The Neotia University

ज्ञानम् आत्म प्रदीपाय

Fundamentals of Plant Pathology
Practical Manual
Course No-CC-AGP213
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Introduction:

The subject Plant Pathology under the discipline Agriculture is an important branch. This branch is deals with the detection and diagnosis of agriculturally important diseases, symptoms, their causal agents, favourable environment and management aspects. Being an applied science and detailed study of plant diseases, laboratory work under this subject is indispensable.

Keeping this view, ICAR has framed a syllabus for practical classes of each courses of Plant Pathology for undergraduate students. Therefore, we have tried to prepare a laboratory manual for the course of “Fundamentals of Plant Pathology” for benefits of the students. In this manual, we have tried to arrange topics and contents in such a manner that students will get an easy understanding of the principles and methods of the experiment in advance. The content of this manual is framed according to the syllabus of the course- “Fundamentals of Plant Pathology” as mentioned in ICAR Fifth Dean Committee. In this manual, the syllabus for practical of this course has divided into fifteen lessons.

Content

Name _

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Section _

Year _

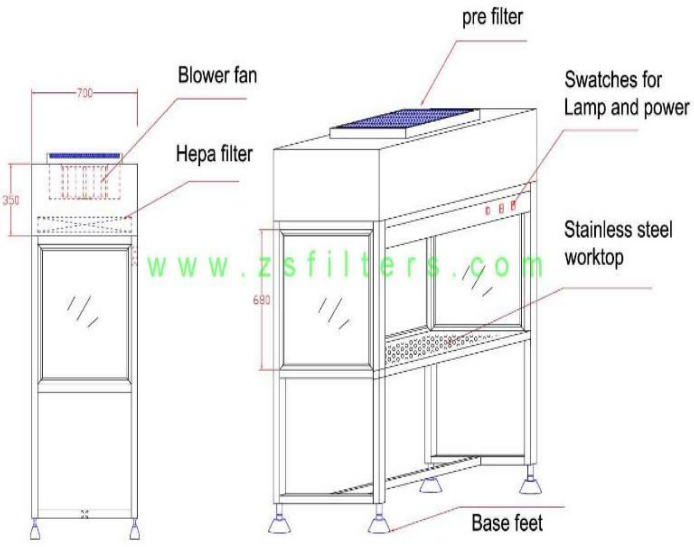
Studies on the utility of different instruments commonly used in Plant Pathology Laboratory

Objective: To familiarize the students with general plant pathological equipment like compound microscope, autoclave, laminar air flow, incubators and hot air oven

1. B.O.D. INCUBATOR

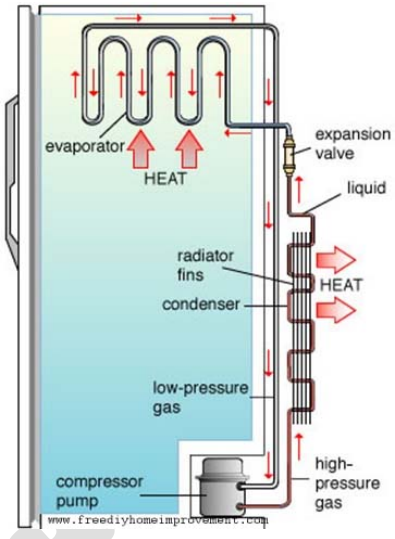
The biologically oxygen demand (BOD) incubator is a chamber in which temperature could be maintained below and above room temperature (5-50 ° C, with an accuracy of ± 1° C) **for growth and multiplication of various microorganisms**. Its body is constructed of double insulated walls. It has both heating as well as cooling devices (Compressor) that are controlled by thermostat and relay systems. Uniformity of temperature at every point of inner chamber is maintained by a blower and the whole system operates electrically.

2. LAMINAR AIR FLOW CABINET



Laminar air flow cabinet is aspecial type of cabinet with a covered working table in front. Mild air free from any particle (0.3 micron or large) blow over the working table outward which **provides a clean and germ free space (Aseptic sterilized) where we can handle any pure micro-organism easily with little chance of contamination**. A unique filter named High efficiency particulate air (HEPA) filter is used to blow aseptic air. Outside air is sucked through pre-filter into the cabinet by a motor which again blows that air through the HEPA filter over the working table. Working area is illuminated by fluorescent light. A **UV light is also fixed over the working table which is put on 15-20 minutes before working with a view to kill any microorganism and just at the time of working it is switched off.** Some common tools required during working in the laminar flow cabinet are inoculation needles, foreceps, marker, sprit, sprit lamp, surface sterilizing chemical, distilled water, culture medium, culture, cork borer, lighter/match box, Absorbent cotton.

3. REFRIGERATOR /DEEP FREEZE



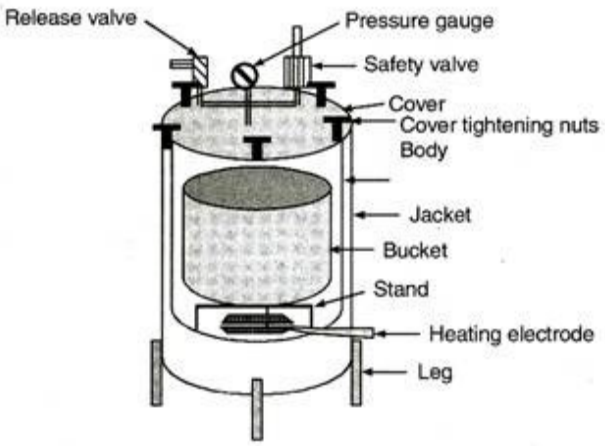
Refrigerator that we use in our houses to store perishable foods and goods, is the same instrument used to store microorganism cultures for longer time because at low temperature (0-5 ° C) all life process slow down. Whereas deep freeze has all specification similar to refrigerator except that temperature is maintained below 0 ° Cnbetter insulated than normal refrigerator. Deep freeze is used mostly to store precise chemicals (bio-chemicals) that may be spoiled at above 0 ° C and living microorganisms for long period necessary precautions.

4. AUTOCLAVE / STEAM STERILIZER

An autoclave is used for sterilization of media.Autoclave/Steam sterilizer is an equipment which is used to sterilize (to kill all the microorganisms) various materials specially media under saturated steam heating. Domestic pressure cookers (are good example of such sterilizer. Body cylinder and lid with rubber gasket and closing devices) of autoclave is made up to steel or other metal/alloy which can tolerate high pressure and it is leak proof. Pressure cookers works at a fixed pressure of 15 pound per square inch (p.s.i.), whereas laboratory autoclaves have systems to regulate pressure (pressure gauge and pressure release valves) simultaneously the temperature. They are mostly electrically operated and water boils by submerged water heater fitted at the bottom of a heavy walled closed vessel. Autoclaves are of two types:-

(i) Vertical autoclave.

(ii) Horizontal autoclave.



Various safety devices are fixed with autoclaves such as pressure gauge, pressure release valves, safety valves, water level indicator etc.

Principle: The principle of autoclave is that the water boils at about 100 ^{o}C, depending upon the vapour pressure of the atmosphere. If the vapour pressure is increased, the temperature will be increased.

Construction: Constructed with 2 metallic walls, made up of stainless steel or copper. Surface of the autoclave is covered with heavy lid, provided with pressure meter to measure the pressure of water vapour, a safety valve and an exhaust valve.

Working: Sterilization in autoclave is accomplished generally at is done 121^{o}C temperature coupled with 15lb pressure for 15 min.

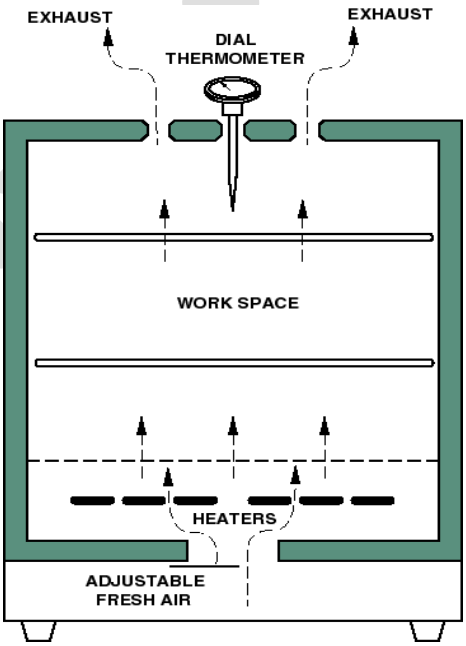
Precautions

- Autoclave should not be overloaded.
- All the air must be removed from within the autoclave before closing the exhaust valve by keeping the outlet valve open until a jet of continuous air comes out of it.
- Sterilization time must be counted not from the time it is switched on but from the time the required pressure is built up.
- Ensure that there is sufficient water in the autoclave before switching on.
- At the end of the sterilization period, allow the steam pressure to drop to zero and only then open the lid.

5. HOT AIR OVEN

It is a type of sterilizer, primarily used for sterilization of glass goods by using dry heat.

Principle: The principle of working of it depends on increasing amount of temperature that facilitates oxidation process. This is used to sterilize the glass goods those are not damaged or burnt in high temperature.



Construction: It consists of an insulated double walled cabinet, made up of stainless steel or aluminium and is designed to prevent heat conduction from inside the chamber. It is fitted with a motor and fan from the backside or bottom. The fan circulates hot air inside of the chamber and thus increasing the temperature for sterilization. A thermostat is also fitted to control the temperature at desired level. There are perforated shelves inside the chamber that are used for keeping the goods and perforation helps to pass the air for proper circulation.

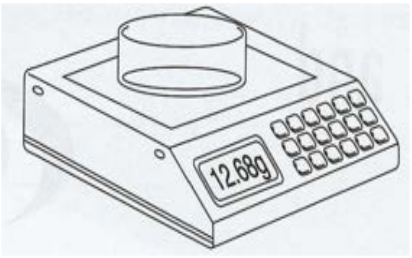
Working: Temperature range of 160-180^{o}C for 1 to 1½ hour is the working principle of Hot air oven.

Commonly used temperatures at different duration are 180^{o}C for 1 hour exposure, 170^{o}C for 2 hours exposure, 140^{o}C for 4 hours exposure, 120^{o}C for 12 to 16 hours exposure.

Precautions

- Exposure time is counted from the time when objects to be sterilized have reached the desired temperature in the oven.
- Calibrated glass should not be sterilized in hot air oven since the expansion and contraction can cause changes in the graduations.
- Rubber goods and culture media should not be sterilized in the hot air oven.
- After the sterilization process, the oven and its contents should be allowed to reach the ambient temperature before opening the door to prevent breakage and recontamination by cool air rushing into the chamber.

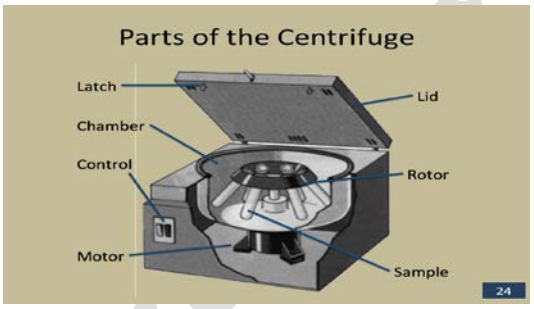
6.WEIGHING BALANCE



Many types of balances such as common double pan balance, top leading single pan electrical balance, chemical balance, analytical/ digital balance *etc.* are available for weighing different materials with accuracy. Presently balances are available to weigh a material as low as 0.0001gm paving very high precision and sensitivity.

7.CENTRIFUGE

Centrifuge is a device used to separate various suspended particles of different densities in liquid through centrifugal force. It has a motor driven rotor with several positions to hold different sized tubes and which revolves at high speed (measured by r.p.m.- revolution per minute)-speed may be as low as 3000 r.p.m. to as high as 15000 r.p.m. or even more depending upon the types of centrifuge.



8.WATER BATH

Water bath is a tool is used to maintain a very stable temperature. Temperature of the water bath is thermostatically controlled. There is a tank water heater that usually installed in basement of the water bath and used to heat the water. Water of the water bath is circulated by stirrer to maintained uniform temperature.

Miscellaneous goods:

9. Dissection box

It is a box which contains all the necessary equipments to perform various activities in a laboratory It mainly consists of 1.needle 2. forceps(blunt, sharp) 3.sissors 4.brush 5.coverslips 6.watch glass 7.blade 8.slide 9.dropper

10. Beaker: A cylindrical container with flat bottom. Different sizes of beaker used to prepare the mixtures of liquid.

11. Conical Flask: a bottle, usually of glass, having a rounded body and a narrow neck, used especially in laboratory experimentation

12. Measuring cylinder: A long, cylindrical, narrow container marked with lines to signify the measurement and used to measure the liquids volume.

13. Test tubes: A hollow tube with one end closed, made up of thin glass, used for maintenance of pure cultures, conducting of in vitro experiments in labs.

14. Petri Dish: A Petri dish (alternatively known as a Petri plate or cell-culture dish) is a shallow transparent lidded dish that biologists use to culture cells, such as bacteria, fungi etc.

15. Test tube rack: A plastic holder used to hold test tubes or dry them.

16. Magnifying glass: lens that produces an enlarged image of an object.

17. Microscope slide and cover slip: A microscope slide is a flat, rectangular shaped glass with 75*26 mm^2 size and 1 mm thick. Slide is used to prepare the specimen for microscopic study. Cover Slip is small, thin and square shaped glass piece used to cover the specimen on Slide.

18. Pipette or Dropper: A slender tube graduated with measurement, used in a laboratory for measuring and transferring of liquids.

19. Bunsen Burner: A type of gas burner, used in laboratories for sterilization of small equipment, providing aseptic zone, provided with nonluminous flame.

20. Glass Stirring Rod: A glass, cylindrical-like stick that is used to mix solutions in a beaker. Also referred to as a "Swizzle Stick"

21. Cork borer: A cork borer, often used in a pathology laboratory, is a metal tool for cutting a fungal disc along with media

22. Spatula: A spatula is a metallic spoon that is used for lifting of chemicals during measurement.

23. Inoculating needle/loop: It is thin needle with 4-5 cm length, made up of nichrome wire or Tunsgten. It is used to transfer the microbial culture and to inoculate the fresh media.

Video link-

https://www.youtube.com/watch?v=KXtGkIXMCQU

https://www.youtube.com/watch?v=z3ptW4Y-9rk

https://www.youtube.com/watch?v=LSu8YmW4mhM

Utility of Compound Microscope

Objective: Used for clear visualization and understanding of pathogens structures in a magnified version.

i) Construction of Compound microscope: It consisted with body, assembled lens and illuminator

a) The body is assembly of body tube, base, arm, stage, diaphragm and adjustment screws (fine and coarse). Base provides the support to whole microscope. Arm is helpful for carrying the microscope and supporting the lens system. Coarse adjustment (rough focussing) and fine adjustment (exact focussing) screws are used to bring the specimen into focus. Upper part of body tube composed with ocular lens and lower part with 2 or 3 movable objective lens. The part of body tube that holds the objectives called nosepiece. Stage is a platform that provides space for keeping the prepared slides with specimen. Stage is associated with clips to hold the slide in right place. Condenser used to collect and pass the light rays towards specimen. A metallic diaphragm is assembled near the base of the microscope and below the stage to control the intensity of light.

b) The lens system assembled with eye piece, objectives and condenser. Mostly used eyepieces are 1X, 2X, 5X and 10X and objective lenses are 10X (low power), 40X (high power) and 100X (oil-immersion) objectives.

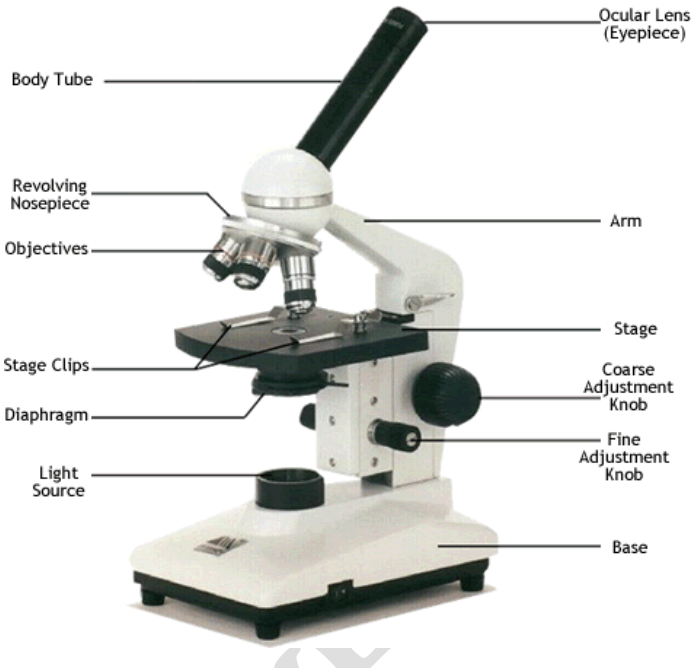
c) Artificial light source or daylight is generally used as a source of illumination. Generally, a concave mirror is used to trap the light at maximum intensity which will be reflected then towards the specimen.

ii) **Principle of working:** Basic principles of magnification, resolving power and illumination are used to work with a compound microscope.

Use of compound microscope

- Place the prepared microscopic slide at the centre of the stage.
- Adjust the light intensity by rotating the mirror or by using the artificial illuminating source. Adjustment of light should be used in such a manner that maximum amount of light will be passes through the specimen.
- Keeping the eye on the eyepiece, gradually lower down the body tube by rotating the coarse adjustment screw till the rough view of the spores/fungi will be seen.

- Now, by adjustment of fine screw, make sharp focus of the specimen. Also adjust the light intensity by adjusting the diaphragm and condenser until the specimen will get optimum light intensity.



- After seeing the specimen under low power objective (10X), it should be shift to and focussed under high power objective by rotating the nosepiece.
- By looking through the eyepiece, slowly raise the body tube with coarse screw to obtain accurate focus. Then by adjusting the fine screw, the image will be brought into final focus.

Video link:

https://www.youtube.com/watch?v=SUo2fHZaZCU

https://www.youtube.com/watch?v=eZX9U15F5Q8

Preparation of culture media for growing microorganisms

Objectives: Multiplication of desired strains of microorganism (fungi / bacteria) to study them in the laboratory.

Heterotrophs like fungi and bacteria generally derive energy tor their growth and multiplication from organic substrate bound chemical energy (Chemo-organotrophs). Some fungi and bacteria could be grown easily in the laboratory in substrates (known as culture medium) which provides all the essential elemental constituents like carbon, nitrogen, phosphorus, sulfur, potassium etc. and also provide the energy required for the body. Art of growing microorganisms artificially in the laboratory is known as 'Culture'. Potato dextrose agar media is used for growing of fungi and Nutrient agar media is commonly used for growing of bacteria.

Composition of common Media:

a)Potato Dextrose agar medium (Natural medium)for fungi

Peeled potato	200.0g
Dextrose	20.0g
Agar-agarpowder	20.0g
Distilled water	1 L
pH	5.6 at 25 ^{o}C

b)Nutrient agar medium (Natural medium)for bacteria

Peptone	5.0g
Beef extract/Yeast extract	3.0g
Sodium Chloride	5.0g
Agar-agar powder	20.0g
Distilled water	1 L
pH	6.8 at 25 ^{o}C

The media compositions mentioned above are solid media. Agar powder is the common ingredient used in all the media to make the media solid but transparent. Agar agar produced from red algae and it is a mixture of two components

(polysaccharides)-agarose and agaropectin. Agar powder has unique characters. It melts at near about 100 ^{O}C and solidifies at 42 ^{O}C in water based substrates. It does not contribute any nutrient element in the medium and does not break down normally by bacteria and other organisms or chemically (except at very low pH).When agar is not added in the medium it remains liquid which is known as broth or liquid medium. Depending on the purpose of studies choice of medium varies.

Materials required: Beaker, Measuring cylinder, conical flask, Non-absorbent cotton, Brown paper/aluminium foil, Rubber bands, Microwave, Autoclave, Weighing machine, Spatula. Also chemicals as listed in **Table A** and **Table B** above.

Methodology: Prescribed or fixed amount of ingredients need to be dissolved into appropriate volume of water for preparation of culture medium:

a. **Preparation of potato dextrose agar medium (PDA)** is done with the following steps-

i) Peeled potato pieces boiled in about doubled quantity of water till potato pieces become soft but not disaggregated. The liquid portion is the decoction which is used to prepare the medium.

ii) After separating of the liquid portion, the volume of the decoction is made upto requisite quantity.

iii) Suitable pH for culturing of fungi is about 5.0 to 6.0. Adjustment of pH of the culture solution to a suitable value is done by adding mild solution of HCI or NaOH.

iv) Ingredients are added and boiled with agar to dissolve at 100 ^{O}C on water bath or heater.

v) Dissolved medium at definite volume is then distributed into suitable glass culture tubes or conical flasks and pluged with non-absorbent cotton followed by wrapping with aluminium foil or brown paper.

vi) Sterilization of the medium is done at 15 p.s.i.for 15 mins (121 °C for 15 mins) in autoclave.

vii) After sterilization, media is brought out from autoclave and stored at cool and clean place.

viii) At the time of use, solidified media is molten and poured into Petri plates (sterilized).

b. Preparation of Nutrient Agar Media:

i) Prescribed amount of ingredients is added into appropriate volume of distilled water.

ii) Heating is done to dissolve the ingredients completely

iii) After plugging media is sterilized at15 p.s.i. for 15 mins (121°C for 15 mins) in autoclave.

Observation and Conclusion:

Potato dextrose media was prepared and stored for future use. After sterilization, Media was in liquid form, color of the media was light brown-deep brown and 20-30 minutes after it was started to become solidified as color was gradually changed from brown to yellowish white.

Fig.14.1 Culture media

Video link:

https://www.youtube.com/watch?v=lmhSv6-T7EQ

https://www.youtube.com/watch?v=h_DCpIRTCrc

Isolation of fungi and bacteria from diseased plant parts

Objective: To learn the isolation process of the fungal and bacterial plant pathogens from infected and diseased plant parts

Materials required:

Infected plant parts / samples, Inoculating needle, Blotting paper, A sharp razor, Few sterile Petri plates, Sterilized media, Spirit, Spirit lamp, Absolute alcohol, 0.1% Mercuric chloride, Hot air oven, Laminar flow, Incubator.

Procedure for isolation of fungi from infected plant tissue:

- Infected sample showing characteristic visible symptoms should be used for isolation.
- After washing the tissues thoroughly in sterile water, the infected tissues along with adjacent small, unaffected tissue are cut into small pieces (2-5 mm^2) by using flame-sterilized scissors.
- Thereafter they are transferred to sterile petri dishes containing 0.1% mercuric chloride or 1%sodium hypochlorite used for surface sterilization of plant tissues for a period of 30-60 s.
- Then put these pieces onto the surface of the sterile paper towel/blotting paper to take away excess water.
- The sterilized pieces are aseptically transferred to Petri dishes containing medium like potato dextrose agar (PDA) supplemented with streptomycin sulphate @ of 3-5 pieces of tissues per Petri plate.
- Incubated the plates at room temperature (25-27^oC) for 7 days that may favour the pathogen development
- A portion of mycelium developing in the nutrient medium is transferred to the agar slants for **purification** and storage for further examination.

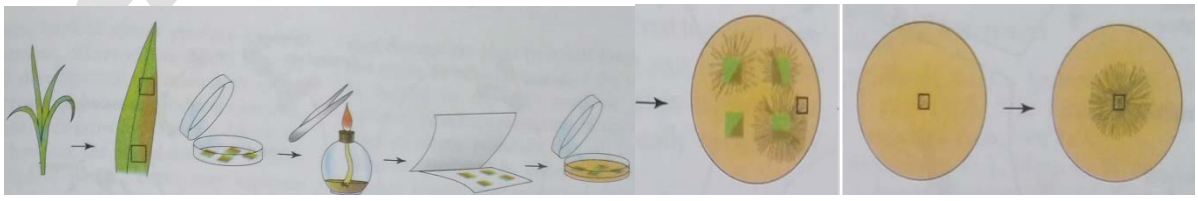


Fig: isolation process of fungi from plant tissue

Procedure for isolation of bacteria from infected plant tissue:

- Infected leaf tissue showing typical symptoms with healthy parts were cut into small pieces under aseptic condition
- Then sterilized the cut leaf bits with 1% sodium hypochlorite for 1 min. and washed subsequently (2-3 times) in sterile water to remove traces of sodium hypochlorite.
- Placed the leaf bits on to a sterilized glass slide and chopped into small pieces using sterilized surgical blade.
- Allow 2-3 minutes to have sufficient oozing of bacterial cells from the cut ends of the diseased tissue, then took a loopful of bacterial suspension and streak it onto nutrient agar.
- The inoculated plates were incubated at 28 C for 36-72 hours in inverted position.
- After incubation, observed the plate and pick single colony, streaked onto nutrient agar.
- After incubation, observed the plate and pick single colony, streaked onto the nutrient agar slant and incubate it again.

Video link:

https://www.youtube.com/watch?v=6L9le2fl75M

https://www.kirkhousetrust.org/pathology

https://www.youtube.com/embed/F92GEhJaubc

https://www.youtube.com/watch?v=zCLIhs4pMkY

https://www.youtube.com/watch?v=UE4s8HsCiY4

Observation and Conclusion:

Studies on the procedure for proving of pathogenicity of a pathogen (Koch's Postulates)

Koch's postulates

German microbiologist, Robert Koch (1887), identified a set of three conditions which had to be satisfied to establish that a particular organism is the causative agent of a particular disease. These conditions are known as Koch's postulates. Later on, a fourth rule was appended by E. F. Smith (1905). The rules are briefly stated below:

1) **The suspected causal organism must be associated with the disease producing specific symptom(s).**
2) **The suspected causal organism must be isolated in purified form from infected part.**
3) **When a healthy susceptible host is inoculated with the pure culture of pathogen, symptoms of the original disease must develop**
4) **The pathogen re-isolated in purified form from the inoculated plants must be same as that was isolated earlier in step-2**

These rules of proof are called as Koch's postulates.

Materials Required: All materials needed for isolation of the pathogen, pure culture of the pathogen, needle, razor blade, inoculating needle, absorbent cotton, cello tape, sterile water, water sprayer, healthy plant, polythene packet.

Experimental steps to establish an organism as plant pathogen

Followings are the steps to prove that the organism isolated from infected plant parts is responsible for the development of the original disease symptom(s).

Step I: isolation of pathogen from diseased part in purified form.

As discussed in earlier lesson

Step II: Inoculation of a susceptible host

1. Grow healthy host plant in soil for 3 weeks from where the pathogen was isolated in step I.
2. Prepare inoculum suspension with spores collected from pathogen grown on agar plate
3. Spray spore suspension on to host plants until run-off or inoculate the host by any other suitable methods
4. Place large, clear plastic bag over inoculated plants to retain high humidity for 24 hours and keep the inoculated plant in dark place.

5. Remove plastic bag from the inoculated plants and leave them in normal light/dark conditions at room temperature for 7 days.

N.B. Method of inoculation may change depending on the type of pathogen e.g. for bacterial disease pin/needle prick method may be followed

Step III: Comparison with original symptom(s) and re-isolation of pathogen from inoculated and infected plants.

Observe the types of symptoms produced by the inoculated plants and compare with the original symptoms. The symptoms produced by the inoculated plant must be same with the original symptoms observed in diseased plant. Re-isolate the pathogen in purified form from inoculated plants following the usual methods of isolation of a plant pathogen as discussed and demonstrated earlier.

Step IV: Confirmation of pathogenicity by establishing similarity between the re-isolated pathogen with the previously isolated suspected pathogen.

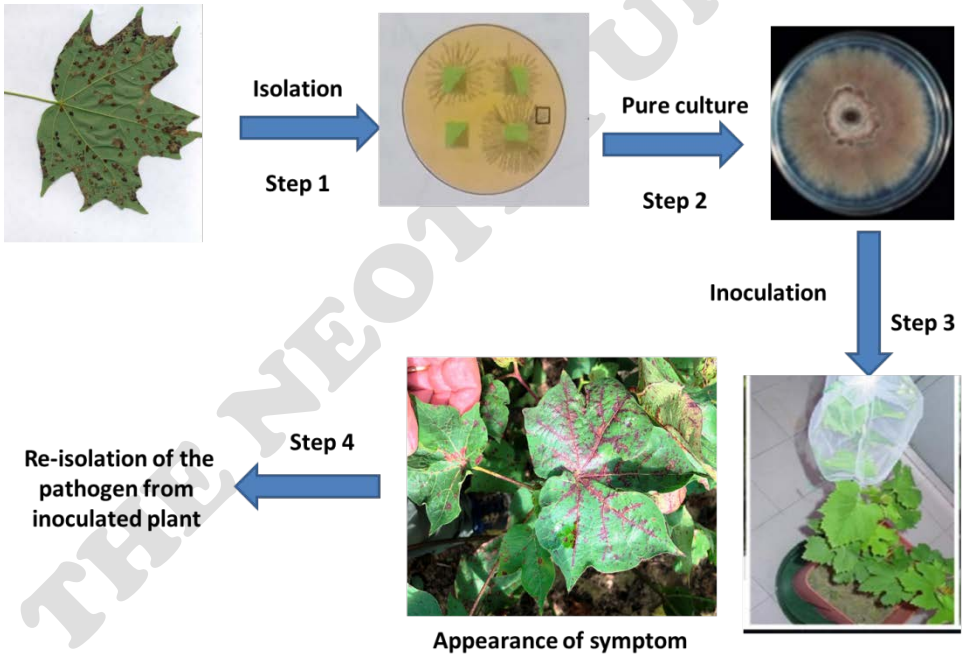


Fig: Procedure of Koch's postulates

Video link:

https://www.youtube.com/watch?v=ipsw28kET-I

Study on symptoms of different plant diseases

Objectives: To gather knowledge about the common disease symptoms of different agricultural crops.

A plant disease is the abnormal phenomenon of plant health that leads to altered appearance and physiolological activity of a plant. Plant diseases caused aberrations in normal physiological activities which in turn affect other activities too. It does not occur instantly like injury, changes in plant system occur over time. Diseases not only reduce yield but also deteriorate the quality of the seeds. Symptoms are the visible reflections of the infected plants manifested by continuous irritation of plant pathogens. It can be the changes of leaf color, shape, size or any other morphological and physiological disturbances whereas, signs are the physical evidence of the pathogen like bacterial ooze, fungal mycelia, sclerotia etc. Symptoms and signs help plant disease identification.

Few examples of signs and symptoms of common diseases incited by fungi, bacteria and viruses are mentioned here:

Fungal disease symptoms:

Spot: It is sharply defined lesion of diseased tissue; appear either on leaf or fruits.

Lesion: A localized area of diseased tissue.

Blight: Spots that formed after coalescing of numerous small spots. In advance stage, the rapid decline and death of young plant parts occurs. This term generally used to signify the health condition of infected plant. (e.g. Botrytis blight, blossom blight).

Blotch: This term indicates irregular dead areas of leaf tissue. Infected area are larger than the spot.

Rot: Rotted condition of a plant parts is manifestation of disintegrated cell wall and cell membrane due to some bacteria or fungi that produce pectinolytic enzymes.

Wilt: Due to water stress condition or clogging of xylem vessels with fungal mycelia or expolysaccharides of bacteria, plants become droopy, pale and died later on. e.g. Fusarium wilt, Bacterial wilt of solanaceous vegetables by *Ralstonia solanacearum*.

Plant droops due to water stress; can be systemic (xylem) or due to root rot e.g. Fusarium wilt of banana.

Dieback: In this symptom, progressive drying and death of shoots from tender tips is observed. This symptom may be caused by a root or stem related disease, insect injury, nematode feeding and a number of abiotic factors, such as winter injury or moisture stress. e.g. chili dieback

Scorch: Foliar leaf tissues, specially the leaves shows burning symptoms along the marginal parts or tips.

Shot-hole: Spotted portion of leaf tissue will be dried, become papery and fall off. Which will gives an appearance of numerous small holes on the leaf surface. E.g. Cercospora leaf spot.

Patches and decline: These terms often used in association with grasses (turf, grain crops). e.g. patches caused by *Rhizoctonia* sp.

Anthracnose: Firstly, symptoms of a**nthracnose** appear as small, irregular faded yellow or brown **spots**. These spots darken as they age and may also expand, covering the whole leaf and become sunken with numerous small dot like fruit bodies embedded on this**.** e.g. Chili anthracnose by *Colletotrichum capsici*.

Rust: It is a fungal disease that produces dusty appearance due to exposure of spores and spore bearing structures from broken epidermis. Colors of the spores may be orange, yellow, brown, black or white and called pustules when form cumulatively. e.g. Black rust of wheat (*Puccinia graminis tritici).*

Smut: A fungal disease of cereals in which parts of the ear change to black powder Form black powdery spore masses that resemble soot or smut. e.g. Loose smut of wheat caused by *Ustilago tritici.*

Downy **mildew:** Initially symptoms appear as yellow colored small spots on the upper surface of the foliage. On the reverse side of the spotted areas, massive mycelia growth can be seen with spores and sporophores growing downwardly. As the leaf spot dies, the fluffy growth darkens to grey in colour. e.g. Downy mildew of cucurbits caused by *Pseudoperonospora cubensis*.

Powdery mildew: It is a common disease of **cucurbits**. A **powdery mildew** disease gives white powdery appearance on the affected plant parts. Infested areas become pale yellow in color due to interference with photosynthesis. e.g. Powdery mildew of cucurbits caused by *Sphaerotheca fuliginia*.

Pustule: Pustules are the small dotted blister like appearance, appear cumulatively. Over the mature pustules, epidermis becomes ruptured, helps to expose the spores of the pathogen. E.g. rust pathogen.

Conk (also Conch): This refers to fruiting bodies of the wood rotting fungi.

Damping-off: Mostly in nursery beds of the vegetables, seeds decayed after sowing due to infection of soil borne fungi. Either seedling not emerged or become infected at the soil line after emergence. E.g. *Pythium* sp, *Phytophthora*sp and *Fusarium* sp.

Symptoms of bacterial diseases:

Canker: It is sunken, necrotic areas on twig, leaves, stem or trunk of plant**, surrounded by corky dead tissues.** e. g. Citrus canker caused by *Xanthomonas campestris pv. citri*

Vascular discoloration: Darkening of the plant's vascular elements.

Scab: Crust like rough brown colored area on the surface of a plant organ. e.g. Potato scab caused by *Streptomyces scabies.*

Other symptoms are-

- Leaf spots and blights – water soaked, greasy (Bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae*)
- Soft rots of fruits e.g. soft rot of Potato caused by *Pectobacterium carotovorum* pv. *carotovorum*
- Leaf curling
- Etiolation
- Virescence
- Sheperd's crook stem ends on woody plant

Symptoms of viral diseases:

Dwarfing or stunting: Reduced growth of plants.

Mosaics: Light green, yellow or white areas intermingled with green–leaves or fruits. Symptom of certain viral diseases of plants are characterized by intermingling patches of normal green and light green or yellowish colors. e.g. Bean golden mosaic virus.

Chlorosis: This is results of loss of chlorophyll. Due to destruction or breakdown of chlorophyll, green leaf tissue become white or pale yellow.

Ring spots: Concentric rings of chlorotic or necrotic tissues on leaves, stems and fruits. e. g. Papaya ringspot virus

Curling: Rolling, cupping, or puckering of leaves. e.g. Tomato leaf curl virus.

Necrosis: Dead plant tissue. Color may be brown, black, tan, or grey. e.g. Tobacco necrosis virus

Mottling: Different shades of color will be mingled on the surface of infected tissue.

Rugose: The blistered or warty crinkled foliar parts of an affected plant e.g. Potato rugose mosaic virus

Vein banding: Infected leaves show banded areas with darker region along veins and chlorotic areas at interveinal regions. This symptoms generally induced by viruses.

Vein clearing: Vein tissues become chlorotic due to destruction of chlorophyll. It is mainly the result of virus infection.

Witches' broom: Excessive proliferation of foliages of vegetative branches gives a broom like appearance. Generally caused by viroids. E.g. Potato withes broom.

Distortion: Malformed plant tissues

Exercise:

Materials required:
Pen, marker pen, record book, polythene packet, rubber band

Work out:

1. Visit the field and by random sampling method collect the samples.
2. Keep the sample into polythene packet containing a wet cotton swab and close the mouth of the polythene packet with rubber band.
3. During collection, record the important information like; name of the plant, date of collection, location, symptom of the collected plant parts, name of the suspected causal agent etc.

Serial no.	Date of collection	Host plant	Plant part collected	location	Symptom	Causal agent

Video link:

https://www.youtube.com/watch?v=WaBouSHmAZk

https://www.youtube.com/watch?v=MQgDQwX6upE

https://www.youtube.com/watch?v=lCPW1lOxXIo

https://www.youtube.com/watch?v=Tj9WShQ1nRU

https://www.youtube.com/watch?v=QakXfQNu1Ms

Studies on the techniques of Collection and preservation of disease specimen

Objectives: For better understanding of diseases with special reference to the symptoms, diseased specimen will be collected and preserved in the laboratory.

Materials required for dry and wet/ colour preservations:

A small knife, razor blade, magnifying lens, polythene bags, cotton, distilled water, rubber bands/cotton threads, small towels, pencil, paper tag, a pocket book, blotting paper, a blank album with butter paper bag, formaldehyde 40%, glacial acetic acid, alcohol, zinc chloride, glycerine, heater, borosil, 500/1000ml beaker, specimen bottles, marker pen.

Methodology:

A. Collection of diseased specimen:

Plant diseases are recognized by the symptoms that are produced in plants. Diseased symptoms in plants may be of different types and various plant parts like leaf, stem, fruit, flower, root, seeds etc. are affected.

Collection of diseased leaves:

In case of leaf spots of different sizes, shapes and colours, the diseased leaf samples should be collected in bulk. Insert the diseased sample, immediately after collection, in a polythene packets containing small water-soaked cotton or cloth piece and tied the mouth of polyethylene packets with rubber band or cotton thread or dip cut end of samples in water of a beaker so that the samples do not roll or dry till processing is over.

Collection of diseased stems:

Stem samples showing various symptoms should be collected in the similar way as leaf samples are collected.

Collection of roots:

Root samples showing various disease symptoms are collected along with rhizosphere soil. More root and soil samples from adjoining area may also be collected if necessary. If the target crop grows in water logged condition then soil along with water is needed to collect sometimes.

Collection of fruits and seeds:

Diseased fruits and grains should be collected in 10-15 numbers and 100-150 numbers, respectively.

During collection of diseased plant sample, the points to be recorded are: (i) Name of the host (ii) Locality (iii) Date (iv)Soil type (v) Extent of damage (vi) Crop variety (vii) Manures and fertilizers applied and (viii) Control measures adopted, if any.

B. Preservation of diseased specimen:

Preservation means killing or ceasing the growth of an organism in or on the substrate where it grows. Preserved diseased materials are valuable working material as well as worthy reference material for demonstration.

Diseased specimen collected from the field can be preserved in dry form in an album or in wet form in FAA (Formalin-Acetic Acid-Alcohol): 5 ml: 5 ml: 90 ml of 70% alcohol or in 4% formaldehyde solution for long periods.

Materials required for preservation:

Dry preservation: Diseased sample, blotting paper, polythene packet, label or sticker, pen/marker

Wet preservation: Diseased sample, glass jar, clean water, formaldehyde, acetic acid, 70% alcohol, label.

Coloured wet preservation: Diseased sample, glass jar, clean water, copper sulphate granule, glacial acetic acid, 4%formalin, blotting paper, heater, stirrer, saucepan.

Methods of preservation:

1) **Dry preservation:** This is mostly done for foliar diseases.

- Collect the representative, good quality leaf samples and press carefully in the fold of blotting paper with proper labelling.
- After pressing between blotter sheets, dehydrate the samples in hot air or in the sunlight for few days.
- After drying, label and pack the specimen with disinfectant and store it for long time.

2) **Wet preservation:** Collected specimen is kept in preservative after fixation.

- Collect the samples (leaf, fruit, stem, root) from field
- Prepare Formalin-acetic acid-alcohol (FAA) solution that is a good fixative to to restore the natural shape of the material by checking its dehydration.
- Make the solution by mixing 5 ml Formaldehyde, 5 ml acetic acid and 90 ml 70% alcohol for 100 ml of FAA (1:1:9).
- Pour the cleaned sample into the solution for long time preservation.

Note-For general purposes 4% solution of formaldehyde (40%) in water is a good preservative.

3) **Coloured preservation:**

- Collect the samples (leaf, fruit, stem, root) from field

- Wash the collected sample with clean water.
- Prepare the solution by mixing copper acetate-glacial acetic acid-water
- Boil the sample with solution of copper acetate-glacial acetic acid-water till green colour of the plant part reappears.
- Then wash this material for several hours under running tap water.
- Now after proper drying of the sample with blotting paper, pour it within 4% formaldehyde solution with proper seals.

Observation and conclusion:

Video link:

https://www.youtube.com/watch?v=d8tNws_ZK4U

https://www.youtube.com/watch?v=TWQhP5IAgWU

Protocol of gram staining

Objectives: To learn the techniques of gram staining for identification of plant pathogenic bacteria

Gram staining is a technique by which the bacteria can be differentiated on the basis of their cell wall compositions. Steps of this technique was formulated by Christian Gram in 1988

Materials required:

1. 2 pieces clean microscopic slide
2. Inoculation loop
3. Solution of Bacteria
4. Sterilize water
5. Crystal violet
6. 95% alcohol
7. Iodine solution
8. Safranin
9. Spirit lamp
10. Cover slip
11. Balsam oil
12. Compound microscope

Methods:

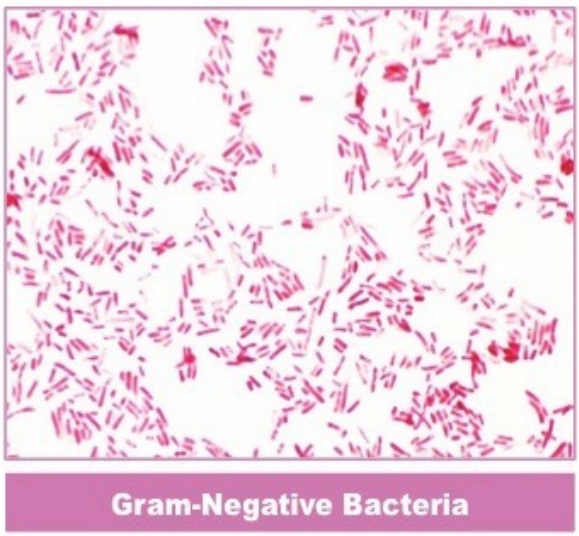
1. Take a clean, grease free slide.
2. Now smear of suspension on the clean slide will be prepared by taking a loopful of sample.
3. Once the liquid has evaporated, heat fixed the bacteria to the slide by quickly passing the slide through the flame 3 times (for 1 second each).
4. Cover the fixed bacterial smear with a few drops of **crystal violet** solution and allow it to dry for 1 minute.
5. **Rinse off** the crystal violet with distilled water.
6. Add a few drops of **iodine solution** to the slide, and allow standing for one minute.
7. **Rinse off** the iodine solution with distilled water.
8. Add **decolorizing agent** to the slide by holding the slide over a cup of water and add the decolorizing agent drop by drop.
9. Add a few drops of **counterstain** (safranin) to cover the bacteria and allow standing for 1 minute.
10. **Rinse off** the safranin with distilled water.
11. Blot dry with blotting paper
12. After adding one drop of canada balsam oil and placing a coverslip on it, examine your slide under the microscope.

Video link:

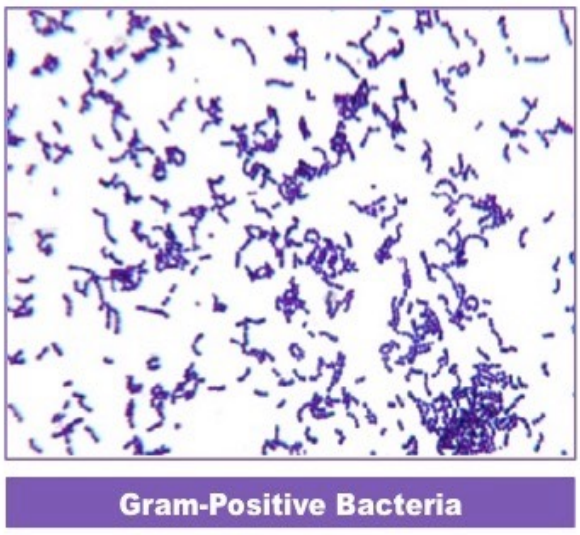
https://www.youtube.com/watch?v=FassELAQa1Y

https://www.youtube.com/watch?v=AZS2wb7pMo4

Observation and conclusion:



Gram-Negative Bacteria



Gram-Positive Bacteria

Identification of fungal vegetative bodies and their modifications

Objective: To observe different types of thallus and mycelial modifications of fungus

The vegetative bodies of fungi are thaloid (*i.e* devoid of root, stem andleaf) and are of three types *viz.* (a) Plasmodial (b) Non-filamentous (c) Filamentous. A unit of filamentous structures is called hypha. Mycelium is the collection of hypha. Thallus is the collection of mycelia. Mycelium may be colourless (hyaline) or pigmented but is transparent to make visible the internal cell organelles. Type of thallus and some modifications of fungal vegetative body are mentioned here:

Plasmodial thallus: Plasmodial vegetative body does not have any cell wall and thus has no definite shape and size. The multinucleate dark **plasmodial** mass can be seen in hypertrophied host cell. Plasmodial masses take the shape of cell *(e.g. Plasmodiophora brassicae)* **(Fig-A)**

Unicellular thallus: Non-filamentous and filamentous vegetative bodies have cell wall with definite shape and size. The **unicellular** vegetative body of *Saccharomyces* sp. (Yeast) and the daughter cells develops in chain (form Pseudo-hypha) from the unicellular mother cell by budding. Sometimes bud cells remain attached to the mother cell forming chains resembling mycelium which is called as pseudo-mycelium. **(Fig-B)**

Multicellular thallus: Observe the filamentous thread like structures. Filamentous vegetative body may be septate i.e. multicellular with cross walls, or aseptate, *i.e.* without septum /cross wall. e.g. aseptate mycelia of the genus Pythium/ Phytophthora septate mycelia of the genus Rhizoctonia. **(Fig-C)**

Hyphal modification and mycelial aggregation: Filamentous hyphae are sometimes modified and/or aggregated to perform various specific functions. Some examples are as follows:

Hyphalmodification:
Chlamydospores: Round,swollen,thick and smooth walled, terminal or intercalary hyphal structures called chlamydospores are produced to withstand adverse conditions**(Fig-D)**

Rhizoids: Root like sometimes branched structures, thinner than main vegetative hyphae to anchor with the substrate and absorb nutrients **(Fig-E)**

Sclerotium:
Slerotia (plural) or sclerotium (singular) are the hard coated, melanised resting structures of fungi; formed by aggregation of mycelia. (**Fig.F**)
Sporophores:
This is specialized hyphal branches which bear the spores. Conidia bearing structures are called conidiophores sporangia bearing structures are called sporangiophore. **(Fig-E)**

Pycnidium:
Physical appearance of pycnidia on diseaed lesion is black dot like. Pycnidium is globose to subglobose, flask shaped hollow fruit body. Many conidiophores are lined on its inner surface. Pycnidium usually contains an ostiole or opening, small papillae to long beak or may be completely closed. The walls of pycnidium are thick, formed from loosely interwoven mycelia but others are stromatic. Pycnidia producing fungal genera are *Phyllosticta sp., Phoma sp., Macrophomina sp*.**(Fig-G)**

Acervulus:
Saucer shaped or flat stromatic pseudo-parenchymatous structure composed of complex aggregation of mycelia was observed. Many short conidiophores closely packed together developed from the underlined stromatic structure, may or may not intermingled with short, bristle like, pointed, sterile, black colored, septate setae. Acervuli are neither surrounded by any hyphal wall nor do they possess any definite opening. Acervuli producing fungal genera are *Colletotrichumsp, Gloesporium sp*.**(Fig-H)**

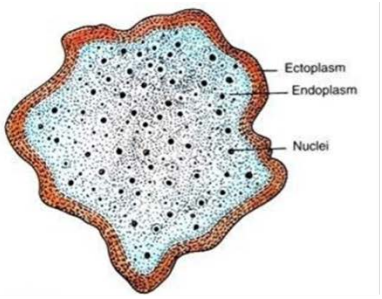
Sporodochium:
A pad or cushion shaped stromatic structure formed by compact or loose aggregation of mycelia was observed. Simple or branched conidiophores in compact mass are produced on the surface of stromatic structure. Sporodochium produced by fungus *Fusarium* sp.**(Fig-I)**

Materials required: Microscope, Prepared microscopic slide

Work out

1. Observe the prepared slide (of different thallus and mycelial modifications) under low and high magnification objective lens of the microscope.
2. Draw the representative diagram of each structure.

Observation and conclusion:



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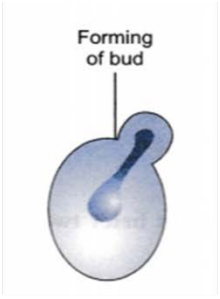

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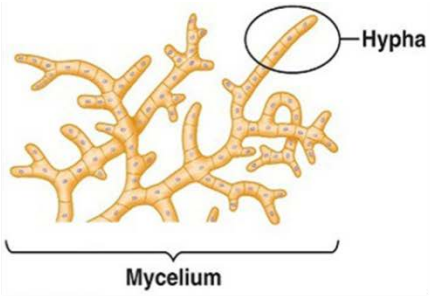

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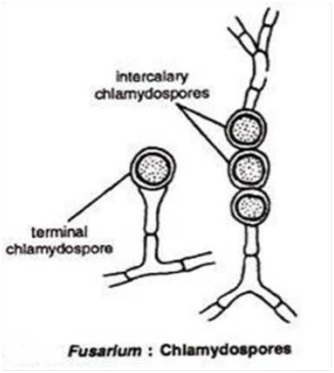

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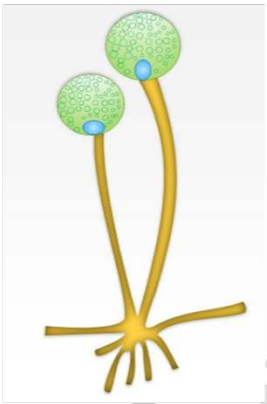

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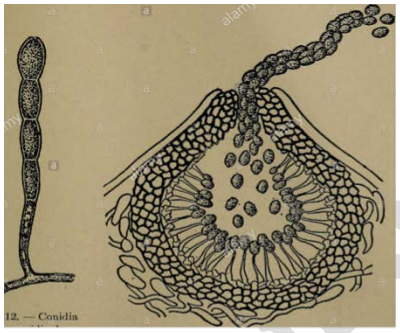

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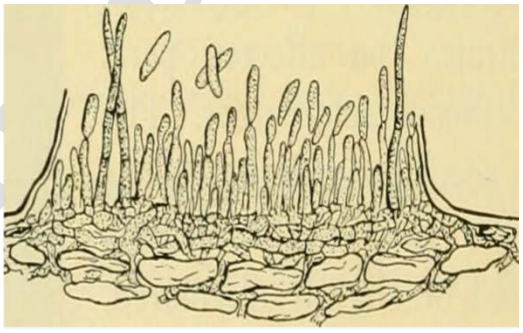

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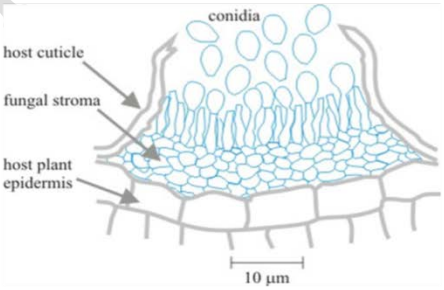


I

Studies on fungal reproductive structures: Asexual and sexual reproduction

Objective: To acquire the clear concepts of different fungal reproductive structures within microscopic field.

Reproduction is the formation of individual/progeny having typical characteristic to the species following sequence of processes. In fungi reproduction are of two types–sexual and asexual. Sexual reproduction involves fusion of two compatible nuclei brought into a cell by fusion of two protoplasm(plasmogamy)to form zygote followed by meiosis but in asexual reproduction these three steps *i.e* plasmogamy, karyogamy and meiosis are lacking. Asexual reproduction may be either vegetative (simply apart of vegetative body which developed by fragmentation, budding, fission, chlamydospores formation *etc.* And grows into a thallus) or asexual by producing small reproductive units *i.e* spores (seeds).The unit of fungal reproductive structure is spore.

Asexual spores:

Sporangia and sporangiophores:

Sporangia are round or oval shaped balloon like asexual spores and sporangiophores are the sporangia bearing structures. E.g. sporangia and sporangiophores of *Pseudoperonospora cubensis* (Fig:A)

Conidia and conidiophores: Conidia are also the asexual, sac like, cylindrical spores and conidiophores are conidia bearing structure. E.g. Conidia and conidiophores of *Helminthosporium* sp. (Fig: B)

Diploid sexual spores:

Resting Spore: Resting spore is nothing but the zygote produced by copulation of two equal (iso) or unequal (aniso) motile or planogametes. E.g. *Synchytrium* sp. (Fig: C)

Zygospore: This is produced from fusion of two morphologically similar gametangia. The two gametangia come in contact and fuse entirely. E.g. zygospore of *Mucor* sp., *Rhizopus* sp. (Fig: D)

Oospore: Oospore is produced by the fertilization of non-motile female gamete in oosphere by male gamete. The male (antheridium) and female (oogonium) gametangia come in close contact. The male nucleus from antheridium migrates into oogoniun through a fertilization tube and fuses with the female nucleus in the oosphere. E.g. *Pythium* sp. (Fig:E)

Haploid sexualspore: Haploid sexual spores are produced either endogenously within a structure or exogenously on the surface of a structure.

Ascospore: Ascospores are endogenously produced haploid sexual spores and the structure in which these spores are formed is called ascus. Usually there are 8 ascospores in an ascus.

Cleistothecium: Cleistothecium is a completely closed ascocarp or fruitbody and has dark coloured, thick pseudoparenchymatous wall. Cleistothecia are formed on the leaf surface and may contain one (e.g *Sphaerotheca* sp., *Podosphaera* sp.) or many asci (e.g. *Erysiphe* sp.,*Uncinula* sp.). (Fig:F)

Perithecium: The perithecium is a flask shaped partially closed structure with an opening at the tip (ostiole). It has pseudoparenchymatous wall. Cylindrical club shaped asci, each containing 8 ascospores, originate from the base of perithecium and is directed towards the ostiole.There are fine hairs developed from the inner wall of the ostiole knowing as periphyses. Examples of perithecia producing fungi are *Neurospora* sp.,*Claviceps* sp.(Fig:G)

Apothecinm: The apothecium is a flat, cup or a saucer shaped opened fruit body. Asci containing ascospores are produced in a layer (hymenium) on the upper surface of the fruit body. Example of apothecium producing fungi is *Ascobolus sp*. (Fig:H)

Basidiospore: Basidiospores are exogenously produced haploid sexual spores and the structure on which these basidiospores are produced is called basidium. There are generally four basidiosporers on ·a basidium. (Fig:I)

Materials required: Microscope, Prepared microscopic slide

Workout:

1. Place the prepared slide of (sporangia and sporangiophore, conidia and conidiophore, resting sporangia of *Synchytrium* sp., zygospores, ascospores within different ascocarps and basidiospores) different asexual and sexual spores.
2. Observe the structures under low and high magnification objective lens of the microscope.
3. Draw and record the representative diagram of each structure.

Video Link:

http://www.youtube.com/watch?v=dM_g_p4h6CM

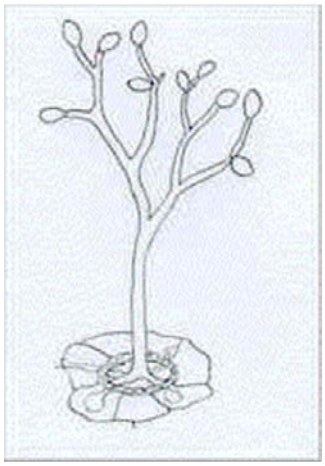
http://www.youtube.com/watch?v=qDwgSWDqKoQ

https://www.youtube.com/watch?v=A1uQ5rXmbO8

https://www.youtube.com/watch?v=VVuYGkk_I8s

Observation and conclusion:

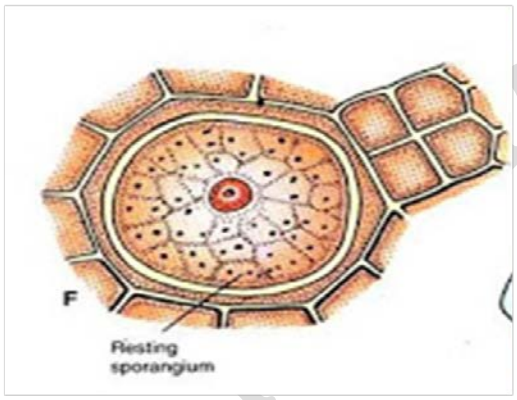
Sporangia and sporangiophore



A

Conidia and Condiophore

B



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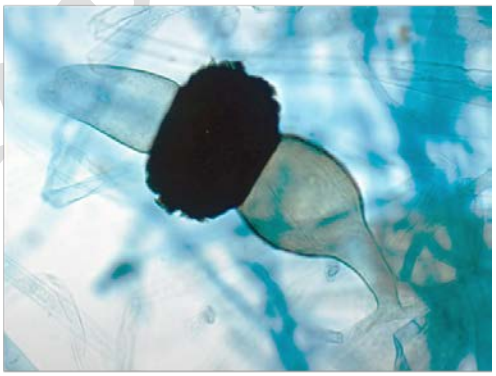

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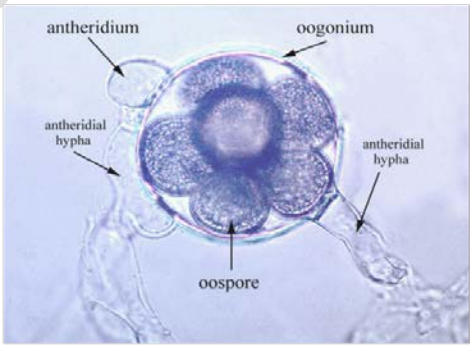

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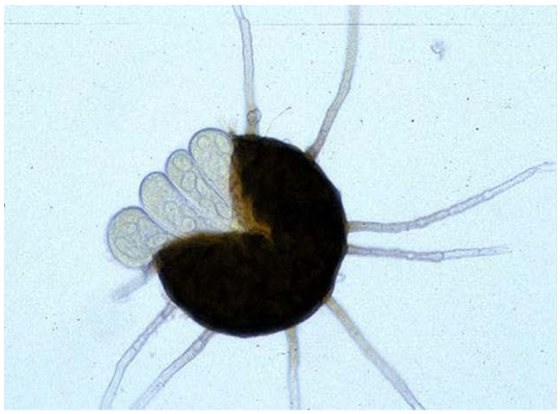

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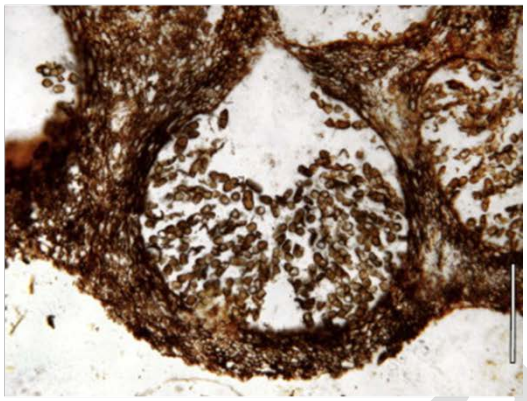

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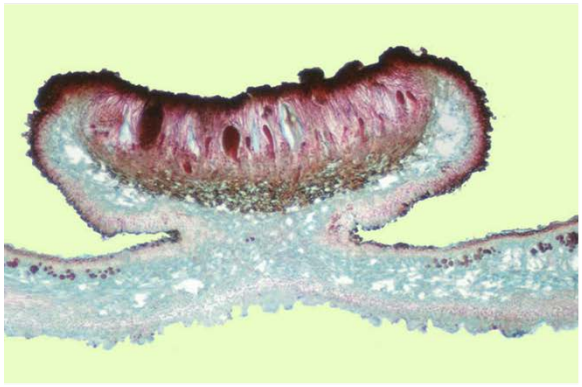

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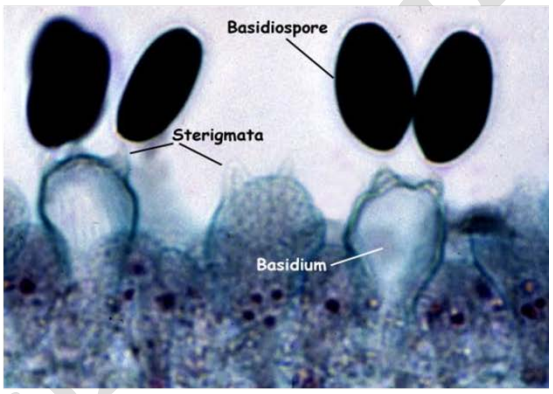


I

Studies on some plant pathogenic genera of fungi:

Objectives: To know the morphological characteristics of different fungi along with their asexual spores (conidia/sporangia) and spore bearing structures (conidiophore/ sporangiophore)

Morphological description of some genus are mentioned here-

***Helminthosporium* spp:**

The pathogen is a facultative parasite. Its mycelium is olivaceous brown to dark brown, septate and highly branched. Conidiophores are straight, long. Conidiophores produce conidia successively from its continuously growing tip. Conidia are dark olivaceous brown, cylindrical straight or slightly curved widest at the middle and have broadly rounded ends. Conidia have many transverse septations. E.g. *H. Oryzae* caused brown spot disease of rice (Fig: E)

***Alternaria* spp:**

The genus *Alternaria* is a facultative parasite. Its hyphae are dark coloured, septate and develop many branches, it reproduces by the production of conidia. Conidiophores are dark, septate, branched or unbranched. The conidia are dark brown, oval to obclavate in shape and are mostly borne in chains. The conidia may have beak at the tip. The conidia have both longitudinal and transverse septations and are termed as muriform spore or dictyospores. (Fig:F)

e. g. early blight disease of potato caused by *Alternaria* spp.

Pyricularia oryzae:

The mycelium is well developed and consists of branched septate, intercellular or intracellular hyphae. Theconidiophores are simple, long, slender, septate or aseptate and usually unbranched. Groups of conidiophores come out through stomata. Conidia are pale brown, pyriform, two septate and three celled. Each conidium remains attached with the conidiophores by papilla like hilum. (Fig:G)

Diseases caused by *Pyricularia* spp. are blast of rice–*Pyricularia oryzae*

Stemphylium spp:

The dark conidiophores produce a single apical spore (conidium) through a pore and then continue to growth rough the pore, thus dislodging the spore. Successive spore production and re-growth give the conidiophores a noded appearance. The spores are dark brown and are divided by several longitudinal and transverse septa. (Fig: H)

Diseases caused by *Stemphyliurn* spp. are black stalk rot and tip blight of onion-*S. botryosum;* leaf blight of gram- *S. sarciniformae;* leaf spot of wheat- *S. tritici*

***Rhizopus spp*:**

Observe thin, highly branched, coenocytic, hyaline, mycelia. You could also see sporangiophore which is erect, long, unbranched, specialized structure develops from vegetative hypha. They produce rhizoids at the junction of two aerial stolons meet and from the point where unbranched, tuff of sporangiophores develop. Brown sporangiophores in cluster develop from point opposite to the place from where rhizoids emerge. Sporangiophore bears globose, columellate multi-spored head/ structure terminally, known as sporangium. The peripheral fertile region of this globose sporangium contains densely crowded oval to circular light brown sporangiospores. The central sterile portion of the sporangium that is continuous with the sporangiophore is called columella. Columella attached to the sporangiophore. (Fig:C)

Rhizopus sp cause soft rot of sweet potato - *Rhizopus sp,* Fruit rot of Jack fruit - *Rhizopus artocarpii*

***Phytophthora* spp:**

Observe the sporangiophores of *P. infestans* which are sympodially branched, hyaline, aseptate, comes out tuff through stomata. They are of indeterminate type (i.e. the growth of sporangiophore continues even after the formation of sporangium), have characteristic nodal swelling at the point from where sporangium *is* developed. Sporangia are lemon-/ pear-shaped, papillate, deciduous (i.e. sporangia leave sporangiophores after their formation) (Fig:A)

Diseases caused by *Phytophthora* spp. are late blight disease of potato-*Phytophthora infestans;* leaf blight of colocasia -*P. colocasiae;* fruit and stem rot of pointed gourd-*Phytophthora cinnamomi.*

Peronospora spp:

Observe well differentiated, dichotomously branched, hyaline, aseptate sporangiophore. Sporangiophore branching starts from it supper one third part, branches at acute angle; the tip of sporangiophore is pointed and bears sporangium singly. Sporangiophore stops growing at maturity, produces and liberates a crop of sporangia of same age simultaneously. Sporangia are non-papillate, hyaline, mostly round or oval, deciduous, always germinate by the production of germ tube. (Fig: B)

Diseases caused by *Peranospora* spp. are downy mildew of crucifers–*P. parasitica;* downy mildew disease of pea-*P. pisi.*

***Albugo spp*:**

Observe well differentiated, unbranched, club shaped, stout, short, hyaline, aseptate, sporangiophores produced under the host epidermis. Round/spherical shape sporangia are produced in chain from the tip of sporangiophore. (Fig: D)

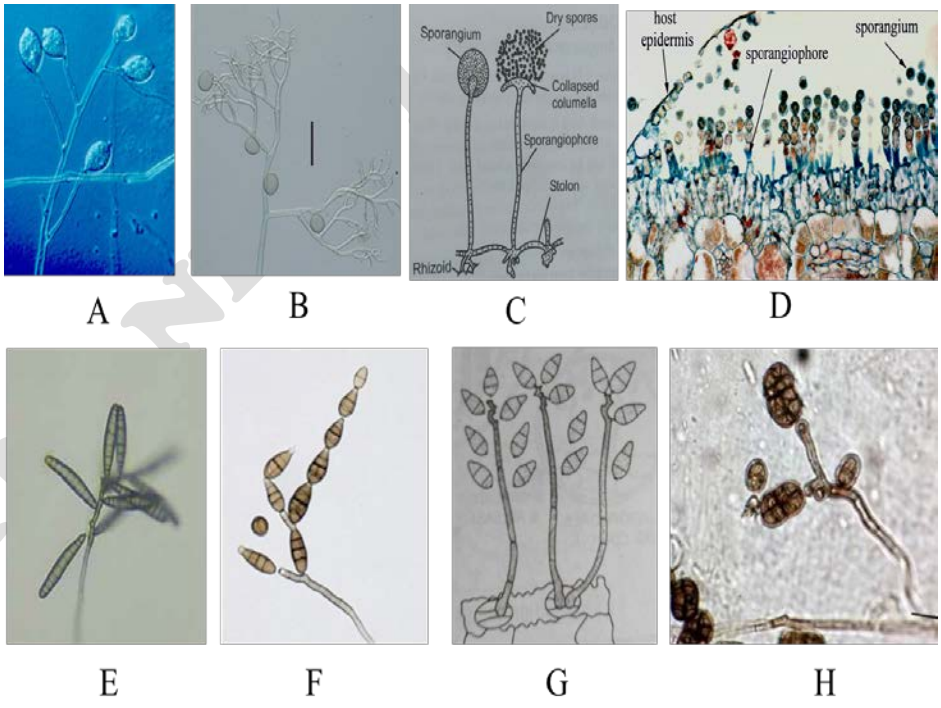
Diseases caused by *Albugo* spp. are white rust of ciucifers– *A.candida*, white rust of amaranthus–*A. bliti*.

Materials required: Microscope, Prepared microscopic slide

Work out:

1. Place the prepared slide of different fungal genera with the conidia/sporangia and conidiophore/sporangiophore
2. Observe the structures under low and high magnification objective lens of the microscope.
3. Draw and record the representative diagram of each structure.

Observation and conclusion:



Study on mechanical transmission of plant viruses

Objectives: To learn the *in vitro* process of mechanical transmission of plant viruses

Mechanical transmission is the most common way of plant virus transmission. Healthy plants are become infected only when they come in touch with the infected plant or become injured. Experimentally, mechanical transmission of plant viruses can be proven by sap inoculation method. *In vitro* mechanical transmission can be done by Leaf rub, Cotton swab, Pinprick, Microinjection.

Materials required: Inoculum, host plants, additives like Tween 80, abrasive material like carborandum powder.

Procedure:

☐ Preparation of inoculum:

1. Collect a suspected plant with prominent symptom of tobacco mosaic virus.
2. After washing it the plants/plant parts, extract the crude juice of the infected leaves. Add Phosphate buffer (pH: 7-8) to retain the infectivity and stability of the virus in extracted juice.
3. Use of additives like Triton X-100 or Tween 80 in extracted juice to help in release of virus particles from cell components.
4. Then filtered the cell sap using filter paper.

☐ **Inoculation of healthy host:**

1. Choose suitable hosts plant like *Nicotiana spp., Chenopodium spp., Cucumis sativus, Gomphrena sp., Datura spp, Phaseolus vulgaris* and the plant should be healthy *i.e* free from symptom.
2. Use abrasives like carborandum powder to make wounding on leaf surface before application of inoculum.
3. After creation of successful wounding inoculum (infected plant cell sap), rub it on the injure area of leaves with help of absorbent cotton plug.
4. Then cover the inoculated area with cotton swab
5. Observe & record the symptom at 10 days after inoculation

Video link:

https://www.youtube.com/watch?v=UZoQKtu_dOA

https://www.youtube.com/watch?v=RJB__d_xKjI

Observation and conclusion:

Sampling and extraction of nematodes from plant material

Objectives: To diagnose the presence of nematode in infested plant material.

a) Sampling techniques of nematode infested samples:

Sampling can be carried out at random or systematically. Theoretically, random sampling is best as it excludes bias; by sampling in a haphazard and criss-cross way samples can be considered as independent observations. In practice, however, sampling is usually carried out in a systematic way because it requires much less time.

Materials required:

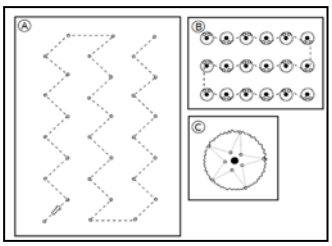
- Polypropylene packets
- Aluminium tag
- Marker pen
- Rubber bands
- Khurpi/ Auger

Procedure:

- Diagnose the infested plants
- Collect the samples from middle of an infested field, the surrounding plants showing poor development and healthy plants outside the patch
- Collect Root, stem, and leaf material and cores from the surrounding soil in the following method-

a. Marked a 10 x 10 metre area consisting of approximately 50 cores
b. Make 10 cores for each replication of each sample with the help of an auger (10 cm length and 1.7 cm diameter)
c. Make 5 replications for each sample and collect > 1 kg soil

- Record all symptoms and signs of infested plants
- Pour the sample into a polythene packet, close the packet and marked with date of collection



Different patterns of sampling: a. on fallow land b. in an orchard c. for a single plant/tree (From Baker et al, 1978)

b) Process of extraction of nematodes from plant materials

Extraction methods for plant material are usually based on nematode mobility. These methods vary depending on whether or not the samples have been cut in smaller pieces to speed up crawling out of nematodes. The blender centrifugal flotation method is the only method, apart from picking nematodes under a microscope, which does not make use of nematode mobility, making it suitable for the extraction of swollen endo-parasitic stages and eggs.

Materials Required:

- Dissecting microscope
- Petri dish
- Dissecting needles
- Pair of forceps
- Handling ('fishing') needle
- Painting brush
- Clean water

Procedure:

1. Wash the collected plant material carefully
2. Make section of the sample with dissecting needles and forceps, under dissecting microscope
3. Place the dissecting plant materials into a Petri dish filled with clean water
4. Picked the emerging, crawling out nematodes from the suspension with a handling needle or painting brush under dissecting microscope
5. Preserved the crawled nematodes

Video: Sampling

https://www.youtube.com/watch?v=U7x0xHoKqC8

https://www.youtube.com/watch?v=dKsjI4Rgabg

Video: Extracting

https://www.youtube.com/watch?v=GrBrbJtvCfc&t=99s

Extraction of nematodes from soil sample by Decanting method

Objective: To learn the process of nematode extraction from soil samples

Materials Required

- Beaker (2- 1000 ml)
- Stirring rod
- Plastic bowls of ±4 litres
- Decanting tray
- Extraction sieve (ø 16 cm)
- Clamping ring for securing the nematode filters
- 2 nematode filters or equivalent
- Watch glass (ø 6 cm)
- Extraction dish
- Clean water

Procedure

1. Take a sample (50 gm) following the guidelines in lesson 12 and put it in a beaker and add approximately 1 litre of water.

2. Make uniform suspension of water and soil by continuous stirring with a glass rod. After 15 seconds, decant the supernatant into a bowl.

3. Repeat the stirring and decanting process for another 2 times and discarded the sediment finally.

Cleaning the suspension with nematode filters:

4. Attach 2 nematode extracting filters with a clamping ring and place it into a extraction sieve. Spray the water to the filter with sprayer to remove air bubbles in between the filters.

5. Take a decanting tray filled with water and place the sieve in it

6. Place a watch glass on the filters.

7. Pour the suspension carefully from the bowl to the watch glass on the filter.

8. After pouring all the suspension, remove the watch glass carefully and rinse it

9. Lift the sieve and remove the clamping ring.

10. Take an extraction dish containing sufficient water (100ml) in an area free of vibrations.

11. Place the well-drained sieve in the extraction dish. Care should be taken during placing of sieve into dish to avoid contamination of dirt from filter. The dish should be covered with thin cloth to check evaporation and dust particle falling in.

12. Like this condition, extraction dish should be allowed to standby for 16-48* hours. Then, remove the sieve and pour the nematode suspension into a 100 ml beaker.

Video link:

https://www.youtube.com/watch?v=yk5XxTtCbPc

Observation and conclusion:

Techniques of permanent mounting of nematodes

Objectives: To make the permanent microscopic slides of plant parasitic nematodes.

Permanent mounting in glycerine can be permanently stored and used for future reference. Permanently mounted slides can be stored for long time.

Materials Required:

- Cobb slide
- Square cover slip (25 x 25 mm)
- Circular cover slip (18 mm)
- Two pieces of cardboard (25 x 25 mm)
- Pair of forceps
- Handling ('fishing') needle
- Painting brush (No. 1)
- Glass fibre
- Dehydrated glycerine
- Ethanol 96%, Glyceel (nail varnish)
- Dissecting needle with flattened and sharpened point
- White spirit

Procedure: Permanent slide of mounted nematode will be prepared with following standard protocol:

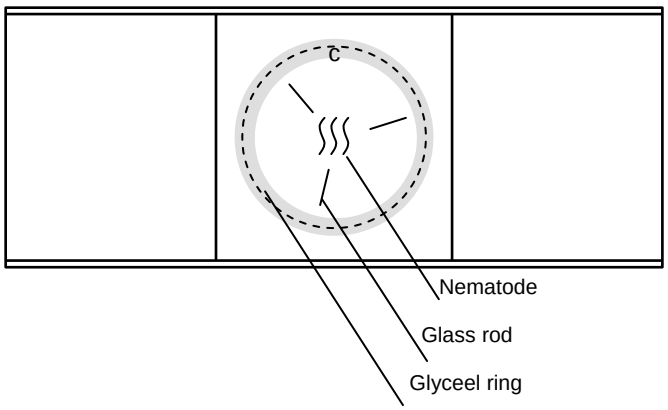
Using of a dissecting microscope, transfer the nematodes to a drop of anhydrous glycerine on a square shaped cover slip inserted into a Cobb slide. Transfer the nematodes by using fishing needle in such a way that they settle at the bottom of the droplet

Arrange 3-4 pieces of glass fibre (1 mm long) on the bottom of glycerine droplet and around the nematodes

Place a cover slip with help of a pair of forceps

Remove the excess glycerine with filter paper

Encircle the round cover slip with glyceel. The glyceel needs to dry for one night; after that, the mount can be studied. Fold the sides of the mount-holder and use the pieces of cardboard to write specifications on with waterproof ink.



Observation and conclusion:

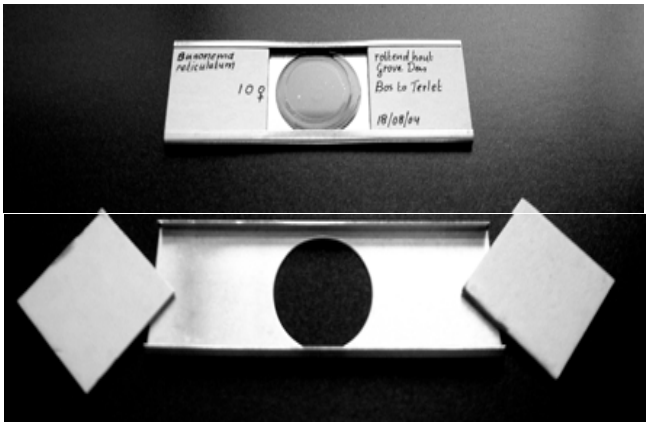


Fig:Prepared Cobb slide, and an aluminium holder with two cardboard pieces.

Video link

https://www.youtube.com/watch?v=OeyY6tbTI9I

Preparation of fungicide spray solutions for field application

Objective: To prepare the fungicidal formulations like solutions, slurries and pastes and their applications.

The objective of the fungicide application is to keep the plant disease under check. Fungicides are principle materials that are need to be dissolved into water to make uniform solution for distribution over large spraying area. There are different types of formulations of the fungicides in market. Most commonly used formulations are as under:

a) Solid

- **Dusts-** Dusts are free flowing powders containing technical material in the range of 2 to 10 per cent and inert carrier.
- **Granule-** Granule products contain technical material in the range of 3 to 10 per cent and granule base.
- **Water dispersible powders or Wettable Powders (WP) -**Water dispersible powders or Wettable Powders (WP) are free flowing powders containing technical material mostly in the range of 25 to 75 per cent, and contain wetting and dispersing agents and carriers.

b) Liquid

- **Water soluble liquids (SL) -** Water soluble liquids (SL) are liquid formulations based on technical material which are insoluble in water and contain 36 to 85 per cent technical material and solvent.
- **Emulsifiable concentrates (EC) -** Emulsifiable concentrates (EC) are liquid formulations based on technical material which are not soluble in water and contain 25 to 80 per cent technical material, solvent and emulsifier.
- **Fumigant-** Fumigant formulations used for indoor application for storage of grains.

Materials required:

- Fungicides of different formulations
- Water
- Bucket
- Sprayer

Procedure:

1. Calculate the amount of required fungicides

2. Mix the measured quantity of fungicides into small amount of water i.e. 5-10L of water
3. Add the required quantity of water to make the volume as to be sprayed
4. Add little amount of sticker (in case of rainy days)
5. Keep stirring of the chemical suspension with wooden rod so that the fungicidal suspension does not settle down
6. Each fungicide formulations are available in market is labelled with the amount of active ingredient (a.i) either weight by volume or weight by weight basis. As for example, 50% WP Captan and 75EC Propiconazole. Therefore, calculation is needed to make working solution before spraying.

Video link:

https://www.youtube.com/watch?v=DQTcCBSffGI

Few examples of calculation:

1) 25 EC, **ABC** fungicide is recommended to spray at 1.5 kg a.i/ ha. Find out quantity of formulation and water required to spray 3 ha area at 500 L/ha?

Answer- Spray solution volume required for 3 ha area = 3 x 500 = 1500 liters a.i.

For 3 ha, a.i required = 1.5 x 3 = 4.5 kg. a.i. of ABC fungicide

25 EC means there is 250 gm a.i in 1000 gm of the formulation.

That is,

250 gm a.i in 1000 gm formulation

4500 gm (4.5 kg) =1000 x 4500/250

= 18000 gm or 18 kg

Assuming the specific gravity as one, formulated fungicide 18 liters is needed be sprayed.

Volume of solution needed = 1000 liters

Volume of pesticide to be mixed = 18 liters

Volume of water needed = (1000-18)=982 liters

2) Fungicide ABC 35 Ec is recommended for spraying at 0.07% concentration solution. How much formulation is required to be added to water to spray one hectare area with 100 liters water?

Answer-

1% =1/100

0.1% =1/1000

0.01% =1/10000

0.07% =7 /10000, or 7ml in 10000 ml (10 L)

So, 70 ml (a.i) in 100 L

As 35 EC is 35 ml a.i in 100 ml formulations

So, 70 ml a.i will be in 100 x 70/35= 200 ml formulation.

Exercise:

Q1. How many gms of active ingredient are there in a 10 kg bag of a 50WP Captan? (Hint: For dry formulations, active ingredient is measured in percentage active ingredient by weight of product)

Q2. How many Chlorothalonil 75%WP are needed to supply 15 gms of active ingredient?

Q3.Chlorothalonil is mixed in 1 litre of water. Calculate the strength of the solution of Chlorothalonil in ppm.

Q4. A farmer applied 1500 g a.i. Carbendazim/ha. Calculate the dose of Bavistin (50 WP) in g/lt he applied in the field. (Volume of water for spray 500 lt/ha).

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