Guide for Good Plant Clinic Practices and Services

This project has been funded with support of the European Commission. This publication reflects the views only of the author, and the Commission cannot be held responsible for any use which may be made of the information contained therein.

Table of Contents

Introduction

Plant clinics serve as the foundational means for diagnosing plant diseases and pests, as well as for monitoring and surveillance, official plant health controls, and emergency and contingency planning in the phytosanitary area. This set of guidelines has been created as part of the Project Proposal Deliverable. The guidance document is based on OECD, EPPO standards and procedures for identifying pests and diseases, as well as ISPM 27 Diagnostic Protocols for regulated pests of the International Plant Protection Convention.

Chapter One: Definitions

Definition in this document are taken from the Regulation (EU) 2016/2031 of the European Parliament of the Council of 26 October 2016 on protective measures against pests of plants, amending Regulations (EU) No 228/2013, (EU) No 652/2014 and (EU) No 1143/2014 of the European Parliament and of the Council and repealing Council Directives 69/464/EEC, 74/647/EEC, 93/85/EEC, 98/57/EC, 2000/29/EC, 2006/91/EC and 2007/33/EC and in accordance with ISPM 5 of the International Plant Protection Convection.

1. **Harmful organisms** mean animals, plants, fungi, bacteria, viruses, and Viruses–like organisms that can at any stage of their development be harmful to plants and plant products or contaminate their place of growing.
2. **Non-harmful organisms** are animals, plants, fungi, bacteria, viruses, and Virus–like organisms that are not harmful to plants.
3. **Non-Infectious Plant Diseases** are phenotypic changes in the plants that are not caused by harmful organisms and cannot be transmitted from plant to plant.
4. **Plants** shall be considered to mean living plants and living parts thereof, including seeds.
5. **Living Parts of Plants** shall be considered to include:
   - fruit, in the botanical sense, other than that preserved by deep freezing,
   - vegetables, other than those preserved by deep freezing,

Co-funded by the Erasmus+ Programme of the European Union
New Professional Diploma in Plant Clinic and Phytosanitary Technologies (PRO-DPCP)
609550-EPP-1-2019-1-BG-EPPKA2-CBHE-JP

- roots, tubers, corms, bulbs, rhizomes,
- cut flowers,
- branches with foliage,
- cut trees retaining foliage,
- plant tissue cultures.

6. **Seeds** shall be considered to mean seeds in the botanical sense, other than those not intended for planting
7. **Plant products** shall be considered to mean products of plant origin, unprocessed or having undergone simple preparation, in so far as these are not plants;
8. **Planting** shall be considered to mean: any operation for the placing of plants to ensure their subsequent growth, reproduction, or propagation.
9. **Plants intended for planting** shall be considered to mean:
   - plants that are already planted and are intended to remain planted or to be replanted after their introduction, or
   - plants that are not planted at the time of introduction, but are intended to be planted thereafter.
10. **Plant passport** shall be considered to mean: an official label that gives evidence that the provisions of this Directive related to plant health standards and special requirements are satisfied, and which is standardized at a national level for different types of plants or plant products and is prepared and issued by the responsible official authority in a country.

11. **Plant products'** means unmanufactured material of plant origin and those manufactured products that, by their nature or that of their processing, may create a risk of the spread of quarantine pests.

12. **Wood** shall only be considered a plant product if it fulfills one or more of the following criteria:

(a) it retains all or part of its natural round surface, with or without bark;

(b) it has not retained its natural round surface due to sawing, cutting, or cleaving.

(c) it is in the form of chips, particles, sawdust, wood waste, shavings, or scrap, and has not undergone processing involving the use of glue, heat pressure, or a combination thereof to produce pellet, briquettes, plywood, or particle board.

(d) it is, or is intended to be, used as packaging material, whether or not it is actually in use for transport of goods.

13. **Planting** means any operation for the placing of plants in a growing medium, or by grafting or similar operations, to ensure their subsequent growth, reproduction, or propagation
14. **Plants for planting** means plants intended to remain planted, to be planted, or to be replanted
15. **Other object** means any material or object, other than plants or plant products, capable of harboring or spreading pests, including soil or growing medium
16. **Competent authority** means the central authority or authorities of a Member State, or, where applicable, of a third country, responsible for the organization of official controls and other official activities, or any other authority to which that responsibility has been conferred, by Union legislation on official controls
17. **Lot** means several units of a single commodity, identifiable by its homogeneity of composition, origin, and other relevant elements, forming part of a consignment
18. **Trade unit** means the smallest commercial or other useable unit applicable to the marketing stage concerned, which may be the subset or the whole of a lot
19. **Professional operator** means any person, governed by public or private law, involved professionally in, and legally responsible for, one or more of the following activities concerning plants, plant products, and other objects: planting; breeding; production, including growing, multiplying and maintaining; introduction into, and movement within and out of, the Union territory; making available on the market; storage, collection, dispatching, and processing
20. **Registered operator** means registered professional operator

21. **Authorized operator** means a registered operator authorized by the competent authority to issue plant passports
22. **Final user** means any person acting for purposes that are outside that person's trade, business, or profession who acquires plants or plant products for personal use
23. **Test** means an official examination, other than visual, to determine if pests are present or to identify pests
24. **Treatment** means a procedure, whether official or non-official, for the killing, inactivation, or removal of pests, or for rendering those pests infertile, or for the devitalization of plants or plant products
25. **Incidence** means the proportion or number of units in which a pest is present in a sample, consignment, field, or other defined population
26. **Establishment** means the perpetuation, for the foreseeable future, of a pest within an area after entry
27. **Eradication** means the application of phytosanitary measures to eliminate a pest from an area
28. **Containment** means the application of phytosanitary measures in and around an infested area to prevent the spread of a pest
29. **Quarantine station** means any official station for holding pests, plants, plant products, or other objects in quarantine
30. **Confinement facility** means any facility, other than quarantine stations, where pests, plants, plant products, or other objects are kept under confinement conditions
31. **Traceability code** means a letter, numerical or alphanumerical code that identifies a consignment, lot, or trade unit, used for traceability purposes, including codes referring to a lot, batch, series, date of production, or professional operator documents
32. **Phytosanitary measure** means any official measure having the purpose to prevent the introduction or spread of quarantine pests or to limit the economic impact of regulated non-quarantine pests

Chapter Two: The Role of the Plant Health Clinic in Egypt

Managing plant health problems is a continuous challenge for farmers and extension workers. Regular crop losses and compromised quality are caused by biotic factors like pests and diseases, as well as abiotic factors such as low soil fertility. The diagnosis of these issues is complicated by a range of causes and symptoms, making it challenging to pinpoint the root of the problem. To choose the most effective management options, careful consideration is necessary.

In many cases, technical support services can be insufficient, leaving extension providers unable to assist all farmers in need. To address this issue, plant health clinics (PHCs) have emerged as a practical solution that enables plant health specialists to work closely with extension workers, providing farmers with valuable advice on how to manage various plant health issues. These clinics often operate differently and offer a range of services, with some institute-based clinics, equipped with laboratory facilities to identify pests and pathogens and providing management guidance through extension intermediaries. Most smallholder farmers are unlikely to know of such clinics or are unable to contact them directly.

The primary topic is the extension-based Plant Health Clinics (PHCs), which are designed to directly assist farmers. These clinics are conveniently located in public areas near the communities where farmers live and work.

Plant health clinics are a service that responds to demand and advises as part of everyday extension activities. They are most effective when they are part of an overall plant health system in the country.

The Independence of the Plant Health Clinics

The Plant Health Clinics must be an independent and well-organized unit providing independent plant health expertise to the professionals' users, organizations, and farmers. Based on this expertise sufficient policy-making decisions can be made and implemented.

The Emergency Contingency obligations of the plant health Clinic

Plant health clinics play a crucial role in managing phytosanitary outbreaks. They should be included in the pest-specific contingency plans to provide stakeholders with essential information on the biology of the specific pest, its introduction, detection, and spread. Additionally, they should provide references to sources of further information. This pest-specific information should be part of the emergency measures taken in the phytosanitary contingency planning as well as in the National pest eradication programmes.

Reporting obligations of the Plant Health Clinic units to the official authorities in accordance with *ISPM 17 Pest reporting and ISPM 19 Guidelines on lists of regulated pests* of International Plant Protection Convention. The guide to National Reporting Obligations.

Chapter Three: Principals for the Functioning of the Plant Health Clinic

General Principals of the Identification of Non-harmful and Harmful Organisms in Plants

The General principles of the Identification of Plant diseases focus on the two main principles – Macroscopic principle and microscopic principles.

The Macroscopic principles aim to make an overall assessment of the Plant's growth to estimate if the growth of the plant is normal or if some abnormalities are observed.

The abnormal symptoms of the plants may occur such as the number of affected plants, it could be linked to the species of the plants, to the location of the sick plants discovered for example are they located in a field, glasshouse, landscape, forest, indoors in a house, etc. to the abnormal symptoms could also be linked to the soil type, to water contents in the soil, etc. Next in the assessment procedure is to define how many plants are observed with abnormal symptoms. In this case, the plant health expert should examine whether there is new growth of the plant parts or the whole plant, are there formation of new tissues or plant organs.

As part of the macroscopic assessment, the observations must be done on plant parts that have been discovered affected. In this assessment plant health experts should observe very carefully the plant organs for damages. This includes the leaves, stem, root, buds, or the flowers of the plant. Examining the leaves, the plant health expert should define first which leaves are affected -are these the developed leaves versus new growth of leaves, are these top leaves versus bottom leaves, etc. After this step is made the next step of the plant health expert should focus on the origin of the damages observed on the plant. In this step, it should be defined whether the origin of damages observed on plants has been caused by insects, disease, or technical damages caused by climate or agricultural or cultivation practices.

Next on the macroscopic assessment is the observation of the stem of the plant. In this assessment, the plant stem should be observed for the presence of cankers, wounds, discoloration, etc. which may be caused by insects, disease, or technical damages caused by climate or agricultural or cultivation practices. In this stage of the examination, the plant health expert should do a cutting into the stem and look at the cambium tissues to find whether this cambium tissue is green or discolored and whether there is a movement of the tissue's liquids, or it is dry.

Next, the examination procedure is to check the health of the plant buds to define whether are they live, green, and viable.

The roots are the next organ of the plant which is subject to macroscopic assessment. This assessment includes the observation of the plant root for discoloration, lesions, stunting, malformed, bites from larvae or insects, poor development and growth and to define the origin of the damages on the affected root such as damages from larva, insects, disease, climate, soil characteristics or mechanical damages caused by the agricultural machinery.

The final stage of the macroscopic assessment is the overall assessment of the affected or diseased/affected plant. From this overall assessment should be concluded was a gradual or sudden death of the plant could result from environmental stress, the presence of harmful organisms, misapplied plant protection products or medicament treatment, etc.

The result of the assessment the plant health expert should be able to define the origin of harmful or non-harmful organisms in plants.

General Principals of the Good Laboratory Practice

Good Laboratory Practice (GLP) ensures the generation of high-quality and reliable test data related to the safety of industrial chemical substances and preparations. The principles have been created in the context of harmonizing testing procedures for the Mutual Acceptance of Data (MAD).

The Principles of Good Laboratory Practice (GLP) is a managerial quality control system covering the organizational process and the conditions under which non-clinical health and environmental studies are planned, performed, monitored, recorded, reported, and retained (or archived). The Principles of GLP are followed by test facilities carrying out studies to assess the health and environmental safety of chemicals and chemical products which may also be of natural or biological origin and, in some circumstances, may be living organisms.

The Principles of GLP define the responsibilities of test facility management, study director, study personnel, and quality assurance personnel that are operating within a GLP system, and minimum standards concerning the suitability of facilities and equipment to perform studies, the need for standard operating procedures, documentation of raw data, study reports, the archiving of records, etc. For more information, please see (OECD, 2023)[1]

Good Experimental Practice (GEP)

The aim of Good Experimental Practice (GEP) is to ensure that high-quality trials are carried out. The quality trials will ensure that results can be used by different authorities based on mutual recognition, zonal evaluation, etc. GEP is concerned with the management of efficacy evaluation trials and with the conditions under which trials should be planned, taken, assessed, recorded, and interpreted so that their results should be comparable and reliable. GEP relates to aspects of staff qualifications, use of suitable equipment and facilities, protocols, modes of

[1] Principles of Good Laboratory Practice (GLP)

operation, and recording of results. In 1992, the first version of the EPPO Standard PP 1/181 Conduct and reporting of efficacy evaluation trials, including good experimental practice was adopted. As it is a core element in the EPPO program on efficacy evaluation, it has been revised on several occasions. Further details on GEP can be found in EPPO Standard PP 1/181[2] and PP1/152(4) - Design and analysis of efficacy evaluation trials (Revision: 4)[3].

Principles of the Good Plant Protection Practice (GP)

EPPO Standards on Good Plant Protection Practice (GPP) are intended to be used by National Plant Health authorities, for the regulation of, and advisory services related to, the use of plant protection products.

Please see EPPO Standards PP2 - Good Plant Protection Practice and EPPO standards PP2/002(2)-Potato, PP2/003(2)-Glasshouse lettuce, PP2/004(2)-Allium crops, PP2/005(1) -Rodent control for crop protection and on farms, PP2/006(1)-Hop, PP2/007(1)-Vegetable brassicas, PP2/008(1)-Rape, PP2/009(1)-Strawberry, PP2/010(1)-Wheat, PP2/011(1) -Barley, PP2/012(1) -Beet, PP2/013(1) -Ornamentals under protected cultivation, PP2/014(1)-Pea, PP2/015(1)-Tobacco, PP2/016(1)-Farm grassland, PP2/017(1)-Maize, PP2/018(1)-Pome fruits, PP2/019(1) -Rye, PP2/020(1)-Mushrooms, PP2/021(1)-Sunflower, PP2/022(1)-Umbelliferous crops, PP2/023(1)-Grapevine, PP2/024(1)-Oat, PP2/025(1)-Leguminous forage crops, PP2/026(1) -Ribes and Rubus crops, PP2/027(1)-Citrus + erratum, PP2/028(1)-Cotton, PP2/029(1) -Solanaceous crops under protected cultivation, PP2/030(1)-Outdoor solanaceous crops, PP2/031(1)-Cucurbits under protected cultivation, PP2/032(1) -Outdoor cucurbits, PP2/033(1)-Stone fruits

[2] *PP1/181– Conduct and reporting of efficacy evaluation trials, including good experimental practice The latest version can be accessed from the EPPO database on PP1 Standards*
[3] PP1/152(4)-Design and analysis of efficacy evaluation trials. Revision: 4. Available at: https://pp1.eppo.int/standards/PP1-152-4

Chapter Four: The Importance of Identification of Non-Infectious Plant Diseases and Harmful Organisms in Plants at the Plant Health Clinic

The Identification of Non-Infectious Plant Diseases and Harmful Organisms in Plants plays a key role and helping plant health experts make the exact diagnosis of the illness or damage to the plants.

Making accurate Diagnoses of the plants can prevent the further spreading of plant pests and diseases that could result in the complete distortion of crop production and lead to global disasters like hunger, poverty, migration to search for food, etc. In general, the plant health diagnosis helps to prevent the appearance and mass spreading of plant pests and diseases, and economic losses and contributes to protecting the plant's health, food security, food sovereignty, and food safety and quality.

Plant diagnostic and plant health tests itself are playing a crucial role at every step of plant pest and disease management – Diagnosis, Monitoring, Screening, and Prognosis.

Diagnosis - The diagnostic test detects a possible condition or confirms the lack of one. Due to climate change and transboundary ways of spreading harmful organisms to plants plant diseases and pests need to be studied over and over for not just their nature but also their stage or degree of development and harmfulness. Diagnostic tests form the framework with which plant health experts can better assess the effectiveness of the chosen pesticide treatment in stopping the progression of pests and diseases on plants.

Monitoring - Some harmful organisms on plants with viral origin cannot be cured, but with the ways of technological development it can be early detected the chosen pesticide treatment, or an increase the plant immunity, the crises situations in the future can be avoided.

The monitoring steps and checks are to be taken to assess if the plant health situation can be controlled. Monitoring can look for a decrease or increase in plant health, in turn, to control the plant health status in the field crops.

Screening on the plant production fields - Some plant diseases or pests in the initial stages may show minimum or even no symptoms at all. That is why the screening is to be used.

Screening is used for the assessment of the current or future predictions of harmful organisms on the plants that do not yet present any symptoms for a particular illness or damage (like larvae in the plant roots or stems), to find out if the harmful process has begun to develop quietly. These tests are applied to the larger or neighboring fields and are therefore affordable and easily accessible by all the stakeholders in the agricultural production process. Screening doesn't diagnose but only identifies possible fields that might have been under harmful organisms' invasion.

Prognosis and signalization of the pests and plant diseases appearance - the Prognosis is the process that goes far ahead and helps the plant health experts to assess the likelihood of the appearance and developing stages of plant pests and diseases. The prognosis allows the plant health expert to use prognosis models and to predict in the near or distant future the threat of harmful organisms on the plants, and to them to take necessary precautionary measures at a much earlier time than it is required.

Chapter Five: Identification of Non-Infectious Plant Diseases, Non-Harmful and Harmful Organisms to Plants. Way of distribution. Methods of Analysis. Good Practices in the Plant Health Clinic. Laboratory equipment.

Part A: Identification of Non-Infectious Plant Diseases on the Plants

I. Identification of Non-Infectious Plant Diseases

The identification of non-infectious plant disease follows at first the general principles of identification.

1. Identification of Non-Infectious Plant Diseases. Ways of distribution. Methods of identification. Good Practices in Plant Health Clinic. Laboratory Equipment

Once the diagnosis has been made by the plant health expert, a plant sample needs to be taken and tested at the plant health clinic in compliance with the principles of GEP and GLP.

Identification of Non-Infectious Plant Diseases. Taking and Structuring of Samples for Plant Clinical Examination

Taking and Structuring of Samples for Plant Clinical Examination is performed by the plant health expert. The structuring and sampling have to be taken in compliance with GEP and to be tested at the plant health clinic under the GLP conditions.

Ways of distribution: non-transmissible ways of distribution and shortage of macro and microelements in the soil.

Methods Used for Identification of Non-Infectious Plant Diseases in Plants

The methods of identification in the Plant health clinic are: Macroscopic analyses, and microscopic analyses described in General Methods for Identification

Good Practices in Plant Clinic and Laboratory Equipment:

- Good practice is after the examination procedure the plant sampling material is disposed safely.

Co-funded by the Erasmus+ Programme of the European Union
New Professional Diploma in Plant Clinic and Phytosanitary Technologies (PRO-DPCP)
609550-EPP-1-2019-1-BG-EPPKA2-CBHE-JP

- IPPC Guide to Pest Risk Communication A guide for national plant protection organizations on communicating with stakeholders about pest risks
- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

Part B: Identification of Harmful Organisms - Infectious Plant Diseases caused by Phytopathogens in Plants.

Identification of Harmful Organisms - Infectious Plant Diseases caused by Phytopathogens in Plants

The identification of Infectious Plant Diseases should follow the general principles of identification and assessment of EPPO and IPPC. Specific and detailed assessment and analysis must be performed as follows:

The symptoms of the disease can be identified by the symptoms shown or expressed on the leaves, stems, or roots of the plants. Visual observation and examination of the whole plant, organs, or part of the plants and microscopic examination are required.

2.1. Identification of Harmful Organisms - Infectious Plant Diseases caused by Phytopathogens of *Viral and Virus–like/Mycoplasmas Origin* in Plants. Methods of identification. Good Practices in Plant Health Clinic. Laboratory Equipment

Identification of Harmful Organisms – Infectious Plant Diseases caused by Phytopathogens of Viral and Virus-like Origin and Taking and Structuring of Samples for Plant Clinical Examination

In case of suspicion of viral or viral-like phytopathogens, the examination should be performed carefully and in a sterile environment. The symptoms of the disease can be found on single parts or organs of the plant (early stage of infection) or symptoms on the whole plant and internal systems of the plant. The expression of the symptoms can be diagnosed in combination of visual observations, and macroscopic and microscopic analyses.

Visual observation and examination of the whole plant, organs, or part of the plant that includes the leaves, stems, roots, and fruits of the plant:

***Leaf Spot**:* spots of dead tissue on the foliage; the size, shape, and color may vary with the causal agent, but are usually limited to a small portion of the leaf.

***Leaf Blotch**:* dead areas of tissue on the foliage, irregular in shape, and larger than leaf spots.

***Wilt**:* drooping of leaves or shoots, often due to lack of water.

Stunting: reduced plant growth.

Chlorosis: yellowing of normally green tissues due to lack of chlorophyll.

Suspicious for specific harmful organisms like Chlorosis: in this case, the plant health expert should look for a pattern like nutritional, herbicide, virus, etc. Also to find out whether the whole leaf is affected by environmental, cultural, chemical, or genetic agents or factors. And to find out if are there irregular spots caused by viruses, insects, chemicals, etc.

Vascular Discoloration: darkening or streaking of the plant's vascular elements.

Symptoms that can be observed under obligatory microscopic analysis: This step is necessary if there is a need for further examination or the phytopathogen origin cannot identified. In this case, the plant health expert has to start by examining affected parts with a dissecting scope and move to a compound light microscope, if necessary. It should look for fruiting structures which are the symptoms of the disease agent or evidence of insects or insect activity.

The examination should start firstly by observing the:

Plant Leaves and the Stems: the following symptoms should be searched for: leaf spots, lesions or cankers, fungal growth. In case fungal growth is found, or if lesions a looking like a bacterial pathogen, then a slide and examine under a compound scope. It should be searched for fungal spores, or any other characteristics of a fungus, or bacterial ooze.

In case the fruiting structures are found, crush it and make a slide, to examine it with a compound scope.

In case no spores are mature, then place the plant tissue or plant part in a moist chamber to force spore formation or maturation in order r to induce sporulation of any fungi that might be shown later.

Roots/Crown: in case the roots or crown are off-colored, then crush the roots or make a section of crown tissues. Then a slide needs to be prepared for examination under a compound scope. The plant health expert should look and search for the presence of pathogen structures such as oospores, bacterial cells bacterial streaming, etc.

In case no fungi or bacteria are found under the microscope, look for soluble salt problems. Soil type and management or cultural practices may be the reason for the affected plant parts.

In case there is no direct evidence of a biotic agent but the plant health expert still thinks that a fungus or bacteria is most commonly the reason for infection and it is linked with the problem observed, then cultures on PDA, Acid PDA, or H2O agar have to be prepared after a thorough washing of the plant tissue in running tap water for 15-20 minutes.

Ways of distribution: transmissible vector plant diseases, transmissible non-vector plant diseases.

Methods used for Identification of Harmful Organisms - Infectious Plant Diseases caused by - Phytopathogens of Viral and virus-like Origin in Plant Clinic

Visual Macroscopic Method and Microscopic Method

Preparation of water liquid microscopic sample

Using a transparent sticky type

Co-funded by the Erasmus+ Programme of the European Union
New Professional Diploma in Plant Clinic and Phytosanitary Technologies (PRO-DPCP)
609550-EPP-1-2019-1-BG-EPPKA2-CBHE-JP

2.1.2.3 Biological Method – Rules of Koch

2.1.2.4 Serologic Method

- **Drop agglutination reaction**
- **Precipitation tests**
- **Fluorescence in-situ hybridization**
- **Use of Indicator Plants** - the plant samples are placed in conditions of high relative humidity to provoke the formation of spores in case the harming agent is of fungal origin.
- **Isolation of the harmful organism in artificial culture medium** (agar-agar, common nutrient media - meat-peptone broth (MPB), meat-peptone agar (MPA), meat-peptone gelatin (MPA)
- **Artificial infection with an isolate from a sick on a healthy plant**
- **Electron microscopy**- Immune Electric Microscopy and/or Immune Fluorescence Microscopy
- **ELISA Method**
- **PCR Method** - DNA and RNA analyses

2.3. Structuring of Samples for plant clinical examination from Living Parts of Plants in compliance with GEP and GLP see chapter

Working environment: sterile and/or nonsterile environment, wearing protective clothes, protective glasses and gloves, etc.

2.1.3. Good Practices and Working Environment in the Plant Clinic. Laboratory Equipment

Good practices: EPPO Standards –

- ***PM 7 Diagnostics and ISPM 27 Diagnostic protocols for regulated pests of the International Plant Protection Convention***

- International Plant Protection Convection - Managing Relationships with Stakeholders - A guide to stakeholder relations for national plant protection organizations
- IPPC Guide to Pest Risk Communication A guide for national plant protection organizations on communicating with stakeholders about pest risks
- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

Working environment: sterile and/or nonsterile environment, wearing protective clothes, protective glasses and gloves, etc.

Laboratory equipment: PCR, Microscopes, Magnifying glasses, etc.

2.2. Identification of Harmful Organisms - Infectious Plant Diseases caused by Phytopathogens of *Bacterial Origin*. Methods used for Identification. Good Practices in Plant Clinic. Laboratory Equipment

2.2.1. Identification of Harmful Organisms - Infectious Plant Diseases *caused by Phytopathogens of Bacterial Origin.* Taking and Structuring of Samples for plant clinical examination

Symptoms that can be defined using macroscopic and microscopic analyses are:

Visual observation and examination of the whole plant, organs, or part of the plants that include:

Leaf Spot*:* spots of dead tissue on the foliage; the size, shape, and color may vary with the causal agent, but are usually limited to a small portion of the leaf.

Leaf Blotch*:* dead areas of tissue on the foliage, irregular in shape, and larger than leaf spots.

Leaf Blight*:* dieback of a major portion of a plant, especially young, growing tissues.

Scorch*:* browning and death of indefinite areas along the leaf margins and between the veins.

Wilt*:* drooping of leaves or shoots, often due to lack of water.

Canker: localized dead stem tissue, often shrunken and discolored.

Stunting: reduced plant growth.

Gummosis: exudation of sap or gum from wounds or other openings in the epidermis.

Gall: swollen plant tissue that may be induced by insects, fungi, bacteria, or nematodes.

Chlorosis: yellowing of normally green tissues due to lack of chlorophyll.

Suspicious for specific harmful organisms expressing symptoms of Chlorosis:

Check for a pattern if it is available then it could be nutritional, phytotoxicity, or viral organism. If the entire leaf is affected most common could be environmental, cultural, chemical, or genetic expression or pathogenic organisms.

If irregular spots are present the plant health expert should focus on viruses, insects, chemical pathogens, or harmful organisms.

Here EPPO Standard PM7 - Diagnostic protocols for regulated pests – bacterial harmful organisms should be taken into account and followed by the plant health expert[4].

***In the case of Necrosis*:** death of tissue.

C. IF Necrosis: How extensive is it (chemical, cultural, environmental, disease)? Look for cankers if the entire branch is dead. Is the entire leaf brown, just portions, or the margin? Any pattern of necrosis? Spots (possibly insects, frost, disease).

Dieback: a large portion of dead tissues; usually used for woody ornamentals.

Mildew: white or grayish fungal growth on the surface of plant tissues, usually leaves or tender shoots.

Vascular Discoloration: darkening or streaking of the plant's vascular elements.

[4] EPPO Standards on phytosanitary measures. PM7 - Diagnostic protocols for regulated pests. Available at: https://gd.eppo.int/standards/PM7/

Damping-Off: collapse and decay of seedlings before or after emergence from the soil.

Witches' Broom: abnormal development of multiple secondary shoots, forming a broom-like effect. D. IF Abnormal Growth: Leaves twist and curl (chemical, insect, disease, mechanical, environmental, frost). Symptoms on stem or witches' broom (disease, insect). Whole plants are stunted from root damage, poor management, environmental, disease, and insects.

E. IF Missing or Damaged Parts: Bark missing could be deer, rodents, insects, or mechanical injury. Swelling on trunk: burlap/string still attached to shrub or tree. Leaves with holes or notches or skeletonized: could be disease, insect, or physiological factors. Roots: look at color, for any distortion, look at structure, growth, and size (chemical, insect, disease, culture).

Microscopic symptoms and signs can be performed by the plant health expert in the plant health clinic when the macroscopic observations are done but there is still a need for additional examination to be carried out.

The plant health expert should start by examining affected parts with a dissecting scope and move to a compound light microscope, if necessary. The plant Health expert should search for the presence of fruiting structures known as signs of the disease agent or evidence of insects or insect activity.

In case Leaves/Stems are infected, the plant health expert should start with an examination of the leaf spots, lesions, or cankers and **look for fungal growth**.

If **fungal growth is present**, or if lesions look bacterial, make a slide and examine under a compound scope; look for spores, any characteristics of a fungus, or bacterial ooze.

IF fruiting structures are present, crush, make a slide, and examine with a compound scope.

If no spores are mature, place tissue or plant parts in a moist chamber to force spore formation or maturation or to induce sporulation of any fungi that might be present.

Symptoms on the Roots/Crown are expressing the plant health expert should focus on the following symptoms:

Co-funded by the Erasmus+ Programme of the European Union
New Professional Diploma in Plant Clinic and Phytosanitary Technologies (PRO-DPCP)
609550-EPP-1-2019-1-BG-EPPKA2-CBHE-JP

In case of off-colored, crush roots or section crown tissues and make a slide with a scalpel and examine under a microscope for typical pathogen structures such as oospores, bacterial cells bacterial streaming, etc.

In case there are no fungi or bacteria present, the plant health expert should look for soluble salt problems and should check the soil type and management or cultural practices.

If there is no direct evidence of a harmful organism affecting a plant, but a plant health expert suspects that a fungus or bacteria may be the cause of the problem, they should set up cultures on Potato Dextrose Agar (PDA) to test for yeasts and molds. This can be supplemented with acid or antibiotics to inhibit bacterial growth, or Acid PDA, or H2O agar can be used after thoroughly washing the plant tissue in running tap water for 15-20 minutes.

2.1.2. Methods used for Identification of Harmful Organisms - Infectious Plant Diseases *caused by Phytopathogens of Bacterial Origin* in Plant Clinic

- ***Isolation of the harmful organism in artificial culture medium (agar***-agar, common nutrient media - meat-peptone broth (MPB), meat-peptone agar (MPA), meat-peptone gelatin (MPA);
- ***Artificial infection with an isolate from a sick on a healthy plant***
- ***Electron microscopy***
- ***ELISA Method***
- ***PCR Method -*** *DNA and RNA analyses*

Working environment: sterile and/or nonsterile environment, wearing protective clothes, protective glasses and gloves, etc.

2.1.3. Good Practices in Plant Clinic and Laboratory Equipment –

- International Plant Protection Convection - Managing Relationships with Stakeholders - A guide to stakeholder relations for national plant protection organizations
- IPPC Guide to Pest Risk Communication A guide for national plant protection organizations on communicating with stakeholders about pest risks

- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

3.3. Identification of Harmful Organisms - Infectious Plant Diseases *caused by Phytopathogens of Fungal Origin*. Methods used for Identification. Good Practices in Plant Clinic. Laboratory Equipment

3.3.1. Identification of Harmful Organisms - Infectious Plant Diseases *caused by Phytopathogens of Fungal Origin* and Taking and Structuring of Samples for Plant Clinical Examination

Symptoms that can be defined using macroscopic and microscopic analyses are performed with full visual observation and examination of the whole plant, organs, or part of the plants that include:

Leaf spot is characterized by the appearance of dead tissue spots on the foliage, which may differ in size, shape, and color based on the causal agent, but typically impact only a small area of the leaf.

Leaf blotch refers to dead areas on foliage that are larger than leaf spots and have an irregular shape.

Scorching appears as brown areas along leaf margins and between veins***.***

"Wilt" refers to the drooping of leaves or shoots, which is often caused due to lack of water.

Canker refers to a condition where a localized part of a stem or branch dies, resulting in the tissue shrinking and changing color***.***

Stunting refers to the condition in which the growth of plants is reduced***.***

Gummosis refers to the process of exudation of sap or gum from wounds or other openings in the epidermis.

Rust caused by certain fungi appears as orange or reddish-brown pustules on leaves, or as galls and cankers on stems.

Galls are swollen plant tissues that can be caused by various organisms such as insects, fungi, bacteria, or nematodes.

Chlorosis is a condition that causes the normally green plant tissues to turn yellow due to a lack of chlorophyll. In the case of identifying chlorosis caused by specific harmful organisms like viruses or insects, a plant health expert should look for a known pattern such as nutritional deficiencies or herbicide exposure. Additionally, they should check whether the entire leaf is affected by environmental, cultural, chemical or genetic factors and whether there are any irregular spots present, which may indicate the presence of viruses, insects or chemical damage.

Necrosis refers to the death of plant tissue, which can occur due to various reasons such as injury, infection, abiotic factors (sun burn) or lack of plant fluids supply. In case of Necrosis a specific check should be performed on the extensity whether is it chemical, cultural, environmental, disease and other reason. It should be closer look paid for cankers if the entire branch is dead or whether the entire leaf brown, or some pieces of the leaf and portions, or the margin and et. The plant health expert should search for known pattern of necrosis like spots that might be caused by insects, frost, disease or other.

Dieback: a substantial portion of dead tissues usually used for woody ornamentals.

Mildew: is white or grayish fungal growth on the surface of plant tissues, usually leaves or tender shoots.

Vascular Discoloration refers to darkening or streaking of the plant's vascular elements.

Damping-Off: collapse and decay of seedlings before or after emergence from the soil.

Witches' Broom: abnormal development of multiple secondary shoots, forming a broom-like effect.

Microscopic symptoms and signs is the next step that plant health expert should take in case the macroscopic observations required additional examination. First examination starts by examining affected parts with a dissecting scope and move it to a microscope. A fruiting structures whish are the signs of the disease harmful organism or evidence of insects or insect activity should be observed.

Microscopic symptoms and signs on the Leaves/Stems are performed if there is a leaf spots, lesions, or cankers then the plant health expert should search for fungal growth. If fungal growth is present, or if lesions look bacterial, a slice of the tissues should be examined under a microscope and to search for spores, or any other characteristics of a fungus, or bacterial ooze. In case fruiting structures are present, crush, make a slide, and examination under a microscope should be performed. If no spores are mature, then the plant health exert should place a tissue or plant parts in a moist chamber to force spore formation or maturation or to induce sporulation of any fungi that might be present.

Microscopic symptoms and signs on the Roots/Crown: IF there is off-colored, crush roots or section crown tissues are found the plant health expert should make a thin slice of the tissue and examine it under microscope for pathogen structures such as oospores, bacterial cells bacterial streaming, etc.

In case that there is no fungi or bacteria found the look should be focused on soluble salt problems or search at the soil type and management or cultural practices. In situation that there is no other direct evidence of a biotic agent but the plant health expert still has a suspicion of fungal or bacterial infection, then he/she should set up cultures on Potato Dextrose Agar (PDA) or Acid PDA, or H2O agar after a thorough washing of the plant tissue in running tap water for 15-20 minutes.

3.3.2. Methods used for Identification of Harmful Organisms - Infectious Plant Diseases *caused by Phytopathogens of Fungal Origin* in Plant Clinic

- ***Wet chamber method*** - the plant samples are placed in conditions of high relative humidity to provoke the formation of spores in case the harming agent is of fungal origin.
- ***Isolation of the harmful organism in artificial culture medium (agar***-agar, common nutrient media - meat-peptone broth (MPB), meat-peptone agar (MPA), meat-peptone gelatin (MPA).
- ***Artificial infection with an isolate from a sick on a healthy plant***
- ***Electron microscopy***
- ***ELISA Method***
- ***PCR Method -*** *DNA and RNA analyses*

Working environment: sterile and/or nonsterile environment, wearing protective clothes, protective glasses and gloves, etc.

3.3.3. Good Practices in Plant Clinic and Laboratory Equipment

- International Plant Protection Convection - Managing Relationships with Stakeholders - A guide to stakeholder relations for national plant protection organizations
- IPPC Guide to Pest Risk Communication A guide for national plant protection organizations on communicating with stakeholders about pest risks
- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

Part C: Identification of Non-Harmful Organisms – Natural enemies of pests on Plants (Invertebrate biological control)

Invertebrates, such as insects, mites or nematodes, feeding or antagonizing on harmful organism can be used as one form of natural pest control. In order to better distinguish them

Co-funded by the Erasmus+ Programme of the European Union
New Professional Diploma in Plant Clinic and Phytosanitary Technologies (PRO-DPCP)
609550-EPP-1-2019-1-BG-EPPKA2-CBHE-JP

from other categories of biological pest control, they are usually referred to as invertebrate biological control agents (IBCAs)[5].

EPPO Standards – PM 6 Safe use of biological control should be applied.

4: Identification of Non-Harmful Organisms - Natural enemies of pests in Plant Clinic

4.1. Identification of Non-Harmful Organisms **Natural enemies of pests** from *Arthropoda Species* and Taking and Structuring of Samples for plant clinical examination

4.2. Methods used for Identification of Not Harmful Organisms - **Natural enemies of pests** from *Arthropoda Species*.

4.3. Good Practices in Plant Clinic - Working Environment and Laboratory Equipment.

5. Identification of Non-Harmful Organisms – Natural enemies of pests from *Nematoda Species* and Taking and Structuring of Samples for plant clinical examination

5.1. Methods Used for Identification of Not Harmful Organisms Pests on the Plants from *Nematoda Species*

5.2. Good Practices in Plant Clinic and Laboratory Equipment

- International Plant Protection Convection - Managing Relationships with Stakeholders - A guide to stakeholder relations for national plant protection organizations
- IPPC Guide to Pest Risk Communication A guide for national plant protection organizations on communicating with stakeholders about pest risks
- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

[5] European Commission (2024). Invertebrate biological control agents (IBCAs) against plant pests

6. Identification of Non-Harmful Organisms – Natural enemies of pests from *Acarida Species* and Taking and Structuring of Samples for plant clinical examination

6.1. Methods Used for Identification of Not Harmful Organisms Pests on the Plants from *Acarida Species*

6.2. Good Practices in Plant Health Clinic and Laboratory Equipment

- International Plant Protection Convection - Managing Relationships with Stakeholders - A guide to stakeholder relations for national plant protection organizations
- IPPC Guide to Pest Risk Communication A guide for national plant protection organizations on communicating with stakeholders about pest risks
- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

Part D: Identification of Harmful Organisms - Pests on the Plants

7. Identification of Harmful Organisms - Pests on the Plants from Class Insecta. Taking and Structuring of Samples for plant clinical examination

7.1. Methods Used for Identification of Harmful Organisms - Pests on the Plants from *Arthropoda Species*

7.2. Good Practices in Plant Health Clinic and Laboratory Equipment

- International Plant Protection Convection - Managing Relationships with Stakeholders - A guide to stakeholder relations for national plant protection organizations
- IPPC Guide to Pest Risk Communication A guide for national plant protection organizations on communicating with stakeholders about pest risks
- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

8. Identification of Harmful Organisms - Pests on the Plants from Class Nematoda. Taking and Structuring of Samples for plant clinical examination

8.1. Methods Used for Identification of Harmful Organisms - Pests on the Plants from *Nematoda Species*

8.2. Good Practices in Plant Health Clinic and Laboratory Equipment

- IPPC Guide to Pest Risk Communication A guide for national plant protection organizations on communicating with stakeholders about pest risks
- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

9. Identification of Harmful Organisms - Pests on the Plants from Class Arachnida. Taking and Structuring of Samples for plant clinical examination

9.1. Methods Used for Identification of Harmful Organisms - Pests on the Plants from *Acarida Species*

9.2. Good Practices in Plant Health Clinic and Laboratory Equipment

- IPPC Guide to Pest Risk Communication A guide for national plant protection organizations on communicating with stakeholders about pest risks
- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

10. Identification of Harmful Organisms - Pests on the Plants from *Gastropoda \slugs & snails)*. Taking and Structuring of Samples for Plant Clinical Examination

10.1. Methods Used for Identification of Harmful Organisms - Pests on the Plants from *Mollusca Species*

10.2. Good Practices in Plant Health Clinic and Laboratory Equipment

Section E: Identification of Harmful Organisms – Weeds and Parasitic Plants on the Plants

11. Identification of Harmful Organisms – Weeds and its Seeds. Methods and Good Practices

11.1. Identification of Weeds and its Seeds. Taking and Structuring of Samples for plant clinical examination

11.2. Methods Used for Identification in Plant Clinic

11.3. Good Practices in Plant Health Clinic and Laboratory Equipment

- IPPC Guide to Pest Risk Communication A guide for national plant protection organizations on communicating with stakeholders about pest risks
- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

12. Identification of Harmful Organisms-Parasitic Plants. Methods and Good Practices in Plant Health Clinic

12.1. Identification of Weeds and Taking and Structuring of Samples for plant clinical examination – Visual methods are applicable and observations of weed's seeds under microscope

12.2. Methods Used for Identification in Plant Clinic – Visual methods are applicable and observations of weed's seeds under microscope

12.3. Good Practices in Plant Health Clinic and Laboratory Equipment

- IPPC Guide to Pest Risk Communication A guide for national plant protection organizations on communicating with stakeholders about pest risks

- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

Section F: Identification of Harmful Quarantine Organisms on Plants. Methods and Good Practices in Plant Health Clinic

- International Plant Protection Convention standard - ISPM 11 on Pest risk analysis for quarantine pests is fully applicable in this chapter
- EPPO A1 and A2 list of pests recommended for regulation as quarantine pests
- EPPO Standards – PM 7 Diagnostics

Part A: Harmful Quarantine Organisms- Quarantine Plant Diseases

13.1. Identification of Harmful Quarantine Organisms-Quarantine Plant Diseases. Taking and Structuring of Samples for plant clinical examination - ISPM

13.2. Methods Used for Identification in Plant Clinic

13.3. Good Practices in Plant Health Clinic and Laboratory Equipment

Part B: Harmful Quarantine Organisms-Quarantine Pests on the Plants

13.4. Identification of Harmful Quarantine Organisms-Quarantine Pests. Taking and Structuring of Samples for plant clinical examination

13.5. Methods Used for Identification in Plant Clinic

13.6. Good Practices in Plant Health Clinic and Laboratory Equipment

- IPPC Guide to Pest Risk Communication A guide for national plant protection organizations on communicating with stakeholders about pest risks
- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

Part C: Harmful Quarantine Organisms- Quarantine Weeds and its Seeds

13.7. Identification of Quarantine Weeds and its Seeds. Taking and Structuring of Samples for plant clinical examination

13.8. Methods Used for Identification in Plant Clinic

13.9. Good Practices in Plant Health Clinic and Laboratory Equipment

- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

Section G: Identification of Harmful Organisms-Invasive Species in Agricultural Fields of Plants. Methods and Good Practices in Plant Health Clinic

- EPPO Standards – PM 7 Diagnostics are applicable in this chapter is applicable.

14.1. Identification of Harmful Organisms-Invasive species in Agricultural Fields of Plants.

4.2. Methods Used for Identification in Plant Clinic – Visual methods and identification standards of EPPO and IPPC are applicable.

4.3. Good Practices in Plant Health Clinic

- IPPC Guide to Pest Risk Communication A guide for national plant protection organizations on communicating with stakeholders about pest risks
- Communication and exchange of information and know-how in and throughout the Plant Health Clinics Network

Section H: Identification of Harmful Organisms to Plants in Soil. Methods and Good Practices in Plant Health Clinic

In this section the following standards are applicable

- EPPO Standards – PM 7 Diagnostics are applicable
- International Plant Protection Convention standard - ISPM 11 on Pest risk analysis for quarantine pests is fully applicable in this chapter
- EPPO A1 and A2 list of pests recommended for regulation as quarantine pests

Annexes

Annex 1: Submission form for plant diseases samples – *The unified submission form template is a subject to be developed by the partnering Universities in Egypt*

Annex 2: Submission form for plant pests' samples - *The unified submission form template is a subject to be developed by the partnering Universities in Egypt*

Annex 3: Submission form for soil samples *The unified submission form template is a subject to be developed by the partnering Universities in Egypt*

Annex 4: Submission form for weed plants and seeds - *The unified submission form template is a subject to be developed by the partnering Universities in Egypt*

References

Douglas, D. S. (2023, December 11). *Diagnosis of Plant Health Problems.* (1. H. Department of Plant Pathology and Ecology, Ed.) Retrieved December 11, 2023, from The Connecticut Agricultural Experiment Station: https://portal.ct.gov/CAES/Fact-Sheets/Plant-Pathology/Diagnosis-of-Plant-Health-

Problems#:~:text=Start%20by%20examining%20affected%20parts,cankers%2C%20look%20for%20fungal%20growth.

OECD. (2023, December 11). *OECD Principles of Good Laboratory Practice (GLP) and GLP Compliance Monitoring.* Retrieved from Principles of Good Laboratory Practice (GLP) and GLP Compliance Monitoring: https://www.oecd.org/chemicalsafety/testing/overview-of-good-laboratory-practice.htm

The final version of this document in implementation of WP1, task 1.4. of the project has been prepared by Dafinka Grozdanova, Agricultural University-Plovdiv.