

Identification of Plant Diseases: A short review

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Abstract:

As per FAO (2000) reports, 67% of population are involved in Agriculture, and alone contributing to 34% of overall GDP. Diseases in Plants causes major losses therefore it's quite evident that proper monitoring and disease detection is necessary for sustainability. According to several reports, in the period 2001-2003, Global potential loss varied to about 29% in soybean, 50% in wheat to about 80% in Cotton. This short synopsis will be acting a small referral piece of document to give an idea about the common Fungal, Viral, Bacterial diseases. This shall also be discussing about some of the conventional techniques for disease detection in plants viz.; Microarray, PCR, ELISA etc.

1. Introduction :

Diseases in plants are the major reasons of losses in Agricultural sectors. Fungal, viral, bacterial pathogens alongwith pest infestations are a major reason for the Plant diseases and damages. USA alone reports around 50,000 parasitic and non- parasitic plant diseases. Accidental introduction of plant vectors or infected plant species are believed to be the entry points of the microbial pathogens. Ornamental Plants are believed to one of the major hosts of such pathogens, as well. Crop losses due to pathogens is believed to sum up to a whooping figure of 33 billion dollars every year in USA. The study of plant pathogens and diseases, it's identification, scouring

strategies is the need of the hour. Understanding the biology. Morphology, life cycle of the commonly devouring pathogens: fungi, virus, bacteria is verily essential.

Fungi family is alone, the reason behind 85% of plant diseases [1]. They are a group of achlorophyllous, thalloid, eukaryotic, heterotrophic, spore forming organisms. They are cosmopolitan in origin and could be unicellular, as well as multicellular and can remain associated with algae as well as roots of higher plants, named as Lichens and Mycorrhiza. Their cell walls are made of Chitin, with the exception of Oomycetes. They are often broadly classified into : Oomycetes, Zygomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes, with the last three variants posing major threats to the Plants species. Who doesn't remember of the infamous Bengal Famine of 1942-43. killing a population of almost 2.1 - 3 million people approximately, for it was caused by massive crop failure, as a result of *Helminthosporium oryzi*, causing the Brown leaf spot of Rice, is a major pathogenic variant under Deuteromycetes or fungi imperfecti. Some other major pathogenic ones are *Albugo candida*, that causes the White Rust of Crucifer/Mustard or *Phytophthora infestans*, the causative agent of Late Blight of Potato, also the reason behind Irish famine (1845-47).

Viruses are smallest of the pathogens and can only be seen with an electron

microscope [2,3]. Virus discovery itself surfaced with TMV- Tobacco Mosaic Virus , which infects *Nicotiana tabacum* (Tobacco Plant), belonging to the family Solanaceae, commonly characterized by Mosaic like pattern on leaves. Viruses are obligate parasites, possesses either single/double stranded DNA/RNA, and can be either enveloped/capsulated or non-enveloped. Capsid is the protein coat of virus, made up of Capsomeres. Viruses can multiply only within an host, are non cellular and crystallisable. Viruses are transmitted to healthy plants by insects, mostly aphids and whiteflies, mites, fungi, nematodes and humans [3].

Bacteria (Eubacteria) are unicellular Prokaryotes with 70s ribosomes. Bacterial cell envelope is mainly comprised of Glycocalyx, Cell wall, Plasma membrane. They require outside agents for dispersal. Mostly by splashing of irrigation water, or by wind, and also by human contact or via tools used in agricultural purpose. Genera *Pseudomonas*, *Xanthomonas* from *Pseudomonadaceae* family, *Agrobacterium*, *Corynebacterium*, *Erwinia* are the most plant pathogenic bacterial variants [4,5].

After the onset of pathogen attack, several symptoms surface, and what immediately should come handy is the verification by disease identifying techniques. Nowadays the most widely used techniques include : ELISA (Enzyme Linked Immuno Sorbent Assay) for identification of proteins, PCR , real time PCR for identifying the Nucleic acid sequences of the pathogens [6].

2. Identification :

Molecular techniques employed these days include Immunofluorescence (IF), Flow cytometry, Fluorescence in situ

Hybridization (FISH), DNA Microarrays, and the most frequently used ones being Enzyme Linked Immuno Sorbent Assay (ELISA) and PCR , real time PCR [7]. While ELISA is associated with the identification of the microbial antigen, PCR, on the other hand, employs in identification of a specific DNA band in Gel Electrophoresis, thereby confirming the presence of microbe [8,9]. However, certain limitations are associated with the molecular techniques, with them being time-consuming and labor-intensive [10], and the requirement of an elaborate commitment, in terms of extraction, collecting, storing, proper handling of collected molecular specimen , consumable reagents, tailored distinctly for each variant of pathogen viz; sequence specific primers, thereby losing it's candidacy for a preliminary screening tool [11,12].

Rapidity, specificity, sensitivity to detection are the must needed calibres for the candidature of detection too. Various spectroscopic and imaging techniques like fluorescence imaging, multispectral or hyperspectral imaging, infrared spectroscopy, fluorescence spectroscopy, visible/multiband spectroscopy and nuclear magnetic resonance (NMR) spectroscopy have been employed [13] in the near past, for the detection of symptomatic and asymptomatic plant diseases, identification of plant stresses, nutrient deficiencies in plant. Fluorescence Spectroscopy has been employed in the detection of stress caused from Citrus Canker, caused by bacteria *Xanthomonas citri*-*X. axonopodis* pv. *Citri* [14]. There are differential protocols to differentiate Chlorosis stressed leaves from Citrus Canker afflicted ones, as well as from the healthy leaves, as well. Further, assessment in the near infra red regions (NIR) of the electromagnetic spectra, together with the visible region has shown to reflect more light on the physiological stress, related to disease conditions of plant,

as seen in the case of fire blight disease of plant. Spectral Reflectance has also been employed to identify viral infection causing cause grapevine leafroll disease, Sclerotinia rot disease in celery [15,16], Verticillium wilt in cotton canopy, with almost upto 75% efficacy. Scientists are of the opinion that, spectral reflectance in the wavelength range from 400 to 1300 nm, gave maximum insight on plant condition, than that would do by pondering over the entire Visible, IR range i.e., upto 2500 nm, with some other works further narrowing down the range from 780 to 1300 nm, again. Fluorescence imaging has also been used to monitor leaf diseases, generally equips a xenon or halogen lamp, as a UV light source for fluorescence excitation, while fluorescence at distinct wavelengths are recorded with the charge coupled device (CCD)-based camera system, with some common targets of the spectra being green (520–550 nm), blue (440 nm), red (690 nm), far red (740 nm), and near infrared (800 nm)(Lenk and Buschmann, 2006)(Lenk et al., 2007). This techniques has been effectively used to study the progress of TMV infected Tobacco plants, identification of yellow rust (*Puccinia striiformis*) in winter wheat [17,18], while the latter also employed the joint utility of another technique called Hyperspectral imaging, in association with multispectral fluorescence imaging. In Hyperspectral Imaging, the spectral reflectance of each pixel is captured for a wide range of wavelengths in the EM spectra, with the wavelengths including the visible and infrared regions of the spectra, as well. This novel imaging technique has been used to assess quality of citrus fruits,[19,20] to develop a machine vision system model, in an attempt to assess food quality, as it has been used in detection of bruises in apples [17,18]. However it's of the opinion that certain regions provide unique information about the plant- IR

region for physiological status of plant, visible region for pigmentation .

Volatile organic compounds (VOC) profiling of plants have also proved to be a novel technique to analyse the physiological condition of the plant, which is further reflected, based on the physico-chemical parameters, that affects the plant's functioning viz; humidity, temperature, light, soil condition, and fertilization, growth and developmental stage of the plant, insects, and presence of other herbs [20,21]. Some commonly isolated plant volatiles include terpenoids, fatty acids such as methyl jasmonate, phenylpropanoids, benzenoids, amino acid volatiles, N,S containing volatiles etc. [20-22]. VOCs change while a plant is infested with pathogenic attacks i.e., in diseased conditions, which can be therefore targeted for screening [22]. The two commonly based techniques for VOC profiling are Gas Chromatography based techniques and Electronic nose based techniques. Electronic nose utilizes a series of gas sensors to detect a wide array of Organic compounds, while GC-MS utilizes qualitative and quantitative analysis to assess, identify volatile metabolites released by plants in differential physiological conditions [20-22] has used Electronic nose to understand, distinguish the post-harvest fungal diseases. Extensive studies have been done to profile a wide series of pathogens e.g.; *Phytophthora infestans*, *Pythium ultimum*, *Botrytis cinerea* etc., that infect potato tubers or ones affecting onion bulbs viz.; soft rot causing *Erwinia carotovora*, or basal,neck rot causing *Botrytis allii* [17,18,20].

3. Classification of Plant Pathogens

Whittaker in 1969 described the Five Kingdom classification: Monera (or

Prokaryotes), Protista, Animalia, Plantae and Fungi. Fungi, Bacteria, Viruses comprise the majority of Plant pathogens. According to further various studies [20-22], there are about 4300 valid genera and 70,000 species of Fungi, living as saprophytes, and amidst them, about 8000 species live as plant pathogens [22]. However, they are divided into three kingdoms and eleven phyla. There are over 80 species of bacteria which cause plant diseases, but they can majorly be categorised into three major categories : 1.

wilting, as in cucumber wilt, 2. necrotic blights, rots, and leaf spots, as in, fire blight, delphinium black spot, soft rot of iris, 3. overgrowth or hyperplasia, as in crown gall or hairy root [18-22]. There are over 850 described species of plant viruses, as approved by ICTV (International Committee on Taxonomy of Viruses). Scheme of virus and viroid classification has been described [11,20,21] based on genus, family, type of nucleic acid, presence of envelopes, shapes of virions, type species etc

Fig. 1

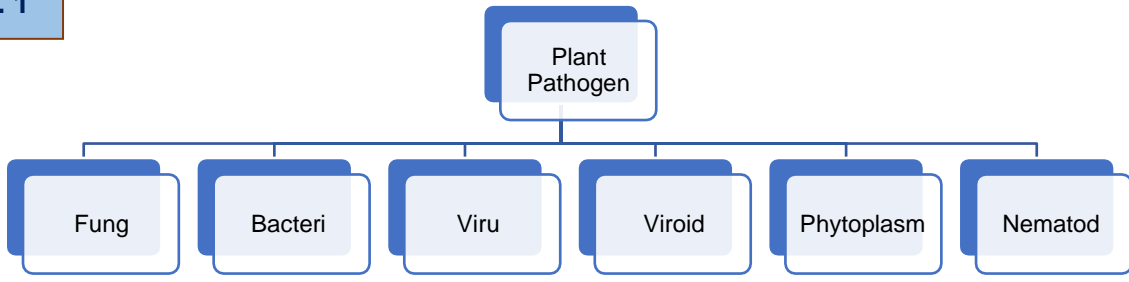


Fig. 2

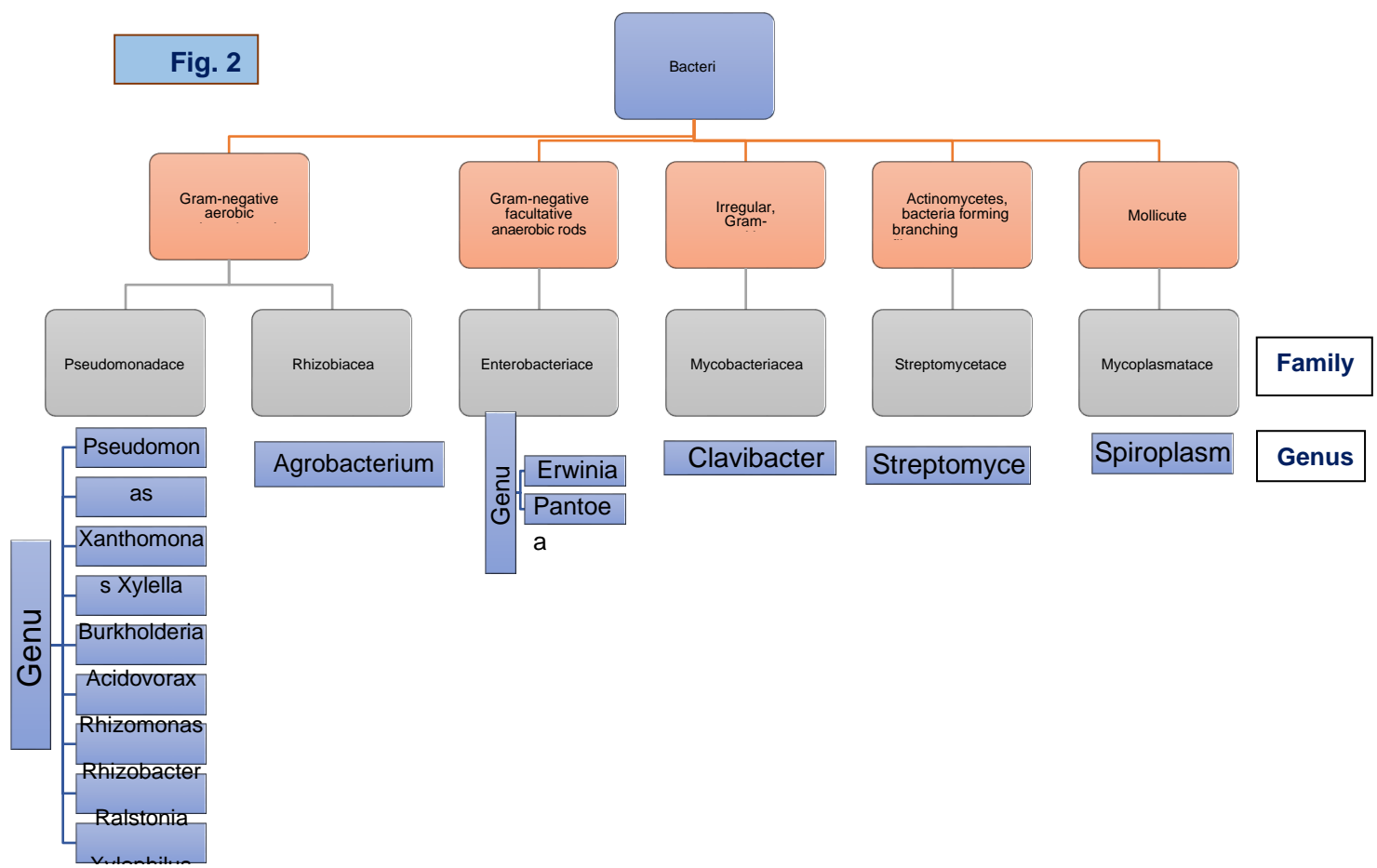


Fig. 3

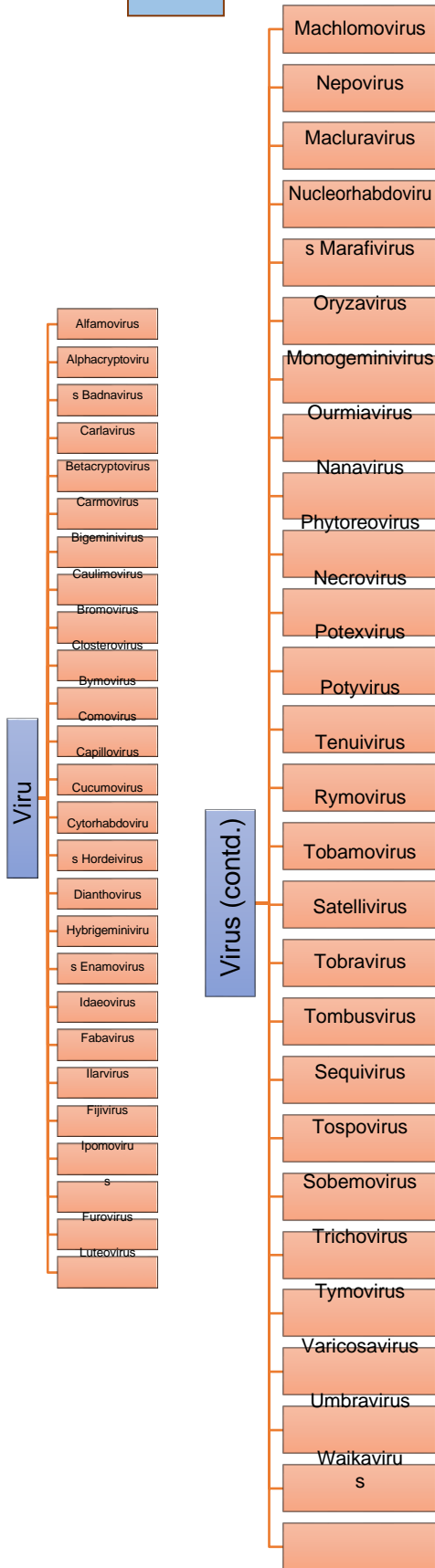


Fig. 4

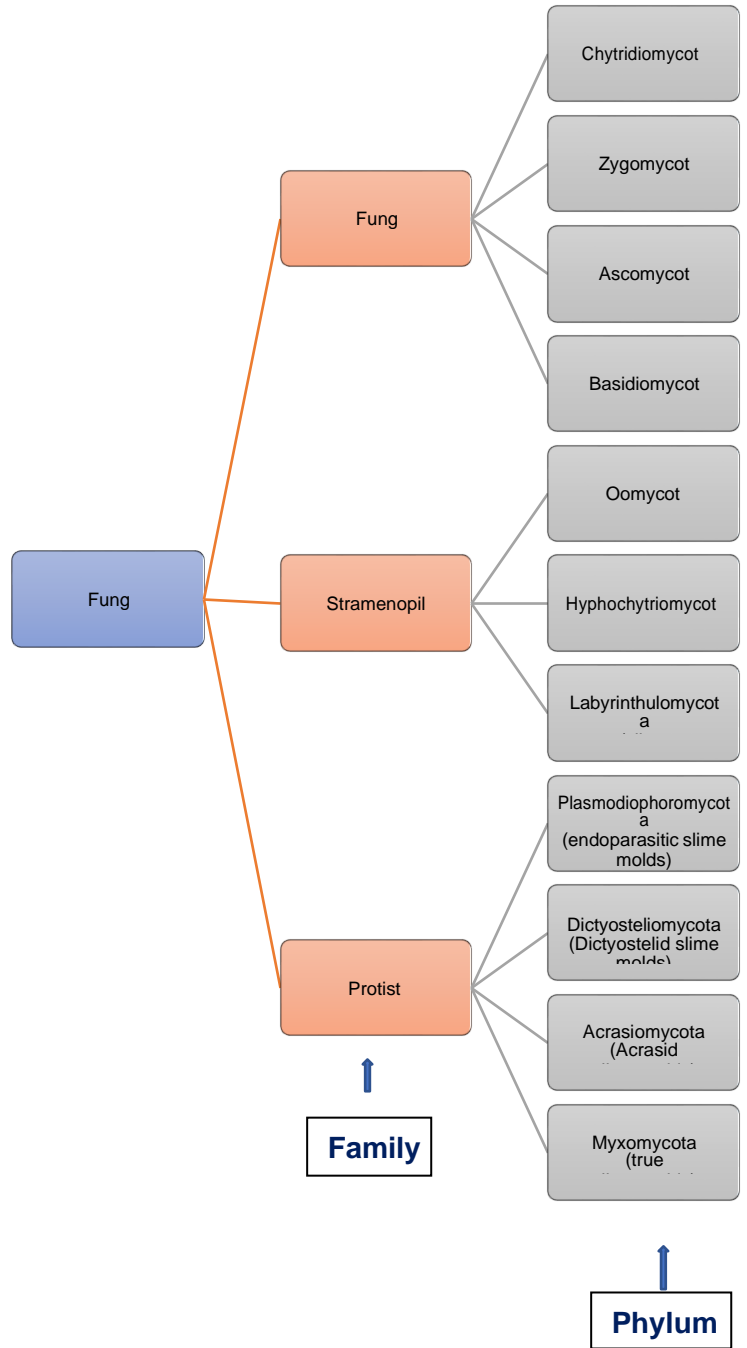
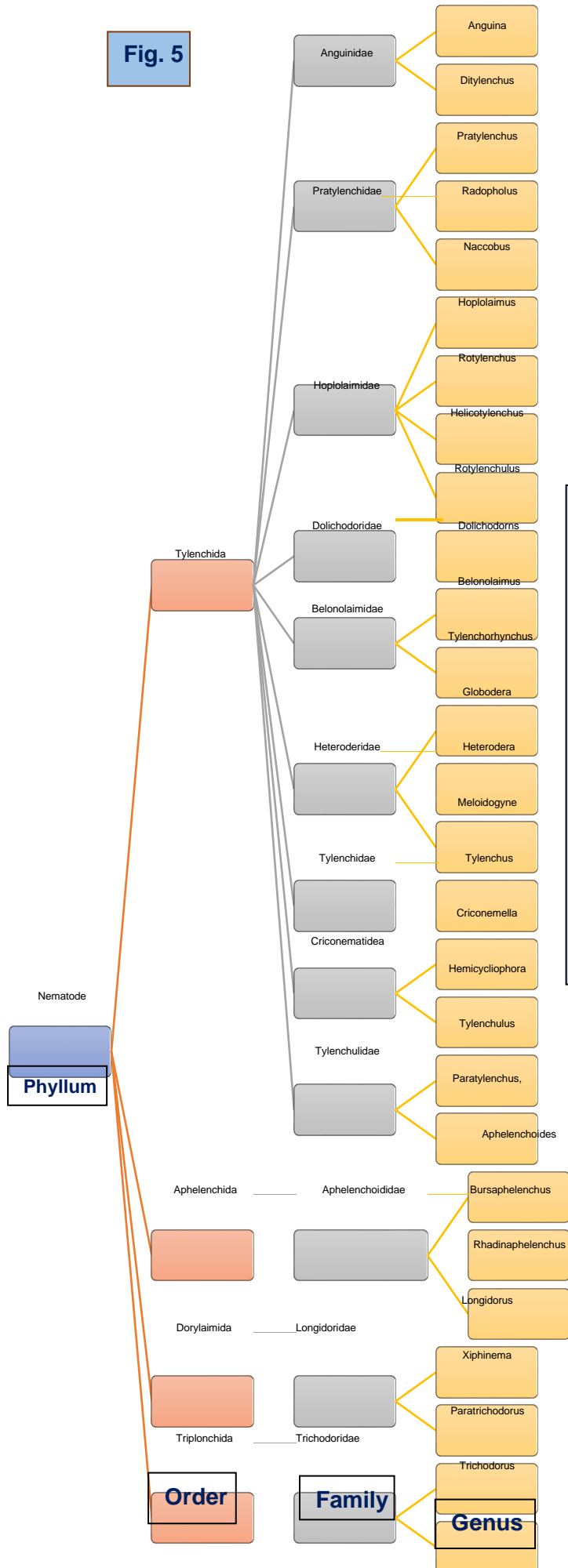


Fig. 5



Key:

- ✓ **Fig. 1** - Classification of Plant Pathogens
- ✓ **Fig. 2** – Classification of Bacteria (Brenner and Farmer III, 2015)
- ✓ **Fig. 3** – Classification of Virus (Mayo and Pringle, 1998), (Disease, 2008)
- ✓ **Fig. 4** – Classification of Fungi (Hawksworth, 1991)
- ✓ **Fig. 5** : Classification of Nematodes (Disease, 2008)

4. Some common Plant Diseases:

4.a. Fungal Diseases :

Fungi, the reason behind 85% of plant diseases, are known to cause several diseases in plants, some prominent ones being Black Knot of plum, cherry (Prunus), Blackleg of crucifers, Black Mildew. Rose Blackspot, Blights in cucumbers, watermelon, muskmelon, squash, Purple Blotch of onions, potato leaf Blotch, etc. It has also been found to be causing Cankers and Diebacks e.g., Balsam Fir Canker, Dieback of Elm. Club Root of cabbage, crucifers, finger and toe disease of brussels, sprouts, cabbage, cauliflower, mustard, radish etc. is caused mainly by *Plasmodiophora brassicae*. Damping off for the destruction of young seedling can also be caused by fungi species [20-22] living saprophytically on the upper layers of soil viz.; *Pythium debaryanum*, *P. mastophorum*, *Rhizoctonia solani*, *Aphanomyces*, *Botrytis*, *Cylindrocladium*, *Diplodia*, *Fusarium*, *Macrophomina*, *Helminthosporium*, *Sclerotium rolfsii*, *Fusarium equiseti*, *Phytophthora* etc.[22]. Downy mildews / false mildews are Oomycetes protrude through stomata, and their sporangia make white, blue, gray patches on leaves, as seen in *Bremia lactucae*. causing Downy Mildew of lettuce, or *Peronosclerospora sorghi* causing Downy Mildew of sweet corn or *Phytophthora phaseoli* causing Downy Mildew of lima bean and several other members of *Peronosclerospora sp.*, *Phytophthora sp.* or *Sclerospora sp.* causing similar manifestations in several other crops, vegetations. Several members of Ascomycetes, Deuteromycetes viz.; *Zygomycetes sp.*, *Cribropeltis sp.*, *Aureobasidium sp.*, *Microthyriella sp.*, *Mycosphaerella sp.*, *Rodotorula sp.* etc. are known to frequently cause a popular manifestation in fruits, characterised

mainly by blemishes or symptoms of rot diseases called Fruit Spots, as often seen in pome fruits, especially Apple. Apart from the Bacterial family of Rhizobiaceae, comprised of the widely popular *Agrobacterium sp.*, members of the fungal community i.e.; Basidiomycetes, Deuteromycetes have also been reported to manifest gall like or Hyperplastic growth in plants. Leaf Curls by members of genus *Taphrina*, Leaf Spots by members of *Septoria* (about 1000 sp. solely) [11-14,17], *Cercospora sp.*, *Mycosphaerella sp.*, Leaf Scorch (colour change without consuming) are popular fungal manifestations in leaves. Similarly. White Rusts by the members of genus *Albugo sp.*, Wilt diseases by members of *Fusarium sp.*, Rust by *Uredinales*, an order of Basidiomycetes, Scabs by *Elsinoë* or its anamorph *Sphaceloma*, Rots by various species of *Fomes*, *Polyporus*, and other shelving or bracket fungi, Scurfs in potatoes and sweet potatoes are also some widely studied Fungal diseases [19-21]

4.b. Viral Diseases:

There are several groups of viruses, as also shown in the Fig. 2, [20-24] known to cause around 250 widely reported disease manifestations in Plants. It would be sane to discuss briefly about some major groups. Members of The Carlavirus groups are Carnation Latent Virus (CLV), Chrysanthemum mosaic virus, Hop mosaic virus, Poplar Mosaic Virus (PopMV), Potato Virus M (PVM), Potato Virus S (PVS), mostly known to cause Chlorotic local lesions, systemic mottle, recurving of leaf margins, stunting, necrotic streaks etc. They are widely screened serologically (ELISA), or by Immune Electron Microscopy (IEM), Bioassays. Apple chlorotic leaf spot virus (ACLSV), Beet Yellow virus (BYV), Citrus tristeza virus

(CTV) etc. are members of the ssRNA containing Closterovirus group, exhibiting symptoms like dark green leaf mottle in pomes, ring mosaic for ACLSV, vein clearing and yellowing, 'pinpoint spots' by BYV, reddish purple colourations in some cases etc.. Potato leaf roll virus (PLRV) of the Luteovirus Group produces smaller tubers, induces rolling and pale yellowing of leaflets, stunting of plant growth, chlorosis. Members of the Nepovirus, Potexvirus, Potyvirus, Tobamovirus groups are also among the well studied ones, known to frequently induce diseases in the plants, alongwith minor groups like Alfaalfa Mosaic virus, Dianthovirus, Bromovirus, Caulimovirus, Comovirus, Geminivirus, Hordeivirus groups [20-22].

4.c. Bacterial Diseases :

Most common Plant pathogens come from Rhizobiaceae, Coryneform Group Mycobacteriaceae, Enterobacteriaceae, Pseudomonadaceae, Xanthomonas, Mycoplasmataceae families mostly. *Agrobacterium sp.* are widely known for causing hypertrophies or galls in the roots, stems of plants viz.; *Agrobacterium rhizogenes*. causing Hairy Root disease in pomes, *Agrobacterium tumefaciens* causing Crown Gall disease, *Agrobacterium rubi* causing Cane Gall of brambles, on blackberry, raspberries. *Clavibacter sp.* are known to cause several manifestations in plants [18-21] such as, Yellow Gum Disease on western wheat grass, Bacterial Canker of Tomato, Bacterial Wilt of bean, Bacterial Ring Rot of potato etc. *Erwinia* are gram negative bacteria from the family of Enterobacteriaceae, widely known for occurrence of Bulb Decay on onion (*cloaceae* species), Fire Blight in Rosaceae (*amylovora* species), along with *Pantoea sp.*, widely known for Bacterial Wilt of

corn, Stewart's Disease, Bulb Decay of onion, Leaf Spot of dracaena etc. Members of Gram Negative Pseudomonas group too exhibit their manifestations in the form of Bacterial Spot of cereals, grasses, corn, Bacterial Leaf Spot, Brown Spot of orchid, Bacterial Wilt of carnation etc., viz.; *Pseudomonas syringae pv. Apii* causing Bacterial Leaf Spot, Bacterial Blight of celery. Most species of Xanthomonas are plant pathogens causing Necrosis viz.; *Xanthomonas campestris* causing Black Rot of crucifers, Bacterial Blight, Wilt, Stump Rot, Horse-Radish Leaf Spot, Bacterial Leaf Spot on cabbage and radish etc. They are known to enter via Hydathodes, further advancing to vascular system, leading to vein blackening, leaf tissue browning, with early infections leading to stunting and late infections resulting in defoliation [16, 20].

5. Concluding Remarks :

Plant Diseases are the major reason of economic losses in agriculture. Proper monitoring of healthy, diseased populations and development of a reliable method for disease detection is necessary. Among the conventional methods used these days, ELISA has been the most trusted one. But, keeping an eye on the dynamics of fast changing world, mutations, there is a need to evaluate a software based solution for easy detection, classification of plant diseases. The use of AI, Machine Learning methods such as Artificial Neural Networks (ANNs), Decision Trees, K-means, k nearest neighbours, and Support Vector Machines (SVMs) have been gradually explored, and is the need of the present era, to error proof the process [18,20-22].

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