Traditional and current-prospective methods of agricultural plant diseases detection: A review

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Abstract. As it is known, a significant part of the yield of agricultural crops is lost due to harmful organisms, including diseases. The article reveals the data on the widespread types of plant diseases (rot, wilting, deformation, the formation of tumors, pustules, etc.) and their symptoms. Early identification of the pathogen type of plant infection is of high significance for disease control. Various methods are used to diagnose pathogens of disease on plants. This article discusses the review of the literature data on traditional methods for diagnosis of plant pathogens, such as visual observation, microscopy, mycological analysis, and biological diagnostics or the use of indicator plants. Rapid and reliable detection of plant disease and identification of its pathogen is the first and most important stage in disease control. Early identification of the cause of the disease allows timely selection of the proper protection method and ensures prevention of crop losses. There are a number of traditional methods for identifying plant diseases, however, in order to ensure the promptness and reliability of diagnostics, as well as to eliminate the shortcomings inherent in traditional diagnostics, in recent years, new means and technologies for identifying pathogens have been developed and introduced into practice. As well as the article provides information on such innovative methods of diagnosis of diseases and identification of their pathogens, which are used widely in the world today, such as immunodiagnostics, molecular-genetic (and phylogenetic) identification, mass spectrometry, etc.

1. Introduction

The Food and Agriculture Organization (FAO) launched the United Nations’ “International Year of Plant Health (IYPH)” for 2020 which aims to raise global awareness on to end hunger, reduce poverty, protect the human health and environment, and maintain plant health in economic development [1, 2].

The plants are under constant and increasing threat from pests and diseases. According to the Secretariat of the International Plant Protection Convention (IPPC) under the FAO, if no any actions and measures are taken in a timely manner against pests and diseases, the situation can lead to tragic and difficult consequences. Today, global food crops are lost from 20% to 40% due to pests and diseases [3], with damage reaching 14.1% by plant diseases, accounting for $ 220 billion in annual agricultural trade losses [4].
Presently, about 83% of known plant infectious diseases are caused by fungi, 9% by viruses and phytoplasmas, and more than 7% by bacteria [5]. In order to implement timely and effective control measures against these diseases, it is important to diagnose them, to detect the species composition of pathogens. Delay in diagnosis and failure to take control measures can lead to large crop losses and a sharp decline in product quality.

There is a lot of information in the history of agricultural science that the mass infestation of crops with diseases has led to tragic consequences. Such catastrophic consequences are rare nowadays due to the availability of modern, more reliable methods of prevention and protection of plants from the mass development of diseases (epiphytosis) in a particular area, however plant diseases still cause severe damage to agriculture today. For example, phytophthora incidence may result in the loss of half or more of the potato crop, while the tomato may not yield at all. Due to rust diseases of wheat and other grain crops, in most cases 30-40% of the crop is lost, and 10-15% due to powdery mildew. The loss of yields of many vegetable products is also observed due to diseases. In this case, the damage of phytopathogenic organisms is direct and indirect. These losses are not limited only to a decrease in sales revenue. There are such plant diseases with which the use of contaminated products poses a risk to humans and farm animals. For example, some fungi belonging to the Fusarium family, which grow on the grains of cereals, poison flour and bread made from it resulting danger to human. Ergot, fusarium wilt, and some other diseases can cause poisoning and even death of cattle [6].

Assessment of damage and crop loss is done in different specifications for different diseases. However, regardless of the level of specifications in damage assessment, it is emphasized that urgent measures should be taken to prevent crop loss as much as possible. The effectiveness of the system for combating diseases of agricultural crops often depends on the rapid, reliable and accurate detection of the plant pathogen, that is, disease causative agent, and thus on the proper diagnosis of the disease by its symptoms [7].

2. Common types of plant diseases

As a result of influence of pathogenic microorganisms, a number of disease symptoms occur due to pathological processes in plants. These include rotting, withering or wilt, staining, formation of powder and galls, tumors and warts, mummification, deformation (leaf curling, twisting, wrinkling, etc.), gommos or mucus leakage, and etc.

Decay is a pathological process caused by the breakdown and softening of plant tissue, which is mainly caused by fungi from the genera Fusarium, Sclerotinia, Rhizopus, Pythium, Rhizoctonia, etc. [8], as well as bacteria such as Pectobacterium carotovorum, Xanthomonas campestris [9].

Wilting or withering is a common type of disease. The cause of plant withering is damage to its conductive system. The wilt pathogen penetrates into the root system of the stem, causing the vessels to become clogged, releasing toxins from itself, and these toxins cause necrosis in the vein walls. As a result of this, the plant's water supply is disrupted and withers. Wilt can be caused by fungi and bacteria. Wilt caused by fungi is called tracheomycosis, caused by bacteria is called trexeobacteriosis. Plant withering can also occur due to adverse environmental conditions (drought, root injury, etc.) [6, 10].

Tumors or galls are the result of the growth of damaged tissue under the influence of the pathogen. They appear in various organs of plants - roots (cabbage stalks), tubers (potato cancer), root crops (beet root cancer) and others. The reason for their appearance is an increase in the volume of infected cells (hypertrophy) or an increase in the number (hyperplasia). Sometimes both processes occur together. Tumors, warts, and galls are typical signs of diseases caused by fungi, bacteria, viruses, and nematodes [6].

Pustules are round or oval-shaped powder composed of mycelium and spores of fungi of various sizes and colors. Pustules form inside a leaf or other organ tissue and then emerge. Examples are rust diseases (white birch rust disease - Melampsoridium betulae, wheat brown rust - Puccinia triticina and yellow rust - Puccinia striiformis).
Deformation is a deformity or distortion of the infected plant organ. Leaf curling, threadlike leaves, wrinkles (wrinkling) on the leaves, thickened or stiff leaves (doubling), fruit ugliness - all these are examples of deformation. The cause of the deformation is the flow of nutrients or disruption of the processes of assimilation, the failure of tissue elements to grow at the same rate (smooth), etc. For example, the reason for the appearance of wrinkles on the leaves and their curling is that the mesophyll and leaf veins do not grow smoothly, and the reason for the leaves to form a threadlike shape is that only the leaf veins do not grow smoothly. Leaf curl is due to the fact that the leaves are overfilled with starch, which occurs as a result of disruption of the conduction system and leakage of assimilates. Deformations are typical symptoms of diseases caused by fungi, bacteria and phytoplasmas [6].

Although the symptoms of the diseases mentioned and described above are different, their number is much less than the number of common plant diseases. That is, the same disease symptom can occur as a result of different factors (fungi, bacteria, or non-infectious diseases). This situation makes it difficult to diagnose diseases and accurately identify, detect their pathogens. Therefore, it is not appropriate to base the diagnosis on a single symptom.

3. Traditional methods of diagnosis and detection of plant disease pathogens

Diagnosis of plant diseases is improving day by day. New means and technologies are being developed and introduced to ensure the reliability and rapidity of diagnostics. However, not having lost the significance, the traditional diagnostic methods allow to diagnose plant diseases in a timely, rapid and high-accurate manner with the created tools and technologies. This serves for proper and timely decision-making on the control of diseases of agricultural crops.

A number of diagnosis methods are used in plant pathology research. These may include visual observation, microscopy, mycological diagnosis, biological diagnosis or testing in indicator plants, immunological diagnostics, molecular genetic identification, mass spectrophotometry and etc. [6, 7, 11, 12, 13, 14, 15, 16, 17, 18].

We will study below traditional diagnostic methods that are widely used in phytopathological research.

3.1. Visual observation

Visual observation of the plants is initially carried out at the place where the plant is grown, in which the diseased plant is compared with a healthy plant. In this case, plant height, color, leaf shape, leaf density on the branches, changes in the root system, and more others are observed. If there are foci of disease (necrosis or spots on the stems, leaves, etc.), the presence of mycelium, sporangiophora or sclerotia is observed using a magnifying glass, if any, it indicates the presence of a pathogenic fungus.

By visual observation, it is often possible to determine the primary pathogenesis of a nematode, fungus, bacterium, or virus, depending on the signs present in the plant sample. If there are characteristic signs in the sample, it will be possible to make a tentative diagnosis. For example, the bacterium Agrobacterium tumefaciens stimulates the proliferation of host plant tissue and forms tumors, which can be easily seen. Powdery mildew disease causes light powder in the tissues of plant organs, while nematodes (Meloidogyne) form “root nodes” in the thin roots of the plant [19]. When observing, it is also important to look at the general condition of the plant (standing, turgidity), if it is in a state of withering, then the problem is in the conductive tissues or roots. These diseases can be caused by phytopathogenic fungi (tracheomycoses) and bacteria (tracheobacteriosis).

Visual observation in many cases does not allow for a definitive diagnosis. That is, drawing conclusions based on only one morphological feature reduces the accuracy of the diagnosis. To increase the reliability of the diagnosis, microscopy and other studies should be performed too.

3.2. Microscopy

For an accurate diagnosis high-precision microscopes are an important and widely used instrument. After visual observation, the infected parts of the plant are brought to the laboratory for microscopic
observation. A stereomicroscope (binocular) is used for primary observation of disease symptoms in plant materials. By observing the infected parts of the plant under a microscope, it is often possible to reach a diagnostic conclusion by looking at the hyphae, microsclerosis, conidiophores, conidia, and bacterial cell clusters of pathogenic fungi.

However, in adverse weather conditions, fungi may not produce spores, and even fungal mycelium may not be present on the affected surface [11]. In addition, there may be no real pathogen in the dead tissue, but saprophytes or secondary parasites (invaders) may form colonies [19]. In such cases, visual observation and initial microscopy are insufficient to determine the presence of the pathogen. In this case, additional mycological examination ("moist chamber" technique, separation of pure culture, etc.) is used.

3.3. Mycological diagnosis
In the ‘moist chamber’ method, sick plant parts are placed in a high-humidity chamber (Petri dishes, etc.) and incubated. In this case, due to favorable conditions, fungi in the infected tissues develop and begin to manifest themselves. The ‘moist chamber’ method is performed as follows: blotting filter paper is laid out sterile Petri dishes, sterile glassware is placed on its top, then infected plant parts (infected fruit cut pieces, leaves, roots, etc.) are placed on the glass, Petri dish is closed with cover and incubated at 24-28°C (for Phytophtora species 17-20°C). By observing the hyphae, macro and microconidia obtained by this method under a microscope, it is possible to identify a pathogenic fungus or a fungal organism up to the level of genera and species.

However, visual observations, microscopy, and "moist chamber" methods may not allow an accurate detection of some phytopathogens. Many phytopathogens (e.g., Ustilago spp.) remain latent (dormant or latent) for a period of time and do not manifest themselves. On the other hand, many pathogens produce symptoms that are similar to each other. Another problem is that some pathogenic fungi do not form spores even in a moist chamber [11].

Pure cultures isolation method. Cultivation of phytopathogenic microorganisms in artificial nutrient media is an important, relatively easy, and common (classical) method widely used by any researcher in mycological research. Every laboratory for the diagnosis of plant diseases should have equipment, kits for the preparation and storage of nutrient media, as well as substances to add to the nutrient media.

Detection of a pathogenic fungal species by their mycelium or spores may not always give a positive result. In some detection techniques, the shape, color, and other morphological characteristics of the colonies in the nutrient media were used as markers to identify the species. Therefore, in many cases it is necessary to isolate the pure culture of the fungus [11, 20]. Pure culture can be isolated from soil, plant roots, and the infected surface parts of the plant. A binocular microscope, a sterile darning needle, an alcohol lamp, and a sterilized standard or selective or semi-selective agar medium are required for the isolation of pure culture from the infected tissue of the plant.

For the isolation of most fungi belonging to ascomycetes, wort agar, PGA nutrient media are used, while for oomycetes, an agar medium based on corn, rye or pea flour is often used. Also, when incubating in a moist chamber, favorable conditions must be created for the growth of the pathogen to be isolated and for the formation of spores. For example, moist chamber incubation should be carried out at a temperature of 16–18°C to isolate Phytophthora infestans, which cause phytophthora in potatoes, and 24–25°C to isolate members of the Alternaria family [11]. Separation of pure culture from soil, plant roots and damaged surface parts of the plant is detailed in several literature [11, 20].

After isolation of pure culture, the macromorphological and cultural characteristics of the pathogen in the nutrient medium are determined on the basis of micromorphological features identified using microscopy (using identifiers) or by molecular-genetic identification. In this method it is necessary to carry out Koch’s postulates in determining the type of pathogen. That is, Koch’s postulates include the following steps: isolating the pathogen, infecting the healthy plant with it, re-isolating the pathogen from the artificially infected plant, and proving that it is compatible with the symptoms of the pathogen isolated from the infected plant under natural conditions [6].
3.4. Biological assays or indicator plant tests.
This method is widely used in the detection of phytoviruses and phytoplasmas, along with other diagnostic methods. Biological diagnosis is performed on indicator trees and herbaceous plants. Mechanical inoculation or injection is used to transmit infection to indicator plants. In this case, the leaves with symptoms of the disease are rubbed into a healthy indicator plant, or a standard buffer suspension is obtained and injected. Also, the method of artificial infestation by micro-grafting is used on indicator trees and shrubs. Such testing on indicator plants is carried out under strictly isolated conditions. In order to carry out diagnostic work on indicator trees, healthy seedlings should first be prepared from virus-free mother material [21, 22, 23].

Young, intensively growing plants are selected to induce virus infection. Dicotyledon indicator plants are infected mechanically for testing when they form 2-3 true leaves. In some cases it can be carried out (in beans - for the test of alfalfa mosaic virus) on primordial leaves and cotyledons (in cucumbers - for the test of tobacco necrosis virus) or first leaves of seeds. Monocotylidon (grain crops) plants are infected when they form one or two leaves and when they are grown in dark condition for 3 days, their susceptibility to virus increases [23].

Although this method has a number of advantages (no need to use expensive equipments for diagnostics, ease of implementation), its main disadvantage is the long waiting time and the need for special isolated cultivation chambers. The waiting period for herbaceous indicator plants can last 30-60 days and from 45 days to 2 years in trees [22, 24].

Other disease-susceptible varieties or wild relatives of the plant under investigation may be used as indicator plants. Today there are more than 600 indicator plants belonging to 43 families, 173 genera. For example, plant indicators of potato X-virus are Gomphrena globosa and Datura stramonium, for tobacco mosaic virus Nicotiana glutinosa and Datura stramonium, for plum orca virus Chenopodium foetidum and Nicandra physaloides species [25]. 2-3 days after infestation, indicator plants are observed; the observation in herbaceous indicator plants is 4 weeks. During this time, the symptoms of viral disease develop on the plant under the testing. There are common conditional signs that indicate symptoms of viral disease on indicator plants: Chl - chlorosis, Dis - distortion, En - enation, IV - interroot, L – local reaction (appearance of symptoms on an infected area), LeAb – leaf shedding, LLN – local infestation, LeChl – leaf chlorosis, LeDis – leaf distortion, LeN – leaf necrosis, M – mosaic, N - necrosis, NQL – necrotic oak pattern, Ri - ring, S – systematic reactions, Sp - spotting, StN – stalk necrosis, Stu - stunting, Y - yellowing, VN – vein necrosis, VC – vein discoloration, LeCu – leaf curl [23].

4. Prospective modern methods of identification of phytopathogenic organisms
Diagnosis of plant diseases is improving day by day. New means and technologies are being developed and introduced to ensure the reliability and rapidity of diagnostics. However, not having lost the significance, the traditional diagnostic methods allow to diagnose plant diseases earlier in a rapid and high-accurate manner with the created tools and technologies. This serves for proper and timely decision-making on the control of diseases of agricultural crops.

A number of traditional diagnostic methods are used in phytopathological research. These methods include visual observation, microscopy, mycological diagnosis, biological diagnosis or testing in indicator plants, and more.

It should be noted that the diagnosis of the disease on the basis of external symptoms in the host plant is not always reliable. Because the symptoms of many diseases are consistent with the symptoms of physiological disorders caused by external influences, and some phytopathogens can cause disease with asymptomatic or weakly characteristic symptoms at the beginning of development. Long duration of the process of diagnosing diseases using indicator plants method, the need for special isolation chambers are a disadvantage of this method, and the use of this method in cases where pesticides are used reduces the reliability of the diagnosis.

In addition, the same symptom can occur as a result of different factors (fungi, bacteria, or non-infectious diseases). This situation makes it difficult to diagnose diseases and accurately identify their
pathogens. It can therefore be concluded that it would not be correct to base the diagnosis on a single symptom.

Therefore, there is a need for each method in phytopathological studies. The need to address existing problems in the traditional diagnosis of plant diseases has led to the introduction of radically new methods of detecting and identifying the presence of pathogens in plant pathology.

In phytopathology, a number of promising modern methods are used to identify the pathogens of plant diseases. These methods include immunological diagnostics, molecular-genetic identification, mass spectrophotometry and others [7, 11, 13, 14, 26, 27]. We will review below modern-prospective diagnostic methods widely used in phytopathological research.

4.1. Immunological diagnostic methods

This method is based on a special reaction of antigen-antibodies. It detects the presence of small molecular compounds, viruses and their quantitative indicators.

Immunodiagnostics, that is serological methods include immunofluorescence, immunoblotting, serological special electron microscopy, Enzyme-linked immunosorbent assay (ELISA) and many other methods [11]. Initially, serological methods were used mainly for the identification of viruses [23, 28], but in recent years they have also been used to diagnose other pathogens in plants, especially fungi, oomycetes, bacteria and phytoplasmas [28, 29, 30, 31, 32].

Although there are many methods of immunodiagnostics, among them enzyme-linked immunosorbent assay (ELISA) is relatively sensitive and specific, allowing a reliable diagnosis.

In this method, an enzyme is attached to specific antibodies to a specific virus and a complex called 'conjugate' is obtained. The sap of a plant infected with the virus is added to the conjugate. Enzyme-labeled antibodies react with the virus-antigen. As a result of the adsorption of the antigen by the antibodies, the enzyme attached to the antibody becomes inactive. The enzyme in antibodies that do not react with the virus remains active. The substrate affected by this enzyme is then filled. The amount of enzyme involved in the reaction is determined using colorimetry (measuring the color change of the product). The presence of the virus and its concentration are determined by comparing the decrease in enzyme activity in experimental and control options [6].

In enzyme-linked immunosorbent assay, it is based on two different reactions, namely immunochemical and enzymatic reactions. In an immunochemical reaction, an antibody binds to a pathogen antigen, while in an enzymatic reaction, a substance is taken into account in the result of an immunological reaction under the action of an enzyme, and the sample color changes as a result of the enzymatic reaction. The substance affected by the enzyme is called the substrate, and the substance obtained under the action of the enzyme is called the product of the enzymatic reaction. Chemically bound (conjugated) horseradish peroxidase, beta-galactosidase, alkaline phosphatases are used as antibodies [10, 11, 33].

There are several specific methods of solid phase ELISA, which include direct, indirect, sandwich ELISA.

Immunochromatographic analysis (LFIA-Lateral Flow Immunoassay) is an immunochemical diagnostic method based on the principle of thin-layer chromatography, which involves the reaction between the antigen and the corresponding antibody. This is done through special test strips or test cassettes [34, 35].

Lateral-flow immunochromatographic analysis test is a paper-based platform that determines the quality and quantity of a substance in a complex mixture, in which a sample is poured into the test strip and the result is displayed in 5-10 minutes. The use of test equipment has been expanded due to its low cost in production, ease of use, and ability to obtain rapid results [36]. Using the lateral flow immunochromatographic analysis test (LFA-Lateral Flow Assay), various biological samples can be examined for saliva [37], serum [38], blood [39], and other fluids. To date, lateral-flow immunochromatographic analysis tests are also widely used in the diagnosis of plant diseases [40, 41, 42].
Immunochromatographic analysis is easy to verify on a test strip, where a liquid sample or extract poured into a test strip trough moves along polymer lines to which molecules can interact with the solution without external influences. In the test strips of lateral-flow immunochromatographic analysis, the sample and conjugate pad and membranes are attached to the back card. The conjugate pad contains a specific antibody (antibody specific to the pathogen to which the colored or fluorescent fragments, colloidal gold, and latex microspheres are attached) that is sensitive to the sample being tested, and the sample fluid binds to the antibody and passes the detection zone along the line. Biological components (mainly antibodies or antigens) are immobilized on a porous membrane composed of nitrocellulose. Their role is to react with an analyte (biological object under investigation) bound to a conjugated antibody. The result on the test strip indicates that the samples are moving correctly, and the coloring of the control line indicates that the result of the test sample is positive, i.e. warns of the presence of pathogens in the material [43, 44]. The principle of this "ladder bars" analysis is that the immobilized antibody is followed by a stepwise colorometric conjugate-antigen complex, in which the number of streaks appearing on the strip is directly proportional to the concentration of the substance (analyte) being examined [45, 46].

4.2. Molecular - genetic identification

In the diagnosis of plant diseases, it is important to determine their systematic location, because without determining the species composition of the pathogen, it is impossible to develop a control measure against it. The traditional approach in the diagnosis of plant diseases is based mainly on the detection of disease symptoms, morphological features of the pathogen (microscopy). Although these methods are important and necessary, but for their high-quality implementation, certain conditions, in particular, the disease symptoms of the pathogen must be clearly manifested. In addition, there is a need for a specialist with extensive experience in performing identification. Also, the similarity of two or more disease symptoms also makes traditional identification more difficult. Often the emergence of new forms of pathogens for diagnosis, including new non-specific symptoms, also interferes a definitive diagnosis. In recent years, certain difficulties and ambiguities in the implementation of traditional diagnostics and identification have necessitated the use of modern molecular-genetic methods that provide high accuracy, specificity, relative simplicity and high quality.

There are many methods of molecular-genetic identification of pathogenic organisms (viruses, phytoplasmas, bacteria, fungi, etc.) in plants, which include nucleic acid hybridization, polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR), real-time PCR and DNA microchips [7, 11].

The polymerase chain reaction was invented in 1983 by the American biochemist Kerry Mullis (Nobel Laureate in 1993), and this invention revolutionized molecular biology and medicine [47]. PCR is based on the multiplication (amplification) of a certain part of DNA under the influence of enzymes in artificial conditions. In this case, millions of copies of that fragment are formed only when a sequence of nucleotides that meet a certain condition is present in the DNA matrix. Under normal PCR conditions, fragments no more than 3000 pairs of nucleotides are amplified [48, 49]. PCR was first used in the diagnosis of plant diseases in 1991 by Rasmussen and Wolf [50] to identify the bacterium Pseudomonas syringae pv pisi, and has since been widely used for its superiority over traditional methods of plant disease diagnosis [51]. PCR-based diagnostics are specific, sensitive, efficient, rapid, and universal [52].

By sequencing a specific section of the genome of phytopathogenic organisms, the genera and species of pathogen can be identified with the reference to a database [11].

DNA barcoding is a method of molecular identification using short genetic markers in DNA to determine whether an organism belongs to a particular taxon [53]. For molecular identification of phytopathogenic fungi, molecular markers such as RAPD (Random amplification of polymorphic DNA), ITS (Internal Transcribed Spacer), IGS (Intergenic spacer), TEF-1α (translation elongation factor 1-α), BT-3 (beta-tubulin), COI (cytochrome oxidase I) are used [11, 54, 55, 56, 57, 58, 59]. The Internal Transcribed Spacer (ITS), the main genetic marker for fungi, is an intergeneral interval in a tandem repeating gene cluster of ribosomal DNA, an intermediate sequence of about 600 pairs of nucleotides,
18S (a small subunit of rDNA - SSU) and 28S (large subunit of rDNA LSU) is adjacent to subunits [60, 61]. The length of TEF-1α [56, 62], which is a secondary genetic marker for fungi in DNA-based taxonomy or DNA barcoding, is 66–3000 bp in sequences at GenBank [62].

The 16S rRNA gene [11, 63, 64, 65, 66, 67] is mainly used to identify bacteria by nucleotide sequences, as well as DNA gyrase ‘B’ subunit (gyrB), RNA polymerase (rpoB, rpoD, etc.), a combination of heat shock protein-dependent genes (cpn60, hsp70, etc.) and other markers are proposed [68, 69, 70].

Identification of phytopathogens by nucleotide sequences (in traditional PCR) involves a series of steps, including sampling, pure culture isolation, genome DNA isolation, molecular marker selection for PCR, and primer formation (based on the selected genetic marker), conducting polymerase chain reaction, isolation of DNA fragments by electrophoresis and DNA purification, determination of nucleotide sequences, etc. [11, 57].

Once the nucleotide sequence is determined, the DNA sequences are edited and rectified. This work is done in special programs. These programs include Sequencing Analysis (Applied Biosystems), BioEdit (Ibis Biosciences, USA), UGENE (UniPro, Russia) and ClustalX. Comparison of the nucleotide sequences of the studied samples with the nucleotide sequences interpreted from genetic databases is performed using the BlastN algorithms in the international resource NCBI (National Center for Biotechnology Information, USA) (http://www.ncbi.nlm.nih.gov) [57, 71, 72]. In addition to the NCBI resource for comparing nucleotide sequences, there are many international databases on molecular biology, genetics, and biomedicine, including EMBL (The European Molecular Biology Laboratory), ENA (European Nucleotide Archive), DDBJ (DNA Data Bank of Japan), FungiDB (Fungal and Oomycetes Genomics Resources), MycoBank (Centraalbureau voor Schimmelcultures fungal biodiversity center) and others.

4.3. MALDI-TOF mass spectrometry
Matrix-assisted laser desorption ionization – time of flight mass spectrometry - MALDI-TOF MS) - has been developed as a rapid, accurate and cost-effective method for routine identification of a wide range of microorganisms [73, 74].

MALDI-TOF mass spectrometry is a desorption method of “soft” ionization, which is the ionization of a small fragmentation of the analyte using a laser pulse absorbing matrix [75]. The matrix consists of crystallized molecules that reduce the destructive properties of laser radiation and ionize the analyte. As a matrix, synaptic acid, α-cyano-4-hydroxycinnamic acid (α-CHCA) and 2,5-dihydroxybenzoic acid (DHB) is used [76].

MALDI-TOF mass spectrometry is widely used for the analysis of non-volatile high-molecular compounds (peptides, proteins, carbohydrates, oligonucleotides, synthetic polymers, organic complex compounds, etc.) [77, 78, 79].

The process of identification of microorganisms in MALDI-TOF mass spectrometry involves the following sequences: a pure culture of a bacteria, fungi, or fungal organism is isolated (either by direct inoculation in a solid agar medium or by cultivation in a liquid medium; if they grown in fluid medium, the colonies are mixed with buffer solutions, centrifuged and dipped, the initial supernatant is removed); the cleaned culture is anointed on a metal tablet cell (96-cell metal tablet) and dried; the matrix-liquid is anointed on the dried culture in a cell in a metal tablet, then mixed and dried; the metal tablet is placed in the equipment mold [27, 80, 81]. Once the device is turned on, the effect of the laser light on the cells in the metal tablet begins, and a set of spectra specific to each species appears in the computer program, which acts as a specific “fingerprint” of a particular species. The program displays which type the sample belongs to by comparing the spectra available in the database.

Today, the leading manufacturers of MALDI-TOF mass-spectrometry microorganism identification systems include Bruker Daltonics (USA), Shimadzu (Japan) and bioMérieux (France).

In the database of the BrukerMALDI Biotype Microorganism Identification System of Bruker Daltonics company there are about 7000 mass spectra of species and strains of microorganisms and this amount is constantly replenished (https://www.bruker.com), while in SARAMIS (Spectral ARchive
And Microbial Identification System) database of AXIMA Microorganism Identification System of Shimadzu company there are more than 50000 spectra of biological objects (https://www.shimadzu.com). It is obvious that the main focus of manufacturers of all these equipments in MALDI-TOF mass-spectrometry microorganism spectrum bases is on clinically important bacteria and fungi [13, 74, 80, 81]. However, in recent years, these bases are constantly replenished with specific spectra of phytopathogenic microorganisms, and their number is growing from year to year [27, 82, 83, 84].

In a study conducted in France [85], 62 isolates of the genus Fusarium belonging to 9 species were identified in molecular analysis (TEF-1 gene sequence) and by MALDI-TOF. While 57 isolates of many recorded species (Fusarium solani, F.oxysporum, F.verticilloides, F.proliferatum, F.dimerum) confirmed by molecular identification (http://www.ncbi.nlm.nih.gov/BLAST/) (92%) were correctly identified. 4 species (F.chlamydosporum, F.equiseti, F.polyphialidicum, F.sacchari) that were not present in MALDI-TOF database, were not identified. Molecular-genetic and MALDI-TOF identification were consistent in 5 of the 6 isolates whose morphological and molecular identities did not match. This short-term method, based on mass spectral diagnosis, can be used as a valuable tool in the identification of representatives of the fusarium family at the species level. The database should be enriched only with spectra of unusual species. Also, 99.8% of clinically significant gram-negative bacteria, 98.2% of species [86], 95.5% of gram-positive bacteria, 92.8% of species [87] and 92.5% of fungi [88] were correctly identified.

5. Conclusion
Early detection of infection on plants is of crucial significance in the control and elimination of diseases (as well as in recording quarantine diseases of plants and in their eradication).

Although each of the methods for diagnosing plant diseases and identifying pathogens has its own specific shortcomings, these methods do not lose their importance in diagnosis. Because in most cases, traditional methods are sufficiently informative, cheap, universal, give reliable results, do not require special expensive equipments. According to the recommendations of leading mycologists and phytopathologists, in many cases the results of the use of molecular identification methods are insufficient, and they are of course required to be used in conjunction with classical morphological identification (microscopy, study of macro- and micromorphological markers, artificial infestation of host plants, and implementation of the Koch’s triad). The extensive introduction and application of new promising, modern, rapid and reliable methods does not mean that traditional methods should be ignored.

The use of modern prospective methods in the diagnosis of plant diseases, such as ELISA, lateral flow immunoassay, molecular-genetic identification, MALDI-TOF mass spectrometry, guarantees the quality and rapidity of diagnosis and allows for short-term decision-making.

Regardless of the method of detection, rapid, accurate and reliable identification of the type and isolates of the pathogen is a key requirement for the development of disease control systems. Plant disease diagnostic laboratories (Research Institutes, Plant Quarantine Inspection and Plant Clinic Laboratories) are required to provide systematic and professional services for rapid, reliable identification of phytopathogens in field or laboratory samples from observed areas, to detect the causes of disease spread and to control these diseases.

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