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## Use of pre-sowing soil microbiological analysis to predict the spread of sugar beet root rot

**Abstract.** *Aphanomyces cochlioides* and *Rhizoctonia solani* are two of the main soil phytopathogens of sugar beet, which lead to substantial losses in yield and quality. Since disease control capabilities are complex and pathogen monitoring is important for predicting risks, it is of great importance in rural production to create available methods for preliminary field assessment. The purpose of the study was to introduce a predictive methodology for assessing the potential of soils for their suitability for growing sugar beet in the system of short-rotation crop rotations. As an indicator plant, sugar beet seeds were used, which are not characterised by genetic resistance against these diseases. The possibility of using the pre-growing disease index (PPDI), which has a gradation from 0 to 100 and covariance with the actual yield, sugar content and gross sugar yield per hectare, is tested. The pre-sowing PPDI value is compared with the actual yield from each specific field to really assess the possibility of using the PPDI index to characterise yield losses on production crops. It is determined that with an increase in the PPDI value, the gross yield of sugar beet, sugar content, and gross sugar yield decreased. The results of studies show that one unit of PPDI causes a loss of gross yield at the level of 0.24 t/ha and a decrease in the sugar harvest by 0.018 t/ha. That is why, to manage the risks of growing sugar beet, it is necessary to assess the phytopathogenic load of the soil. It is established that the most harmful and permanently identified phytopathogens are *Rhizoctonia solani* and *Fusarium* spp, which indicates the need to select hybrids that are characterised by resistance or high tolerance to these pathogens. The information obtained can be used for timely identification of infection risks, development of effective management strategies, and improvement of agricultural techniques for growing sugar beet to reduce crop losses and increase the efficiency of agricultural products

**Keywords:** *Aphanomyces cochlioides*; *Rhizoctonia solani*; *Fusarium* spp.; phytopathogenic load; gross yield; sugar content

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## INTRODUCTION

Sugar beet cultivation in Ukraine has several strategic tasks: providing the internal market with sugar, increasing export potential, and restoring soil fertility. Thus, beet farming is a socially and economically important branch of agriculture, which provides jobs, determines food security, and forms foreign exchange earnings in Ukraine. One of the promising management strategies for these cultivated plants is the use of pre-sowing soil microbiological analysis. This method can be an effective tool for predicting and preventing the spread of root rot, which is especially important for ensuring high quality and quantity of the crop. It is important to focus on quick detection and assessment of the risk of developing these diseases in soils to take timely and adequate measures to control them. Such an analysis can become a key element in the system of predicting and managing the risk of diseases, which will provide farmers with the opportunity to respond in time to potential threats to the sugar beet crop. The introduction of pre-sowing soil microbiological analysis in agricultural practices can help optimise agricultural techniques and provide stable conditions for growing this important agricultural crop.

According to data of I. Karas *et al.* (2019), in the conditions of Western Ukraine, root rot of various etiologies (*Rhizoctonia solani*, *Aphanomyces cochlioides*, *Fusarium spp*) cause substantial damage to sugar beet crops, and crop losses can be up to 80%, especially when growing sugar beet in a short-term crop rotation. The most common type of rot is fusarium rot, which substantially reduces the productivity of sugar beet roots. Thus, fungi of the *Fusarium spp.* genus affect the sugar beet throughout the growing season. They cause not only rotting of the root system of seedlings, fusarium rot, necrosis of vascular-fibrous bundles and leaves of sugar beet but also fusarium jaundice.

V. Rafiei *et al.* (2023) investigated various subgroups of *R. Solani*, enabling the identification of the AG2-2IIIB group, the representatives of which cause maximum harm in the cultivation of sugar beet. The interaction of pathological systems of *R. Solani* and *C. beticola* were also examined, which indicates early infection of sugar beet plants with cercosporium, if the plants were previously affected by rhizoctoniosis.

A study by Å. Olsson *et al.* (2019) demonstrated a negative correlation between the content of calcium ions and the development of root rot caused by *A. Cochlioides*. The importance of liming in terms of the availability of elements in the soil and the effect of calcium on the damage of sugar beet plants by root rot is demonstrated. In other paper, Å. Olsson *et al.* (2023) analysed the long-term effects of structured lime and ground limestone on the stability of soil aggregates, the risk of loss of mobile phosphorus, and the degree of soil disease damage in winter wheat, rapeseed, and sugar beet. The positive effect of lime use was demonstrated only on sugar beet due to a decrease in the level of root rot damage compared to the control; no effect was identified for winter wheat and rapeseed yield decreased.

Notably, *R. solani* and *A. cochlioides* have common features, which consist both in the possibility of infecting both sugar beet seedlings and plants at later stages of development. The role of microorganisms in the loss of agricultural products after harvesting is a key subject of the study by N.S. Bist *et al.* (2020). This review draws attention to the influence of microorganisms on the quality and shelf life of agricultural products. The study highlights the importance of understanding the interaction of microorganisms with collected products to effectively solve the problems of losses after harvesting in the agricultural sector. In addition, both pathogens remain in the soil for a long time, and mild warm winters lead to the preservation of the virulent pathogen and an increase in the level of pathogenicity. However, the pathogens themselves differ substantially. Taxonomically, they are not related, and the distribution of zoospores of *Aphanomyces* substantially depends on the high humidity of the soil. In addition, pathogens of *Rhizoctonia spp* have a much wider range of host plants, while *Aphanomyces spp* is a specific pathogen for sugar beet. Root rot of grain crops caused by phytopathogenic fungi is a serious problem for agricultural production. N. Grebenikova *et al.* (2018) investigated the causes and mechanisms of root rot development in cereals that cause phytopathogenic fungi. The types of fungi that most often cause this disease are analysed, and the factors that contribute to the spread of root rot in grain crops are also

examined. The importance of this phenomenon for crop production is emphasised, and possible approaches to the control and prevention of root rot are being developed to ensure the stability and high yield of grain crops. Virulence factors in the interaction of a phytopathogen with a plant are an important topic that was considered by J. Pontes *et al.* (2020). In this context, the molecular and biological factors that determine the ability of phytopathogens to cause diseases and overcome the plant's defence mechanisms are considered. The study focuses on identifying and understanding these virulence factors, which may contribute to the development of new strategies for controlling phytopathogens and improving plant disease resistance.

Available means of limiting the spread of both pathogens are the following agrotechnological techniques: seed treatment with several fungicides, the use of tolerant hybrids, early sowing dates. However, none of these mechanisms of influence on the spread of pathogens guarantees 100% effectiveness, especially in the presence of both pathogens in the soil, and therefore, it is necessary to predict the risks of the development and spread of phytopathogens.

The purpose of the study was to introduce a predictive methodology for assessing the potential of soils from the point of view of suitability for growing sugar beet in short-term crop rotations, considering the problems of root rot, for making timely and correct management decisions on growing sugar beet in a particular field.

## MATERIALS AND METHODS

These studies were initiated to support sustainable sugar beet production, which includes mandatory soil testing for nematodes. One of the issues of stable sugar beet cultivation is obtaining complete information about soil phytopathogens, namely the number and species composition of phytopathogens, which will allow making managerial decisions on sugar beet cultivation in the field. The resulting indicator of growing risk assessment is the pre-growing disease index (PPDI), which ranges from 0 to 100 and is based on the previously proposed development risk assessment *Aphanomyces* due to the level of moisture in the shoots (Schneider *et al.*, 1978; Lindgren *et al.*, 1995).

*Microbiological analysis.* The applied use of the study was tested on 45,000 hectares of short-term crop rotation over a 3-year period. A total of 1,890 soil analyses were performed. Soil samples were taken in the fields of the private enterprise "Western Bug" within the Lviv, Ternopil, and Chernivtsi regions during 2021-2023. More than 490 fields were analysed, from which 1,980 samples were taken one year before sugar beet sowing after the precursor was collected. The selection was conducted from a horizon of 10-15 cm from several points within the field. One average sample included samples from 10 sampling points each, which is averaged over 30 ha of the field.

Each soil sample was placed in 2 plastic containers with a diameter of 10 cm and kept at a temperature of 20°C for 4 days. Afterwards, 25 seeds of the Akazia KWS hybrid were added into these containers, which are not resistant to root rot. After the emergence of seedlings, daily monitoring was conducted for the presence of symptoms of infection. Any shoots with symptoms were transferred to potato-dextrose agar, where pathogens were identified by the nature of mycelium growth (Schneider *et al.*, 1978; Park & Grau, 1992). The duration of the test was 4 weeks. The index was calculated using the formula:

$$DI = \frac{[4*IS+3*IS2+2*IS3+IS4]}{[4*PE]*100}, \quad (1)$$

where *DI* – infection rate, *IS<sub>j</sub>* – number of infected plants per week; *PE* – total number of plant emerged.

Index values indicate the level of risk of developing diseases, namely, a value of 30-45 indicates a moderate risk of the disease, a value greater than 45 indicates a high risk, and an indicator less than 30 – a low risk.

*Statistical analysis – comparison of PPDI value with actual yield.* The pre-sowing PPDI value was compared with the factual yield from each specific field to assess the possibility of using the PPDI index to characterise yield losses on production crops. For this task, all areas were divided into three risk categories (low, moderate, and high) based on PPDI and the following indicators were identified: gross yield, sugar content and sugar harvest per hectare, which were

used to evaluate the performance of the field in each category (Windels *et al.*, 2009). Covariance analysis (ANCOVA) was used to assess the relationship between variable yield parameters and PPDI among fields over different years. ANCOVA is a special type of regression analysis, it is a covariance analysis model in which both fictitious and quantitative explanatory variables can be applied. ANCOVA was performed for each yield parameter separately, where the line was suitable for each harvest year, and it was checked whether the slopes of the lines differed substantially. The study was conducted according to the Convention on Biological Diversity (1992)

and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973).

## RESULTS AND DISCUSSION

It was established that the most common soil phytopathogen that causes root rot are the representatives of the *Fusarium* ssp genus, which caused 24.07% of root rot manifestations. The least harmful among the identified pathogens were representatives of the *Phytium* ssp. genus – 2.05%. In turn, the presence of a high titer of phytopathogens in any case negatively affected the degree of root rot damage to sugar beet roots (Table 1).

**Table 1.** Frequency of occurrence of soil pathogens in soil samples

Pathogen	Infection index, %	Root rot, %
<i>Aphanomyces cochlioides</i>	9	2.18
<i>Phytium</i> ssp.	21	2.05
<i>Rhizoctonia solani</i>	53	21.93
<i>Fusarium</i> ssp	19	24.07

Source: compiled by the authors

As the PPDI value increased, the gross sugar beet yield, sugar content, and gross sugar yield decreased. Thus, fields characterised by a lower risk of root rot based on the

PPDI value (14.17) on average had a 9.8 t higher root yield and a 1.93 t/ha higher gross sugar yield compared to high-risk fields (PPDI value 59.67) (Table 2).

**Table 2.** Average PPDI values and yield patterns

Risk category	PPDI	Gross yield, t/ha	Sugar content, %	Gross sugar harvest, t/ha
Low	14.17	74.5	17.24	12.84
Medium	38.96	69.8	16.94	11.82
High	59.67	64.7	16.87	10.91

Source: compiled by the authors

The correlation between the PPDI index value and gross yield is -0.9973, PPDI and sugar content -0.9574, between PPDI and gross sugar yield, it is -0.9998, which, in turn, indicates a close inverse linear relationship between these indicators. Based on the above, it can be argued that with an increase in the PPDI value, all indicators of the crop structure decrease (gross yield, gross sugar yield per ha, and sugar content). These results showed that the PPDI test can accurately predict the potential risks of

root rot of various etiologies. Summarising the results obtained, it can be stated that with each unit of increase in the PPDI index, the yield in the fields under study decreased by 0.24 t/ha. A similar trend is typical for the gross sugar harvest per ha, which causes a change in the sugar content per unit of PPDI in the range of 0.018 t/ha. In addition, the identified high values of correlation coefficients between PPDI and gross yield, sugar content and gross sugar yield indicate a pronounced interdependence

of these parameters. This highlights the importance of using PPDI to predict sugar beet yield and quality. The results indicate a systematic decrease in all aspects of sugar beet yield with increasing PPDI values. This close relationship allows identifying the potential risks of root rot exposure to sugar beet cultivation, allowing farmers to take effective measures to reduce crop losses and improve product quality. The PPDI test can serve as an effective tool for accurately predicting the potential risks of root rot in sugar beet, considering its impact on all aspects of yield. Thus, the ability to manage this risk can be improved by timely and accurate determination of PPDI values in the soil before cultivation.

These studies also identified some features of ecology and pathogenicity of the fungi. Firstly, a high presence frequency of *R. Solani* in soils was established, which confirms its widespread distribution among the examined samples for several years. It was detected in 53% of soil samples, but the percentage of root rot was 21.93%, compared to *Fusarium* spp, which was 24.07%. Compared to other pathogens, such as *Fusarium* spp, *R. Solani* was less frequent but resulted in a higher percentage of root rot. This factor, together with PPDI values, indicates a high risk of root rot, which can affect the gross yield and sugar content of sugar beet.

In turn, the development of root rot caused by *Aphanomyces* was not stable over the years and averaged 9% of the analysed samples. Given that the development of this phytopathogen mainly depends on the level of soil moisture, epiphytotic occur only under favourable conditions. Low stability in the development of root rot caused by *Aphanomyces* indicates the influence of climatic and soil conditions on the spread of this phytopathogen. In particular, its development was less stable in the context of years, and the impact on yield was less severe compared to *R. Solani*. This indicates that meteorological and agroclimatic conditions affect the activity of *Aphanomyces* and can determine the degree of its negative impact on the sugar beet culture.

Of particular importance for production is the ability to predict the types of root rot that can affect crops and substantially affect yields, which can be done using the PPDI test since it

is able to predict the risks of epiphytotic development and potential yields. In addition, it is important to note the PPDI test's ability to identify different pathogens simultaneously and to determine their titer in soil samples. The test allowed demonstrating a higher level of pathogenicity of *R. solani*, a more harmful causative agent of the disease for commercial sugar beet crops, compared to *A. cochlidioides*.

Notably, the PPDI test proved to be not only an effective tool for predicting the risks of root rot but also the ability to simultaneously determine the titer of various pathogens in the soil. This allows accurately determining the threat level from individual fungal pathogens and taking timely measures to control them. Finally, the low detection rate of *Pythium* spp points to their less substantial role in reducing yields in western Ukraine, as it is identified only in a limited number of samples and with a low percentage of root rot. Conditions for harmful effects of *Pythium* spp, in particular, *Pythium aphanidermatum*, can only be conducted under certain favourable soil temperature conditions during the growing season of plants, which limits their importance in this region.

The main task that all sugar beet producers are trying to solve is to increase the sugar harvest per unit area of sowing. A.K. Nurmukhammedov & O.M. Nevmerzhitska (2010) established that an important condition for increasing the profitability of beet cultivation is the search for disease-resistant and productive hybrids with increased sugar content and adaptability to local conditions. They focused on developing state-of-the-art disease and pest control systems, which is another area that helps maintain crop sustainability and health. It was determined that the use of biotechnologies in the breeding process also opens up new opportunities for creating hybrids with improved properties, such as resistance to stressful conditions and increased yields. Generalising, the development of modern sugar beet hybrids and the improvement of agricultural cultivation techniques are key factors for achieving the goal of increasing the sugar collection from each unit of the sown area and ensuring the stability of growing this crop in different climatic conditions.

The microbiological composition of the soil varies depending on the location of the soil. J. Yuan *et al.* (2020) examined the composition of healthy and affected soils, samples of which were taken from eight different countries. They identified that soil bacterial and fungal communities were clearly divided between sick and healthy soil samples that came from six crops in nine countries or regions. Alpha-diversity was consistently higher in the fungal community of healthy soils. Whereas the microbiomes of diseased soil contained more *Xanthomonadaceae*, *Bacillaceae*, *Gibberella*, and *Fusarium oxysporum*, the microbiome of healthy soil contained more *Streptomyces Mirabilis*, *Bradyrhizobiaceae*, *Comamonadaceae*, *Mortierella*, and non-pathogenic fungi *Fusarium*. In Turkey, in the province of Konya, M. Avan *et al.* (2021) differentiated 71 isolates of *Rhizoctonia* spp. with different levels of virulence. Based on virulence, isolates were divided into four categories: (I) 11 isolates: non-pathogenic, (II) 15 isolates: low-virulent, (III) 6 isolates: moderately virulent, and (IV) 39 isolates: high-virulent

Currently, due to the achievements of breeding, it has been possible to reduce the impact of rhizomania by using sugar beet hybrids that are resistant and tolerant to the pathogen but genetic resistance exists only for certain races of the pathogen, which, in turn, does not give full guarantees for the protection of crops from these diseases (Pavli *et al.*, 2011; Aleagha & Farzadfar, 2023). In a study by C.E. Windels *et al.* (2009), it was determined that the pathogen *R. solani* is widespread and reaches a high level of harmfulness. It is identified during the growing season and leads to a loss of yield of up to 50%.

Aphanomycete root rot, caused by the pathogen *A. cochliformis*, is also present in the region and especially progresses in wet years, in places where water stagnates, or with over-compacted soil. The presence of both pathogens in crops simultaneously is increasingly being identified, which is becoming a problem in short-term crop rotations. In turn, C.A. Strausbaugh (2020) confirmed that the interaction of two pathogens increases the likelihood of root rot damage to sugar beet. Thus, researchers used several variants of a combination of pathogens

*Rhizoctonia solani* and *Leuconostoc* spp. and strains of one pathogen to inoculate root crops, which, as a result, led to an increase in root rot compared to treatments with only one type or strain of pathogen.

There are many methods for determining pathogens in the soil, which are constantly being improved. These are methods that can already be called classical and are based on the use of monoclonal antibodies or polymerase chain reaction (PCR) (Cahill, 1999). J.R. Botkin *et al.* (2022) indicate that for the diagnosis of the presence of *A. cochliformis* in the soil, a 4-week biological analysis of the soil and a 2-day culture analysis are used. However, molecular biological approaches allowed the development of an accelerated method for detecting this pathogen using qPCR, using specific primers for the mitochondrial DNA of the pathogen. This method allows assessing the harmfulness of aphanomycete rot using the disease severity index (DSI). However, this method allows assessing the presence or absence of risks of developing only one pathogen. M. Aragona *et al.* (2022) proposed a high-throughput sequencing (HTS) method for identifying pathogens in soil. This method allows applying a different approach to disease control strategies, considering pathogens in their environment and deciphering the complex interaction between microorganisms and cultivated crops. However, the authors believe that for the widespread implementation of this method, it is necessary to further examine and improve the economic and technical aspects.

R.M. Harveson *et al.* (2014) indicate a close relationship between the PPDI index and gross yield and sugar content of sugar beet for the US conditions. For comparison, index 1 in the current study causes a decrease in yield by 0.24 t/ha against 0.27 t/ha and a gross sugar yield of 0.018 t/ha against 0.049 t/ha for the United States. In general, the trend of dependence of the PPDI value and the main indicators of the crop structure remains.

The use of PPDI is a universal method for assessing the suitability of sugar beet cultivation and risk management since it is possible to simultaneously evaluate the entire complex of soil phytopathogens presented in soil samples.

These approaches are used in the assessment of fields in the state of emergency “Zakhidny Bug” and farmers who grow sugar beet for LLC “Radekhivsky tsukor”.

## CONCLUSIONS

The results of the study proved the effectiveness of the PPDI test in predicting the risk of root rot of various etiologies on sugar beet since the test allows for the simultaneous identification of the risk of developing several phytopathogens in one sample. A close inverse linear relationship between PPDI and gross yield, sugar content, and gross sugar yield per ha was also established. It is determined that the most harmful and permanently identified phytopathogens are *Rhizoctonia solani* and *Fusarium* ssp, which indicates the need to select hybrids that are characterised by resistance or high tolerance to these pathogens.

It was established that the most common soil phytopathogen that causes root rot were the representatives of the *Fusarium* ssp genus, which caused 24.07% of root rot manifestations. The least harmful among the identified pathogens were representatives of the *Phytium* ssp. genus – 2,05%. As the PPDI value increased, the gross sugar beet yield, sugar content, and gross sugar yield decreased. Thus, fields characterised

by a lower risk of root rot based on the PPDI value (14.17) had an average 9.8 t higher root yield and a 1.93 t/ha higher gross sugar yield compared to high-risk fields (PPDI value 59.67).

Further scientific research may be aimed at improving the methodology of pre-sowing analysis, in particular, expanding the range of analysed microorganisms and clarifying their role in the development of root rot. It is also important to consider the interaction between different types of soil microorganisms and their impact on the pathogenicity of fungi, which can open up new opportunities for developing effective disease control strategies. Additional aspects of the research may include examining the impact of agricultural practices and various varieties of sugar beet on the structure of the soil microbiota and their interaction with pathogens. Expanding the geographical scope of research can also make an important contribution to understanding the variability of this phenomenon in different climatic and soil conditions.

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## CONFLICT OF INTEREST

None.

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## **Використання передпосівного мікробіологічного аналізу ґрунту для прогнозування поширення кореневих гнилей цукрових буряків**

**Анотація.** Однією з основних ґрунтових фітопатогенів цукрового буряка, що призводять до значних втрат врожайності та якості, є *Aphanomyces cochlioides* і *Rhizoctonia solani*. Оскільки можливості контролю захворювань є складними, а моніторинг патогенів є важливим для прогнозування ризиків, то створення доступних методів для попередньої оцінки полів набуває великого значення в сільському виробництві. Метою дослідження було впровадження прогностичної методики для оцінки потенціалу ґрунтів щодо їх придатності для вирощування цукрових буряків в системі короткоротаційних сівозмін. В якості рослини-індикатора використовувалося насіння цукрового буряка, яке не характеризується генетичною стійкістю проти зазначених захворювань. Протестовано можливість використання індексу передвирощувальних захворювань (PPDI), який має градацію від 0 до 100 і коваріацію з фактичною врожайністю, цукристістю та валовим збором цукру з гектара. Для реальної оцінки можливості використання індексу PPDI для характеристики втрат врожайності на виробничих посівах, нами було здійснено порівняння передпосівного значення PPDI з фактичною врожайністю з кожного конкретного поля. Встановлено, що зі збільшенням значення PPDI знижувались валова урожайність цукрового буряка, цукристість та валовий збір цукру. Результати досліджень показали, що одна одиниця PPDI обумовлює втрати валової врожайності на рівні 0,24 т/га та зменшення волового збору цукру на 0,018 т/га. Саме тому, для управління ризиками вирощування цукрових буряків, необхідно проводити оцінку фітопатогенного навантаження ґрунту. Встановлено, що найбільш шкодочинними та постійно ідентифікованими фітопатогенами є *Rhizoctonia solani* та *Fusarium spp.*, що вказує на необхідність підбору гібридів, які характеризуються стійкістю або високою толерантністю до вказаних збудників. Отримана інформація може використовуватися для своєчасного виявлення ризиків зараження, розробки ефективних стратегій управління та вдосконалення агротехнік вирощування цукрових буряків з метою зменшення втрат врожаю та підвищення ефективності агропродукції

**Ключові слова:** *Aphanomyces cochlioides*; *Rhizoctonia solani*; *Fusarium spp.*; фітопатогенне навантаження; валова врожайність; цукристість