, Cd, Zn and other metals were identified and indicated a decrease in the viability of test cultures. A. Acharya *et al*. (2023) also analysed the global status and prospects of phytoremediation of heavy metals in soils, in particular emphasising the need for field studies and the use of indicator plant species for effective toxicity assessment and soil remediation. These studies prove that unauthorised landfills are a stable source of heavy metal accumulation in the soil, and phytotesting based on the growth and development of indicator plants effectively reflects the level of phytotoxicity. Despite this, in the Ukrainian context, there is a lack of localised phytotoxic assessments of soils near illegal landfills using modern methods and test cultures. According to research by S. Albert &

E. Bloem (2023), bioindication methods allow for the assessment not only of the presence of individual chemicals, but also of the actual toxicity of mixtures, including their bioavailability and possible synergistic or antagonistic interactions. This gives an advantage over traditional analytical methods, which often do not reflect the full complex toxic impact of pollutants in natural soil conditions.

Such an assessment is more relevant when studying the impact of local sources of pollution, which are characterised by complex, often unstable combinations of toxicants. In addition, soil acts as an important reservoir of organic carbon, which is a critical element of the global carbon cycle. In their work, P. Borrelli *et al*. (2020) emphasise the importance of soil resources in the context of climate change and land use change, noting that the ability of soil to accumulate carbon not only helps maintain fertility but is also one of the leading natural mechanisms for adapting to climate challenges. At the same time, current trends in the intensive use of soil resources in agricultural production are causing accelerated degradation of the soil cover. As noted by O.H. Tarariko *et al*. (2017), such degradation poses a direct threat not only to global food security but also to the long-term sustainable development of humanity.

The aim of the study was to assess the phytotoxic properties of soils contaminated with heavy metals as a result of unauthorised landfills, using biotesting of indicator plants to determine the level of environmental risk to agroecosystems and the potential for subsequent phytoremediation.

MATERIALS AND METHODS

Soil samples were collected in the third decade of July 2024. The research focused on two unauthorised landfills located in the Chernihiv and Cherkasy regions. The first landfill (Object 1) is located within the Nizhyn district, Bakhmach territorial community of the Chernihiv region (51°09'45'N, 32°42'14'E), 17 km from the city of Bakhmach. The Object covers an area of 2.25 hectares. The second landfill (Object 2) is located within the Uman district, Kniazha-Krynytska territorial community of the Cherkasy region (49°06'22'N, 29°44'18'E), 4 km from the village of Kniazha Krynytsia. Its area is 0.2 hectares.

Soil sampling scheme. Soil samples were taken within technologically polluted areas, directly near landfills, at a depth of 5-20 cm. For each of the sites, sampling was carried out in three directions, selected according to the spatial location of the landfill, possible directions of pollutant migration and safe access. At Object 1, samples were taken to the north, northeast and southeast. In each direction, three samples were taken at a distance of 5 metres from the landfill body. At Object 2, sampling was carried out to the west, north and south, also with three samples in each direction at a distance of 5 metres from the landfill body. The control (background) point was established at a distance of about 30 metres from the landfill boundary, in an area that had not been directly affected by human activity. The control point area is characterised by the absence of signs of economic activity, which allows these samples to be used for comparative analysis.

Analytical determination of heavy metal content. Laboratory studies of soil samples were carried out at the State Institution "Derzhgruntohorona". The content of heavy metals (Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}) was determined by atomic absorption spectrophotometry (AAS) using the AAS KVANT-2 AT device, which is highly accurate and widely used in soil chemistry. The samples were prepared by extracting mobile forms of metals with a buffer ammonium acetate solution with a pH of 4.8. After filtration of the extracts, the metal concentrations were determined spectrophotometrically in accordance with the requirements of current national standards:

- DSTU 4770.6:2007 (2007) – copper (Cu);
- DSTU 4770.2:2007 (2007) – zinc (Zn);
- DSTU 4770.3:2007 (2007) – cadmium (Cd);
- DSTU 4770.9:2007 (2007) – lead (Pb).

This approach made it possible to obtain an objective quantitative assessment of the level of soil contamination with potentially toxic elements.

Bioindicative methods: Phytotesting. To assess soil phytotoxicity, *Raphanus sativus d.* var. *oleifera* Metrg (ISO 22030:2005, 2005), a representative of the *Brassicaceae* family, was selected as the biotest object. This species is widely used in biotesting due to its high sensitivity to heavy metals, rapid germination and ease of cultivation in laboratory conditions. Oil radish is recommended by ISO 11269-2:2012 (2012) as a standard test Object for assessing soil phytotoxicity. Other plants were not used in this study because oilseed radish

provides sufficient sensitivity and representativeness for primary environmental monitoring. The involvement of additional species is possible at later stages, but is not critically necessary in this study.

Biotesting methodology. Radish seeds were pre-disinfected in a 1% potassium permanganate solution for 15 minutes, followed by rinsing with distilled water. The biotest was carried out in 10 cm diameter Petri dishes with filter paper moistened with water extract from the soil (ratio 1:2). Ten seeds were sown in each dish and incubated in a thermostat at a temperature of 22-24 °C for 7 days. Each sample was analysed in two replicates. After the exposure, the following parameters were evaluated: percentage of germinated seeds, root length, and stem length. The results were interpreted according to the ISO 11269-1:2012 (2012) toxicity scale.

To assess the condition of the soil cover, nine soil samples were taken from each of the studied objects (Object 1 and Object 2). The selection was carried out in three directions, with three samples in each direction, which ensured the spatial representativeness of the sample. The phytotoxicity study was carried out for each sample in two repetitions, which are further referred to in the text as (1) and (2). This scheme was implemented for samples from both objects – Object 1 and Object 2.

Processing of results and analytical approach. To process the obtained data, methods of variational statistics were used with the calculation of mean values, standard deviation and coefficients of variation. Statistical analysis was performed using Microsoft Excel software.

General methodological concept. A comprehensive approach combining analytical (chemical) and bioindication (phytotesting) methods made it possible to reliably assess the ecotoxicological state of the soil cover near unauthorised landfills. The selected methods ensured the sensitivity, representativeness and reproducibility of the results, which is critically important for environmental monitoring and further justification of measures for soil rehabilitation.

A correlation analysis was performed to quantitatively assess the relationship between the content of heavy metals in the soil and the phytotoxic effect on the test Object *Raphanus sativus* var. The calculations used the concentrations of mobile forms of heavy metals (Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}) and the corresponding biometric indicators of seedlings (root length, stem length). The calculations were performed in Microsoft Excel. The analysis revealed a statistically significant correlation between the level of soil contamination with lead, cadmium and copper and the germination indicators of oil radish. This confirmed the impact of toxicants on the initial stages of plant ontogenesis, which allowed the results to be used as an indicator of environmental risk in the studied areas. Table 1 below shows the sampling scheme and sample labelling.

Table 1. Procedure for selecting soil samples and their labelling

Name of Objects	Direction	Labelling of samples	Distance from the landfill body, m
Object 1			
	North	I,1	5
		I,2	10
		I,3	15
	North-East	II,1	5
		II,2	10
		II,3	15
	South-East	III,1	5
		III,2	10
		III,3	15
Object 2			
	West	I,1	5
		I,2	10
		I,3	15
	North	II,1	5
		II,2	10
		II,3	15

Table 1. Continued

Name of Objects	Direction	Labelling of samples	Distance from the landfill body, m
	South	III,1	5
		III,2	10
		III,3	15

Source: developed by the authors

Thus, the following studies were conducted: the content of mobile forms of Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, mg/kg was determined in selected soil samples; the level of acute toxicity was established using a biotest object, the experiment was laid for a period of 7 days *Raphanus sativus d.* var. *oleifera* Metrg (ISO 22030:2005, 2005); the morphological composition of the waste was determined. The experimental studies of plants complied with institutional, national and international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

The results obtained indicate a decrease in the germination rate and energy of oil radish on soil samples taken near unauthorised landfills compared to control samples. Despite the fact that the concentrations of heavy metals (Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}) did not exceed the established maximum permissible values, a pattern was identified: soil samples with lower germination rates of the phytotest culture showed higher relative concentrations of these elements. This indicates the potentially negative impact of heavy metals, even within acceptable concentrations, on the biological activity of the soil. Therefore, phytotesting results can be a reliable indicator of hidden or cumulative soil toxicity, which emphasises the advisability of a comprehensive approach to assessing the condition of soil cover in areas adjacent to landfills.

Determining the morphological composition of waste

The settlement in Chernihiv region faces a problem in the area of solid waste management, which manifests itself in the growing negative impact of waste on the environment and the health of people and animals, in particular stray dogs living near landfills. It should be noted that plastic waste (21%) and paper waste (23%) predominate in the waste structure.

The work of A. Illiash *et al*. (2023) provides an analysis of the morphological composition of household waste in several regional centres of Ukraine. According to their data, fluctuations in the proportions of plastic (9-29%) and paper (2.5-14%) largely correlate with the socio-economic level of the settlements. The results of this study (plastic – 21%, paper – 23%) coincide with this range, confirming the representativeness and comparability of data on the impact of the private sector and the level of urbanisation. The article by I.V. Satin & O.S. Panchenko (2021) develops an improved methodology for analysing the morphological composition of waste, broken down into specific fractions, including paper, polymers, organic matter, etc. The authors note that in private residential areas, the share of paper was 7-14%, and the share of polymers was 9-22%. The figures obtained in this study (paper – 23%, plastic – 21%) slightly exceed the upper limit of the published values, which may be due to the active growth in the use of plastic packaging. A pattern has also been observed that the larger the rural population and private sector, the slightly higher the percentage of organic and food waste. It should also be noted that in recent years, emissions of plastic packaging products and plastic food packaging, mainly polyethylene, have increased, accounting for 16% at this landfill (Fig. 1).

Figure 1 shows the characteristics of waste in the settlement of Kniazha Krynytsia, Cherkasy region. The landfill is mainly polluted with waste generated by the local residents living nearby. The main components are: plastic waste (28%) – the largest portion of the waste. Plastic is a long-lasting environmental pollutant, as it takes hundreds of years to decompose. It can enter the soil and water, causing ecological problems. Paper and cardboard (27%) – a significant part of the waste consists of paper products, which decompose relatively easily and can be recycled. This indicates a large proportion of packaging materials in the waste. Polyethylene (17%) – one of the most persistent

forms of plastic, which pollutes the environment and causes serious ecological issues. Glass (16%) – does not decompose naturally, but is well-suited to recycling. Its considerable share indicates consumption of products in glass containers. Rubber waste (7%) – such as old tyres, which are hazardous to the environment as they decompose very slowly and can release toxic substances. Organic waste (2%) – decomposes the quickest, but may produce unpleasant odours and attract rodents and insects. Iron, textiles, and wood (1% each) – although their share is small, they also require proper disposal or recycling. Below is the morphological composition of waste for Object 2 (Fig. 2).

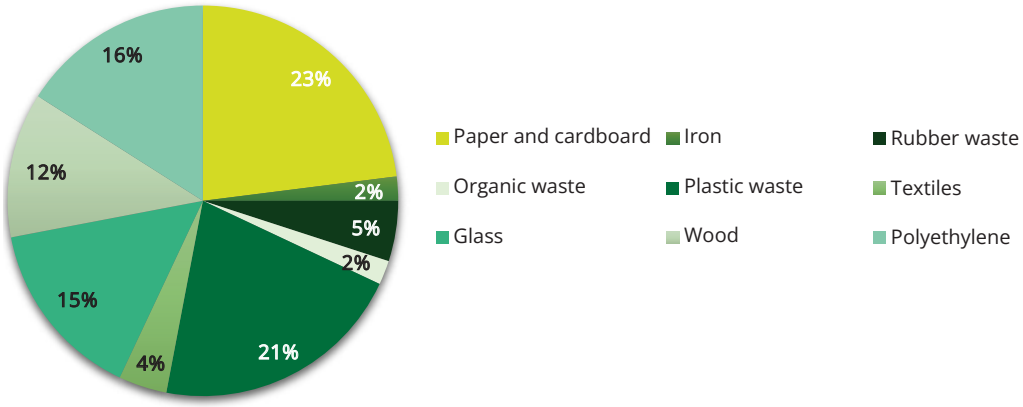

Figure 1. Average morphological composition of selected waste samples Object 1, %
Source: developed by the authors

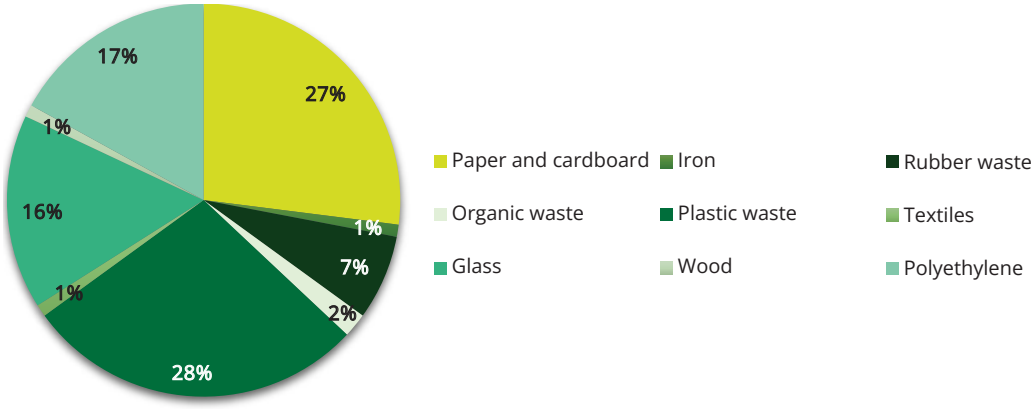

Figure 2. Average morphological composition of selected waste samples Object 2, %
Source: developed by the authors

The morphological analysis of municipal solid waste (MSW) at Object 2 showed that synthetic materials dominate the waste stream (Fig. 2). Plastic waste makes up the largest fraction at 28%, including single-use packaging and containers. This indicates excessive plastic consumption and a poor system for waste separation. Paper and cardboard follow at 27%, representing a valuable recyclable material. Polyethylene accounts for 17%, mainly consisting of bags and films. Glass makes up 16%; it is an inert component that can be recycled to save raw materials and energy. Rubber waste – 7%, which can include worn-out tyres and other technical materials. Smaller fractions include organic waste (2%), which can produce methane and foul odours, and iron, textiles, and wood (1% each).

Metals are recyclable, wood can be biodegraded or used for energy, while textiles are particularly difficult to dispose of due to synthetic additives. The long-term accumulation of these materials at unauthorised landfills can cause toxic compounds to migrate into the soil, altering its chemical and toxicological properties. Therefore, a soil study was conducted around the Object to assess the potential environmental impact. Table 2 provides the results from soil samples at Object 1.

Table 2. Content of mobile forms of heavy metals in soils (mg/kg), Object 1, third decade of July 2024

Sample Code	MPC mg/kg	North			North-East			South-East		
		I,1	I,2	I,3	II,1	II,2	II,3	III,1	III,2	III,3
Cu, mg/kg	3.0	0.675	0.42	0.355	0.559	0.465	0.48	0.208	0.55	0.425
Zn, mg/kg	23	1.854	0.211	0.391	1.925	1.129	0.434	2.262	0.849	4.761
Cd, mg/kg	0.7	0.203	0.248	0.286	0.215	0.225	0.179	0.153	0.25	0.175
Pb, mg/kg	6.0	2.14	1.543	2.245	0.8	0.764	1.59	1.25	1.805	1.472

Note: MPC – maximum permissible concentration
Source: developed by the authors

Contamination by heavy metals is one of the main environmental problems affecting soil quality, water resources, and ecosystem health. Heavy metals can accumulate in the biosphere and influence microorganisms, plants, and higher trophic levels, including humans. Table 2 shows that in all samples the concentration of Cu (copper) does not exceed the maximum permissible concentration (MPC) of 3.0 mg/kg. Copper is known to be an essential micronutrient for plants; however, its excess may lead to toxic effects such as disruption of growth and development of root systems. At all sampling points, Zn (zinc) is below the MPC of 23.0 mg/kg, indicating no significant technogenic pollution. Zinc is necessary for enzyme function in plants and microorganisms, although high concentrations may cause phytotoxicity. All measured values of Cd (cadmium) are below the MPC of 0.7 mg/kg, which suggests a natural origin of the metal. Cadmium is a highly toxic element, and its accumulation in soils can disrupt plant metabolism and adversely affect organisms. The concentration of Pb (lead) in all samples is lower than the MPC of 6.0 mg/kg, indicating no large-scale anthropogenic contamination. Lead is hazardous to the environment and human health as it can accumulate in plants, enter food chains, and cause neurotoxic effects.

According to V. Laptiev *et al*. (2024), the concentration of Cu and Zn in the plant biomass of *Erigeron canadensis* in Dnipro significantly exceeded background values, even though the total content of these metals in the soil had not yet exceeded regulatory limits. This corresponds with the findings of the present study: it was observed that the concentrations of Cu and Zn in the soils did not exceed the MPC but were higher in the samples with lower germination rates of radish. The research demonstrates that even permissible levels of heavy metals affect bioavailability and accumulation in plants.

Similar conclusions were drawn in the work of N. Chernykh *et al.* (2021). Their study of soils near the Sharra landfill (Tirana, Albania) revealed that, despite Cd and Pb levels being clearly within norms, accumulation of heavy metals in *Trifolium repens* was already recorded, which reduced germination. Unlike the present study, where the main focus was on phytotoxic effects through reduced germination, the aforementioned work emphasised the translocation of metals into plant tissues. Thus, the results of this study support the observations of other authors regarding the risks of hidden toxic effects of heavy metals even within regulatory limits.

Differences in the content of heavy metals are observed across the three sampling directions, although none of the recorded levels exceed the maximum permissible concentrations. In Direction I, the highest maximum concentrations of Pb (2.245 mg/kg) and Zn (1.854 mg/kg) were recorded, which may indicate either the local natural origin of these metals or a minor influence of anthropogenic factors. In Direction II, the values of heavy metals remain relatively stable, without sharp fluctuations, which may suggest the absence of

pronounced sources of contamination. At the same time, in Direction III, the highest values of Zn (4.761 mg/kg) and Pb (1.805 mg/kg) were observed compared to other directions. This is likely due both to the natural properties of the soil and the influence of local sources of pollution, in particular transport infrastructure or industrial facilities.

From Table 2, a preliminary conclusion can be drawn about the experiment at Object 1. All measured concentrations of heavy metals in soil samples do not exceed the maximum permissible concentrations, which indicates the environmental safety of the studied area and the absence of significant anthropogenic load. The highest values of zinc and lead were recorded in Direction III, which may point to local peculiarities of the geochemical composition of the soil or the presence of potential minor sources of contamination, although all indicators remain within permissible limits. Overall, all sampling points demonstrate similar results without significant deviations, which further confirms the absence of considerable technogenic impact. At the same time, given the ability of heavy metals to accumulate in the soil environment, it is advisable to continue monitoring studies and environmental risk assessments, particularly in the context of their potential entry into food chains. In general, the results of the study indicate a stable ecological condition of the area and no signs of critical soil contamination by heavy metals. Table 3 presents the results of soil samples from Object 2.

Table 3. Content of mobile forms of heavy metals in soils (mg/kg), Object 2, third decade of July 2024

Sample Code	MPC mg/kg	Western			Northern			Southern		
		I,1	I,2	I,3	II,1	II,2	II,3	III,1	III,2	III,3
Cu, mg/kg	3.0	0.429	0.405	0.62	0.635	0.412	0.49	0.18	0.115	0.67
Zn, mg/kg	23	1.234	0.723	0.873	8.097	3.788	1.467	0.425	0.135	0.128
Cd, mg/kg	0.7	0.37	0.324	0.253	0.135	0.18	0.265	0.108	0.188	0.205
Pb, mg/kg	6.0	0.903	1.041	0.832	2.125	2.395	1.575	0.668	0.705	0.68

Note: MPC – maximum permissible concentration
Source: developed by the authors

Soil contamination by heavy metals is one of the key environmental issues affecting ecosystem functioning, biodiversity, and the state of the agroecological environment. The conducted studies (Table 3) showed that the concentrations of the analysed metals (Cu, Zn, Pb, Cd) in the soils of the studied area do not exceed the maximum permissible concentrations (MPC), which indicates the overall environmental safety of the territory. The highest level of Zn (8.097 mg/kg) was recorded in Direction II, Pb (2.395 mg/kg) also in Direction II, Cd (0.37 mg/kg) in Direction I, while Cu (3.0 mg/kg) remained within the norm across all directions. The spatial distribution of metals suggests the influence of local natural factors (such as soil type, pH, organic matter content) or a weakly expressed anthropogenic impact (transport emissions, small-scale waste burning or accumulation). Although the concentrations of metals do not exceed MPC, even minor accumulation may lead to long-term changes in the structure of the microbiocenosis, a reduction in phytobiomass, and toxic effects on root systems, particularly under conditions of increased bioavailability (e.g., in acidic soils). This could threaten the decline of agronomic fertility and increase the risk of heavy metals entering food chains.

The obtained results may serve as a basis for environmental risk assessment, taking into account the spatial characteristics of contamination, as well as for the development of effective reclamation measures – in particular, the selection of phytoremediation methods according to the level of metal bioavailability. In addition, the findings may be applied in planning the locations of sorting stations or other waste management facilities: areas with the lowest concentrations of toxicants may be prioritised for further development with minimal environmental burden, whereas directions with localised increases in concentrations require additional monitoring. The concentrations of heavy metals in soil do not exceed MPC, which confirms the ecological safety of the studied areas. The highest levels of Zn and Pb were observed

in Direction II, likely linked to natural or anthropogenic factors. All sampling points demonstrate stable indicators without critical deviations.

Since heavy metal concentrations do not exceed permissible levels, their presence in soils is primarily attributed to natural processes, such as mineral weathering. However, possible anthropogenic factors include transport emissions, industrial sources, and the use of agrochemicals. Although soil concentrations remain below MPC, their levels in the biosphere may change under the influence of natural and human-induced processes. The accumulation of cadmium and lead in soils may lead to their entry into food chains, negatively affecting human and animal health. It is also important to consider the bioavailability of metals, as even low concentrations may be toxic to microorganisms and plants.

The results indicate that concentrations of heavy metals (Cd, Cu, Pb, Zn) in the studied soils do not exceed the established maximum permissible concentrations (MPC), pointing to the relative ecological safety of the study area. The spatial distribution of metals demonstrated certain local fluctuations, which may be attributed to both natural factors (geochemical features, soil properties) and anthropogenic influences (transport, local unauthorised landfills, use of agrochemicals). Nevertheless, even at concentrations below MPC, heavy metals can accumulate in biota, creating risks for agroecosystems, biodiversity, and human health through their transfer into food chains. In this regard, emphasis should be placed not only on total concentrations but also on bioavailability, which determines the actual impact on living organisms. To reduce risks and plan further environmental protection measures, it is recommended to:

1. Regular monitoring of soils for heavy metal content over time, particularly in areas where higher concentrations of Pb and Zn were detected. Such an approach will enable timely identification of changes in the chemical composition and assessment of pollution trends.

2. Study of metal bioavailability using modern biotests and chemical fractionation analyses. This will make it possible to determine which fraction of the elements is potentially hazardous to plants and microorganisms, even at low overall concentrations.

3. Assessment of soil parameters such as pH, organic matter content, granulometric composition, and cation exchange capacity, which directly influence the mobility of metals. For instance, soil acidification may significantly increase the toxicity of heavy metals.

4. Establishment of sorting stations and control over unauthorised waste disposal. The results may be used to identify priority areas for land reclamation or reconnaissance when planning the development of environmental infrastructure.

5. Conducting environmental risk assessments with the use of models of contaminant migration in the soil – plant system. This will allow prediction of the long-term impact on the agroecosystem and ensure scientifically sound planning of agricultural land use.

6. Involvement of local communities in environmental monitoring and awareness campaigns on household waste management, since local pollution is most often caused by indirect impacts of domestic activities.

Thus, the results of the study are not merely descriptive but may serve as a basis for decision-making in the fields of environmental monitoring, land reclamation, and the organisation of waste management infrastructure. The practical application of the obtained data will help to reduce environmental pressures and preserve soil resources for sustainable use. The concentrations of heavy metals recorded in both data sets do not exceed the maximum permissible concentrations, which indicates a relatively stable environmental condition of the soil cover in the study area. At the same time, the detected increase in zinc (Zn) content in direction II (Table 3) may indicate the presence of a local anthropogenic source of pollution, which requires further observation and clarification of its spatial distribution. A slight increase in lead (Pb) concentrations in the same direction may also be an indicator of the influence of anthropogenic factors, in particular transport or industrial activity. Additional monitoring studies will allow assessing the spatial and temporal dynamics of heavy metal distribution in the soil, as well as identifying potential pathways for their migration in the environment. In general, the soil is characterised by a satisfactory ecological state, but the detected local anomalies in Zn and Pb content in direction II justify the need for further monitoring.

Study of acute toxicity levels using oil radish as a biotest Object conducted at Object 1

Northern direction. In each of the three directions, ten seeds were sown in two replicates. The table below presents the number of germinated seeds relative to the total number of seeds sown. Number of germinated seeds: I,1(1) – 3 out of 10, I,1(2) – 8 out of 10, I,2(1) – 7 out of 10, I,2(2) – 6 out of 10, I,3(1) – 3 out of 10, I,3(2) – 8 out of 10. The highest germination rate was recorded in directions I,1(2) and I,3(2) (8 germinated seeds each), which may indicate a lower level of toxicants in the soil compared to other directions. In contrast, the lowest germination rates were recorded in directions I,1(1) and I,3(1) – only 3 seeds each.

In the work of K. Phoungthong *et al.* (2016), the effect of leachates from inert materials obtained by leaching ash waste from waste incineration plants on the germination of wheat seeds (*Triticum aestivum* L.) was investigated. It was found that at extreme pH values, as well as at elevated heavy metal content in leachates, a decrease in seed viability and inhibition of root growth were observed. These findings show that even moderate levels and dependence on the environment can affect seed viability. This is similar to the results of the study: in areas with lower germination rates (I,1(1); I,3(1)), seed development was also inhibited despite normal heavy metal content.

The study by S. Hazratqulov *et al*. (2024) examined how the treatment of organic waste reduces the availability of heavy metals, which stimulates plant growth, particularly root growth. The authors argue that even in the presence of metals in the soil, bioavailability determines the effect on germination. The data obtained in the study support this view: at points with better germination (I,1(2); I,3(2)), the bioavailability of toxicants is likely to be lower, although the total metal content was within normal limits. Below is an analysis of the phytotoxicity of oil radish in direction I of Object 1 (Fig. 3).

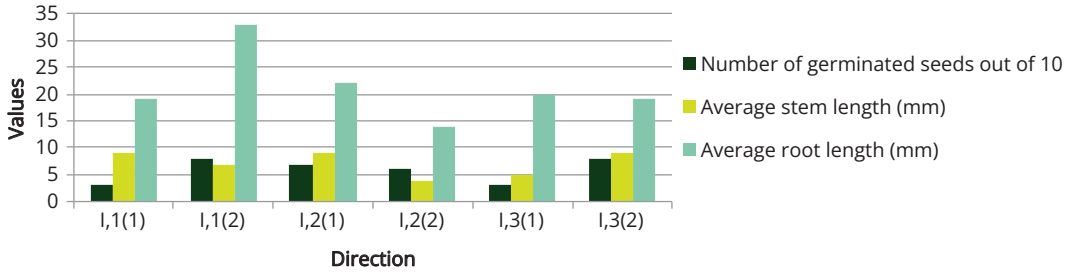

Figure 3. Bioindication of soil contamination with seed germination
Source: developed by the authors

Within the framework of biotesting of oil radish in different directions of Object 1, variability in germination and morphometric indicators of plants was revealed (Fig. 3). The lowest values of stem and root length, as well as a reduced number of germinated seeds, were recorded in direction I,3(1), indicating an increased level of soil phytotoxicity at this point. In contrast, better growth indicators were observed in direction I,1(2), which may indicate a lower level of contamination. A decrease in germination was observed in directions I,1(1) and I,3(1), indicating an increase in heavy metal content at these points. These are areas close to the source of pollution, as these samples also had an accumulation of debris before it was removed to the general waste. A significant reduction in root and stem length indicates plant stress at the cellular level. The situation is particularly critical in direction I,1(1), which has the lowest average morphometric values.

North-Eastern direction. Within the study, the phytotoxicity of soils was assessed using bioindication approaches, in particular, the reaction of seed germination and morphometric parameters of seedlings. Ten seeds were sown in each experimental direction (II,1(1); II,1(2); II,2(1); II,2(2); II,3 (1); II,3(2)). Variability in seed germination and growth indicators was established, indicating different toxicological quality of soils in the study area.

The maximum germination values (70%) were recorded in areas II,1(1) and II,1(2), indicating a relatively low level of phytotoxicity in these areas.

The lowest germination rates (30%) were found in direction II,2(2), indicating significant growth inhibition under the influence of existing pollutants. At the same time, the maximum morphometric parameters of stems (27.2 mm) and roots (8.2 mm) were found in direction II,3(2), indicating optimal growth conditions. The worst indicators of root system development (3.2 mm) were also recorded in direction II,2(2), which, combined with low germination rates, indicates the presence of biologically active toxic substances even in the absence of significant exceedances of the maximum permissible concentrations of heavy metals. Such approaches are relevant in the context of increasing anthropogenic transformation of landscapes and the need to restore degraded areas. Below is an analysis of the phytotoxicity of oil radish in direction II of Object 1 (Fig. 4).

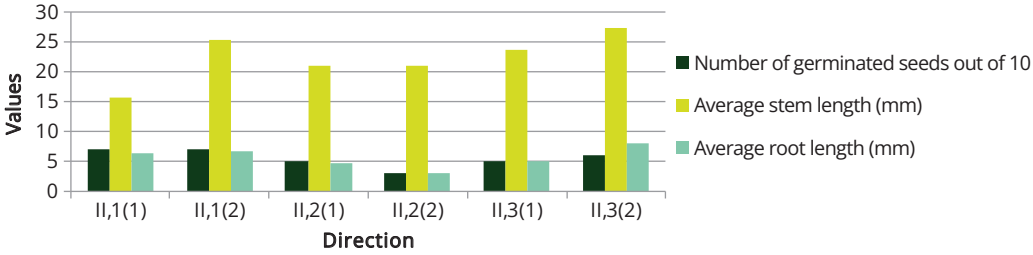

Figure 4. Morphometric parameters and germination
Source: developed by the authors

In direction II (Fig. 4), the overall seed germination rate was relatively stable; however, root length remained shorter than stem length, indicating a slowdown in root system development. This morphological response may suggest the chronic action of pollutants that do not critically affect germination but inhibit normal plant growth. Bioindication has proven to be an effective method for assessing spatial variation in soil contamination. Based on a combination of indicators – seed germination, root length and stem length – it can be concluded that: the most contaminated area is II,2(2); the least contaminated, in terms of phytotoxicity, are II,1(2) and II,3(2); the direct correlation between root length and contamination confirms the sensitivity of the root system to toxicants, especially heavy metals that suppress meristematic tissue growth.

South-Eastern direction. Number of germinated seeds: III,1(1) – 7 out of 10; III,1(2) – 6 out of 10; III,2(1) – 10 out of 10; III,2(2) – 10 out of 10; III,3(1) – 8 out of 10; III,3(2) – 9 out of 10. The lowest germination rates were observed in samples III,1(1) and III,1(2), which may indicate increased anthropogenic pressure, despite only minor levels of heavy metals in these samples. In contrast, samples III,2(1) and III,2(2) showed 100% germination, suggesting relatively better soil quality or lower levels of contamination.

Average stem length (K): III,1(1) – 18.1 mm, III,1(2) – 11.0 mm, III,2(1) – 34.0 mm, III,2(2) – 36.3 mm. Average root length (C): III,1(1) – 6.4 mm, III,1(2) – 3.7 mm, III,2(1) – 24.5 mm, III,2(2) – 18.4 mm. The low values of germination and morphometry in directions III,1(1) and III,1(2) may indicate a deterioration in the ecological state of soils. Improved values in III,2(1) and III,2(2) suggest potentially lower contamination. Longer stems and roots in these samples are a sign of better metabolic activity and the absence of phytotoxic substances. From an ecotoxicological perspective, the most contaminated samples are III,1(1) and III,1(2), as confirmed by low germination percentages and morphometric parameters. Conversely, samples III,2(1) and III,2(2) demonstrate high levels of germination and growth, indicating relatively clean soil conditions in these directions.

The results of the bioindication study confirm the relevance of including phytotesting in systems of ecological monitoring and soil assessment. The obtained indicators may be used to substantiate ecological risk assessment, to plan reclamation measures, or to organise waste sorting facilities with due consideration of toxicant bioavailability and their impact on biodiversity. The findings also highlight that chemical analysis of element content alone does not provide a

complete picture of ecological threats. Phytotesting makes it possible to determine the bioavailability of toxicants and their effects on living organisms, which is particularly important for assessing real ecological risks. The practical significance of the study lies in the possibility of zoning the territory according to degrees of phytotoxicity. The spatial variations observed in seed germination and morphometric characteristics of plant development may serve as a basis for ecological mapping of the area and for identifying zones with increased toxic pressure.

The obtained results allow for the formulation of recommendations regarding the further use of such areas. Specifically, plots with low germination and significant suppression of growth processes (e.g., site II,2(2)) should be temporarily excluded from intensive agricultural use or subjected to restricted use under special agrotechnical regimes. To reduce ecotoxic pressure, phytomelioration measures should be applied, including the cultivation of plant species tolerant to contamination, the introduction of organic ameliorants, and the implementation of biological remediation. Particular attention should be given to reclamation planning. Plots that exhibit high levels of phytotoxicity should be identified as priorities for reclamation, since even relatively low pollutant concentrations in the soil are associated with suppression of plant viability. The study results are also relevant for assessing the suitability of these territories for infrastructure development, particularly for waste-sorting stations. The presence of phytotoxic manifestations indicates potential risks linked to the long-term use of such areas for waste management facilities.

Study of acute toxicity levels using a biotest Object conducted at Object 2

Western direction. The highest germination rate is observed in samples I,1(1) and I,1(2), with 9 and 8 out of 10 seeds, respectively. The lowest level is in samples I,2(2) and I,3(2), with only 5 germinated seeds each, indicating potential soil contamination. Below is an analysis of oil radish germination in direction I of Facility 2 (Fig. 5).

The percentages shown in Figure 5 do not reflect the absolute percentage of germination for each sample (out of 10 seeds), but rather the relative share of each sample in the total number of germinated seeds in the western direction. For example, for sample I,2(2), 5 seeds out of 39 total seeds in this direction germinated, which is 13% – this is the percentage shown in Figure 5.

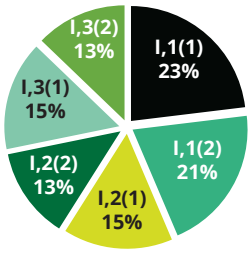

Figure 5. Percentage of germinated seeds in the Western direction
Source: developed by the authors

The biotest conducted confirmed the variability of oil radish seed germination shown in Figure 5 and indicates different levels of phytotoxicity of the samples studied. The highest biological activity of the soil was observed in the first sample I,1(1), where the highest percentage of germinated seeds was recorded. On the other hand, some samples showed inhibition of germination, which may be due to the presence of pollutants. In general, the results indicate an uneven distribution of toxicity within one direction, which indicates localised soil contamination. The average values of stem and root lengths indicate a negative impact of contamination in directions I,2(1), I,2(2) and I,3(2), where the lengths are significantly lower compared to control samples I,1(1) and I,1(2). Below is an analysis of the phytotoxicity of oil radish in direction I of Object 2 (Fig. 6).

Samples I,1(1) and I,1(2) can be considered reference samples or the least contaminated, as they show high germination and morphometric characteristics (Fig. 6). The decrease in germination rates in samples I,2(2) and I,3(2) indicates probable increased soil contamination, especially with heavy metals or residual agrochemicals. The results confirm the advisability of further analysis of the chemical composition to confirm the ecotoxicological status of these samples.

Southern direction. According to the data obtained, the number of germinated seeds was: III,1(1) – 4 out of 10, III,1(2) – 7 out of 10, III,2(1) – 5 out of 10, III,2(2) – 10 out of 10, III,3(1) – 3 out

of 10, III,3 (2) – 5 out of 10. There is variability in germination, which indicates the heterogeneity of the impact of pollution factors at different sampling points. Morphometric indicators of plants: stem length (K) varies from 18 to 142 mm. The maximum stem length values (over 100 mm) were observed in samples III,2(2) and III,3(1), which may indicate local areas with relatively better conditions for seedling development; root length (C): varies from 2 to 36 mm. Shorter root lengths (<10 mm) are recorded in most samples, which is an indicator of the presence of toxic substances in the soil that inhibit the development of the root system.

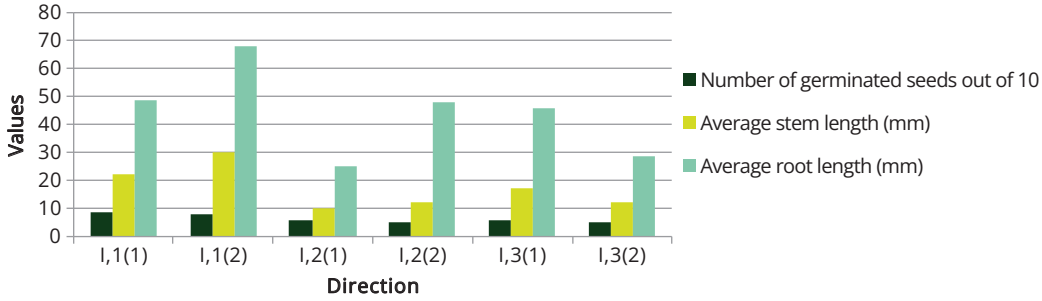

Figure 6. Bioindication of soil contamination based on seed germination
Source: developed by the authors

In general, shorter roots compared to stem length indicate an increased toxicant load, as the root system is more sensitive to pollutants. A high percentage of seeds that did not germinate also confirms the presence of toxic stress. he situation is particularly critical in samples III,3(1) and III,3(2), where the number of germinated seeds was the lowest (3-5 out of 10), which may indicate the level of soil contamination. Below is an analysis of the phytotoxicity of oil radish in direction III of Object 2 (Fig. 7).

The results of biotesting indicate differences in the phytotoxicity of soil samples in direction III in Figure 7. There is a general trend towards reduced seed germination and suppression of growth parameters (stem and root length) at certain points, which may indicate the presence of toxicants in the soil. At the same time, some samples show better growth indicators, which indicates local heterogeneity of contamination. Data analysis shows significant heterogeneity of soil contamination in the southern direction. The main indicators of contamination are a decrease in root length, a decrease in the number of germinated seeds, and a disruption in the ratio between stem and root development. The signs of stress indicate the need for soil remediation measures in this area.

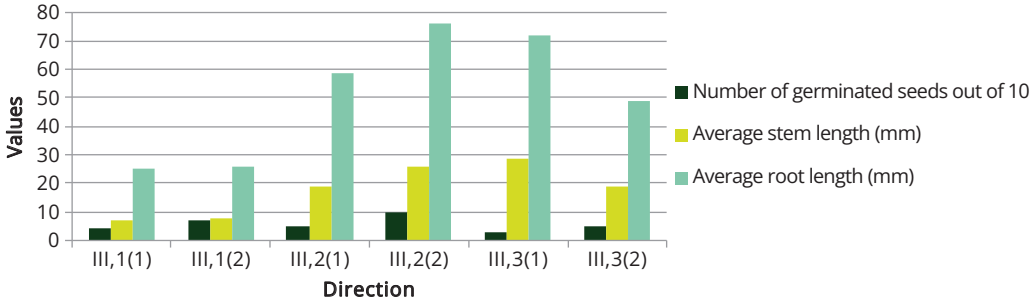

Figure 7. Bioindication of soil contamination based on seed germination
Source: developed by the authors

Northern direction. In each variant of the experiment, 10 seeds were sown, but since the experiment was conducted in two replicates, the total number of seeds was 20. Therefore, the analysis of germination took into account the total indicators of both replicates. Seed germination: II,1 – 11 out of 20 germinated seeds (5 + 6) = 55% germination, II,2 – 6 out of 20 seeds germinated (2 + 4) = 30% germination, II,3 – 8 out of 20 seeds germinated (3 + 5) = 40% germination. Thus, the best germination was observed at points II,1, indicating a relatively lower level of soil contamination. The worst germination rates (only 30%) were recorded at points II,2. Analysis of the average values of stem length (K) and root length (C) for each point: I,1 (average values): Stem (K): ~33 mm Root (C): ~25 mm, II,2 (average values): Stem (K): ~6 mm, Root (C): ~5 mm, II,3 (average values): Stem (K): ~26 mm, Root (C): ~25 mm (Fig. 8).

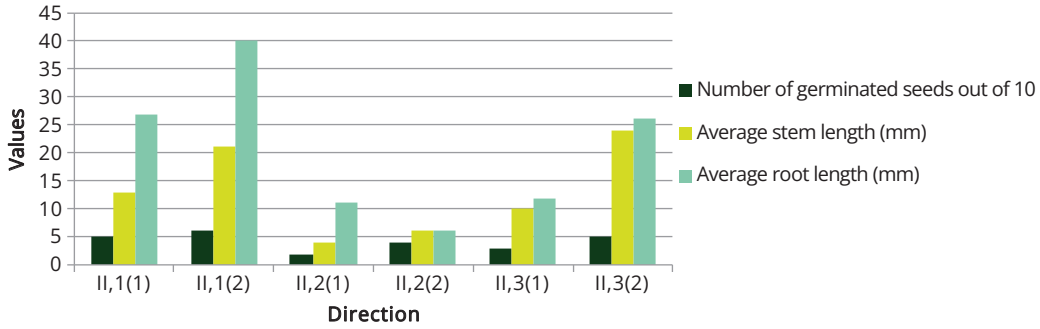

Figure 8. Bioindication of soil contamination based on seed germination
Source: developed by the authors

The morphometric indicators of seedlings at points II,2 are significantly lower, which indicates a strong inhibition of growth processes shown in Figure 8, most likely due to the presence of toxic substances in the soil. The length of the root and stem is on average reduced by 4-5 times compared to II,1. Low seed germination energy and suppressed seedling development at points II,2 may indicate the presence of significant concentrations of toxicants or pesticide residues in the soil. The reduction in stem length indicates a general suppression of photosynthetic activity in seedlings. In turn, the indicators in II,3 are intermediate: although germination is insignificant, morphometric characteristics indicate the adaptation of some plants to the present contaminants. This may indicate a less toxic but still disturbed chemical composition of the soil.

Similar results were observed in a study by J. Bae *et al*. (2016), who studied the effect of heavy metals (Zn, Pb, Ni, Cu, Cd) on the germination of *Ambrosia artemisiifolia* and legume seeds. The authors noted that even moderate concentrations of these metals significantly reduced germination energy and inhibited seedling development, with the root being particularly sensitive. This is consistent with the results of this study, where root length is one of the most affected indicators in contaminated sites. In a study by I. Sanjosé *et al*. (2021) using *Salsola vermiculata* as an example, it was found that an increase in the concentration of Cd, Cu, Pb and Zn in the soil led to a delay in germination and a decrease in seedling biomass. In particular, the authors point out that the most contaminated samples showed a decrease in stem length, which, in their opinion, is a consequence of a general decrease in photosynthetic activity. These results are similar to the observations at points II,2 of this study, where stem growth inhibition was also found, which can be interpreted as a decrease in photosynthetic efficiency due to toxic load. Thus, the results of both analysed studies confirm the hypothesis of the negative impact of heavy metals on seed germination and morphometric characteristics of seedlings, which is consistent with the conclusions of the present study. The only difference is the type of test objects used in the studies (in I. Sanjosé *et al.* – a steppe plant, in J. Bae *et al*. – weeds and legumes), but the general trends remain similar.

Thus, the generalisation of the data presented indicates a clear trend of inhibition of seed germination and initial plant growth under the influence of heavy metals. Common features are a decrease in germination energy and a reduction in root and stem length, reflecting the overall toxicity of the soil and a decrease in photosynthetic activity. The results obtained confirm the relevance of data from other authors and reinforce the argument in favour of using phytotesting to assess soil contamination.

Correlation analysis of *Raphanus sativus d.* var. *oleifera* Metrg germination under conditions of soil contamination with heavy metals

The study found a statistically significant correlation between the level of soil contamination with heavy metals (lead, cadmium, copper) and the germination rates of oil radish. Details are given in the table below for Object 1 (Table 4).

Based on the results of the analysis, the following conclusions can be drawn: high concentrations of Cu^{2+} do not cause a sharp decrease in seed germination, but may slightly reduce the number of germinated seeds. Higher concentrations of Zn^{2+}) theoretically may promote better root and stem growth, but the number of germinated seeds does not increase significantly. Excess Zn^{2+} can be toxic, but in this experiment, growth is observed. Even in small concentrations, Cd^{2+} negatively affects germination and growth, reducing the number of germinated seeds and the length of the root and stem. Pb^{2+}, like Cd^{2+}, inhibits seed germination and plant growth. Higher concentrations lead to a decrease in the number of germinated seeds.

Table 4. Correlation analysis of *Raphanus sativus d.* var. *oleifera* Metrg germination by Object 1

Copper (Cu^{2+})				
Sample Mark	**Concentration Cu (mg/kg)**	**Root Germination (R). mm**	**Stem Germination (S). mm**	**Number of Germinated Seeds. pcs**
I,1	0.675 (highest)	I – repeat – 19 II – repeat – 33	I – repeat – 9 II – repeat – 7	I – repeat – 3 II – repeat – 8
III,1	0.208 (lowest)	I – repeat – 19 II – repeat – 31	I – repeat – 7 II – repeat – 17	I – repeat – 7 II – repeat – 6
Zinc (Zn^{2+})				
Sample Mark	**Concentration Zn (mg/kg)**	**Root Germination (R)**	**Stem Germination (S)**	**Number of Germinated Seeds. pcs**
III,3	4.761 (highest)	I – repeat – 20 II – repeat – 31	I – repeat – 10 II – repeat – 9	I – repeat – 4 II – repeat – 9
I,2	0.211 (lowest)	I – repeat – 22 II – repeat – 7	I – repeat – 7 II – repeat – 9	I – repeat – 7 II – repeat – 6
Cadmium (Cd^{2+})				
Sample Mark	**Concentration Cd (mg/kg)**	**Root Germination (R)**	**Stem Germination (S)**	**Number of Germinated Seeds. pcs**
I,3	0.286 (highest)	I – repeat – 20 II – repeat – 19	I – repeat – 5 II – repeat – 9	I – repeat – 3 II – repeat – 8
III,1	0.153 (lowest)	I – repeat – 19 II – repeat – 31	I – repeat – 7 II – repeat – 17	I – repeat – 7 II – repeat – 6
Lead (Pb^{2+})				
Sample Mark	**Concentration Pb (mg/kg)**	**Root Germination (R)**	**Stem Germination (S)**	**Number of Germinated Seeds. pcs**
I,3	2.245 (highest)	I – repeat – 20 II – repeat – 19	I – repeat – 5 II – repeat – 9	I – repeat – 3 II – repeat – 8
II,2	0.764 (lowest)	I – repeat – 29 II – repeat – 11	I – repeat – 3 II – repeat – 3	I – repeat – 5 II – repeat – 3

Source: developed by the authors

It has been determined that heavy metals (especially Cd^{2+} and Pb^{2+}) have a toxic effect on seed germination, reducing both the number of germinated seeds and the length of the root and stem. Cu^{2+} and Zn^{2+} in moderate concentrations can slightly stimulate growth, but their excess also inhibits germination. The presence of heavy metals in the soil is a serious environmental

problem, as they reduce plant viability, which can lead to reduced yields and ecosystem disruption. When determining the level of acute toxicity, it was found that the lowest germination rates of the biotest Object were recorded at a distance of 5 metres from the landfill body at the northern point, namely 30% seed germination, where the Zn^{2+} level was 1.854 mg/kg. The highest germination energy level was observed in the sample at a distance of 15 metres from the landfill body at the south-eastern point, namely 100% seed germination, which is 92% higher than the germination of plant roots and 76% higher than the germination of plant stems from the control experiment.

According to M.O. Alotaibi (2024), studies of the effect of cadmium (Cd) and lead (Pb) on the germination of *Cymbopogon comosum* seeds showed a marked inhibition of germination, as well as a change in the protein profile of seedlings, confirming the toxic effect of these metals at concentrations above a certain threshold. This conclusion is consistent with the results of the present study – a low germination rate (30%) was recorded at the point with elevated Zn^{2+} content along with Cd^{2+} and Pb^{2+}, indicating the inhibitory effect of heavy metals even at relatively low concentrations.

Author M. Sharifi-Rad (2017) investigated the effect of cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni) and zinc (Zn) on the germination and development of *Hyssopus officinalis* in laboratory conditions at concentrations of 50, 100 and 150 µM. The results showed that all metal stressors significantly inhibited germination, reducing the germination rate, root and stem length, and biomass, with the order of inhibition strength being Cr > Cd > Cu > Ni > Zn. This partly coincides with the observations: moderate levels of Zn could stimulate the development of individual indicators (for example, at the southern point there was 100% germination), but at Zn values ~1.85 mg/kg, growth inhibition was noted. The table below shows the correlation analysis for Object 2 (Table 5).

Table 5. Correlation analysis of *Raphanus sativus d*. var. *oleifera* Metrg germination by Object 2

Copper (Cu^{2+})				
Sample Mark	**Concentration Cu (mg/kg)**	**Root Germination (R), mm**	**Stem Germination (S), mm**	**Number of Germinated Seeds, pcs**
III,3	0.67 (highest)	I – repeat – 72 II – repeat – 49	I – repeat – 29 II – repeat – 19	I – repeat – 3 II – repeat – 5
III,2	0.115 (lowest)	I – repeat – 59 II – repeat – 76	I – repeat – 19 II – repeat – 26	I – repeat – 5 II – repeat – 10
Zinc (Zn^{2+})				
Sample Mark	**Concentration Zn (mg/kg)**	**Root Germination (R)**	**Stem Germination (S)**	**Number of Germinated Seeds, pcs**
II,1	8.097 (highest)	I – repeat – 27 II – repeat – 40	I – repeat – 13 II – repeat – 21	I – repeat – 5 II – repeat – 6
III,3	0.128 (lowest)	I – repeat – 72 II – repeat – 49	I – repeat – 29 II – repeat – 19	I – repeat – 3 II – repeat – 5
Cadmium (Cd^{2+})				
Sample Mark	**Concentration Cd (mg/kg)**	**Root Germination (R)**	**Stem Germination (S)**	**Number of Germinated Seeds, pcs**
I,1	0.37 (highest)	I – repeat – 49 II – repeat – 68	I – repeat – 22 II – repeat – 30	I – repeat – 9 II – repeat – 8
III,1	0.108 (lowest)	I – repeat – 25 II – repeat – 26	I – repeat – 7 II – repeat – 8	I – repeat – 4 II – repeat – 7
Lead (Pb^{2+})				
Sample Mark	**Concentration Pb (mg/kg)**	**Root Germination (R)**	**Stem Germination (S)**	**Number of Germinated Seeds, pcs**
II,2	2.395 (highest)	I – repeat – 11 II – repeat – 6	I – repeat – 4 II – repeat – 6	I – repeat – 2 II – repeat – 4
III,1	0.668 (lowest)	I – repeat – 25 II – repeat – 26	I – repeat – 7 II – repeat – 8	I – repeat – 4 II – repeat – 7

Source: developed by the authors

The results of the studies showed that an increase in the content of Cu^{2+}, Zn^{2+} and Pb^{2+} in the soil negatively affects the germination of oil radish (*Raphanus sativus* var.), which indicates the phytotoxic effect of these metals even at concentrations that do not exceed the MPC. The lowest germination rate (30%) was recorded in the northern area near the landfill, where the concentration of Pb^{2+} was 1.575 mg/kg. In the control sample, 100% germination was observed.

This pattern is confirmed by the studies of K. Phoungthong *et al*. (2016), who also found inhibition of wheat (*Triticum aestivum* L.) germination under the influence of soluble forms of heavy metals, especially copper and lead, in waste leachates. The authors found that even without exceeding the regulated limits for metal content in ash residue extracts, there was a significant decrease in the energy of germination and growth of primary roots, which is consistent with the results obtained in this study. In addition, a similar approach to studying phytotoxicity was applied by S. Hazratqulov *et al*. (2024), who assessed the impact of heavy metals in soils stabilised with organic waste. According to their data, the greatest growth inhibition occurred at high bioavailability of lead and zinc, and the use of bioamendments significantly reduced phytotoxicity. Unlike their work, no soil remediation was carried out in this study, but the results obtained regarding the sensitivity of germination to Pb^{2+} and Zn^{2+} confirm that even under natural conditions there is a high risk of phytotoxic effects of these elements near unauthorised landfills. Thus, a summary of the available literature data and the results of this study indicates a stable negative reaction of plant biotests to elevated concentrations of heavy metals, in particular Pb^{2+}, Cu^{2+} and Zn^{2+}, even at relatively low levels of contamination, which is characteristic of areas with long-term exposure to landfill waste.

The results of the study, which demonstrate the impact of heavy metals (in particular Cu^{2+}, Zn^{2+}, Pb^{2+}) on the phytotoxicity of soils in the area of unauthorised landfills, can serve as an important basis for planning measures for recultivation and the organisation of sorting stations. Firstly, identifying the points with the most negative impact of heavy metals on plant development allows for the targeted identification of priority areas for reclamation. The use of biotests, as in this study, helps to assess the real biological effect of pollution, which is critical for the selection of adequate restoration technologies (e.g., phytoextraction, amendment application, use of biological preparations). This information contributes to the optimisation of resources and the improvement of the effectiveness of measures. Secondly, data on specific toxic metals that dominate the pollution indicate the need to sort waste according to its composition to prevent the further spread of toxicants. The organisation of sorting stations with a focus on the removal of metal and hazardous components from waste can significantly reduce the level of heavy metals in waste, reducing the risk of soil contamination. Thus, the results obtained are a valuable scientific basis for the development of a comprehensive waste management system that combines preventive measures with technical solutions to minimise phytotoxic effects and improve the ecological condition of areas near landfills.

CONCLUSIONS

The study showed that the soil samples taken near the landfill did not contain heavy metal concentrations exceeding the maximum permissible concentrations (MPC). Despite the absence of toxicologically hazardous levels of contamination, the results of phytotoxic testing showed a decrease in seed germination, indicating an inhibitory effect on growth processes. Thus, for Object 1, the lowest seed germination rate (3 out of 10 in each repetition) was recorded at points with the highest concentrations of cadmium (0.286 mg/kg) and lead (2.245 mg/kg), indicating their impact on phytotoxicity. A similar trend was observed for Object 2: at the highest content of Zn (8.097 mg/kg) and Pb (2.395 mg/kg), the number of germinated seeds decreased to 5-6 out of 10, while with a decrease in these concentrations, germination increased to 8-10 seeds. In addition, the reduction in the length of roots and stems of seedlings at elevated concentrations of heavy metals confirms the presence of a negative effect even within acceptable levels. For example, at the lowest Zn content (0.128 mg/kg), the root length reached 72-73 mm, while at the highest content, it was only 27-40 mm.

The results obtained confirm the feasibility of combining chemical analysis with bioindication

methods in assessing soil condition, particularly in terms of ecotoxicology. It is planned to continue regular monitoring of soils in areas adjacent to solid waste landfills, using phytotesting as a sensitive tool for early diagnosis of potential environmental threats. The results of the study are of great practical and scientific importance for further improvement of approaches to environmental monitoring of soil condition in areas affected by landfills. The proposed phytotesting methodology can be integrated into the system for evaluating the ecotoxicity of soil cover as a sensitive indicator of early changes in the soil environment that are not always detected by standard chemical analysis. The use of a bioindication approach allows the detection of hidden forms of pollution caused by the complex influence of factors that do not exceed regulatory indicators but still have a negative impact on living organisms. Prospects for further research include expanding the number of research sites, conducting seasonal monitoring, and studying the synergistic effects of several pollutants simultaneously in order to comprehensively assess the phytotoxic potential of soils in areas affected by solid waste landfills.

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Несанкціоновані сміттєзвалища як джерело забруднення ґрунтів важкими металами: фітотоксичний аспект

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Анотація. У статті розглянуто питання біотестування ґрунту як ефективного індикаторного підходу до оцінки екологічного стану земель, що зазнають антропогенного навантаження з боку локальних джерел забруднення. Об'єктами дослідження були зразки ґрунту, відібрані поблизу потенційно небезпечних техногенних ділянок, а саме з територій сміттєзвалищ. Проведено оцінку токсичності за допомогою фітотестів культури редьки олійної (*Raphanus sativus d*. var. *oleifera* Metzg.), що виявилася чутливим індикатором наявності шкідливих речовин у ґрунтовому середовищі. Паралельно здійснено аналітичне визначення вмісту важких металів (Cd, Pb, Zn, Cu) у ґрунтах. Результати фітотестування засвідчили зниження схожості насіння порівняно з контрольними зразками, що є свідченням токсичної дії ґрунтового середовища. Також спостерігалося зменшення енергії проростання та пригнічення росту первинної біомаси. Виявлено тісний кореляційний зв'язок між високим вмістом металів і пригніченням ростових параметрів культури редьки олійної, що вказує на взаємозалежність між хімічними показниками забруднення та біологічною відповіддю організмів. Зокрема, спостерігалося зменшення довжини проростків, що свідчить про біологічну активність важких металів. У деяких зразках було зафіксовано 30 % проростання, що свідчить про критичний рівень токсичності. Біотестування в поєднанні з хімічним аналізом дозволило виявити ділянки з найбільш критичним екологічним станом. Запропонований підхід може бути корисним для екологічних служб, органів місцевого самоврядування та установ, що займаються землеустроєм, для оперативного моніторингу стану ґрунтів та виявлення «гарячих точок» забруднення. Методика може бути адаптована для моніторингу промислових зон, зон санітарного нагляду, а також територій поблизу транспортних магістралей. Результати дослідження підтверджують доцільність використання інтегрованих підходів до моніторингу, що включають як аналітичні методи, так і біоіндикаційні тести. Такий підхід забезпечує більш об'єктивну оцінку ризиків для довкілля та може бути використаний для планування заходів з реабілітації забруднених територій, збереження родючості ґрунтів і сталого землекористування

Ключові слова: екотоксикологія; індекс проростання; біомоніторинг; ризики для агроекосистем; тест-рослини; токсикологічна чутливість

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Assessment of the effect of graphene oxide on morphometric parameters of common cabbage (*Brassica oleracea*) for different processing methods

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Abstract. The growth of the world's population and the reduction of fertile soil areas due to global climate changes require advanced scientific solutions to increase the yield of major food crops to ensure food security. The purpose of the study was to investigate the effect of graphene oxide nanoparticles at different concentrations and processing methods on the linear and weight parameters of common cabbage seedlings (*Brassica oleracea*) of the ultra-early variety 'Iyunska'. Physiological research methods were used to determine the morphometric parameters of plants and seed germination. Nanostructured colloidal solutions in the concentration range of 20, 50, and 70 μg/mL and two treatment methods – seed priming and root application – were used to evaluate the effect of graphene oxide. It was found that graphene oxide stimulated the germination of cabbage seeds when soaking the seeds in solutions of all the concentrations under study. The highest seed germination rate was observed in the group where a solution with a nanoparticle concentration of 20 μg/mL was used for priming. It was in this group that the seed germination rate was 83%, which significantly exceeds this indicator in the control group – 56%. Root application of graphene oxide solutions of similar concentrations did not cause significant changes in the specified indicator, which remained within the control values. In this paper, it was proved that the morphometric parameters of cabbage seedlings reacted more positively to the action of nanoparticles during seed priming in contrast to the application by irrigation. Priming cabbage seeds with different concentrations of graphene oxide caused an increase in the linear characteristics of seedlings, which was most pronounced mainly at a concentration of 20 μg/mL: the total length of plants increased by 32.8%, the length of the STEM by 26.8%, the average length of the roots by 37.7% compared to the control group. The average plant weight and root weight showed the greatest growth when treated with seeds in a graphene oxide solution with a concentration of 70 μg/mL. The only indicator that reacted negatively

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to nanoparticle priming is the weight of the seedling stem, which decreased at all concentrations. The use of carbon nanoparticles in crop production is a promising area of agricultural technologies, which can provide not only high seed germination rates, but also stimulate the growth and development of food crops with properly selected concentrations and processing methods

Keywords: carbon nanoparticles; nanopriming; plants; seed germination; growth rates

INTRODUCTION

Recent global trends in the agricultural sector have led to an increase in interest in innovative approaches in agricultural technologies that would improve the growth, increase the yield and stress resistance of food crops without negatively affecting the quality and safety of final products. Cabbage is an important food crop that is in high demand in domestic and international markets. It is grown in both northern and southern countries. The area occupied by cabbage is estimated by FAOSTAT to be 2.6 million hectares (Food and Agriculture Organisation of the United Nations, 2025). Cabbage consumption differs significantly in quantitative indicators in different countries of the world, which is conditioned by the culinary traditions of the population. It is used fresh for cooking, and in processed form (fermentation, canning).

According to L. Ray *et al*. (2021), extracts and phytocomponents isolated from *B. oleracea* var. *capitata,* has antitumour, antihypertensive, anticholesterolemic, antioxidant, anti-inflammatory, antibacterial, anticoagulant, and haepatoprotective effects. J.J. Uuh-Narvaez & M.R. Segura-Campos (2021) indicated that cabbage is a food product that can be used in food therapy, and as a preventive ingredient for type 2 diabetes. The main bioactive compounds present in cabbage varieties are phenolic acids, flavonoids, anthocyanins, and fibre, which help inhibit amylolytic enzymes responsible for converting carbohydrates into sugars.

As noted by O. Statilko *et al*. (2024), different varieties of cabbage differ in the composition of biologically active compounds. Thus, for varieties *B. oleraceae* var. *capitata* f. *rubra* it is characterised by a high content of phenolic compounds, in particular anthocyanins, and for varieties *B. oleraceae* var. *capitata* f. *alba* – glucosinolates. The level of these compounds also varies depending on the variety. According to W. Wu *et al*. (2021), ten glucosinolates were found in red cabbage, including 4-methoxyglucobrasicin, neoglucobrasicin, glucoalisin, glucobrasicin, glucoraphanin, glucoiberin, progoitrin, gluconapine, and sinigrin, have antibacterial, anti-inflammatory, and antioxidant properties. Y. Gelaye & E. Tadele (2022) noted that the cultivation of cabbage and its productivity depends on many factors, in particular, soil fertility, proper agronomic practices – pinching, thinning. According to research by M. Saba *et al*. (2025), organic cabbage cultivation is becoming increasingly popular, as it has higher levels of essential nutrients, antioxidants, and phytochemicals than traditionally grown counterparts. This is achieved by not using chemicals to treat plants and fertilise the soil.

J.D. da Silva Fonseca *et al*. (2024) determined that the rapid development of nanotechnologies leads to an increase in the popularity of using metal and nonmetal nanoparticles in crop production, in particular, as pesticides, nanofertilisers, and elicitors. Nanoparticles (NP) obtained from organic substances by "green" synthesis can improve nutrient absorption, soil quality, seed germination, and plant growth. L. Xuan *et al*. (2023) indicated the ability of metal nanoparticles to pose a potential threat to the environment due to accumulation in soils and bio-objects and public health as a result of their migration in food chains. Risks of using carbon nanoparticles, according to N. Xin *et al*. (2023), still being studied. Information on the optimal application rates of carbon nanoparticles, their agronomic efficiency, influence on the physiology of agricultural crops, and the biochemical quality of soil is very limited.

Undoubtedly, graphene/graphene oxide has significant application potential in various industries and medicine, as it has a number of advantages. However, according to the study by A.N. Ghulam *et al*. (2022), its exposure to the environment can affect the soil microbiota, plants, and animals. That is why it is important to find out the consequences of this effect, since graphene nanoparticles are of increasing interest for use in agricultural technologies. According to X. Zhang *et al*. (2022), in scientific studies, there

are mixed data on the effect of these nanoparticles on the plant body. Some researchers note the positive effects of graphene on the growth and development of various plant species, while others note the toxic effect, which manifests itself in the form of physical (mechanical damage and blocking) and physiological and biochemical effects (activation of the development of reactive oxygen species, a decrease in the activity of antioxidant enzymes, and effect on the photosynthetic apparatus of plants).

Elucidating the mechanisms and consequences of graphene exposure on plants will weighing all the benefits and risks of its use and making appropriate decisions on the further use of these nanoparticles in the agricultural sector. The purpose of the study was to evaluate the effect of graphene oxide on the morphometric parameters of common cabbage (*Brassica oleracea*) varieties 'Iyunska' by soil application and seed priming.

MATERIALS AND METHODS

The research was conducted in the educational and scientific laboratory "Biochemistry and phytobiotechnology" at the Department of Physiology, Plant Biochemistry, and Bioenergetics of the National University of Life and Environmental Sciences of Ukraine in May-June 2024. *Object of research* was a common cabbage (*Brassica oleracea*) of the ultra-ripe variety 'Iyunska'. Seeds were sown in a universal substrate of the following composition: riding peat, lime flour, perlite, river sand; macronutrients: nitrogen (N) – 80-140 mg/L, phosphorus (P_2O_5) – 100-150 mg/L, potassium (K_2O) – 140-180 mg/L, trace elements. A 3% solution of hydrogen peroxide (H_2O_2) and 0.006% solution of boric acid (H_3BO_3) were used for pre-sowing seed treatment. Pre-sowing seed treatment was carried out according to the following scheme (Fig. 1). Further treatment of seeds and plants was carried out with colloidal solutions of graphene oxide.

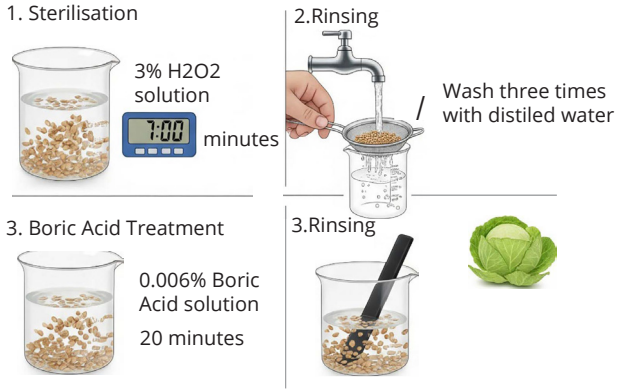

Figure 1. Pre-sowing treatment of cabbage seeds

Source: created by the authors

Synthesis and characterisation of structured graphene oxide nanoparticles. Samples of highly stable water-soluble dispersed graphene oxide (GO) nanoparticles at an initial concentration of 4 mg/mL were provided by Graphenea S.A. (San Sebastian, Spain). The authors confirmed the structural organisation and stability of the samples using high-resolution microscopy and dynamic light scattering. The studies used an aqueous solution of GO in the concentration range: 20, 50, and 70 µg/mL.

Experiment scheme. Cabbage seeds were planted in 7 trays, each of which had 24 wells, 6 seeds in one well: Group 1 – control (without NP treatment); Group 2 – seeds were pre-soaked (priming) in a solution of graphene oxide with a concentration of 20 µg/mL; Group 3 – seeds were pre-soaked in a solution of graphene oxide with a concentration of 50 µg/mL; Group 4 – seeds were pre-soaked in a solution of graphene oxide with a concentration of 70 µg/mL; experimental groups 5, 6, 7 – cabbage seedlings were watered with graphene oxide solution at concentrations of 20, 50, 70 µg/mL, respectively, on days 5 and 10, 5 mL in each well. In addition, all plants were watered at intervals of 4 days with water in the amount

of 10 mL per well. The graphene oxide concentrations and application intervals used in the study were selected experimentally, having previously analysed existing scientific data and conducted appropriate pilot studies.

Plants were examined at the stage of 2 leaves (20 days from the moment of planting seeds in the ground). Seed germination was evaluated, and morphometric studies of plants with the determination of such indicators as plant length, stem, root, leaves, weight of plant, stem, and roots. The sample of plants in the study consisted of 30 seedlings from each study group. The results obtained were processed statistically using generally accepted methods of variational statistics by Yu.I. Prylutskyi *et al*. (2017) and using the Microsoft Excel 2010 software suite. Changes in $P \leqslant 0.05$ were considered statistically significant. The plant research was conducted in accordance with institutional, national, and international guidelines and compliance with the Convention on Biological Diversity (1992) standards.

RESULTS AND DISCUSSION

Carbon nanomaterials have the potential to improve seed germination, nutrient availability, and stress resistance. Increasing crop yields is impossible without developing sustainable agricultural methods, which require additional research into the mechanisms underlying the effects of carbon nanoparticles on plant growth and development. Seed germination is an indicator that is mainly determined by the quality of seed material, and also depends on the cultivation technology, soil and climatic conditions, and other factors. The results of the study showed that the germination rate of cabbage seeds varied significantly in the experimental groups (Fig. 2).

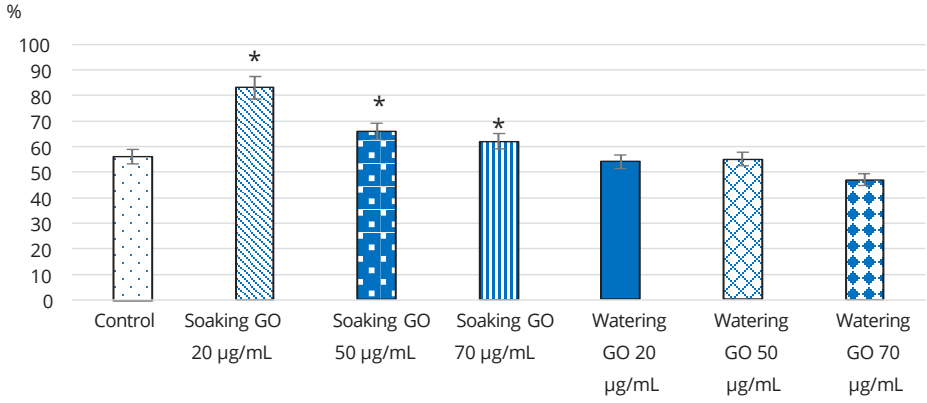

Figure 2. Seed germination (%) of cabbage (*Brassica oleracea*) on Day 20 of cultivation by various methods of treatment with graphene oxide nanoparticles

Note: * $p < 0.05$ compared to the control
Source: created by the authors

It was found that the highest percentage of seed germination of 83% was observed in the Group 2, where the seeds were primed (soaked) in a colloidal graphene oxide solution with a concentration of 20 µg/mL. This figure was significantly higher than in the control group (56%), in which the plants were watered only with water. Groups 3 and 4 also showed higher germination rates, namely 66% and 62%, in which seeds were primed with graphene oxide solutions with concentrations of 50 and 70 µg/mL. It is characteristic that in groups 5, 6 and 7, where graphene oxide was introduced by irrigation, the seed germination rate did not exceed that in the control group of plants (Fig. 2). Cabbage plants grown from seeds pretreated with graphene oxide solutions at concentrations of 20, 50, and 70 µg/mL had, on average, a longer overall length than the control group. The greatest increase in this indicator (32.8%) was observed at a low graphene oxide concentration of 20 µg/mL, while nanoparticle concentrations of 50 and 70 µg/mL were slightly lower, but also stimulated linear plant growth by 23.5% and 19.5%, respectively, compared to the control (Fig. 3).

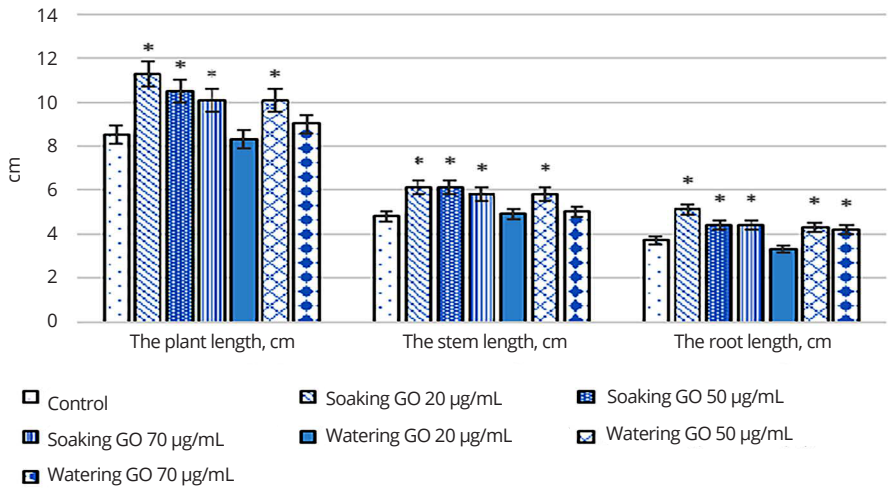

Figure 3. Morphometric parameters (cm) of cabbage (*Brassica oleracea*) on Day 20 of cultivation by various methods of treatment with graphene oxide nanoparticles
Note: * $p < 0.05$ compared to the control
Source: created by the authors

Plant groups in which plants were treated with graphene oxide nanoparticles by irrigation at concentrations of 20 and 70 μg/mL did not have a statistically significant difference in length compared to plants in the control group. However, watering with a colloidal solution with a concentration of 50 μg/mL also stimulated plant growth, since their length exceeded the same parameter of the control group by 19.0%, respectively (Fig. 3). In addition, the length of the STEM and root of plants was estimated by various processing methods. In the groups where seeds were primed with graphene oxide solutions with concentrations of 20, 50, and 70 μg/mL, the average stem length increased by 26.8%, 26.9%, and 20.6%, respectively, compared to plants in the control group. In the experimental groups where graphene oxide root treatment was used by double irrigation, a 21.6% increase in stem length was observed only at a nanoparticle concentration of 50 μg/mL, while no statistically significant changes were observed at other concentrations compared to the plants of the control group (Fig. 3).

According to the data obtained (Fig. 3) experimental groups of cabbage plants in which seeds were primed with graphene oxide nanoparticles with concentrations of 20, 50, and 70 μg/mL showed an increase in the average root length by 37.7%, 19.1%, and 18.1%, respectively, compared with the control group. Those plants that were treated with nanoparticle solutions by irrigation were not characterised by statistically significant changes in root length at concentrations of 20 and 70 μg/mL, and only at concentrations of 50 μg/mL, an increase of 15.5% was noted. Changes in the average length of cabbage leaves were also noted, and depending on the method of treatment with nanoparticles, they were different. In particular, in the groups where seeds were pre-soaked in graphene oxide solutions with concentrations of 20 and 50 μg/mL, the average leaf length was 13.0 and 21.1% longer, respectively, compared to the control group (Fig. 4).

In the experimental groups where irrigation was used, on the contrary, a decrease in the average leaf length of 20 and 70 μg/mL was noted by 13.3% (Fig. 4). Soaking seeds in graphene oxide solutions significantly affected the mass of plants. In the control group, the average plant weight was 213.4 mg. In the groups where seeds were primed with colloidal solutions of graphene oxide with concentrations of 20, 50, and 70 μg/mL, this indicator increased by 23.4%, 59.1%, and 68.5%, respectively. However, no statistically significant changes were observed in the groups with root application of nanoparticle solutions in the specified concentration range compared to the control (Fig. 5).

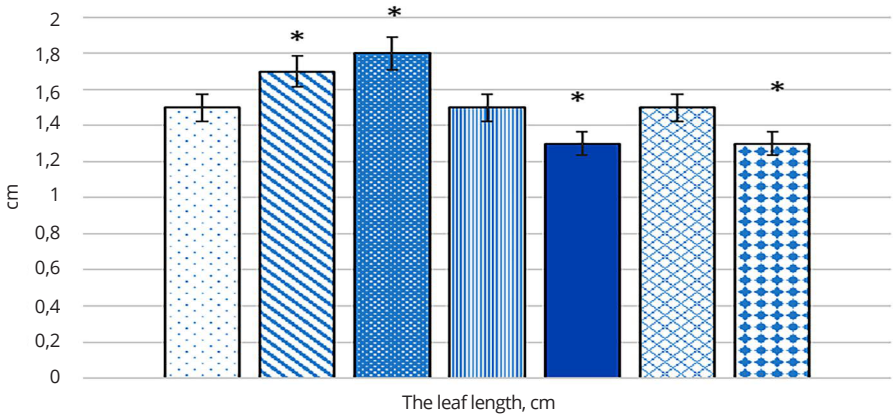

Figure 4. Average length (cm) of cabbage leaves *Brassica oleracea*) on Day 20 of cultivation by various methods of treatment with graphene oxide nanoparticles
Note: * $p < 0.05$ compared to the control
Source: created by the authors

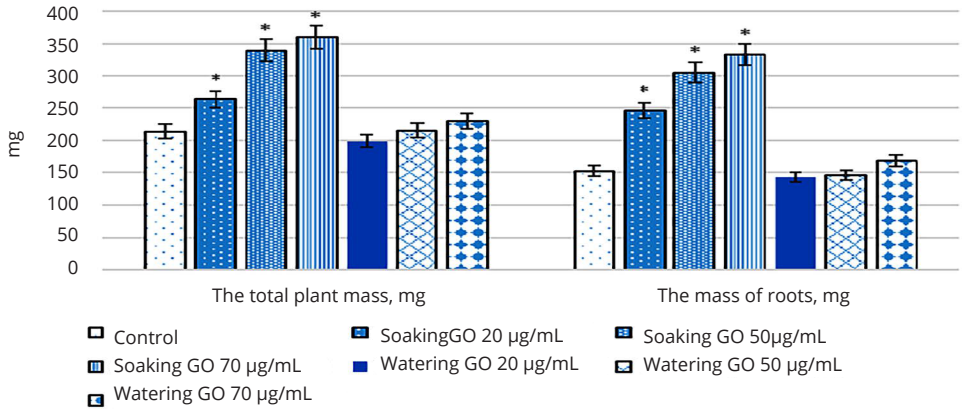

Figure 5. Total weight and root weight (mg) of cabbage seedlings (*Brassica oleracea*) on Day 20 of cultivation by various methods of treatment with graphene oxide nanoparticles
Note: * $p < 0.05$ compared to the control
Source: created by the authors

In addition to the total weight of the plant, the weight of the ground and underground parts of cabbage seedlings was also estimated. According to the results obtained (Fig. 5) the weight of roots differs significantly in the experimental groups. In the control group, the average root weight was 152.6 mg. In the experimental groups where cabbage seeds were primed with graphene oxide solutions with concentrations of 20, 50, and 70 µg/mL, this indicator increased by 1.61, 2.0, and 2.2 times, respectively, compared to the control. No significant changes in this indicator were observed in the experimental groups where irrigation with similar nanoparticle solutions was used compared to the control group. The results of comparing the stem weight showed that cabbage seedlings whose seeds were previously soaked in a graphene oxide solution with a concentration of 20 µg/mL had a stem weight 79.3% lower than that of the control group plants. Similar changes, but less pronounced, were observed in the experimental groups where solutions with concentrations of 50 and 70 µg/mL were used, namely, a decrease in the weight of the ground part of seedlings by 43.1% and 56.6%, respectively (Fig. 6).

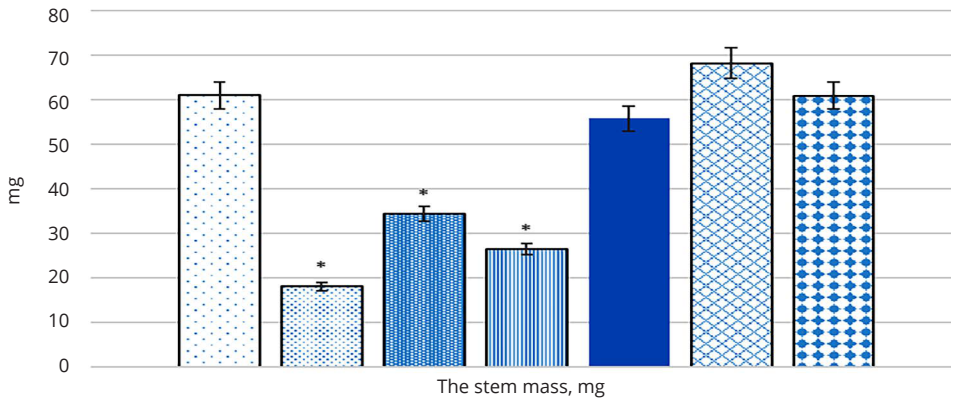

Figure 6. Weight of cabbage seedling stems (*Brassica oleracea*) on Day 20 of cultivation by various methods of treatment with graphene oxide nanoparticles, cm
Note: * $p < 0.05$ compared to the control
Source: created by the authors

No statistically significant changes in stem weight compared to the control were observed in the groups with root application of nanoparticle solutions in the specified concentration range (Fig. 6). Thus, graphene oxide affects seed germination and morphometric parameters of cabbage seedlings (*Brassica oleracea*), in particular, the total length of plants and the length of the stem, root and leaves, and the total weight of plants and the weight of ground and underground parts. The degree of this effect depends on several factors, namely, the concentration of the colloidal solution of nanoparticles and the method of their application.

The study by S. Kondratenko *et al*. (2023) and this study on the effects of graphene oxide share a common goal – to improve productivity of *Brassica oleracea* by using external biologically active factors and assessing their impact on the morphometric parameters of plants. Seed germination is one of the critical points that ultimately determines the quantity and quality of the resulting crop. According to M.T. MacDonald & V.R. Mohan (2025), advanced agricultural technologies involve the use of seed priming to increase its germination and stress resistance. According to M.S. Rhaman *et al*. (2021), seed priming is a physiological method that involves first moistening the seeds and then, usually, drying them, which helps to optimise metabolic processes before germination, increases the percentage and rate of germination, improves plant growth and yield both under optimal conditions and under the action of biotic or abiotic stresses. S. Chakraborti *et al*. (2022) and M.T. MacDonald & V.R. Mohan (2025) note that seed priming can be carried out either with ordinary water or with solutions containing various substances of both biological and chemical origin. Both studies demonstrate innovative approaches to stimulating the growth and development of food crops in the context of contemporary challenges to food security.

As indicated by S. Chakraborti *et al*. (2022), substances of biological origin used for bio-priming include: bacteria, fungi, seaweed extract, humic and fulvic acids, chitosan. Therewith, A. Shelar *et al*. (2021) and M.T. MacDonald & V.R. Mohan (2025) report that chemical priming is carried out using organic acids (ascorbic, salicylic, pyroline), inorganic salts (sodium chloride, potassium nitrate, calcium chloride, calcium sulphate), osmotically active substances (sugar, polyethylene glycol, glycerol, manitglycinbetaine sorbitol), benzimidazoles, synthetic phytohormones, antioxidants, etc. According to A. Shelar *et al*. (2021), recently, seed nanopriming involving engineered nanoparticles, in particular chitosan nanoparticles with copper/zinc or lignin loaded with gibberellic acid, metal nanoparticles, metalloids, and carbon, has become increasingly common. As the results showed, priming seeds with colloidal solutions of graphene oxide of various concentrations (20, 50, and 70 μg/mL) can significantly increase the germination rate of cabbage seeds. The greatest increase in the percentage of seed germination was

noted at the lowest studied concentration. The results obtained were confirmed by other studies.

According to the results obtained by I. Vera-Reyes *et al*. (2024), graphene oxide accelerated seed germination and seedling growth of *S. lycopersicum* at concentrations up to 200 mg of L^{-1} due to an increase in the content of reactive oxygen species (ROS), which led to rapid seed germination and higher germination rates. N. Yan *et al*. (2024) note that priming peanut beans with graphene oxide at a concentration of 400 mg L^{-1} causes activation of the carbon and nitrogen metabolic pathways in seeds. As indicated by L. Yang *et al.* (2024), a possible mechanism of effective action of nanopriming on seed germination is stimulation of aquaporin expression and activation of amylase formation.

The ability of graphene to penetrate the seed shell is also proven, which, according to M. Zhang *et al*. (2015), can disrupt the integrity of the seed coat and thus facilitate water absorption, which ultimately ensures faster germination and higher germination rates. Thus, summing up the analysed literature data, it can be argued that graphene oxide promotes the germination of plant seeds, in particular cabbage, due to the following main mechanisms: accumulation of reactive oxygen species in seeds; increased activity of specific enzymes in places of starch accumulation and activation of a number of metabolic pathways; expression of genes, in particular aquaporin; development of mechanical pores in the seed coat by nanoparticles.

However, A. Srivastava *et al*. (2021) note that seed priming affected not only the germination process, but also the preservation of the stimulating effect and increased stress resistance during plant ontogenesis. According to N. Louis *et al*. (2023), priming-induced epigenetic changes can persist and may further affect the priming-induced transgenerational stress memory of plants. According to the results obtained, soaking cabbage seeds in graphene oxide solutions not only improved its germination, but also contributed to linear plant growth, exceeding similar morphometric parameters of cabbage seedlings of those research groups in which nanoparticles were used by irrigation. However, a decrease was noted in plants obtained from primed seeds compared to the control of only one indicator – the raw weight of stems, which did not change in the experimental groups with watering.

Similar effects of graphene on plant growth have been observed by other researchers. According to M. Zhang *et al*. (2015), tomato seedlings grown from graphene-treated seeds had slightly less biomass accumulation than the control, but had significantly longer stems and roots than the control. Z. Zhou *et al*. (2023) noted that graphene oxide at concentrations of 20, 80, and 140 $mg \cdot l^{-1}$ increased the relative growth rate of *I. pseudacorus* and their dry weight, which, according to the researchers, is associated with improved regulation of photosynthetic electron transfer in PSII, the activity of endogenous endothelial cells, and the efficiency of light energy conversion. However, according to X. Xiao *et al*. (2022), various forms of graphene oxide negatively affected photosynthesis in *Brassica napus* L. plants. Reduced graphene oxide reduced the chlorophyll content and activity of the Rubisco enzyme. Graphene oxide directly affected chloroplast membranes, photosystems, and F-type ATPases, damaged photosystems and chloroplast structure, and inhibited carbon fixation.

However, the T. Lopes *et al*. (2021) found that GO nanoparticles prevent the development of abiotic stress in plants. Thus, graphene oxide in combination with a bacterium *Rhizobium* sp. applied to maize plants, increased osmotic and antioxidant protection under drought conditions. According to L. Zhao *et al*. (2022), graphene oxide increased the activity of protective enzymes in soybean plants, hormone content, and the expression of drought-related genes. M. Mazhar *et al*. (2025) noted that soybean plants treated with graphene oxide nanoparticles at concentrations of 50 and 100 ppm showed better growth parameters, reduced oxidative stress, increased activity of antioxidant enzymes (peroxidase, superoxide dismutase, catalase), and reduced arsenic accumulation in seeds under the action of the corresponding stress factor. Thus, the results of the study, and data from other authors, indicate a predominantly positive effect of graphene oxide nanoparticles on the growth and development of agricultural plants. It should be noted that the use of OG nanoparticles by seed treatment is more effective.

CONCLUSIONS

Seed germination and morphometric parameters of cabbage seedlings (*Brassica oleracea*) were studied by various methods of treatment with a

nanostructured aqueous solution of graphene oxide. It was found that colloidal solutions of graphene oxide nanoparticles in the concentration range of 20, 50, and 70 μg/mL during seed priming improve the germination of cabbage seeds, while the lowest studied concentration of graphene oxide showed the best results. The root introduction of NP did not affect the similarity index, which indicates the importance of choosing the method of using nanoparticles to obtain a specific effect of exposure.

It should be noted that other studied indicators of cabbage seedlings also reacted more to the action of graphene oxide during seed priming and did not change much during their root application. In particular, there was an increase in such indicators of cabbage seedlings as the total length of plants (19.5-32.8%), stem length (20.6-26.9%), average length of roots (18.1-37.7%) and leaves (13.0-21.1%), average plant weight (23.4-68.5%, and average root weight (1.6-2.2 times) after soaking cabbage seeds in graphene oxide solutions, while each of them had variable changes depending on the nanoparticle concentration. The only indicator that decreased with both methods of applying graphene oxide was stem weight, which requires further research to determine the cause of this effect. Thus, seed priming has a pronounced effect on the linear and mass parameters of seedlings, and in addition, this method is more convenient for use in crop production due to the ease of reproduction, economical use of nanoparticles, and reducing the risk of soil contamination with carbon nanoalotropes. Research in the chosen area is promising for the development of crop production, increasing the yield and stress resistance of agricultural crops in the conditions of total changes in climatic conditions and soil quality. But given the contradictory results regarding the effectiveness of various methods of graphene oxide applications obtained in this study and by other researchers, it is necessary to further investigate the mechanisms of influence of these nanoparticles and optimise practical methods of application with the selection of appropriate concentrations.

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CONFLICT OF INTEREST

None.

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Оцінка впливу оксиду графену на морфометричні показники капусти городньої (*Brassica oleracea*) за різних способів обробки

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Анотація. Зростання кількості населення у світі та зменшення площ родючих ґрунтів у зв'язку з глобальними кліматичними змінами вимагають сучасних наукових рішень щодо збільшення врожайності основних продовольчих культур з метою забезпечення продовольчої безпеки. Метою роботи було дослідити вплив наночастинок оксиду графену за різних концентрацій та способів обробки на лінійні і вагові показники сіянців капусти городньої (*Brassica oleracea*) ультраннього сорту 'Іюнська'. У роботі використовували фізіологічні методи дослідження з визначенням морфометричних показників рослин та схожості насіння. Для оцінки впливу оксиду графену використовували його наноструктуровані колоїдні розчини у діапазоні концентрацій 20, 50, 70 мкг/мл та двох способів обробки – праймінгом насіння та кореневим внесенням. Встановлено, що оксид графену стимулював схожість насіння капусти при замочуванні насіння в розчинах всіх досліджуваних концентрацій. Найвищий показник схожості насіння відмічали у групі, де для праймінгу був використаний розчин з концентрацією наночастинок 20 мкг/мл. Саме в цій групі схожість насіння становила 83 %, що суттєво перевищує цей показник в контрольній групі – 56 %. Кореневе внесення розчинів оксиду графену аналогічних концентрацій не викликало суттєвих змін зазначеного показника, який залишався в межах контрольних значень. У роботі доведено, що морфометричні показники сіянців капусти більш позитивно реагували на дію наночастинок за праймінгу насіння в противагу внесенню способом поливу. Праймінг насіння капусти різними концентраціями оксиду графену обумовлював збільшення лінійних показників сіянців, яке було найбільш вираженим переважно за концентрації 20 мкг/мл: загальна довжина рослин збільшилась на 32,8 %, довжина стебла на 26,8 %, середня довжина коренів на 37,7 % порівняно з контрольною групою. Середня маса рослин і маса коренів показали найбільше зростання за обробки насіння в розчині оксиду графену концентрацією 70 мкг/мл. Єдиним показником, який негативно відреагував на праймінг наночастинками, є маса стебла сіянців, яка зменшилась за всіх концентрацій. Використання наночастинок вуглецю у рослинництві є перспективним напрямком агротехнологій, що може забезпечити не лише високі показники схожості насіння, а й стимулювати ріст і розвиток продовольчих культур за правильно підібраних концентрацій та способів обробки

Ключові слова: вуглецеві наночастинки; нанопраймінг; рослини; схожість насіння; показники росту

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Detection and identification of soy viruses by molecular diagnostics methods

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Abstract. Viral plant diseases are one of the main causes of soybean crop losses in Ukraine, and the lack of systematic monitoring and limited use of modern diagnostic methods make it difficult to effectively control the spread of viral pathogens. The purpose of the study was to assess the prevalence of major viral pathogens in soybean crops in the Central Forest-Steppe of Ukraine. A combination of serological analysis and molecular method was used to detect soybean mosaic virus, soybean vein necrosis virus, and alfalfa mosaic virus. The results showed that the overall infection rate of soybean crops was 40.7% according to real-time polymerase chain reaction data with reverse transcription. The species composition of pathogens showed clear differentiation: soybean mosaic virus was detected in 20.0% of samples, soybean vein necrosis virus – in 8.0%, alfalfa mosaic virus – in 7.3%. The real-time reverse transcription polymerase chain reaction method showed higher sensitivity compared to the enzyme-linked immunosorbent assay method, revealing an additional 13 positive samples. The most significant discrepancies between the methods were observed when detecting alfalfa mosaic virus, where the molecular method detected 50% more positive samples. Regional analysis revealed the highest infection rate in the Cherkasy region (44.0%), with the maximum prevalence of soybean mosaic virus (22.0%). A moderate correlation was found between symptom severity and threshold cycle values for soybean mosaic virus (r = -0.62), whereas for soybean vein necrosis virus and alfalfa mosaic virus, the correlation was statistically insignificant. The results confirmed the high prevalence of viral infections among soybean crops in the study region. The results obtained will allow stating phytosanitary services, breeding institutions, and agricultural producers to implement regional-differentiated strategies for protecting soybeans, which will reduce crop losses and increase the effectiveness of phytosanitary measures

Keywords: serological analysis; polymerase chain reaction; enzyme immunoassay; co-infection; phytosanitary monitoring

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INTRODUCTION

Soybeans (*Glycine max* L.), as a strategically important leguminous crop, faces significant threats from viral pathogens that cause crop losses and degrade product quality. In the context of growing global demand for vegetable protein and the need to ensure food security, effective control of viral diseases of soybeans is becoming a priority for the agricultural sector of Ukraine. A particular threat is the ability of viruses to spread rapidly, form new strains and form mixed infections, which complicates phytosanitary control. The lack of monitoring systems and the limited use of molecular diagnostic methods necessitates a comprehensive study of the prevalence and identification of the main viral pathogens of soy in Ukrainian agrocoenoses.

According to a recent study by L. Mishchenko *et al*. (2022), soybean crop losses from viral diseases can reach 30-70%, depending on the region and pathogen. The study included the investigation of seed and transmissible viral diseases in various soybean genotypes. The influence of viral infections on crop productivity in Ukraine was established. The results showed the need for constant monitoring of the phytosanitary condition of crops. On the territory of Ukraine, which is one of the leading soybean producers in Europe, the phytosanitary situation remains tense, as evidenced by the monitoring data of the most dangerous soybean and potato viruses, conducted by A. Dunich *et al*. (2024), presented the results of diagnostics of the main viral pathogens of soy on the territory of Ukraine. The study included analysis of samples from different regions of the country. The researchers noted the need to find sources of virus resistance in soybeans.

International studies show increased attention to the investigation of soy viral pathogens, which is conditioned by their rapid spread and significant economic losses. The study by A. Hameed *et al*. (2022) was devoted to a comprehensive analysis of environmental interactions in the SVNV system (soybean vein necrosis virus) – plant thrips, where key factors contributing to the rapid spread of the virus have been identified. However, the issue of regional features of SVNV distribution in Ukraine remains unexplored, which is one of the key aspects of this study. Studies of co-infections of viral pathogens are important. J. Zhou & I.E. Tzanetakis (2020) proved the ability of SVNV to move systematically in soybean plants in the context of co-infection with the bean pod spot virus, using state-of-the-art molecular detection methods. However, the question of the prevalence and features of the course of mixed infections in Ukrainian agrocoenoses remains poorly understood. The genetic aspects of viral pathogens were investigated by O.A. Abdalla *et al*. (2020), who found a significant variety of AMV isolates (alfalfa mosaic virus) in Saudi Arabia, which explains the difficulty of controlling this pathogen. Despite this, the issue of genetic variability and biological features of AMV in Ukraine requires additional study. The problem of seed transmission of viruses is reflected in the paper by D.N. Bhagwatkar *et al*. (2025), where the role of seed transfer *Carlavirus vignae* was established in the development of soybean diseases in India. For Ukrainian conditions, similar studies are limited, which makes it necessary to investigate seed transmission of viruses in local conditions.

Diagnostic methods are presented in the paper by M.G. Elmore *et al*. (2022), who demonstrated the potential of metagenomic sequencing to detect a wide range of viruses. However, the practical implementation of this method in Ukraine is difficult due to technological limitations, which led to the choice of more affordable RT-qPCR methods in this study. The development of SVNV management tools is presented in the study by C. Zambrana-Echevarría (2021), but their adaptation to the conditions of Ukraine requires additional research, considering local characteristics. The study by S. Zambrana-Echevarría (2021) was dedicated to developing tools for SVNV management and tobacco streak ilarvirus in soy. The study included the development of virus detection methods and control strategies. The researcher proposed an integrated approach to the management of these pathogens. Given the complexity of three-way interactions between viruses, their carriers, and host plants, A. Hameed (2020) investigated three-way interactions between SVNV, thrips, and soybean plants. The study included laboratory and field studies of these complex relationships. The results demonstrate the influence of various factors on the effectiveness of virus transmission.

The purpose of this study was to assess the prevalence of major soybean viruses in agrocoenoses of the Central Forest-Steppe of Ukraine. To

achieve the goal, the following tasks were set: to take samples of soybean plants with symptoms of viral damage from different regions of the Central Forest-Steppe of Ukraine; to perform serological analysis using the DAS-ELISA method for primary screening for the presence of SMV, SVNV, and AMV; to conduct molecular identification of viruses using RT-qPCR and compare the effectiveness of serological and molecular diagnostic methods.

MATERIALS AND METHODS

Selection and preparation of plant material. The selection of plant material was carried out in the period from June to August 2024 on soybean crops of the 'Annushka' variety at the agricultural enterprises of three regions of the Central Forest-Steppe of Ukraine: Cherkasy (private enterprise "Luvais"), Poltava (limited liability company "Agris"), and Vinnytsia (private joint stock company "Podillia Foods Company"). Approximately 100 hectares of crops were surveyed using the route method, from which plants suspected of having a viral infection were selectively sampled. The criterion for selection was the presence of at least one of the characteristic symptoms of the virus: a pronounced mosaic colouring of the leaves in combination with a wrinkled surface of the leaf blade, a strong deformation – fern-like or corrugated edges of the leaf, local necrotic spots of brown or black colour, and general shortening of internodes and curling of the upper leaf tiers. The total number of individual leaf samples selected was 150 units (50 samples from each region), which was approximately 5-7 plants from each hectare of the surveyed area. Sampling was carried out in sterile disposable gloves using disinfected scissors to avoid cross-contamination between samples.

Each selected sample, consisting of two or three upper leaves with a growth point, was prepared for further analysis. After visual evaluation, each sample was divided into two identical parts to apply different diagnostic methods. The first part of the leaves without grinding was placed in a separate sterile plastic container with a capacity of 50 mL with perforation for gas exchange. These containers were placed in a portable refrigerator – a cooler bag with cold storage batteries, where the temperature was maintained in the range of +2°C to +4°C. Under such conditions, the samples were transported to the laboratory, where they were stored in a refrigerator at a stable temperature of +4°C and a relative humidity of 80-90% for no longer than 48 hours before the enzyme-linked immunosorbent assay, which allowed maintaining the stability of viral antigens.

The second part of the sample intended for molecular diagnostics was subjected to instant cryogenic fixation. For this purpose, the leaves were individually immersed in a container of liquid nitrogen for 30-40 seconds, which provided an instant stop of all enzymatic processes and prevented RNA degradation. Next, cryogenically treated samples were individually packed in pre-sterilised foil bags and placed for long-term storage in a freezer with forced air circulation at a stable temperature of -80°C (Thermo Scientific, USA). This storage regime guaranteed the preservation of the integrity of nucleic acids throughout the entire study period. Before RNA extraction, the samples were transported to the laboratory in dry ice containers that maintained a temperature of -79°C.

Serological analysis by DAS-ELISA. Serological analysis was performed under laboratory conditions by two-stage enzyme immunoassay (DAS-ELISA) in accordance with the standard protocols given in the manufacturer's instructions. Commercial kits were used to detect soybean mosaic virus (SMV), soybean vein necrosis virus (SVNV), and alfalfa mosaic virus (AMV) from "Loewe Biochemica" (Germany). Extraction of plant antigens was performed by homogenising 0.1 g of leaves in 1 mL of soluble buffer (extraction buffer) included in the kit. The sandwich complex was created on 96-hole tablets for ELISA (Nunc, Denmark). Incubation with primary and secondary antibodies, and with a conjugate (alcalic phosphatase), was performed in a thermostat at 37°C. The tablet was washed using a small laboratory microplate washer. The substrate p-nitrophenyl phosphate (pNPP) was used for visualisation. Incubation with the substrate was carried out in the dark at room temperature of 23°C for 60 minutes. The reaction was stopped by adding 3M NaOH. Optical density (OD) at a wavelength of 405 nm was measured on a vertical spectrophotometer for microplates "Multiskan GO" (Thermo Fisher Scientific, Finland). The sample was considered positive if its optical density value exceeded the arithmetic mean of the negative control values by at least three times.

Molecular diagnostics by RT-qPCR. Molecular diagnostics were performed in the laboratory using real-time reverse transcription and polymerase chain reaction (RT-qPCR). Total RNA was isolated from 100 mg of cryogenically crushed leaf tissue using the "Ukrbiopreparat" kit (Ukraine) in accordance with the manufacturer's instructions. The concentration and purity of the obtained RNA preparations were evaluated using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). The A260/A280 ratio for all samples was in the range of 1.8-2.0. Reverse transcription was performed using the "Reverta-L" kit (Ukraine) in a reaction volume of 20 µl containing 1 µg of total RNA, random hexameric primers, and M-MuLV polymerase. The reaction was performed according to the following programme: 10 minutes at 25°C, 60 minutes at 42°C, and 10 minutes at 70°C to inactivate the enzyme.

Real-time polymerase chain reaction was performed in a CFX96 Touch amplifier (Bio-Rad, USA). The 25 µl reaction mixture contained 1X PCR buffer (polymerase chain reaction), 2.5 mm $MgCl_2$, 200 µM each dNTP, 0.2 µM forward and reverse primer, 0.5 µl intercalating dye SYBR Green I (Ukraine), 1 unit Taq-DNA (deoxyribonucleic acid)-polymerase (Ukraine) and 2 µl matrix cDNA. Species-specific primers were used to detect viruses, the sequences of which were taken from literature sources. To detect soybean mosaic virus primers SMV-F (5'-CGA-GCA-AGC-AGT-TCA-AGA-CC-3') and SMV-R (5'-TCT-GCC-ATA-ACC-ATG-GAC-GA-3') were aplied, described in the paper by B. Xue *et al*. (2021). To detect soybean vein necrosis virus (SVNV) used primers SVNV-F (5'-ATG-GGT-TTG-GGA-AGA-AGG-TG-3') and SVNV-R (5'-TCA-AGC-AAC-TCC-AGC-ACC-at-3') published in the paper by C. Groves *et al*. (2016). For detecting alfalfa mosaic virus applied primers (5'-ATGCTACCCAGGCATGTATATTT-3') and (5'-GCTGCATCTTTCGCCAGAA-3') that amplify the capsid protein gene region.

The amplification protocol included initial denaturation at 95°C for 3 minutes, followed by 40 cycles, each consisting of denaturation at 95°C for 15 seconds, primer annealing at 60°C for 20 seconds, and DNA synthesis at 72°C for 30 seconds. After amplification, melting curves were constructed by slow heating in 0.5°C increments from 65°c to 95°C to confirm the specificity of the synthesised PCR products. In each run, negative control without matrix (no template control, NTC) and positive control were used. The sample was considered positive if the amplification threshold cycle (Ct) was less than 35, and there was a clear peak on the melting curve with a melting point identical to the positive control. Experimental plant studies, including the collection of plant material, were in accordance with institutional, national, and international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992).

Quantitative analysis of viral load and statistical data processing. To quantify the viral load, the threshold cycle values were analysed. Quantitative calibration was performed using a series of ten-fold dilutions of recombinant plasmids containing target regions of viral genes. A standard curve with a correlation coefficient $R^2 > 0.98$ was constructed for each virus. The linearity range for all detected viruses was from 10^1 to 10^8 copies/µl. The threshold cycle values were interpreted as follows: Ct < 30 indicated a high concentration of the virus ($\geqslant 10^5$ copies/µl), Ct 30-35 indicated an average concentration (103-10 copies/µl), and Ct > 35 indicated a low concentration of the virus (< 103 copies/µl). Samples with Ct > 40 were considered negative. Quantitative analysis was performed using the CFX96 Touch analyser software (USA), with automatic calculation of the virus concentration based on standard curves. Each sample was analysed in three repetitions, and the mean Ct value was used for further calculations. Statistical processing of the obtained data was performed using Microsoft Excel 2019 and Statistica 12.0 software suite (USA). Differences between the frequency of virus detection by different methods were estimated using the nonparametric criterion χ^2 (chi-square). The difference was considered statistically significant at the significance level $p < 0.05$.

RESULTS

Results of serological analysis by DAS-ELISA

Serological analysis by DAS-ELISA showed a significant level of infection of soybean crops with viral pathogens in the study region. Out of a total of 150 samples analysed, a positive response to the presence of at least one of the three viruses under study was recorded in 48 samples, which is 32.0%

of the total sample size. The average optical density (OD_{405}) of positive samples was 0.85 ± 0.23, which significantly exceeded the negative control value (0.12 ± 0.04). In all positive samples, the optical density value exceeded the positivity threshold (0.36) by at least 3 times. The results of serological analysis by the DAS-ELISA method are presented in Table 1.

Table 1. Results of serological analysis of soybean samples by DAS-ELISA method

Research area	Total number of samples	SMV-positive	SVNV-positive	AMV-positive	General infection rate
Cherkasy	50	11 (22.0%)	4 (8.0%)	3 (6.0%)	18 (36.0%)
Poltava	50	9 (18.0%)	3 (6.0%)	3 (6.0%)	15 (30.0%)
Vinnytsia	50	7 (14.0%)	5 (10.0%)	3 (6.0%)	15 (30.0%)
Total	150	27 (18.0%)	12 (8.0%)	9 (6.0%)	48 (32.0%)

Note: the sum of individual infections may exceed the total number of infected samples due to the presence of mixed infections that were not detected by the DAS-ELISA method
Source: compiled by the authors based on the conducted research

A detailed analysis of the species composition of pathogens revealed a clear differentiation in the prevalence of individual viruses. The most common pathogen was soybean mosaic virus (SMV), which was identified in 27 samples, accounting for 18.0% of the total number of plants tested. The average optical density for SMV-positive samples was 0.92 ± 0.18. Soybean vein necrosis virus (SVNV) took the second place in prevalence – it was detected in 12 samples (8.0%), with an average value of OD_{405} = 0.76 ± 0.21. The least common among the pathogens under study was alfalfa mosaic virus, which was detected in 9 samples (6.0%) with an average optical density of 0.68 ± 0.15.

Statistical analysis using the χ^2 criterion did not reveal statistically significant differences in the frequency of virus detection between different study areas ($p > 0.05$), which was confirmed by the following indicators: total infection varied between 30.0-36.0%, the prevalence of alfalfa mosaic virus was 6.0% in all three regions, and the detection rate of soybean mosaic virus has only minor differences between regions (from 14.0% to 22.0%). However, analysis of the distribution of infection by region revealed certain trends. In the Cherkasy region, the highest overall infection rate was observed – 36.0% (18 positive samples out of 50), while in the Poltava and Vinnytsia region this figure was 30.0% (15 positive samples out of 50 in each region). The distribution of certain types of viruses by region also showed certain regional features: SMV was most common in the Cherkasy region (22.0%), while SVNV was more often detected in the Vinnytsia region (10.0%).

It was noted that the colour intensity (OD_{405}) of positive samples correlated with the severity of visual symptoms in the field. Samples with pronounced mosaic and leaf deformity symptoms showed OD_{405} values above 1.0, while samples with mild symptoms had values in the range of 0.4-0.7. In 6 samples (4.0% of the total), OD_{405} values close to the threshold level (0.36-0.42) were observed, which could indicate the initial stages of infection or a low titre of the virus in plant tissues.

Effectiveness of molecular diagnostics and comparative analysis of methods

Molecular diagnostics using real-time reverse transcription and polymerase chain reaction (RT-qPCR) showed an advantage in sensitivity compared to DAS-ELISA serological analysis. The overall detection rate of viral pathogens was 40.7% (61 positive samples out of 150), which is 8.7 percentage points higher than the indicator obtained by the ELISA method. A detailed analysis of the detection efficiency of individual viruses showed that the advantage of RT-qPCR was most pronounced for detecting alfalfa mosaic virus (AMV), where the molecular method detected 50% more positive samples (12 vs. 8), and for soybean vein necrosis virus, where the difference was 8.3% (13 vs. 12).

Quantitative analysis of threshold cycle values (Ct) showed a high titre of viruses in infected plants. The Ct range for positive samples ranged

from 24.3 to 32.7 cycles. The lowest Ct values (higher virus concentration) were observed for SMV – an average value of 26.5 ± 2.3, which correlated with pronounced symptoms of mosaic and leaf deformity. For SVNV, the mean Ct value was 28.1 ± 3.1, and for AMV – 30.4 ± 2.8, which indicates a lower concentration of these pathogens in plant tissues.

Statistical analysis using the χ^2 criterion revealed a statistically significant difference between the effectiveness of the DAS-ELISA and RT-qPCR methods ($\chi^2 = 8.45$; $p < 0.01$). The RT-qPCR method revealed an additional 13 positive samples that showed negative results in serological analysis. The detection of 4 samples with Ct values in the range of 31.5-32.7 was indicative, which indicates a low concentration of the virus, insufficient for detection by the ELISA method (Table 2).

Table 2. Comparative characteristics of the effectiveness of DAS-ELISA and RT-qPCR methods

Parameter	DAS-ELISA	RT-qPCR	Difference
General detection	48/150 (32.0%)	61/150 (40.7%)	+13 samples (+8.7%)
SMV	27/150 (18.0%)	29/150 (19.3%)	+2 samples (+1.3%)
SVNV	12/150 (8.0%)	13/150 (8.7%)	+1 sample (+0.7%)
AMV	8/150 (5.3%)	12/150 (8.0%)	+4 samples (+2.7%)
Mean Ct	-	26.5-30.4	-

Note: (-) in the DAS-ELISA column for the "mean Ct" parameter indicates that the enzyme-linked immunosorbent assay method does not provide for determining the threshold cycle; (-) in the "Difference" column for the "Mean Ct" parameter, conditioned by the lack of comparable data in the DAS-ELISA method
Source: compiled by the authors based on the conducted research

The analysis in Table 2 demonstrates the advantages of the molecular diagnostic method. The most significant increase in sensitivity is observed for detecting alfalfa mosaic virus, where the polymerase chain reaction method detected 50% more positive samples. Overall detection of viral infections increased by 8.7% when using the molecular method, which confirms its higher effectiveness for comprehensive monitoring of soy viral diseases. Analysis of regional samples showed that the differences between the methods were most pronounced in the Vinnytsia region, where RT-qPCR detected 6 positive samples more than ELISA, while in Cherkasy and Poltava region this difference was 4 and 3 samples, respectively. This may indicate regional features of the development of viral infections and the level of accumulation of the virus in plants. The melting curves of all positive samples showed clear peaks with characteristic melting points: 82.5°C ± 0.5°C for SMV, 85.2°C ± 0.3°C for SVNV, and 79.8°C ± 0.4°C for AMV, which confirms the specificity of amplification and the absence of non-specific PCR products. Atypical Ct values (more than 34 cycles) were observed in 5 samples, which, however, had characteristic melting curves and were classified as weakly positive. The results confirm that the RT-qPCR method is a sensitive tool for early diagnosis of soy viral infections, especially in cases of low virus concentrations and for detecting AMV, which shows the lowest titres in plant tissues.

Spread of viral infections and detection of mixed infections

A detailed analysis of the obtained results of molecular diagnostics allowed establishing a complex structure of the spread of viral infections among soybean crops in the study region. The overall infection rate determined by RT-qPCR was 40.7% (61 samples out of 150), which indicates the accumulation of viral pathogens in soybean agrocoenoses. The regional distribution of infected samples showed clear territorial features: the highest level of infection was registered in the Cherkasy region (44.0%, 22 samples), a slightly lower indicator was observed in the Vinnytsia region (40.0%, 20 samples), and the lowest number of infected plants was found in the Poltava region (38.0%, 19 samples). The value of the study is the detection of mixed infections that could not be reliably identified by the serological method (Table 3).

Table 3. Regional distribution of viral infections and mixed infections according to RT-qPCR data

Type of infection	Cherkasy region (n = 50)	Poltava region (n = 50)	Vinnytsia region (n = 50)	Total (n=150)
SMV	11 (22.0%)	9 (18.0%)	10 (20.0%)	30 (20.0%)
SVNV	4 (8.0%)	3 (6.0%)	6 (12.0%)	13 (8.7%)
AMV	3 (6.0%)	4 (8.0%)	4 (8.0%)	11 (7.3%)
SMV + SVNV	3 (6.0%)	2 (4.0%)	2 (4.0%)	7 (4.7%)
SMV + AMV	1 (2.0%)	1 (2.0%)	1 (2.0%)	3 (2.0%)
General infection rate	22 (44.0%)	19 (38.0%)	20 (40.0%)	61 (40.7%)

Source: compiled by the authors based on the conducted research

Molecular diagnostics revealed 10 cases of co-infections, which is 6.7% of the total number of analysed samples. The most common combination was SMV and SVNV, which was recorded in 7 samples (4.7%). Less common was SMV and AMV co-infection was detected in 3 samples (2.0%). Analysis of the threshold cycle (Ct) values for samples with mixed infections showed a pattern: in 8 out of 10 cases, the dominance of one of the viruses was observed, which was manifested in Ct values that differed by 3-5 cycles. Soybean mosaic virus showed the highest prevalence in the Cherkasy region (22.0%), which is associated with favourable climatic conditions for the development of aphids carrying this virus. Soybean vein necrosis virus was most common in the Vinnytsia region (12.0%), which is probably conditioned by the presence of reservoir plants and favourable conditions for thrips development. Alfalfa mosaic virus showed the most uniform distribution between regions (6.0-8.0%), which may indicate a stable circulation of this pathogen in the agrocoenoses of the region.

Another aspect of the study was the analysis of the relationship between the type of infection and the severity of symptoms. In samples with mixed infections, significantly more pronounced symptoms of the lesion were observed: intense mosaicism, severe deformation of leaf blades, necrotic spots, and pronounced dwarfism of plants. The mean Ct value for samples with mixed infections was 25.8 ± 1.9, which indicates higher viral concentrations compared to monoinfections (28.3 ± 2.4). The geographical distribution of mixed infections also had its own characteristics. The highest frequency of co-infections was observed in the Cherkasy region (8.0%), where 4 cases of mixed infections were detected. In the Poltava and Vinnytsia regions, this figure was 6.0% and 6.0%, respectively. This may indicate a more intensive epiphytotic process in the Cherkasy region, which requires additional research to identify the causes and factors of such differentiation. The results highlight the importance of considering mixed infections when assessing the phytosanitary condition of soybean crops and developing protection measures, since co-infections can significantly enhance the pathogenic effect and lead to more significant crop losses.

Correlation between symptoms and infection intensity and diagnosis specificity

A detailed analysis of the relationship between the clinical manifestations of viral infections and laboratory indicators of infection intensity revealed patterns that are important for practical diagnosis. For SMV, a moderate negative correlation was observed between the severity of symptoms and Ct values ($r = -0.62$; $p < 0.05$), which indicates a clear relationship between the severity of clinical manifestations and the concentration of the virus in plant tissues. Samples with a pronounced mosaic (4-5 points) showed Ct values in the range of 24.3-26.8, while plants with mild symptoms (1-2 points) had Ct values of 28.5-31.2. The strongest correlation was observed for mosaic symptoms ($r = -0.58$) and leaf blade deformity ($r = -0.61$), while the association with necrotic spots was less pronounced ($r = -0.42$).

For SVNV, the correlation between symptoms and Ct values was weak and statistically insignificant ($r = -0.34$; $p > 0.05$). This may indicate a more complex pathogenesis of this virus, where clinical manifestations depend not only on the concentration of the virus, but also on other factors, such as the age of the plant, environmental conditions or

characteristics of the variety. Samples with characteristic vein necrosis had Ct values in a wide range from 26.8 to 32.4, which confirms that there is no direct association between visible symptoms and viral concentrations. For AMV, correlation analysis showed the weakest association between symptoms and Ct values ($r = -0.28$; $p > 0.05$). This may be conditioned by the fact that AMV causes atypical or mild symptoms on soybean, and the fact that the pathogen may be latent or cause symptoms only under certain environmental conditions. Of interest is also the analysis of samples with mixed infections, where a significantly stronger correlation was observed between the total symptom score and the mean Ct values ($r = -0.71$; $p < 0.01$). This confirms the synergistic effect in co-infection, when the presence of two viruses leads to more pronounced symptoms at the same concentrations of pathogens.

The specificity of the developed PCR system was carefully analysed using a detailed study of melting curves. For SMV, clear peaks were observed with a melting point of 82.5°C ± 0.5°C, which corresponds to the expected amplicon size of 233 bp. For soybean vein necrosis virus, the melting point was 85.2°C ± 0.3°C, which is typical for a longer fragment of nucleocapsid protein. For AMV, peaks with a melting point of 79.8°C ± 0.4°C were recorded, which corresponds to a fragment of the capsid protein gene. All melting curves showed a single-modal character with no side peaks, which indicates high amplification specificity and the absence of primer dimers or non-specific products.

Control experiments confirmed the high reliability of the molecular method. All negative controls ($n = 15$) showed no amplification for 40 cycles, while controls without a matrix (NTC, $n = 12$) also remained negative, which excludes the possibility of contamination. Positive controls for each virus ($n = 9$) showed stable Ct values with a coefficient of variation of less than 3.2%, which confirms the reproducibility of the method. An additional confirmation of specificity is the fact that Ct values correlated with the results of serological analysis: samples with low Ct values (high virus concentration) usually showed a strong positive response in ELISA, while samples with high Ct values were often weakly positive or negative in the serological test. This pattern was particularly clear for SMV, where the correlation between OD values in ELISA and Ct values in RT-qPCR was $r = -0.59$ ($p < 0.05$).

DISCUSSION

The results obtained regarding the high prevalence of viral infections among soybean crops in the Central Forest-Steppe of Ukraine are consistent with research data from other regions of the world. The overall infection rate of 40.7% detected by RT-qPCR correlates with the results of studies in India, where viral infections also pose a serious problem for soybean producers, as indicated in by N. Sandra *et al*. (2021). The high prevalence of SMV may be due to its ability to transmit seeds and effective transmission by aphids, which is confirmed by studies of the genetic structure of populations of this virus, which were conducted by M. Usovsky *et al*. (2022). A long period of preservation of the viability of the virus in seeds and a wide presence of vectors in agrocoenoses contribute to maintaining a high level of infection.

The advantage of the RT-qPCR method over DAS-ELISA in detecting viral infections is confirmed in current studies. Current approaches to virus screening using eDNA-based electronic samples show promise for global monitoring of soy viral diseases, as noted in the paper by M.R. Ribeiro-Junior *et al*. (2025), and is consistent with the need to introduce more sensitive diagnostic methods in the phytosanitary control system. Detection of mixed infections in 6.7% of the samples under study is important for understanding the epiphytotic process. Studies of interactions between different viruses in soybean plants have shown that co-infections can significantly affect disease development and crop productivity. Combinations of viruses with different transmission mechanisms are dangerous, which complicates control measures, as noted by S. Tatineni & G.L. Hein (2023).

The regional features of the spread of viruses identified in the study were confirmed in international scientific works. Differences in the prevalence of viruses between Cherkasy, Poltava and Vinnytsia regions are consistent with data from studies in Bangladesh, where significant regional variability in the prevalence of soymean viral pathogens was also observed, as indicated by M.F. Khatun *et al*. (2025). In contrast to the study in Indonesia conducted by E. Uge *et al*. (2023), which revealed a clear correlation between climatic

conditions and the prevalence of mosaic viruses, this study failed to establish a direct relationship between climatic parameters and infection rates, which may be conditioned by the relative uniformity of climatic conditions in the Central Forest-Steppe of Ukraine in comparison with various climatic zones of Indonesia, and differences in the species composition of vectors and their ecological features. However, the identified trends in the regional distribution of individual viruses, in particular, the increased prevalence of soybean vein necrosis virus in the Vinnytsia region, may be of practical importance for the development of differentiated plant protection measures. The detection of AMV in soybean agrocoenoses in the Central Forest-Steppe of Ukraine is consistent with the global trend in the spread of this pathogen. H.A. Hobbs *et al*. (2012) recorded the appearance of AMV on soybeans in North Dakota, indicating the virus' ability to adapt to new geographical areas. The latest data by R. Dawoud (2025) highlighted the significant potential of AMV for genetic variability, which explains its successful circulation in various agroecosystems. The high frequency of detection of AMV in Ukrainian soybean crops may be associated with a wide range of host plants and the ability of the virus to persist for a long time in plant residues, which justifies the need for constant monitoring and development of specialised control measures.

Correlation analysis between the severity of symptoms and Ct values confirms the complexity of visual diagnosis of viral infections. The moderate negative correlation for SMV (r = -0.62) is consistent with current studies using artificial intelligence to identify soy diseases by K. Zhang *et al*. (2021). However, the lack of a statistically significant correlation for SVNV and AMV indicates limited visual assessment methods, which is not fully consistent with the results of studies of automated diagnostic systems by K. Zhang *et al*. (2021), which demonstrated high accuracy for various types of viral lesions. This is conditioned by the more complex pathogenesis of SVNV and AMV, and their ability to cause atypical symptoms depending on the physiological state of the plant. The need to introduce a comprehensive system of protection measures was confirmed by the results of breeding studies. The high level of infection of soybean crops with viral pathogens in Ukraine is consistent with the need to use molecular markers and genomic selection in breeding programmes, which was considered by F. Lin *et al*. (2022). Identification of SMV resistance genes in international studies by D. Wang *et al*. (2022) and J. Chu *et al*. (2021) confirmed the prospects of creating sustainable varieties for the conditions of Ukraine. The study by D. Wang *et al*. (2022) presented a comprehensive analysis of the genetic loci responsible for SMV resistance. The researchers identified a key QTL (quantitative trait locus) on chromosome 13, which explains up to 68% of the phenotypic variability of viral resistance. The value is that the researchers were able to map a new Rsv4 gene that provides resistance to a wide range of SMV isolates. The study by J. Chu *et al*. (2021) complemented these results by focusing on studying the Rsv1 gene in various soy genetic populations. The researchers found that allelic variations in this gene cause different levels of resistance to SMV, with the most effective alleles providing immunity to 95% of known virus isolates.

The use of RNA interference to control SMV demonstrated the effectiveness of this approach, which is fully consistent with the results of the study by H. Luan *et al*. (2020), where transgenic plants with the Vma12 gene turned off showed increased resistance to SMV. However, the effectiveness of RNA interference for controlling SVNV and AMV remains poorly understood, unlike SMV, for which specific control strategies have been developed. The use of viral vectors for induced gene silencing opens up new horizons in the fight against soy viral infections, which is confirmed by the innovative study by M. McCaghey *et al*. (2021). The researchers demonstrated that a vector based on the bean pod spot virus can effectively deliver constructs for RNAi-mediated silencing of oxaloacetate-acetylhydrolase genes in *Sclerotinia sclerotiorum*, which led to a significant reduction in the development of soy disease. This approach is useful for controlling viral infections, as it allows purposefully inhibiting the replication of viruses or disrupt their life cycles, creating resistance at the molecular level. The advantage of this technology is the ability to quickly adapt to new strains of viruses by changing siRNA sequences, which is important in the context of constant evolution of viral pathogens.

Adevanced multi-mix approaches radically change the methodology of breeding virus-resistant

soybean varieties, which is fully confirmed in the study by A. Bisht *et al*. (2023). Comprehensive analysis of transcriptomics, proteomics, and metabolomics reveals not only individual resistance genes, but also entire networks of molecular interactions that form systemic resistance to viral infections. However, according to S. Rahman *et al*. (2023), the introduction of these advanced technologies in the breeding process requires significant investment in equipment and training. In the context of Ukraine, these restrictions are particularly relevant, which makes it necessary to gradually introduce omics technologies – first with a focus on transcriptome analysis as the most accessible method, with further expansion to full-scale multi-omics studies. The use of genomic selection to improve virus resistance is confirmed in international studies, but its practical implementation requires adaptation to local conditions and pathogenic complexes. The use of high-throughput sequencing to detect new and emerging viral pathogens is fully consistent with the findings of R. Fowkes *et al*. (2021), where this method helped to identify turnip yellows virus and soybean dwarfism virus in peas in the UK.

The study by M. Solomiichuk & M. Pikovskyi (2021) examined the use of *Pseudomonas fluorescens* and stimulating substances to fight pathogens, improving the productivity of soybeans. In turn, this study focused on detecting mosaic viruses and soy necrosis using molecular methods, in particular PCR. Both studies contribute to reducing soybean crop losses by using different approaches to pathogen control. The effectiveness of the applied diagnostic methods is confirmed in contemporary studies on soy pathology. The use of a combination of serological and molecular methods to detect viral infections is consistent with the approaches described for the diagnosis of fungal and oomycete soy pathogens in B. Hosseini *et al*. (2023), which notes the importance of combining different methods for accurate pathogen identification. However, in contrast to the diagnosis of fungal diseases, where real-time PCR and enzyme immunoassay methods are widely used, for viral pathogens, molecular methods show higher efficiency, which is confirmed by the detection of an additional 13 positive samples using RT-qPCR compared to DAS-ELISA. Biotechnological approaches to creating virus-resistant soybean varieties show promise in global research. Development of a guide RNA delivery system using the ALSV virus for genomic editing proposed by Y. Luo *et al*. (2021) is consistent with the need to develop innovative methods to protect against SMV, which is identified as the dominant pathogen in this study. However, in contrast to the experimental conditions of the study by Y. Luo *et al*. (2021), the practical application of such technologies in Ukraine requires further research. The use of CRISPR/Cas systems to create virus resistance is a promising area, but its effectiveness in controlling SVNV and AMV remains poorly understood. The molecular mechanisms of natural resistance to SMV described in international studies may explain the high prevalence of this virus in Ukraine. Detection of an NLR receptor that recognises the cylindrical SMV inclusion protein in the study by J. Yin *et al*. (2021) offers a molecular explanation of the mechanisms of resistance, which is partially consistent with the data obtained on the high prevalence of SMV (20.0%). However, in contrast to the experimental conditions of the study, J. Yin *et al*. (2021), where specific resistance genes were studied, the presence of functional alleles of these genes may be limited in Ukrainian soybean varieties. This makes it necessary to introduce marker-associated breeding for introgression of SMV resistance genes in Ukrainian varieties.

The conducted study confirmed the effectiveness of molecular diagnostic methods for detecting soybean viral pathogens in Ukraine. The results obtained substantiate the need to introduce an integrated approach to phytosanitary monitoring, combining serological and molecular methods. The identified regional features of the spread of viruses and the frequency of mixed infections form the scientific basis for the development of differentiated plant protection measures aimed at reducing crop losses and improving the efficiency of soybean production in Ukraine.

CONCLUSIONS

Based on the conducted comprehensive study on the detection and identification of soybean viruses by molecular diagnostics methods, a high level of prevalence of viral infections among soybean crops in the Central Forest-Steppe of Ukraine was established. Serological analysis by the DAS-ELISA method revealed a total infection rate of 32.0%, and the

highest rates were observed in the Cherkasy region (36.0%). Among the three viruses studied, soybean mosaic virus was the most common – 18.0%, which indicates the need to strengthen phytosanitary control over this particular pathogen.

The RT-qPCR method showed a statistically significant 8.0% increase in sensitivity, detecting an additional 12 positive samples, and when detecting alfalfa mosaic virus, the number of positive samples detected increased by 50%. This justifies the feasibility of using RT-qPCR as the main method for accurate diagnosis, especially in the early stages of infection. For the first time in the study area (Central Forest-Steppe of Ukraine), mixed infections were identified with a frequency of 6.7% of the total number of analysed samples. SMV + SVNV co-infection was the most common (4.7%). The identified mixed infections highlighted the complexity of the epiphytotic situation and the need for a comprehensive approach to diagnosis. Correlation analysis revealed varying degrees of association between visual symptoms and laboratory parameters for individual viruses. For soybean mosaic virus, a moderate negative correlation ($r = -0.62$) was established between the severity of symptoms and threshold cycle values, which indicates the possibility of using visual assessment for preliminary diagnosis. Regional analysis revealed differentiation in the spread of viruses: the highest total infection was recorded in Cherkasy region (44.0%), and the maximum prevalence of soybean vein necrosis virus – in Vinnytsia region (12.0%), which may be due to climate features and the presence of vectors.

The conducted study has certain limitations, among which the regional specifics of the sample are highlighted, covering only three regions of the Central Forest-Steppe, the lack of monitoring of the dynamics of virus accumulation during the growing season, and the limited diagnosis of the three main viruses without considering other potentially dangerous pathogens. The prospects for further research include expanding the geography of monitoring to other agroclimatic zones of Ukraine, studying the influence of climatic factors on the development of viral infections, and developing multiplex PCR systems for simultaneous detection of a wider range of pathogens.

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Виявлення та ідентифікація вірусів сої методами молекулярної діагностики

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Анотація. Вірусні хвороби рослин є однією з основних причин втрат врожаю сої в Україні, а відсутність системного моніторингу та обмеженість застосування сучасних методів діагностики ускладнюють ефективний контроль поширення вірусних патогенів. Метою роботи була оцінка поширеності основних вірусних патогенів у посівах сої Центрального Лісостепу України. Використано комбінацію серологічного аналізу та молекулярного методу для детекції вірусу мозаїки сої, вірусу некрозу жилок сої та вірусу мозаїки люцерни. Результати показали, що загальний рівень інфікованості посівів сої становив 40,7 % за даними полімеразної ланцюгової реакції у реальному часі з зворотною транскрипцією. Видовий склад патогенів демонстрував чітку диференціацію: вірус мозаїки сої виявлено у 20,0 % зразків, вірус некрозу жилок сої – у 8,0 %, вірус мозаїки люцерни – у 7,3 %. Метод полімеразної ланцюгової реакції у реальному часі з зворотною транскрипцією показав вищу чутливість порівняно з методом імуноферментного аналізу, виявивши додаткові 13 позитивних зразків. Найбільш значні розбіжності між методами спостерігалися при виявленні вірусу мозаїки люцерни, де молекулярний метод виявив на 50 % більше позитивних зразків. Регіональний аналіз виявив найвищу інфікованість у Черкаській області (44,0 %), з максимальною поширеністю вірусу мозаїки сої (22,0 %). Встановлено помірну кореляцію між виразністю симптомів та значеннями порогового циклу для вірусу мозаїки сої (r = -0,62), тоді як для вірусу некрозу жилок сої та вірусу мозаїки люцерни кореляція була статистично незначущою. Висновки підтвердили високий рівень поширеності вірусних інфекцій серед посівів сої в регіоні дослідження. Отримані результати дозволять державним фітосанітарним службам, селекційним установам та агровиробникам впровадити диференційовані за регіонами стратегії захисту сої, що дозволить зменшити втрати врожаю та підвищити ефективність фітосанітарних заходів

Ключові слова: серологічний аналіз; полімеразна ланцюгова реакція; імуноферментний аналіз; ко-інфекція; фітосанітарний моніторинг

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