Identification of plant diseases and distinct approaches for their management

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Abstract

Background: Globally in the agricultural industry the major loss faced by is due to plant diseases. Various pathogens are responsible for causing plant bacterial and viral diseases, the treatment of them is very important in order to bring out the best quality and quantity of the agricultural yield. Before Technology came into practice the plant disease were identified by visual examination, the main symptoms such as curling of leaves and change of colour were observed. With advancements in science the microscopic examination for more clarity regarding diseases came into existence.

Main body: The distinct methods involve use of nucleic acids and serological assays were implemented to study bacterial and viral characteristics of the infecting pathogen. Traditionally, seed coating and mulching techniques were more common among farmers to generate better quality of the crops and prevent plants from any disease but currently new innovative methods are used. Microbial bio control agents are now one of the widely used approach in which microbial species are used to eliminate or inhibit the growth of pathogens in order to reduce the severity of the infection. Similarly, like microbial agent’s different chemicals are present in order to kill the pathogens. These chemicals are classified as bactericides, fungicides and nematicides which suppress the plant infection caused by bacteria, fungal and nematodes, respectively.

Conclusions: In the forthcoming years, the development of more innovative agricultural-related techniques is prime that will help in increase of the yield and provides resistance to plants. Some of them are developed earlier but there is still need to develop more pathogen-resistant species for example in case of silencing of genes with insertion of a viral segment.

Keywords: Plant diseases, Bio control agents, Pathogens, ELISA, FISH

Background

The major loss is faced by the agricultural industry is due to plant diseases. Various pathogens are responsible for causing plant bacterial and viral diseases, the treatment of them is very crucial. Earlier, on the basis of the natural phenomenon, it was assumed that evolution was the only process to deal with pathogens in which plants themselves undergoes the process of evolution either they develop resistance or sensitivity to the pathogens attacked. However, with time and introduction of the new techniques things are now different and changed (Fang and Ramasamy 2015). The developments in science and technology implemented various new methodologies for the treatment of the plants. From the beginning of the 1765 new methodologies were adopted, when the identification of various cryptogrammic parasites was done. Earlier, the plant diseases were identified by visual examination of the various parts of the plants in which the curling of leaves, change of colour from red to yellow and spots either brown or white (Lopez et al. 2003). With scientific progression, the microscopic examination came to effect in order to observe and study various pathogens...
involved in cause of a disease (Belet Chane and Boyraz 2019).

Main text
Traditional approaches to plant disease management
In 1929 H.H. Whetzel articulated the development of some basic principle that can be followed for the treatment of the plant diseases that are avoidance, exclusion, eradication, elimination and destruction. The main focus of these principles was on protection, resistance and therapy in which the use of toxicants was there in order to prevent infection (Spadaro and Gullino 2005). Despite of the advantages there are various shortcomings of these principles such as, they were failed to provide dynamics of the diseases depicting severity of it with time. Traditionally, farmers used to identify the disease by visual examination after that they used to follow some traditional methods (Fig. 1) for the plant treatment which are discussed as follows:

I. **Seed treatment**—In this method, the seeds were soaked in warm cow's milk for approximately several hours, than they were treated with cow's ghee and dung (Shanti et al. 2010).
II. **Removal of ulcers and patches**—Initially, a sharp knife is used to remove the ulcers from the affected plant than a paste is prepared with help of cow ghee and Embelia ribes (Chitarra and van den Bulk 2003).
III. **Prevention from sun scorching**—In order to prevent rusting of coffee leaves and blight of tea due to blister shading of the plants was done (Bürling et al. 2011).
IV. **Suppression of the soil-borne pathogens**—Use of raised beds and ridges. For example, in Mexico for the treatment of the *Pythium* and *Phytophthora* which are soil-borne pathogens, the chinampas or floating garden were used (Bonaterra et al. 2007).
V. **Suppression of the air and seed-borne disease**—Planting was done across the direction of the wind so that the airborne pathogens do not intervene with the plants (Garrett et al. 2010).

Innovative approaches for the detection of plant diseases
Presently, some basic molecular techniques are used for the detection of the various diseases. These molecular diseases are categorized under two approaches, i.e. serological assays and nucleic acid-based approaches. The serological assays are used to detect various bacterial and fungal pathogens, the techniques require monoclonal and polyclonal antibodies in order to detect pathogens (Singh et al. 2013). The approaches like restriction fragment length polymorphism (RFLP) and loop-mediated isothermal amplification can also be utilized for the detection purpose (Jones 2009). Additionally, different direct and indirect methods can be used such as western blot, polymerase chain reactions, immunofluorescence, fluorescence in situ hybridization, thermography, hyperspectral techniques, fluorescence imaging and biosensors (Barnes and Szabo 2007).

**Fig. 1** Traditional principles of pathogen attack control

Traditional principles were adopted by the farmers in order to control the pathogen attack on the plants and to prevent any further spread of the disease. These methods allow selection of a particular time or seasonal which reduces resistance of a plant to a pathogen. These principles were finalised by the US National Academy of Science, each principle plays a significant role in prevention of the disease either by preventing the introduction of the inoculum or by eliminating or inactivating it.
Some of the molecular techniques are discussed as follows

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Some of the molecular techniques are discussed as follows

I. **Enzyme linked immune sorbet assay**—This technique was introduced in 1970 and is effectively used for the detection of the viruses in plants. It has been used for the detection of the grapevine virus and to detect **Xylella fastidiosa** which is a causative agent of the Pierce’s disease. In this approach, the detection is based on antibody—antigen interaction, the reaction obtained from it is observed as a change in colour in the assay (Aslam et al. 2017). This change in colour in the assay depicts whether a reaction has occurred or not, i.e. how an antibody reacted towards antigen attached with a specific epitope on its surface. Specifically, an epitope is regarded as an antigenic determinant which is capable of eliciting an immune response.

II. **Fluorescence in situ hybridization (FISH)**—It is one of the most well-established approaches used for the detection of the bacteria and fungi in case of the plants. It works in combination with the microscopy as well as hybridization which helps in the extraction of the target genes from the plant samples with help of their DNA probes. Cytogenetic is involved in this technique in order to detect and locate specific DNA sequence present in the genome (Gailliet and Rouanet 2015). The probes used are labelled with haptene due to their specific binding characteristics as they only bind to the sequence which shows high percentage of the similarity (Fig. 2).

III. **Western blots**—A Plum pox virus was detected from **Nicotiana benthamiana** with the help of the
immune-blotting technique. In this process, different parts of plant were infected with the PPV capsid protein and after 21 days of the inoculation the Plumpox virus was detected. This method was effectively used for the detection of the plant virus characteristics and virus-particle protein interactions (Bashan and Holguin 1998).

IV. *Nucleic acid-based techniques*—Among various different techniques the polymerase chain reaction is used for the detection of the plant pathogens. In the processes, the one common approach used is enrichment of the pathogen in media selective before the final application of the molecular techniques (Spadaro and Gullino 2004). In case of PCR techniques the common targets are the ribosomal RNA genes and intervening sequences from the bacterial, fungal and nematode pathogens (Zhang et al. 2005). The nucleic acid techniques offer great advantages for the process of detection of distinct pathogens some of them are discussed as follows.

- For the detection of various obligate parasites which are present in a complex environmental conditions (Lievens et al. 2006).
- Detection of the organisms whose growth rate is too slow and the chances of the contamination are more (Nutter and Schultz 1995).
- They are widely used to identify the species that are morphologically similar to the non-pathogenic organisms (Mehta and Rosato 2001).
- Molecular detection of the pathogens having regulatory concerns can be detected with help of various nucleic acid-based techniques for example, *Phytophthora ramorum*, *Xanthomonas citri* and *Tilletia indica* (Martinelli et al. 2014).
- With the help of the nucleic acid techniques, the quantification of the pathogen biomass from the host cell can be done. Moreover, the geographic origin of the pathogens with historical origin can also be identified (Nutter and Schultz 1995; Mehta and Rosato 2001).
- Co-PCR can be used for the detection of the viruses and bacteria. In case of plants in a single reaction the detection can be done, thus this will reduce any chances of the contamination during the analysis of the pathogens. When a Co-PCR is coupled with a colorimetric technique of the detection then the sensitivity for the virus detection increases. Hence, making it easy for the highly unstable viruses to get detected in one go (Martinelli et al. 2014).
- Reduction of time and cost due to M-PCR approach. As in cases where multiple pathogens are responsible for the infection of a single plant with help of nested and multiple PCR technique. It will be easy to detect multiple causative agents at a single time (Koczula and Gallotta 2016).

Pathogen control

In order to meet the demand of the population, there is a need to control the plant diseases so as to maintain equilibrium between the quality and quantity of the food commodities. Various approaches are used for the control of the disease with help of some chemicals or by biological methods with help of microbes. Each method has their own mode of action for instance such as:

- **Biological control agents**—They suppress the growth of the pathogen by inducing resistance to plants or by modulating the conditions required for the growth of a pathogen in the host body (Garnsey et al. 1993).
- **Chemical control**—The use of chemical pesticides and insecticides was abundant with their great positive effect rate. These chemicals result in causing environment and agricultural pollution so in order to preserve the environment various norms have been implemented by the government against such chemical pesticides. Consequently, now researchers are more focussed towards the development of the different microbial control agents in bulk against all plant species so as to control the plant infections.
with an alternative to chemical methods (Whipps 2001).

Biological control agents
As the name defines itself, i.e. microbial biological control agents means use of distinct microbes which includes nematode, bacteria, virus, live predatory insects etc. for the control of the plant infection. In study of the plant pathology, the suppressing of the pathogens with microbial antagonists and use of host-specific pathogens for mitigate weed describes the term BCA, i.e. biological control agents (Beale and Pitt 1995). The extracts from the microbes can be fermented and used as BCAs, formulating such mixtures is very easy. Natural products are used and they provide multiple effects on the host and the pathogen.

• Mechanism of Action—Each microbial control agent has its own specific mechanism of action but some basic modes include mimicking the pathogens, reducing the nutrient content in host plant so that the growth of pathogen can be hindered (Weller 2007). Additionally, inducing resistance to plants, directly targeting the metabolic pathway of the pathogens and decreasing or increasing the temperature within the host plant are other modes used. The prime role of each BCA is to control the infection (Dun-chun and Lian-hui 2016) (Fig. 3).

• Interactions between plant and pathogens—Plants and pathogens undergo various interactions either specific or non-specific in nature throughout their life cycle. Some interactions benefit both pathogen and its host while other benefit one either host or pathogen and some do not benefit either. Different types of interactions are mutualism, commensalism, neutralism, e.g. Mucuna pruriens, Desmodium uncinatum, amensalism, competition, predations by predators for example, Rodolia cardinals, Neodusmetia sangwani, Philodromus cespitum and parasitism by parasites, e.g. Ichneumonid wasps, Braconid wasps (Acosta-Leal et al. 2011). Each plays their own significant role in control of the plant pathogens such as stimulation of host defense, consumption of pathogen biomass which ultimately led to death of pathogen, increase nutrition quality of plant and some creates an inability between the interactions of the population dynamics of plant species to the pathogen (Table 1) (Sundin et al. 2016).

• Two component system

1. A two component system mechanism can be implemented as it helps to inhibit the secondary growth of the pathogen thus acting as a great defence system, for instance Pseudomonas aurefaciens strain 30–84 is a biocontrol agent and has widely been used for all diseases related to wheat (Köhl et al. 2019).

2. In the process of the two systems, when a pathogen attacks on the roots it results in increasing the oozing process in roots which causes increase in growth of the bacteria in the infection region (Wiesel et al. 2014).

3. Therefore, in pseudomonas strain 30–84 the switching on of the PCA pathway occurs due to increase levels of the signal molecule, i.e. N-acyl-L-homoserine lactone (HSL), the activated PCA pathway increase in production of the PCA which inhibits the further growth of the bacteria (Nicot et al. 2011).

4. The strain 30–84 of Pseudomonas aurefaciens primarily does not decrease the severity of the infection but secondarily inhibit the growth of pathogen. Similarly, various fungal pathogens have been eliminated with help of the biocontrol agents such as actinoplanes species can treat Pythium ultimum present in soil environment affecting table beet plant, treatment of Pythium aphanidermatum with help of the Pseudomonas aureofaciens strain 63–28 and inhibition of growth of the Rhizoctonia solani which affects tall fescue plant with treatment of Stenotrophomonas maltophilia C3 (Timms-Wilson et al. 2005).

5. The mode of action of bacterial species is by creating pores on the body of the pathogen, lysis of the fungal cell wall, degradation of the pathogens cell wall enzymes and completely inhibiting the metabolic pathway of the pathogens. Thus, resulting in complete elimination of the disease-causing agents (Kloepper et al. 1980).

• Siderophores—hey are compounds which are high in affinity towards iron-chelation; they are produced by bacteria and fungi. They play a great role in transport of iron across the cell membranes of the plants (Louws et al. 1999). Directly or indirectly these siderophores provides protection to the plants against various infectious pathogens for example, pyochelin protects tomato from Pythium. Some examples of biocontrol species of microbial agents with their interactions towards specific pathogens are mentioned in Table 2.
Advantages of microbial bio control agents over chemical agents

- Environmental friendly in nature and are effective even in small quantities.
- Decomposition of them is not an issue (Audenaert et al. 2002).
- They are target specific in nature. Thus, a specific microbe acts against a particular target or pathogen. Moreover, they do not affect other organism’s even humans (Audenaert et al. 2002).
- Use of biological material is a cost-effective manner to control plant diseases in long term.

Chemical control

Similarly, like microbial agent’s different chemicals are present in order to kill the infection-causing pathogens, these chemicals are classified as bactericides to kill bacteria, fungicides they destroy fungal spores causing infection and nematicides completely kill nematodes. Chemicals can be sprayed directly over plant surfaces

Table 1  The detail of roles and the type of interactions

| Interaction | Role | References |
|-------------|------|------------|
| Mutualism   | Stimulate host defense, improve nutrition in plants | Dodds and Rathjen (2010) |
| Commensalism| Presence of such relationship creates challenges for pathogens, absence of it led to decrease in the severity of the infection | Eilenberg et al. (2001) |
| Predators   | They consume pathogen biomass for their sustenance, e.g. protists, mesofauna and microarthropods | Jones and Dangl (2006) |
| Neutralism  | It creates an inability between the interaction of the population dynamics of plant species to the pathogen | Juroszek and von Tiedemann (2011) |
| Competition | Led to decrease in growth of infectious microbes | Coakley et al. (1999) |
| Parasites   | Involves the use of hyperparasites that led to parasitize the plant pathogens | Scherm (2004) |

Fig. 3  Mode of action of microbial control agents
such as fruits, flowers and soil (Kliot et al. 2014). Each chemical control agent work on several mechanisms such as some of them inhibits the cell membrane ergosterol biosynthesis and prevents the development of the fungus (Table 3). Moreover, they supress the growth of the fire blight and frost forming bacteria on plant surface, break the DNA strands and results in loss of helical structure of the infectious organism present over plant surfaces. Some of them improves the water solubility in plants and effects the pathogens growth. Few nematicides are organophosphate in nature and thus inhibit the acetylcholine esterase synthesis which is required for the normal functioning of a plant (Nilsson 1995).

Although the chemicals are very effective in broad range to kill the microorganisms and control the plant disease from spreading, but there are some disadvantages of these chemicals. They affect the fertility of the soil, growth of the plants and the crops are consumed by humans and the chemicals indirectly affects them (Smith 1903). They induce immune toxicity in case of humans and thus resulting in suppression of the immune system. Consequently, give rise to autoimmune diseases. The

### Table 2 Examples of bio control agents and pathogens

| Biocontrol specie | Microbial agent | Pathogen | Interaction | Plant | References |
|-------------------|-----------------|----------|-------------|-------|------------|
| Bacteria          | *Comamonas acidovorans* | *Magnaporthe poae* | Bacteria biocontrol—fungal pathogen | Kentucky bluegrass | Nicot et al. (2011) |
| Fungi             | *Idriella bolleyi* | *Bipolaris sorokiniana* | Fungi biocontrol—fungal pathogen | Barley | Timms-Wilson et al. (2005) |
| Fungi             | *Trichoderma harzianum T-22* | *Pyrenophora triticis-repentis* | Fungi biocontrol—fungal pathogen | Wheat | Timms-Wilson et al. (2005), Kloepper et al. (1980) |
| Fungi             | *Pythium oligandrum* | *Verticillium dahlia* | Fungi biocontrol—fungal pathogen | Pepper | Gaffney et al. (1994) |
| Bacteria          | *Pseudomonas species* | *Fusarium oxysporum* | Bacteria biocontrol—fungal pathogen | Tomato | Louws et al. (1999) |
| Fungi             | *Trichoderma harzianum BAFC 742* | *Sclerotinia sclerotiorum* | Fungi biocontrol—fungal pathogen | Soybean | Kloepper et al. (1980) |
| Bacteria          | *Pseudomonas fluorescens WCS358* | *Fusarium oxysporum* | Bacteria biocontrol—fungal pathogen | Radish | Nicot et al. (2011) |

### Table 3 Different groups and their uses are discussed as follows

| Chemical name | Group | Uses and mode of action | References |
|---------------|-------|-------------------------|------------|
| Azoxystrobin  | Fungicide | Effective against powdery and downy mildews fungus | Nilsson (1995) |
| Acibenzolar-S-methyl | Fungicide | Used for Crops and vegetables | Nilsson (1995) |
| Blight ban    | Bactericide | Supress the growth of the fire blight and frost forming bacteria on plant surface | Pothier et al. (2011) |
| Avermectin    | Nematicide | Improves plants thermal stability and has greater water solubility | Bargabus et al. (2002) |
| Carbendazim   | Fungicide | Controls mould, spot, mildew and scorch. It affects the biosynthesis of the DNA during the process of the fungal cell division | Garnsey and Cambra (1991) |
| Allyl isothicyanate | Nematicide | Broad spectrum in nature widely effective against numerous soil-borne pathogens | Bargabus et al. (2002) |
| Cephalosporins | Bactericide | Inhibit cell wall synthesis of pathogens | Pothier et al. (2011) |
| Difenconazole  | Fungicide | Inhibits the cell membrane ergosterol biosynthesis and prevents the development of the fungus | Nilsson (1995) |
| Vancomycin     | Bactericide | It inhibits trans peptidase synthesis which further prevents elongation consequently destroys the pathogen | Pothier et al. (2011) |
| Cyanogen       | Nematicide | Kills plant parasites | Kollerova et al. (2008) |
| Myclozolin     | Fungicide | Prevents the growth of the pathogens | Bargabus et al. (2002) |
| Metronidazole  | Bactericide | Break the DNA strands and results in loss of helical structure of the infectious organism present over plant surface | Nilsson (1995) |
| Diamidafos     | Nematicide | Organophosphate in nature and thus inhibits the acetylcholine esterase synthesis which is required for the normal functioning of a plant | Kollerova et al. (2008) |
| Telithromycin  | Bactericide | Interferes with the protein synthesis of the bacteria and prevents its growth | Bargabus et al. (2002) |
chemicals led to killing of some useful organisms such as chemicals effect the rate of the honeybee pollination, loss of birds and contaminating water streams led to death of aquatic animals (Cambra et al. 2000). Due to their disadvantages now research is more focused towards the introduction of the bio control agents. That will benefit the society economically and agricultural sector for the production of hybrid quality of products with better yield.

Conclusions
Different pathogens are responsible for causing plant diseases and due to them the major loss is faced by the agricultural industry. Earlier, the plant diseases were identified by visual examination the main symptoms such as curling of leaves and change of colour were observed. Moreover, traditional principles were adopted by the farmers in order to control the pathogen attack on the plants and to prevent any further spread of the disease. But as soon as technology came into existence different approaches were discovered to diagnose plant disease such as ELISA, FISH, western blotting and use of nucleic acids. They are widely due to less time consuming and effective results in regard to the type of the pathogen with proper identification. For prevention of further disease spread now the more focus is on microbial biological control agents (MBCAs) that suppress the growth of the pathogen by inducing resistance to plants or by modulating the conditions required for the growth of a pathogen in the host body. The use of chemical pesticides and insecticides was abundant with their great positive effect rate but these chemicals result in causing environment and agricultural pollution so in order to preserve the environment various norms have been implemented by the government against such chemical pesticides. Consequently, now researchers are more focussed towards the development of the different microbial biological control agents in bulk against all plant species so as to control the plant infections with an alternative to chemical methods. Furthermore, the development of transgenic plants with transformed genes and use of variety of biosensors for the determination of plant diseases is the current focus in the forthcoming times. In addition, use of nanoparticles for the development of biosensors as they can improve the working capacity of a biosensor. The development of more such biosensors is under process in order to cover wide range of plant disease detection.

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SGS reviewed and interpreted the Plant diseases and their management. LK collected and analysed the various approaches of plant disease management and was a major contributor in writing the manuscript. Both authors read and approved the final manuscript.

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Abbreviations
BCA: Biological control agents; CymMV: Cymbidium mosaic virus; DTBIA: Direct tissue blot immunosassay; ELISA: Enzyme-linked immunosorbent assay; FISH: Fluorescence in situ hybridization; FAO: Food and agricultural organization; ITS: Internal transcribed spacers; MBCAs: Microbial bio control agents; ORSV: Odontoglossum ringspot virus; PCR: Polymerase chain reaction; PPV: Plumpox virus; RFLP: Restriction fragment length polymorphism.
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