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Diagnosis and management of important diseases of solanaceous vegetables

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Abstract
Solanaceous vegetable crops play a significant role in human nutrition and to cope with malnutrition, especially as sources of vitamins like vitamin C, A, E, thiamine, niacin, pyridoxine, folacin, minerals and dietary fibre. They are termed as Protective Foods because these are rich source of minerals, vitamins and phytonutrients. Phytochemicals found in solanaceous vegetables like lycopene in tomato and peppers, nasunin in brinjal are strong antioxidants and are thought to reduce the risk of chronic disease. Monoculture of these crops consequently leads to increased spectrum of insect-pests and diseases. The losses in crop yield due to pathogen infections range between 20% and 40%. On average, pathogen-induced losses of potato, tomato are estimated to be around 24% (Oerke, 2006). Early and accurate detection and diagnosis of plant diseases is key factor for reduction of losses and spreading of diseases (Balodi *et al*., 2017). Conventional or traditional methods of disease detection include methods of visual examination, culturing or plating, biochemical tests etc. These methods are reliable but on the other hand they are very time consuming (Ray *et al*., 2017). On the Other hand, advance method such as Serological and molecular methods have high sensitivity and specificity and are less time consuming (Sankaran *et al*., 2010). Early and accurate detection and diagnosis assess the effectiveness of application of cultural, physical, biological and chemical management.

Keywords: Diseases, solanaceous vegetables, vitamin

1. Introduction
The term "Solanaceous crops" generally refer to plants in the nightshade family. Solanaceae has more than 90 genera and nearly 3,000 species distributed throughout the world. The Solanaceous family of vegetable mainly include 4 vegetables potato, tomato, eggplant, capsicum and chilli. Tomato is fourth most popular fresh market vegetable behind potatoes and onions. Over 90% of India's exports in fresh products go to west Asia and East European markets). India is major producer, consumer and exporter of chilli in the world. Mid hills of HP is leading suppliers of capsicum during off season. Potato is one of the four major food crops of the world after rice, wheat and maize. Important cash crop produces more dry matter, well balanced protein and more calories from less unit area of land and time than other major food crops. Brinjal is considered as poor man's vegetable because it is popular amongst small scale farmers and low-income consumers. India is the second largest producer in the world.
Monoculture of these crops consequently leads to increased spectrum of insect-pests and diseases. Pathogens being microscopic in nature are difficult to diagnose thus have adverse effect in crop losses throughout the world. The losses in crop yield due to pathogen infections range between 20% and 40% (Davino *et al.*, 2015) [14]. On average, pathogen-induced losses of potato, tomato are estimated to be around 24% (Oerke, 2006) [15]. In order ensure agricultural sustainability, advanced disease detection and prevention in crops is highly important so that disease induced damages in crops during growth, harvest can be minimized and productivity is enhanced. Farmers experience great difficulties in switching from one disease control policy to another. The naked eye observation of experts is the traditional approach, this method can be time consuming, expensive and inaccurate. The crop losses can be minimized by applying pesticides or its equivalent to combat the effect of specific pathogens, if diseases are correctly diagnosed and identified early.

2. The need for plant disease diagnosis
Diagnosis is one of the most important aspects of a plant pathologists training. Without proper identification of the disease- and disease-causing agent, disease control measure can be waste

of time and money. Proper disease diagnosis is therefore vital. Early and accurate detection and diagnosis of plant diseases is key factor for reduction of losses and spreading of diseases. Plant disease diagnosis assesses the effectiveness of application of cultural, physical, chemical, or biological methods of containing the pathogens. It also assesses pathogen infection in plant materials in breeding programs. To detect and identify new pathogens rapidly to prevent further spread. Diseases diagnosis helps to study pathogenesis and gene functions and to resolve the components of complex diseases incited by two or more pathogens.

3. Important diseases of solanaceous vegetables

Common name of the diseases along with causal organisms

3.1 Tomato

- Buckeye rot: *Phytophthora nicotianae* var. *parasitica*
- Early blight: *Alternaria tomatophila*
- Alternaria leaf spot: *Alternaria alternata*
- Septoria leaf spot: *Septoria lycopersici*
- Fusarium wilt: *Fusarium oxysporum* f.sp. *lycopersici*
- Bacterial canker: *Clavibacter michiganensis*
- Bacterial wilt: *Ralstonia solanacearum*
- Tomato yellow leaf curl: *Tomato yellow leaf curl virus* (TYLCV)
- Tomato mosaic: *Tomato mosaic virus* (ToMV)

3.2 Potato

- Late blight: *Phytophthora infestans*
- Black scurf: *Rhizoctonia solani*
- Wart: *Synchytrium endobioticum*
- Common scab: *Streptomyces scabies*
- Black leg: *Pectobacterium carotovorum* sub sp. *atrosepticum*
- Ring rot: *Clavibacter michiganensis*
- Potato leaf roll virus: Potato *leaf roll virus* (PLRV)
- Potato virus X: *Potexvirus*

3.3 Capsicum and chilli

- Phytophthora blight: *Phytophthora capsici*
- Cercospora (Frogeye) leaf spo: *Passalora capsicicola*
- Anthracnose: *Colletotrichum acutatum, C. gloeosporioides*
- Fusarium wilt: *Fusarium oxysporum* f. sp. *capsici*
- Bacterial wilt: *Ralstonia solanacearum*
- Bacterial spot: *Xanthomonas euvesicatoria*
- Chili leaf curl: *Begomovirus; Chili leaf curl virus*
- Pepper veinal mottle: Pepper *veinal mottle virus* (PVMV)

3.4 Brinjal

- Phomopsis blight and fruit rot: *Phomopsis vexans*
- Cercospora leaf spot: *Cercospora melongenae*
- Little leaf: Phytoplasma

4. Different methods of diagnosis of diseases

- Visual Examinations
- Cultural and Morphological method
- Biochemical methods
- Serological methods
- Molecular methods

4.1 Visual examinations

4.1.1 Tomato

- **Buck eye rot:** Visual symptoms in buck eye rot appears as water-soaked light brown discoloured spots which increase readily showing concentric dark brown rings slightly resembling the markings on a buckeye. The lesions rapidly enlarge and in 3-4 days whole of the fruit surface turns dark brown and feels soft to touch. In warm and humid weather, white flocculent superficial growth of the fungus consisting of sporangia and sporangiophore also develops on the diseased fruits.
- **Alternaria leaf spots:** Dark brown spots with concentric rings develop on the leaves, which give target board effect, the most characteristic symptom of the disease. Lower leaves are attacked first. On fruits dark brown to black spots appear near the stalk end. Spots are small, circular scattered, dark brown spots. Older spots are surrounded by yellow halo. The affected leaves dry prematurely.
- **Fusarium wilt:** The disease was reported for the first time from Pusa (Bihar) India by Butler in 1918. Disease is important in those areas where soil temperature remain high during crop season and in polyhouses. The disease first appears in the field as yellowing of the lower leaves. Affected leaves die prematurely and the symptoms continue to appear on successively younger leaves. Browning of the walls of the vascular bundles
- **Bacterial wilt:** The petiole of the older leaves droops down and the leaves show epinasty symptoms accompanied by yellowing and stunting of whole plant. There is a typical browning of the vascular tissues of roots and stems.
- **Bacterial Spot:** The pathogen produces lesions on all above ground parts of the plant - leaves, stems, flowers and fruit. On leaf small, circular to irregular, dark lesions appear, which may be surrounded by a yellow halo. The lesions may increase in size to a diameter of 3-5 mm. Infected leaves develop a scorched appearance. When spots are numerous, foliage turns yellow and eventually dies, leading to defoliation of the lower portion of the plant.
- **Leaf curl:** The major symptoms include chlorosis of leaflets and reduction in their size accompanied by curling inwards. Significant reduction of nodes and inter nodal lengths occurs giving the plant a bushy appearance. In advanced stages of infection severe stunting and partial to complete sterility occurs. Infected plants bear few or no fruits.

4.1.2 Potato

- **Late Blight:** Most destructive disease of potato throughout the world. First recorded in the beginning of nineteenth century in the Andes Mountains of South America, it moved from this place to Europe around the year 1842 and established in Ireland. It caused severe epidemic in 1845-46, resulting in famous Irish famine Four million people were directly affected by this epidemic and many (approx. 1.5 million) among them faced death with millions migrating to other parts of the world. In India, the disease was first reported from Nilgiri Hills in between 1870 and 1880. In Himachal Pradesh, disease was first reported from Kumarsain (Shimla) district by Butler in1903. Initially small water-soaked lesions develop near the tips and margins of the leaves

which grow into large, brown to purplish black, necrotic lesions. During morning hours, whitish downy growth of the pathogen can be seen on the underside of the leaves. Light brown to dark brown lesion appears on stem and petioles which may elongate later and girdle the affected parts. Since the disease is polycyclic in nature, the entire crop in the field may be killed in one or two weeks and field give blighted appearance. The tuber show irregular, small to large areas of brown to purplish skin which extend deep into the internal tissue. The infected tuber is often invaded by secondary pathogens resulting in to soft rot of tubers.

- **Early Blight:** On older leaves dark brown oval or angular spots surrounded by chlorotic zone, which may extend much beyond the lesion due to the presence of toxin "alternaric acid" produced by the pathogen. Spots enlarge become irregular and cover entire part of the leaf lamina. Concentric ridges appear on the necrotic tissue giving them target board appearance. Dark brown lesions are produced on stem and petioles, which break at the point of infection. On the tubers, the lesions are slightly dark, sunken and round to irregular in shape, with time infected tubers show dry rot symptoms.
- **Stem canker:** Growing tips of sprouts show browning, sunken, brown necrotic spots are also observed on the sprouts. Severely affected sprouts are killed.
- **Black scurf:** Presence of black crust on tubers due to the formation of sclerotia. These are normally on the skin and not damage tuber inside. Black scurf phase is more common than stem canker in India.
- **Potato Wart:** The disease is endemic to Darjeeling area. The plant cells multiply rapidly at the infection site and produce hypertrophied tissue masses and beads like projections on the stems or stolons. Warts are normally soft, pulpy, spherical and similar in colour to tubers.
- **Bacterial Wilt and Brown Rot:** The disease damage the crop in two ways:
  (i) Premature wilting and death of the plants thereby total loss of the yield.
  (ii) Causing rot of tubers in storage and transit.
- Another indirect loss is spread of the disease through planting of healthy-looking tubers harvested from infested fields. In India, losses up to 80% have been recorded under extreme conditions. Bacterial wilt poses a serious restriction to seed/ processing potato production. The earliest symptom is slight wilting of leaves on top branch during clear sunny days. The leaves show drooping due to loss of turgidity followed by total unrecoverable wilt. In advanced stages of wilt, the basal cut end of the stem may show dull white ooze on squeezing. In tubers, two types of symptoms: vascular rot and pitted lesions on surface. Vascular rot: vascular tissues of transversely cut tuber show water-soaked brown circles. Later brown rot, bacterial mass may ooze out from eyes may carry soil glued with the ooze. Lesions on tuber surface: Lesions produced due to infection through lenticels (skin pore). Initially, water-soaked spot develops which enlarges in the form of pitted lesion.
- **Potato Leaf Roll Virus:** Potato leaf roll is one of the most prevalent viral diseases of potato in India. There is rolling of upper leaves. Infected leaves remain upright and turn pale yellow in colour. Rolled leaves are leathery, stiff and brittle. Marginal interveinal chlorosis of leaflets is visible on upper leaves

4.1.3 Pepper

- **Phytophthora blight:** Disease affect all the aerial parts-leaves, stem, branches, growing shoots, fruits.
- **On Leaves:** First symptoms appear as water-soaked bleached spots on any portion of the leaf. Under humid conditions diseased areas enlarge covering entire leaf resulting in premature leaf fall. On stem and shoots: On stem and shoots dark green water-soaked areas appears which turn dark brown to black and later give blighted appearance. Whitish mycelium is also sometimes visible on the affected portion. On fruit: small water-soaked spots appear on the fruits and the flesh below the skin become soft and usually there is a distinct line of demarcation between the invaded tissue and healthy. Whitish mould appears on the rotten fruits under humid conditions. Completely rotten fruits may fall down on the ground
- **Anthracnose:** This is a serious disease of seed crops in those areas where high moisture and moderate temperature conditions are there. Symptoms appear in two phases

1) Die-back: Necrosis of tender twigs from the tip backwards. The entire plant or branch may wither away. The twigs become straw coloured in advanced stages of the disease. Large numbers of black dots (acervuli of the fungus) are seen scattered all over the necrotic parts of the plants.

2) Anthracnose and ripe fruit rot: Appearance of small, circular, yellowish to pinkish, sunken spots on the skin of the fruits which spread in the direction of long axis. As the fruit matures, these spots become brownish to black. Severely infected fruits look straw coloured and bear numerous dots like acervuli in concentric rings. On seed: The seeds produced in such fruits are discoloured and covered with mycelial mat.

- **Cercospora leaf spot:** Scattered, circular spots with brown borders and light coloured, faded or grayish central part which resemble the frogeye, so the disease is also called as frog-eye leaf spot. Severely infected leaves may turn yellow and fall down prematurely.
- **Pepper veinal mottle virus:** Mottle virus causes mosaic mottling, distortion and filiformy of the leaves leading to bushy appearance of the infected plants.

4.1.4 Brinjal

- **Phomopsis blight:** On Leaves: Circular grey spots with light-coloured centers appear on the leaves. In late stages, the lighter portion is studded with numerous black pycnidia. Affected leaves turn yellow and fall down pre maturely. Symptoms on fruit: Pale sunken spots, which later enlarge and cover the entire fruit surface. A large number of dots like pycnidia also develop on such spots. If infection of fruits takes place through calyx, the whole fruit becomes mummified due to dry rot.
- **Little leaf:** Reduction in leaf size which have shortened petioles and leaf lamina. There is production of a greater number of branches and all this results in stunted and bushy appearance of the plants. Normally the flowers are not produced but in case these are produced, these remain green.

4.2 Cultural and Morphological Methods

In cultural method, the specimen is cultured by spreading a small portion of it on an agar medium. The media is then incubated in a warm, moist environment and examined. The fungi are identified based on colony morphology and colour and presence of septate or non-septate hyphae and fruiting. *Phytophthora nicotianae var. parasitica* (buck-eye-rot of tomato) was cultured by Chaudhary *et al* and they maintained it on CMA medium in the petriplates. Then the culture was incubated at 25 °C for 10 days till uniform fluffy growth was obtained. After ten days of mycelial growth, the density of fungal inoculum was observed. The fungal mycelium was hyaline, coenocytic with sympodially branched sporrangiophores, had swelling at the nodes and produced lemon-shaped, papillate sporangia (López *et al.*, 2001) [13]. IN case of bacteria liquid and solid (agar) growth media are commonly used for culture of the bacterium. On solid agar medium, individual bacterial colonies are usually visible after 36 to 48 hours of growth at 82.4 °F (28 °C). Two main colony types differing in morphology can be distinguished: colonies of the normal or virulent type that are white or cream-colored, irregularly-round, fluidal, and opaque; and colonies of the mutant or non-virulent type that are uniformly round, smaller, and butyrous. A semi-selective medium, called modified SMSA medium, has been developed for detection of *R. solanacearum* in water and soil samples, and in plant extracts. On this medium, typical bacterial colonies appear fluidal, irregular in shape, and white with pink centers after 2 to 5 days incubation at 82.4 ºF (28 ºC) (Patrice *et al.*, 2009)

4.3 Biochemical methods

- **Gram staining:** In this method, thin smear of the suspension on a glass slide is prepared. Cover the smear with Hacker's ammonium oxalate crystal violet for 1 minute and then wash in tap water. Flood the slide with Gram's iodine solution and allow remaining for 1 minute. Wash the slide in tap water and blotting dry. Decolourise with alcohol until only faint violet colour remains in the solvent. Wash the slide in tap water and blot dry. Gram positive bacteria stain blue (VIOLET) and gram-negative bacteria red in colour. Characterization of *Ralstonia solanacerum* causing bacterial wilt of brinjal were performed using gram staining test and results showed that all of the isolates of *R. solanacearum* did not retain violet colour i.e., the isolates retained counter stain (pink colour). Therefore, all isolates of *R. solanacearum* representing each group are gram negative and straight or curved rod shaped (Osdaghi *et al.*, 2016) [16]
- **Flagella staining:** Flagella are organs of bacterial cells responsible for motility. The principal involved in staining flagella is to get a heavy deposition of stain on them with the help of mordants like tannic acid, potassium aluminium sulphate and mercuric chloride. This makes the width of these organs more and renders them visible. After that the slide is treated with 1% methylene blue for 1 minute. The slide is washed under water, air dry and observes under oil immersion. Examine the whole smear and select a field showing maximum number of bacterial cells with flagella. In smear preparation usually motile cells tend to accumulate at margin of the smear and therefore, it is advisable to examine bacterial cells at smear margins for flagella. (Balodi *et al*., 2017) [2].
- **KOH Solubility test:** In this method, on a glass slide mix a loopful of bacteria from a week-old colony in a drop of 3% KOH aqueous solution for not more than 10 seconds. For mixing a toothpick can be used instead of loop. Raise the toothpick a few centimetres from the glass slide. The gram-negative test of *R. solanacearum* was also confirmed by Potassium hydroxide solubility test. The positive test indicated by a elastic thread or viscious thread observed when loop raised from the bacterial solution by toothpick a few centimetres from glass slides in case of all group of isolates of *R. solanacearum* indicating that all groups of *R. solanacearum* isolates are gram negative (Osdaghi *et al.*, 2016) [16].

4.4 Serology Based Methods

4.4.1 ELISA (Enzyme linked immunosorbent assay)

ELISA, first employed in the 1970s, is by far the most widely used immunodiagnostic technique because of its high throughput potential. In this method, the target epitopes (antigens) from the viruses, bacteria are made to specifically bind with antibodies conjugated to an enzyme. The detection can be visualized based on colour changes resulting from the interaction between the substrate and the immobilized enzyme. The performance of ELISA can be improved greatly with the application of specific monoclonal and recombinant antibodies which are commercially available. For plant disease detection, tissue print-ELISA and lateral flow devices that enable detection have been fabricated for on-site detection. However, the sensitivity for bacteria is relatively low (105–106 CFU/mL). (Lopez *et al*., 2001) [13]. There are

4.4.2 Generally two types of ELISA test

4.4.2.1 Direct-ELISA

Direct ELISA is a plate based immuno assay intended for the detection and quantification of an antigen from within a complex biological sample. Antigen is immobilized onto the wells of a polystyrene plate. An enzyme conjugated primary antibody specific for the target antigen is added to the wells which directly binds to the antigen. A respective enzyme substrate is added, which upon reaction with the enzyme, produces a visible colorimetric output that can be measured by a spectrophotometer. It has less sensitivity and less specificity.

4.4.2.2 Indirect-ELISA

In Indirect ELISA there is addition of another step to the direct ELISA procedure. In this method, antigen of choice is immobilized on the wells and an unlabeled primary antibody specific for it is added and binds to the antigen. Subsequently, an enzyme-conjugated secondary antibody is added which binds to the primary antibody. A colourless substrate is then introduced to the sample which reacts with the enzyme conjugate and produces a measurable product, Sensitivity is more while specificity is less.

4.4.2.3 Serologically specific electron microscopy (SSEM)

Serologically specific electron microscopy technique combines the specificity of serological properties with the morphology of virus particles visualized in electron microscope. This process involves initially selectively entrapping of virus particles on copper grids coated with specific antibodies. The antibody coated grids are washed with buffer and floated on drops of extracts from virus infected plant tissue at room temperature for 3-4h, after washing grids are stained with 1% uranyl acetate. Finally,

grids are dried and examined under electron microscope. Due to its higher specificity and rapidity, it has a wide application in detection of viruses present in low titer in plants. This method is highly useful in detecting potato virus A in potato plant (Singh *et al*., 2013) [3].

4.4.2.4 Tissue Blot Immuno Assay (TBIA)

In tissue blot immuno assay, the samples consist of preparation of infected plant tissues and can be used to detect virus antigens in plant tissues such as leaf, stem, bulb etc. The tissues are cut with razor blades and pressed on the membrane to transfer the virus particles or protein. Tomato spotted wilt virus (TSWV) was detected in tissue blots from infected leaves and stems. The presence of tomato spotted wilt virus antigen in blot of infected tissues was evidence by the development purple colour when primary antibodies were omitted from the reaction mixture, tissue blots from infected plants tissues developed purple colour. The healthy control leaf and stem blots did not develop purple colour (Singh *et al*., 2013) [3].

4.4.2.5 Immunofluorescence

Immunofluorescence method includes fluorescence microscopy-based technique for pathogen detection. For this technique, generally plant samples are fixed to microscopic slides in thin tissue sections. Immunofluorescence is a method that allows *in situ* localization of the pathogens. There are generally two types of fluorescence.

1. **Direct immunofluorescence:** It involves detection of pathogen by conjugating a fluorescent dye with specific antibody to visualize the distribution of target molecule (antigen) throughout the sample.
2. **Indirect immuno fluorescence:** In indirect immuno fluorescence antigen was allowed to react with unlabeled antibody which is then detected by specific antibody labeled with fluorescent dye. (Singh *et al*., 2013) [3].

4.5 Molecular methods

4.5.1 PCR (Polymerase chain reaction)

Based on the fidelity of DNA hybridization and replication, PCR was initially used for highly specific detection of diseases caused by bacteria and viruses. Polymerase chain reaction, PCR technology, is an efficient and cost-effective way to copy or amplify small segments of DNA or RNA. The technique is fast and highly specific. It can be used to detect trace amounts of fungal DNA from environment samples before symptoms occur. It therefore allows the implementation of early disease control methods. This technique utilizes specific oligonucleotide primers which are designed based on nucleic acid sequences that are diagnostic for the pathogen. Primers developed are based on more specific sequences such as the argk-tox gene of Pseudomonas syringae PV. phaseolicola which encodes a gene involved in phaseolotoxin biosynthesis and can be used to identify bacteria that possess this trait (Martinelli *et al*., 2015) [14]. DNA extracted from the leafhoppers that are potential vectors of phytoplasmal diseases can be PCR amplified using phytoplasmal specific primers to identify which species are true vectors. A number of techniques have been developed to improve the reliability, efficiency and cost effectiveness of PCR based techniques such as multiplex PCR kits capable of detecting more than one pathogen present in a particular plant or soil sample. These multiplex systems are combination of primers that give different size fragments for each of the species and it is further developed by using primers that fluoresce at different wavelengths for different pathogens. Kits are commercially available to amplify *Fusarium oxysporum* in tomato using primer sets FOR1-F/FOR1-R and FOR2-F/ FOR2-R (Cai, *et al*., 2014) [4].

4.5.2 RT- PCR (Reverse transcriptase-PCR)

Reverse transcriptase-PCR is a laboratory technique combining reverse transcription of RNA into DNA (in this context called complementary DNA or cDNA) and amplification of specific DNA targets using polymerase chain reaction (PCR). It is primarily used to measure the amount of a specific RNA. In this variant of PCR, firstly RNA is reversely transcribed into cDNA by random primers and reverse transcriptase (RT) enzyme and then amplified by conventional PCR method. It is generally used for the detection of RNA containing viruses like retro viruses and reported to be used for the quantification of *leaf curl virus* in tomato. RT-PCR procedures were effectively used to amplify the coat proteins gene for Potato virus Y (James, 1999) [9]

4.5.3 Nested- PCR

Nested PCR is used to increase the specificity of DNA amplification. It is a modification of conventional PCR in which two pairs of PCR primers are used to amplify a fragment. First pair of primer amplifies a fragment similar to a standard PCR. Second pair of primer (nested primers) binds inside the first PCR product fragment to allow amplification of a second PCR product which is shorter than the first one. Nested PCR has advantage over conventional PCR in having low probability of non-specific amplification. Detection of pathogen *Colletotrichum gloeosporioides* in pepper has been reported by using nested PCR. (Lau *et al*., 2017) [11]

4.5.4 Multiplex PCR

Multiplex PCR was proposed to enable simultaneous detection of different DNA or RNA by running a single reaction. It is a variant of PCR which is used for amplification of multiple targets in a single PCR experiment. In this assay, more than one target sequence can be amplified by using multiple primer pairs in a reaction mixture. Different fragments that were simultaneously amplified were then detected on the basis of their molecular sizes on the agarose gels. Generally, up to eight primer pairs can be used in a standard multiplex reaction. Multiplex PCR technique has been used for the simultaneous detection of fungal pathogens, such as *Fusarium oxysporum*, *Phytophthora nicotinae* and *Phytophthora cactorum* (Aslam *et al.*, 2017) [1]

4.5.5 LAMP (Loop Mediated Isothermal Amplification)

This technology was developed by Notomi. It is a very sensitive, easy and time efficient method. The LAMP reaction proceeds at a constant temperature using a strand displacement reaction. In this technology, *Bst* DNA polymerase and a set of four primers (two inner and two outer primers) are used, which recognize total six unique sequences on the targeted DNA. The first inner primer containing sense and antisense sequences in the DNA will hybridize the target sequence and initiate DNA synthesis. After that, the outer primer carries out the standard-displacement DNA synthesis and produces a single stranded DNA which works as a template for the second inner and outer primers producing a DNA molecule with a loop structure. The amplified product can be detected by naked eye as a white precipitate or a

yellow-green colour solution after addition of SYBR green dye to the reaction tube. The final product of the amplification will lead to a stem loop DNA with several inverted repeats of the target. The reaction process proceeds at a constant temperature using Strand displacement reaction. Amplification and detection of gene can be completed in a single step, by incubating the mixture of sample primers, DNA polymerase with strand displacement activity and substrate at constant temperature (about 65 ºC). In the target gene, the F3c, F2c and the F1c regions at the 3' side and the B1, B2 and B3 regions at the 5' side are the distinct regions. There is no need for a step to denature double stranded into a single stranded form. It provides high amplification efficiency with DNA being amplified 10^{9}-10^{10} times in 15-60 minutes. Because of its high specificity, the presence of amplified product can indicate the presence of the target gene. The high specificity and rapidness made LAMP a widely used technique for late blight disease detection in potato (Donso and Valenzuels, 2018) [5].

4.5.6 Fish (Fluorescent in-situ hybridization)

It is a type of molecular detection technique, which is applied for bacterial detection in combination with microscopy and hybridization of DNA probes and target gene from plant samples. FISH is a hybridization technique used for the detection of plant pathogens. Due to the presence of pathogen-specific ribosomal RNA (rRNA) sequences in plants, recognizing this specific information by FISH can help detect the pathogen infections in plants. This technique uses 16S or 23S rDNA oligonucleotide probes labeled with fluorescent dye in combination with fluorescence microscopy. A FISH probe recognizes the pathogens in the plant tissues/cells which are fixed in a microscopic slide and thus hybridize with target gene of pathogen in the sample. This probe-target hybridization thus can be visualized by fluorescent light. The high affinity and specificity of DNA probes provide high single-cell sensitivity in FISH, because the probe will bind to each of the ribosomes in the sample. Early and successful detection of soil borne pathogen *Sclerotium rolfsii* causing southern blight of tomatoes using whole cell hybridization has been reported by means of FISH technique (Mijri *et al*., 2009).

5. Management

5.1 Cultural control

Follow long crop rotation up to 2-3 years using crops like maize, cereals, garlic, onion, cabbage. Do not rotate with other solanaceous crops so as to avoid soil borne pathogens. Rogue out infected plants and destroy them by burning so as to avoid spore spread and other infecting material. Use of healthy planting material can take care of almost 80% of bacterial wilt problem. Movement of soil should be avoided from one site to another to reduce the risk of moving pathogens which overwinter in soil. Some pathogens can only enter the host through wounds; situations that promote plant injury should be avoided. Apply pine needle/grass mulch on the field floor to create a barrier between the host and soil borne inoculums. Weed control is important for the management of viral diseases. Weeds may be alternate / collateral hosts for many important vegetable viruses. Green manuring or bio fumigation with *Brassica* spp. may reduce the bacterial inoculum in soil as they emit volatile compound such as iso-thiocynate which act as antagonist against pathogens (Lazarovits *et al*., 2001) [12].

5.2 Physical and Mechanical control

The plants should be stake erect, foliage and fruit should be removed to a height of 15-20 cm in tomato to avoid moist and stagnant air conditions. In order to reduce dispersal of soil borne pathogens between fields, stakes and farm equipment should be decontaminated. All field equipments should be disinfect by dipping in or washing them either with 3% trisodium phosphate or calcium hypochloride (1%) solution (Somasekhara and Gowda, 2015) [15]. Soil sterilization at 50-60 ºC for about 30 min kills the all soil borne pathogens as the pathogens are exposed to high temperature. De-haulming of the crop before the aphid's cross critical level to enforce rigid control of the insect vector in potato (Osdaghi *et al*., 2016) [16]. Some diseases like bacterial wilt of tomato (55 ºC for 10-30 min), anthracnose diseases of capsicum (45 ºC for 15 min and 53 ºC for 4 min can be treated by hot water treatment by immersing infected seeds in hot water at recommended temperature and time (Bhatnagar *et al*., 2013) [3].

Use of resistant cultivars

Cultivars such as Sweet 72, Marglove, IPA-5, Rio Fuego have been found resistant against septoria leaf spot. Wild relative species *S. pimpinellifolium* have been found to be resistant to Bacterial Canker. In potato varieties like Kufri Giriraj, Kufri Jyoti, Kufri Gridhari, Kufri Himalini, Kufri Kanchan have been found to be resistant to Late blight (Joseph *et al*., 2011) [10]. Wild species such as *Solanum phureja* and *S. chacoense* which can be exploited for breeding resistant cultivars against early blight and Cv. Kufri Sindhuri provides good resistance against this disease in plains. Some potato lines *viz*., Rondo, Edina, Mira from European countries have shown considerable resistance to wart.

In pepper the source of resistance belongs to pungent peppers which can be exploited for breeding resistant var. against *Phytophthora blight* and cultivars such as Waxy Glove, Stal Cristal, Kingkun, Szechwan etc. have shown resistance (Joseph *et al*., 2011) [10]. Some Pepper varieties like Punjab Lal, Perennial and Guhati Black are resistant to mosaic viruses. In brinjal Pusa Dwarf, Annamalai vars. are resistant to phomopsis blight and cultivar such as Manjari Gota, Junagarh sel., Black round, Arka Nidhi is moderately resistant to cercospora leaf spot (Zehra *et al*., 2017) [23]

5.3 Biological control

The use of bio control agents in disease management is increasing, especially among organic growers. These products are considered safer for the environment. Bacterial antagonists such as *Pseudomonas florescens, P. glumae, P. cepacia* and *Bacillus* spp. have also been known to reduce bacterial wilt disease incidence (Raghu *et al*., 2018) [18]. Seed treatment with *Bacillus subtilis* reduces bacterial spread of bacterial canker. Examples of commercially available bio control agents include the fungi *Trichoderma viride* and *Gliocladium virens*, an *actinomycete Streptomyces griseoviridis*, and a bacterium *Bacillus subtilis*. (Nagendra, 2018) [6]. Bacteriophages have been found to be an effective bio control agent for managing bacterial spot-on tomato. Use of bio control agents like *Pseudomonas* spp., *Trichoderma* spp. also reduces infection of wilt pathogen. (Patel and Saraf, 2017) [17].

5.4 Chemical control

When all the previous methods are not effective and pathogens cause destructive loss of the crops then we should go for chemical control. Depending on the kind of pathogens

they affect, the chemicals are called fungicides, bactericides, viricides, Physical mode of action of fungicides can be classified into four categories such as Protective, After infection, Pre- symptom, Anti-sporulant (post symptom) (Somasekhara, 2015) [21].

Table 1: Fungal Disease

Disease Name & Causal Organism	Chemical Control
Late Blight Causal Organism: *Phytophthora infestans*	✓ Before the initiation of disease, apply one protective spray of mancozeb (0.25%) followed by metalaxyl + mancozeb (0.25%) at critical stage of disease appearance followed by Bordeaux mixture (4:4:50) or mancozeb (0.3%) or copper oxychloride (0.3%) and repeat at 7-10 days interval.
Cercospora leaf spot Causal Organism: *Cercospora melongenae, C. egenulae, C. solnigena*	✓ With the initiation of the disease, spray the crop with carbendazim (0.1%) or combination of mancozeb (0.25%) and carbendazim (0.05%) or copper oxychloride (0.3%) and repeat at 10 to 14 days interval.
Buckeye Rot Causal Organism: *Phytophthora nicotianae* var. *parasitica*	✓ With the onset of monsoon rains, spray the crop with metalaxyl + mancozeb (0.25%) followed by sprays of either mancozeb (0.25%) or copper oxychloride (0.3%) or Bordeaux mixture (4:4:50) and repeat at 7-10 days interval.
Alernaria leaf spots Causal Organism: *Alternaria solani*	✓ And repeat at 10-1 Spray the crop with copper oxychloride (0.3%) or mancozeb (0.25%) or Bordeaux mixture (4:4:50) 4 days interval.
Septoria leaf spot *Causal Organism: Septoria lycopersici*	✓ Seed treatment with captan or thiram (0.3%) before sowing. ✓ With the initiation of disease, spray the crop with carbendazim (0.1%) or mancozeb (0.25%) or copper oxychloride (0.3%) and repeat at 10-14 days interval.
Fusarium wilt Causal Organism: *Fusarium oxysporum* f.sp. *lycopersici*	✓ Treat the seed with carbendazim (0.2%). Drench the affected plants with carbendazim (0.1%).
Black scurf Causal Organism: *Rhizoctonia solani*	✓ The normal unsprouted tubers should be dipped in carbendazim (0.1%) or Monceren 25 SC (0.25%) for 10 minutes.
Phomopsis blight and fruit rot Causal Organism: *Phomopsis vexans*	✓ Seed treatment with carbendazim (0.2%) or thiophenate methyl (0.2%)

Table 2: Bacterial Disease

Disease Name & Causal Organism	Chemical Control
Bacterial Wilt Causal Organism: *Pseudomonas solanacearum*	✓ Soil fumigation with Formalin @ 7% before sowing (Vanitha *et al*., 2009) [22] ✓ Seed treatment with Streptocycline (150 ppm) for 90 minutes ✓ Sterilize the nursery soil with Formalin @ 1% before sowing
Bacterial Canker Causal Organism: *Corynebacterium michiganense*	✓ Give seed dip treatment with Streptocycline (150 ppm) for 90 minutes ✓ Foliar application of copper fungicides (copper oxychloride, copper hydroxide), antibiotics (streptocycline) or their mixture 0.2% + 0.01% are also effective
Bacterial Spot Causal Organism: *Xanthomonas vesicatoria*	✓ Foliar application of copper fungicides (copper oxychloride, copper hydroxide), antibiotics (streptocycline) or their mixture 0.2% + 0.01% are also effective. ✓ Seed treatment with Streptocycline (150 ppm) for 90 minutes

Table 3: Viral Disease

Disease Name & Causal Organism	Chemical Control
Leaf curl Causal Organism: Tomato leaf curl virus	✓ Spray imidacloprid 17.8 SL @ 0.5-0.6 ml/litre water at 15 days interval (as the disease is transmitted by white fly) (Zehra, 2017) [23]
Spotted wilt virus Causal Organism: Tomato spotted wilt virus	✓ Same chemical control (the virus is transmitted by thrips in persistant manner)
Mosaic Causal Organism: Brinjal Mosaic Virus	✓ Spray imidacloprid 17.8 SL @ 0.5-0.6 ml/litre water at 15 days interval (as virus is transmitted by aphids).

6. Conclusion

- Early detection of plant diseases is crucial for development of management strategies.
- Need is there for high-throughput, specific, reliable and sensitive technologies for plant disease detection.
- Most of the molecular based methods are there which met these criteria but requirement of experienced staff, specialized laboratories makes these methods time consuming and laborious.
- The recent advancement in the area of PCR based approaches further extended its versatility
- Assays like RT-PCR, nested PCR, LAMP are among the detection options that provides rapid data analysis with specificity.
- However, major challenge for the development of these kind of technologies remains their cost effectiveness and affordability.

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