B.Sc. III Sem-V

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Theory paper - XIII : plant pathology-I

Unit-I. Fundamentals of plant pathology :-

=> scope, Importance, History and advancement of plant pathology, Classification of plant diseases on the basis of causal organism and symptoms, Field and laboratory diagnosis - Isolation of plant pathogens from infected plant parts, soil and air, pure culture technique, Koch's postulates for pathogenicity.

=> phytopathology (phyton - plant; pathos - suffering; loges - knowledge) is the study of the diseases of plants and covers the entire field of biological and scientific activity concern with this complex phenomenon. Thus, it is concern with nature, development and control of plant diseases.

Def -

Disease - It is an interaction among the host, parasite and environment.

Dictionary - Any departure from health, presenting marked symptoms, malady, illness, disorder. (serious problem)

Diseased plants are distinguished by changes in their morphological structures or physiological processes, which are brought about by unfavorable environment or by parasitic agencies.

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Plant pathology has following four objectives :-

(A) Etiology / Aetiology :-

It deals with the study of causal disease factor. This is the study of living, and nonliving and environmental causes of plant diseases.

(B) Pathogenesis —

It is the actual mechanism of disease development. It deals with process of infection and colonization of host by pathogen. This phase involves host-pathogen interaction.

(C) Epidemiology :

It is the spread of pathogen within crop areas. It is actually the interaction of crop, pathogen and environment (It deals with the dispersal of pathogens).

(D) Control :-

This concerns with the development of suitable methods for controlling the diseases. It has objective of reducing loss in yield.

Broadly, plant pathology deals with learning and understanding the nature of a disease and also with diagnosis and control of plant diseases.

Diseases of individual plants are relatively unimportant. It is a community aspect. In human, the Doctor's main target is to cure individual by treatment, whereas, plant pathologist never bothers about individual plant

In plant pathology, most methods are preventive whereas in human pathology, these are curative.

⇒ Scope and significance (Importance) of plant pathology :-

Plant pathology is concerned with all aspects of plant diseases and thus has a much wider scope.

Importance

There has been rapid rise in world population. To meet the food needs of these people and of the additional millions to come in next few years, all possible methods to increase the world food supply are being used. These include improved methods of cultivation, increased use of fertilizers, use of improved varieties and improved crop protection.

Plant diseases are important as they cause damage to crop plant and plant products. There are several historical and present examples of losses caused by diseases. Wheat, jowar, rice, corn, potato and legumes are source of staple food in different parts of world. With the advancement in human societies, need for fibre, shelter, paper, rubber, drugs etc. increased tremendously. The plants yielding these products suffer from severe losses (each year) due to pests and diseases.

Scope → Plant pathologists study the diseases caused by fungi, bacteria, viruses, mycoplasma →

parasitic higher plants, nematodes etc. They also study plant disorders caused by excess, imbalance or lack of certain physical or chemical factors such as moisture, temperature and nutrients.

Other areas of science are also important to plant pathology. Plant pathology utilizes the basic knowledge of botany, mycology, bacteriology, virology, nematology, plant anatomy, plant physiology, genetics, biochemistry, horticulture, soil science, forestry, chemistry, physics, meterology and many other branches of science.

scope

Importance - The purpose of plant pathology is to develop control over all plant diseases. The goal is to save the produce which is destroyed by diseases. Thus, the plant pathologist must acquire and use knowledge from all disciplines of science. As long as man must eat, the plant pathologist will find a job to do - to ensure enough food, fibre & and fuel for all.

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⇒ History of plant pathology:-

A Greek philosopher, Theophrastus (370 BC.) (Father of Botany) was the first botanist to study the diseases of plants. He studied the diseases of trees, cereals and legumes. He was not aware that diseases are caused by microorganisms. No definite opinion about cause of disease was made for nearly 2000 years after the time of theophrastus.

A Dutch scientist A. V. Leeuwenhoek (1665) invented the microscope and discovered the bacteria for the first time. Micheli (1729) made extensive study of fungi and their reproductive structure. He proved that, the fungi reproduce by their spores. Tillet (1755) published an important paper on bunt of wheat. He tested the effect of dusting (Smut ball smut.) seeds with the black mass present in diseased kernels. He also observed that, seed treatment with some chemical prevents the disease. He believed that the bunt disease was caused by some poison present in the Black powder.

A French Botanist Prevost (1807) proved that the micro-organisms. In case of bunt of wheat, he studied germination of spores by mixing with healthy seeds. The healthy seeds were found to be diseased. Thus, the credit for discovering life cycle of bunt fungus goes to him. Prevost also demonstrated the control of smut by seed treatment with $CuSO_4$. Unfortunately his work was neglected for about 40 years.

In 1840, Tulsane brothers of France made illustrated drawings of smuts, rusts and Ascomycetes. They also confirmed the findings of Prevost.

Irish famine - During 1830-1845, in England, Ireland and continental Europe, late blight of potato was severely spread. Potato was staple food of Irish people. The potato crop was severely destroyed by the disease. This was a very tragic event in the history of Ireland. This event caused a famine called 'Irish famine'. Over one million people died by starvation and 1½ million people migrated to other countries. No definite opinion about the causal organism of late blight of potato was made. Though, many committies and commissions were formed to submit report about the disease.

The detailed information about late blight of potato was given by a German scientist Anton de Bary (1888). He found that causal organism of the disease is phytophthora infestans which is a fungi. He also studied rusts, smuts, downy mildews and sorts. He gave the heteroecious nature of wheat rust fungus Puccinia graminis tritici and proved that it completes its life cycle on two hosts i.e. wheat and barberry. He also suggested role of enzymes in tissue disintegration. 'De Bary' initiated the work in physiological plant pathology.

Brefeld of Germany (1875) was first to develope techniques of growing microorganisms in pure culture

Discovery of Bordeaux mixture:-

The downy mildew of grapes caused by Plasmopara viticola was first reported in Europe in 1878. The disease spread rapidly. Prof. Millardet studied the disease very thoroughly. In 1882, he noticed that, to avoid the grapes from being stolen, the farmers treated the vines on roadsides by a mixture of $CuSO_4$ and lime. It was interesting to note that, the treated vines were healthy whereas untreated vines were defoliated. This fact suggested to Millardet a means of curing the disease. He thoroughly tested the efficacy of $CuSO_4$ and lime in controlling the downy mildew of grapes. In May 1885, Millardet gave details of spraying with $CuSO_4$ & lime. He formulated a mixture known as Bordeaux mixture. This mixture was continued as a best and successful fungicide for many other diseases.

Bacteria as Pathogens-

Burrill (1878) was first to describe a bacterial disease in plants. He showed that the fireblight of apple and pears is caused by a bacterium Erwinia amylovora. Smith (1890) used the best available methods in the study of bacterial diseases of plant & mentioned many other plant diseases caused by bacteria.

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Viruses as phatogens –

A German scientist Adolf mayer (1886) worked on a serious disease of tobacco (tobacco mosaic) showed that, the juice from infected plants, when applied to healthy plants, could produce the disease.

In 1892, a Russian scientist Iwanowsky showed that, the causal of tobacco mosaic could pass thsaugh those filters that retained the bacterial cells and he suggested that, viruses are smaller than bacteria.

Beijerinck (1898) suggested that, the causal organism of tobacco mosaic is a living fluid which he called viruses.

An Important discovery about viruses was published in 1935 by Stanley. He showed that the causal agent of tobacco mosaic can be crystallized and suggested that, viruses are not living because no living form can be crystallized.

In 1936, Bawden and his co-workers in England, found that, crystalline powder of viruses contain nucleic acids and protein.

In the same year, electron microscope was invented and it was possible to see viruses particles in infected cells. It was also discovered that, the nucleic acid is actual infecting agent.

⇒ Mycoplasma as pathogen :-

In 1898, Nocard and Roux discovered

some other agent of diseases in animals which was similar to viruses in size, but which could be cultured on artificial media. This was known as mycoplasma. (Mycoplasma mycoides, the causal agent of Pneumonia had been cultured by that time)

In 1967, Japanese scientist discovered that, mycoplasmas are responsible for most yellow diseases of plants. Since then, more than 15 diseases, which were supposed to be viral, in nature, were found to be related with mycoplasma.

⟹ History of plant pathology in India:-

In India, the Science of plant pathology was developed with the begining of universities of Culcutta, Madras and Bombay in 1857. More emphasis was given on study of fungi rather than the diseases caused by them. plant pathology as a subject was started in 1930 at University of Allahabad, Lucknow and Madras. Before 1850, the Indian fungi were sent to England for identification. Later on, Cunningham & Berkeley started identification in India itself. K. R. Kirtikar was the first Indian mycologist, who collected and identified fungi in India. To promote the study of fungi, British Govt. established the 'Imperial Agricultural Reserch Institute' at Pusa (Bihar) in first decade of 20th century. After a serious earthquake in Bihar, It was shifted to Delhi in 1934. Now, this institute →

It is known as "Indian Agricultural Research Institute (IARI)". The first Imperial mycologist in this Institute was E. J. Butler.

E. J. Butler :-

Butler worked in Imperial (IARI) for about 20 years. He initiated extensive study of Indian fungi and the diseases caused by them. He is considered as 'father of modern plant pathology' in India. He gave detailed account of many diseases of rice, sugarcane and rust of cereals. In addition to this work, he also trained a team of young Indian pathologists, who took over his work after his departure to England. In 1918, he wrote a classic book named "Fungi and diseases in plants". This book is still used. Butler left India in 1920 and joined as a first director of "Imperial mycological Institute" in England. The present name of that institute is "common wealth mycological Institute (CMI)

T. F. Dastur :-

Dastur was a colleague of Butler, he was the first Indian pathologist, who made detailed study of fungi and plant diseases. He worked on the genus 'phytophtho-ra' which causes disease to potato & castes.

B. B. Mundkur -

Mundkur worked on disease control

of cotton wilt by developing resistant varieties. It resulted in reduction of losses from disease in Maharashtra to a greater extent. He also identified and classified a large number of smut fungi. He established the Indian phytopathological society in 1948 and also started a research journal 'Indian phytopathology'. He wrote a text book entitled, "Fungi and plant diseases."

Dr. K.C. Mehta :-

Dr. Mehta was Prof. at Agra college. He made an outstanding contribution by discovering disease cycle of wheat rust in India and found that, its life cycle is completed on two hosts i.e. wheat growing in plain area and barberry growing in hilly region.

J.C. Luthra and Associates :-

They were from Punjab. They developed solar heat treatment of wheat seeds to control loose smut. At that time, no fungicide was discovered and thus solar heat treatment was the only protective measure of the crop. They soaked the wheat seeds in water for half an hour and spread on floor in the open sunlight for 6 hours (10 am to 4 pm).

R.N. Tandon :-

Tandon was professor at University of Allahabad. He trained many

Mycologist who contributed much to the knowledge of physiology of fungi causing fruit rot diseases.

S.N. Dasgupta & T.N. Sadasivan :-

S.N. Dasgupta in Lucknow and T.N. Sadasivan in Madras worked on biochemistry of host-parasite relationship

G. Rangaswami -

worked on bacterial plant pathogens in India. He wrote a book "Diseases of crop plant in India".

⇒ M.J. Thirumalachar -

Thirumalachar worked at Hindustan Antibiotics limited, at pimpsi, pune. He successfully introduced use of antibiotics in plant diseases control. Auseofungin and streptocyclin are the antibiotics developed by him.

S.P. Raychaudhari :-

Raychaudhari worked in IARI. He studied mycoplasma disease of plants.

Teaching of Plant Pathology in India :-

Teaching of plant pathology is started in Madras University for P.G. later on Allahabad & Lucknow University started P.G. courses in plant pathology.

With the Establishment of Agricultural University in 1916 teaching for pathology, bacteriology, mycology, virology & nematology has become important part of graduate & P.G. Programme of learning, teaching & research.

CSIR consial of scientific & industrial Research.

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Research Institutes in Plant pathology :-

IARI - Indian Agricultural Research Institute, New Delhi.

ICAR - Indian Council of Agricultural Research, New Delhi.

→ Central potato Research Institute, Simla (HP)
→ Central Rice Research Institute, Cuttack (Orissa)
→ Central Tobacco Research Institute Rajahmundry
→ Sugarcane Research Institute, Coimbatore (T.N.)
→ National Research center for Pomegranate, Solapur

Research Publications in plant pathology

1. Indian Phytopathology
2. Indian Journal of Mycology & plant pathology
3. Indian Journal of Agricultural ~~Research~~ Sciences.
4. Current science
5. Current Agriculture
6. Journal of Biological control
7. Agricultural Research
8. Review of plant pathology

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Classification of plant diseases on the basis of causal organisms -

On the basis of causal organisms plant diseases are classified into two categories.

(a) Non-infectious / Non-parasitic diseases
(b) Infectious or Parasitic diseases.

(a) Non infectious or Non parasitic diseases :-

These are the diseases in which no living ~~object~~ organism is associated. Therefore, they are not transmitted from the diseased plant to the healthy plant called as non-infectious disease since no parasite is associated with these diseases, they are also called non parasitic diseases.

These diseases occur due to following reasons.

(1) Adverse soil conditions, - such as unbalanced soil moisture, poor soil structure, poor O2 supply, nutritional imbalance or deficiency etc.
(2) Adverse climatic conditions, - such as unfavourable atmospheric temperature, light deficiency, excess of humidity, rain, wind, storm, snow fall etc.
(3) Defective Agricultural Practices such as excess use of chemicals like fertilizers, pesticides fungicides, ~~etc.~~ weedicides etc.
(4) Industrial & other chemical pollutants of the atmosphere

Temperature is the most important cause of injury to plants. Excessive heat kills plant tissues, especially of non succulent plant parts. Freezing (effect of frost in

① Necrosis of mango fruit - Boron deficiency.
② Black heart of potato - Unfavourable O_2 content
③ Khaira of paddy - Zn deficiency

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sub-tropical and temperate areas) brings about death of tissues through formation of ice-crystals with in the cells and in the intercellular spaces resulting in injury to the plasma membrane (young succulent parts have high water content and are susceptible to damage by frost).

Inadequate light retards development of chlorophyll, while, excessive bright light destroys chlorophyll. High humidity is favorable for the sporulation of pathogen and hence indirectly affects the disease development. But the dry conditions cause harm to tissues.

There is chemical injury to crop due to faulty application of fungicides, pesticide and weedicides.

The air in industrial areas or near brick kilns may contain impurities like sulphur dioxides, hydrogen sulphides, chlorine etc. These substances cause ~~many~~ many disease to the plants

The fog damage is also serious to crops.

Other atmospheric impurities are ethylene, hydrogen fluoride, nitrogen dioxide and aldehydes.

Unfavorable soil moisture directly causes injury to plant roots. Low moisture causes physiological wilting, while high moisture result in reduced O₂ supply, accumulation of CO_2 & other toxic metabolites produced

by anaerobic bacteria accumulates affecting the plant. Soil structure affects water holding capacity and aeration. This can retard growth of roots if soil is hard and compact. This results in stunted growth of plant. Imbalance of mineral elements in soil is also responsible for non-infectious diseases.

Some common examples of non infectious diseases

① Necrosis of mango fruit - Boron deficiency
② Black heart of potato - Unfavourable O2 content
③ Khaira of paddy - Zn deficiency.

(b) Infectious or Parasitic Diseases :-

The diseases caused by living organisms or viruses are called as infectious or parasitic diseases. Association of some pathogen is essential with such diseases. These diseases are always infectious and are transmitted from diseased plant to healthy plants in field. These diseases are classified on the basis of causal organism, such as fungi, bacteria, mycoplasma, nematodes or by viruses).

① Fungal diseases :-

The diseases caused by fungi are called as fungal diseases. The fungi are chlorophyll lacking thallophytes. They contain a network of filaments called mycellium. Asexual reproduction takes place by spores.

Sexual reproduction takes place by fusion of gametes resulting in formation of sexual spores. The pathogenic fungi enter into plant system by direct penetration, through natural openings (stomata, lenticels etc) through wounds, buds, root hairs etc.

Some examples of fungal diseases of plant are rust of jawar, rust of bajara, whip smut of sugarcane etc.

ii) Bacterial Diseases -

The disease caused by bacteria are called bacterial diseases. Bacteria are unicellular prokaryotic organisms. They lack true nucleus. plant pathogenic bacteria are mostly rod shaped non motile, or motile. Their multiplication take place by binary fission. Some bacteria produce endospores under unfavorable conditions. Sexual reproduction takes place by transformation, conjugation and transduction.

Bacteria are less effective than fungi as plant pathogens. They cannot directly penetrate into the host & need some natural opening or wound for penetration. The bacterial diseases spread through the agency of air, water and insects

e.g. Citrus canker, angular leaf spot of cotton, bacterial blight of pomegranate etc.

(iii) Diseases of Mycoplasma :-

Mycoplasma are very small, unicellular non-motile prokaryotes. They lack cell wall and have a triple-layered plasma membrane.

They are resistant to penicillin and inhabited by tetracyclines. They are transmitted through leaf hoppers, aphids, thrips, mites etc.
e.g. little leaf of brinjal, grassy shoot of sugarcane etc.

(iv) Diseases of Nematodes :-
The nematodes are natural fauna of soil and water. They belong to Animal kingdom. Many of them are pathogenic to plants. They secrete enzymes and toxins which react with host plant and cause stunted growth, chlorosis, root knots and some other symptoms. Root knot is the widely observed disease caused by the nematodes.
e.g. Root knot of tomato, brinjal, pomegranate etc.

(v) Viral Diseases :-
Disease caused by virus called viral disease. Viruses are very simple, Ultramicroscopic, obligate parasite. They are considered to be on border line of living and nonliving. Viruses do not have enzyme systems like living organism. They can multiply only within living cells. Many viruses are pathogenic to plants and cause disease. Plant viruses are transmitted through contact or insects, some viruses are transmitted by nematodes, mites, leaf hoppers, aphids etc.
e.g. Yellow vein mosaic of Bhendi; leaf curl of tomato; chilli, Tobacco mosaic etc.

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☆ Classification of Plant Diseases on the basis of symptoms :-

'Evidence of disease shown by the host organism is called symptom.'

or

'Expressions of diseased condition is called symptom.'

On the basis of symptoms, plant diseases are classified as follows.

① Mildews - These are the diseases in which the pathogen grows superficially on the host surface. The growth may be white, grey, brown or purple in colour. The mildews are of two types, as follows.

ⓐ Downy mildews:

In these diseases, the growth of pathogen is in the form of a tangled, cottony layer. This growth consists of sporangiophores & sporangia of the pathogen.

e.g. Downy mildew of grapes, Downy mildew of bajara etc.

ⓑ Powdery mildews:-

In these diseases the growth of pathogen is white and powdery. The growth consists of conidiophores and conidia of the pathogen.

e.g. powdery mildew of black gram, powdery mildew of peas etc.

② Rusts:-

These are the diseases with rusty symptoms. The rust appears in the form of

small pustules, which burst open and spores are liberated. The postules may be brown, yellow or black in colour.

e.g. Black/stem rust of wheat, rust of jawar etc.

③ White rust / blisters :-

These diseases occurs mostly on plants of cruciferae (Brassicaceae) family. These appears numerous white coloured, blister-like postules, which burst open & sporangia are liberated.

e.g. White rust of mustard, white rust of Achyranthus etc.

④ Smuts -

The word 'smut' means a sooty or charcoal like powdery mass. In smut disease the affected plant shows a black powdery mass. Smut is developed mostly in floral organs, particularly ovaries.

e.g. Whip smut of sugarcane, loose smut of wheat etc.

⑤ Blight :-

The term 'blight' means burnt appearance. In these diseases, there is sudden death of plant parts such as leaves or fruits. The dead parts turns brown or black & soon disintegrates.

e.g. early blight of tomato, late blight of potato, bacterial blight of pomegranate etc.

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(6) Damping off :-

In these diseases, stem is attacked near ground level. The affected position become constricted and weak. Thus, it becomes incapable of bearing the load of upper position. As a result, the seedling get collapsed and dies.

eg. Damping off of mustard, Damping off of brinjal etc.

(7) Wilt -

In wilt diseases, the entire plant dries. The drying effect is seen first in some of the leaves, later, the entire plant gradually or suddenly dries up. The wilting is due to plugging of xylem vessels by the pathogen. The wilting due to a disease (pathogen) is different than physiological wilting, in which the plant recovers as soon as there is supply of water

eg. Wilt of tur, wilt of gram etc.

(8) Spot :-

In these diseases, cells are killed in definate areas. The dead cells turn brown & these areas appear as brown spots. The spots are more common on leaves. However, they do also occur on stem and fruits. The spots on fruits may be superficial or deep.

e.g. leaf spot (tikka) of ground nut, leaf spot of jowar etc.

(9) Cankers -

In these diseases, necrotic spots

are developed on stem, leaves and fruits. The surface of the spot is rough and corky. Canker symptoms are most common in perennial plants

eg. Citrus canker, Tomato stem canker

(10) Rot :-

In rot diseases, the infected tissues dies decomposes rapidly & turns brown. This condition is brought about by those fungi which are able to dissolve cell wall of host cells. Rot is caused to plant parts like root, stem, fleshy leaves, flowers fruits etc.

eg. soft rot of tomato, fruit rot of pomegranate etc.

(11) Growth abnormalities :-

(a) Overgrowth :-

In some diseases, there is abnormal increase in size of the affected plant part. The overgrowth may be due to following processes

i) Hypertrophy - It is abnormal increase in size of the plant part due to increase in size of cells.

ii) Hyperplasia :- It is increase in size of of the plant part due to increase in number of cells

The overgrowth may be galls, cusls etc.

(i) Galls - These are globose or irregular-shaped outgrowths developed on the infected parts. The galls may be fleshy or woody.

eg. Root knot of tomato, pomegranate.

(ii) Cust :- This is curling of leaves due to localized growth. It results in premature dropping of leaves.

(b) - Dwarfing :-

There is inhibition of growth resulting in dwarfing. Entire plant or same plant part is dwarfed e.g. stunt of maize, little leaf of brinjal etc.

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Plant Disease Diagnosis :-

Since the plant pathogens are microscopic, a student of plant pathology should know how to use and care the microscope. He should have a thorough knowledge of fundamental microbiological procedures such as handling the microorganism, their isolation, making pure cultures & studying their physiology & biochemistry. The important procedures used/followed in studying plant diseases are as follows.

Field observations/Field Diagnosis -

The field with diseased crops should be visited frequently. During field trips, one should carry with him hand lens, paper bags, containers for carrying specimens; a knife, a spatula, forceps, scissors, diary/note book and conventional tool to dig out plants, soil samples are to be taken, sterile containers, soil moisture & pH kits should be carried. If the organism is to be brought into culture on a artificial media in field, as if laboratory is far away from the field, equipments such as a spirit lamp, match box, alcohol, culture media in test tube (slants), inoculating needles, cotton, labels etc should be carried.

In the field observations should be made on some aspects of disease, such as whether it occurs on young as old plants, on young leaves as old leaves and other parts on the same plant, on the parts near ground level as away from the ground. The distribution pattern of the disease

should also be noted. An approximate estimate of damage should also be made by taking random counts. The occurence of the disease in neighbouring fields should be examined. The past history of the field, including crop variety, occurrence of the disease, previous crops, treatments given to the crop, rainfall etc. should be recorded in the field note book.

The diseased plant / plant parts should be examined carefully in field. (and a description of the disease should be recorded). In case of leaf spots, their appearance under lens, their shape, color, margin, whether found on both (upper & lower) surfaces, should be noted. Some samples of diseased plant parts should be collected & placed in paper bags. To determine the name of the host, samples of stem, leaves, flowers and fruits should collected.

★ Laboratory observations / lab. Diagnosis –

(1) Identification of pathogenic organism :-

As soon as the specimens are brought to the laboratory, they should be spread on blotters and pressed. the blotters should be ~~spread~~ changed at frequent intervals till the specimens dry. Preliminary observations are made by scrapping infected tissue and mounting in water & observed under microscope. Free hand transverse sections of infected tissue may also be taken

& mounted on slide and examined. Permanent slides may also be made.

The temporary mounts and permanent slides may be examined under microscope. Sometimes the specimens may have to be incubated in a moist chamber for 2-3 days during which the pathogen sporulates and morphological features of the pathogen can be noted.

One should know the procedure for measuring the microorganisms. The length & width of the microorganisms are measured by the general procedures. In case of fungal pathogens, various features such as thickness of hyphae, length of sporangiophore, no. of septa in conidiophores and conidia etc are recorded

In laboratory techniques to study plant pathogens include - culturing pathogenic organisms, making their pure culture and appling Koch's postulates.

(2) Culturing the Pathogenic organisms-

In order to learn about the cultural characters and physiology, the pathogenic organism should be cultured in vitro

(a) Preparation of culture medium :-

microorganisms are cultured in the laboratory on different media which contains a variety of substances. Most microorganisms require about 1% of carbon source in

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the form of sugars, less than 0.5% of nitrogen source in the form of salt or yeast extract and small quantities of phosphate, sulphur, potassium, magnesium and traces of Ca, Fe, Zn, Mn & Mo. These nutrients are supplied to the microorganisms through the culture media. The media are classified into non-synthetic (natural) & synthetic media. Non-synthetic media are natural with unknown molecular structure (cooked vegetable agar, corn meal agar etc). The synthetic media consist of chemicals of known molecular structures (e.g. nutrient agar). The media may be liquid or solid. In order to solidify the liquid media 2% agar is added. The procedure used for preparing lab. media is to dissolve the known quantities of chemicals in measured quantity of water, adjust the pH to the required level by adding 0.1N HCl or 0.1N NaOH, pour in test tubes or flasks & plug with cotton. For preparing solid medium 2% agar is dissolved in the liquid medium by slow heating with constant stirring.

(b) Sterilization:-

It is the process of killing all type of living organisms in a given substance. Sterilization of culture media is accomplished by exposure to moist heat under pressure in an autoclave. The inoculating needle, scalpel, forcep and other metallic instruments are sterilized by

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heating over a flame for a few seconds. Glassware such as petriplates, conical flask pipettes are sterilized at 180°c for about 1 hrs by keeping in hot air oven.

(c) Isolation of plant pathogenic organisms:-

The pathogenic organism is isolated by agar plate method. The infected host tissue is cut into small pieces & transferred to sterile petridishes containing 0.1% $HgCl_2$ for surface sterilization. After half minute, these pieces are transferred to petridish containing sterile distilled water and washed thoroughly to free them from the chemicals. These surface sterilized pieces are then transferred to suitable agar media in petridishes under aseptic conditions. The petridishes are incubated at suitable temperature (20 to 27°c) and examined daily for the growth of pathogen (fungal hyphae or bacterial colonies) developing from the tissue are transferred into agar slants and allow to grow.

The plant pathogenic organism present in soil may be isolated by dilution plate method. Soil samples are added in water and different dilutions are obtained. One ml of each dilution is added to separate petridishes. To these plates suitable melted and cooled agar medium is added, mixed throughly and allowed to set. The petridishes are inoculated at desired temperature

→ The fungi which are obligate parasites, are brought into culture on healthy plants and grown under controlled conditions.

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and observed daily for growth of the microorganisms.

(d) → Purification and maintenance of the culture:

The isolated pathogens are transferred to agar slants incubated at required temperature and allowed to grow. These are maintained as pure cultures on suitable media by periodic sub culturing.

(3) Koch's postulates - (कोच के अभिगृहीत)

Only the isolation of an organism from a diseased tissue does not prove that it is pathogenic. The isolated organism must be examined for its pathogenicity. In case of animal pathogens, in 1882, Koch formulated certain procedures to be followed, which are also adopted for plant pathogens. These procedures are in the form of 4 postulates, known as Koch's postulates. The postulates are as follows:-

① The micro-organism must always be associated with the disease.

② The microorganism must be isolated and brought into pure culture.

③ When host is inoculated with the isolated microorganism, the disease should appear with characteristic symptoms.

④ The microorganism must be reisolated from the experimental host and must be identical with the original organism.

To study the pathogenicity, the leaf stem or other parts are inoculated with

pure culture of the test organism. The host tissues are washed & surface sterilized with 0.1% $HgCl_2$ soln. Then washed thoroughly with sterilized water. The test organism is applied to sterile surface of host plant by transferring the culture with sterile forcep or spraying the suspension with the help of hand sprayer. For bacterial pathogens, host tissue should be wounded.

After inoculation, the plants are transferred to humid chambers & incubated for 24-48 hrs. observations are then made frequently on appearance of disease symptoms.