B.Sc. III Year (Theory)
Semester –VI
Paper XX (C)

(Microbiology and Disease Management)

45L

Unit-1 Credit-1

1. Microbiology

Microorganisms in biological world, their classification and features of different groups (03)

2. **Microbial techniques**:

a. Microscopy – simple, compound and electron microscope

b. Micrometry – Principle, working and uses

c. Staining – common stains used in pathology,their preparation and significance, (cotton blue and Gram's Stain)

d. Sterilization of glasswares and media (06)

3. Culture media for isolating plant pathogen

Industrial application of microorganisms - organic acids, alcohol,milk products, antibiotics and biopesticides (06)

Unit-2 Credit-2

Disease management:

1. Preventive methods: field sanitation, use of clean planting material, crop rotation, trap crops, time of sowing, planting distance and tillage (02)

2. Control methods –

a. Seed treatment: concept, objective, traditional and modern methods of seed treatment (02)

b. Soil sterilization: concept, objectives and methods (02)

c. Fungicides: Definition, classification and ideal characteristics of fungicides, study of fungicides with respect to active ingredients, formulations, methods of application, mode of action and uses (08)

i. Sulphur fungicides – Inorganic – Wettable sulphur, Organic – Thirum

ii. Copper fungicides

iii. Mercuric chloride – Agrosan – GN

iv. Heterocyclic nitrogenous compounds – Captan

v. Benzene compounds – Dexon

vi. Antibiotics – Streptomycin and Aureofungin

vii. Systemic – Bavistin and Vitavax

d. Pesticides:Nicotin,Neem and pyrethrum (01)

e. Rhodenticides – Zinc phosphoid (01)

f. Nematicides- Nemagon, Propoxar (01)

g Weedicides- 2,4-D (01)

h. Biological control- definition, need, examples and role (02)

Plant quarantine (01)

3. Control measures and environment: pollution due to chemicals, residual

effects, toxicity, safe measures, colour code, antidote, symptoms of
45
poisoning, precautions in using pesticides (03)

4. Pesticide application equipments: principle and working –pneumatic air pump knapsack sprayer, mist blower and duster, types of nozzles (03)

5. Plant clinic: Concept, objective and need (01)

6. Recent techniques in plant pathology: Genetically modified organisms (GMO's), B.T.Cotton, Pheromones (02)

1. MICROORGANISMS

There are evidences to believe that microorganisms were the first of the multitude of biological forms to appear on this planet. The other biological forms are believed to have appeared on earth several million years later. By the time, man learnt to cultivate plants for his benefit, about 10000 years ago, microorganisms had already multiplied in number and spread universally. They became associated with both animate and inanimate objects. Their continuous association with various objects, particularly with other biological systems, has resulted in certain adaptation and special features among them. Such features have caused evolutionary changes to result in different forms and types of organisms. The association between plants and microorganisms has become increasingly complex. In the process of evolution, such organisms which were not virulent enough to compete with others disappeared, while the most virulent ones survived. Such changes continue to take place in a never-ending sequence.

One of the human activity is the domestication of plants and animals. When he had sown the seeds in soil, microorganisms are already there to influence them; when grains were harvested and stored, some of the organisms from the field accompanied them to the bins. Thus the continued association between microorganisms and plants in various stages of growth has created certain types of inter-relationship. Some examples of close association are the mycorrhiza-pine root relationship, the legume root nodule – bacteria relationship, and various types of pathogen- host plant relationships.

Taxonomy and Classification of Microorganisms:

In the broadest sense the term microorganisms refers to all minute or microscopic organisms including fungi, bacteria, viruses, protozoa, microscopic algae and nematodes. The specific discipline governing the study of microorganisms is called microbiology, which has several specialized branches dealing with specific groups. Thus, mycology deals with the study of fungi, bacteriology with the study of bacteria, virology with the study of viruses, protozoalogy with the study of protozoa, algology with the study of algae and nematology with the study of nematodes. The taxonomy of these diversified groups of microscopic organisms is not limited to either Plant or Animal kingdom, as some of them closely resemble plants while others resembles animals. For example, fungi are considered as lower plants while protozoa have animal-like characteristics. For the present purpose these organisms are listed under the existing international system of classification of plants and animals.

Whittaker (1969) proposed a five-kingdom system and placed fungi in a separate kingdom, coordinate with plants and animals. The two primitive kingdoms are the Monera (Prokaryotes) and Protista (Unicellular eukaryotes). Whittaker proposed that three nutritionally distinct lines have developed from the protista which characterized the three kingdoms. They are: 1) The Plant kingdom, characterized by photosynthesis, 2) The Animal kingdom, characterized by ingestive nutrition; and 3) The fungi, in which nutrition is absorptive.

Main Groups of Microorganisms:

I) Bacteria: They are the true prokaryotes. The group include true bacteria (eubacteria) and related organisms i. e. archeobacteria, blue-green bacteria, mycoplamsas, rekettsiae and chlamydiae.

They are prokaryotes. Reproduction is by simple fission, nutritional processes are very diverse. Many can thrive in both oxygen rich and oxygen free environments. Growth occurs over a wide range of temperatures and usually at a neutral pH. They occur in almost every terrestrial and aquatic environment.

The rekettsiae, chlamydiae and mycoplasmas are also prokaryotes. The rekettsiae and chlamydiae are intercellular parasites of eukaryotes. The rekettsiae are tiny rods that are transmitted by arthropods and multiply only in living cells. The chlamydiae are among the smallest bacteria which were formerly considered viruses. The mycoplasmas are the smallest organisms that can be cultivated outside living tissues. Some are involved in lung disorders.

Intracellular parasites of prokaryotes is a group of small, highly motile bacteria, that adhere to the host wall, penetrate through to the periplasm where they replicate, causing eventual lysis of cell. Organisms of this type are called bdellovibrios and perhaps occuyr in soil.

The blue green bacteria are typical prokaryotes. They evolve oxygen during photosynthesis and have chlorophyll a. they are very ancient. They are perhaps major primary producersin the world's oceans and many are ecologically important as fixers of atmospheric nitrogen.

II) Actinomycetes: Majority of them are mycelia. They are Gram positive prokaryotes, a large group of filamentous bacteria which show branching pattern just like those of fungi. They produce spores when grow in culture. They are easily isolated from soil. Most of these produce antibiotics and so are of great value to the pharmaceutical industry.

Ex. *Streptomyces, Micromonospora, Actinoplanes, Streptosporangium, Actinomyces, Streptococcus, Lactobacillus, Leucomonas, Staphylococcus, Micrococcus, Sarcina.*

III) Protozoa: They are unicellular, eukaryotic animals and are non photosynthetic. They obtain their food by phagocytosis and are without true cell wall. They are involved in many blood and tissue diseases. They are motile.

1. The Protozoa exhibits amoeboid movement or flagellar movement or ciliary movement.
2. Food taken by phagocytosis of solid particles such as bacteria.
3. Generally they lack cell wall.
4. Degree of specialization in single cells.

Some workers also study some slime molds and water molds along with protists. They have been traditionally studied by mycologists. However, they are protists phyllogenetic ally. The slime molds form like fungi, macroscopic fruit bodies. But their feeding phase is amoeboid. They live on surface of decaying vagatation. There are two main groups: a) the cellular slime molds, and b) acellular slime molds.

The water molds are of two main types, the oomycetes and the chytrids, originally included among the fungi. They are shown phyllogenetically protists because they form flagellated motile cells. However, their nutritional processes and vegetative ststes appear more like fungi than protists.

IV) Algae: They are pigmented eukaryotes microscopic to gient. The chief characters are as follows:

1. The process of photosynthesis occurring in chloroplasts. The pigments are variety of chlorophylls and carotenoids with chlorophyll a.
2. They exhibit a wide range of morphological types.
3. Many are motile by flagella.

4. Cell wall characteristically of polysaccharide made of components as pectin, cellulose or xylan. Wall of some red seaweeds contain the agar gel.
5. Reproduce sexually or asexually showing very rare complicated life cycles.

V) Fungi:

1. Heterotrophic eukaryotic microbes obtaining their food in a soluble form by uptake through plasma membrane in a manner similar to prokaryotes.
2. Have thick cell wall made of polysaccharides with chitin.
3. Motile stages absent, never form flagella.
4. Mycelia may be coenocytic or septate.
5. Mycelium made up of individual filaments, the hyphae. Some are unicellular (Yeast).
6. Asexual reproduction by variety of spores.
7. Sexual reproduction is also produced. In some large fruit bodies are produced. Life cycles may be simple or complex.

VI) Viruses: They are acellular, infectitious agents. They have no associated metabolism. They have no such activities except replication that takes place in cytoplasm of living cells. They consist of fragments of nucleic acid surrounded by a layer of protein. They causes important diseases.

Viroids are minute infectious agents of plants which are composed of single naked RNA molecules. Viroid RNAs are very unusual they are circular, uniquely folded and so small that the largest one so far described is only 371 nucleotides long about $1/10^{th}$ of the size of the smallest RNA virus. Viroids are known to cause diseases to plants only.

Prions or slow viruses are a group of recently discovered infectious particles. Prions appear to be proteins or glycoproteins with no associated nucleic acid of any kind. Since replication of prion-protein occurs by normal process, this implies that these proteins may be able to bring about the production of their own requisite mRNA.

2. MICROBIAL TECHNIQUE

A) MICROSCOPY

There were no suitable means of magnifying invisible organisms prior to the 17th century. Anton Van Leeuwenhock is honoured for providing the first accurate report on occurrence of bacteria with the help of his single lens microscope of simplest possible design. He could make lenses and using them to build magnifying glasses to provide a magnification of about 200 times.

Robert Hooke had used a compound microscope in 18th century, but these were incapable of good performance due to defects. During the 18th century these defects were gradually overcome by the following refinements:

1. Corrected eye piece and object lenses.
2. A condenser to focus light on the object
3. A thin glass cover slip to place over a liquid drop on a glass slide so that object within the liquid could be viewed in a flat plane
4. The oil-immersion lens to increase resolving power.

Later there took place many refinements in microscopy during the last century. At present different kinds of microscopes are available.

I) Simple or Dissecting Microscope:

It is a simple microscope consists of only one lens unit. This lens unit may even be an ordinary magnifying glass. Dissecting microscope is used either for dissecting the material or for less magnification i. e. only 5X, 10X or rarely 20X. It is mainly used for taxonomic studies, embryo separation etc.

A dissecting microscope consists of a basal foot and a limb. The stage made up of a simple glass plate, is attached to the limb. For light adjustment purposes a mirror is attached to the limb under the stage. Mirror can be moved vertically with the help of an adjustment screw at the tip of the limb is present a folded arm, on which a lens of definite magnification is fitted. Folded arm is moved to keep the lens in the desired position on the stage. The material to be viewed is placed on the stage. The eye is placed close to the lens. Folded arm is tilted to bring the lens over material. Light is adjusted by movement of the mirror. Focusing is done with the help of adjustment screw.

II) Compound Microscope:

It consists of two or more lens systems. At the top present the ocular lens. It can be turned around or may be removed. At the top of ocular lens is written 5X or 10X signifying the 5 times or 10 times magnification respectively. Just below the ocular is a body tube, the bottom end of which contains a circular piece, called nose piece. It contains three lenses called objective lenses. Nose piece can be rotated to change the position of objectives. The flat platform present below the objectives is called stage. On the arm of the microscope are

present two knobs called coarse adjustment knob and fine adjustment knob. Out of the three objectives, the shortest is the low power objective. It has the largest lens but its magnifying power is least. On the objective may also be written 10X. It means if a 10X ocular lens is used the magnification is 10 X 10 =100. The other objective is high power objective. Its magnification is equal to the number written on it multiplied by the power of ocular i.e. 5X or 10X. The third objective is called oil immersion. Generally it contains a black band around the lower end. Use a drop of oil on the slide at the time of studying with the oil immersion objective. Just below the stage is the condenser. Its function is to gather light from the mirror and direct it to the objective lens. Condenser may be lowered or raised by the knob present on the side of the microscope beneath the stage. Condenser contains a shutter called Iris diaphragm. Just below the condenser is present a mirror having its one surface flat and other con cave. Use the concave surface in the day light. Flat surface of the mirror is used when electric lamp is applied.

III) Electron Microscopy:

The main point is the unusual short wavelength of the electron beams, substituted for light energy. The wavelength of about 0.005nm increases the resolving power of the instrument to fraction of nanometer. It makes possible to see viruses and large molecules clearly.

Two types of electron microscopes are in use today:

1) Transmission Electron Microscopy (TEM):

This is used to see the fine structure of cells. Ultra-thin sections of the objects are prepared by embedding or freezing the specimen and sectioning it with a diamond or glass knife. Sections are floated in water and picked up on a wire grid. They are stained with a heavy metal to make certain part dense, and inserted in the vacuum chamber. A 10000 volt electron beam is focused on the section and manipulated by magnetic lenses. A photograph prepared from the image may be enlarged with enough resolution to achieve a total magnification of over 20 million times. Objects as small as 1.0 nm may be observed.

2) Scanning Electron Microscopy (SEM):

This microscopy allows surfaces of objects to be seen in their natural state without staining. The specimen is put in to the vacuum chamber and covered with a thin coating of gold to increase electrical conductivity and thus forms a less blurred image. The electron beam then sweeps across the object building on image line by line as in a TV camera. As electrons strike the object they knock loose showers of electrons that are captured by a

detector to form the image. Magnifications with this microscopy are limited to about 75000-100000 diameters.

B) MICROMETRY:

Micrometry is the measurement of the microorganisms. Since microorganisms can be seen only under a microscope, a suitable scale for their measurements should be somewhere in the microscope itself. Usually the micrometer is of two types a) ocular micrometer and b) stage micrometer.

a) Ocular micrometer: Ocular micrometer serves as a scale or rule. Ocular micrometer is simply a disc of glass upon which are lines. These are usually 100 equally spaced divisions marked 0 to 10 upon ocular micrometer. When placed in the ocular (eye piece) the ruled lines superimpose certain distance markers on the microscope field. However, the scale on ocular micrometer does not have any standard value. We can find out the value of one division of this unknown scale by calibrating it with a known scale. Thus actual value of one division of ocular micrometer is found by using stage micrometer.

b) Stage micrometer: Stage micrometer is a microscope glass slide having in its center a known (1mm) distance etched in to 100 equally spaced divisions. This 1 mm (1000µm) distance is encircled and mounted by a cover glass. Thus each division of stage micrometer is equal to 0.01m or 10 µm. the distance of each division of stage micrometer becomes correspondingly enlarged under high power and oil emulsion objectives of the microscope.

Ocular micrometer is there fore calibrated under different objective lens system of the microscope. By determining how many divisions of ocular micrometer superimpose a known distance on the stage micrometer, we may find out the exact value of 1 division of ocular micrometer in the microscope field. Once calibrated, the ocular micrometer can be used to measure the size of various microbes in terms of length, breadth and diameter.

Procedure:

1. It is noted that which objective lens is in use on the microscope
2. Remove the ocular (eye piece) lens and insert the ocular micrometer on the circular shelf.
3. Remove the eye piece and mount in the microscope and observe, there will be seen scale lines ocular micrometer in sharp focus, the lines and distance will remain unchanged under different objectives.
4. Mount the stage micrometer on the microscope stage and bring its scales in the centre of microscope field under a sharp focus. This is done first low power objective there after high and oil immerson objectives.

5. The eye piece is rotated in such a way that the ocular scales and stage micrometer scales are parallel.
6. The stage micrometer is moved so that the first division marks of the two scales are in line. One can now see how many divisions on the eye piece as well as on the stage micrometer scale correspond to each other.
7. Since 1 division on the stage micrometer equal to 10 µm one can find the value of one division of the eye piece scale.
8. Calculate the value of one division of ocular micrometer for different objective.

e. g. 100 divisions of stage micrometer = 1mm = 1000 µm

1 division of stage micrometer = 0.01mm = 10 µm

If 40 divisions of ocular micrometer = 60 divisions of stage micrometer

= 60 X 10 µm = 600 µm

1 division of ocular micrometer = 15 µm.

e.g. 6 occular divisions = 8 stage micrometer divisions.

6 occular divisions = 0.8mm

1 occular division = $\frac{0.8}{6}$ mm = $\frac{0.8 \text{ X } 1000}{6}$ = 13.3 µm

Thus the microscope is calibrated for different combinations of eye piece and objective lens and kept for record.

Use: Having calibrated the eye piece scale for all the objective lenses on the m icroscope, one can use it to measure the dimensions on cellular and sucellular structures e.g. Bacteria, fungi, epidermal cells.

C) STAINING IN PLANT PATHOLOGY

1) Simple stain technique: A small amount of bacteria is placed in a drop of water on a glass slide, and then air dried it. The slide is passed through a flame in a process called heat fixing which fixes the slides, kills most organisms and prepares them for staining. Now the slide is flooded with a basic dye such as crystal violet or methylene blue for a minute. The positive charged dye is attracted to the bacterial cytoplasm which has a negative charge and staining takes place. This is effective for vegetative cells, the stain do not easily penetrate spores.

2) Negative stain technique: It is opposite to simple stain technique. Bacteria are mixed on a slide with an acidic dye such as Congo red or black stain negrosin. The mixture is smeared across the face of the slide and allowed to air dry, because the stain carries negative charge. The stain gathers around the cell, since a chemical reaction has not takes place, heat fixing has been avoided, and the cell appear less shrivelled or distorted. They often appear large than stained cells and more natural.

3) Gram stain technique: A Danish scholor Christian Gram in 1884 deviced a differential staining procedure, which differentiate between two kinds of bacteria Gram positive and Gram negative bacteria. The procedure is called Gram staining technique.

1. A thin smear of bacteria is prepared on the slide.
2. To the smear crystal violet solution is applied for 30 seconds.
3. The slide is gently rinsed in clean water and iodine solution is applied for 30 seconds.
4. This in turn rinsed off.
5. If the slide is examined, all cells would be deeply stained and appear blue purple.
6. Then 95% ethyl alcohol is applied and this is renewed until all but the thickest parts of the smear have ceased to give off the dye.
7. This usually takes from 20 seconds to one minute.
8. Microscopic examination of the slide will reveal that Gram positive bacteria retain the violet iodine combination (retaining and, blue-purple colour after alcohol wash).

Whereas Gram negative ones loose the blue purple colour after alcohol wash. Those species retain the stain are called Gram positive whereas those which yield the stain to alcohol are called Gram negative bacteria.

	I	II	III	IV
	Crystal violet	Iodine	Alcohol wash	Saffranin
Gram +ve cell				
	I	II	III	IV
Gram –ve				
	Purple	Blue purple	Loses stain	Orange red

Then there is applied counter stain a dye of some contrasting colour i.e. eosin (red), saffranin (red), light green. Exact of these colours the Gram negative species the cells become clearly visible. It has been postulated that since Gram negative bacteria have relatively high lipid content in their cell walls, the alcohol dissolve the lipid that allows the leakage of crystal violet-iodine complex. The Gram positive bacteria with less lipid in their cell wall, less susceptible to the action of alcohol.

1) Cotton Blue:

Aniline Blue 0.1 gm
Phenol 25 gm
Glycerine 25 cc
Lactic acid 25 cc
Distilled water 25 cc

This stain is used for staining various fungi.

2) Lactophenol:

This mounting media can be prepared by adding equal quantity of phenol, lactic acid, glycerine and distilled water.

Phenol 25gm
Lactic acid 25 cc
Glycerine 25 cc
Distilled water 25 cc

This medium is used for staining of various fungi.

D) METHODS OF STERILIZATION:

Sterilization is the complete destruction or removal of all living organisms from the object being sterilized. Experiment designed to prove or to disprove spontaneous generation depended upon two general principles:

1) The complete sterilization of a suitable growth medium so that no living organisms exist at the start of the experiment.

2) The design of the vessel of a type that it is impossible for microbes to enter from outside.

If these two principles are strictly followed and conditions are otherwise suitable for multiplication of microbes, any growth occurring must be the result of spontaneous generation.

The attainment of sterility:

The usual method depends upon heat treatment. However, it was soon realized that microorganisms vary widely in their resistance of heating. Bacteria require higher temperature and some also produce heat stable spores. Thus boiling at normal pressure was insufficient to kill these spores and therefore autoclave was designed to increase the pressure and temperature. Sealing of flask was not proper as oxygen could no longer enter the vessel. It is necessary to include some kind of filter to prevent the entry of microbes but not of air. This led to the development of the cotton wool plug that was soon adopted by microbiologists. Some of the currently used sterilisation methods are summerised below:

1) Heat: For general sterilisation a time and temperature that kill organisms including heat-resistant spores is used. The methods generally adopted are as follows:

a) Wet heat in autoclave: the usual method is a time of 30 min. at a pressure of 1.05kg/cm^2 that will give a temperature of 121^{0}C. this is the best practical method.

b) Tyndallisation: This is a course of three periods of boiling at 100^{0}C for 30 min. at daily intervals.

c) Dry heat: This is done in a dry oven, where a temperature of 160^{0}C for two hours is required.

2) Filtration: The liquid or gas to be sterilized is passed through a filter with porosity sufficient to remove any microorganism in suspension. The cotton wool is used for gases. For liquids, a variety of filters are available made of materials such as cellulose nitrate. This method is very useful for sterilisation of liquids containing heat liable components.

3) Radiation: Ultra violet light is very effective in sterilisation of air. For solids, gamma rays or X-rays from radioactive cobalt are used. Ionizing radiation is often used to sterilize plastics and other heat liable materials.

4) Chemicals: Many chemicals are lethal to microbes. Hypochloride solution and phenolic derivatives are used as general laboratory disinfectants. Similar chemical is gaseous ethylene oxide. However, these may not cause sterilisation under some conditions.

3. INDUSTRIAL APPLICATIONS OF MICROORGANISMS

Activities of microorganisms are very important to almost every sector of concern to mankind. Microorganisms are useful in agriculture, forestry, food, industry, medicines and environment. The scope of microbiology has enlarged manifold. Application of microorganisms in the industries are discussed below.

1) Organic acids: several organic acid including acetic, glucanic, citric, itaconic, gibberellic and lactic acids are produced commercially through microbial transformation.

Gluconic acid is produced by various bacteria including *Acetobacter* spp and several fungi such as *Aspergillus* and *Penicillium* spp. *A. niger* converts glucose to gluconic acid in a single enzymatic reaction (glucose oxidase). Fermentation is carried out at 30^0C with aeration and agitation. Gluconic acid is recovered by adding calcium hydroxide to form crystalline calcium gluconate. Free gluconic acid can be recovered by adding the acid.

Citric acid is also produced by *A. niger*. Citric acid is used as food additive, especially in soft drinks, as a metal chelating and sequestering agent and as a plasticizer. It is essential to limit the growth of the fungus so that high levels of citric acid can accumulate. The medium contains molasses, ammonium nitrate, $MgSO_4$ and potassium sulphate. Acid is added to lower pH. Metals added to the medium are removed from solution by cation exchange resins.

Itaconic acid is used as a resin in detergents. The transformation of citric acid by *Aspergillus terreus* can be used for commercial production of itaconic acid. Fermentation process involves a well aerated mineral salts medium at a pH below 2.2. At higher pH this microbe degrades in itaconic acid. Low levels of trace metals must be used to achieve acceptable product yield.

Gibberrellic acid and related gibberellins are important growth regulators of plants. Commercial production helps in boosting agriculture. The source of this acid is fungus *Gibberella fujikuroi*. This can be produced commercially using aerated submerged cultures. A glucose mineral salt medium incubation at 25^0C and slightly acidic pH are used for fermentation.

Lactic acid is used as preservative in foods, in leather production and textile industry. Various other forms of lactic acid are also used for other purpose. Fermentation is carried out by *Lactobacillus, Streptococcus* and *Leuconostoc* spp. The typical medium contains 10-15% glucose other sugar, 10% $CaCO_3$ to neutrlise the lactic acid formed and ammonium phosphate and nitrogen sources in trace. Carbohydrate sources used for fermentation;

temperature of 45-50^{0}C and pH 5.5-6.5. Agitation is needed without aeration. Process is complete within 5-7 days with approximately 90% of the sugar conversion to lactic acid.

2. Alcohol:

Alcoholic fermentation have been well known since ancient time, through little was known of the nature of the process. Generally *Saccharomyces* are used to produce various types of alcoholic beverages. The process relies on alcoholic fermentation conversion of sugar to alcohol by microbial enzymes.

i) Beer: The fermentation of beer is usually a batch process. In some countries the production of beer is carried out in a continuous flow through process. Beer is a product of the fermentation of barley grains by yeast. Barley seeds are allowed to germinate. During germination, amylase covert starch to sugar most of which is maltase. The process is called malting and the digested grain as malt. The next step is mash the grain with water and removes the fluid portion called the wort. Dried petals of vine, *Humulus lupulus* called hops are then added to the wort to give it flavor, colour and stability. Hops also prevent contamination of the wort, due to presence of two antimicrobial substances in petals. At this stage the fluid is filtered out and yeast is added in large quantities.

The yeast commonly used in fermentation of wort is one of the many strains of *Saccharomyces cerevisiae* developed by brewers. Some yeasts give a uniform cloudiness to beer and are carried to the top of the fermentation vat by foaming CO_2. Other yeast ferments the beer more slowly and produces a light beer with less alcohol. These yeasts are known as bottom yeasts and their product as lager beer.

Generally one week time is needed for normal fermentation. After a week the young beer is transformed to vats for primary and secondary aging. It may takes about six months more. For canning and bottling the beer is to be pasteurized at 140^{0}F for 13 min. to kill the yeast or may be filtered to remove the yeasts. Some yeast is used to seed new wort, the rest for animal feed or pressed to tablets for human consumption. Alcoholic content of beer is roughly 4 pre cent.

ii) Wine:

Wine fermentations are carried by controlled cultures of *Saccharomyces ellipsoidseus*, a variety of *S. cerevisiae* or with the strains of yeast naturally occurring on grapes. The former is common in U.S.A. and the latter in Europe.

Wine is made from ripe fruits, fruit juice or plant extract such as dandelions. Fermentation usually begins with crushing of the fruit to produce must. Sulpher dioxide may be added to control the process. In natural fermentation SO_2 is not used and the yeast begin to

digest suger. Oxygen may be supplied to promote aerobic growth of the yeast. However, anaerobic conditions are established later. Alcohol production occurs within few days, though aging may take month or years. During this period secondary fermentation develops flavor, aroma and bouquet of the wine. Red wine becomes red as alcohol extract the colour of grape skin. For red wine fermentation is carried out at 24-27^{0}C for 3-5 days and white wine takes 1-2 weeks at 10-21^{0}C. Additional CO_2production yields champagne and other sparkling wines which are naturally carbonated. Sherry wines result from inoculation with special yeast to have unique flaours. In dry wines most or all of sugar is metabolized, where as in sweet wines, fermentation is stopped before entire sugar is consumed. The strongest natural wines have about 16 per cent alcohol. Most table wines average about 10-12 per cent alcohol, with fortified wines reaching 22 per cent alcohol. In fortified wines brandy or other spirits are added to produce port, sherry and cocktail wines. For mass production, the wine is pasteurized, filtered and bottled.

iii) Distilled spirits:

They contain more alcohol than beer or wine. Alcoholic content is shown by a proof number which is the twice the actual percentage of alcohol. The process of distilled spirit begins with same type as for wine and beer, except that after the fermentation process the alcohol is collected by distillation to allow higher concentration of alcohol. The raw product is first fermented by *Saccharomyces*, then aged and finally matured in casks. At this time the process differs. The alcohol is concentrated by a distillation apparatus using heat and vacuum. During maturing, flaours from the chemicals as aldehydes, ethers and volatile acids are added. The alcohol content is then standardised by diluting it with water before bottling. There are four basic types of spirits: brandy is made from fruit juice; rum from molasses; whisky frommalted cereal grains; scotch from barley and bourbon from corn. The neutral spirit as vodka is made from potato starch and left unflaoured and jin flavoured with juniper oil.

3. Milk product:

Many products are made through microbial fermentation of milk, including buttermilk, yogert and many cheeses. Fermentation is primarily carried out by lactic acid bacteria. The differences in the flavour and aroma of the various dairy products are due to additional fermentation products that may be present in very low concentration.

i) Buttermilk, sour cream, kefir and koumis: Different products are produced by using different strains of lactic acid bacteria as shorter cultures and different fractions of whole milk as the starting substrate.

Sour cream uses *Streptococcus cremoris* and *S. lactis* for producing lactic acid and *Leuconostoc cremoris* for characteristic flavour. Butter is normally made by churning cream that has been soured by lactic acid bacteria. *Streptococcus cremoris* or *S. lactis* is used to produce lactic acid rapidly and *Leuconostoc citrovorum* produces necessary flavours. Kefir and koumis popular in Europe are fermentation products of *S. lactis, S. cremoris* other *Lactobacillus* spp and yeast.

ii)Yogert: It is made by fermenting milk with a mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* at 40^0C. Flavour is due to accumulation of lactic acid and acetaldehyde.

iii) Cheese: Cheese consist of milk curd that have been separated from the liquid portion of the milk (whey). The curdling of milk is done by enzyme rennin and lactic acid bacterial starter cultures. Cheeses are classified as soft (high water 50-80% content), semihard (about 45% water) and hard (less than 40% water). They are also classified as ripened and unripened. Cottage and cream are soft unripened cheese; Brie, camembert and limburger are soft, 1-5 months ripened cheeses; blue, brick, gorgonzola, Monterey, muenster and Roquefort are semisoft, 1-12 months ripened cheeses, whereas cheddar and Colby are hard, 3-12 months ripened cheeses.

Natural production of cheese involves lactic acid fermentation with various mixtures of *Streptococcus* and *Lactobacillus* spp used as a starter cultures. The flavour results from use of different microbial starter cultures, varying incubation times and conditions and the inclusion or omission of secondary microbial species late in the process. Ripening involves additional enzymatic transformation after the formation of cheese curd. Various fungi are also used in the ripening of different cheeses. The unripened cheese is inoculated with fungal spores. Blue cheese is produced by *Penicillium* spp. Roquefort cheese is produced by using *P. roquefortii* and camembert and brie by using *P. camembertii* and *P. candidum*.

4. Antobiotics:

There are thousands of antibiotics produced by microbes in nature. Alexander Fleming (1928) observed some substance from molds inhibiting growth of bacteria and concluded that active principle from *Penicillium* possesses antibacterial properties. He called this substance as penicillin. Rene Dubos in 1939 indicated that soil bacteria could produce antibacterial chemicals. Howard Florey and the German biochemist Ernst Boris Chan reisolated penicillin and carried out careful trials with highly purified samples. In 1940 their successful attempts were published in 1945. Fleming, Florey and Chan received Nobel prize

for discovery and development of penicillin. Then a term antibiotic was introduced in medicine.

i) Penicillin: Among large group of penicillin darivatives, Penicillin G or benzyl penicillin is the most popular. Other types are penicillin F or penicillin V. all have the same basic structure with a beta-lactin nucleus and several attached groups.

Penicillins are active against a variety of Gram positive bacteria including *Streptococci* and *Staphylococci*. Penicillin functions during the synthesis of bacterial cell wall. There are two major drawbacks. One the anaphylactic reaction in allergenic cases causes swelling about the eyes or wrist, itchy skin etc. Second the evolution of some penicillin resistant bacteria that produce an enzyme, penicillase. This converts penicillin in to harmless penicilloic acid. Modern penicillins are produced from *Penicillium notatum* and *P. chrysogenum*.

ii) Semisynthetic Penicillin: In 1950 the betelactum nucleus of the penicillin molecule was identified and synthesised. Then various groups could be attached to this nucleus, creating a number of new penicillins now thousands of penicillins are prepared by semi synthetic process. Ampicillin is less effective against Gram positive cocci, but valuable against Gram negative rods. It can be taken orally and absorbed from the intestine. Amoxicillin and penicillin are useful to treat urinary tract infections. Carbeicillin is used for *Pseudomonas* and *Proteus* infections of urinary tract. Others are methicillin, nafcillin and oxacillin which are resistant to penicillase.

iii) Cephalosporins: They were developed in 1960. Cephalosporin C was isolated from the blue mold, *Cephalosporium*. A number related semi synthetic drugs developed from it are cephalexin, cephalothin, cephazolin and cephaloridine. These are alternatives to penicillin and are effective for Stephylococcal boils or wounds, Streptococci and bacterial pneumonia and urinary tract infection by Gram negative bacteria.

iv) Streptomycin (an aminoglycoside): This was discovered by Selman A. Waksman. It was produced from *Streptomyces griseus*. This drug was made available in 1947 and Waksman

received Nobel prize in 1952 in medicine. Streptomycin in combination with isoniazid is important for treatment of tuberculosis; Gram negative infections as plague, brucellosis are also treated.

v) Other aminoglycoside antibiotics: Gentamycin is the first drug to be given for Gram negative bacteria. It is combined with carbencillin for *Pseudomonas* infections, with ampicillin for *Staphylococcus* infections of intestine and with cephalosporin for staphylococcal disorders. Neomycin is now used as eye bacterial conjunctivitis or other Gram negative infections. Neomycin (Neosporin) combined with polymyxin are useful for variety of skin infection by bacteria. All aminoglycoside are derived from the species of *Streptomyces*.

vi) Chloremphenicol: this is the first broad spectrum antibiotic discovered. It was isolated in 1947 by Ehrlich, Burkholder and Gotlieb. It inhibits a wide variety of Gram positive and Gram negative bacteria as well as several rekettsiae and fungi. It was originally isolated from the metabolites of *Streptomyces venezualae*. Its advantage is that it prevents haemoglobin incorporation into the R.B.C.-aplastic anemia. Die to its accumulation in blood of new borne child; it causes a toxic reaction and sudden breakdown of cardio vascular system- grey syndrome.

vii) Tetracyclines: They are also broad spectrum antibiotics. They include naturally occurring Chlorotetracyclin and oxytetracyclin isolated from species of *Streptomyces*. They may be taken orally, though they have side effect problem. They are used in Gram negative infections as brucellosis, plague, cholera, for primary atypical pneumonia, as substitutes for penicillin in syphilis, anthrax, gonorrhea and pneumonia and therapy of some protozoan infections as amoebiasis.

viii) Other antibiotics: Erythromycin obtained from *Streptomyces* is useful for primary atypical pneumonia, staphylococcal and streptococcal infections and syphilis. Vancomycin also a product of *Streptomyces* is given intravenously against Gram positive infections. Other antibiotics are rifampin for leprosy and tuberculosis; clindamycin and lincomycin are active against Streptococci, Staphylococci and other Gram positive organisms.

Bacitracin and polymyxin are obtained from *Bacillus* spp. Bacitracin is used as ointment for staphylococci and the polymyxin for Gram negative bacilli. Streptomycin a product of *Streptomyces* became popular in 1970 as substitute for penicillin in case of gonorrhea.

ix) Antifungal antibiotics: Nystatin a product of the *Streptomyces* is used as cream or ointment for infection of oral cavity, vagina or intestine due to *Candida albicans*. Grisofulvin

is used for fungal infections of skin, hair and nails. It is effective against ringworm and aczema. This is a product of *Penicillium*. For serious systemic fungal infections, amphotericin B is used. It is effective for organisms of histoplasmosis, blastomycosis, cryptococcosis etc.

5. Biopesticides:

Several microbes are being developed as suitable biopesticides for management of insect and nematodal pests. Some fungi have good potential of their use as bionematicides to control nematodal pests of vegetables, fruits and cereal crops.

In order to minimise the use of chemicals in agriculture, biological control methods are being developed. Many of the microorganisms are used as pesticides to protect plants from the pests. Microbial populations can be used directly for controlling pests. Preparations of antagonistic microbial populations are called microbial pesticides. A microbial pesticides should be harmless to man and other valued plants and animal populations.

Microbial Insecticides:

The greatest commercial impacts of biocontrol agents have been made in the insecticide market. The most successful biocontrol agent so far been the insecticidal bacterium, *Bacillus thuringiensis*, whosae sales in forestry, agriculture and public health were much higher. Viruses, bacteria and fungi have been used as microbial insecticides.

a) Viral Insecticides:

Pathogenic viruses possess the potential for use as pesticidal agents. They attack insects and other arthropods the most commonly used viruses are, i) nuclear polyhedrosis viruses (NPV), ii) cytoplasmic polyhedrosis viruses (CPV), iii) granulosis viruses (GV). Pathogenic baculoviruses have been found principally for Lepidoptera, Hymenoptera and Diptera.viruses have been used in attempts to control outbreak of several insect pests including gypsy moth, Douglas fir tussock moths, pine caterpillers, red banded leaf rollers (pest of apple), spuce budworms, codling moths, alfalfa caterpillers, cabbage white butterflies, cabbage loopers, cotton bollworms, corn earworms, tobacco budworms, tomato worms etc. An interesting example of the use of viral pesticides is the attempt to control rabbit populations in Australia with myxoma virus.

b) Bacterial Insecticides:

There are several bacterial pathogens of insects that are being used at present as insecticides. They includes endospore forming *Bacillus* and *Clostridium* spas well as non endospore forming species of *Pseudomonas, Enterobacter, Proteus, Serratia* and *Xenorhabdus*. Of these *Bacillus thuringiensis* has been most extensively used. Commercial

preperations of *B. thuringiensis* are registered by more than 12 manufacturers for use in several agricultural crops, forest trees and ornamentals for control of various insect pests. This bacterium has been testedsuccessfully against more than 150 insect species. Four separate toxins are produced by *B. thuringiensis*. Several vegetable insect pests have been managed by this bacterium. About 16 formulations based on exo- and endotoxins are used in U.S.A., France, Germany, USSR and Czechoslovakia. Some registered products are Thuricide, Sporcine, Condor, Cutlass, Foil and Invade, mostly in USA. In India also trials have been made for thuricide against insect pests of lac, cruciferous crops and white grubs of sugarcane.*Bacillus thuringiensis* has potential to control mosquito vectors of malaria. In India trials have been made for *B. thuringiensis¸ B. papillae* and *Serratia mariscens* to control insect pests of sugarcane.

c) Fungal Insecticides:

Fungal insecticides could became most common and effective means of control of pests in some countries, chiefly in USSR. Products of entomogenous fungi have been used for insects of field crops, forest trees as well as horticultural and vegetable crops. Several preparations have been produced, formulated and used commercially in USSR, Brazil, Cuba and Israel. Different kinds of formulations have been developed and applied in different ways. The most studies on entomogenous fungi have been concerned with species of the genera *Aschersonia,Beauveria, Metarrhizium, Verticillium, Hirsutella, Coelomomyces* and *Entornophthora*.

Microbail Nematicides:

Most studies have been made with fungal nematicides. The fungi of genera *Arthreobotrys, Dactylaria, Dactylella* and *Monachrosporium* have been studied in trials to control nematode genera *Meldogyne, Heterodera* and *Rotylenchulus* attacking mostly vegetables crops. These nematodes cause cyst and root knot diseases. There have been some limitations in the use of nematophagous fungi for the control of nematodes. It is difficult to manage fungi so that periods of nematode migration and trap formation coinside. Another group of fungi are found ideal nematicides. These are opportunistic fungi such as *Verticillium, Chlamydosporium, Dactylella oviparasitica* and *Paecilomyces lilacinus* that also attacks eggs and young females of cyst and root knot nematodes.

Microbial Herbicides:

Fungi could be suitable for herbaceous weeds. In classical strategy, weed parasitic fungi (mostly rust fungi) are introduced. Several products of fungi have been used on commercial scale in different parts of the world.

CULTURAL METHODS OF DISEASE CONTROL

Cultural methods of disease control aim at reducing insect population or inoculums potential of pathogens; or preventing damage due to pests; encouragement of healthy growth of plants; preventing attack by changing various agronomic practices. The efforts involves except adjustment in the cropping system. Operations in these methods are directed towards field sanitation; clean cultivation; crop rotation; adjustment in the dates of sowing and planting distances; water and fertilizer management; tillage practices.

A) Field sanitation

It is an essential measure to reduce the inoculums of the pathogen to minimize the possibilities of the appearances of epiphytotics or epizootics. Measures adopted for field sanitation includes: a) destruction of crop residues, stubbles and self sown tillers; b) use of eradicant sprays where complete destruction of crop residue is not possible; c) eradication of affected parts and plant parts; d) eradication of weeds or other plants serving as alternate hosts in the off season and growing season; and e) tillage practices which will reduce the inoculums or lead to damage and destruction of resting stages of pathogen or eggs of insects.

a) Destruction of crop residues: Destruction of crop residues like dry leaves, sticks, stubbles, ear head or other plant parts results in the elimination of sites of hibernation and shelter for insects in the off-season. Quite often these plant parts are infected with pests. It has been observed that leaf blight (*Helminthosprium oryzae*) of rice is carried out in the stubbles. Infection of *Sclerotium rolfsii* on jute is carried over in the foot and root in the stubbles of the jute plant. Sugarcane stubbles left over in the field help to carry over red rot fungus. Rice stem borer insect *Scirpophagus incertula* and others are supposed to hibernate in the rice stubbles.

In many cases, diseased planting materials left in the field serve as sources of infection as in the case of late blight of potato where piles of refuses of rejected tubers later become an important source of infection. The proper disposal of straw of *Brassica napus* early in spring results in the infestation of painted bug. Pink roll worm (*Pectinophore gossypiella*) can be destructed by damaging cotton debris. It may be emphasised that crop residues constitute important source of infection and need to be eliminated.

b) Use of eradicant sprays: In the floor of orchards there may be substantial number of leaves. Effective disposal of them is not feasible. They constitute sources of infection in the next season. In such cases the use of an eradicant spray has been found to be useful. For ex. In the scab disease of apple, phenyl mercuric chloride (25gms of mercury/400 litres of water) is sprayed on the fallen leaves to reduce infection of ascospores.

c) Eradication of affected plant parts and plants: eradication schemes have been undertaken in a number of cases to solve the sudden appearance of a disease in an area. Physical removal of entire diseased plant is not the only method of eradication. Removal of affected plant parts may also reduce the inoculums as in canker of apple caused by *Nectria* spp and in similar cases including citrus canker. Removal of smutted inflorescences constitutes an important method of control in whip smut of sugarcane (*Ustilago scitaminae*) and also recommended to corn smut (*U. maydis*). The removal of branches and twigs of mango infected with angiospermic partial parasite *Loranthus* gives good results. Systemic destruction of the affected plant or part in the proper manner to keep down the population is resorted to reduce the damages caused by fruit flies infesting cucurbits, mango, guava, peach etc. and many tissue borers of plants.

d) Destruction of weeds, alternative, alternate or collateral hosts: Weeds or uneconomic unrelated plants harbour the pathogens and insects in the off-season when host plants are not available. Alternate hosts are necessary for completion of life cycle of number of fungi. It may not be possible in all cases to get rid of such uneconomic plants but in some cases good results may be obtained. Destruction of *Malva perviflora, Althea rosea, Malvastrum* spp during April-June reduces carry over of spotted boll worm of cotton. Eradication of *Sorghum haplense* keeps the population of sugarcane mites low.

In many cases weeds are perennial and too many weeds may be involved and it may not be possible to achieve any significant results. However, a clean cultivation should be aimed at to reduce inoculums and insect population and keep down chances of infection.

e) Tillage: Summer ploughing and upturning of the top layer of soil exposes the soil to summer heat resulting that the fungi and insects in the soil are destroyed to a considerable extent.

B) Use of clean planting materials:

Use of disease and pest free planting material is also an important method of clean cultivation, as many pathogens, nematodes and some insect pests are carried over in seeds and planting materials. In viral diseases, the most important practical measure is the use of virus free planting materials. The absence or presence of a very low level of initial inoculums is definitely helpful in delaying or suppressing the incidence of pests. This can be easily achieved by use of clean planting materials which may be considered as a major sanitation measure.

C) Crop rotation:

Rotation of crops or change in sequence of cropping pattern is in use for a very long time. In many cases this practice results in much less incidence of pests. It is most useful

against diseases caused by fungi and nematodes. Crop rotation is essentially a preventive measure and effected mainly on the succeeding crop.

The main object is to disrupt the continuity in the availability of host plant resulting that pathogen will face starvation and decline in their population is caused. Crop rotation is a very effective method of control of root diseases in field crops. In many cases, a break for one year by a non-susceptible host may be sufficient, particularly where the pathogens are soil inhabitant. For crop rotation, knowledge of life history with reference to host range, perenation, longivity of resting structures etc. is essential. The organisms should not be capable of remaining alive in the soil for a number of years and rotation should not include susceptible crop. It can not be practiced where pathogens are typical soil inhabitants and can live for a long time without any host.

D) Trap crops or secondary crops:

In many cases, an early cultivation in small area of a susceptible crop ahead of the main crop to draw the insects and to destroy them to reduce the damage is practiced. The crop is termed as trap or secondary crop which must be highly susceptible to the pests and should be destroyed before the main crop. Instead of growing ahead, they may be grown along with main crop and must be destroyed in time. Bhendi (*Abelmoschus esculantus*) is often sown with cotton to attract cotton jassid and spotted boll worm and the plants should be destroyed before the insect migrate to the cotton. Arhar (*Cajanus cajan*) may be used as a trap crop in mixed cropping with a cotton to remove attack of cotton grey weevil which shows preference to arhar in relation to cotton. Planting of wild beets with sugar beet is useful in reducing attack of beet nematode *Heterodera rostochiensis*.

E) Adjustment of sowing or planting:

Many plants are susceptible to attack of pests during a limited period and serious attack will result if the population or inoculums build-up takes place during that period. Adjustment in date of sowing may be profitably practiced to circumvent attack of pest by avoiding the peak period of attack. This circumvention can be effected in relation to space also. Plants may be grown in areas, where vectors are either absent or not active. This is taken advantage of in obtaining tubers free from virus diseases in growing potato in aphid free areas. Similarly seed multiplication in dry areas is often practiced to have a low level of seed borne infection by pathogenic fungi which are more active in humid areas.

F) Planting distance:

Spacing of plants in the cultivation may affect the intensity of disease incidence. Very close spacing of rice plants results in a more humid microclimatic condition which favours incidence of foliage blights brown plant hopper. Spacing at wider intervals has been found to be beneficial for avoiding attack of pests. Early spread of black rot of cabbage (*Xanthomonas campenstris*) takes place by plant to plant contact may be checked by avoiding planting at close distance. In respect to insect attack it has been claimed that in some cases close spacing may be beneficial. A balance has to be maintained between planting distance for maximum yield and consequent effect on microclimatic conditions favouring pests.

G) Tillage practices:

Depth of seedling sometimes affects seedling blight and damping off. Deep ploughing may cause delay in the emergence of seedlings, which may be vulnerable to pre-emergence damping off. Early emergence results in early lignifications of tissues which become resistant to attack of soil borne pathogens. On the other hand deep ploughing has been claimed to be beneficial for control of gram wilt. Deep ploughing may burry insects too deep in the soil for emergence. Many insect pests have been reported to be controlled to a large extent by deep ploughing.

SEED TREATMENT

Many pests during the absence of dormancy of the host perpetuate in the seed or propagative organs till a new host is found. Hence the treatment of seeds to get rid of infection and to secure healthy plant materials constitutes one of the major measures of plant protection. The application of fungicides to seeds has two fold effects: a) control of diseases caused by seed borne infection, b) protection of germinating seeds or seedlings from the attack of soil-borne pathogens. Appropriate treatment of seeds can get rid of the seed borne pathogens and can control to a large extent diseases. Incorporation of a protective chemicals on the surface of the seed can reduce the chances of infection consequently harmful effects of many soil borne pathogens.

The introduction of systemic fungicides for seed treatment has added further possibilities: a) control of pathogens located deep inside the seeds, which are inaccessible to other seed treating chemicals and b) control of air borne infection at a later stage of growth of crop, the toxicant being systemically translocated to aerial parts. There is no single method or material which can be universally recommended for treatment of seeds. These methods have to be chosen in accordance with the modes of perpetuation of pests. Broadly speaking, they may be divided in to three categories: a) mechanical, b) chemical and c) physical.

Mechanical Method:

In many cases due to infection there may be an alteration in size, shape and weight of seeds by which it is possible to detect the infected seeds and separate them from the healthy ones. In ergot disease, infecting sclerotia are large in size and lighter than grains. They may be separated out by sieving or floatation. In tendu disease of wheat caused by *Anguina tritici*, galls due to infection in grains can be separated by floatation. In many seed borne infections, infected seeds are usually smaller in size and lighter in weight. They may be separated out by gravity grading, floatation or sifting through sieves as may be convenient. Such mechanical separation eliminates infected materials to a large extent and in some cases (ergot and tendu diseases) this is the only method.

Chemical Method:

Disinfection of seeds by chemicals was common for a long time. In 1809, Prevost carried out his classical work on the use of copper sulphate for the treatment of cereal seeds infested with spores of bunt fungi. Chemical treatment also afford protection to the seeds and young seedlings in the early stages of growth from soil borne fungi by sterilizing the small

amount of soil around the seed and keeping it free from organisms during germination and early stages of seed establishment.

Very recently attempts have been made to control deep seated infections by the use of systemic fungicides for the control of: a) pathogens situated within the seed and previously inaccessible to chemicals, and b) air borne diseases using the dressing as a reservoir of fungicide during the growth of the crop at least in the early stages. Success of the use of systemic fungicides as seed dressing is now clearly established. Eoxathin and Carboxin are widely used for the control of deep seated infections of loose smut (*Ustilago nuda*). By use of pyrimidine ethirimol as seed treatment control of powdery mildew has been effected.

Treatment of seeds by chemicals may be affected by: a) steeping in liquid, b) dry seed treatment, or c) slurry treatment. Steeping in liquid may be done in buckets. Dry seed treatment usually carried out in rotary or gravity fed seed dressers. In dry seed dressing, powder which is applied in very fine from adheres to the surface of seeds. Slurry treatment in which the chemical is applied in the form of a thick soup, so that during the process of treatment slurry get deposited on the surface of the seeds in the form of a thin paste which dries up. Seeds treated with dry dust may be stored for a long period so also slurry treated seeds. But seeds treated by steeping in liquid can not be stored. Seeds treated with chemicals must be stored in dry and treatment should be done one week before sowing. In case of treatment with liquids, treatment has to be done immediately before sowing. Liquid treatment is usually followed for vegetative propagating stocks. Storing under damp conditions after treatment has been reported to damage the viability of seeds. Hence proper storage of treated seeds has to be ensured.

Organomercuric chemicals are called broad spectrum seed treating fungicides. They have shown effectiveness against a number of diseases in a m\number of different crops. Alternative chemicals include Chlorobenzene, Benzimidazole compounds like Captan, Carboxin. Many chemicals used for the seed treatment are poisonous and toxic to men and animals and they should be used with caution. Chemical treated seeds should not be used for consumption by men and animals. Sacks, bags or other containers used for storing treated seeds should not be used for other purposes before thorough cleaning. Inhalation of fumes or dust during the process of treatment should be avoided. Extreme care should be taken to avoid the skin coming into contact with these chemicals. Seed treatment should always be carried out in the open. Instructions given by the manufacturers regarding safety, dosage, and handling should be strictly followed.

Physical Method:

Seeds and planting materials can be subjected to heat treatment in order to eliminate certain internally borne pests including nematodes. Temperature and period of treatment will vary with the infection or pest concerned. The use of heat as an agent of disinfection was first made by Jensen who attempted to control the internal infection of diseased tubers of potato affected with late blight (*Phytophthora infestans*). It was noted that internal mycelium could be killed by a four hour treatment at 40^0C.

Jensen's method of hot water treatment (1897) is more widely known and accepted for the control of loose slut of wheat, barley and oats. In this practice, seeds are to presoaked for four hours at 20-30^0C, during which period, dormant mycelium develops actively and becomes more vulnerable to exposure in hot water at 50-52^0C for a few minutes. Seeds then dried very carefully before they can be used for sowing.

In India, solar energy has been utilized as a means of disinfection of seeds attacked with loose smut. In this process, seeds are to be presoaked for four to five hours in shallow vat conataining water during night. During this period the seeds remain immersed in water. Then they should be taken out, the excess water drained off and the seeds spread to dry in the sun on a clean floor. At the end of the day the seeds are expected to be dry. They should be stored in clean airtight containers. This process is simple and suitable for bulk treatment, but can only be adopted in summer.

The practicability of use of hot air treatment for the control of virus in the propagating stocks was first suggested by Kunkel in peach yellows. It was claimed that peach can be cured of yellows by warm water treatment. It was also noted that plants or planting materials kept at 35^0C for a fortnight can be cured of infection. Attempts were made to control virus diseases of sugarcane and strawberry runners by hot air treatment. In the 1950s, hot air treatment was used by Dr. Kassanis to eliminate potato leaf roll virus.

Seed treatment particularly by chemicals has become popular. It is advocated to have disease-free seeds and planting materials. The cost of treatment is very low in terms of potential benefits and methods of treatment are simple. At present, treatment of seeds is adopted as routine measure by the organizations dealing with seeds.

SOIL TREATMENT

Soil treatment includes those methods which are used for the possible destruction of pests, harmful fungi, bacteria and nematodes, insects and weed seeds. Methods may be classified as physical and chemical. Certain other methods such as agronomic practices or mechanical or mechanical and biological methods also adopted. Many harmful soil microflora colonise in the soil are responsible for causing diseases in plants are very difficult to be get rid of. Control of them poses a serious problem and in many instances may be a limiting factor in the growth of the crop. Soil contains both harmful and beneficial organisms. The fertility of soil and availability of nutrients to large extent is dependent on the biological activity of the beneficial microorganisms. Hence soil treatment should aim at the destruction of only harmful organisms while the beneficial ones will not be affected.

Various methods of soil treatment may be classified as follows:

1) Physical methods: a) Steam sterilisation; b) Hot-air sterilisation; c) Electrical sterilisation.
2) Chemical methods: a) Volatile chemicals; and b) Non-volatile chemicals.

1) Physical methods:

Physical methods of soil sterilisation involve use of heat in dry or wet form to kill the destructive organisms. In tropical countries, exposure of soil to the heat of the sun in summer gives at least partial control of soil borne organisms. Soil sterilisation with heat has its own advantages, as it does not leave any toxic residue and soil can be used immediately as soon as it cools. Amongst some of the disadvantages of physical treatments are the inconvenience, cost of heating installations in many cases and the possibility of accumulation of soluble salts that may be injurious to plant growth.

Steam is considered to be the efficient source of heat. Most of the heat resistant fungi, bacteria and viruses capable of inciting plant diseases are inactivated by a 30 minutes exposure to temperature 60^0C. Most weed seeds are inactivated at 82^0C and nematodes at 49^0C. The majority of the beneficial micro-organisms can survive the temperature that inactivates the pathogenic ones. To secure the beneficial effects of soil sterilisation, the soil should be maintained at a temperature of 80-90^0C for one hour. Various methods of soil treatment by heat are available. Sterilisation by steam is effected by the passage of the steam, at high or low pressure generated from a boiler in to the soil. A mixture steam and air has also been recommended for the reduction for the reduction of cost. Small quantities of soil in glasshouses can be sterilized by heating in the flat pan maintaining the correct temperature. Methods of heating the soil with electric heating coils are also available and practiced in some countries.

2) Chemical methods:

Soil treatment b y the application of chemicals is a simple phenomenon. The chemicals are cheap; have a power of good penetration; harmless and non-injurious to the plants. But most of the chemicals used for the sterilisation of soil do not possess all the desired properties. The methods of application of pesticides for the soil treatment depend on the characteristics of the chemical and machinery used for the purpose. Gases (methyl bromide), liquids (carbon disulphide, formaldehyde) are introduced by injecting them at set points through injectors. Soil particles in the form of dust are incorporated into the soil through plowing. Most of the pesticides have to be applied before planting or sowing as they act as general sterilants. Some fungicides like Maneb, Zineb, Ziram or Copper compounds are not phytotoxic at lower concentration and they may be applied as drenches in the standing crop.

Volatile soil treating chemicals:

These chemicals are often known as fumigants. They are follows:

a) Chloropicrin: Nitrochloroform or trichloronitromethane (CCl_3NO_2) is a colourless non-inflammable, poisonous liquid. It is effective for the control of a wide range of soil borne pests. It is to be applied as a preplanting treatment, as it is extremely toxic to living plants and care should be taken so that no chemical is split near any living plant. It is marketed as liquid or as an aerosol formulation.

It is injected into the soil with special injection equipment. About 3 ml of the liquid has to be injected at a depth of 13 cm on 26 cm centre grids throughout the bed. After the treatment soil should be wet with water an entire area should be covered with plastic sheet for 24 hours. Treated soil should not be used for planting or sowing at least for 10 to 14 days. Longer periods may be needed in cold rainy weather.

b) Methyl bromide (CH_4Br): Methyl bromide is not injurious to the plants and has a greater penetration rate. It is odourless, but very dangerous to the operators and all precautions need to be taken. Methyl bromide is a gas which is marketed as aerosols or in cylinders. It may also available in liquid form. Soil Treated with methyl bromide should be covered for 24-48 hours and seven days aeration should be allowed before planting. Treatment with methyl bromide may reduce germination of some seeds. Such soil should be planted with garlic, cornation or salvia.

c) Vapam ($C_2H_4NS_2Na,2H_2O$): It can be applied to the soil mixed with water with the help of a power sprayer at the rate of 500cc/10m^2. After treatment soil should be watered and kept for two or three weeks before use for planting. It leaves no toxic residue in the soil. It is very effective against pests, weed seeds and nematodes.

d) Mylone: 3,5-dimethyltetra hydro1,3,5,2H-thiadiazine-2-thione ($C_2H_{10}S_2N_2$): It is applied at the rate of 150kg/ha either mixed with sand and applied with fertilizer, spreader or may be applied uniformly with a sprinkling can or power sprayer as suspension in water. A period of two or three weeks should be elapse between treatment and planting. It is effective against weeds and nematodes.

e) Formaldehyder: It is used as 1% solution at the rate of 4 litres per 10 sq. m. After treatment soil should be watered and covered for 24 hours. After removal of cover soil should be raked for two to three days.

f) Allyl alcohol: It is used to control weed seeds and nematodes.

g) Carbon disulphide (CS_2): It is cheap, volatile and has good insecticidal properties. Carbon disulphide enters the soil by a simple process of diffusion.

Non-volatile soil treating chemicals:

Carbmates (Zineb, Ziram, Maneb) have been suggested as promising chemicals for giving protection against soil borne fungi. These chemicals may be used as dust or drench. As dust, 15% dust to be applied at the rate of 1kg/500m^2 or liquid 0.05% solution at the rate of 4lit/10m^2.

Nitrobenzenes- pentachloronitrobenzene has been found to be effective against a number of fungi. It may be applied as a mix, surface application, and transplant solution. It is available as dust with active ingredient varying from 10-40% and for spray as 75%WP. Doses for dust vary from 10 to 40kg/ha depending upon strength, crop and disease. Spray concentration vary from 0.1 to 0.5% depending upon the time of application, crop and disease.

Copper compounds- copper fungicides at the rate of 25 per cent dilution of spray strength have been found to be promising in control of phycomycetes fungi when applied as drench. Such applications may be made even with standing crop in the soil, as the fungicides are not phytotoxic. Trenches filled with lime, surrounding the dead stumps have been found to be useful in the prevention of spread of root infecting fungi in tropical plantation crop.

Chlorinated hydrocarbons- Gamma-BHC, DDT, aldrin etc. have been applied successfully for the control of insects in the form of dust at the time of soil preparation prior to planting. While gamma-BHC and DDT do not persist for very long time, cyclodiene insecticides like aldrin or dieldrin decompose very slowly and there may be accumulation in the soil.

Some systemic fungicides like thiabendazole, benomyl have shown promise for soil treatment. Cultural and biological methods may also be adopted for getting rid of pests in the soil.

SULPHER FUNGICIDES

Elemental sulpher has been in use as a fungicide for a long time even today it is one of the best for the control of powdery mildew diseases. The sulpher fungicides can be classified as follows:

Sulpher fungicides

Inorganic Organic

Elemental sulpher Lime sulpher Carbamate fungicides

Dust Wettable

The elemental sulpher fungicides are available in two types of formulations viz. sulpher dust and wettable sulpher.

Mechanically ground sulpher have a tendency to form small aggregates which may be overcome by the addition of small amount of inert material such as Kaolin or lead arsenate. The fungicidal efficiency of a sulpher dust depends on the fitness of its particles, a high proportion should pass a 200 or 300 mesh sieve and still finer division is preferable. In recent years, wettable sulpher have become more popular. They form uniform suspension in water and are meant for use as sprays. This can be possible in two forms viz. paste or powder. Any form of sulpher can be made in to wettable sulpher by grinding with protective colloidal materials such as sulphite lye, casein, bentonite clay etc. There are many proprietary products consisting of wettable sulpher which are available in India.

The mechanism of action of elemental sulpher has been a subject of interest for many years. Mach and Portelle (1884) first suggested that sulpher dioxide accounted for the fungicidal activity of sulpher. According to direct action theory, sulpher acts as a hydrogen acceptor in metabolic systems and disturbs the normal hydrogenation and dehydrogenation reactions in the cell. Sulpher fungicides emit sufficient vapour to prevent the growth of fungus spores at a distance of several mm from deposits on leaves.

Phytotoxicity: In warmer climate (above 80^0F) severe burning is caused on cucurbits when sulpher is used for the powdery mildew control. Apples treated with sulpher in semi-arid areas may develop lesions on sun exposed sides. Sulpher placed on stigma of apple blossoms exhibits pollen germination. It has been observed that lime sulpher when applied on green leaves considerably reduces the photosynthesis.

Disease control: Both elemental sulpher and lime sulpher have been widely used as fungicide for the control of different types of diseases, particularly powdery mildew and leaf spot diseases. Whereas elemental sulpher has been used as residual fungicide, lime sulpher has been mainly used as a contact fungicide.

Organic sulpher (Carbamate):

The carbamate fungicides form a very important group among fungicides. Most of these are foliage fungicides, while some are used for soil and seed treatments. All the carbamate fungicides available commercially are derivatives of dithiocarbamic acid. This acid has the structural formula:

Thiram: Thiram is coined name for tetramethylthiuram disulphide or bis (dimethylthiocarbamoyl) disulphide. The structural formula is as under:

It is sold in market under the names such as Arasan, Hexathir, Nomersan, Panoram 75, Pomasol, Spotrete, Tersan 75, Thiram, Thiride, Thylate, TMTD (TMTDS) etc. The molecular weight is 240.4. It is unstable in the presence of acids. It is white coloured substance which is essentially insoluble in water, slightly soluble in alcohol and ether and completely soluble in acetone and chloroform.

It is toxic if consumed orally. It has also been fond to be irritating to nose, throat and skin. It is one of the most effective seed protectants and is less phytotoxic. It is used for seed treatment either as dry powder or as slurry. Generally, the rate of application for dry seed dressing is about 0.25%. It is also used for treating the soil and the rates of application vary between 15-25 kg per hectare. Some of the diseases controlled by a thiram are: stem gall of coriander, damping off, smut, neck rot of onion, black rot of sugar beet, anthracnose and stem rot of tobacco, seedling diseases of cotton and many other soil borne diseases.

Thiram is decomposed in humus sandy soils at pH 3-4 after 4-5 weeks and in soil at pH 7.0 after 14-15 weeks. In extreme alkaline soils, initial fungicidal effect is delayed. On seed, thiram is degraded if treated seed is stored and hence a compensatory dose is required.

COPPER FUNGICIDES

The fungicidal activity of copper sulphate was first recognised by Prevost (1807). Today copper fungicides are used widely in many countries. The copper fungicides have been used control of many vegetables, fruit and flowering plant diseases. They have been commonly used in plantation crops like tea, rubber, coffee etc.

Copper fungicides

Copper sulphate

Bordeaux Mixture | Bordeaux paste | Burgundy mixture | Cheshunt compound | Chaubattia paste

Copper Oxychloride (Fytolan, Blitox 50) | Cuprous oxide (Perenox)

Bordeaux mixture:

It is commonly used fungicide. The common formula for mixture is 4:4:50 or 5:5:50 ie. 1.5 kg of $CuSO_4$ and 1.5 kg of lime in 200 liters of water. It is prepared in following manner:

a) CuSO4 is powdered and dissolved in 100 liters of water.

b) Quick lime is slaked with small quantity of water is mixed with 100 liters of water.

c) These two solutions are poured in to a third wooden container, mud pot or brass vessel through a strainer to retain large particles of unslaked lime and other contaminants.

d) The mixture is tested for free copper by placing a knife blade, a brown coating of copper will be seen on the surface indicating excess of copper which is phytotoxic. In such a case additional quantity of lime should be added for neutralisation.

e) The chemical reaction involved in Bordeaux mixture is:

$$CuSo_4 + Ca(OH)_2 \rightarrow Cu(OH)_2 + CaSO_4$$

The mixture is neutral or slightly alkaline and is not injurious. The cupric hydroxide is the active principle when soluble and is toxic to spores and sporelings. The fungicidal action is due to soluble copper. It is used in downy mildews, late blight of potatoes and areca nuts, coffee rusts, various leaf spot diseases, anthracnose etc. Its phytotoxic effect is characterised by chlorosis followed by brown or purple flecks on the leaves or fruits.

Bordeaux paste:

The content of Bordeaux paste is similar to that of Bordeaux mixture. It is primarily used for tree wound dressing to prevent fungal attacks e.g. in the control of stem bleeding disease of coconut. It is normally prepared by adding one lb each of copper sulphate and lime in one gallon of water.

Burgundy mixture:

This was introduced by Mason (1887) in Burgundy (France) in place of Bordeaux mixture in which lime is replaced by sodium carbonate crystals. This preparation was developed at that time good quick lime was not easily available in Europe. A 4:4:50 formula has been reported to satisfactory. It is slight less effective than Bordeaux mixture.

Cheshunt compound:

This compound was suggested by Bewlay in 1921. It contains two parts of $CuSO_4$ and 11 parts of ammonium carbonate. These are well powdered and mixed and are stored in air tight receptacle for 24 hours before using. One ounce of mixture is dissolved in a little hot water and the solution is made up to two gallons with cold water. It is used to drench nursery beds for the control of damping off.

Chaubattia paste (copper corbonate preparation):

This paste was developed at the Government Fruit Research Station, Chaubattia in Almora district of U. P. This was developed as wound dressing fungicide to be applied to pruned parts apple, pear and peach trees. The paste is prepared by mixing copper carbonat (800gm) and red lead (800gm) in 1 liter of lanolin or raw linseed oil and mixed in glass or chinaware pot. It is not washed easily by rain.

Copper oxychloride preparation:

This is low injurious copper fungicide not found to be effective as Bordeaux mixture. It has been formulated both as wettable powder and as a ready-to-use dust. Copper oxychloride ($CuCl_2.3Cu(OH)_2$) is readily formed by the action of air on cupric chloride solutions or scrap copper. Formulations containing 4 to 50% metallic copper in the form of copper oxychloride are available in India. Copper oxychloride containing 4-12% metallic copper is used for dusting and 50% metallic copper for spraying. Some of the commercial preperations are: Blimix 4%, Blitox 50, Cupramar, Micop D-06, Micop W-50, Fytolan and Blue copper '50'.

Cuprous oxide preparation is another low soluble copper recommended for spraying 50% copper in water (100 gallons).

MERCURIC CHLORIDE (Agrosan-GN)

Mercurial compounds have been known for their fungicidal and bactericidal properties. These have been used mostly for treating the seeds to protect them from fungal attack. Some compounds had been used as fruit and foliage protectants. The first important commercial product was "Uspulum". Organomercurals are very widely used for seed treatment. Uspulum contained 16.1% metallic mercury in the form of crystal mercuric cyanide ($CHO(CH)_3C_6HK_3HgCN$). The general structure of these darivatives is R-Hg-X. where R-hydrocarbon with or without substitute groups, X- an acid radical such as chloride, nitrate, acetate, benzoate, gluconate etc.

R is usually ethyl or phenyl group. Some of the important compounds are Agrosan GN(phenyl mercury acetate and ethyl mercury chloride mixture). Such chemical can be used either as dips or dust. Rate of application is 0.25% for wet seed treatment 2.5-6%. The site of fungicidal activity of mercury either as vapour or ion. Organomercurals are more toxic than inorganic mercurals. Seed injury is considered one of the hazards in the use. Organomercural fungicides are mainly used to control externally seed borne diseases since these compounds are able to eradicate the inoculums from the seed.

HETEROCYCLIC NITROGENOUS COMPOUNDS

CAPTAN:

Captan is the common name adopted for N-trichloro-methyl-thio-4-cyclohexane-1,2-dicarboximide. It is a heterocyclic nitrogenous compound. Kittleson (1952) prepared this compound and first reported its fungicidal activity. Hence in the beginning, it was called Kittleson's killer. The structural formula of captan is:

Captan is commercially sold under different names such as Captan 50W or Captan 75W, Esso fungicide 406, Orthocide 406, Vancide 89 etc.

Hochstein and Cox (1956) suggested that captan competes with cocarboxylase (Thiamine pyrophosphate) for site on coenzyme free carboxylase in the decarboxylation of pyruvate. Captan mainly acts as protectant, but in some cases it is claimed to have acted systemically. It is not commercially effective against rusts, powdery mildew and downy mildew. It is degraded when exposed to sunlight. It is incompatible with all alkaline materials since it gets decomposed at higher pH. It is incompatible with lime sulpher and Bordeaux mixture. Animals feed with captan treated grains have shown no harmful effects.

BENZENE COMPOUNDS: DEXON

Chemically Dexon is sodium p-dimethylaminobenzene-diazosulphonate. Its structural formula is:

Its empirical formula is $C_8H_{10}N_3NaSO_3$ and the molecular weight is 209. It is an odourless, yellowish powder. It is 2 to 3% soluble in water, soluble in ethanol and methanol, but insoluble in benzene and ether. It has an acute oral LD_{50} of 60mg/kg body weight for rats. It is compatible with PCNB. It shows a little phytotoxicity at the recommended rates. At 100 to 200 ppm it has inhibitory to root elongation and nodulation in certain legumes. It is available under the trade name Dexon as a 5% granular and 70% WP formulation.

It is the most promising diazo compound developed for plant disease control. It is fairly specific in protecting germinating seeds and growing plants from seedd and soil borne phycomycetes like *Pythium* spp., *Aphanomyces* spp and *Phytophthora* spp. The aqueous solution is not stable in presence of sunlight. It shows no significant effect on the associated saprophytic microflora of soil. In glasshouse conditions 10 or 20 ppm soil drench per week gave good control of *Phytophthora* root rot of avocado seedlings, while 40 ppm per week application was phytotoxic. Under field conditions, use of 100 ppm dexon suspension at the rate of 1 gal/sq.ft. per month with irrigation water resulted in significant recovery of already infected plants.

It is fungistatic in action. Germination of sporangia, zoospores and chlamydospores of *Phytophthora cinnamomi* was inhibited at 100 to 500 ppm, where as the formation of sporangia and chlamydospores was inhibited effectively at as low as 5 to 10 ppm concentrations. It is recommended and used as seed treatment with PCNB for sugarbeets and cotton seed and also as soil drench at 30 to 100 ppm at planting.

In sensitive fungi it inhibits respiration. In the mitochondria of *Pythium* it inhibits oxidation of nicotinamide adenine dinucleotide ($NADH_2$).

ANTIBIOTICS

There has been a continuous search for antibiotics for the control of plant diseases. According to Martin, more than 340 antibiotics have been found to be of no practical value because of their instability and undesirable properties. Only a few have been found to be promise.

1) Streptomycin:

It is obtained from culture filtrates of certain strains of *Streptomyces griseus*. Its chemical structure is that of a glycoside in which a glycone streptidine is linked to N-methyl glucosamine through an unusual sugar streptose. It is strongly basic in character and is marketed as sulphate or hydrochloride. A formulation of strepromycin mixed with oxytetracycline (Terramycin) is marketed under the name Agrimycin. Streptomycin is effective against both Gram-positive and Gram-negative bacterial plant pathogens but they do not show any toxicity against true fungi. It is systemic in nature, but due to its possible phytotoxicity application in desirable concentration is limited. Streptomycine has been successfully used against bacterial seed borne pathogens at 100 ppm or more. There is possibility of development of strains of bacteria resistant to streptomycin. Addition of oxytetracycline retards the development of resistant strains; hence the use of Agrimycin is advocated. Streptomycin and Agrimycin are compatible with lead arsenate, DDT, aldrin, fixed copper fungicides, ferbam, parathion, wettable sulpher and zineb but not with gamma-BHC, chlorden or glyodin.

2)Aureofungin:

It is produced in submerged cultures of *Streptomyces cinnamomeus* var. *terricola* in the Hindustan Antibiotics, India (1964). Chemically it belongs to a new aromatic subgroup among heptaene- the aromatic moiety being N-methyl-p-amino aceptophenone and mycosamine.

It is insoluble in water, but soluble in alkaline solutions, hence soap solution of this antibiotic (1gm in 10 ml water) can easily be prepared. It is unstable in the presence of moisture and light. It has been claimed to be a broad-spectrum antifungal antibiotic systemic in nature. It is non-toxic to warm blooded animals. It has been found to be effective in controlling several fungal diseases. Normal concentration of use is 5-10 mg/1000 ml.

In spite of the possibility of effective use of antibiotics in the control of plant diseases, very few practical applications in wide scale have been made. But in japan, there has been a tremendous development in the use of antibiotics for the control of plant diseases.

SYSTEMIC FUNGICIDES

Systemic fungicides act as direct chemotherapeutants. Interest in chemotherapy and use of systemic fungicides was stimulated by the availability of diverse organic compounds possessing specificity for action as fungicides. Hundreds of chemicals have been tried and periodic reviews on the progress of development of systemic fungicides and chemotherapy have been published.

1) Bavistin (Carbendazim):

Bavistin is the trade name of Carbendazim (methyl-2-benzimidazole carbamate or MBC). The chemical structure is:

The empirical formula is $C_9H_9N_3O_2$ and molecular weight 191.2. It is a whitish grey powder having a faint acrid odour. Its solubility is less than 10 ppm in oil, 400 ppm in ethyl alcohol, 300 ppm in acetone and 100 ppm in chloroform. In water its solubility is 28 ppm at pH 4, 8 ppm at pH 7 and 7 ppm at pH 8. It is very stable compound, not decomposing at 50^0 C for two years. However, it decomposes in the presence of acids or alkalies. It is one of the safer fungicides having a LD_{50}(oral) for rats of more than 6400mg/kg body weight. The maximum tolerable residue in food stuff is 18.75 ppm. However, tolerance from 0.1 ppm to 7 ppm has been fixed depending on the fruit, vegetable and cereals. It is found to be non-phytotoxic. It shows a broad spectrum fungitoxic activity being effective against Ascomycetes, fungi imperfecti and various Basidiomycetes. It is not effective against Phycomycetes and bacteria. With this, beneficial side effects like stimulation of growth, flowering and yield of plant as well as reduction of mite population on the treated hosts have been reported. It is used as spray, seedling dip (5-10 min.), seed dressing, soil drench, or as post harvest treatment of fruits (dip for ½-1 min.).

2) Vitavax:

This is oxathiin compound developed by Van Schmeling and Kulka in 1966. Two oxathiin compounds were developed a) 2,3-dihydro-5 carboxianilido-6-methyl-1,4, oxathiin (or 5,6-dihydro-2-methyl 1,4,oxathiin-3-carboxianilide) called vitavax and b) 2,3-dihydro,5-carboxianilido-6-methyl-1,4, oxathiin-4,4 dioxide (DCMOD) known as plantavax. Vitavax is water soluble and do not show any phytotoxic properties on crops. It exhibits a remarkable selectivity in the antifungal spectrum. Vitavax have been found to be active against number of

pathogenic fungi. Selectivity in action has been attributed to the degree of uptake and binding. Vitavax is not stable when applied to the soil and complete degradation may take place in 10-30 days. Vitavax have shown promise as a seed dressing or soil drench for the treatment of cereals against loose smut which are internally seed borne and *Rhizoctonia* disease of cotton and sugarbeet. Vitavax have been employed against control of smuts and also for control of rusts of cereals and vegetables. The mode of action is considered to be the interference with synthesis of protein, RNA and DNA in rapidly metabolising cells. They have been found to inhibit mitochondrial respiration. Rate of use is 50-250gm/50kg of seed treatment and 10 per cent dust in soil treatment.

PESTICIDES

Nicotine:

Nicotine is used against pierce sucking insects such as aphids, whiteflies, leaf hoppers and thrips. If it affects humans and other warm blooded mammals, then logically it affects small insects. Nicotine comes from a plant and as found mainly in tobacco, tomato, potato, eggplant and green peppers. Nicotine can also be found on the leaves of coca plants. Like other natural insecticides, nicotine is diluted and used as a spray against insect pests. With today's technology, nicotine as a natural insecticide is also combined with other chemicals to become a stronger pesticide against insects. Nicotine may infect the environment and ecosystems. Nicotine as a natural insecticide is regulated by the government. As of now, the insecticide is usually sold in less than a 50% liquid concentrate, it is diluted in water and used as a spray. On the home front gardeners can create their own spray. All they need to do, it is steep a cup of tobacco in some water, after about 12 hours, strain it and then compare it as a spray. The idea is to spray the plants you want protected from those pesky insects that want to feed on it. Nicotine works very well with caterpillars and aphids.

There are sometimes some side effects when working with pesticides – natural insecticides is no different. In one study, some worms that became resistant to nicotine as a natural insecticide actually became bigger and faster than those worms that fed on plants with normal nicotine levels. But the natural insecticide is still effective with other insect pests. The green peach aphid is an example of an insect that repels against nicotine.

It is important to point out that nicotine as a natural insecticide is very toxic to humans. Nicotine can actually be absorbed through the skin. It is best to use gloves when handling nicotine. The good thing about nicotine when used as a natural insecticide is that it is highly biodegradable, and can be used on crops. Because of this, when using nicotine as a natural insecticide, it is also good to know that it can harm other plants such as roses.

When it comes to humans we should remember that many people have died because of nicotine in cigarette smoking. Nicotine in pesticides has also been used as a suicidal means. Young children have been rushed to the ER due to ingesting nicotine pesticides by accident. Nicotine is a great natural insecticide to use against insects, but we should handle it with great respect.

Instead of homemade nicotine insecticide, it is probably better to purchase products with nicotine as a natural insecticide. For example, Black Leaf is a popular brand of pesticide that includes nicotine in its 40% concentrated.

Neem:

Neem pesticides plays a vital rope in pest management and hence widely been used in agriculture. There has been as evident shift all over the world from synthetic pesticide to non synthetic ones, this is because of the wide spread awareness of the side effects on the plants as well as on other living organisms. This is a great opportunity for neem pesticide manufacturers to cash on the growing popularity of herbal pesticides. Azadirictin is the main ingredient used to manufacture bio pesticides.

Parts of neem used: Neem oil and extract are known to posses germicidal and anti-bacterial properties which are useful to protect the plants from different kinds of pests. One of the most advantages of neem pesticide is that they do not leave any residue on the plants. Neem pest control is very beneficial for proper crop and pest management.

Benefits of neem pesticides:

- It also helps to nourish and condition the soil.
- It is environmental friendly.
- It is non-toxic.
- It can be used in combination with other pesticide and oil for more efficacy.
- Instead killing of pests, it affect the life cycle of the pests.
- Anti feed out properties found in neem compounds help to protect the plants.
- Pests generally do not develop a resistance to neem pesticide.
- These are water soluble and help in the growth of the plants.
- Acts as pest repellent.
- Acts as pest reproduction controller.

Pyrethrum:

Pyrethrum (*Crysenthemum cincerariaefolium*) of family Asteraceae is a perennial African plant. The plant is economically important as a natural source of insecticide. The flowers are pulverized and the active components, a group of esters called pyrethrins ($C_{21}H_{28}O_3$ or $C_{22}H_{28}O_5$) contained in the seed cases are extracted and sold in the form of an oleoresin. This is applied as a suspension in water or oil, or as a powder. Pyrethrin attacks the nervous system of all insects and inhibits female mosquitoes from biting. When not present in amounts fatal to insects, they still appear to have a repellent effect. They are harmful to fish, but are far less toxic to mammals and birds than many synthetic insecticides and are non persistant, being biodegradable and also breaking down easily on exposure to light. They are considered to be amongst the safest insectides for use around food.

Kenya produced 90% (over 6000 tons) of world pyrethrin in 1998, in Tasmania production is increasing. Pyrethroides are synthetic insecticide based on natural pyrethrum: an example of one is permethrin. A common formulation of pyrethrin in preperations containing the synthetic chemical Piperonyl Butoxide: this has the effect of enhancing the toxicity to insects and speeding the effects when compared with pyrethrins used alone. These formulations are known as synergized pyrethrins.

RHODENTICIDE

Zinc phosphide:

It is rhodenticide. Its chemical group is inorganic phosphide. Empirical formula is Zn_3P_2

Zn

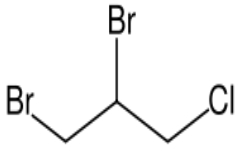
P Zn P

Zn

A rhodenticide of high mammalian toxicity in the presence of dilute acid will decompose to liberate phosphine. It is a grey powder of high melting point which sublimes when heated in the absence of oxygen. Practically it is insoluble in water and ethanol. It is soluble in benzene and carbon disulphide. It is soluble when dry but decompose slowly in moist air. It react violently with acids with decomposition to the spontaneously inflammable phosphine gas. Vapour pressure is very low. Phosphine odour detectable at 1.5-3.0 ml/m^3 depending on its purity. Mice, rats, ship rats, field mice, ghopers, ground squirrels, prairies dogs mainly controlled. Under exposed conditions toxicity is lost in about two weeks. Its mode of action is probably decomposes phosphine in the stomach and is absorbed both as phosphine and phosphide. It has toxic action on the heart, liver and kidneys. Death occurs from heart and kidney failure. After massive dose, death may occur in 70 minutes, with smaller doses, death may be delayed from 24 hours up to 2-3 days. A dose of 5 gm has caused death to man. It is very toxic substance. Keep dry and away from acids of all kinds.

NEMATICIDES

Nemagon (1,2-Dibromo-3-chloropropane)

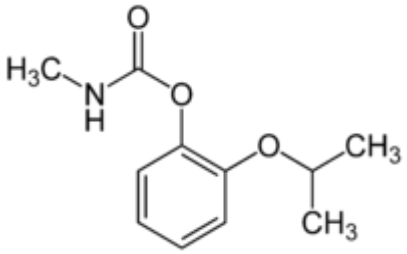


1,2-Dibromo-3-chloropropane, (dibromochloropropane) better known as DBCP, is the active ingredient in the nematicide Nemagon, also known as Fumazone. It is a soil fumigant formerly used in American agriculture. In mammals it causes male sterility at high levels of exposure. After discovery of its deleterious health effects on humans, the compound was banned from use in 1979 by the United States Environmental Protection Agency (EPA). The continuing presence of the chemical as a contaminant in ground water remains a problem for many communities for years after end of use.

Until 1977, DBCP was used as a soil fumigant and nematocide on over 40 different crops in the United States. It fights pests that attack the roots of fruit trees and boosts the weight of harvests by 20 percent. From 1977 to 1979, EPA suspended registration for all DBCP-containing products except for use on pineapples in Hawaii. In 1985, EPA issued an intent to cancel all registrations for DBCP, including use on pineapples. Subsequently, the use of existing stocks of DBCP was prohibited. DBCP is used as an intermediate in the synthesis of organic chemicals.

Human exposure to DBCP could result from the ingestion of contaminated drinking water and food.Human exposure could also result from inhalation and / or skin contact with the product.In the past, release of DBCP to the environment occurred primarily from its fumigant and nematocide uses; because of the cancellation of all DBCP uses, environmental exposure is expected to decline with time.

Propoxur



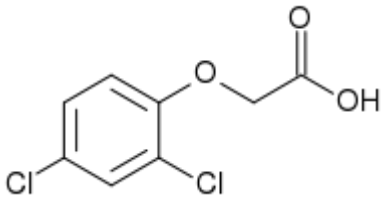
- Propoxur (Baygon) is a carbamate insecticide and was introduced in 1959.
- Propoxur is a non-systemic insecticide with a fast knockdown and long residual effect used against turf, forestry, and household pests and fleas.

- It is also used in pest control for other domestic animals, *Anopheles* mosquitoes, ants, gypsy moths, and other agricultural pests. It can also be used as a molluscicide.
- Several US states have petitioned the Environmental Protection Agency (EPA) to use propoxur against bedbug infestations, but the EPA has been reluctant to approve indoor use because of its potential toxicity to children after chronic exposure.
- Carbamate insecticides kill insects by reversibly inactivating the enzyme acetylcholinesterase.
- It rapidly breaks down in alkaline solution.
- Propoxur is highly toxic to many bird species, but its toxicity varies by the species.
- It is moderately too slightly toxic to fish and other aquatic species.
- Propoxur is highly toxic to honeybees.

WEEDICIDES

2,4-Dichlorophenoxyacetic acid (2,4-D)

2,4-Dichlorophenoxyacetic acid (2,4-D) is a common systemic pesticide/herbicide used in the control of broadleaf weeds. It is the most widely used herbicide in the world, and the third most commonly used in North Americ. 2,4-D is a synthetic auxin (plant hormone), and as such it is often used in laboratories for plant research and as a supplement in plant cell culture media such as MS medium.



2,4-D was developed during World War II by a British team at Rothamsted Experimental Station, under the leadership of Judah Hirsch Quastel, aiming to increase crop yields for a nation at war. When it was commercially released in 1946, it became the first successful selective herbicide and allowed for greatly enhanced weed control in wheat, maize (corn), rice, and similar cereal grass crops, because it only kills dicots (broadleaf plants), leaving behind monocots (grasses).

2,4-D is a member of the phenoxy family of herbicides. 2,4-D is a synthetic auxin, which is a class of plant hormones. It is absorbed through the leaves and is translocated to the meristems of the plant. Uncontrolled, unsustainable growth ensues, causing stem curl-over,

leaf withering, and eventual plant death. 2,4-D is typically applied as an amine salt, but more potent ester versions exist as well.

2,4-D is primarily used as an herbicide. It is sold in various formulations under a wide variety of brand names. 2,4-D can be found in lawn herbicide mixtures such as "Weed B Gon MAX", "PAR III", "Trillion", "Tri-Kil", "Killex" and "Weedaway Premium 3-Way XP Turf Herbicide". All of these mixtures typically contain three active ingredients: 2,4-D, mecoprop and dicamba. Over 1,500 herbicide products contain 2,4-D as an active ingredient.

2,4-D is most commonly used for:

- Weed control in lawns and other turf
- Control of weeds and brush along fences and highway and railroad rights of way
- Conifer release (control of broad-leaf trees in conifer plantings)
- Grass hayfields and pastures
- Cereal grains corn and sorghum (occasionally)
- As a synthetic auxin analog

2,4-D continues to be used, where legal, for its low cost. However, where municipal lawn pesticide bylaws exist, such as in Canada, alternatives such as corn gluten meal and vinegar-based products are increasingly being used to combat weeds.The LD_{50} determined in an acute toxicity rat study is 639 mg/kg. Single oral doses of 5 and 30 mg/kg body weight did not cause any acute toxic effects in human volunteers. This chemical has been associated with the risk of amyotrophic lateral sclerosis.

The amine salt formulations can cause eye damage (blindness) on contact; ester formulations are considered non-irritating to the eyes. One study found that occupational exposure to 2,4-D caused male reproductive problems, including dead and malformed sperm. Concerns regarding neurotoxicity have been voiced with increased sensitivity to amphetamine and thus concerns of increased risk of drug addiction among those exposed. It is possible that 2,4-D causes cancer in humans.

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