**Semester – VI**

**16MBU602B MICROBES IN SUSTAINABLE AGRICULTURE AND DEVELOPMENT (4H – 4C)**

**Instruction Hours/week: L: 4 T: 0 P: 0** **Marks:** Internal: **40** External: **60** Total: **100**

**End Semester Exam:** 3 Hours

**Unit I – Soil Microbiology**

Soil as Microbial Habitat, Soil profile and properties, Soil formation, Diversity and distribution of microorganisms in soil, Mineralization of cellulose, hemicelluloses, lignocelluloses, lignin and humus, phosphate, nitrate, silica, potassium

**Unit II – Microbial Activity in Soil and Green House Gases**

Carbon dioxide, methane, nitrous oxide, nitric oxide – production and control

**Unit III – Microbial Control of Soil Borne Plant Pathogens**

Biocontrol mechanisms and ways, Microorganisms used as biocontrol agents against Microbial plant pathogens, Insects, Weeds.

**Unit IV – Biofertilization, Phytostimulation, Bioinsecticides**

Plant growth promoting bateria, biofertilizers – symbiotic (*Bradyrhizobium*, *Rhizobium*, *Frankia*), Non Symbiotic (*Azospirillum*, *Azotobacter*, Mycorrhizae, MHBs, Phosphate solubilizers, algae), Novel combination of microbes as biofertilizers, PGPRs.

**Unit V – Secondary Agriculture Biotechnology**

Biotech feed, Silage, Biomanure, biogas, biofuels – advantages and processing parameters, Advantages, social and environmental aspects, Bt crops, golden rice, transgenic animals.

**SUGGESTED READINGS**

1. Agrios GN. (2006). Plant Pathology. 5th edition. Academic press, San Diego.
2. Singh RS. (1998). Plant Diseases Management. 7th edition. Oxford & IBH, New Delhi.
th
3. Glick BR, Pasternak JJ, and Patten CL (2010) Molecular Biotechnology 4 edition, ASM Press,
4. Atlas RM and Bartha R. (2000). Microbial Ecology: Fundamentals & Applications. 4th edition. Benjamin/Cummings Science Publishing, USA.
5. Maier RM, Pepper IL and Gerba CP. (2009). Environmental Microbiology. 2nd edition, Academic Press.
6. Barton LL & Northup DE (2011). Microbial Ecology. 1st edition, Wiley Blackwell, USA.
7. Campbell RE. (1983). Microbial Ecology. Blackwell Scientific Publication, Oxford, England.
8. Coyne MS. (2001). Soil Microbiology: An Exploratory Approach. Delmar Thomson Learning.
9. Altman A (1998). Agriculture Biotechnology, Ist edition, Marcel decker Inc.

# LECTURE PLAN

## UNIT1

| Duration | Topic | Reference |
|---|---|---|
| 2 | Soil as Microbial Habitat, Soil Flora | T1:2-6 |
| 2 | Soil profile and properties | T2:23-32 |
| 1 | Soil formation | T2:35-42 |
| 1 | Diversity and distribution of microorganisms in soil | T1:8-43 |
| 8 | Mineralization of cellulose, hemicelluloses, lignocelluloses, lignin and humus, phosphate, nitrate, silica, potassium | T1-89-125 |
| 1 | Unit Revision | |
| | **Total hours: 15** | |

## UNIT2

| Duration | Topic | Reference |
|---|---|---|
| 1 | Production and control of carbon dioxide | W1 |
| 1 | Production and control of methane | T3: 217-223 |
| 1 | Production and control of nitrous oxide | T4: 271-276 |
| 1 | Production and control of nitric oxide | |
| 1 | Unit Revision | |
| | **Total hours: 05** | |

## UNIT3

| Duration | Topic | Reference |
|---|---|---|
| 3.5 | Biocontrol mechanisms and ways | T5: 489-491 |
| 3.5 | Microorganisms used as biocontrol agents against Microbial plant pathogens | T5: 492-501 |
| 1.5 | Microorganisms used as biocontrol agents against Insects | T6: 504-507 |
| 1.5 | Microorganisms used as biocontrol agents against Weeds | T6: 507-509 |
| 1 | Applications and advantages | |
| 1 | Unit Revision | |
| | **Total hours: 12** | |

**UNIT4**

| Duration | Topic | Reference |
|---|---|---|
| 1 | Plant growth promoting bateria, | T3: 249-254 |
| 1 | biofertilizers | |
| 1.5 | symbiotic (*Bradyrhizobium, Rhizobium, Frankia*), | T3:260-288 |
| 1.5 | Non Symbiotic (*Azospirillum, Azotobacter*, Mycorrhizae, MHBs, Phosphate solubilizers, algae), | |
| 2 | Novel combination of microbes as biofertilizers, | |
| 1 | PGPRs | T3: 241-254 |
| 1 | Unit Revision | |
| | **Total hours: 09** | |

## UNIT5

| Duration | Topic | Reference |
|---|---|---|
| 1 | Biotech feed | W2 |
| 1 | Silage, Biomanure | |
| 1 | biogas, biofuels | |
| 1 | social and environmental aspects, Bt crops | W3 |
| 1 | golden rice | |
| 2 | transgenic animals | T1:361-374 |
| 1 | Unit Revision | |
| 1 | Discussion on previous years question papers | |
| | **Total hours: 09** | |

T1: Barton LL & Northup DE (2011). Microbial Ecology. 1st edition, Wiley Blackwell, USA

T2: Agrios GN. (2006). Plant Pathology. 5th edition. Academic press.

T3: Altman A (1998). Agriculture Biotechnology, Ist edition, Marcel decker Inc

T4: Coyne MS. (2001). Soil Microbiology: An Exploratory Approach. Delmar Thomson Learning

T5: Singh RS. (1998). Plant Diseases Management. 7th edition. Oxford & IBH, New Delhi.

W 1: https://www.ncbi.nlm.nih.gov/pubmed/25746902

W2: https://www.slideshare.net/BatoolAbbas1/role-of-biotechnology-in-animal-feed

W 3: https://www.frontiersin.org/articles/10.3389/fpls.2015.00283/full

**Syllabus**

**Unit I – Soil Microbiology**

Soil as Microbial Habitat, Soil profile and properties, Soil formation, Diversity and distribution of microorganisms in soil, Mineralization of cellulose, hemicelluloses, lignocelluloses, lignin and humus, phosphate, nitrate, silica, potassium

**Soil formation:**

Soil forms continuously, but slowly, from the gradual breakdown of rocks through weathering.

Weathering can be a physical, chemical or biological process:

- Physical weathering—breakdown of rocks from the result of a mechanical action. Temperature changes, abrasion (when rocks collide with each other) or frost can all cause rocks to break down.
- Chemical weathering—breakdown of rocks through a change in their chemical makeup. This can happen when the minerals within rocks react with water, air or other chemicals.
- Biological weathering—the breakdown of rocks by living things. Burrowing animals help water and air get into rock, and plant roots can grow into cracks in the rock, making it split.

The accumulation of material through the action of water, wind and gravity also contributes to soil formation. These processes can be very slow, taking many tens of thousands of years. Five main interacting factors affect the formation of soil:

- parent material—minerals forming the basis of soil
- living organisms—influencing soil formation
- climate—affecting the rate of weathering and organic decomposition
- topography—grade of slope affecting drainage, erosion and deposition
- time—influencing soil properties.

Interactions between these factors produce an infinite variety of soils across the earth‘s surface.

**Parent materials**

Soil minerals form the base of soil. They are produced from rocks (parent material) through the processes of weathering and natural erosion. Water, wind, temperature change, gravity, chemical interaction, living organisms and pressure differences all help break down parent material.

The types of parent materials and the conditions under which they break down will influence the properties of the soil formed. For example, soils formed from granite are often sandy and infertile whereas basalt under moist conditions breaks down to form fertile, clay soils.

**Organisms**

Soil formation is influenced by organisms such as plants, micro-organisms (such as bacteria or fungi), burrowing insects, animals and humans.

As soil forms, plants begin to grow in it. The plants mature, die and new ones take their place. Their leaves and roots are added to the soil. Animals eat plants and their wastes and eventually their bodies are added to the soil.

This begins to change the soil. Bacteria, fungi, worms and other burrowers break down plant litter and animal wastes and remains, to eventually become organic matter. This may take the form of peat, humus or charcoal.

**Climate**

Temperature affects the rate of weathering and organic decomposition. With a colder and drier climate, these processes can be slow but, with heat and moisture, they are relatively rapid. Rainfall dissolves some of the soil materials and holds others in suspension. The water carries or leaches these materials down through the soil. Over time this process can change the soil, making it less fertile.

**Topography**

The shape, length and grade of a slope affect drainage. The aspect of a slope determines the type of vegetation and indicates the amount of rainfall received. These factors change the way soils form.

Soil materials are progressively moved within the natural landscape by the action of water, gravity and wind (for example, heavy rains erode soils from the hills to lower areas, forming deep soils). The soils left on steep hills are usually shallower. Transported soils include:

- Alluvial (water transported)
- Colluvial (gravity transported)
- Aeolian (wind transported) soils.

**Time**

Soil properties may vary depending on how long the soil has been weathered. Minerals from rocks are further weathered to form materials such as clays and oxides of iron and aluminium.

**Soil Forming Processes:**

The collective interaction of various soil-forming factors under a different set of conditions sets a course to certain recognised soil-forming processes. The ultimate result of soil formation is profile development. Specific profile features develop under particular soil-forming processes.

Some important soil formation processes are described as follows:

**Podsolisation:**

It is a type of eluviation in which humus and sesquioxides become mobile, leach out from upper horizons and become deposited in the lower horizons. This process is favoured by cool and wet climate. It requires high content of organic matter and low alkali in the parent material. The process increases the proportion of silica, sesquioxide in A-horizon and accumulation of clay, iron and aluminium in B-horizon.

**Laterisation:**

In this process, silica is removed while iron and alumina remain behind in the upper layers and usually there are no well-defined horizons. Laterisation is favoured by rapid decomposition of parent rocks under climates with high temperature and sufficient moisture for intense leaching, such as found

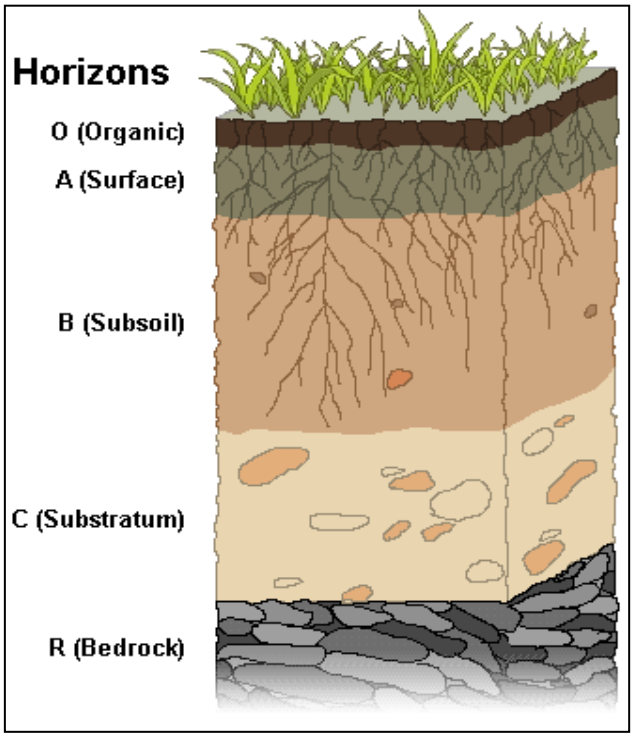
in the tropics. The soil formed in this process is acidic in nature. Podsolisation and laterisation produce soils that belong to the pedalfer (iron accumulating) group.

**Calcification:**

In this process, there is usually an accumulation of calcium carbonate in the soil profile. Such soils belong to the group called pedocal (calcium accumulating). This process is favoured by scanty rainfall and alkali in parent material.

**Hydromorphic Profile Development:**

Such soil forming process occur under impeded drainage conditions when certain horizons become saturated and percolation is restricted. Anaerobic conditions develop and more chemical reduction process set in. Marsh, bog, swamp, muck and peat soils are produced. Under fluctuating ground water level and under monsoon climatic conditions soils develop under alternating oxidation and reduction conditions leading to the formation of yellow brown, or rusty mottling.




**Soil profile**

The arrangement of horizons in a soil is known as a soil profile.

Horizon**,** a distinct layer of soil, approximately parallel with the land surface, whose properties develop from the combined actions of living organisms and percolating water. Most soils have three major horizons - organic horizon (O) on the surface, the surface horizon (A), the subsoil (B), the substratum (C), The master horizon, E (eluviation) and hard bedrock, which is not soil, uses the letter R.

Soil horizons differ in a number of easily seen soil properties such as color, texture, structure, and thickness. All these properties are used to define types of soil horizons.

O) Organic surface layer: Litter layer of plant residues, the upper part often relatively undecomposed, but the lower part may be strongly humified.

A) Surface soil: Layer of mineral soil with most organic matter accumulation and soil life. Additionally, due to weathering, oxides (mainly iron oxides) and clay minerals are formed and accumulated. It has a pronounced soil structure. But in some soils, clay minerals, iron, aluminium, organic compounds, and other constituents are soluble and move downwards. When this eluviation is

pronounced, a lighter coloured E subsurface soil horizon is apparent at the base of the A horizon. A horizons may also be the result of a combination of soil bioturbation and surface processes that winnow fine particles from biologically mounded topsoil. In this case, the A horizon is regarded as a "biomantle".

B) Subsoil: This layer has normally less organic matter than the A horizon, so its colour is mainly derived from iron oxides. Iron oxides and clay minerals accumulate as a result of weathering. In a soil, where substances move down from the topsoil, this is the layer where they accumulate. The process of accumulation of clay minerals, iron, aluminium and organic compounds, is referred to as illuviation. The B horizon has generally a soil structure.

C) Substratum: Layer of non-indurated poorly weathered or unweathered rocks. This layer may accumulate the more soluble compounds like $CaCO_3$. Soils formed *in situ* from non-indurated material exhibit similarities to this C layer.

R) Bedrock: R horizons denote the layer of partially weathered or unweathered bedrock at the base of the soil profile. Unlike the above layers, R horizons largely comprise continuous masses (as opposed to boulders) of hard rock that cannot be excavated by hand. Soils formed *in situ* from bedrock will exhibit strong similarities to this bedrock layer.

**The fundamental processes that develop a profile are described as follows:**

**Humification:** Helps in the formation of the surface humus layer, called A-horizon. The percolating water passing through this humus layer dissolves certain organic acids and affects the development of the lower A-horizon and the B-horizon.

**Eluviation and Illuviation:**

Development of profile horizons is mainly dependent on the amount and nature of movement of water in the soil. Eluviation (wash out) is the process of removal of constituents by percolation from upper layers to lower layers. This layer of loss is called eluvial and is designated as the A-horizon. The eluviated products move down and become deposited in the lower horizon which is termed as the illuvial (wash in) or B-horizon. Mechanical eluviation removes finer suspended fractions of soils, producing textural profiles characterised by a coarser-textured A-horizon and a finer-textured B-horizon that sometimes develops into a hard pan.

**SOIL PROPERTIES:**

**Physical properties of the soil:**

(1) Soil separates and texture,
(2) Structure of soil,
(3) Weight and soil density,
(4) Porosity of soil,
(5) Permeability of soil,
(6) Soil colour,

(7) Temperature of soil, and
(8) Soil Plasticity, Compressibility and Erodibility

**1. Soil Separates:**

Mineral fraction of soil consists of particles of various sizes. According to their size, soil particles are grouped into the following types.

| Sl No. | Name of soil particle | Diameter range of soil particles in millimetre |
|---|---|---|
| 1 | Course particles or gravels | >2.00 |
| 2 | Course sands | 2.00-0.20 |
| 3 | Fine sands | 0.20-0.02 |
| 4 | Silts | 0.02-0.002 |
| 5 | Clays | <0.002 |

The particle types are generally called 'soil separates' or 'soil fractions'.

Amount of soil separates is determined by a process known as mechanical analysis. In this process, soil sample is crushed and screened through a 2 mm round hole sieve. The screened soil is then homogeneously dispersed in water and allowed to settle. In suspension, particles of largest dimensions will settle first and those of smaller dimensions will settle afterwards. Individual soil separates are identified on the basis of their respective diameter ranges. Soil separates (sand, silt and clay) differ not only in their sizes but also in their bearing on some of the important factors affecting plant growth, such as, soil aeration, workability, movement and availability of water and nutrients.

Important characteristics of different soil separates are as follows:

Sand:

- This fraction of soil consists of loose and friable particles of 2.20 to 0.02 mm diameter.
- These particles, although inactive, constitute the framework of the soil.
- When coated with clay, these sand particles take very active part in chemical reactions. Sands increase the size of pore spaces between soil particles and thus, facilitate the movement of air and water.

Silt:

- It consists of soil particles of intermediate sizes between sand and clay (diam range 0.02 to 0.002 mm).
- Silt, when wet, feels plastic but in dry state feels like flour or talcum. Coarse silt shows little physicochemical activities but finer grades play important role in some chemical processes. Silty soil has got larger exposed surface area than the sandy soil.
- Silty soils contain sufficient quantities of nutrients, both organic and inorganic.
- Soils rich in silt possess high water holding capacity. Such soils are good for agriculture.

Clay:

- This soil fraction contains smaller particles than silt (below .002 mm diameter) which exhibit plasticity and smoothness when wet and hardness when dry.

- Owing to their smallest size and colloidal nature, the clay particles expose extremely large surface area. They take very active part in physicochemical reactions of the soil.
- Clay soils have fine pores, poor drainage and aeration and thus they have highest water holding capacity. The clay acts as store house for water and nutrients.

**Soil Textures:**

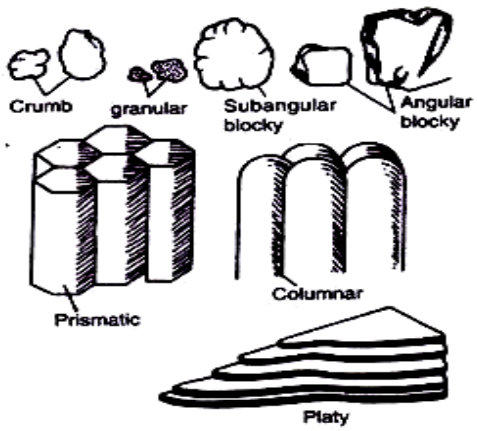
The relative percentage of soil separates of a given soil is referred to as soil texture. Texture of soil for a given horizon is almost a permanent character, because it remains unchanged over a long period of time.

The common textural classes, as recognized by USDA (U.S. Department of Agriculture) are given in the following table. These classes are recognized on the basis of relative percentage of separates; sand, silt and clay.

**Table 23.2. Soil textural classes**

| Soil classes or Textural name | Range in relative percentages of soil separates | | |
|---|---|---|---|
| | Sand | Silt | Clay |
| Sandy soil | 85—100 | 0—15 | 0—10 |
| Loamy sand | 70—90 | 0—30 | 0—15 |
| Sandy loam | 43—80 | 0—50 | 0—20 |
| Loam | 23—52 | 28—50 | 7—27 |
| Silt loam | 0—50 | 50—88 | 0—27 |
| Silt | 0—20 | 88—100 | 0—12 |
| Sandy clay loam | 45—80 | 0—28 | 20—35 |
| Clay loam | 20—45 | 15—53 | 27—40 |
| Silty clay loam | 0—20 | 40—73 | 27—40 |
| Sandy clay | 45—65 | 0—20 | 35—45 |
| Silty clay | 0—20 | 40—60 | 40—60 |
| Clay | 0—45 | 0—40 | 40—100 |

**2. Structure of Soil:**




Sand, silt and clay are found in aggregated form. Arrangement of these soil particles on certain defined patterns is called soil structure.

The natural aggregates of soil particles are clod peds whereas an artificially formed soil mass is called clod.

Soil structure also reveals the colour, texture and chemical composition of soil aggregates. Soil structure is influenced by air moisture, organic matter, micro-organisms and root growth. When many particles or peds are aggregated into cluster, a compound particle is formed.

**Soil Structure is described under the following three categories:**

**A. Type:**

This indicates the shapes or forms and arrangement of peds. Peds may be of various shapes, such as granular, crumb, angular blocky, sub angular blocky, platy and prismatic. Different types of peds and their properties are described.

**Table 23.3. Types of peds**

| Type of ped | Properties |
|---|---|
| (1) Granular | Small, spheroidal and nonporous. |
| (2) Crumb | Small, porous and spheroidal. |
| (3) Angular blocky | Block-like with sharp ends, one end may be pointed. |
| (4) Subangular blocky | Block-like but bounded by other aggregates. |
| (5) Platy | Plate like, sometimes plates are overlapped. |
| (6) Prismatic or Columnar | Prism like but without rounded surface. |

| **B. Size Class:** | **C. Grade:** |
|---|---|
| **These are as follows:**<br>(i) Very fine or very thin<br>(ii) Fine or thin<br>(iii) Medium<br>(iv) Coarse or thick<br>(v) Very coarse or very thick | This indicates the degree of distinctness of peds.<br>It is described under the following four categories:<br>**(i) Structure less:**<br>Peds not distinct, i.e., cement or sand like condition.<br>**(ii) Weak:**<br>Peds distinct and rarely durable.<br>**(iii) Moderate:**<br>Peds moderately well developed, fairly durable and distinct.<br>**(iv) Strong:**<br>Peds well developed, quite durable and distinct |

**3. Density and Soil Weight:**

Density of soil is the mass per unit volume. It is expressed in terms of gm per cubic centimeter. Average density of the soil is 2.65 gms per cubic centimeter. Density of soil varies greatly depending upon the degree of weathering.

For this reason soil density is expressed in two generally accepted forms

(i) Particle density or true density; and

(ii) Bulk density.

**(i) Particle density:**

Density of solid portion of soil is called particle density. It is sum total of densities of individual organic and inorganic particles. Average particle density of organic soil varies from 1.2 to 1.7 gms per ml.

**(ii) Bulk density or apparent density:**

Dry weight of unit volume of soil inclusive of pore spaces IS called bulk density. It is expressed in terms of gm per ml or lbs per cubic foot.

**4. Porosity of Soil:**

The spaces occupied by air and water between particles in a given volume of soil are called pore spaces. The percentage of soil volume occupied by pore space or by the interstitial spaces is called porosity of the soil. It depends upon the texture, structure, compactness and organic content of the soil. Porosity of the soil increases with the increase in the percentage of organic matter in the soil. Porosity of soil also decreases as the soil particles become much smaller in their dimension because of decrease in pore spaces. It also decreases with depth of the soil. The pore spaces are responsible for better plant growth because they contain enough air and moisture.

Depending upon the size pore spaces fall into two categories.

**These are:**

(1) Micro-pore spaces (capillary pore spaces)

(2) Macro-pore spapes (non-capillary pore spaces)

Capillary pore spaces can hold more water and restrict the free movement of water and air in soil to a considerable extent, whereas macro-pore spaces have little water holding capacity and allow free movement of moisture and air in the soil under normal conditions.

**5. Permeability of Soil:**

The characteristic of soil that determines the movement of water through pore spaces is referred to as soil permeability. Soil permeability, because it is directly dependent on the pore size, will be higher for the soil with large number of macro-pore spaces than that for compact soil with a large number of micro-pore spaces (capillary spaces).

**6. Soil Colour:**

Soils exhibit a variety of colours. Soil colour may be inherited from the parental material (Le., lithochromic) or sometimes it may be due to soil forming processes. The variations in the soil colour are due to organic substances, iron compounds, silica, lime and other inorganic compounds.

The O and A horizons tend to be dark because of their abundant organic material. The E horizon, if present, may be almost white, owing to the leaching of iron and aluminium oxides. The B horizon shows the most dramatic differences in colour, varying from yellow-brown to light red-brown to dark red, depending upon the presence of clay minerals and iron oxides. The horizons may be light coloured due to their carbonates, but they are sometimes reddish as a result of iron oxide accumulation. The original parent material, if rich in iron, may produce a very red soil even when there has been relatively little soil profile development. Well-drained soils are well aerated (oxidizing conditions), and iron oxidises to a red colour. Poorly drained soils are wet, and iron is reduced rather than oxidised. The colour of such a soil is often yellow.

**7. Soil Temperature:**

The chief sources of soil heat are solar radiations and heat generated in the decomposition of dead organic matters in the soil and heat formed in the interior of earth. The soil temperature greatly affects the physico-chemical and biological processes of the soil. Temperature of soil depends upon the temperature of atmospheric air and on moisture content. It is controlled by climate, colour of soil, slope, and altitude of the land and also by vegetation cover of the soil.

**8. Soil Plasticity, Compressibility and Erodibility:**

Soil plasticity is a property that enables the moist soil to change shape when some force is applied over it and to retain this shape even after the removal of the force from it. The plasticity of soil depends on the cohesion and adhesion of soil materials. Cohesion refers to the attraction of substances of like characteristics, such as, that of one water molecule for another. Adhesion refers to the attraction of substances of unlike characteristics. Soil consistency depends on the texture and amount of inorganic and organic colloids, structure and moisture contents of soil.

**Compressibility:**

It refers to the tendency of soil to consolidate or decrease in volume. The compressibility is partly a function of elastic nature of soil particles and is directly related to settlement of structures. With the decrease in the moisture contents soils gradually tend to become less sticky and less plastic and finally they become hard and coherent. Plastic soils have great cohesion force. It is only because of cohesion property the moist clay soils frequently develop cracks when they become dried. Coarse materials such as gravels and sands have low compressibility and the settlement is considerably less in these materials as compared to highly compressible fine grained organic soils.

**Erodibility:**

It refers to the ease with which soil materials can be removed by wind or water. Easily eroded materials include unprotected silt, sand and other loosely consolidated materials, Cohesive soils (with more than 20% clay) and naturally cemented soils are not easily removed from its place by wind or water and, therefore, have a low erosion factor

**Chemical Properties of soil:**

**1. Cation Exchange Capacity (CEC)**

Some plant nutrients and metals exist as positively charged ions, or “cations”, in the soil environment. Among the more common cations found in soils are hydrogen ($H^+$), aluminum ($Al^{+3}$), calcium ($Ca^{+2}$), magnesium ($Mg^{+2}$), and potassium ($K^+$). Most heavy metals also exist as cations in the soil environment. Clay and organic matter particles are predominantly negatively charged (anions), and have the ability to hold cations from being “leached” or washed away.

The adsorbed cations are subject to replacement by other cations in a rapid, reversible process called “cation exchange”.

The “cation exchange capacity”, or “CEC”, of a soil is a measurement of the magnitude of the negative charge per unit weight of soil, or the amount of cations a particular sample of soil can hold in

an exchangeable form. The greater the clay and organic matter content, the greater the CEC should be, although different types of clay minerals and organic matter can vary in CEC.

Cation exchange is an important mechanism in soils for retaining and supplying plant nutrients, and for adsorbing contaminants. It plays an important role in wastewater treatment in soils. Sandy soils with a low CEC are generally unsuited for septic systems since they have little adsorptive ability and there is potential for groundwater.

**2. Soil pH**

By definition, "pH" is a measure of the active hydrogen ion (H+) concentration. It is an indication of the acidity or alkalinity of a soil, and also known as "soil reaction".

The most important effect of pH in the soil is on ion solubility, which in turn affects microbial and plant growth. A pH range of 6.0 to 6.8 is ideal for most crops because it coincides with optimum solubility of the most important plant nutrients. Some minor elements (e.g., iron) and most heavy metals are more soluble at lower pH. This makes pH management important in controlling movement of heavy metals (and potential groundwater contamination) in soil.

In acid soils, hydrogen and aluminum are the dominant exchangeable cations. The latter is soluble under acid conditions, and its reactivity with water (hydrolysis) produces hydrogen ions. Calcium and magnesium are basic cations; as their amounts increase, the relative amount of acidic cations will decrease.

Factors that affect soil pH include parent material, vegetation, and climate. Some rocks and sediments produce soils that are more acidic than others: quartz-rich sandstone is acidic; limestone is alkaline. Some types of vegetation, particularly conifers, produce organic acids, which can contribute to lower soil pH values. In humid areas such as the eastern US, soils tend to become more acidic over time because rainfall washes away basic cations and replaces them with hydrogen. Addition of certain fertilizers to soil can also produce hydrogen ions. Liming the soil adds calcium, which replaces exchangeable and solution H+ and raises soil pH.

Lime requirement, or the amount of liming material needed to raise the soil pH to a certain level, increases with CEC. To decrease the soil pH, sulfur can be added, which produces sulfuric acid.

**Mineralization**

Mineralization is the decomposition of the complex chemical compounds in organic matter, by which the nutrients in those compounds are released in soluble inorganic forms that may be available to plants and microbes.

Decomposition is the process by which organic substances are broken down into simpler organic matter by living organisms. Degradation of a substance by chemical or physical processes.

**Factors affecting mineralization in soil:**

Various environmental and other factors affects rate of mineralization in soil by micro organsims, some of them are;

**i. Addition of available Nitrogen:**

Addition of inorganic nitrogen compounds such as ammonia, nitrite or easily decomposable nitrogen compounds like amino acids and proteins increase the rate by microorganisms. Microorganisms require both carbon and nitrogen for biosynthesis of their cellular materials. Therefore, microbial mineralization cannot occur without nitrogenous sources.

**ii. Temperature:**

Mineralization can occur from temperature near freezing to above 65 °C because both psychrophiles and thermophiles are involved in decomposition. But rate of biopolymer mineralization is maximum in mesophilic range of temperature of 25-30 °C.

**iii. Aeration:**

In anaerobic soil, anaerobic bacteria decompose biopolymers and in aerobic soli mainly fungi and aerobic bacteria take part in decomposition of biopolymers. Rate of biopolymers is higher in aerobic soil.

**iv. Moisture:**

Excessive moisture brings anaerobic condition in soil. Therefore, rate of decomposition is slower in water logged soil.

**v. pH:**

In neutral to alkaline soil, bacteria and actinomycetes mainly take part in decomposition.

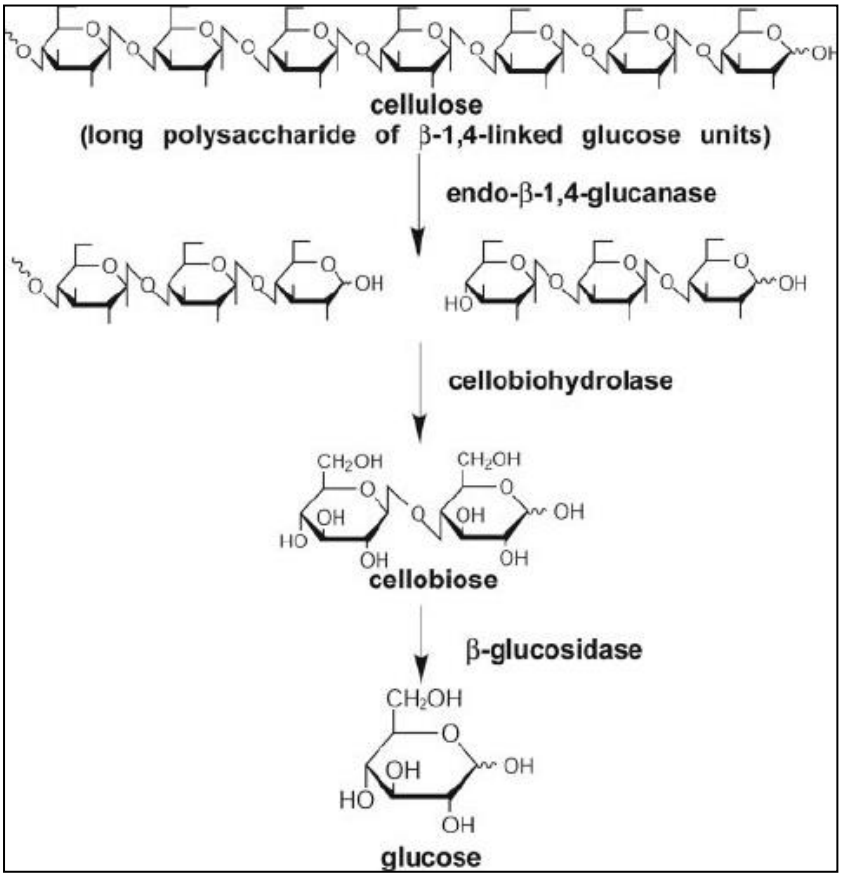
In acidic soil, fungi are dominant and rate of decomposition is slightly higher in acidic soil than alkaline and neutral.

**CELLULOSE:**

- Cellulose is found in cell wall of plant cell.
- Cellulose is a linear polymer of β-D-glucose in which glucose units are linked together by β-1,4-glycosidic bond. It is the most abundant organic matter found in nature. In plant it occurs in association with lignin and hemicellulose.
  Examples:
- Bacteria: *Bacillus, Cellulomonas, Clostridium, Cytophaga, Polyangium, Pseudomonas* etc
- Fungi: *Aspergillus, Alterneria, Fomes, Fusarium, Myrothecium* etc
- Actinomycetes: *Micromonospora, Nocardia, Streptomyces, Streptosporangium* etc

**Mechanism of cellulose mineralization:**

Pathway of cellulose mineralization follows series of enzymatic reactions. Enzymes responsible for cellulose mineralization are cellulase. Cellulase is a complex of three enzymes.




| Aerobic degradation | Anaerobic degradation |
|---|---|
| • 90 to 90 % of cellulose mineralization.<br>• End products are carbon dioxide and water.<br>• *Trichoderma reesei* is commercially successful and prevalent aerobic microorganism. | • 5 to 10 % of cellulose mineralization.<br>• End products are carbon dioxide, methane water.<br>• Example: *Clostridium thermocellum.* |

**HEMICELLULOSE:**

Hemicelluloses are a group of complex heteropolysaccharides made up of various sugars (D-xylose, D-glucose, D-mannose, D-galactose, and L-arabinose) and sugar acids (D-glucuronic and D-galacturonic acids), depending on the plant species.

Endo-1,4-β-xylanase and 1,4-β-xylosidase produces xylose. Xylan esterases, coumaric esterases, ferulic esterases, arbionfuranosidase, α-1-methyl glucuronidase produces xylose and mannose. Acetyl esterases, α-glucuronidase, β-xylosidase, endomannases, endoxylanases etc.

Examples: *Phanerochaete chrysosporium, Trichoderma reesei, Thermotoga, S. Viridosporus, Polyporus, Aspergillus* etc

**LIGNIN:**

Lignin is a class of complex organic polymers that form key structural materials in the support tissues of vascular plants and some algae. Lignins are particularly important in the formation of cell walls,

especially in wood and bark, because they lend rigidity and do not rot easily. Three monolignol monomers are precursors, all of which are methoxylated to various degrees: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol.

Enzymes involved in lignin degradation can generally be divided into two main groups: lignin-modifying enzymes (LME) and lignin-degrading auxiliary (LDA) enzymes. LDA enzymes are unable to degrade lignin on their own yet are necessary to complete the degradation process.

LME produced by different microorganisms are classified as phenol oxidase (laccases) and heme containing peroxidases (POD), namely lignin, manganese and multifunctional (versatile) peroxidise. Auxiliary enzymes allow the lignin degradation process through the sequential action of several proteins which may include oxidative generation of $H_2O_2$. This group includes glyoxal oxidase, aryl alcohol oxidases, pyranose 2-oxidase, cellobiose dehydrogenase and glucose oxidase.

Examples: *Phanerochaete chrysosporium,* white-rot fungus *Polyporus, Aspergillus, Agaricus, Chaemotium thermophilum* etc

**LIGNOCELLULOSE:**

Lignocellulose refers to plant dry matter (biomass), so called lignocellulosic biomass. It is the most abundantly available raw material on the Earth for the production of biofuels, mainly bio-ethanol. It is composed of carbohydrate polymers (cellulose, hemicellulose), and an aromatic polymer (lignin). These carbohydrate polymers contain different sugar monomers (six and five carbon sugars) and they are tightly bound to lignin.

Glycoside hydrolase, esterases and lyases are collectively called as carbohydrate active enzyme complex involved in the mineralization of lignocelluloses.

Examples: *Cellulomonas, Clostridium, Erwinia, Fibrobacter, Butyrivibrio, Acetivibrio, Thermobifida* etc.

**NITRATE:**

Mineralization is the process whereby organic matter, organic fertilizers, and some slow-release fertilizers are broken down or transformed by soil microorganisms to supply plants with available ammonium and nitrate.

Mineralization is a three-step process involving aminization, ammonification, and nitrification. Aminization and ammonification are stages of the mineralization process in which proteins, amines, and amino acids (usually from organic matter or humus) are converted to ammonium, a source of nitrogen utilized by plants. Mineralization is described in the following equation:

$$R\text{-}NH_2 + H_2O \text{ ---------- } > NH_4^+ + R\text{-}OH + \text{energy}$$

Organic nitrogen Ammonium nitrogen

Following mineralization, ammonium nitrogen ($NH_4^+$) is absorbed by plants or undergoes further transformation to become nitrate ($NO_3^-$). Ammonium nitrogen is the preferred nitrogen source because additional energy is required to transform nitrate into usable forms by plants and because ammonium nitrogen is less vulnerable to leaching and denitrification losses. Ammonia monooxygenase (AMO), Hydroxylamine oxidoreductase (HAO) and Nitrite oxidoreductase (NXR) are the three enzymes

involved in the transformation of ammonium nitrogen to nitrate nitrogen, referred as nitrification, is described as follows:

$NH_4^+ + O_2$ ---------- > $NO_2 + 2H+$ ---------- > $NO_3^- + H_2O$

Ammonium Nitrite nitrogen Nitrate nitrogen

Nitrification depends on environmental conditions that favor soil microbiological activity. Warm temperatures, adequate soil moisture, and soil oxygen are necessary for this activity. However, nitrification does not readily occur in extreme temperatures (e.g., below freezing or above 105°F), in saturated or poorly aerated soil, in excessively dry soil, or in soil with a low pH (<4.8). Under such unfavorable conditions, microorganisms do not perform nitrification and ammonium may accumulate. Ammonium nitrogen also may become toxic to turf grasses when they are grown under cool, low-light conditions that minimize nitrification.

Nitrate nitrogen is readily soluble in water and may be repelled by negatively charged exchange ions of soil components. Therefore, unless grasses rapidly utilize this form, it may be lost through leaching if excessive moisture is applied.

Examples: *Nitrosomonas, Nitrosospira, Nitrosococcus*; *Nitrobacter, Nitrospina, Nitrospira, Nitrococcus* etc.

**PHOSPHATE:**

Phosphorus is one of the most important constituent of several important compounds always present in organisms. It occurs both in organic (nucleic acids, nucleoproteins, phospholipids, etc.) and inorganic (phosphate) forms in the living organisms. Animals possessing bones have large amount of phosphorus in its inorganic form.

However, phosphorus is added to soil through chemical fertilizers, excrete and organism- residues. Though there is plenty of phosphorus present in the soil in unavailable inorganic forms, most of the plants obtain it only as orthrophosphate ions (soluble inorganic forms).

The cycle of phosphorus can be well studied under following two heads:

1. Mineralization: Conversion of Organic Phosphorus into Insoluble Inorganic Phosphates:

Many soil microorganisms produce enzymes that attack many of the organic phosphorus compounds in the soil and release inorganic phosphate. This process is comparable to the mineralization of organic nitrogen compounds. The enzymes involved in these reactions are collectively called 'phosphatases' which have a broad range of substrate specificity.

2. Solubilization: Conversion of Insoluble Inorganic Phosphates into Soluble Inorganic Phosphates:

The availability of phosphorus depends on the degree of solubilization by various organic and inorganic acids produced by microorganisms in soil. These are the solubilized form of insoluble inorganic phosphates which are taken in by the plants.

Fungi, e.g., *Aspergillus, Penicillium, Fusarium* are the most important of the soil microorganisms which produce substantial amounts of these acids; others are the bacteria, namely, *Bacillus, Pseudomonas, Micrococcus, Flavobacterium,* etc.

$$Ca_3(PO_4)_2 + H_2SO_4 \longrightarrow Ca_2H_2(PO_4)_2 + CaSO_4$$

(Insoluble calcium phosphate) (Sulfuric acid) (Calcium-monohydrogen phosphate fairly soluble in water)

$$Ca_2H_2(PO_4)_2 + H_2SO_4 \longrightarrow CaH_4(PO_4)_2 + CaSO_4$$

(Calcium-dihydrogen phosphate; highly soluble in water)

The availability of phosphorus in soil to plants depends of several reversible pathways:

Bacteria: Bacteria convert plant-available phosphate into organic forms that are then not available to plants. Although other bacteria make phosphate available by mineralisation, the contribution of this is small.

Adsorption: Inorganic (and available) phosphorus can be chemically bound (adsorbed) to soil particles, making it unavailable to plants. Desorption is the release of adsorbed phosphorus from its bound state into soil solution.

pH: Inorganic phosphorus compounds need to be soluble to be taken up by plants. This depends on the acidity (pH) of the soil. If soils are less than pH 4 or greater than pH 8. Maximum mineralization is observed in the pH range 6.0 to 7.0. Lower pH results tied up of phosphorous with other compounds (Al and Fe), whereas, at higher pH phosphorous precipitates in presence of calcium.

**HUMUS:**

Humus is the organic residue in the soil resulting from decomposition of plant and animal residues in soil, or it is the highly complex organic residualmatter in soil which is not readily degraded by microorganism, or it is the soft brown/dark coloured amorphous substance composed of residual organic matter along with deadmicroorganisms. On average humus is composed of Carbon (58 %), Nitrogen (3-6 %, Av.5%), acids -humic acid, fulvic acid, humin, apocrenic acid, and C: N ratio 10:1 to 12:1.

Functions/Role of Humus:

1.It improves physical condition of soil
2.Improve water holding capacity of soil.
3.Serve as store house for essential plant nutrients
4.Plays important role in determining fertility level of soil
5.It tend to make soils more granular with better aggregation of soil particles
6.Prevent leaching losses of water soluble plant nutrients
7.Improve microbial/biological activity in soil and encourage better development of plant-root system in soil
8.Act as buffering agent i.e. prevent sudden change in soil PH/soil reaction
9.Serve as source of energy and food for the development of soil organisms

10.It supplies both basic and acidic nutrients for the growth and development of higher plants
11.Improves aeration and drainage by making the soil more porous

Humus is mainly composed of two major groups, they are
I. Humic group
II. Non-humic group

I. Humic group: The humic substances make up about 60-80 % of the soil organic matter. On the basis of resistance to degradation and of solubility in acids and alkalis, humic substances have been classified into five chemical groupings.
Fulvic acid, Humic acid and Humin: Contain uronides, simple carbohydrates and their sugars, phenolic glycosides, tannin and other organics, also rich in N and P.

II. Non humic group: It comprises about 20-30% of the organic matter in soils. They are less complex and less resistant to microbial attack than those of humic group. They are comprised of specific organic compounds with definite physical and chemical properties.
E.g.: a. Polysaccharides- Polymers with sugar like structures. They are effective in enhancing soil aggregates stability.

b. Polyuronoids- They are not found in plants but have been synthesized by the soil microbes and held as part of the organisms body tissues.

c. Organic acids and protein like materials.

**Possible Questions**

**Part - C (8 marks)**

1. Detail account on distribution of microbes in soil
2. Describe in detail about soil properties.
3. Justify soil as microbial habitat.
4. List the cellulose degrading microbes and describe the microbial action on cellulose
5. Detail account on soil formation
6. Detail account on diversity and distribution of microorganism in soil
7. Write the characteristics, role and function of soil humus
8. Describe about the mineralization of lignocelluloses.
9. Describe about the mineralization of phosphorus.
10. Describe about the mineralization of Hemicelluloses.
11. Discuss in detail about mineralization of silica.
12. Describe about the mineralization of potassium.
13. Describe about the mineralization of lignin.

# KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: III B.Sc MB COURSE NAME: Microbes in Sustainable Agriculture and Development

COURSE CODE: 17MBU602B UNIT: I BATCH-2017-2020

| Sl.No | Questions | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | The number of microorganisms greater in the | Red soil | Clay soil | Rhizosphere soil | Bulk soil | Rhizosphere soil |
| 2 | What does top soil contains _____ | Minerals | Mixer of minerals and humus | Humus | Weathered rocks | Mixer of minerals and humus |
| 3 | Which of the following is capable of oxidising sulfur to sulfate | *Thiobacillus thiooxidans* | *Desulfotomaculum* | *Rhodospirullum* | *Rhodomicrobium* | *Thiobacillus thiooxidans* |
| 4 | The root exudates increase the availability of | Nutrients | Temperature | Moisture | Ph | Nutrients |
| 5 | _____ contains soil, clay and silt | Clayey soil | Sandy soil | Loamy soil | Red soil | Loamy soil |
| 6 | The ---------- that greatly help in weathering of the rock. | Lichen | Virus | Mycorrhizae | Bacteria | Lichen |
| 7 | Howmany years takes for the formation of 1 inch of top soil | 100 yrs | 200- 500 yrs | 20 years | 8- 10 years | 200- 500 yrs |
| 8 | In methane gas ,each carbon atom is bonded with---------- | Two hydrogen atoms | 3 oxygen atoms | 4 hydrogen atoms | 2 oxygen atoms | 4 hydrogen atoms |
| 9 | _____ is not the role of regular soil organisms | Organic matter decomposition | Breakdown of toxic compounds | Inorganic transformations | Convert the light energy to chemical energy | Convert the light energy to chemical energy |
| 10 | Most soil protozoa are flagellates or amoeba, having their dominant mode of nitrogen as ingestion of | Bacteria | mold | Yeast | Actinobacteria | Bacteria |
| 11 | The population of algae in the soil generally _____ | Greater than bacteria and fungi | Lesser than bacteria and fungi | Equal to bacteria | Higher than all other microbes | Greater than bacteria and fungi |
| 12 | Organism utilize carbon from $CO_2$ for their cellular synthesis | Heterotrophs | Autotrophs | Chemotrophs | None of these | Autotrophs |
| 13 | _____ play a key role in the transformation of rock to soil | Cyanobacteria | Pectin decomposing bacteria | Nitrifying bacteria | De-nitrifying bacteria | Cyanobacteria |
| 14 | Red soil contains large amount of _____ | Magnesium | Iron | Iron oxide | Carbon | Iron oxide |
| 15 | In carbon cycle flow of energy is _____ | Bidirectional | Linear | Cyclic | Irreversible | Linear |

| | | | | | | |
|---|---|---|---|---|---|---|
| 16 | The physical structure of soil is improved by the accumulation of ____ | Mold mycelium | Minerals | Water | Nutrients | Mold mycelium |
| 17 | _____ is the not common microbial pathogens of soil | To inject BT toxin into roots | *Bacillus anthracis* | *Clostridium perfringens* | *Clostridium botulinum.* | *Clostridium botulinum.* |
| 18 | Loss of fertile topsoil is _____ | Erosion | Reformation | Formation | Physical weathering | Erosion |
| 19 | Soil gases generally have a high proportion of | Methane | Oxygen | Carbon dioxide | $NO_2$ | Carbon dioxide |
| 20 | Humus and the smallest particles of rock form the | A-horizon | B-horizon | C-horizon | Bedrock | C-horizon |
| 21 | Which of the following fungi on infecting crop roots can improve their uptake of _____ | *Saccharomyces cerevisiae* | VAM fungi | *Candida* | *Aspergillus* | VAM fungi |
| 22 | Which of the following is not the biofertilizer | *Azatobacter* | *Azospirillum* | *Rhizobium* | *Clostridium* | *Clostridium* |
| 23 | Organism can serve as their sole source of carbon called as _____ | Autotrophs | Phototrohs | Chemotrophs | Phototrophs | Autotrophs |
| 24 | The phenomenon of commensalism refers to a relationship between organisms in which | One species of a pair benefits | Both the species of a pair benefit | One species of a pair is more benefited | Both the species not benefited | One species of a pair benefits |
| 25 | Which of the following organism is known to grow on the surface of freshly exposed rocks | Green algae | Diatoms | Cyanobacteria | Yeast | Cyanobacteria |
| 26 | Which of the following mentioned ghgs has the highest atmospheric lifetime? | Carbon tetrafluoride | Nitrous oxide | Methane | CFU | Carbon tetrafluoride |
| 27 | The organisms responsible for the characteristic musty or earth odor of a freshly plowed field is _____ | Actinomycetes | Bacteria | Fungus | Algae | Actinomycetes |
| 28 | The organism responsible for the characteristics musty and earthy odor of freshly ploughed field | Nocardia | Streptomyces | Micromonospora | All of these | All of these |
| 29 | Which of the following greenhouse gas is contributed by cattle farming? | Nitrous oxide | Methane | Carbon monoxide | $NH_4$ | Methane |
| 30 | Waterlogging can be expected in soil which is rich in | Sand | Clay | Silt | Humus | Humus |
| 31 | Bedrock is absent in which soil | Black soil | Red soil | Alluvial soil | Forest soil | Alluvial soil |
| 32 | Saline soil is also known as | Solon chalk | Solonetz | Sodochalk | None of these | Solonchalk |

| | | | | | | |
|---|---|---|---|---|---|---|
| 33 | Red color of the soil is due to | Hematite | Goethite | Glauconite | Magnemite | Hematite |
| 34 | Which important greenhouse gas other than methane is provided in agricultural fields | SO | Nitrous oxide | Ammonia | Oxygen | Nitrous oxide |
| 35 | The degradation of complex molecules in soil by fungi for utilization by bacteria | Neutralism | Mutualism | Commensalism | Antagonism | Commensalism |
| 36 | Fungi produces which of the following inhibitory toxic product | Cyanide | Fatty acid | Methane | Sulfides | Cyanide |
| 37 | Nitric oxide formula is | NO | $NO_2$ | $NO_3$ | None of these | NO |
| 38 | What is called for the movement of surface litter and topsoil from one place to another? | Soil submerge | Soil degradation | Soil erosion | Soil pollution | Soil erosion |
| 39 | What is used to convert wastelands into the agricultural lands? | Check dams | Water purifier | Rain harvesters | Gradonies | Gradonies |
| 40 | The four main bases in soil are | Calcium, Magnesium, Potassium and Sodium | Vitamin A to D | Nitrogen, Phosphorus, Iron and Potassium | Magnesium, Vandium, Iron and Aluminum | Calcium, Magnesium, Potassium and Sodium |
| 41 | The layer next to the upper zone is poor in certain _____ | Inorganic constituents | Organic constituents | Minerals | Salts | Inorganic constituents |
| 42 | The _____serve as store house for essential plant nutrients | Humus | Biofertilizer | Biomannure | Mannure | Humus |
| 43 | The breakdown of rocks from the result of a mechanical action is called _____ | Physical weathering | Chemical weathering | Both A and B | Biological weathering | Physical weathering |
| 44 | An accumulation of calcium carbonate in the soil profile is called _____ | Humification | Calcification | Laterization | Erosion | Calcification |
| 45 | Enzymes are involved in ligninolysis by white-rot fungi | Peroxidase and laccase | Lipase | Phosphatase | Nitrogenase | Peroxidase and laccase |
| 46 | The _____can degrade/mineralize a wide variety of toxic xenobiotics | Fungi | White rot fungi | Actinomycetes | *Bacillus* | White rot fungi |
| 47 | The _____prevent sudden change in soil ph/soil reaction | Biomannure | Mannure | Humus | Biofertilizer | Humus |
| 48 | The _____ is the major constituent of | Zinc | Phosphate | Nitrogen, | Iron | Phosphate |

| | | | | | | |
|---|---|---|---|---|---|---|
| | nucleic acids in living system, that element is added to the soil in the form of chemical fertilizers | | | phosphorus, iron and potassium | | |
| 49 | Fungi like _____ have the ability to survive in the absence of o2 and good cellulolytic sps | *Merulius* | *Aspergillus* | *Penicillium* | *Fusarium* | *Merulius* |
| 50 | The _____improves aeration and drainage by making the soil more porous | Biofertilizer | Biomannure | Mannure | Humus | Humus |
| 51 | The aerobes convert simple sugars to $CO_2$, the anaerobes responsible for _____ | Hydrogen | Acid | Inorganic acid | Organic acid | Organic acid |
| 52 | Good starch decomposer is _____ | *E.coli* | *Micromonospora* | *Klebsiella* | *Fusarium* | *Micromonospora* |
| 53 | Amylase are the enzymes responsible for _____ breakdown | Protein | Hemicellulose | Lignin | Starch | Starch |
| 54 | A water soluble polymer of galacturonic acid with methyl ester linkages | Pectin | Pectic acid | Protopectin | Inulin | Pectin |
| 55 | The process in which organic matter becomes saturated with silica is called _____ | Humification | Calcification | Laterization | Silicification | Silicification |
| 56 | Soil contains a mixture of grain sizes, the soil is called _____ | Loam | Granules | Clay | Rock soil | Loam |
| 57 | The _____ is a layer of partially altered bedrock. | Top soil | Bottom soil | C horizon | B horizon | C horizon |
| 58 | The root nodule of legumes contain pink pigment which has high affinity for oxygen is | Nod haemoglobin | Leghaemoglobin | Haemoglobin | Bacterial haemoglobin | Leghaemoglobin |
| 59 | Which one is green manure/biofertilizer? | Sesbania | Rice | Oat | Maize | Sesbania |
| 60 | In plants, the strains of which one of the following bacterium initiates to the formation of galls? | *Agrobacterium* | *Rhizobium* | *Pseudomonas* | *Ralstonia* | *Agrobacterium* |

**Syllabus**

**Unit II – Microbial Activity in Soil and Green House Gases**

Carbon dioxide, methane, nitrous oxide, nitric oxide – production and control

## GREENHOUSE GASES

Greenhouse gases influence the climate because they interact with flows of heat energy in the atmosphere. The main greenhouse gases that are affected by human activities and are relevant to agriculture are carbon dioxide ($CO_2$), methane ($CH_4$), and nitrous oxide ($N_2O$).

These gases vary in their potency or effect on the atmosphere, with methane being 21 times more potent than carbon dioxide, and nitrous oxide being 310 times more potent than carbon dioxide. This means that one molecule of methane has the same effect on global warming as 21 molecules of carbon dioxide.

Methane warms the planet 84 times as much as carbon dioxide over a 20-year period. The reason some gases are more potent than others is a complex combination of factors including among other things, the gas's longevity in the atmosphere and its molecular structure which determines its ability to trap or reflect heat.

Movement of the materials from one reservoir to another may be driven by physical agents like wind or gravitational energy. It may also be due to chemical energy, e.g., when the water body reaches saturation-the reservoir is chemically full and therefore, no longer can hold it as such. Then the material usually is precipitated out. The average time for which a material (molecule of a substance) remains in a reservoir is known as its residence time.

Various materials including different nutrients and metals move in the ecosystem in a cyclic manner. The major reserves or storage compartment of the materials are known as reservoirs. When the major reservoir of a nutrient is in the atmosphere, it is known as a % of the atmosphere. gaseous cycle, e.g., nitrogen cycle, which has its reservoir in the form of nitrogen. When the reservoir is in the earth's crust or sediments, it is known as a sedimentary cycle e.g., phosphorus cycle-which has its reserve as phosphate rocks. Sulphur cycle is an example of an intermediate type, which has reservoir both in soil and the atmosphere

Nutrients like carbon, nitrogen, sulphur, oxygen, hydrogen, phosphorus etc. move in circular paths through biotic and abiotic components and are known as biogeochemical cycles. Water also moves in a cycle, known as hydrological cycle. The nutrients to move through the food chain and ultimately reach the detritus compartment (containing dead organic matter) where various microorganisms carry out decomposition. Various organically bound nutrients of dead plants and animals are converted into inorganic substances by microbial decomposition that is readily used up by plants (primary producers) and the cycle starts afresh.

### Carbon dioxide

The carbon cycle is the biogeochemical cycle by which carbon is exchanged among the biosphere, pedosphere, geosphere, hydrosphere, and atmosphere of the Earth. It is one of the most important cycles of the earth and allows for carbon to be recycled and reused throughout the biosphere and all of its organisms.

The following major reservoirs of carbon interconnected by pathways of exchange:

i. The atmosphere.

ii. The terrestrial biosphere, which is usually defined to include fresh water systems and non-living organic material, such as soil carbon.

The oceans, including dissolved inorganic carbon and living and non-living marine biota.

iv. The sediments including fossil fuels

v. The Earth's interior, carbon from the Earth's mantle and crust is released to the atmosphere and hydrosphere by volcanoes and geothermal systems.

**Carbon is released into the atmosphere in several ways:**

i. Through the respiration performed by plants and animals. This is an exothermic reaction and it involves the breaking down of glucose (or other organic molecules) into carbon dioxide and water.

ii. Through the decay of animal and plant matter. Fungi and bacteria break down the carbon compounds in dead animals and plants and convert the carbon to carbon dioxide if oxygen is present, or methane if not.

iii. Through combustion of organic material which oxidizes the carbon it contains, producing carbon dioxide (and other things, like water vapour). Burning fossil fuels such as coal, petroleum products releases carbon dioxide. Burning agro fuels also releases carbon dioxide

iv. Volcanic eruptions and metamorphism release gases into the atmosphere. Volcanic gases are primarily water vapour, carbon dioxide and sulphur dioxide.

Carbon is transferred within the biosphere as heterotrophs feed on other organisms or their parts (e.g., fruits). This includes the uptake of dead organic material (detritus) by fungi and bacteria for fermentation or decay.

vi. Most carbon leaves the biosphere through respiration. When oxygen is present, aerobic respiration occurs, which releases carbon dioxide into the surrounding air or water. Otherwise, anaerobic respiration occurs and releases methane into the surrounding environment, which eventually makes its way into the atmosphere or hydrosphere (e.g., as marsh gas or flatulence)

**Circulation of carbon dioxide:**

i. Plants absorbs the carbon dioxide from the atmosphere.

ii. During the process of photosynthesis, plants incorporates the carbon atoms from carbon dioxide into sugars.

iii. Animals, such as the rabbit eat the plants and use the carbon to build their own tissues, chain the carbon content

iv. Through the food chain, carbon is transferred into foxes, lions etc.

v. The animals return carbon dioxide into the air when they breathe, and when they die, since the carbon is returned to the soil during decomposition.

Soil organic carbon (SOC) is the main constituent of soil organic matter (SOM). SOM is formed by the biological, chemical and physical decay of organic materials on the soil surface and below the ground. Basically, soil organic matter (SOM) is composed of anything that once lived, including:

 organic bits and pieces of plant and animal remains in various stages of decomposition, sloughed off cells and tissues of soil organisms, and substances from plant roots and soil microbes.

 living soil microbes (bacteria, fungi, archaea, nematodes and protozoa) and plant roots. If we weighed all of the organisms found in soil, soil microbes would comprise about 90-95% of that weight.
 humus, a chemically stable type of organic matter composed of large, complex organic carbon compounds, minerals, and soil particles. Humus is resistant to further decomposition unless disturbed by a change in environmental conditions. If undisturbed, humus can store soil carbon for hundreds to thousands of years. This makes humus a very important carbon sink.
 charcoal (biochar), incompletely burned plant material. Charcoal can remain undecomposed in the soil for decades to centuries.

The carbon balance within soil is controlled by carbon inputs from photosynthesis, carbon losses by respiration and carbon storage in humus

**Control:**

- The first strategy is to reduce emissions of carbon dioxide. This can take the form of reducing usable energy consumption or of substituting non-carbon based fuels for carbon-based fuels.
- The second strategy is to negate the damages of emissions of carbondioxide. This can take the form of introducing the carbon into places where it does less damage (such as the deep oceans), or of using counteracting forces to offset the effects (this would be such factors as using stratospheric dust to cool the earth, changing the albedo by putting gauze over the arctic,(or by painting roads or roofs white or by other means). The second approach, then, relies .on the inhomogeneities in nature to minimize the impact without influencing the actual emissions.
- A third approach would be to use other processes to clean out the carbon dioxide from the atmosphere ex post. This approach would rely on the possibility that removing the carbon from the air by a natural or industrial process is cheaper than refraining from putting the carbon in the atmosphere in the first place. Two possibilities here are simply growing trees and locking the carbon in the trees, or removing the carbon from the air by an industrial process.

**Methane**

Methane is a chemical compound with the chemical formula $CH_4$. It is a group-14 hydride and the simplest alkane, and is the main constituent of natural gas. Methane is a greenhouse gas that is 23 times more effective at trapping heat than carbon dioxide. Methanogenesis or biomethanation is the formation of methane by microbes known as methanogens. They are unique in terms of metabolism and energy conservation, are widespread in different habitats and show a high diversity in morphology and physiological parameters. Different methanogenic reactions are catalyzed by unique sets of enzymes and coenzymes. While reaction mechanism and energetics vary between one reaction and another, all of these reactions contribute to net positive energy production by creating ion concentration gradients that are used to drive ATP synthesis.

Known methanogenic archaea belonged exclusively to the phylum *Euryarchaeota and* were classified 7 orders, namely *Methanococcales, Methanobacteriales, Methanosarcinales, Methanomicrobiales* and *Methanopyrales.*

1. Hydrogenotrophic methanogenesis: Hydrogenotrophic methanogenesis from $H_2$ and $CO_2$ is found in almost all methanogenic orders with the exception of the *Methanomassiliicoccales*. Due to its broad distribution it is postulated that this type of methanogenesis is the ancestral form of methane production. Organisms that

produce methane via $H2/CO_2$ methanogenesis include *Methanosarcina barkeri*, *Methanobacterium thermoautotrophicum*, and *Methanobacterium wolfei*. These organisms are typically found in anaerobic environments.

2. Aceticlastic methanogenesis: Methane formation from acetate, called aceticlastic methanogenesis, can be found only in the order *Methanosarcinales*

3. Methylotrophic methanogenesis is the methane formation from different methylated compounds such as methanol, methylamines or methylated thiols, is found in the orders *Methanomassiliicoccales*, *Methanobacteriales* and *Methanosarcinales*.

Methanogens show not only a wide diversity in regard to their habitats but are also highly diverse in terms of morphology, temperature optimum, pH and osmolarity. The shapes of methanogens can be coccoid as for *Methanococcus*, *Methanosphaera* or *Methanococcoides*, long or short rods as for *Methanobacterium* or *Methanobrevibacter*, or rods in chains as for *Methanopyrus*. *Methanoplanus* has a plate-shaped morphology and *Methanospirillium*, as the name says, a spirally shape. *Methanosarcina* are irregularly shaped cocci, most often arranged to sarcina cell packages.

Many methanogens have a mesophilic temperature spectrum, as, e.g. *Methanosarcina*, *Methanobacterium*, or most *Methanococcus*. However, thermophilic and even hyperthermophilic methanogens are known, like *Methanothermobacter thermautotrophicus* or *M. jannaschii* which grow at temperatures of up to 75 and 86 °C, respectively. Even growth up to 110 °C is possible in hot environments as shown for the hyperthermophilic strain *M. kandleri*. In contrast, also cold-loving methanogenic strains could be isolated. One example is the methanol-converting archaeon *Methanolobus psychrophilus*, which grows optimally at 18 °C and shows still metabolic activity at 0 °C. Although most methanogens grow optimally around neutral pH, some, which are halophilic or halotolerant, show also an adaptation to alkaline pH.

**Control:**

1. Anaerobic "digesters" utilize microorganisms to decompose cattle manure within a huge container. The resulting biogas can be harvested and used for "free" electricity production, rather than be expelled into the atmosphere.
2. Control of the environmental acetate concentration
3. Control of the environmental $H_2$ concentration

**Nitrous oxide ($N_2O$)**

Globally agriculture contributes a significant lot of the total green house gas, consisting of $N_2O$ (84 %) as the major trace constituent. The global emission of $N_2O$ from Indian agricultural activities is 0.23 %. Annual emission of $N_2O$ is increasing at a rate of 50 ppb and nearly 70 % of this emission, both direct and indirect, is believed to be from the agricultural sector. Though $N_2O$ contributes to 9 % of total green house gas emission, its global warming potential is 300 times higher than $CO_2$.

Natural production of nitrous oxide is from microbial activity in soils and in the ocean and after nitrous oxide production by the microbes the gas goes to the atmosphere. Human production of nitrous oxide is primarily due to combustion of fossil fuels, biomass burning, industrial production of nitric acid, and application of fertilizers to agricultural crops.

$N_2O$ is produced naturally from soil through major microbial metabolic pathways such as nitrification and denitrification. Nitrification occurs when soil $NH_4$-N is available and environmental conditions (such as temperature and moisture) are favourable for the nitrifier population. During oxygen deficient conditions, microorganisms use nitrate as the electron acceptor for the anaerobic oxidation process known as denitrification. The later generally occurs in wet soils where diffusion of oxygen is inhibited by saturated condition inside the soil aggregate (compaction).

**Nitric oxide (NO)**

Neither nitric oxide nor nitrogen dioxide is greenhouse gases, although they are important in the process of creation of tropospheric ozone which is a greenhouse gas. There are several sources of nitrous oxide, both natural and anthropogenic (human), to the atmosphere with many of these sources difficult to measure. Because of this, there is general agreement that the atmospheric sources and sinks of nitrous oxide are difficult to bring into balance.

Nitrification is the process by which ammonia ($NH_3$) or ammonium ($NH_4^+$) is converted to nitrate ($NO_3^-$). Nitrification is the net result of two distinct processes: oxidation of ammonium to nitrite ($NO_2^-$) by nitrosifying or ammonia-oxidizing bacteria and oxidation of nitrite ($NO_2^-$) to nitrate ($NO_3^-$) by the nitrite-oxidizing bacteria. Nitrification is an important step in the nitrogen cycle in soil. Nitrification is an aerobic process performed by small groups of autotrophic bacteria and archaea.

Nitrification is a process of nitrogen compound oxidation (effectively, loss of electrons from the nitrogen atom to the oxygen atoms):

1. $2\ NH_4^+ + 3\ O_2 \rightarrow 2\ NO_2^- + 2\ H_2O + 4\ H^+$ (Nitrosomonas)
2. $2\ NO_2^- + O_2 \rightarrow 2\ NO_3^-$ (Nitrobacter, Nitrospina, Nitrococcus, and Nitrospira)
3. $NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$
4. $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$

Both of these processes are extremely energetically poor, which leads to very slow growth rates for both types of organisms.

Denitrification is a microbially facilitated process where nitrate ($NO_3^-$) is reduced and ultimately produces molecular nitrogen ($N_2$) through a series of intermediate gaseous nitrogen oxide products. Facultative anaerobic bacteria perform denitrification as a type of respiration that reduces oxidized forms of nitrogen in response to the oxidation of an electron donor such as organic matter. The preferred nitrogen electron acceptors in order of most to least thermodynamically favorable include nitrate ($NO_3^-$), nitrite ($NO_2^-$), nitric oxide (NO), nitrous oxide ($N_2O$) finally resulting in the production of dinitrogen ($N_2$) completing the nitrogen cycle. Denitrifying microbes require a very low oxygen concentration of less than 10 %, as well as organic C for energy.

The process is performed primarily by heterotrophic bacteria (such as *Paracoccus denitrificans* and various pseudomonads), although autotrophic denitrifiers have also been identified (e.g., *Thiobacillus denitrificans*). Denitrifiers are represented in all main phylogenetic groups.[3] Generally several species of bacteria are involved in the complete reduction of nitrate to $N_2$, and more than one enzymatic pathway has been identified in the reduction process

## Half reactions [ edit ]

Denitrification generally proceeds through some combination of the following half reactions, with the enzyme catalyzing the reaction in parentheses:

- $NO_3^- + 2\ H^+ + 2\ e^- \rightarrow NO_2^- + H_2O$ (Nitrate reductase)
- $NO_2^- + 2\ H^+ + e^- \rightarrow NO + H_2O$ (Nitrite reductase)
- $2\ NO + 2\ H^+ + 2\ e^- \rightarrow N_2O + H_2O$ (Nitric oxide reductase)
- $N_2O + 2\ H^+ + 2\ e^- \rightarrow N_2 + H_2O$ (Nitrous oxide reductase)

The complete process can be expressed as a net balanced redox reaction, where nitrate ($NO_3^-$) gets fully reduced to dinitrogen ($N_2$):

- $2\ NO_3^- + 10\ e^- + 12\ H^+ \rightarrow N_2 + 6\ H_2O$

## Possible questions

## PART-B (2 MARKS)

1. Define De-nitrification process
2. Discuss the key steps in nitrification process
3. Name the five process in nitrogen cycle
4. Define methanogenesis
5. Name the organism involved in nitrogen cycle
6. Write about the carbon , methane and nitrous oxide distribution
7. Write about the microorganism mediating function in soil
8. Write about co2 circulation

## PART-C (8 MARKS)

1. Compare the specific roles of nitrifying bacteria, decomposers and denitrifying bacteria in the plant/soil ecosystem.
2. Explain why soil microbes are critical to carbon storage in soil.
3. Describe at least one way the nitrogen cycle and the carbon cycle are interconnected in soil.
4. Explain why soil microbe diversity is important to building rich soil.
5. Justify the green house gases influence the climate change
6. Detail account on denitrification process
7. Discuss in detail about the microbe , soil and carbon relation
8. Detail note on Methane production and control
9. Brief account on green house gas and its effect
10. Detail account on carbon production and control
11. Discuss the steps in involved in carbon release to atmosphere

# KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: III B.Sc MB COURSE NAME: Microbes in Sustainable Agriculture and Development

COURSE CODE: 17MBU602B UNIT: II BATCH-2017-2020

| Sl.No | Question | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | _____ catalyze conversion of atmospheric nitrogen to ammonia | Kinase | Hydrogenase | Nitrogenase | Phosphatase | Nitrogenase |
| 2 | Conversion of nitrite to nitrate is carried out by _____ | *Nitrosomonas* | *Nitrosococcus* | *Nitrobacter* | *Clostridium* | *Nitrobacter* |
| 3 | Denitrification is done only by microorganisms, usually by which one of the following | Facultative anaerobes | Obligate aerobe | Phototrophic aerobe | Microaerophilic | Facultative anaerobes |
| 4 | Denitrification is a microbially facilitated process of _____ | Nitrate degradation | Nitrate assimilation | Nitrate oxidation | Nitrate reduction | Nitrate reduction |
| 5 | The phenomenon of commensalism refers to a relationship between organisms in which | One species of a pair benefits | Both the species of a pair benefit | One species of a pair is more benefited | Both the species not benefited | One species of a pair benefits |
| 6 | Soil gases generally have a high proportion of | Methane | Oxygen | Carbon dioxide | No2 | No2 |
| 7 | Fixed nitrogen is lost through the process of nitrogen cycle through _____ | Nitrification | Denitrification | Humification | Calcification | Denitrification |
| 8 | Waterlogging can be expected in soil which is rich in | Humus | Sand | Clay | Silt | Humus |
| 9 | While the use of_____ may reduce the methane emission | Fermented organic fertilizer | Chemical fertilizer | Manure | Inorganic substance | Fermented organic fertilizer |
| 10 | In the rice field methane is produced in the soil layer with depths of _____ | 2–10 cm. | 2–50 cm. | 2–25 cm. | 2–40 cm. | 2–25 cm. |
| 11 | What common gas has an extremely high global warming potential | Carbon dioxide | Methane | Nitric oxide | Nitrous oxide | Methane |
| 12 | Methane remains in the atmosphere for about _____ | 4 yrs | 3 yrs | 5 yrs | 8 yrs | 12 yrs |
| 13 | Globally, over 60% of total methane emissions come from _____ | Chemical agriculture | Farming | Animal activities | Human activities | Human activities |
| 14 | _____ is the key emitting sector of methane emissions, responsible for about 40% | Industry | Hospital | Textiles | Agriculture | Agriculture |
| 15 | Methane warms the planet _____ as much as carbon dioxide over a 20-year period | 84 times | 100 times | 50 times | 20 times | 84 times |
| 16 | The _____ is not the source of methane emission | coal mining | Organic farming | Oil and gas production | Biomass burning | Organic farming |

| | | | | | | |
|---|---|---|---|---|---|---|
| 17 | improved livestock feeding strategies can reduce of 20% of global _____ | Cooling | Warming | Carbon emission | Methane emission | Methane emission |
| 18 | _____ from coal mining and the oil and gas sector could be reduced by over 65% by preventing gas leakage | Metane emissions | Cooling | Warming | Carbon emission | Metane emissions |
| 19 | The presence of methane in the atmosphere can also affect the _____ | Sulphur oxide | Nitric oxide | Nitrous oxide | Tropospheric ozone | Tropospheric ozone |
| 20 | Upgrade wastewater treatment with gas recovery and overflow control will control the _____ | Metane emissions | Cooling | Warming | Carbon emission | Metane emissions |
| 21 | Methane does not cause direct harm to _____ | Human health | Environment | Ponds | Water bodies | Human health |
| 22 | Reducing_____ emissions can deliver energy, safety, and local air and water quality benefits | Nitrogen | Methane | Oxygen | Ozone | Methane |
| 23 | To reduce ozone pollution, need to control emissions of _____ | Nitrogen | Methane | Oxygen | Ozone | Methane |
| 24 | _____reduces nitrate to nitrite at the expense of nad(p)h | Nitrate reductase | Reductase | Oxidase | Nitrate oxidase | Nitrate reductase |
| 25 | 1-electron transfer from nad(p)h to nitrite resulting in _____ formation | Nitrogen | Formic oxide | Nitrous oxide | Nitric oxide | Nitric oxide |
| 26 | The conversion of amino acids to ammonium by soil decomposers is called_____ | Ammonification | Mineralization | Deamination | Both a and b | Both a and b |
| 27 | To fix one molecule of nitrogen _____ | 6 ATP molecules are required | 12 ATP molecules are required | 16 ATP molecules are required | 20 ATP molecules are required | 16 ATP molecules are required |
| 28 | Conversion of nitrite to nitrate is carried out by _____ | *Nitrosomonas* | *Nitrosococcus* | *Nitrobacter* | *Clostridium* | *Nitrobacter* |
| 29 | All of the following are examples of negative symbiosis_____ | Amensalism | Competition | Commensalism | Parasitism | Competition |
| 30 | The reservior for nitrogen is _____ | The atmosphere | Rocks | Ammonia | Nitrates | The atmosphere |
| 31 | Degree of compost maturity can be assesed by _____ | Infrared tehnique | Germination test | Both a and b | Mpn test | Both a and b |

| | | | | | | |
|---|---|---|---|---|---|---|
| 32 | Which one of the following nacterium peodices nodule in alfalfa | *Bradyrhizobium* sp. | *Rhizobium* aggregatum | *Rhizobium* leguminosarum | *Rhizobium* melliloti | *Rhizobium* melliloti |
| 33 | In non leguminous plant, nodules are formed by which one of the following | *Anabaena* | *Frankia* | *Ralstonia* sp. | *Sinorhizobium* | *Frankia* |
| 34 | Which one of the following component is the limiting and critical for soil | Carbon | Nitrogen | Oxygen | Phosphorous | Phosphorous |
| 35 | Which of the following is a classical example of a rhizobial species having biovars | *Rhizobium borbori* | *Rhizobium leguminosarum* | *Rhizobium lupini* | *Rhizobium vignae* | *Rhizobium leguminosarum* |
| 36 | Which of the following compound is known as the most resistant to microbial degradation during organic matter decomposition | Cellulose | Chitin | Hemicellulose | Lignin | Lignin |
| 37 | Which of the following forms symbiotism in soyabean crops | *Azotobacter paspali* | *Bradyrhizobium* | *Nostoc* | *Rhizobium* | *Bradyrhizobium* |
| 38 | In1888, a dutch microbiologist beijerinck succeeded in isolating which one of the following bacterial strain from root nodules? | *Bradyrhizobium japonicum* | *Rhizobium leguminosarum* | *Sinorhizobium meliloti* | *Nostoc* | *Rhizobium leguminosarum* |
| 39 | Ammonia produced in the bacteriod needs to be transported to the plant through which one of the following membrane_____ | Lipid membrane | Periplasmic membrane | Symbiosome membrane | Cytoplasmic membrane | Symbiosome membrane |
| 40 | Which one of the following is the first species of rhizobia, identified in 1889 | *Rhizobium borbori* | *Rhizobium leguminosarum* | *Rhizobium leucaenae* | *Rhizobium lupine* | *Rhizobium leguminosarum* |
| 41 | The fixation of the inert atmospheric elemental nitrogen by microorganisms through a reductive process accounts for about_____ | 60% | 70% | 90% | 50% | 70% |
| 42 | $CO_2$ production appeared to be strongly controlled by _____ | Temperature | Moisture | pH | Salinity | Temperature |
| 43 | Soil $CO_2$ emissions comprise an important component of _____ | Biogeochemical cycle | Sulphur cycle | Nitrogen cycle | Carbon cycle | Carbon cycle |
| 44 | The present globally averaged concentaration of atmospheric $CH_4$ is _____ | 1.75ppm | 2.755ppm | 2.5ppm | 1.5ppm | 1.75ppm |
| 45 | The concentaration of atmospheric ch4 is increasing globally with a rate of _____% per year. | 0.8 | 8 | 9 | 0.9 | 0.9 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 46 | Methane emission rate from the rice field significantly reduced by _____ | Fermented organic fertilizer | Chemical fertilizer | Use of so42-containing fertilizer | Both a & c | Both a & c |
| 47 | 30%, of the produced methane is emitted to the atmosphere through _____ | Wheat plant | Rice plant | Tomato plant | Vegetable crops | Rice plant |
| 48 | $H_2CO_2$- dependent methanogenesis dominates upto _____, $CH_4$ production in sediments of lakes, marinebights and peat bogs. | 50 | 25 | 10 | 100 | 100 |
| 49 | Ozone is responsible for about _____premature respiratory death | 1000 | 2 million | 10000 | 1 million | 1 million |
| 50 | Pedogenesis otherwise called as _____. | Soil erosion | Soil formation | Soil accumulation | Soil deposition | Soil formation |
| 51 | Sand and silt are the products of physical and chemical weathering of the _____ | Rock | Parent rock | Volcanic rock | Igneous rock | Parent rock |
| 52 | The clumping of the soil textural components of sand , silt and clay causes _____. | Accumulation | Erosion | Aggregates | Formation | Aggregates |
| 53 | An accumulation of calcium carbonate in the soil profile is called _____ | Humification | Calcification | Laterization | Erosion | Calcification |
| 54 | Enzymes are involved in ligninolysis by white-rot fungi | Peroxidase and laccase | Lipase | Phosphatase | Nitrogenase | Peroxidase and laccase |
| 55 | The _____can degrade/mineralize a wide variety of toxic xenobiotics | Fungi | White rot fungi | Actinomycetes | Bacillus | White rot fungi |
| 56 | The _____prevent sudden change in soil ph/soil reaction | Biomannure | Mannure | Humus | Biofertilizer | Humus |
| 57 | The _____ is the major constituent of nucleic acids in living system, that element is added to the soil in the form of chemical fertilizers | Zinc | Phosphate | Nitrogen, phosphorus, iron and potassium | Iron | Phosphate |
| 58 | Fungi like _____ have the ability to survive in the absence of o2 and good cellulolytic sps | Merulius | Aspergillus | Penicillium | Fusarium | Merulius |
| 59 | The _____improves aeration and drainage by making the soil more porous | Biofertilizer | Biomannure | Mannure | Humus | Humus |
| 60 | Bedrock is absent in which soil | Black soil | Red soil | Alluvial soil | Forest soil | Alluvial soil |

**Syllabus**

**Microbial Control of Soil Borne Plant Pathogens**

Biocontrol mechanisms and ways, Microorganisms used as biocontrol agents against Microbial plant pathogens, Insects, Weeds.

## Biocontrol agents

- The term "biological control" and its abbreviated synonym "biocontrol" have been used in different fields of biology
- In plant pathology, the term applies to the use of microbial antagonists to suppress diseases as well as the use of host-specific pathogens to control weed populations. More broadly, the term biological control also has been applied to the use of the natural products extracted or fermented from various sources.
- These formulations may be very simple mixtures of natural ingredients with specific activities or complex mixtures with multiple effects on the host as well as the target pest or pathogen.
- The organism that suppresses the pest or pathogen is referred to as the biological control agent (BCA).
- "The use of natural or modified organisms, genes, or gene products, to reduce the effects of undesirable organisms and to favour desirable organisms such as crops, beneficial insects, and microorganisms".

## Types of interactions

Throughout their lifecycle, plants and pathogens interact with a wide variety of organisms. These interactions can significantly affect plant health in various ways.

In order to understand the mechanisms of biological control, it is helpful to appreciate the different ways that organisms interact.

- Mutualism
- Protocooperation
- Commensalism
- Neutralism
- Competition
- Amensalism
- Parasitism
- Predation
- Syntrophism

**Mutualism** is an association between two or more species where both species derive benefit. Sometimes, it is an obligatory lifelong interaction involving close physical and biochemical contact, such as those between plants and mycorrhizal fungi. However, they are generally facultative and opportunistic. These types of mutualism can contribute to biological control, by fortifying the plant with improved nutrition and/or by stimulating host defences

Protozoan-termite:

- Protozoan-termite relationship is the classical example of mutualism in which flagellated protozoan lives in the gut of termites.
- Theses flagellated protozoan feeds on diet of carbohydrates acquired as cellulose or lignin by their host termites, metabolize into acetic acid which is utilized by termites.

Lichens:

- Lichens are excellent example of mutualism.
- They are the association of specific fungi and certain genus of algae. In lichen, fungal partner is called mycobiont and algal partner is called
- Phycobiont is member of cycanobacteria ad green algae (*Trabauxua*).
- Because phycobionts are photoautotrophs, the fungus get its organic carbon directly from algal partner, in turn fungi protects the phycobiont from extreme conditions and also provide water and minerals to algae.

Paramecium-Chlorella:

- *Paramecium* (protozoa) can host *Chlorella* (algae) within its cytoplasm.
- The algae *Chlorella* provide the protozoan partner with organism carbon and $O_2$, in turn protozoa provide protection, mortility, $CO_2$ and other growth factors.
- The presence of *Chlorella* within *Paramecium* helps to survive protozoa in anaerobic condition as long as there is sufficient light.

**Protocooperation** is a form of mutualism, but the organisms involved do not depend exclusively on each other for survival. Many of the microbes isolated and classified as BCAs can be considered facultative mutualists involved in protocooperation, because survival rarely depends on any specific host and disease suppression will vary depending on the prevailing environmental conditions.

- Association of *Desulfovibrio* and *Chromatium*: it is a protocooperation between carbon cycle and sulfur cycle.
- Interaction between $N_2$-fixing bacteria and cellulolytic bacteria such as *Cellulomonas*

**Commensalism** is a symbiotic interaction between two living organisms, where one organism benefits and the other is neither harmed nor benefited. Most plant-associated microbes are assumed to be commensals with regards to the host plant, because their presence, individually or in total, rarely results in overtly positive or negative consequences to the plant.

*Flavobacterium* (host) and *Legionella pneumophila* (commensal):

- *Flavobacterium* excrete cystine which is used by *Legionella pneumophila* and survive in aquatic habitat.

Association of *Nitrosomonas* (host) and *Nitrobacter* (commensal) in Nitrification:

- *Nitrosomonas* oxidize Ammonia into Nitrite and finally *Nitrobacter* uses nitrite to obtain energy and oxidize it into Nitrate.

**Neutralism** describes the biological interactions when the population density of one species has absolutely no effect whatsoever on the other. Related to biological control, an inability to associate the population dynamics of pathogen with that of another organism would indicate neutralism. In contrast, antagonism between organisms results in a negative outcome for one or both.

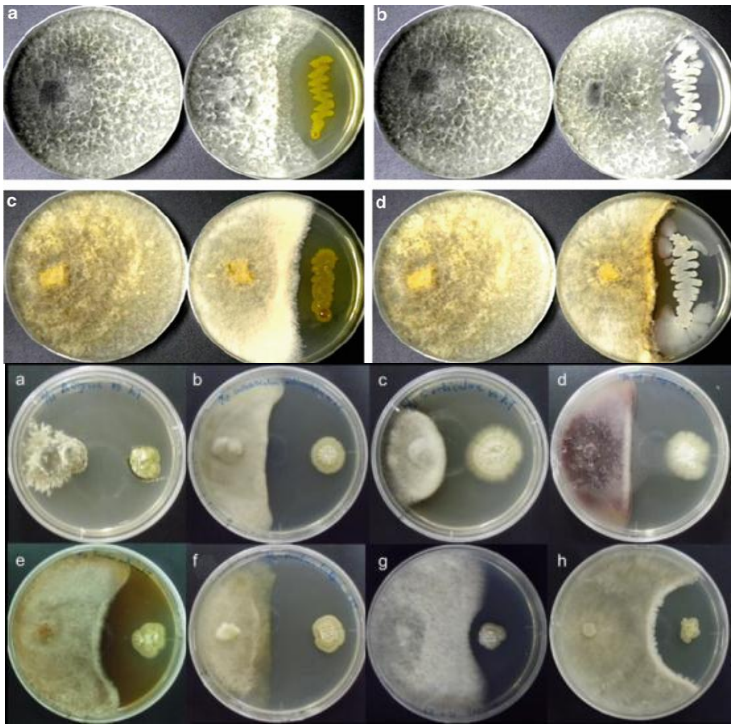
- The tarantulas living in a desert and the cacti living in a desert

**Competition** within and between species results in decreased growth, activity and/or fecundity of the interacting organisms. Biocontrol can occur when non-pathogens compete with pathogens for nutrients in and around the host plant. The competition represents a negative relationship between two microbial populations in which both the population are adversely affected with respect to their survival and growth. Direct interactions that benefit one population at the expense of another also affect our understanding of biological control.

- Competition between *Paramecium cadatum* and *Paramecium aurelia:* Both species of Paramecium feeds on same bacteria population when these protozoa are placed together. *P. aurelia* grow at better rate than *P. caudatum* due to competition

**Amensalism (antagonism):**

When one microbial population produces substances that are inhibitory to other microbial population then this inter population relationship. It is a negative relationship. The first population which produces inhibitory substances are unaffected or may gain a competition



and survive in the habitat while other population get inhibited. This chemical inhibition is known as antibiosis.

Lactic acid produced by lactic acid bacteria in vaginal tract:

Lactic acid produced by many normal floras in vaginal tract is inhibitory to many pathogenic organisms such as *Candida albicans*.

Skin normal flora:

Fatty acid produced by skin flora inhibits many pathogenic bacteria in skin

*Thiobacillus thiooxidant*:

*Thiobacillus thioxidant* produces sulfuric acid by oxidation of sulfur which is responsible to lowering of pH in the culture media which inhibits the growth of most other bacteria.

**Parasitism** is a symbiosis in which two phylogenetically unrelated organisms coexist over a prolonged period of time. In this type of association, one organism, usually the physically smaller of the two (called the parasite) benefits and the other (called the host) is harmed to some measurable extent. The host-parasite relationship is characterized by a relatively a long period of contact which may be physical or metabolic.

Viruses:

Viruses are obligate intracellular parasite that exhibit great host specificity.

There are may viruses that are parasite to bacteria (bacteriophage), fungi, algae, protozoa etc.

*Bdellovibrio*:

*Bdellavibrio* is ectoparasite to many gram negative bacteria.

The parasite *Bdellovibrio* penetrate the outer membrane of its host and enters periplasmic space but not inside host cytoplasm.

The activities of various hyperparasites, i.e., those agents that parasitize plant pathogens, can result in biocontrol. And, interestingly, host infection and parasitism by relatively avirulent pathogens may lead to biocontrol of more virulent pathogens

**Predation** refers to the hunting and killing of one organism by another for consumption and sustenance. Phenomenon when one organism (predator) engulf or attack other organism (prey). The prey can be larger or smaller than predator and this normally results in death of prey. Normally predator-prey interaction is of short duration.

e.g. protists, and mesofauna, e.g. fungal feeding nematodes and microarthropods, that consume pathogen biomass for sustenance.

Protozoan-bacteria in soil:

Many protozoans can feed on various bacterial population which helps to maintain count of soil bacteria at optimum level

*Bdellovibrio, Vamparococcus, Daptobacter* etc are examples of predator bacteria that can feed on wide range of bacterial population.

**Syntrophism**

It is an association in which the growth of one organism either depends on or improved by the substrate provided by another organism. In syntrophism both organism in association gets benefits.

*Lactobacillus arobinosus* and *Enterococcus faecalis*:

In the minimal media, *Lactobacillus arobinosus* and *Enterococcus faecalis* are able to grow together but not alone. The synergistic relationship between *E. faecalis* and *L. arobinosus* occurs in which *E. faecalis* require folic acid which is produced by *L. arobinosus* and in turn *lactobacillus* require phenylalanine which is produced by *Enterococcus faecalis*.

**Mechanisms of biological control**

Direct antagonism results from physical contact and/or a high-degree of selectivity for the pathogen by the mechanism(s) expressed by the BCA(s).

In contrast, indirect antagonisms result from activities that do not involve sensing or targeting a pathogen by the BCA(s). Stimulation of plant host defense pathways by non-pathogenic BCAs is the most indirect form of antagonism.

**Table 1.** Types of interspecies antagonisms leading to biological control of plant pathogens.

| Type | Mechanism | Examples |
|---|---|---|
| Direct antagonism | Hyperparasitism/predation | Lytic/some nonlytic mycoviruses<br>*Ampelomyces quisqualis*<br>*Lysobacter enzymogenes*<br>*Pasteuria penetrans*<br>*Trichoderma virens* |
| Mixed-path antagonism | Antibiotics | 2,4-diacetylphloroglucinol<br>Phenazines<br>Cyclic lipopeptides |
| | Lytic enzymes | Chitinases<br>Glucanases<br>Proteases |
| | Unregulated waste products | Ammonia<br>Carbon dioxide<br>Hydrogen cyanide |
| | Physical/chemical interference | Blockage of soil pores<br>Germination signals consumption<br>Molecular cross-talk confused |
| Indirect antagonism | Competition | Exudates/leachates consumption<br>Siderophore scavenging<br>Physical niche occupation |
| | Induction of host resistance | Contact with fungal cell walls<br>Detection of pathogen-associated, molecular patterns<br>Phytohormone-mediated induction |

***Hyperparasites and predation***

In hyperparasitism, the pathogen is directly attacked by a specific BCA that kills it. In general, there are four major classes of hyperparasites:

- Obligate Bacterial Pathogens,
- Hypoviruses,
- Facultative Parasites, And
- Predators.

*Pasteuria penetrans* is an obligate bacterial pathogen of root-knot nematodes that has been used as a BCA.

Hypoviruses are hyperparasites. A classical example is the virus that infects *Cryphonectria parasitica*, a fungus causing chestnut blight, which causes hypovirulence, a reduction in disease-producing capacity of the pathogen.

However, the interaction of virus, fungus, tree, and environment determines the success or failure of hypovirulence.

There are several fungal parasites of plant pathogens, including those that attack sclerotia (e.g. *Coniothyrium minitans*) while others attack living hyphae (e.g. *Pythium oligandrum*).

A single fungal pathogen can be attacked by multiple hyperparasites. For example, *Acremonium alternatum, Acrodontium crateriforme, Ampelomyces quisqualis, Cladosporium oxysporum,* and *Gliocladium virens* are just a few of the fungi that have the capacity to parasitize powdery mildew pathogens (Kiss 2003).

Microbial predation is more general and pathogen non-specific and generally provides less predictable levels of disease control.

Some BCAs exhibit predatory behavior under nutrient-limited conditions. However, *Trichoderma* produce a range of enzymes that are directed against cell walls of fungi. However, when fresh bark is used in composts, *Trichoderma* spp. do not directly attack the plant pathogen, *Rhizoctonia solani*. But in decomposing bark, the concentration of readily available cellulose decreases and this activates the chitinase genes of *Trichoderma* spp., which in turn produce chitinase to parasitize *R. solani.*

***Antibiotic-mediated suppression***

**Antibiotics** are microbial toxins that can, at low concentrations, poison or kill other microorganisms.

Most microbes produce and secrete one or more compounds with antibiotic activity.

To be effective, antibiotics must be produced in sufficient quantities near the pathogen to result in a biocontrol effect.

Several biocontrol strains are known to produce multiple antibiotics which can suppress one or more pathogens. For example, *Bacillus cereus* strain UW85 is known to produce both zwittermycin and kanosamine. The ability to produce multiple antibiotics probably helps to suppress diverse microbial competitors, some of which are likely to be plant pathogens.

The ability to produce multiple classes of antibiotics, that differentially inhibit different pathogens, is likely to enhance biological control. More recently, *Pseudomonas putida* WCS358r strains genetically engineered to produce phenazine and DAPG(2,4-

Diacetylphloroglucinol) displayed improved capacities to suppress plant diseases in field-grown wheat.

**Table 2.** Some of antibiotics produced by BCAs

| Antibiotic | Source | Target pathogen | Disease |
|---|---|---|---|
| 2, 4-diacetyl-phloroglucinol | *Pseudomonas fluorescens* F113 | *Pythium* spp. | Damping off |
| Agrocin 84 | *Agrobacterium radiobacter* | *Agrobacterium tumefaciens* | Crown gall |
| Bacillomycin D | *Bacillus subtilis* AU195 | *Aspergillus flavus* | Aflatoxin contamination |
| Bacillomycin, fengycin | *Bacillus amyloliquefaciens* FZB42 | *Fusarium oxysporum* | Wilt |
| Xanthobaccin A | *Lysobacter* sp. strain SB-K88 | *Aphanomyces cochlioides* | Damping off |
| Gliotoxin | *Trichoderma virens* | *Rhizoctonia solani* | Root rots |
| Herbicolin | *Pantoea agglomerans* C9-1 | *Erwinia amylovora* | Fire blight |
| Iturin A | *B. subtilis* QST713 | *Botrytis cinerea* and *R. solani* | Damping off |
| Mycosubtilin | *B. subtilis* BBG100 | *Pythium aphanidermatum* | Damping off |
| Phenazines | *P. fluorescens* 2-79 and 30-84 | *Gaeumannomyces graminis* var. *tritici* | Take-all |
| Pyoluteorin, pyrrolnitrin | *P. fluorescens* Pf-5 | *Pythium ultimum* and *R. solani* | Damping off |
| Pyrrolnitrin, pseudane | *Burkholderia cepacia* | *R. solani* and *Pyricularia oryzae* | Damping off and rice blast |
| Zwittermicin A | *Bacillus cereus* UW85 | *Phytophthora medicaginis* and *P. aphanidermatum* | Damping off |

***Lytic enzymes and other byproducts of microbial life***

Many microorganisms produce and release lytic enzymes that can hydrolyze a wide variety of polymeric compounds, including chitin, proteins, cellulose, hemicellulose, and DNA. Secretion of these enzymes by different microbes can sometimes result in the suppression of plant pathogen activities directly.

For example, control of *Sclerotium rolfsii* by *Serratia marcescens* appeared to be mediated by chitinase expression.

While they may stress and/or lyse cell walls of living organisms, these enzymes generally act to decompose plant residues and nonliving organic matter. *Lysobacter* and *Myxobacteria* are known to produce copious amounts of lytic enzymes, and some isolates have been shown to be effective at suppressing fungal plant pathogens. Furthermore, some products of lytic enzyme activity may contribute to indirect disease suppression.

For example, oligosaccharides derived from fungal cell walls are known to be potent inducers of plant host defenses.

The quantitative contribution of any and all of the above compounds to disease suppression is likely to be dependent on the composition and carbon to nitrogen ratio of the soil organic matter that serves as a food source for microbial populations in the soil and rhizosphere.

For example, in post-harvest disease control, addition of chitosan can stimulate microbial degradation of pathogens similar to that of an applied hyperparasite. Amendment of plant growth substratum with chitosan suppressed the root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato.

***Other microbial byproducts also may contribute to pathogen suppression.***

Hydrogen cyanide (HCN) effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms at picomolar concentrations. The production of HCN by certain fluorescent pseudomonads is believed to be involved in the suppression of root pathogens. *P. fluorescens* CHA0 produces antibiotics, siderophores and HCN, but suppression of black rot of tobacco caused by *Thielaviopsis basicola* appeared to be due primarily to HCN production.

Volatile compounds such as ammonia produced by *Enterobacter cloacae* were involved in the suppression of *Pythium ultimum*-induced damping-off of cotton.

***Competition***

To successfully colonize the phytosphere, a microbe must effectively compete for the available nutrients. On plant surfaces, host-supplied nutrients include exudates, leachates, or senesced tissue. Additionally, nutrients can be obtained from waste products of other organisms such as insects (e.g. aphid honeydew on leaf surface) and the soil.

While difficult to prove directly, much indirect evidence suggests that **competition** between pathogens and non-pathogens for nutrient resources is important for limiting disease incidence and severity.

In general, soilborne pathogens, such as species of *Fusarium* and *Pythium*, that infect through mycelial contact are more susceptible to competition from other soil- and plant-associated microbes than those pathogens that germinate directly on plant surfaces and infect through appressoria and infection pegs. The most abundant nonpathogenic plant-associated microbes are generally thought to protect the plant by rapid colonization and thereby exhausting the limited available substrates so that none are available for pathogens to grow.

At the same time, these microbes produce metabolites that suppress pathogens. These microbes colonize the sites where water and carbon-containing nutrients are most readily available, such as exit points of secondary roots, damaged epidermal cells, and nectaries and utilize the root mucilage.

***Induction of host resistance***

Plants respond to a variety of chemical stimuli produced by soil- and plant-associated microbes. Such stimuli can either induce or condition plant host defenses through

biochemical changes that enhance resistance against subsequent infection by a variety of pathogens. Induction of host defenses can be local and/or systemic in nature, depending on the type, source, and amount of stimuli.

The first of these pathways, termed systemic acquired resistance (SAR), is mediated by salicylic acid (SA), a compound which is frequently produced following pathogen infection and typically leads to the expression of pathogenesis-related (PR) proteins. These PR proteins include a variety of enzymes some of which may act directly to lyse invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death.

A second phenotype, referred to as induced systemic resistance (ISR), is mediated by jasmonic acid (JA) and/or ethylene, which are produced following applications of some nonpathogenic rhizobacteria. Interestingly, the SA- and JA- dependent defense pathways can be mutually antagonistic, and some bacterial pathogens take advantage of this to overcome the SAR.

For example, pathogenic strains of *Pseudomonas syringae* produce coronatine, which is similar to JA, to overcome the SA-mediated pathway. Because the various host-resistance pathways can be activated to varying degrees by different microbes and insect feeding, it is plausible that multiple stimuli are constantly being received and processed by the plant. Thus, the magnitude and duration of host defense induction will likely vary over time. Only if induction can be controlled, i.e. by overwhelming or synergistically interacting with endogenous signals, will host resistance be increased.

Table 3. Bacterial determinants and types of host resistance induced by biocontrol agents

| Bacterial strain | Plant species | Bacterial determinant | Type |
|---|---|---|---|
| *Bacillus mycoides* strain Bac J | Sugar beet | Peroxidase, chitinase and β-1,3-glucanase | ISR |
| *Bacillus subtilis* GB03 and IN937a | *Arabidopsis* | 2,3-butanediol | ISR |
| *Pseudomonas fluorescens strains* | | | |
| CHA0 | Tobacco | Siderophore | SAR |
| | *Arabidopsis* | Antibiotics (DAPG) | ISR |
| WCS374 | Radish | Lipopolysaccharide | ISR |
| | | Siderophore | |
| | | Iron regulated factor | |
| WCS417 | Carnation | Lipopolysaccharide | ISR |
| | Radish | Lipopolysaccharide | ISR |
| | | Iron regulated factor | |
| | *Arabidopsis* | Lipopolysaccharide | ISR |
| | Tomato | Lipopolysaccharide | ISR |
| *Pseudomonas putida* strains | *Arabidopsis* | Lipopolysaccharide | ISR |
| WCS 358 | *Arabidopsis* | Lipopolysaccharide | ISR |
| | | Siderophore | ISR |
| BTP1 | Bean | Z,3-hexenal | ISR |
| *Serratia marcescens* 90-166 | Cucumber | Siderophore | ISR |

**Microorganisms used as biocontrol agents Insects**

Entomopathogens are microorganisms that are pathogenic to arthropods such as insects, mites, and ticks.

**Microbial pesticides**

- They come from naturally occurring or genetically altered bacteria, fungi,algae, viruses or protozoans.
- Microbial control agents can be effective and used as alternatives to chemical insecticides.
- The effect by microbial entomopathogens occurs by invasion through the integument or gut of the insect, followed by multiplication of the pathogen resulting in the death of the host, e.g., insects.
- Studies have demonstrated that the pathogens produce insecticidal toxin important in pathogenesis.

Most of the toxins produced by microbial pathogens which have been identified are peptides, but they vary greatly in terms of structure, toxicity and specificity.

Advantages of microbial insecticides

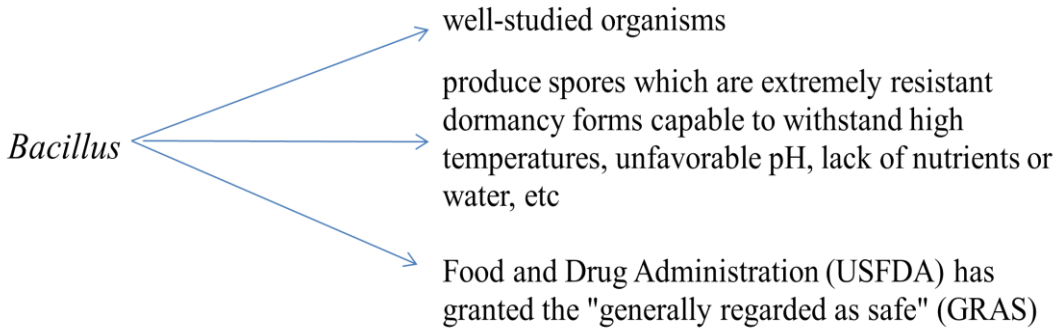
- essentially nontoxic and nonpathogenic to wildlife, humans, and other organisms not closely related to the target pest.
- The toxic action of microbial insecticides is often specific to a single group or species of insects.
- If necessary, most microbial insecticides can be used in conjunction with synthetic chemical insecticides because in most cases the microbial product is not deactivated or damaged by residues of conventional insecticides.
- Because their residues present no hazards to humans or other animals, microbial insecticides can be applied even when a crop is almost ready for harvest.
- They also enhance the root and plant growth by way of encouraging the beneficial soil microflora.

Disadvantages of microbial insecticides

- Because a single microbial insecticide is toxic to only a specific species or group of insects, each application may control only a portion of the pests present in a field and garden.
- Heat, desiccation (drying out), or exposure to ultraviolet radiation reduces the effectiveness of several types of microbial insecticides. Consequently, proper timing and application procedures are especially important for some products.
- Special formulation and storage procedures are necessary for some microbial pesticides.
- Because several microbial insecticides are pest-specific, the potential market for these products may be limited. Their development, registration, and production costs cannot be spread over a wide range of pest control sales. Consequently, some products are not widely available or are relatively expensive.

**Bacterial biopesticides** are the most common and cheaper form of microbial pesticides.

- As an insecticide they are generally specific to individual species of moths and butterflies, as well as species of beetles, flies and mosquitoes.
- Bacteria in biological pesticides survive longer in the open.
- Bacterial insecticides must be eaten to be effective; they are not contact poisons.
- Bacterial pathogens used for insect control are spore-forming, rod-shaped bacteria in the genus *Bacillus*. They occur commonly in soils, and most insecticidal strains have been isolated from soil samples.
- *Bacilli* are present in an extremely large area of environments ranging from sea water to soil, and are even found in extreme environments like hot springs




***Bacillus thuringiensis* (Bt)** is an aerobic, gram positive, spore forming soil bacterium that shows unusual ability to produce endogenous different kinds of crystals protein inclusions during its sporulation

- mainly caterpillars of the Lepidoptera (butterflies and moths) but also mosquito larvae, and simuliid blackflies
- The commercial Bt products are powders containing a mixture of dried spores and toxin crystals.
- They are applied to leaves or other environments where the insect larvae feed. The toxin genes have also been genetically engineered into several crop plants.
- has developed many molecular mechanisms to produce pesticidal toxins; most of toxins are coded for by several cry genes.
- Synthesis of a crystalline inclusion during sporulation, containing proteins known as endotoxins or Cry proteins, which have insecticidal properties.
- The crystal protein inclusions are composed of one or more crystal (Cry) and cytolytic (Cyt) toxins which are also called δ-endotoxins or insecticidal crystal proteins.
- As a pesticide, (BT) accounts for over 90 percent of total share of today's bioinsecticide market and has been used as biopesticide for several decades.

**Viruses**

There are more than 1600 different viruses which infect 1100 species of insects and mites.

- Viral pesticides are more expensive than chemical agents.
- A special group of viruses, called **baculovirus**, to which about 100 insect species are susceptible, accounts for more than 10 percent of all insect pathogenic viruses. genus *Nucleopolyhedrovirus*

- Alkaline condition of insect's midgut dissolves the protein covering and the viral particles are released from the inclusion body. These particles fuse with the midgut epithelial cells, multiply rapidly and eventually kill the host.

Disadvantages

- Many baculoviruses are host specific.
- Therefore they cannot be used to control several different pests. The action of baculoviruses on insect larvae is too slow to satisfy farmers.
- These viral preparations are not stable under the ultraviolet rays of the sun.
- Efforts are being made to encapsulate baculoviruses with UV protectants to ensure a longer field-life.

**Protozoan**

- Infect a wide range of insect hosts
- pathogens can kill their insect hosts
- reduction in the number of offspring produced by infected insects.
- Microsporidia: wide range of insects; slow acting organisms; Frequently they reduce host reproduction or feeding rather than killing the pest outright.

**Fungi**

- Entomopathogenic fungi are important natural regulators of insect populations and have potential as mycoinsecticide agents against diverse insect pests in agriculture.
- These fungi infect their hosts by penetrating through the cuticle, gaining access to the hemolymph, producing toxins, and grow by utilizing nutrients present in the haemocoel to avoid insect immune responses
- Entomopathogenic fungi may be applied in the form of conidia or mycelium which sporulates after application.

| PATHOGEN | HOST RANGE | USES AND COMMENTS |
|---|---|---|
| BACTERIA | | |
| *Bacillus thuringiensis* var. *kurstaki* (*Bt*) | caterpillars (larvae of moths and butterflies) | Effective for foliage-feeding caterpillars (and Indian meal moth in stored grain). Deactivated rapidly in sunlight; apply in the evening or on overcast days and direct some spray to lower surfaces or leaves. Does not cycle extensively in the environment. |
| *Bacillus thuringiensis* var. israelensis (*Bt*) | larvae of *Aedes* and *Psorophora* mosquitoes, black flies, and fungus gnats | Effective against larvae only. Active only if ingested. *Culex* and *Anopheles* mosquitoes are not controlled at normal application rates.. Does not cycle extensively in the environment. |
| *Bacillus thuringiensis* var. *tenebrinos* | larvae of Colorado potato beetle, elm leaf beetle adults | Effective against Colorado potato beetle larvae and the elm leaf beetle. Like other *Bts*, it must be ingested. It is subject to breakdown in ultraviolet light and does not cycle extensively in the environment. |
| *Bacillus thuringiensis* var. *aizawai* | wax moth caterpillars | Used only for control of was moth infestations in honeybee hives. |
| *Bacillus popilliae* and *Bacillus lentimorbus* | larvae (grubs) of Japanese beetle | The main Illinois lawn grub (the annual white grub, *Cyclocephala* sp.) Is NOT susceptible to milky spore disease. |

| | | |
|---|---|---|
| *Bacillus sphaericus* | larvae of *Culex, Psorophora,* and *Culiseta* mosquitos, larvae of some *Aedes* spp. | Active only if ingested, for use against *Culex, Psorophora,* and *Culiseta* species; also effective against *Aedes vexans.* Remains effective in stagnant or turbid water |
| FUNGI | | |
| *Beauveria bassiana* | aphids, fungus gnats, mealy bugs, mites, thrips, whiteflies | Effective against several pests. High moisture requirements, lack of storage longevity, and competition with other soil microorganisms are problems that remain to be solved. |
| *Lagenidium giganteum* | larvae of most pest mosquito species | Effective against larvae of most pest mosquito species; remains infective in the environment through dry periods. A main drawback is its inability to survive high summertime temperatures. |
| PROTOZOA | | |
| *Nosema locustae* | European cornborer caterpillars, grasshoppers and mormon crickets | Useful for rangeland grasshopper control. Active only if ingested. Not recommended for use on a small scale, such as backyard gardens, because the disease is slow acting and grasshoppers are very mobile. Also effective against caterpillars. |
| VIRUSES | | |
| Gypsy moth nuclear plyhedrosis (NPV) | gypsy moth caterpillars | All of the viral insecticides used for control of forest pests are produced and used exclusively by the U.S. Forest Service. |
| Tussock moth NPV | tussock moth caterpillars | |
| Pine sawfly NPV | pine sawfly larvae | |
| Codling moth granulosis virus (GV) | codling moth caterpillars | Commercially produced and marketed briefly, but no longer registered or available. Future re-registration is possible. Subject to rapid breakdown in ultraviolet light. |

**Microorganisms used as biocontrol agents against plant pathogens**

- Plant pathogens causing major damages to crop plants include fungi, bacteria, viruses and nematodes.
- Crop losses are creating a major threat to the food production with about 27 to 42 % loss in global food production attributed to plant disease caused by plant pathogens
- Plant pathogens are attacked with biological control through conservation is accomplished either by preserving existing microbes which attack or compete with pathogens or by enhancing conditions for their survival and reproduction at the expense of pathogenic organisms.
- The rhizoplane and surrounding rhizosphere are significant sources of carbon and photosynthate allocation to this zone can be as high as 40 percent. Thus, along root surfaces, there are various suitable nutrient rich niches attracting a great diversity of microorganisms, including phytopathogens.
- There are several different ways in which a microbial biological control agent can operate against a targeted plant pathogen. Among these are **competition, microbial by-products, induction of plant defenses, and parasitism.**

**Competition for Available Resources**

- To successfully colonize the phyllosphere or rhizosphere, a microbe must effectively compete for the available nutrients supplied in the form of exudates, leachates or senesced tissue.
- Competition for these nutrients and niches is a fundamental mechanism by which an effective biocontrol agent can protect plants from phytopathogens.
- Using this competition approach, control of soil-borne pathogens such as *Fusarium* and *Pythium* that infect through mycelial contact, has been achieved with greater success as compared to other pathogens that directly germinate on plant surfaces.

**Root Colonization and Protection of Infection Sites**

- Root colonization ability of biocontrol agents and potential to survive and proliferate along growing plant roots over a considerable period, in the presence of the indigenous microflora results in intimate associations that directly provide a selective adaptation to plants towards specific ecological niches.
- Also, the ability of biocontrol agent to colonize specific substrates or sites, whether a seed, root, shoot area, stump or fruit surface, provides protection to infection site from pathogen attack.
- However, they are effective only when provided with an additional competitive advantage, such as high initial cell numbers, earlier establishment than the pest or pathogen, or the production of antibiotic substances.
- Understanding root-microbe communication, as affected by genetic and environmental determinants in spatial and temporal contexts, will significantly contribute to improve the efficacy of these biocontrol agents. Once biocontrol agents establish on the site, the

mechanism of antagonism might be competition for nutrients, space, siderophore production, antibiosis, production of hydrolytic enzymes or other active substances.

**Active Metabolites Mediated Suppression of Pathogens**

- Production of active microbial metabolites including **iron-chelating siderophores, antibiotics, biocidal volatiles, lytic enzymes, and toxins** play a verysignificant role in determining the offensive biocontrol activity.

**Antibiotics**

Some examples of antibiotics reported to be involved in plant pathogen suppression include

- 2, 4-diacetyl phloroglucinol against *Pythium* spp.,
- Agrocin 84 against *Agrobacterium tumefaciens*,
- Iturin A against *Botrytis cinerea* and *Rhizoctonia solani*,
- Phenazines against *Gaeumannomyces graminis* var. *tritici.*

**Iron-chelating siderophores**

- Iron is an essential growth element for all living organisms and scarcity of its bioavailablity in soil habitats and on plant surfaces creates a furious competition
- Under iron-limiting conditions, biocontrol agents produce low-molecular-weight compounds called siderophores to competitively acquire ferric ion
- Some bacteria, especially fluorescent pseudomonads, produce siderophores that have very high affinities for iron as compared to fungal siderophores

**Lytic Enzymes**

- Chitinase and -1, 3-glucanase attack on chitin and -1, 3-glucan, major constituents of many fungal cell walls, resulting in its degradation which further kills the pathogens.
- Chitinase produced by *S. plymuthica, Serratia marcescens, Paenibacillus* sp. and *Streptomyces* sp was found to be inhibitory against *Botrytis cinerea, Sclerotium rolfsii, Fusarium oxysporum f. sp. Cucumerinum.*
- Similarly, laminarinase produced by *Pseudomonas stutzeri* digest and lyse mycelia of *F. Solani*.
- 1, 3-glucanase synthesized by *Paenibacillus, B. cepacia* destroy F. *oxysporum, R. solani, S. rolfsii,* and *Pythium ultimum* cell walls

**Induction of Systemic Resistance**

- Certain biocontrol agents show indirect mode of antagonism against the pathogens by inducing a state of plant resistance .
- This induced resistance is of two types representing two distinct pathway responses: **systemic acquired resistance (SAR) and induced systemic resistance (ISR).**
- Typically, SAR is induced by pathogens while ISR is salicylic acid independent and is induced by non-pathogenic bacteria.

- SAR is mediated by a compound called salicylic acid which is frequently produced following pathogen infection that leads to expression of pathogenesis related (PR) proteins such as PR-1, PR2, chitinases, and some peroxidases. These PR proteins can cause lysis of invading cells, reinforcement of cell membranes to resist infections, or induce localized cell death. In contrary, certain biocontrol agents do not induce PR proteins but increase accumulation of peroxidase, phenylalanine ammonia lyase, phytoalexins, polyphenol oxidase, and/or chalcone synthase.
  - A second pathway referred to as ISR is mediated by jasmonic acid (JA) and/or ethylene, which are produced following applications of some nonpathogenic rhizobacteria.
  - ISR was first observed on carnation with reduced susceptibility to wilt caused by *Fusarium* sp. and on cucumber with reduced susceptibility to foliar disease caused by *Colletotrichum orbiculare.*

**Parasitism**

- *Bdellovibrio bacteriovorus* is a predatory bacterium which has the unusual property to use cytoplasm of other Gram-negative bacteria as nutrients
- Predation by *B. bacteriovorus* strains of other plant pathogenic bacteria such as *Agrobacterium tumefaciens*, *Xanthomonas vesicatoria*, *X. campestris* pv. *campestris*, *Erwinia carotovora* pv. *carotovora*, *Pseudomonas syringae* pv. *glycinea*, *P. syringae* pv. *tomato*, *P. marginalis*, and *Erwinia herbicola*

**Table 1: Management of plant pathogens/diseases of important crops by various biocontrol agents**

| Crop | Disease | Pathogen | Possible biocontrol agents |
|---|---|---|---|
| **Cereals** | | | |
| Rice | Blast | *Pyricularia oryzae* | *P. fluorescens Trichoderma* spp. |
| | Bunt | *Neovossia indica* | *T. harzianum, T. viride, T. virens, T. deliquescens* |
| | Sheath blight | *Rhizoctonia solani* | *P. fluorescens* & *P. putida, T. harzianum, T. viride, T. virens, A. niger* AN27 |
| | Brown spot | *Drechslera oryzae* | *T. viride* |
| | Bacterial leaf blight | *Xanthomonas oryzae* | *Bacillus* spp. |
| Wheat | Karnal bunt | *Neovossia indica* | *T. viride, T. harzianum, T. pseudokoningii* & *T. koningii* |
| | Loose smut | *Ustilago segetum* | *T. viride, T. harzianum, T. Koningii, T. lignorum* |
| | Root rot | *S. rolfsii, F. oxysporum,* | *T. harzianum* |
| | Take-all | *Gaeumannomyces graminis var. tritici* | *T. harzianum* |
| Maize | Charcoal rot, Banded Blight | *Macrophomina phaseolina, R. solani* | *Trichoderma* spp. |
| Sorghum | Charcoal rot | *M. phaseolina* | *A. niger* AN27 |
| **Pulses** | | | |
| Pigeon pea | Wilt | *Fusarium udum* | *T. viride, T. hamatum, T. harzianum, T. koningii, B. subtilis* |
| | Seed-borne disease | *Xanthomonas campestris pv. vinae* | *T. viride, T. harzianum* |

| | | | |
|---|---|---|---|
| Chickpea | Wilt | *F. oxysporum* f. sp. *ciceri* | *T. viride*, *T. harzianum*, *T. virens*, *B. subtilis* *A. niger* AN27 |
| | Root rot | *Rhizoctonia solani*/ *M. phaseolina* | *T. viride*, *T. harzianum* |
| | Collar rot | *Sclerotium rolfsii* | *T. viride*, *T. harzianum*, *P. fluorescens* |
| | Grey mold | *B. cinera* | *Trichoderma sp.* |
| | Stem rot | *S. sclerotiorum* | *T. harzianum* |
| Cowpea | Wilt | *F. oxysporum* f. sp. *ciceris* | *T. viride* |
| | Charcoal rot and wilt | *M. phaseolina*, *F. oxysporum* f. sp. *tracheiphilum* | *T. viride*, *T. harzianum*, *T. koningii*, *T. pseudokoningii* |
| Soybean | Dry root rot | *M. phaseolina* | *T. viride*, *T. harzianum* |
| Mungbean | Root rot | *M. phaseolina* | *T. harzianum*, *T. viride* |
| **Oil Seed Crops** | | | |
| Groundnut | Crown rot | *Aspergillus niger* | *T. viride*, *T. harzianum*, *B. subtilis* |
| | Stem & pod rot | *Sclerotium rolfsii* | *T. harzianum*, *Rhizobium* |
| | Late leaf spot | *Phaeoisariopsis personata* | *Penicillum islandicum*, *P. fluorescens* *T. harzianum*, *B. subtilis* |
| | Root and stem rot | *R. solani* | *T. virens*, *T. longibrachiatum* |
| | Rust | *Puccinia arachidis* | *Verticillium lecanii*, *T. harzianum* |
| Castor | Wilt | *Fusarium oxysporum* f. sp. *ricini* | *T. viride*, *A. niger* AN27 |
| | Grey mold | *Botrytis cinerea* | *T. viride*, *P. fluorescens*, |

| Crop | Disease | Pathogen | Possible biocontrol agents |
|---|---|---|---|
| Seasamum | Blight | *Phytophthora* sp. | *T. harzianum*, *T. viride* |
| | Wilt | *F. oxysporum* f. sp. *sesami* | *A. niger* AN27 |
| | Root rot | *M. phasolina* | *Trichoderma* sp., *Gliocladium* sp., *B. subtilis* |
| Sunflower | Blight | *Alternaria helianthii* | *T. virens* |
| | Root/collar rot | *S. rolfsii*, *R. solani*, *S. sclerotiorum* | *T. harzianum*, *T. hamatum* |
| **Vegetables** | | | |
| Bottlegourd | Wilt | *F. oxysporum* | *A. niger* AN27 |
| | Root rot | *R. solani* | *A. niger* AN27 |
| | Collar rot | *Sclerotinia sclerotiorum* | *T. viride*, *T. virens*, *B. subtilis* |
| Cauliflower | Damping off | *Rhizoctonia solani* | *T. harzianum* |
| | | *P. aphanidermatum* | *A. niger* AN27 |
| | Stalk rot | *S. sclerotiorum* | *A. niger* AN27 |
| Chilli | Root rot | *S. rolfsii* | *T. harzianum* |
| | Fruit root and die back | *Colletotrichum capsici* | *T. viride*, *T. harzianum*, *T. konningii*, *T. hamatum*, *T. longibrachiatum*, *T. pileatus* |
| Cucumber | Seedling diseases | *Phytophthora* or *Pythium* sp. | *T. harzianum* |
| | | *Fusarium oxysporum* f. sp. *cucumerinum* | *A. niger* AN27 |
| Egg plant | Wilt, Damping off | *F. solani*, *P. aphanidermatum* | *T. viride*, *T. konningii* |
| | Collar rot | *S. sclerotiorum* | *T. viride*, *T. virens* |

| | | | |
|---|---|---|---|
| Cucumber | Seedling diseases | *Phytophthora* or *Pythium* sp.<br>*Fusarium oxysporum* f. sp. *cucumerinum* | *T. harzianum*<br>*A. niger* AN27 |
| Egg plant | Wilt, Damping off<br>Collar rot | *F. solani, P. aphanidermatum*<br>*S. sclerotiorum* | *T. viride, T. konningii*<br>*T. viride, T. virens* |
| Fenugreek | Root rot | *R. solani* | *T. viride, P. fluorescen* |
| French bean | Root rot | *R. solani* | *T. viride, T. hamatum* |
| Okra | Wilt | *Pythium* spp. | *A. niger* |
| Pea | Seed and Collar<br>White rot | *Pythium* sp., *R. solani*<br>*S. sclerotiorum* | *T. harzianum, T. hamatum*<br>*T. viride* |
| Potato | Black-scurf<br>Charcoal rot<br>Late blight | *R. solani*<br>*M. phaseolina*<br>*P. infestans* | *T. viride, T. viride, B. subtilis*<br>*A. niger*<br>*Trichoderma* sp. |
| Tomato | Damping off and wilt<br>Grey, *B. cinerea*<br>Root Knot-*Meloidogyne incognita, M. javanica* | *F. oxysporum, B. cinerea* f. sp. *lycopersici*<br>*Meloidogyne incognita*<br>*M. javanica* | *T. harzianum, P. fluorescens*<br>*T. harzianum*<br>*T. harzianum* |

**Microorganisms used as biocontrol agents against weed**

- There are two primary fields of application within the study of biological weed control.
- Classical (inoculative) biocontrol involves the release of a relatively small number of control agents. These agents feed on the weed, reproduce and gradually suppress the weed as their population grows. For this approach, arthropods are generally used as control agents.
- Inundative biocontrol In this type of biological control, large quantities of a control agent, generally a pathogen (a bacteria or fungus that causes disease in a weed) are applied to weeds in much the same manner as a chemical herbicide would be.
- The inundative biological control strategy is more relevant to the needs of agriculture and turf management, as it can generally be implemented through the application of inoculum as liquid sprays or solid granules in a similar manner to conventional herbicides
- The classical approach to biological control of weeds involves the introduction of host-specific natural enemies of allien weeds.
- Recently the approaches utilized in bio control programs have been expanded to include two other methods:
  1) Augmentation of natural enemy populations
  2) Application of "biological herbicides".

- *Colletotrichum gloeosporioides* f.sp. *malvae,* introduced for the control of round leaf mallow (*Malva pusilla*) and *C. gloeosporioides* f.sp. *Aeschynomene*

- *Sclerotinia minor* was introduced for the control of dandelion (*Taraxacum officinale*), white clover (*Trifolium repens*) and broadleaf plantain (*Plantago major*) in turf.
- three genera of fungi have received the majority of attention as bioherbicide candidates.
- Three species within the genus *Phoma* have also received attention as potential agents for biological weed control

| Bioherbicide agent | Target weed | Specificity of control agent | Intended system |
|---|---|---|---|
| **Fungal agents** | | | |
| *Colletotrichum gloeosporioides* f.sp. *aeschynomene* | Northern jointvetch (*Aeschynomene virginica*) | Also affects *Sesbania exaltata* Boyette et al. (2011) | Field crops: Rice, soybean |
| *Colletotrichum gloeosporioides* f.sp. *malvae* | Round leaf mallow (*Malva pusilla*) | Lethal effect limited to *Malvaceae* family Mortensen (1988) | Field crops: Wheat, rye, flax, lentil, barley, canola, sunflower, soybean, oats, mustard, sugar beet and buckwheat |
| *Colletotrichum orbiculare* | Spiny cocklebur (*Xanthium spinosum*) | Known pathogen of the *Cucurbitaceae* Harata and Kubo (2014) | Pasture and field crops |
| *Colletotrichum truncatum* | Hemp sesbania (*Sesbania exaltata*) | Pathogenicity reported as limited to *Leguminosae* Boyette (1991) Minor pathogenicity on *Matricaria perforata* Hynes et al. (2010) | Field crops |
| *Phoma chenopodicola* | Lamb's quarters (*Chenopodium album*), Creeping thistle (*Cirsium arvense*), Green foxtail (*Setaria* | Not tested on other species | Field crops such as sugar beet and corn |
| *Sclerotinia minor* | Dandelion (*Taraxacum officinale*), White clover (*Trifolium repens*), and Broadleaf plantain (*Plantago minor*) | Wide host range, predominantly dicot species Melzer et al. (1997) | Turf |
| *Chondrostereum purpureum* strain HQ1 | Re-growth of deciduous trees and shrubs | Wide host range Setliff (2002) | Forestry |
| *Chondrostereum purpureum* strain PFC 2139 | Re-growth of deciduous trees and shrubs | Wide host range Setliff (2002) | Forestry |
| *Puccinia thlaspeos* | Dyer's woad (*Isatis tinctoria*) | *Isatis tinctoria* only EPA (2002) | Ecological management |
| *Alternaria destruens* | Dodder species (*Cuscata* spp.) | Observed to affect several unspecified crop species EPA (2005) | Alfalfa, cranberries, carrots, peppers, tomatoes, eggplant, blueberries, and woody ornamentals |
| *Phytophthora palmivora* | Strangler vine (*Morrenia odorata*) | Weak pathogen of some crop species Ridings et al. (1973) | Citrus orchards |

| | | | |
|---|---|---|---|
| | *viridis*), Annual mercury (*Mercurialis annua*) | | |
| *Phoma herbarum* | Dandelion (*Taraxacum officinale*) | Reported as a potential control agent for *Trianthema portulacastrum* Ray and Vijayachandran (2013) | Turf |

- Biological weed control using bacteria has been suggested to have several advantages over the use of fungi, including more rapid growth of the bioherbicide agents, relatively simple propagation requirements, and high suitability for genetic modification through either mutagenesis or gene transfer.
- *Pseudomonas fluorescens* and *Xanthomonas campestris* have attracted the most attention.
- Among studies into the suppressive effects of *P. fluorescens*, three strains have been investigated in especially great detail, all of which have been observed to inhibit plant growth and/or germination through the production of extracellular metabolites.
- *P. fluorescens*, three strains have been investigated in especially great detail, all of which have been observed to inhibit plant growth and/or germination through the production of extracellular metabolites
- *Pseudomonas fluorescens* strain D7, originally isolated from the rhizospheres of winter wheat (*Triticum aestivum*) and downy brome (*Bromus tectorum)*, has been observed to selectively inhibit growth and germination of a number of grassy weeds. By selective removal of compounds from cell-free filtrates, the growth-inhibiting activity associated with this strain was attributed to a combination of extracellular peptides and a lipopolysaccharide, which were suggested to work in conjunction to express herbicidal activity
- *P. fluorescens* strain WH6 has been observed to affect the germination of a much broader range of plant species, significantly inhibiting germination of all species tested (21 monocot species and 8 dicot species) with the exception of a modern corn (*Zea mays*) hybrid.
- The germination-inhibiting activity of the WH6 strain has been attributed to the production of a compound originally referred to as Germination Arrest Factor (GAF). The active component of GAF has been identified as 4-formylaminooxy-L-vinylglycine, and its biosynthesis has been proposed to begin with the amino acid homoserine.
- This class of compounds, the oxyvinylglycines, has been shown to interfere with enzymes that utilize pyridoxal phosphate as a cofactor, including enzymes involved in nitrogen metabolism and biosynthesis of the plant hormone ethylene
- *P. fluorescens* strain WH6 has been observed to affect the germination of a much broader range of plant species, significantly inhibiting germination.
- The herbicidal compounds produced by this species, referred to as pseudophomin A and B
- The activity of this species is specific to *Poa annua* and *Poa attenuata*, and was not reported to affect other turf species

| | | | |
|---|---|---|---|
| **Bacterial agents** | | | |
| *Pseudomonas fluorescens* strain D7 | Downy brome (*Bromus tectorum*) | Effect is limited to *Bromus tectorum* Kennedy et al. (2001) | Field crops |
| *Pseudomonas fluorescens* strain BRG100 | Green foxtail (*Setaria viridis*) | Not identified | Not specified |
| *Pseudomonas fluorescens* strain WH6 | Inhibits most of the species tested | Non-specific | Not specified |

- Viruses that affect weed species have also been considered as bioherbicide candidates. This strategy is more commonly considered for management of invasive species in broader ecosystems rather than specifically managed areas.
- Viruses have been suggested to be inappropriate candidates for inundative biological control due to their genetic variability and lack of host specificity.
- Tobacco Mild Green Mosaic Tobamovirus for control of tropical soda apple (*Solanum viarum*) and *Araujia* Mosaic Virus for control of moth plant (*Araujia hortorum*).
- Óbuda Pepper Virus (ObPV) and Pepino Mosaic Virus (PepMV) have been proposed as agents to reduce overall populations of the weed *Solanum nigrum*

| | | | |
|---|---|---|---|
| **Viral agents** | | | |
| Tobacco Mild Green Mosaic Tobamovirus | Tropical soda apple (*Solanum viarum*) | Also affects *Capsicum* spp. and *Nicotiana* spp. Font et al. (2009); EPA (2015) | Pastures |
| *Araujia* Mosaic Virus | Moth plant (*Araujia hortorum*) | Also affects *Morrenia odorata, Oxypetalum caeruleum* and *Gomphocarpus* spp. | Ecosystem management |
| Unspecified virus resembling Tobacco Rattle Virus | *Impatiens glandulifera* | Also affects species within *Chenopodium* and *Nicotiana* Kollmann et al. (2007) | Ecosystem management |
| \'Obuda Pepper Virus | *Solanum nigrum* | Wide host range Tobias et al. (1982) | Ecosystem management |
| Pepino Mosaic Virus | *Solanum nigrum* | Wide host range including *Amaranthaceae, Chenopodiacee, Compositae, Convolvulaceae, Malvaceae, Plantaginaceae* and *Solanaceae* Papayiannis et al. (2012) | Ecosystem management |

## Possible Questions

### PART-B (2 MARKS)

1. Mention the stages in mode of action of *Bacillus thuringiensis.*
2. Define Mycophagy
3. Write about the Bt toxin
4. Define nematophagy
5. Name the commercially available biopesticide
6. Write the role of baculovirus in biocontrol
7. Define antagonism
8. Define predators
9. What is parasitism?
10. Write the application of biocontrol in the field
11. What is mycoparasitism?
12. Define pesticide.
13. Write the advantages of pesticides
14. What are the types of pesticides based on microorganisms?
15. Mention the stages in mode of action of *Bacillus thuringiensis.*
16. What are the types of viral pesticides?
17. Give examples of bacterial pesticides.

### PART-C (8 MARKS)

1. Describe the biocontrol of plant pathogens
2. Write detail account on biopesticides.
3. Justify – Baculovirus kills target insect.
4. Explain the biocontrol of weed.
5. Write about mycopesticides
6. Write detail notes on bacterial pesticides
7. Describe the antagonism and its mechanism of biocontrol
8. Discuss the transgenic plant in biocontrol
9. Explain the plant pathogen control mechanism by bacteria
10. What types of viruses are being used to attempt to control insects?
11. What two important bacteria have been used as bioinsecticides?
12. What is biopesticides? Write the mode of action of *Bacillus thuringiensis*?

# KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: III B.Sc MB COURSE NAME: Microbes in Sustainable Agriculture and Development

COURSE CODE: 17MBU602B UNIT: III BATCH-2017-2020

| S.No | Questions | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | _____ converts the protoxin into active toxin. | Nuclease | Urease | Protease | Lipase | Protease |
| 2 | Convertion of protoxin to active toxin require both protease and _____. | Alkaline pH | Urease | Acid ph | Lipase | Alkaline ph |
| 3 | Parasporal crystals sensitive to_____. | Sunlight | Chemicals | Acid ph | Lipase | Sunlight |
| 4 | Most effective and most often utilized microbial insecticides are toxins synthesized from _____ . | *B.amyloliquefaciens* | *B.thuringiensis* | *B.subtilis* | *B.licheniformis* | *B.thuringiensis* |
| 5 | *B.thuringienis* subspecies kurstaki is toxic to | Bugs | Worms | Cabbage worm | Small worms | Cabbage worm |
| 6 | *B.thuringiensis* subspecies *israelensis*kills | Cabbage worm | Black flies | Lepidopteron larvae | All flies | Black flies |
| 7 | The parasporal crystal is _____ . | Active form | Protoxin | Active toxin | Unstable carbohydrate substance | Protoxin |
| 8 | The insecticidal activity of *b.thuringiensis* is contained within a very large structure called _____ . | Parasporal crystal | Parabasal crystal | Perisporal crystal | Sporal crystal | Parasporal crystal |
| 9 | The subunits of the parasporal crystal can be dissociated invitro by treatment with | Alcohol | Ethylene | B-mercaptoethanol | Xylene | B-mercaptoethanol |
| 10 | Parasporal crystals are _____ lived in the environment. | Short | Long | Moderately | Limited | Short |
| 11 | Which one of the following can be used as a biocontrol agent? | Baculovirus | Retrovirus | *A. Niger* | *Penicillium* sp | Baculovirus |
| 12 | Baculovirus are pathogenic to _____. | Tobacco plant | Rat | Diptera | Mice | Diptera |
| 13 | The insect specific neurotoxin disrupts the . | Normal life cycle of insect | Flow of na ions | Sporulation | Flow of chloride ions | Flow of na ions |
| 14 | When the parasporal crystal is ingested by a target insect, the protoxin is activated by _____ . | Lipase | Specific digestive proteases | Laccase | Amylase | Specific digestive proteases |
| 15 | The mode of action of b.thuringiensis toxins ---------- | Damage the plant growth | Affect the plant root | Kills insects during a specific developmental stage | Support the insect to grow | Kills insects during a specific developmental stage |

| | | | | | | |
|---|---|---|---|---|---|---|
| 16 | Chemical insecticides has the following disadvantage | Specificity | Insects become sensitive easily | Beneficial insects being killed | Simple degradation | Beneficial insects being killed |
| 17 | The steps taken to kill insects in plant roots is | To inject bt toxin into roots | To introduce bt gene into cells of root | Introduce bt toxin gene into bacterial species of rhizosphere | Spray the bt toxin | Introduce bt toxin gene into bacterial species of rhizosphere |
| 18 | Methods for biological protection of plants | Transgenic plants | Chemical insecticides | Trimming of plants | Avoid plant damage | Transgenic plants |
| 19 | Baculo virus is a | Parasite | Obligate parasite | Saprophyte | Pathogen | Obligate parasite |
| 20 | Bt toxin is safe because | Persist in the environment | Hazardous to mammals | Does not persist in the environment | Non degradable | Does not persist in the environment |
| 21 | Gene transfer to animal by ---------- | Transformation efficiency | Microinjection | Vector ti | Transduction | Microinjection |
| 22 | The *b. Thuringiensis* subsp ---------- insecticidal protein is highly toxic when injested by mosquito larvae. | *Israelensis* | *kurstaki* | *Tenebrionis* | *aizawai* | *Kurstaki* |
| 23 | Possible attractive host for the expression of mosquitocidal cry genes ---------- | *Bacillus sphacericus* | *B.thuringinsis* | *B.rhizogenes* | *Asticcacaulis excentricus* | *b.thuringinsis* |
| 24 | Protoxin is activated with in the ---------- | Gut | lungs | Respiratory tract | Stomach | gut |
| 25 | The biological insecticides are _____to humans and other animals | Hazardous | Useless | Non-economical | Non-hazardous | Non-hazardous |
| 26 | Most effective pesticide is _____ | Carbamates | Organophosphates | Phosphates | Organochlorines | Carbamates |
| 27 | Laboratory trials showed that engineered p.fluorescens was toxic to | Tomato hornworm larvae | Potato hornworm larvae | Tobacco hornworm larvae | Cabbage hornworm larvae | Cabbage hornworm larvae |
| 28 | Possible attractive host for the expression of mosquitocidal cry genes ---------- | *Asticcacaulis excentricus* | *B.thuringinsis* | *Bacillus sphacericus* | *B.rhizogenes* | *Bacillus sphacericus* |
| 29 | Phyllosphere refers to ____ | Surface of roots | Surface of leaves | Surface of stem | Surface of flowers | Surface of leaves |
| 30 | The _____as a biopesticide is used to control the velvetbeen caterpillar in soybean. | Baculovirus | Retrovirus | Antagonistic organism | Trichoderma | Baculovirus |

| | | | | | | |
|---|---|---|---|---|---|---|
| 31 | Frankia is a _____ | Virus | Bacteria | Actinomycetes | Fungus | Actinomycetes |
| 32 | The phenomenon where one population adversely affects the growth of another population. | Amensalism | Lysis | Predator | Symbiosis | Amensalism |
| 33 | First plant pathogenic nematode was discovered by | Meloidogyne | Heterodera | Anguina | Globodera | Anguina |
| 34 | Release of bromoxynil resistant cotton | 1980 | 1999 | 1995 | 1987 | 1995 |
| 35 | First plant parasitic bacteria was reported by | T.J. Burrill | Needham | Louis pasteur | Leewenhook | T.j burrill |
| 36 | An example of humicant is ------- | Sodium alginate | Glycerol | Acetone | Petroleum ether | Glycerol |
| 37 | The first chemical shown to have herbicidal activity | 2,4 D | Bordeaux mixture | Paraquat | 2,4 DB | Bordeaux mixture |
| 38 | A weed poisonous to animals and human beings | Datura metal | Ammannia bacifera | Uritica sp | Chemopodium | Datura metal |
| 39 | Bacterial leaf blight of rice caused by xanthomonas ryzae can be identified by | Wilting of the plant | Yellowing of leaves | Ooze test | Defoliation | Yellowing of leaves |
| 40 | Fungi which can grow only on living host plant are called | Obligate saprophytes | Obligate parasites | Facultative parasites | Saprophytes | Obligate parasites |
| 41 | Exclusion of plant disease by legislation is known as ---------- | Disease resistance | Plant quarantine | Biological control of plant | Culture control | Plant quarantine |
| 42 | Foliar spray is ---------- | Spraying on roots | Spraying on stem | Spraying on leaves | Spraying on flowers | Spraying on leaves |
| 43 | The most systematic method for classifying weeds is based on---------- | Phylogenetics | Morphology | Life history | Habitat | Phylogenetics |
| 44 | Bacillus thuringiensis produce ---------- | Insecticidal protein | Nematocidal protein | Fungicidal protein | Bactericidal protein | Insecticidal protein |
| 45 | The plant disease control agent include which of the following bacterium initiates the formation of galls | *Agrobacterium* | *Rhizobium* | *Pseudomonas* | *Ralstonia* | *Agrobacterium* |
| 46 | Soil micro organisms influence above ground ecosystem by contributing except which of the following | Plant nutrition | Soil fertility | Soil structure | Soil texture | Soil texture |
| 47 | Insecticides generally attack ---------- | Respiratory system | Muscular system | Nervous system | Circulatory system | Muscular system |
| 48 | The _____ ia act as active fungicide | *Azospirillum* | *Tricoderma viridae* | *Bacillus polymyxa* | *Aspergillus flavus* | *Tricoderma viridae* |
| 49 | Microbial iron chelating compounds _____have very high affinity for fe3+. | Enzymes | Siderophores | Toxic compounds | Ferridoxin | Siderophores |

| | | | | | | |
|---|---|---|---|---|---|---|
| 50 | To fix one molecule of nitrogen _____ | 6 ATP molecules are required | 12 ATP molecules are required | 16 ATP molecules are required | 20 ATP molecules are required | 16 ATP molecules are required |
| 51 | Conversion of nitrite to nitrate is carried out by _____ | *Nitrosomonas* | *Nitrosococcus* | *Nitrobacter* | *Clostridium* | *Nitrobacter* |
| 52 | All of the following are examples of negative symbiosis_____ | Amensalism | Competition | Commensalism | Parasitism | Competition |
| 53 | The phenomenon of commensalism refers to a relationship between organisms in which | One species of a pair benefits | Both the species of a pair benefit | One species of a pair is more benefited | Both the species not benefited | One species of a pair benefits |
| 54 | Which of the following organism is known to grow on the surface of freshly exposed rocks | Green algae | Diatoms | Cyanobacteria | Yeast | Cyanobacteria |
| 55 | Which of the following mentioned ghgs has the highest atmospheric lifetime? | Carbon tetrafluoride | Nitrous oxide | Methane | CFU | Carbon tetrafluoride |
| 56 | The organisms responsible for the characteristic musty or earth odor of a freshly plowed field is _____ | Actinomycetes | Bacteria | Fungus | Algae | Actinomycetes |
| 57 | The organism responsible for the characteristics musty and earthy odor of freshly ploughed field | Nocardia | Streptomyces | Micromonospora | All of these | All of these |
| 58 | Which of the following greenhouse gas is contributed by cattle farming? | Nitrous oxide | Methane | Carbon monoxide | $NH_4$ | Methane |
| 59 | Silage is produced in _____ weather. | Hot | Cold | Optimum | Too hot | Cold |
| 60 | Silage is stable for _____. | 1 yr | 2 yrs | 5 yrs | 3 yrs | 5 yrs |

**SYLLABUS**
**UNIT-IV**

Biofertilization, Phytostimulation, Bioinsecticides

Plant growth promoting bateria, biofertilizers – symbiotic (*Bradyrhizobium, Rhizobium, Frankia*), Non Symbiotic (*Azospirillum, Azotobacter*, Mycorrhizae, MHBs, Phosphate solubilizers, algae), Novel combination of microbes as biofertilizers, PGPRs

**Plant growth promoting bacteria:**

Plant growth-promoting rhizobacteria (PGPR) were first defined by Kloepper and Schrot to describe soil bacteria that colonize the roots of plants following inoculation onto seed and that enhance plant growth. The following are implicit in the colonization process: ability to survive inoculation onto seed, to multiply in the spermosphere (region surrounding the seed) in response to seed exudates, to attach to the root surface, and to colonize the developing root system.The ineffectiveness of PGPR in the field has often been attributed to their inability to colonize plant roots. A variety of bacterial traits and specific genes contribute to this process, but only a few have been identified. These include motility, chemotaxis to seed and root exudates, production of pili or fimbriae, production of specific cell surface components, ability to use specific components of root exudates, protein secretion, and quorum sensing. The generation of mutants altered in expression of these traits is aiding our understanding of the precise role each one plays in the colonization process.

Progress in the identification of new, previously uncharacterized genes is being made using nonbiased screening strategies that rely on gene fusion technologies. These strategies employ reporter transposons and in vitro expression technology (IVET) to detect genes expressed during colonization.

Using molecular markers such as green fluorescent protein or fluorescent antibodies, it is possible to monitor the location of individual rhizobacteria on the root using confocal laser scanning microscopy. This approach has also been combined with an rRNA-targeting probe to monitor the metabolic activity of a rhizobacterial strain in the rhizosphere and showed that bacteria located at the root tip were most active.

**Mechanisms of action**

PGPRs enhance plant growth by direct and indirect means, but the specific mechanisms involved have not all been well characterized. Direct mechanisms of plant growth promotion by PGPRs can be demonstrated in the absence of plant pathogens or other rhizosphere microorganisms, while indirect mechanisms involve the ability of PGPRs to reduce the harmful effects of plant pathogens on crop yield. PGPRs have been reported to directly enhance plant growth by a variety of mechanisms: fixation of atmospheric nitrogen transferred to the plant production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus, and synthesis of phytohormones. Direct enhancement of mineral uptake due to increases in specific ion fluxes at the root surface in the presence of PGPRs has also been reported. PGPR strains may use one or more of these mechanisms in the rhizosphere. Molecular approaches using microbial and plant mutants altered in their ability to synthesize or respond to

specific phytohormones have increased understanding of the role of phytohormone synthesis as a direct mechanism of plant growth enhancement by PGPRs. PGPR that synthesize auxins and cytokinins or that interfere with plant ethylene synthesis have been identified.

**Biofertilizers**

Biofertilizer is defined as the microbial inoculation contains living cells of efficient strain of microorganisms such as cellulolytic $N_2$ fixing or phosphate solubilizing microbes. Biofertilizers increases the fertility and thus enhances the growth of plants. Biofertilizers are used to reduce the use of chemical fertilizers in agriculture. Chemical fertilizers are much harmful to man, whereas the biofertilizers are harmless. The microbial conversion is of two types namely simple organic conversion and complex
conversion. In simple conversion, the insoluble organic substances are directly converted into organic acids or nitrogenase compounds in the soil. In complex reactions, the conversion is carried out by a series of reactions catalyzed by a number of enzymes produced by microorganisms.

Use of biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers. They can be grouped in different ways based on their nature and function.

**Symbiotic relationship:**

Certain plants establish a symbiotic relationship with bacteria, enabling them to produce nodules that facilitate the conversion of atmospheric nitrogen to ammonia. In this connection, cytokinins have been found to play a role in the development of root fixing nodules. It appears that not only must the plant have a need for nitrogen fixing bacteria, but they must also be able to synthesize cytokinins which promote the production of root nodules, required for nitrogen fixation.

**Bradyrhizobium:**

*Bradyrhizobium* is a genus of Gram-negative soil bacteria, many of which fix nitrogen. Nitrogen fixation is an important part of the nitrogen cycle. Plants cannot use atmospheric nitrogen ($N_2$); they must use nitrogen compounds such as nitrates. *Bradyrhizobium* species are Gram-negative bacilli (rod-shaped) with a single subpolar or polar flagellum. They are common soil-dwelling micro-organisms that can form symbiotic relationships with leguminous plant species where they fix nitrogen in exchange for carbohydrates from the plant. Like other rhizobia, many members of this genus have the ability to fix atmospheric nitrogen into forms readily available for other organisms to use. Bradyrhizobia are also major components of forest soil microbial communities, where strains isolated from these soils are not typically capable of nitrogen fixation or nodulation. They are slow-growing in contrast to *Rhizobium* species, which are considered fast-growing rhizobia. In a liquid medium, *Bradyrhizobium* species take 3–5 days to create a moderate turbidity and 6–8 hours to double in population size. They tend to grow best with pentoses carbon sources. Some strains (for example, USDA 6 and CPP) are capable of oxidizing carbon monoxide aerobically.

**Nodule formation:**

Nodules are growths on the roots of leguminous plants where the bacteria reside. The plant roots secrete amino acids and sugars into the rhizosphere. The rhizobia move toward the roots and attach to the root hairs. The plant then releases flavanoids, which induce the expression of *nod* genes within the bacteria. The expression of these genes results in the production of enzymes called nod factors that initiate root hair curling. During this process, the rhizobia are curled up with the root hair. The rhizobia penetrate the root hair cells with an infection thread that grows through the root hair into the main root. This causes the infected cells to divide and form a nodule. The rhizobia can now begin nitrogen fixation. Over 55 genes are known to be associated with nodulation. *NodD* is essential for the expression of the other *nod*genes.[10] The two different *nodD* genes are: *nodD1* and *nodD2*. Only *nodD1* is needed for successful nodulation.

***Nitrogen fixation:***

*Bradyrhizobium* and other rhizobia take atmospheric nitrogen and fix it into ammonia ($NH_3$) or ammonium ($NH_4^+$). Plants cannot use atmospheric nitrogen; they must use a combined or fixed form of the element. After photosynthesis, nitrogen fixation (or uptake) is the most important process for the growth and development of plants.[11] The levels of ureide nitrogen in a plant correlate with the amount of fixed nitrogen the plant takes up.*Nif* and *fix* are important genes involved in nitrogen fixation among *Bradyrhizobium* species. *Nif* genes are very similar to genes found in *Klebsiella pneumoniae*, a free- living diazotroph. The genes found in bradyrhizobia have similar function and structure to the genes found in *K. pneumoniae*. *Fix* genes are important for symbiotic nitrogen fixation and were first discovered in rhizobia species. The *nif* and *fix* genes are found in at least two different clusters on the chromosome. Cluster I contains most of the nitrogen fixation genes. Cluster II contains three *fix*genes located near *nod* genes.

**Rhizobium:**

Rhizobia are symbiotic diazotrophs (prokaryotic organisms that carry out dinitrogen fixation) that form a symbiotic association with legumes. This association is symbiotic in that both the plant and rhizobia benefit. The plant supplies the rhizobia with energy in the form of amino acids and the rhizobia fix nitrogen from the atmosphere for plant uptake. The reduction of atmospheric dinitrogen into ammonia is the second most important biological process on earth after photosynthesis. The actual process of dinitrogen fixation can only be carried out by diazotrophs that contain the enzyme dinitrogenase. Nitrogen is the most critical nutrient needed to support plant growth. Unfortunately, atmospheric dinitrogen (78% of air we breathe) is extremely stable due to triple bonds which can only be broken by energy intensive ways. These include electrical $N_2$ fixation by lightning where oxides of N come to ground with rain, the Haber-Bosch process in industrial fertilizer production, and biological $N_2$ fixation in legumes by bacterial symbionts such as *Rhizobium etli*. Biological fixation of nitrogen was the leading form of annual

nitrogen input until the last decade of the 20th century. It is gaining attention once again as sustainability becomes a central focus to feed a world population of over 7 billion people.

**Nodule formation**:

The actual process of nodulation is a very coordinated effort between the legume and the *Rhizobium* bacteria in the soil. Infection typically occurs in root hairs of legumes. Many rhizobia and host plants are highly specific and legumes can either attract rhizobia to root hairs directly by excretory compounds or by induction of *nod* gene activity in the bacteria. 1. Flavonoids are released by the host root. The flavonoid is at the highest concentration at the root and interacts with the product of bacterial *nodD* gene. The *nodD* gene produces the protein, *nodD*, which is the sensor that recognizes chemicals excreted by host plant roots.

2. Rhizobia colonize the soil in the vicinity of the root hair in response to the flavonoids. This process is autoregulated where favonoids stimulate Nod factor production, which stimulates flavonoid secretion.

3. Response to Nod factors is extremely rapid and the disruption of cell wall happens very quickly. Disruption of crystallization of cell walls take place, thereby allowing entrance by the rhizobia. At the same time Rhizobia multiply in the rhizosphere. The root hair is then stimulated and curls to the side where the bacteria are attached which stimulates cell division in the root cortex.

4. A "shepherd's crook" is formed and entraps the rhizobia which then erode the host cell wall and enter near the root hair tip. An infection thread is formed as rhizobia digest the root hair cell wall. Free- living *Rhizobium* bacteria are converted to bacteroids as the infection elongates by tip growth down root hair and toward epidermal cells.

5. Infection thread branches and heads toward the cortex and a visibly evident nodule develops on the root as the plant produces cytokinin and cells divide. Nodules can contain one or more rhizobial strains and can be either determinant (lack a persistent meristem and are spherical) or indeterminate (located at the distal end of cylindrically shaped lobes). Many infections are aborted due to a breakdown in communication between rhizobia and the host plant leaving nodule number strictly regulated by the plant.

6. Once inside the nodule, rhizobia are released from the infection thread in a droplet of polysaccharide. A plant-derived peribacteroid membrane, which regulates the flow of compounds between the plant and bacteroid, quickly develops around this droplet via endocytosis. This process keeps the microbes "outside" the plant where the rhizobia are intracellular but extracytoplasmic . The loss of the ammonium assimilatory capacity by bacteroids is important for maintaining the symbiotic relationship with legumes.

Nitrogenase is the actual enzyme responsible for conversion of $N_2$ to ammonium. Nitrogenase exists in three forms that include molybdenum nitrogenase, vanadium nitrogenase, and iron nitrogenase. Mo- containing nitrogenase is the most widely studied and is the enzyme utilized by *Rhizobium* . This enzyme actually is made up of two enzymes, dinitrogenase and dinitrogenase reductase. Nitrogenase is regulated by N supply and by $O_2$ which can inhibit nitrogenase. Leghemoglobin, responsible for giving efficitive nodules their pink color, binds oxygen and transfers it to bacterial electron transport chain so that ATP synthesis can occur. Thus, the concentration of free $O_2$ is lower in the nodule. Unfortunately, high amounts

of ATP and oxygen reductant are needed to meet the demands of the enzyme, but at the same time, nitrogenase is oxygen sensitive. This is often refered to as the 'paradox' of symbiotic nitrogen fixation.

**Rhizobium**

*Rhizobium* is a soil habitat bacterium, which can able to colonize the legume roots and fixes the atmospheric nitrogen symbiotically. The morphology and physiology of *Rhizobium* will vary from free-living condition to the bacteroid of nodules. They are the most efficient biofertilizer as per the quantity of nitrogen fixed concerned. They have seven genera and highly specific to form nodule in legumes, referred as cross inoculation group. *Rhizobium* inoculum contains the viable cells of *Rhizobium* which fixes the atmospheric nitrogen when the roots of higher leguminous plants are injected by *Rhizobium*.

**Characters of *Rhizobium*** : This belongs to bacterial group and the classical example is symbiotic nitrogen fixation. The bacteria infect the legume root and form root nodules within which they reduce molecular nitrogen to ammonia which is reality utilized by the plant to produce valuable proteins, vitamins and other nitrogen containing compounds. The site of symbiosis is within the root nodules. It has been estimated that 40-250 kg N / ha / year is fixed by different legume crops by the microbial activities of *Rhizobium*. The percentage of nodules occupied, nodules dry weight, plant dry weight and the grain yield per plant the multistrain inoculant was highly promising.

**Isolation of *Rhizobium*:**

The leguminous plants are uprooted and tested of any nodule is present in the root. The root nodule which are white brown to pink green in color and washed in water in order to eradicate the soil particles. Then a pinkish green nodule is selected and washed in distilled water. The washed root nodule is kept immersed in 0.1 acidified Kcl solution for 5 min. This Kcl is used in a disinfectant to sterilize the contaminate found on the surface of the nodule. Then again wash the nodule to remove the disinfectant. Finally the nodule is immersed in ethyl alcohol and later washed with sterile $H_2O$. The *Rhizobium* is isolated either by washing the nodule in pestle and morter or by cutting the nodule and streaking. The washed juice is collected by a sieve and serially diluted and plated. The nodule is streaked in a solid media to obtain proper growth of the bacteria. The media used for the growth of *Rhizobium* is yeast extract mannitol agar medium. The rhizobial cells from the culture are identified and mass cultured for the preparation of inoculum. The correct strain of Rhizobia is identified by nodule formation, cultural tests, Microscopic observation and staining techniques.

**Mass culture of *Rhizobium*:**

The selected rhizobial strain is cultured in YEMA medium for about 7 days in order to establish better growth. The *Rhizobium* culture is tested. The tested Rhizobial culture is transferred to a large container containing the sterile YEMA medium are incubated at 30 C for 9 days. Sufficient nutrients should be supplied at regular intervals of 24hrs. The rhizobial culture is checked to detect the presence of contaminants in the culture. pH of the medium and the

growth rate are used to determine the presence of contaminants in the culture.

**Carried-based inoculum for storage:**

The term 'carrier' is generally used for a medium that carries the live microorganisms. The use of ideal carrier material is necessary in the production of good quality biofertilizer. Peat soil, lignite, vermiculite, charcoal, press mud, farmyard manure and soil mixture can be used as carrier materials. The neutralized peat soil/lignite are found to be better carrier materials for biofertilizer production The following points are to be considered in the selection of ideal carrier material.

- Cheaper in cost , Should be locally , High organic matter content, No toxic chemicals, Water holding capacity
- The carrier material (peat or lignite) is powdered to a fine powder so as to pass through 212 micron IS sieve.
- The pH of the carrier material is neutralized with the help of calcium carbonate (1:10 ratio) , since the peat soil / lignite are acidic in nature ( pH of 4 - 5)
- The neutralized carrier material is sterilized in an autoclave to eliminate the contaminants.

The cultured Rhizobial cells can be added to the carrier like lignite to store the inoculum. This storage increases the efficiency of the strain. This carrier is used to preserve the inoculum in a viable condition.

**Field Application:**

1. The cultured *Rhizobium* is diluted with $H_2O$ and applied on seeds. The suspension is sprinkled over on seeds. Sucrose solution (10%) is used to enhance the surviving potential of *Rhizobium* on the seed coats.
2. Inoculum is diluted with $H_2O$ and slurry is uniformly mixed with seeds. Then the inoculum is pellatized on the seed coats. The inoculum is protected from the agricultural chemicals and acids and alkaline reaction of the soil. Thus the inoculum is spread over the field along with the seeds during sowing.
3. Pelleting agents like dolomite, gypsum, charcoal rock phosphates are used along with the inoculum. They increase the sedimentation potential of the inoculum on the surface of seeds. It protects the seeds from winter season.
4. The inoculum is stored at 4 $^{\circ}C$ in a refrigerator. The stored inoculum is sprayed over the soil directly to increase the fertility of the soil.

**Frankia**

*Frankia* belongs to Actinomycetes group of N-fixing organisms forming root nodules with non- leguminous plants. *Frankia* strains are heterotrophic aerobes having generation times of 15 or more hours. As a consequence of their filamentous morphology, the growth kinetics of *Frankia* strains generally consist of a stationary phase after transfer, followed by a short 'exponential' phase, and then by a slower increase in biomass over time. Problems typical of growing other filamentous organisms apply to *Frankia* strains. Care must be taken to avoid nutrient and waste gradients across mycelia and a flocs or pellet formation should be avoided by

frequent homogenization.

*Frankia* is a genus of nitrogen-fixing, filamentous bacteria that live in symbiosis with actinorhizal plants, similar to the *Rhizobium* bacteria found in the root nodules of legumes in the family Fabaceae. Bacteria of this genus also form root nodules. This genus was originally named by Jørgen Brunchorst in 1886 to honor the German biologist, Albert Bernhard Frank.[2]Brunchorst considered the organism he had identified to be a filamentous fungus. Becking redefined the genus in – 1970 as containing prokaryotic actinomycetes and created the family Frankiaceae within the Actinomycetales. He retained the original name of *Frankia* for the genus.

*Frankia* is difficult to isolate directly from soil, so most strains originate from root nodules. Two factors limit success, one is that *Frankia* strains grow slowly, and the other is that fast-growing contaminants are common. To minimize the second problem, nodules are disinfected with dilute sodium hypochlorite and then peeled. Vesicle clusters can be separated from plant tissue by differential screening (Benson, 1982) or density centrifugation. Clusters are best pour-plated on a variety of media and followed microscopically until they begin to grow over a period of ten days to three weeks. Monitoring the outgrowth of hyphae microscopically improves the chances of obtaining a monoculture. Contaminants are spatially removed from the slower-growing *Frankia* colony. The medium used in isolating new *Frankia* strains is important but universal, or selective, media have not been reported. Effective mdia range from defined propionate media, to the complex QMod medium of Lalonde and Calvert. Antifungal agents, like cycloheximide or nystatin, can minimize fungal contamination. Most strains of Frankia are able to fix atmospheric nitrogen in pure culture. Presence of nitrogenase in pure culture was demonstrated by acetylene reduction method. Studies show that enzyme is located in the vesicles of Frankia. The structural genes „nif k", „nif D" and „nif H" encoding for nitrogenase have been cloned and sequenced in two Frankia strains. Frankia nitrogenase enzyme strongly resembles to those of other nitrogen-fixing organisms and it also posses an active hydrogen uptake system to maintain low redox potential. Frankia converts ammonia in to glutamine through glutamine synthetase. No glutamate dehydrogenase has been detected in Frankia. Frankia culture contains Fe and Mn containing SOD that protects nitrogenase from O2. If oxygen concentration is below 0.3% nitrogenase activity is present but no vesicles are formed. Nitrogen fixation is readily observed in Frankia during the lag phase. It increases during early logarithmic phase and then declines temporarily. A decrease in N2 fixation is associated with the decelerating phase. Nitrogen fixing activity in nodules of nonlegumenous nodulating plants was first reported using 15N and C2H2 reduction methods by Sloger and Silver (1965). A haemoglobin has been specially detected in the nodules of Myrica, Alnus and Casuarina that protects nitrogenase from oxygen inactivation . Presence of a vanadium nitrogenase, instead of the normal nitrogenase that contains molybdenum in novel vesicles of Frankia that opt for a non-symbotic mode of survival has been reported by Ganesh (1997). An uptake hydrogenase that utilises the hydrogen gas evolved during N2 fixation was found mainly in vesicles, but to some extent also in hyphae of free living Frankia. Nitrogen fixation by actinorhizal plants were estimated with an annual rate of 240-350kg/ha/yr. Nitrogen fixation by Alnus species ranges from 40-360kg/ha/yr. and by Casuarina 58.5 kg/ha/yr.

**Isolation of *Frankia***

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The medium used in isolating new *Frankia* strains is important but universal, or selective media can be used. Effective media range from defined propionate media to the complex QMod medium of Lalonde and Calvert. Antifungal agents, like cycloheximide or nystatin, can minimize fungal contamination. Virtually all *Frankia* strains isolated require no growth factors, and thus grow well in defined minimal medium (FDM). Some are inhibited by undefined media additives such as yeast extract. Another general medium for *Frankia* is defined propionate minimal medium (DPM). Most strains are grown and maintained in liquid culture, and generally grow slowly on solid media.

**Alder**

*Alder* is the common name of a genus of flowering plants (***Alnus***) belonging to the birch family Betulaceae. The genus comprises about 35 species of shrubs, a few reaching a large size, distributed throughout the North temperate zone with a few species extending into Central America, as well as the Northern and Southern Andes. *Alders* are commonly found near streams, rivers, and wetlands.

**Nitrogen fixation**

Alder is particularly noted for its important symbiotic relationship with *Frankia alni*, an actinomycete, filamentous, nitrogen-fixing bacterium. This bacterium is found in root nodules, which is large with many small lobes, and light brown in colour. The bacterium absorbs nitrogen from the air and makes it available to the tree. *Alder*, in turn, provides the bacterium with sugars, which it produces through photosynthesis. As a result of this mutually beneficial relationship, alder improves the fertility of the soil where it grows, and as a pioneer species, it provide additional nitrogen.

**Uses**
The catkins of some alder species have a degree of edibility and may be rich in protein. Reported to have a bitter and unpleasant taste, they are more useful for survival purposes. The wood of certain alder species is often used to smoke various food items such as coffee, salmon and other seafood. Most of the pilings that form the foundation of Venice were made from alder trees.

**Casuarina**

*Casuarina* is a genus of 17 tree species in the family Casuarinaceae, native to Australia, the Indian subcontinent, Southeast Asia, and Islands of the Western Pacific Ocean. They are evergreen shrubs and trees growing to 35 m (115 ft) tall. The foliage consists of slender, much-branched green to grey-green twigs bearing minute scale-leaves in whorls of 5–20. The apetalous flowers are produced in small catkin-like inflorescences. Most species are dioecious, but a few are monoecious. The fruit is a woody, oval structure superficially resembling a conifer cone, made up of numerous carpels, each containing a single seed with a small wing.

**Uses**

The wood of this tree is used commercially for shingles or fencing, and is said to make excellent, hot burning firewood. The wood of this tree is used for building-timber, furniture and tools, and makes excellent firewood. The tree's root nodules are known to fix nitrogen, and it is traditionally prized for its ability to increase the soil's fertility. Its abundant leaf-fall is high in nitrogen.

**Non-Symbiotic:**

**Free living Nitrogen fixers**

Free living and associative nitrogen fixers are important inoculants for non-leguminous crop particularly graminaceous and vegetable crops. Nitrogen fixing bacteria colonizing graminaceous plants can be grouped into three categories.

1. Rhizosphere organism - The species that colonize the root surfaces such as *Azotobacter* sp.
2. Facultative endophytes - Colonize the surface and interior of the roots such as *Azospirillum* sp.
3. Obligate endophytes - Includes *Gluconacetobacter*, *Herbaspirillum* sp. and *Azoarcus.*

**Azotobacter**

Beijerinck discovered an aerobic bacterium capable of fixing molecular nitrogen. Azotobacter species are known to influence plant growth through their ability to fix nitrogen, production of growth promoting substances like IAA, gibberellins or gibberellin like compounds and excretion of ammonia in the rhizosphere through exudates, production of antifungal metabolites and phosphate solubilization. Ecological or agro climatic factors like fertility level, moisture, temperature, acidic and alkaline condition and the carbon content of the soil seem to influence the proliferation of Azotobacter in the soil or in the rhizosphere.

**Characteristics of Azotobacter**

Azotobacter a soil habitant bacterium is a free living, nonsymbiotic nitrogen fixing bacteria. Azotobacter is rod shaped, relatively large organisms measuring 2.0-7.0µ x 1.0-2.5 µ. The cell size and shape vary considerably with species, strains, age of culture and growth conditions. For several species, the vegetative cells may give rise to specialized spherical resting cells known as cysts. Each cyst is produced from a single vegetative cell. Motility in most of the Azotobacter cells is carried out by means of peritrichous flagella. A unique differentiating character of Azotobacter is its ability to form pigments. Azotobacter species are known to influence plant growth through their ability to fix nitrogen, production of growth promoting substances like compounds and excretion of ammonia in the rhizosphere through exudates, production of

antifungal metabolites and phosphate solubilization.

**Factors influencing Azotobacter growth**

**Temperature**: Azotobacter is typical mesophilic bacteria. The optimal temperature they can withstand is between 25 Cand 30 C.

**Humidity**: Requires high humidity. They have a lower intracellular osmotic pressure than fungi and Actinomycetes. Hence the moisture requirements resemble that of higher plants.

**Aeration**: Being aerobic, Azotobacter needs continous supply of oxygen, but unique in its needs.

**pH**: Optimal pH for its growth is near or slightly above neutrality. (7.2-7.6).

**Salts**: The main ecological factor affecting the viability (metabolism) of microorganisms in saline soils is the high salt concentration

**Isolation of Azotobacter**

Azotobacter species are isolated by soil dilution plating method. One gram of soil sample are transferred to 100ml sterile distilled water and mixed thoroughly by shaking the flask for 5 minutes. Serial dilution of the suspension is made using sterilized distilled water. Any one of the nitrogen free agar media specific for Azotobacter is prepared and poured into sterile petriplates. 0.1 ml samples from the appropriate dilutions are spread evenly over cooled agar medium in petriplates. The plates are incubated at 30◦C for 3-4 days. Azotobacter colonies appear as flat, soft, milky and mucoid on agar plates.

**Mass production of Azotobacter**

Jensen's N-free medium is routinely used for the mass multiplication of Azotobacter. For mass production of Azotobacter, the bacterial strain isolated preserved in slants were transferred to liquid broth of selective as well as optimized medium in the rotary shaker for 4 days to prepare starter culture. Later on the starter culture is transferred to the fermenter in batch culture is transferred to the fermenter in batch mode with proper maintenance of 30 C and continuous agitation for 4-9 days. When the cell count has reached to 108-109 cells/ml, the broth is used as inoculants. For easy handling, packing, storing and transporting broth is mixed with an inert carrier material which contains sufficient amount of cells.

**Carrier based medium**

Powdered peat soil, lignite are used as carriers. The Azotobacter prefers 4 C for its long term storage. Sometimes the powdered carriers are neutralized with $CaCO_3$ and autoclaved for proper sterilization. This is mixed with culture and dried in air before storage.

**Applications**

1. **Seed treatment:**

   The cultured inoculum is diluted with $H_2O$ and the seeds are kept dipped in the inoculum for one night. This seeds are sown in the main field. The slurry is directly poured over the nursery bed or in agricultural field.

   The seeds are spread on a polythene bag and the inoculum is sprinkled over the seeds for the mixing of the inoculum with the seeds. The inoculum-coated seeds are then dried in the air before sowing.

2. **Seedling treatment:**

   In this method, the inoculum in diluted with the $H_2O$ and the roots of the seedlings are kept

dipped in the inoculum for about 10-15 min. Paddy field gets benefited by this process.

3. **In paddy field:**

A required amount of inoculum is mixed with farmyard manure. Then this mixture is properly mixed with soil. The resulting carrier based inoculum is directly used in the cultivation of rice. Azotobacter synthesizes biologically active substances such as nicotinic acid, panthothenic acid, pyridoxine, biotin, giberellic acid. These are plant growth promoting substances (PGPS). Azotobacter provides a favorable micro environment to the root system of higher plants and induces the better growth of the roots which participates in the growth of root systems in higher plants.

**Azospirillium**

**Characteristics of Azospirillium**

*Azospirillum* is a free living nitrogen fixing bacteria closely associated with grasses. Azospirillum is a Gram negative, rod-shaped and motile bacteria associated with roots of monocots including important crops such as wheat, corn and rice. Azospirillum bacterium fixes the atmospheric nitrogen and makes it available to plants in nonsymbiotic manner that can replace 50-90% of the nitrogen fertilizer required by plants. The nitrogen source used by *Azospirillum* for their growth is ammonium, nitrate, amino acids and elemental nitrogen. *Azospirillum* sp. is highly adaptable, being able to grow under anaerobic conditions (nitrate used as eletron acceptor), microaerobic (elemental or ammonia used as N source) and fully aerobic conditions (ammonia, nitrate, amino acid or combined N only). *Azospirillum* as a "biofertilizer" is particularly important in agricultural systems where fertilizer inputs are either impractical (rangelands), undesirable (organic farming), or not possible (subsistence agriculture). Experiments on inoculation of crops with *Azospirillum* or other diazotrophs often resulted in enhanced plant growth or nitrogen content under environmental conditions, improve nutrient assimilation, alter root size and function.

This Azospirillum bacterium fixes the atmospheric nitrogen and makes it available to plants in non- symbiotic manner that can replace 50-90% of the nitrogen fertilizer required by plants.

Azospirillum biofertilizer also secretes some fungicides, enzymes but in minute amount. Use of Azospirillum biofertilizer increases the crop production in large scale. We are engaged in manufacturing and marketing of Bio control agents comprise of different types of beneficial Bacterial, Fungal and Viral cultures. Azospirillum is mainly useful for monocot vegetables. Azospirillum is an eco-friendly liquid biological fertilizer formulation containing bacteria, Azospirillum which contain large amount of lipid granules, which enters the cortical cells of the root and fix up atmospheric nitrogen and also produces biologically active substances like vitamins, nicotinic acid, in dole acetic acid, gibberellins etc and helps in better retention of flowers and enhances the plant growth.

Azospirillum is a gram ngative, symbiotic, vibrioid soil bacterium. It occurs in large numbers in association with roots of cereals, grasses and tuber crops. Azospirillum fixes about 20-40 kg of the atmospheric nitrogen under microaerobic conditions. And it increases the vegetative growth and crop yield in many plants.

Azospirillum includes 4 species- Azospirillum lipoferum, A. brasilense, A. amazonese and A. halopraferns. Among them, A. lipoferum and A. brasilense are in common use as biofertilizers.

The production of Azospirillum inoculants involves the following important steps: Azospirillum is present both inside and outside the plant roots. A proper host plant is uprooted from the soil and its root system is excised. The root system is washed with running water to remove the soil particles. It is cut into small pieces of 1cm in length. The root pieces are surface sterilized by dipping them in 0.1% mercuric chloride for one minute and then washed repeatedly with a phosphate buffer.

Semi-solid malic acid medium is formulated, sterilizes and distributed into culture tubes. One or two root pieces are aseptically inoculated and incubated at room temperature for 3-5 days. A white pellicle like layer of Azospirillum appers just below 1-2 mm from the surface of the medium. The medium becomes blue in colour.

Development of blue colour in nitrogen free malic acid medium is the confirmative test for Azospirillum. A loopful of Azospirillum colony in the malic acid medium is transferred to okan‟s medium in culture flasks. The culture flasks are maintained at room temperature for 3-5 days. The bacterial cells grow into a starter culture. And then they are subjected to mass multiplication.

Azospirillum inoculants are mixed with the carrier till it attains 40% of moisture.

Azospirillum fixes the atmospheric nitrogen and releases plant growth promoting substances so the crops grow well and give higher yield. Azospirillum increases the straw yield as well as grain yield in rice, wheat, barley, sorghum, bajra and fodder oats. This treatment saves about 25% of the recommended dose of nitrogen fertilizer for sugarcane. Meantime, it increases the sugar level in the canes. This treatment increases phenolic contents in sorghum. Hence becomes resistant to sorghum shootfly and Antherigona soccata.

**Benefits**

1. Promotion of root hair development and branching
2. Increased uptake of N, P, K and microelements
3. Improved water status of plants and
4. Increased dry matter accumulation and grain yield.

**Isolation of *Azospirillum***

The roots are separated from the plants and thoroughly washed in running tap water. Then transferred into 1 L flask containing 500 mL of sterile tap water and shaken for 30 min. The procedure is repeated three times, after which the same procedure is repeated with distilled water three times. The washed roots are transferred to sterile petridish and are cut into pieces with sterile scissors. Half centimeter long root pieces are surface sterilized in 70% alcohol for 3-5 seconds. The root pieces are repeatedly washed in phosphate buffer (pH 7.0) and then they are plated in semi solid, nitrogen free medium. The plates are incubated at 35 C for 3 days. Characteristic growth of *Azospirillum* is indicated by the formation of white pellicles 2-4 mm below the surface of the medium.

**Mass multiplication of *Azospirillum***

For mass multiplication of *Azospirillum*, the organism is allowed to grow in flasks containing $NH_4Cl$ and malic acid medium and incubated at 35-37∘C for 3 days. When there is good growth,

the broth culture is mixed with the carrier and carrier based inoculum is packed in polythene pouches. The preparation of carrier based inoculant and for inoculating the seed or seedlings with *Azospirillum* culture are allowed to multiply be an important factor in *Azospirillum* culture preparation.

**Carrier for *Azospirillum***

Soil and farmyard manure in the ratio of 1:1 sterilized for 3 hours consecutively for 3 days were found to be best suited as a carrier for *Azospirillum*. The bacterium was able to survive up to 6 months in the soil and farmyard and gave counts of$10^6$ cells/g of carrier materials.

**Application**

1. The cultured *Azospirillum* is diluted with $H_2O$ and applied on seeds. The suspension is sprinkled over on seeds. Sucrose solution (10%) is used to enhance the surviving potential of *Azospirillum* on the seed coats.
2. Inoculum is diluted with $H_2O$ and slurry is uniformly mixed with seeds. Then the inoculum is pellatized on the seed coats. The inoculum is protected from the agricultural chemicals and acids and alkaline reaction of the soil. Thus the inoculum is spread over the field along with the seeds during sowing.
3. Pelleting agents like dolomite, gypsum, charcoal rock phosphates are used along with the inoculum. They increase the sedimentation potential of the inoculum on the surface of seeds. It protects the seeds from winter season.
4. The inoculum is stored at $4^{\circ}C$ in a refrigerator. The stored inoculum is sprayed over the soil directly to increase the fertility of the soil.

**Mycorrhizae:**

**Mechanisms**

The mechanisms by which mycorrhizae increase absorption include some that are physical and some that are chemical. Physically, most mycorrhizal mycelia are much smaller in diameter than the smallest root or root hair, and thus can explore soil material that roots and root hairs cannot reach, and provide a larger surface area for absorption. Chemically, the cell membrane chemistry of fungi differs from that of plants. For example, they may secrete organic acid that dissolve or chelate many ions, or release them from minerals by ion exchange. Mycorrhizae are especially beneficial for the plant partner in nutrient-poor soils.

**Disease, drought and salinity resistance and its correlation to mycorrhizae**

Mycorrhizal plants are often more resistant to diseases, such as those caused by microbial soil- borne pathogens. These associations have been found to assist in plant defense both above and belowground. Mycorrhizas have been found to excrete enzymes that are toxic to soil borne organisms such as nematodes. More recent studies have shown that mycorrhizal associations result in a priming effect of plants that essentially acts as a primary immune response. When this association is formed a defense response is activated similarly to the response that occurs when the plant is under attack. As a result of this inoculation, defense responses are stronger in plants

with mycorrhizal associations.

AMF was also significantly correlated with soil biological fertility variables such as soil fungi and soil bacteria, including soil disease. Furthermore, AMF was significantly correlated with soil physical variable, but only with water level and not with aggregate stability. and are also more resistant to the effects of drought. The significance of arbuscular mycorrhizal fungi includes alleviation of salt stress and its beneficial effects on plant growth and productivity. Although salinity can negatively affect arbuscular mycorrhizal fungi, many reports show improved growth and performance of mycorrhizal plants under salt stress conditions.

**Resistance to insects**

Recent research has shown that plants connected by mycorrihzal fungi can use these underground connections to produce and receive warning signals.Specifically, when a host plant is attacked by an aphid, the plant signals surrounding connected plants of its condition. The host plant releases Volatile organic compounds (VOCs) that attract the insect's predators. The plants connected by mycorrhizal fungi are also prompted to produce identical VOCs that protect the uninfected plants from being targeted by the insect. Additionally, this assists the mycorrhizal fungi by preventing the plant‟s carbon relocation which negatively affects the fungi‟s growth and occurs when the plant is attacked by herbivores.

**Colonization of barren soil**

Plants grown in sterile soils and growth media often perform poorly without the addition of spores or hyphae of mycorrhizal fungi to colonise the plant roots and aid in the uptake of soil mineral nutrients. The absence of mycorrhizal fungi can also slow plant growth in early succession or on degraded landscapes. The introduction of alien mycorrhizal plants to nutrient-deficient ecosystems puts indigenous non-mycorrhizal plants at a competitive disadvantage. This aptitude to colonize barren soil is defined by the category Oligotroph.

**Resistance to toxicity**

Fungi have been found to have a protective role for plants rooted in soils with high metal concentrations, such as acidic and contaminated soils. Pine trees inoculated with *Pisolithus tinctorius* planted in several contaminated sites displayed high tolerance to the prevailing contaminant, survivorship and growth. One study discovered the existence of *Suillus luteus* strains with varying tolerance of zinc. Another study discovered that zinc-tolerant strains of *Suillus bovinus* conferred resistance to plants of *Pinus sylvestris*. This was probably due to binding of the metal to the extramatricial mycelium of the fungus, without affecting the exchange of beneficial substances.

**Phosphate solubilizers:**

Phosphate solubilizing microbes (PSB) are an aggregation of helpful microscopic organisms capable of hydrolysing natural and inorganic phosphorus from insoluble compounds. P-solubilization capacity of the microorganisms is recognized to be a standout amongst the most important traits associated with plant phosphate nourishment. It is for the most part acknowledged that the mechanism of mineral phosphate solubilization by PSB strains is

associated with the release of low sub-atomic weight organic acids through which beneficiary hydroxyl and carboxyl groups chelate the cations bound to phosphate, consequently converting it into soluble forms. In addition, some PSB produce phosphatase like phytase that hydrolyse organic forms of phosphate compounds productively. One or both types of PSB have been acquainted with Agricultural group as phosphate Biofertilizer. Phosphorus (P) is one of the major fundamental macronutrients for plants and is applied to soil as phosphate biofertilizer. On the other hand, an extensive portion of soluble inorganic phosphate which is applied to the soil as chemical fertilizer is immobilized quickly and becomes unavailable to plants. Currently, the principle reason in managing soil phosphorus is to improve crop production and minimize the loss of Phosphorus from soils. PSB have pulled the attention of agriculturists as soil inoculums to enhance the plant growth and yield. The point when PSB utilized with rock phosphate, it can recover around the range of half of the harvest prerequisite of phosphatic fertilizer. The utilization of PSB as inoculants increases the P uptake by plants. Simple inoculation of seeds with PSB gives crop yield reactions equal to 30 kg P205 /ha or 50 percent of the need for phosphatic fertilizers. Currently, distinctive strains of these bacteria has been distinguished for utilizing as a part of biofertilizer, of every one of the three new strains Pantoea agglomerans strain (P5), Microbacterium laevaniformans strain (P7) and Pseudomonas putida strain (P13) has been recently recognized as the remarkably effective insoluble phosphate solubilizer. Additionally, phosphate (P) compounds are capable of immobilizing heavy metals, especially Pb, in contaminated environments through phosphate-heavy metal precipitation. However, most P compounds are not readily soluble in soils so it is not readily used for metal immobilization. Phosphate solubilizing bacteria (PSB) have the potential to enhance phosphate-induced immobilization of metals to remediate contaminated soil. However, there is a limit on the amount of phosphate which can be added to the environment due to the issue of eutrophication.

**Phosphate solubilizing microorganisms**

Phosphorus is a major nutrient required for the growth of plants. There are large reserves of phosphorus in soils but very little amount is available to the plant. There are microorganisms in the soil that can solubilize the unavailable phosphorus and make it available to plant. They are called phosphate solubilizing microorganisms (PSM). A group of fungi associates with the roots of higher plants and mobilize the phosphorus from soil to the plant system. Phosphorus solubilizing microorganisms include various bacterial, fungal and actinomycetes forms which help to convert insoluble inorganic phosphate into simple and soluble forms. Members of *Pseudomonas, Micrococcus, Bacillus, Flavobacterium, Penicillum, Fusarium, Sclerotium* and *Aspergillus* are some of the phosphate-solubilizing micro-organisms. They normally grow in a medium containing insoluble tri-calcium phosphate [Ca3 (PO4)2], apatite, rock phosphate, FePO4 and AlPO4 as sole source of phosphate.

**Occurrence of phosphate solubilizing bacteria**

High proportion of PSM is concentrated in the rhizosphere and they are metabolically more active than from other sources. Usually one gram of fertile soil contains 103 to 1010 bacteria. Soil bacteria are in cocci, bacilli or spiral. Bacilli are common in soil, whereas spirilli are very rare in natural environment. The PSB are ubiquitous with variation in forms and population in different soils. Population of PSB depends on different soil properties. Larger populations of

PSB are found in agricultural and rangelands soils.

**Isolation of phosphate solubilizing microbes**

The initial isolation of phosphate solubilizers is made by using Pikovaskaya medium suspended with insoluble-phosphates such as tri-calcium phosphate. The production of clearing zones around the colonies of the organism is an indication of the presence of phosphate-solubilizing organisms. Several rock phosphate dissolving bacteria, fungi, yeast and actinomycetes were isolated from soil samples of rock phosphate deposits and rhizosphere soils of different leguminous crops. The most efficient bacterial isolates were *Pseudomonas striata, Pseudomonas rathonis* and *Bacillus polymyxa* and fungal isolates as *Aspergillus awamori, Penicillium digitatum, Aspergillus niger* and a yeast- *Schwanniomyces occidentalis*. These efficient micro-organisms have consistently their capability to solubilize chemically-fixed soil phosphorus and rock phosphate from different sources. In addition, these microorganisms were found to mineralize organic phosphorus to soluble form due to enzymatic activity.

The efficient cultures have capacity to solubilize insoluble inorganic phosphate such as rock phosphate, tri-calcium phosphate, iron and aluminium phosphates by production of organic acids. They can also mineralize organic phosphatic compounds present in organic manure and soils. Inoculation of PSM to seeds or seedlings increases the grain yield of crops. The inorganic phosphate solubilization by microbes can be attributed to acidification, chelation and exchange reaction in growth medium as well as to the proton transfer during ammonium assimilation.

**Mass production of phosphate solubilizing microorganisms**

1. A loopful of inoculum is transferred in liquid medium and keeps the flak on rotary shaker for 3-7 days. The content of these flasks called mother culture or starter culture. After sterilization suitable broth is inoculated with the mother culture. Keep the flasks on rotary shaker for 96-120 hours until the viable count per ml reaches to 10 9 /ml cells. Peat or Lignite powder is neutralized by addition of 1% calcium carbonate (CaCO3) and sterilized at 15 lbs pressure for 3-4 hours. The carrier should have high organic matter above 60% and high moisture holding capacity 150 to 200% by weight and provide a nutritive medium for growth of bacteria and prolong their survival in culture.
2. The sterilized and neutralized lignite or peat is mixed with high count broth culture in galvanized trays. After mixing the broth cultures and lignite or peat powder in 1:2 proportion in the galvanized trays then it is kept for curing at room temp (28°C) for 5 to 10 days. After curing, sieved powder is filled in polythene bag as of 0.5 mm thickness leaving 2/3 space open for aeration of the bacteria. Then the bag is packed by sealing.
3. The viable cells count in the carrier based inoculants should be maintained as per ISI specifications. The inoculants shall be stored by the manufacture in a cool place away from direct heat preferably at a temp of 15°C for six months.

**Effect of PSB on Crop Production**

Phosphate rock minerals are often too insoluble to provide sufficient P for crop uptake. Use of PSMs can increase crop yields up to 70 percent. Combined inoculation of arbuscular mycorrhiza and PSB give better uptake of both native P from the soil and P coming from the phosphatic rock Higher crop yields result from solubilization of fixed soil P and applied phosphates by PSB Microorganisms with phosphate solubilizing potential increase the availability of soluble phosphate and enhance the plant growth by improving biological nitrogen fixation enhanced the

number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop. Phosphate solubilizing bacteria enhanced the seedling length, while co-inoculation of PSM and PGPR reduced P application by 50 % without affecting corn yield.

**Algae:**

Blue-green algae are photoautotrophic, prokaryotic algae. They are free living creatures and also known as cyanobacteria. It fixes the atmospheric nitrogen in moist soils. So BGA has been recommended as a biofertilizer. The algae includes unicellular as well as filamentous species. Some of the filamentous forms have specialized cells known as heterocysts. Eg- Nostoc, Anabaena etc. These cells are the site of nitrogen fixation. The species which have heterocystous species. Amng them only a few species can reduce N2 into NH3.

**Cyanobacteria**

*Cyanobacteria*, otherwise called as blue-green algae are ubiquitous in distribution. BGA fixes nitrogen in the soil. BGA such as *Anabena, Polypothium, Oscillotrian* actively fixes the nitrogen in soil. The BGA induces the growth of higher plants with the help vitamin B12; auxins etc and thus they form an effective biofertilizer in agriculture. The blue green algal inoculum may be produced by several methods viz., in tubs, galvanized trays, and small pits and also in field conditions. However the large-scale production is advisable under field condition which is easily adopted by farmers.

**Mass multiplication**

**Preparation of the inoculum in trays:**

*Cyanobacteria* are cultured in open trays exposed to air. The culturing tray is made of Zn or Fe and is filled with sieved nice soil, supper phosphate, sodium molybdate and water to keep the mixture or medium wet. The pH is adjusted neutral. A culture of *Cyanobacteria* is sprinkled over the soil mixture and the tray is kept in the open sunlight for about 10-20 days for proper growth. Regular water is necessary which favor the better growth of *Cyanobacteria* in culture tray. Sometimes mosquitoes bread and the breeding can be stopped by the application of carbofuran. Owing to rapid growth, the *Cyanobacteria* cover the entire surface of soil mixture. The algal biomass is then separated from soil and air dried. The dried biomass is powdered and stored in polythene bags for future use.

**Cyanobacteria culture in open:**

The field is ploughed well and leveled properly for the culture of *Cyanobacteria*. The field is watered in order to facilitate the growth of *Cyanobacteria*. To induce the rapid growth of *Cyanobacteria*, super phosphate is sprayed over the surface of the soil. Clayey soil is prepared to sandy soil for proper and quick multiplication of *Cyanobacteria*. Application of carbofuran prevents the invasion of snails and mosquitoes. When the sufficient growth of *Cyanobacteria* is achieved, the field is ploughed well for the proper mixing of *Cyanobacteria* in the soil. Then the field is used as usual for agriculture.

**Application:**

1. The powdered *Cyanobacteria* mixture is simply spread over the agriculture field.
2. The application of *Cyanobacteria* after one week of transplantation of seedlings of paddy gives

more beneficial result, because *Cyanobacteria* can be able to receive more sunlight.

3. Such paddy plants grow well in the field by consuming the nitrogen fixed by the *Cyanobacteria*.

4. The application of *Cyanobacteria* in the field increases the yield of crops.

**Azolla**

*Azolla* is a free-floating water fern that floats in water and fixes atmospheric nitrogen in association with nitrogen fixing blue green alga *Anabaena azollae*. Rice growing areas in South East Asia and other countries have recently been evincing increased interest in the use of the symbiotic N2 fixing water fern *Azolla* either as an alternate nitrogen sources or as a supplement to commercial nitrogen fertilizers. *Azolla* is used as biofertilizer for wetland rice and it is known to contribute 40-60 kg N ha-1 per rice crop. The agronomic potential of *Azolla* is quite significant particularly for rice crop and it is widely used as biofertilizer for increasing rice yields. The common species of *Azolla* are *A. microphylla, A. filiculoides, A. pinnata, A. caroliniana, A. nilotica, A. rubra* and *A. mexicana*.

**Mass multiplication of *Azolla* under field conditions**

A simple *Azolla* nursery method for large scale multiplication of *Azolla* in the field has been evolved for easy adoption by the farmers.

The potential *Azolla* species are maintained in concrete tanks keeping soil under flooded conditions. Partial shade helps during summer months. From these, *Azolla* is harvested and used as inoculum in bigger size plots or in small ponds generally found in rice growing areas. Its large scale production is carried out in a nicely prepared field divided into small sun plots with good irrigation facility (4-50 sqm plot with 5-10 cm water depth). *Azolla* is inoculated at the rate of 0.5 to 1.0 t/ha. Inoculation with higher doses ensures rapid multiplication. Superphosphate at the rate of 4-8 kg/ha stimulates fern growth. Animal dung (1.0-15 t/ha) or cattle slurry (2000- 3000 t/ha) can also be used in place of P2O5. Insecticides like furadon are applied. Under optimum conditions, *Azolla* forms a thick mat on the water surface in 15-20 days. Two-third of it is harvested and the remaining is left for further multiplication. It again multiples and forms a thick mat in 2-3 weeks. About 100 kg fresh *Azolla* inoculum can be obtained every week from 100 $m^2$ nursery. Superphosphate at the rate of 60 kg/ha can be split into 2-3 doses or added at weeks interval to have better results. If *Azolla* multiplication is good even without addition of P, then there is no need to add it.

**Applications in field**

**Inoculation of *Azolla* to rice crop**

The *Azolla* biofertilizer may be applied in two ways for the wetland paddy. In the first method, fresh *Azolla* biomass is inoculated in the paddy field before transplanting and incorporated as green manure. This method requires huge quantity of fresh *Azolla*. In the other method, *Azolla* may be inoculated after transplanting rice and grown as dual culture with rice and incorporated subsequently.

***Azolla* biomass incorporation as green manure for rice crop**

Collect the fresh *Azolla* biomass from the *Azolla* nursery plot. Then prepare the wetland well and maintain water just enough for easy incorporation. Apply fresh *Azolla* biomass (15 t/ha) to the main field and incorporate the *Azolla* by using implements or tractor.

***Azolla* inoculation as dual crop for rice**

Select a transplanted rice field and collect fresh *Azolla* inoculum from *Azolla* nursery. Broadcast the fresh *Azolla* in the transplanted rice field on $7^{th}$ day after planting (500 kg/ha). Maintain water level at 5-7.5cm. Note the growth of *Azolla* mat four weeks after transplanting and incorporate the *Azolla* biomass by using implements or tranctor or during inter-cultivation practices. A second bloom of *Azolla* will develop 8 weeks after transplanting which may be incorporated again. By the two incorporations, 20-25 tonnes of *Azolla* can be incorporated in one hectare rice field.

**Novel combination of microorganism as biofertilizers:**

Phosphorus (P) is an essential nutrient and limits plant growth in many soils. Since global commercially available P stocks are finite and fertilizer production costs are likely to increase, novel management strategies to secure plant P nutrition are needed. A sustainable approach is to use P-rich biochar derived from the pyrolysis of organic waste such as sewage sludge. However plant P availability in thermally treated waste is usually reduced. Plant P availability of biochar-P can be potentially increased by means of phosphate-solubilizing microorganisms (PSM). PSM-based biofertilizers have been marketed but the mechanisms are poorly understood. The solubilization profiles of phosphate-solubilizing Penicillium strains for biochar-P were determined in vitro in order to select strains that can efficiently solubilize biochar-P and investigate the underlying mechanisms. Based on this screening, P. aculeatum (Pa) was selected and tested in two pot experiments. Wheat inoculation and subsequent persistence of Pa in biochar-amended soil resulted in an improved growth and P uptake by wheat, thus increasing the fertilizer value of biochar. As a major part of P solubilized outside the rhizosphere is adsorbed to charged surfaces, the presence of an active mycelium of arbuscular mycorrhizal (AM) fungi was anticipated to increase plant uptake of PSM- released P outside the rhizosphere. Wheat response to co-inoculation of Pa and the AM fungus Rhizophagus irregularis was studied in a pot experiment at two levels of biochar fertilization. The inclusion of radioactive P in a root-free compartment showed that, despite the overall AM-induced growth depressions, AM transferred considerable amounts of P to the plant. Pa actively colonized the rhizosphere and bulk soil, both in the presence and absence of AM and enhanced root colonization by AM-colonization. On the other hand, AM hyphal length density was not enhanced but also not inhibited by the presence of Pa. This suggests that AM and Pa can be combined without showing antagonistic interactions. The application of biochar at a low rate also increased AM-colonized root lengths. However, these effects were not translated to increased P uptake by wheat. Wheat negative responsiveness to AM may have masked any additive effects. Another aspect that must be taken into consideration before the application of a biofertilizer is the potential effects on the indigenous soil microbial communities, which play a key role in plant nutrient acquisition. Biochar-mediated changes of the physical and chemical properties of soil can also impact the soil microbiome. Therefore a controlled pot experiment in compartmented system was set up and 16S rRNA amplicon sequencing was performed to investigate whether biochar and/or Pa affect the bacteria community structure, and if these changes are transient and if they differ in the

rhizosphere and bulk soil. Biochar added in the bulk soil-compartment stimulated specific groups of taxa both at the early and late growth stage and induced a shift in the community even in the rhizosphere soil situated in a distant compartment, whereas Pa affected the communities at a much lesser extent and only temporarily. Taken together, these results open up for new approaches using P-solubilizing Penicillium fungi to increase the fertilizer value of P-rich biochar, but also call for future studies to investigate variations upon different experimental conditions (use of different waste materials, soils, plant species or even microbial strains). Future work on understanding the mechanisms behind the AM-Pa-biochar interactions will promote the potential of combining these in commercial biofertilizers.

**PGPRs:**

Free-living PGPR have shown promise as biofertilizers. Plant growth promotion, increased yield, solubilization of P (phosphorus) or K (potassium), uptake of N (nitrogen) and some other elements through inoculation with PGPR. In addition, studies have shown that inoculation with PGPR enhances root growth, leading to a root system with large surface area and increased number of root hairs. A huge amount of artificial fertilizes are used to replenish soil N and P, resulting in high costs and increased environmental pollution. Most of P in insoluble compounds is unavailable to plants. N2 -fixing and P-solubilizing bacteria may be important for plant nutrition by increasing N and P uptake by the crop plants, and playing a crucial role in biofertilization. N2 -fixation and P-solubilization, production of antibiotics, and other plant growth promoting substances are the principal contribution of the PGPR in the agro-ecosystems. More recent research findings indicate that the treatment of agricultural soils with PGPR inoculation significantly increases agronomic yields as compared to uninoculated soils. The success and commercialization of plant growth promoting rhizobacterial strains depend on the linkages between the scientific organizations and industries. Numerous work done showed different stages in the process of commercialization include isolation of antagonist strains, screening, fermentation methods, mass production, formulation viability, toxicology, industrial linkages, quality control and field efficacy. Moreover, commercial success of PGPR strains requires economical and viable market demand, consistent and broad spectrum action, safety and stability, longer shelf life, low capital costs and easy availability of career materials.

**Plant growth promotory bioformulations**

Bioformulations are best defined as biologically active products containing one or more beneficial microbial strains in an easy to use and economical carrier material. Most bioformulations are meant for field application, it is essential that suitable carrier materials are used to maintain cell viability under adverse environmental conditions. A good quality formulation promotes survival of bacteria maintaining available population sufficient to exude growth promoting effects on plants [71]. Plant growth promoting rhizobacterial bioformulation refers to preparations of microorganism that may be partial or complete substitute for chemical fertilization, pesticides, offer an environmentally sustainable approach to increase crop production and health.

**Formulation design**

Formulation is the crucial issue for inoculants containing an effective bacterial strain and can determine the success or failure of a biological agent. The use of inoculant formulations

involving carrier materials for the delivery of microbial cells to soil or the rhizosphere is an attractive option. Carrier materials are generally intended to provide a protective niche to microbial inoculants in soil, either physically, via the provision of a protective surface or pore space or nutritionally, via the provision of a specific substrate. Bioformulation of plant growth promoting rhizobacteria should be composed of a superior carrier material such as high waterholding capacity, high water retention capacity, no heat production from wetting, nearly sterile, chemically uniform, physically uniform, nontoxic in nature, easily biodegradable, nonpolluting, nearly neutral pH (or easily adjustable pH), and supports bacterial growth and survival. Apart from these materials, many other synthetic and inert materials, such as vermiculite, ground rock phosphate, calcium sulfate, polyacrylamide gels, and alginate have also been evaluated. Drying is a part of many procedures for development of formulation of microbial inoculants. Remarkably low percentage of endospore formers was observed that survived after drying. One factor which can have a detrimental effect on dried microorganisms over the long term is humidity in the environment; increasing moisture content of the dried sample compromises viability. Storage under vacuum or in an inert atmosphere can prevent this but is costly and unwieldy. The use of each type of inoculant depends upon market availability, choice of farmers, cost, and the need of a particular crop under specific environmental conditions.

**Role of PGPRs in crop production:**

Rice, wheat and maize are the three major staple food crops for world‟s population.

A variety of PGPR‟s participate in interaction with C3 and C4 plants and can significantly increase their yield . Rice crop removes around 16–17 kg N to produce 1 t dry weight of rice including straw. Wheat crop requires about 26–28 kg N to produce 1 t of grain including straw . Maize plants require 9–11 kg N to produce 1 t biomass.

The N requirement of cereals is normally met by fertilization at a rate depending on soil fertility with chemical urea . PGPR inoculant biofertilizers can, in principle, be used to supplement or reduce the use of urea-N

. Those closely associated with rice rhizosphere are Azospirillum , Burkholderia and Herbaspirillum . A free living heterotrophic diazotroph like Azotobacter (A. vinelan-dii and A. chroococcum) uses C from sugar as energy source. Thereare obligatory anaerobic heterotrophs like Clostridia which are only capable of fixing N in the complete absence of oxygen and are usually isolated from rice fields . Their activity in rice may be enhanced with the addition of organic source like straw, presumably as a result of microbial breakdown of cellulose into cellobiose and glucose. Yield of rice can be increased with the application of Azotobacter, Azospirillum lipoferum and Azospirillum brasilense, Azospirillum.

In soil, siderophore production activity plays a central role in determining the ability of different microorganisms to improve plant development. Microbial siderophores enhance iron uptake by plants that are able to recognize the bacterial ferric-siderophore complex. and are also important in the iron uptake by plants in the presence of other metals such as nickel and cadmium. However, it is still unclear if bacterial siderophore complexes can significantly contribute to the iron requirements of the plant. Siderophore production confers competitive advantages to PGPR that can colonize roo[illegible] highly competitive cc[illegible] outcome of competition for different carbon sources that are available as a result of root exudation or rhizodeposition. Among most of the bacterial siderophores studied, those produced

by pseudomonads are known for their high affinity to the ferric ion. The potent siderophore, pyoverdin, for example, can inhibit the growth of bacteria and fungi that present less potent siderophores in iron-depleted media *in vitro*. A pseudobactin siderophore produced by *P. putida* B10 strain was also able to suppress *Fusarium oxysporum*in soil deficient in iron; this suppression was lost when the soil was replenished with iron, a condition that represses the production of iron chelators by microorganisms. Recent studies have demonstrated the suppression of soil-borne fungal pathogens through the release of iron-chelating siderophores by fluorescent pseudomonads, rendering it unavailable to other organisms.

## Possible Questions

### PART-B (2 Mark question)

1. What are the cultural characteristics of *Rhizobium*?
2. What is symbiosis?
3. Why potassium chloride is used in the isolation of *Rhizobium*?
4. Name a few carrier materials used for inoculum storage of *Rhizobium*.
5. Which basis the carrier material is selected?
6. Write two field applications of *Rhizobium*?
7. Write the two factors that limit the use of *Frankia* as biofertilizer.
8. Name the antifungal agents that minimize the fungal growth in the isolation of *Frankia.*
9. What is *Azolla*?
10. Define free living nitrogen fixers.
11. Write the three groups of nitrogen fixing bacteria colonizing graminaceous plants.
12. Write the characteristics of *Azotobacter.*
13. What is rhizosphere?
14. Name the carrier material used for *Azotobacter.*
15. Write the benefits of *Azospirillum* as biofertilizer.
16. What is nitrogen fixation?
17. Differentiate between symbiotic and nonsymbiotic nitrogen fixation.
18. Define siderophore.

### PART-C (8 MARKS)

1. Write an essay on *Rhizobium* as symbiotic nitrogen fixers.
2. Give an elaborate note on the isolation, mass multiplication and field application of *Rhizobium*.
3. Describe the mass multiplication and field application of *Cyanobacteria.*
4. What is biofertilizer? Write the field applications and crop response of biofertilizer?
5. Explain the methods of *Azolla* to rice crop.
6. Describe the isolation, mass cultivation and field application of *Azolla.*
7. Give brief note on *Azotobacter* and *Azospirillum* as non symbiotic microorganism.
8. Describe the mass multiplication, field application of *Azospirillum*.
9. Describe the mass multiplication and field application of *Azotobacter*
10. Write the characteristics and application of *Azotobacter* and *Azospirillum*.

# KARPAGAM ACADEMY OF HIGHR EDUCATION

CLASS: III B.Sc MB COURSE NAME: Microbes in Sustainable Agriculture and Development

COURSE CODE: 17MBU602B UNIT: IV BATCH-2017-2020

| Sl. No. | Questions | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1. | A free-living soil bacteria that is involved in nitrogen fixation. | *Alcaligenes* | *Acetobacter* | *Pseudomonas* | *Azotobacter* | *Azotobacter* |
| 2. | _____ are genes encoding enzymes involved in the fixation of atmospheric nitrogen | *mif* | *nif* | *sif* | *nod* | *nif* |
| 3. | _____ catalyze conversion of atmospheric nitrogen to ammonia | Kinase | Hydrogenase | Nitrogenase | Phosphatase | Nitrogenase |
| 4. | _____ is a typical example of symbiotic nitrogen fixation seen in paddy fields | Azolla-*Anabaena* | Alder-*Frankia* | Legume-*Rhizobium* | Higher plants-*Mycorrhizae* | Azolla-*Anabaena* |
| 5. | Apart from biological nitrogen fixation by microbes, _____ can fix atmospheric nitrogen | Cyclone | Thunder | Raining | Lightning | Lightning |
| 6. | Bacteria that forms root nodules in legume plants | *Rhizobium* | *Azotobacter* | *Azospirillum* | Cyanobacteria | *Rhizobium* |
| 7. | Biological nitrogen fixation was discovered by | Winogradsky | Beijerinck | Pasteur | Koch | Beijerinck |
| 8. | Chemicals produced by the Rhizobia called _____ that cause the colonized root hairs to curl | Pod factors | Nod factors | Sod factors | Mod factors | Nod factors |
| 9. | Example of associative nitrogen fixation | Legume-*Rhizobium* | Rice-*Azospirillum* | Higher plants-Mycorrhizae | Azolla-*Anabaena* | Rice-*Azospirillum* |
| 10. | In Cyanobacteria, nitrogen fixation occurs in terminally differentiated cells known as | Cyanocysts | Nitrocycts | Heterocysts | Homocysts | Heterocysts |
| 11. | In root nodules, _____ bind and regulate the levels of oxygen in the nodule | Teghemoglobin | Peghemoglobin | Leghemoglobin | Hemoglobin | Leghemoglobin |
| 12. | Legume plants belongs to | *Solanaceae* | *Rosaceae* | *Astraceae* | *Fabaceae* | *Fabaceae* |
| 13. | Nitrogenase enzyme consists of | Iron protein | Molybdenum-iron protein | Iron protein and a molybdenum-iron protein | Hemoglobin | Iron protein and a molybdenum-iron protein |
| 14. | Rhizobia are attracted to _____ released by the host legume's roots | Flavonoids | Enzymes | Toxins | Chemicals | Flavonoids |
| 15. | The enzyme nitrogenase is inhibited by _____ | $CO_2$ | Sulfur | Hydrogen | Oxygen | Oxygen |
| 16. | The conversion of nitrogen to ammonia or nitrogenous compounds is called as_____ | Nitrogen assimilation | Nitrogen fixation | Denitrification | Nitrification | Nitrogen fixation |

| | | | | | | |
|---|---|---|---|---|---|---|
| 17. | Symbiotic nitrogen cyanobacteria are present in all except_____ | Anthoceros | Azolla | Cycas | Gnetum | Gentum |
| 18. | All the following are free living nitrogen fixers except_____ | Rhizobium | Azotobacter | Rhodospirillum | Clostridium | Rhizobium |
| 19. | Anabena is a nitrogen fixer present in the root pockets of_____ | Marselia | Salvinia | Pistia | Azolla | Azolla |
| 20. | Splitting of dinitrogen molecule into free nitrogen atom in biological nitrogen fixation is carried out by _____ | Hydrogenase | Nitrogenase | Dinitrogenase | Nitrate reductase | Nitrogenase |
| 21. | Which of the following aid plants in the acquisition of nitrogen from nitrogen gas of the atmosphere? | Bacteria | Algae | Nematodes | Moulds | Bacteria |
| 22. | Which one of the following is nonleguminous | Casuarina | Bacillus | Sesbania | Penicillium | Casuarina |
| 23. | Nif gene is associated with _____ | Rhizobium bacteriod | Arthrobacter | Myrica | Bacillus | Rhizobium bacteriod |
| 24. | What are the cofactors needed for nitrogen fixation? | Cobalt | Molybdenum | Zinc | Copper | Cobalt |
| 25. | Azotobacter chroococcum grows in _____ soil | Acidic | Neutral | Basic | Neutral and alkaline | Neutral and alkaline |
| 26. | Azotobacter are _____ shaped bacterium | Rod | Cocci | Sprillium | Comma | Rod |
| 27. | The incubation period for the isolation of Azotobacter is_____ | 3 Days | 5 Days | 1 Day | 7 Days | 3 Days |
| 28. | Aged cultures of Azotobacter chrococcum form an insoluble _____ colored pigment | Yellow | Red | Black brown | Black | Black brown |
| 29. | The melanin formed by Azotobacter is due to the presence of _____ enzyme | Tyrosinase | Maltase | Trypticase | Pectinase | Tyrosinase |
| 30. | The strains of some Azospirillum fail to grow in the absence of particular nitrogen source _____ with glucose | Peptone | Sodium nitrate | Yeast extract | Ammonium chloride | Yeast extract |
| 31. | Azospirillum amazonense peoduce _____ colored colonies in potato dextrose agar | White | Pink | Red | Black | White |
| 32. | Azosprillium irakense was found in association with _____ roots | Wheat | Rice | Grass | Barley | Rice |

| | | | | | | |
|---|---|---|---|---|---|---|
| 33. | Azospirillum rugosum a new species isolated from _____ soil | Sewage contaminated | Water | Oil contaminated | Waste contaminated | Oil contaminated |
| 34. | _____ is used as carrier for Azospirillum | Farmyard manure | Lignite | Charcoal | Press mud | Farmyard manure |
| 35. | Azospirillum are highly sensitive to _____ | Minerals | Salts | Heavy metals | Sugars | Heavy metals |
| 36. | The symbiotic relationship between funig and higher plants are called _____ | Lichen | Mycorrhiza | Helotism | Mutualism | Mycorrhiza |
| 37. | The advantage of plants in this association is | Food | Protection | Increased mineral absorption and disease protection | | Increased mineral absorption and disease protection |
| 38. | The ectomycorrhizas are commonly formed in _____ | Herbaceous plants | Woody plants | All plants | Grasses | Woody plants |
| 39. | The endomycorrhizas are also called as _____ | Hartig nets | Mat forming mycorrhizas | Vesicular arbuscular mycorrhiza | Intracellular mycorrhizas | Vesicular arbuscular mycorrhiza |
| 40. | The ectomycorrhizas form an intracellular network in root cortex called _____ | Arbuscular | Vescicles | Hartig net | Haustoria | Hartig net |
| 41. | The characteristic feature of VAM is it penetrates plant cell wall and form_____ | Spores intracellularly | Vesicles and dichotomously branched invaginations called arbuscules | Haustoria | Massive spore forming structures intracellularly | |
| 42. | The major advantages of a plant with VAM is _____ | Increased $N_2$ absorption | Increased P absorption | Increased K absorption | Increased Mn absorption | Increased P absorption |
| 43. | The fungal partner in ectomycorrhiza belongs to the class_____ | Basidiomycetes | Ascomycetes | Zygomycees | Every three groups | Every three groups |
| 44. | The symbiosis was described by _____ | Albert Bernhard Frank | Gaind | Nautiyal | Franciszek Kamieriski | Franciszek Kamieriski |
| 45. | Who introduced the term mycorhizae? | Albert Bernhard Frank | Gaind | Nautiyal | Gaur | Albert Bernhard Frank |
| 46. | Nitrogen fixation in rice occurs due to presence of _____ | Nostoc | Azolla | Anabena | Rhizobium | Anabena |

| | | | | | | |
|---|---|---|---|---|---|---|
| 47. | The medium for the growth of Rhizobium is _____ | Yeast extract mannitol agar | Rose bengal agar | Nutrient agar | Malt extract agar | Yeast extract mannitol agar |
| 48. | Except Rhizobium, which on of the following bacteria forms nitrogen fixing nodules in plants | Actinorhiza | Burholderia | Micrococcus | Pseudomonas | Burholderia |
| 49. | The root nodule of legumes contain pink pigment which has high affinity for oxygen is | Nod haemoglobin | Leghaemoglobin | Haemoglobin | Bacterial haemoglobin | Leghaemoglobin |
| 50. | Which one is green manure/biofertilizer? | Sesbania | Rice | Oat | Maize | Sesbania |
| 51. | Foliar spray is | Spraying on roots | Spraying on Stem | Spraying on leaves | Spraying on Flowers | Spraying on leaves |
| 52. | Indole acetic acid and gibberelins are | Hormones of bacteria | Hormones that retard plant growth | Plant growth hormones | Weedicides | Plant growth hormones |
| 53. | Liquid extract of composting by earthworms | Vermiwash | Germiwash | Wormiwash | Liquidwash | Vermiwash |
| 54. | Which bacteria is used as biofertilizer in sugarcane crop? | *Beijerinckia* | *Acetobacter diazotrophicus* | *Bacillus* | *Pseudomonas* | *Acetobacter diazotrophicus* |
| 55. | Which is major component of bordeaux mixture? | Copper sulphate | Sodium chloride | Calcium chloride | Magnesium sulphate | Sodium chloride |
| 56. | In plants, the strains of which one of the following bacterium initiates to the formation of galls? | *Agrobacterium* | *Rhizobium* | *Pseudomonas* | *Ralstonia* | *Agrobacterium* |
| 57. | In 1888, a dutch microbiologist Beijerinck succeeded in isolating which one of the following bacterial strain from root nodules | Bradyrhizobium japonicum | Rhizobium leguminosarum | Sinorhizobium meliloti | Azolla | Rhizobium leguminosarum |
| 58. | Monotropoid mycorrhizae are most commonly found in _____ forest | Boreal | Evergreen | Coniferous | Tidal | Coniferous |
| 59. | The life cycle of _____ mycorrhizae go through a period of time where they are not photosynthetic | Ardutoid | Orchid | Monotropoid | Ericoid | Orchid |
| 60. | The advantage of plants in this mycorrhizae association is _____ | Food | Protection | Increased mineral absorption and disease protection | water | Increased mineral absorption and disease protection |

**SYLLABUS**

**UNIT – V**

## Secondary Agriculture Biotechnology

Biotech feed, Silage, Biomanure, biogas, biofuels – advantages and processing parameters, Advantages, social and environmental aspects, Bt crops, golden rice, transgenic animals.

### Biotech feed

Biotechnology is used to improve live stock production by improving the live stock feed via improving nutrients content and also improved the digestibility of low quality animal feed by using efficient food additives. Biotechnology techniques like genetic engineering are used to produce genetically modified feed ingredients in order to improve nutritional quality and production capability of animal feed. Biotechnolgy is also used to improve the nutritional value of animal feed such as low protein, amino acid or deficiency of certain minerals by adding the efficient feed additives. Nutritional improvement can be done mainly in two ways first by value added feed stuff or by feed additives.

### Value added feed stuff:

### Low Phytate Corn:

Natural phosphorus which is present in plant feed are mostly present in the form of phytate phosphorus, this form of phosphorus cannot be utilized by the live stock, using biotechnology techniques low phyatate phosphorus and high available natural phosphorus containing corn are produced. This type of genetically modified corn also contained more amount of crude protein and also high percentage of crude fat when compared to normal corn. Feeding this genetically modified corn on broiler chicken improved body weight, feed conversion, better feed to egg ratio and decreased abdominal fat content and also linolenic and linolic acid content are increased in the egg yolk.

**Low Oligosaccharide soybean:**

The oligosaccharide Raffinose and stachyose present in the soybean act as antinutritive factor. Genetically modified soybean with concentration of oligosaccharide raffinose and stachyose produced using genetic engineering technique, improved amino acid digestibility and also increased dry matter digestibility.

**Soybean with High Lysine:**

Genetically modified soybean with improved content of lysine, decreased the need of supplemental addition of lysine in animal feed or diet.

Genetically modified crops with improved amino acid profile:

Crops are genetically modified in such a way that the amino acid production is improved in these crops. Using these genetically modified crops as feed decreased the amount of nitrogen excretion in poultry.

**Feed Additive:**

Adding specific and efficient additives to the animal feed drastically improves the digestibility of animals and hence reduces feed cost.

**Enzymes:**

Enzyme are biological catalysts, they help in digesting the feed and also improves the availability of nutrition from feed stuff. Microbial phytase enzyme produced using biotechnology is used as feed additives as these enzymes help in digesting phytic acid, which are present in cereals and oil seeds. Thiss providesigestible phosphorus to the animal stock. This reduces the use of supplemental inorganic phosphorus like dicalcium phosphate in animal feed, and also reduced feed cost.

**Dietary amino acid:** Genetically modified microorganisms are used to produce amino acid in large quantities like tryptophan, threonine and other amino acids. Using all these amino acids as feed additive reduces the required dietary crude protein level in the animal feed by 5% hence reduces the feed cost.

**Advantages:**

1. Developing genetically modified crops with improved nutritional value, decreases the antinutritive factors such as trypsin inhibitor, high fiber content and also limitation of phosphorus content during feed formulation.
2. Less phosphorus content is excreted from the live stock hence this would help in controlling eutrophication.
3. Increases digestibility of low quality animal feed by adding additives to the feed.
4. Drastically reduces the cost of animal feed.
5. Decreases animal waste.

**Silage**

Silage is also made as a chopped, fermented feed source, primarily from annual crops like corn, barley, sorghum, oats, millet, and occasionally canola and wheat. Silage is made by packing the chopped crop into a "pit" and packing it down well so that any oxygen pockets are eliminated. Oxygen pockets encourage spoilage of the feed. Silage and haylage can be interchangeable, especially since haylage or baleage involves the same process of ensiling to preserve feed for livestock. However, silage more relates to annual crops than perennial forages.

Silage is fermented, high-moisture stored fodder which can be fed to cattle, sheep and other such ruminants (cud-chewing animals) or used as a biofuel feedstock for anaerobic digesters. It is

necessary to adopt this method by Indian dairy farmers on large scale in situations like drought or heavy rainfall or scarcity of fodder. Silage making means preservation of chaffed cereal green fodder in anaerobic condition by way of fermentation method. Silage is made either by placing cut green vegetation in a silo or pit, by piling it in a large heap and compressing it down so as to leave as little oxygen as possible and then covering it with a plastic sheet, or by wrapping large round bales tightly in plastic film. Silage can be made from many field crops, and special terms may be used depending on type; oatlage for oats, haylage for alfalfa. Ensilage can be substituted for root crops. Bulk silage is commonly fed to dairy cattle, while baled silage tends to be used for beef cattle, sheep and horses.

## Essential fodder crops for silage making

To prepare best quality silage, cereal green fodder like Green fodder maize, Fodder sorghum, Bajara, Hybrid Napier, Sugar cane tops, Oat, Marwel etc are required. Preference for cereal green fodder (monocotyledons) is due to it has more sugar content than protein, as sugar is utilised in fermentation process to make lactic acid by microorganisms. These cereal fodder crops have hard stem, which takes more time for drying in making hay of these crops, so it is better to use these kinds of crops for making silage than hay.

## Benefits of silage making

There are numerous benefits of having silage as an animal feed. Few of them are as follows:

- Silage is storage system of green fodder which keeps all parts of fodder in appropriate condition for feeding than any other system of storage of fodder.
- For daily cutting, transporting & chaffing of fodder in traditional way requires more labour &time but in case of silage, fodder cutting, transport, chaffing is done at one time only, so it is less labour & time consuming practice. Land under fodder cultivation is emptied, and immediately it is used for plantation of other crops. So farmers' can take more crops in same land in a year against traditional way where land is reserved for fodder until all crops is harvested.
- Silage is prepared in closed & air tight condition so there is no danger of fire (In hay making, dry fodder is stocked & exposed for fire like situation).
- Silage requires less space for storage as it is pressed in pit/tank than hay making.
- Due to lactic acid in silage, it is easily digestible to animals, so energy required for digestion is used for other purposes like milk production etc.
- Important thing behind to adopt silage is in scarcity it provide supply of fodder to dairy animals.

Situations like drought, high rainfall & scarcity of fodder, farmers may use silage for feeding to dairy

animals. (Rain fed area where shortage of green fodder is for March to June & in high rainy area or water logged lands, it is impossible to cultivate or harvest fodder)

- Silage is tasty & flavoured, so it increases appetite of dairy animals.
- Due to treatment of additive for silage, farmers can supply energy, mineral & vitamins to dairy animals.

## Silage preparation

Silage is produced through use of pits or trenches, towers and sacks for small quantities. However, pits are mostly used to prepare silage for large dairy units. The silage pit should be located at a place safe from rodents, away from direct sunlight and with higher elevation or slightly sloppy to avoid rain water entering into the facility. The ideal materials used in silage making should have a moisture content of 60 to 70 per cent or dry matter in the range of 30 to 35 per cent (tested by taking a small bundle of the fodder and wringing with two hands and if no moisture comes out, it is ready to ensile) and a pH below 4.2 for wet forage and below 4.8 for wilted forage. In rainy periods when the fodder is too wet, containing more than 70 per cent water, it is advisable to wilt it in the sun first. Crops such as maize, sorghum, oats, pearl millet, and napier grass are very suitable for ensiling (preserve green fodder). They contain fermentable carbohydrates (sugar) necessary for bacteria to produce sufficient organic acid that acts as a preservative. Though leguminous fodders can also be used, they are rich in proteins and low in sugars making them a bit difficult to ensile. Harvesting maize or sorghum for making silage is ideal when their seeds are soft but not milky when squeezed open. Napier grass, on the other hand, needs to be about a metre high while legumes should have young pods, which are not dry. Apart from molasses, other additives like common salt, formic acid, lime or urea can also be used to enable good fermentation process.

- To start, prepare the pit and then place a big polythene sheet on the floor and walls.
- Cover about a metre of walls so that the forage does not come into contact with soil.
- Chop the fresh forage to lengths of about one inch using a chaff cutter.

- Prepare the first layer by emptying the chopped materials into the plastic lined pit to approximately 15cm high, and spread evenly.
- Then dilute molasses with water at a ratio of about 1:2 and sprinkle evenly over the forage layer using a garden water sprayer.
- Compact the layer by trampling on it using clean boots to force out as much air as possible. This will prevent fungi growth and spoilage. Repeat this process of adding bags of chopped forage, diluted molasses while compacting to expel maximum air out of the material until the pit gets filled in a doom shape.
- After the final filling and compacting, wrap the polythene sheet around the silage and cover the top of the heap with a second sheet to prevent water from running into the silage.
- Finally cover the heap with a thick layer of soil of at least 2ft giving special attention to the edges first as you come towards the middle to keep the air out and to prevent damage of the polythene by rain, birds and rodents. With good sheeting and enough soil on it, the silage can be kept for more than one year.
- It takes about 30 to 40 days for the silage to mature and be ready for feeding. Never open the whole silage pit at once.
- Only one end of the narrow side should be opened a bit. Remove enough material for each day's feeding and cover again. This way air is prevented from entering the silage.
- However, once the pit is opened, use the silage as quickly as possible.

## Storing Silage

Silage must be firmly packed to minimize the oxygen content, or it will spoil. Silage goes through four major stages in a silo:

- Presealing, which, after the first few days after filling a silo, enables some respiration and some dry matter (DM) loss, but stops

- Fermentation, which occurs over a few weeks; pH drops; there is more DM loss, but hemicellulose is broken down; aerobic respiration stops
- Infiltration, which enables some oxygen infiltration, allowing for limited microbial respiration; available carbohydrates (CHOs) are lost as heat and gas
- Emptying, which exposes surface, causing additional loss; rate of loss increases.

## Feeding cows with silage

A dairy cow is fed depending on the body weight or generally be given about 6kg to 15kg of silage per day. It is advisable not to feed silage immediately before or during milking especially when the quality is poor as the milk can easily take the smell of the feeds. During these times, a cow can be fed fresh grass, hay, legumes and concentrates. After feeding silage, the bunks and corners of the feeding troughs should be cleaned immediately to prevent contamination.

## Safety measures

Silos are potentially hazardous. Deaths may occur in the process of filling and maintaining them, and several safety precautions are necessary. There is a risk of injury by machinery or from falls. When a silo is filled, fine dust particles in the air can become explosive because of their large aggregate surface area. Also, fermentation presents respiratory hazards. The ensiling process produces "silo gas" during the early stages of the fermentation process. Silage gas contains nitric oxide (NO), which will react with oxygen ($O_2$) in the air to form nitrogen dioxide ($NO_2$), which is toxic. Lack of oxygen inside the silo can cause asphyxiation. Molds that grow when air reaches cured silage can cause organic dust toxic syndrome. Collapsing silage from large bunker silos has caused deaths. Silage itself poses no special danger.

**Definitions**

Silage has been defined in various ways, but all the definitions have a common element. For example, silage is forage preserved in succulent conditions by partial fermentation in a tight container (Martin *et al.*, 1976). Walton (1983) says that it is feed preserved by acid-producing action of fermentation. Cullison and Lowry (1987)indicate that **silage** is a feed resulting from the storage and fermentation of green or wet crops under anaerobic conditions. This is the definition we will use because it incorporates all important elements from the other definitions. **Haylage** is a silage product made from forage grasses and legumes containing 40 to 60% moisture (Walton, 1983). Elsewhere its definition is given as a product resulting from ensiling forage with about 45% moisture in the absence of oxygen (Heath *et al.*, 1973). For the purposes of this chapter, haylage is defined as silage made from forage crops—grasses (such as orchardgrass, smooth bromegrass, etc.) or legumes (such as red clover, alfalfa, etc). We do this without placing a moisture content restriction on forage because the making of quality silagerequires that the material to be ensiled have a specific range of moisture. This is discussed later in the chapter. **Fodder** is course grasses such as corn, sorghum, and pearl millet harvested whole (with grain intact), cured in the upright position in the field, and used for animal feed.

**SILAGE**

Silage is also called **ensilage**, forage plants such as corn (maize), legumes, and grasses that have been chopped and stored in tower silos, pits, or trenches for use as animal feed. Since protein content decreases and fibre content increases as the crop matures, forage, like hay, should be harvested in early maturity. The green material should be chopped fine enough to assure good packing and the exclusion of air from the mass of chopped material. A high moisture content in the ensiled material facilitates compaction and the exclusion of air. However, excess moisture (above 70 percent) seeps away and carries valuable nutrients with it. Excess moisture in the silo may also interfere with the fermentation processes that produce the best quality silage. Under proper storage conditions the silage ferments slightly and keeps for several months.

**Quality silage making process**


**Plan ahead:** You will need to know when the right time to start silaging so that you get the crop cut at the right stage for the best feed quality possible.

Timing is crucial to get the crop cut at the right stage, harvest soon after, and have someone packing the pit as the loads are coming in. The pit will need to also be covered as soon as possible to avoid losses with spoilage.

You must have the right equipment and enough silage plastic available ahead of time so that you are not scrambling and trying to beat a fast-closing window of opportunity to get your crop in.

If you have not yet found a site for and installed a concrete bunker, or dug into the ground an open three-sided pit designed for storing silage, you will need to have this arranged and completed well in advance of silage-making season. Or, if you do not have a bunker or pit dug out and prepared for proper ensiling, you need to find a place where you can create a silage pile that is well-drained and easily accessible during times when you need to access it without much trouble.

**Assess the crop:** For most cereal crops, the best time to cut is when they are at the soft-dough stage. The majority of the plant should still be green, but with a bit of yellowish tinge, especially on the heads of the plant.

- To test crop stage, squeeze a random kernel between your fingers to see how soft it is. At the soft-dough stage you should get a white, soft paste-like substance coming out from the seeds. If it's more liquid than paste, the crop isn't quite ready yet, but getting very close.

- Corn will be at the same stage when it is ready to be harvested for silage. However, to test if corn is ready, take an ear of corn, tear off the husks and break the cob in half. An old rule of thumb is to look for the "milk line" (the line made where the solid and liquid parts of the kernels divide, and tends to progress from the outer edge of the kernel in towards the cob). This milk line should be half to two-thirds of the way in to the cob (the kernels are 2/3 yellow and 1/3 white, for example).
- Weeds are a bit of a non-issue with a silage crop. It's being made into feed, not being sold for grain, and the animals aren't going to judge if they find a tiny bit of wild buckwheat in with the rest of the feed.

**Cut the crop into swaths.** Unlike with making haylage, the best machine to use to cut a crop with is a swather, not a hay mower. A windrower may be all right, but when cutting a thicker and taller crop like barley or oats, a swather is built for the heavier tonnage you will be getting off cropland than you would a perennial forage stand, usually. Also, a swather will not shred seeds off of the crop like you may find happen more often with a windrower.

- It will be a different story with corn and sorghum, or sorghum-sudan grass. This step will not be needed for this type of crop because the swaths will be to large and difficult for a forage harvester to get through. Instead, these crops will be straight-cut, with a header that is suited for large-stemmed crops like corn. Straight-cutting small-cereals like barley and oats for silage is not an issue and an option to consider. With swathing, though, it actually allows the crop to dry down a bit more than if left standing, allowing you to harvest it at a lower moisture than what you would get if harvesting it as a standing crop.
- Straight-cutting small-cereals like barley and oats for silage is not an issue and an option to consider. With swathing, though, it actually allows the crop to dry down a bit more than if left standing, allowing you to harvest it at a lower moisture than what you would get if harvesting it as a standing crop.
- Silage should be put up at around 60 to 70% moisture for best preservation activity. A higher moisture silage will be more prone to seepage or freezing, making things difficult for transport. Nutrients are also lost with the seepage, particularly nitrogen that has been broken down by microbes in the

silage.

Lower moisture may not guarantee the best fermentation activity, particularly if silage is put up at less than 40 to 45% moisture.

**Allow the swaths to wilt down for about half a day before harvesting.** The forage will need to be dried down to about 60 to 70% moisture before chopping for silage.

- Silage can be put up at higher moisture, but as mentioned above seepage will be an issue. Also, the low temperature fermentation activity can provide a suitable environment for undesirable clostridial bacteria that are prone to cause maladies like listeriosis and botulism.

**Harvest the crop.** Machines called "forage harvesters" like the one in the photo above (which is a "self-propelled" harvester) are used to chop up the swathed forage and feed it out through a long, tall spout that can literally "spit" out the feed at quite a distance.

- The forage harvester's cutter blades will need to be set at the right setting so that the forage is cut at the right chop-length. For small grains, set the blades so that they are cutting up forage between ⅜ inch (0.95 cm) and ½ inch (1.3 cm). Larger crops like corn and sorghum-sudan should be chopped at lengths from ½ inch (1.3 cm) to ¾ inch (1.9 cm).
- Since the forage harvester does not have a storage compartment on it like combine harvesters do, a truck with a silage unit on it, a tractor with a silage wagon, or a large unit designed for collecting silage from the forage harvester–called a "Jiffy wagon"–needs to be used to collect the freshly cut forage.
- The Jiffy wagon, for example, acts as the storage compartment for the forage harvester. Once full, it can be dumped into a truck as shown in the sequence of photos here.

**Take the freshly chopped forage to the pile or pit.** Once the truck or silage wagon is full, the unit will need to be taken to the designated pit or pile area to drop off the load. Make sure the loads are placed as close to each other as possible. When first starting the pile, the first several loads must be place where the pile is going to be. After that they are placed close to the built pile, and dumped in a way that is easy for the person in the "packing unit" to move into a pile; i.e., parallel to the pile, and/or in the same direction the pile will be built up as.

- An exchange is made between the wagons and/or trucks so that the person operating the forage harvester doesn't need to stop and wait every so often. Once the first truck is full, the harvester stops briefly so that the truck can pull away and the second one moves into position. The first truck returns after dropping its load off to get another load, and so the process repeats.

**Pack the silage well**

The silage pile must be packed very well, and should be packed down during and after each harvesting day. In a large operation where several people are employed, it would be beneficial to have one (a brave one that is not afraid of heights especially) stay behind to operate another tractor or large loader that will continuously gather and pack the pile well. Tractors with dual wheels are recommended to provide the best packing power possible.

- Packing is what helps encourage fermentation activity and discourages spoilage. The more the pile is packed down, the less pockets of oxygen there are. Oxygen pockets create spoiled feed; aerobic-loving bacteria turn it into a brown to black slimy mess, that often smells like tobacco or burnt caramel. In other words, instead of fermenting the feed (which is producing a significant amount of acid as a means to preserve the feed), the presence of oxygen decomposes it into a substance equivalent to manure. You don't want feed that is messy and gross like manure (think cow poop). If you don't like the look, feel and smell of it, neither will your animals!
- Silage piles must be longer and wider than they are tall. The higher the pile is built up, the wider the edges will need to be. A concrete bunker will control how wide you can make the pile, though you can pack several feet above, but only so much that the sides are not over-flowing.
- A rule of thumb for pile-size is larger at the base than the top; no less than 12 to 15 feet (3.7 to 4.6 m) wide at the top to prevent roll-overs or slippage from machinery; and silage piles should only be 12 to 15 feet (3.7 to 4.6 m) tall, mainly for farm safety reasons[2].

- The best way to tell if you have done a good packing job is when you try to sink your fingers into the pile. If you only get in so far as your second knuckles of your first three fingers, then the pile has been packed very well, and has potential for being good feed in the winter with minimal spoilage.

**Packaging**

Use the proper plastic recommended for covering silage. Often recommended and used is polyethylene plastic that may be black on both sides or white on one side and black on the other. The cheaper stuff is all black, but the better quality is the black and white plastic.

- Use 6 to 10 milliliters (0.34 fl oz) plastic. This can be found at your local farm and ranch supply store. The heavier the plastic, the more effective it is at keeping oxygen out of the pile and reducing wastage with spoiling.
- The rolls are very heavy. Use a tractor loader with bucket teeth to carry the plastic to the pit so that you can unroll and unfold it.
- A trick to use is to insert a 6 feet (1.8 m) long, heavy iron bar into the roll (like you would hanging a roll of toilet paper on a toilet-paper holder), and fashion thick wire or heavy chain that hangs on the teeth of the bucket. Hang the bar onto this.
- Important: White and black plastic must be used so that the white side is facing out, and the black against the fresh silage in the pit. The white side reflects sunlight and reduces excess heating from the sun, whereas the black side keeps heat inside.
- Trim off extra plastic and use that to cover the edges and sides that the plastic has not covered.

**Weigh the plastic down well**

Use numerous old or recycled tires all over the top part of the pile. Hay bales can also be used to hold down plastic on the sides if the silage pile is not in a bunker.

- Old tires are much more gentler on the plastic because they do not cause punctures. Punctures are a serious danger of feed spoilage.

- All sides and all parts of the pile must be covered and held down well so to ensure the pile properly ensiles and spoilage is minimized.

**Quality baled silage**

**Silage making process**


**Repair any holes immediately.** Holes in the plastic can cause massive spoilage problems over time.

- Spoilage will not be localized, especially if the holes go from a tiny tear to a big rip, especially if wind is a problem.

**Biomanure**

**Bio-compost / Bio-manure**

Soil fertility is seriously impaired with the excessive use of chemical fertilizers. Research conducted to study the fall in grain production indicates that the soil is getting drained of organic carbon because of over use of fertilizers, thus effecting soil fertility.

The crop removes large quantity of plant nutrients from soil, particularly the removal of NPK nutrients at the present level of crop production has been estimated at 125 kg/ha/annum whereas the annual addition is not more than 75 kg resulting in depletion of the nutrient reserve of soil. The excessive reliance on chemical fertilizers and the negligence shown to the conservation and use of organic sources of

nutrients have not only caused the exhaustion of soil of its nutrient reserves but also resulted in soil health problems not conducive to achieving consistent increase in agricultural production. Moreover, Indian soils

are poor in organic matter and in major plant nutrients. Soil organic matter is the key to soil fertility and productivity. In the absence of organic matter, the soil is a mixture of sand, silt and clay. Organic matter induces life into this inert mixture and promotes biological activities. Although the beneficial influence of organic matter on the physical, chemical and biological properties of the soil is widely known, the full appreciation of the same remains largely ignored in modern agriculture. The regular recycling of organic wastes in the soil is the most efficient method of maintaining optimum levels of soil organic matter. Recycling of organic matter in the soil should become a regular feature of modern agriculture. In the traditional agriculture, followed over generations in India, the use of plant and animal wastes as a source of plant nutrient was the accepted practice. The importance and aim of organic manures and green manure crops have failed to be recognized in modern agriculture.

**Composting Process**

- Composting is the biological decomposition of ligno-celluloid organic material into a simple compound, a humus-like end-product called -compost‖. It is a rich source of organic manure. It also helps significantly improve the quality of the soil.
- The process is aerobic which uses various micro-organisms such as bacteria, actinomycetes and fungi to break down the higher organic compounds, like cellulose and lignin's, into simpler substances.
- During composting, the micro-organisms consume oxygen while feeding on organic matter, and multiply. Active composting generates a considerable amount of heat. It also discharges large quantities of carbon dioxide and water vapor into the atmosphere.
- The loss of carbon-dioxide and water vapor reduces the weight of the initial dry organic matter. Thus composting reduces both the volume and the mass of the organic matter.

**Process of Bio manure Formation**

- Press mud is stored in triangular shaped rows known as windrows.

- Spent wash is sprayed on each windrow at specific intervals. The windrows are then turned.

- This helps in homogenizing the entire mass, maintaining uniform temperature and moisture with effective aeration and oxygen supply.
- During the composting process, the temperature goes up to 650-700 C
- Due to the churning of the mixture of press mud and spent wash by aero tillers, oxygen is supplied to bacteria, thereby accelerating the composting process. It also dissipates the excess heat generated.
- Bacteria separate carbon and other complex compounds from press mud and spent wash.
- Enhancing the effectiveness of this process requires 50-60% moisture.

**Composting Cycle**

It takes typically 60 days to complete the composting cycle. During the first five days windrow dressing, moisture reduction and inoculation are completed. From the sixth to the 50th day, the temperature and moisture levels are maintained by spraying spent wash. From the 51st to the 60th day, moisture reduces curing and aging, and the stabilization process gets under way. This again reduces the moisture level.

**Specifications of bio compost**

- Moisture Content : 30%-40%
- Nitrogen : 1.8%-2.5%
- Potassium : 1.5%-2.0%
- Phosphorus : 2.0%-2.6%
- Calcium : 3.0%-4.0%
- Sulphur : 1.0%-1.5%
- Magnesium : 0.8%-1.5%
- Iron : 0.04%-0.06%
- Zinc : 0.025%-0.035%
- Organic carbon : 24%
- Organic Matter : 42%-50%

- Others : 2.0%-2.5%(micro nutrients etc )
- pH : 7.0-7.4
- C: N Ratio : Less than 18

**Unique benefits of bio compost**

- Improves the physical, chemical and biological properties of the soil.
- Improves the soil structure, air circulation and water retention capacity of the soil.
- Retains nutrients and prevents them from leaching away the plant roots.
- Contains both the micro-nutrients (calcium, sulphur, magnesium, iron, etc.) and macro-nutrients (nitrogen, phosphorus, and potash) essential for plant growth.
- Microbes accelerate the breakdown of crop residues in the soil. This improves the texture of the soil and also releases the locked-up elements.
- Increase the yield and quality of field, vegetable, tree and fruit crops.
- Can easily be applied as both base-dressing and top-dressing.
- Improves alkaline and saline/sodic soil.
- Helps the growth of the roots by improving the soil structure.
- Ultimately, it betters the crop yield by improving soil fertility and soil structure.
- Useful for all types of field crops, vegetable crops, orchards, kitchen gardens and flowers.

**EFFECT OF INORGANIC FERTILIZERS AND OTHER AGRO-CHEMICALS ON SOIL AND PLANTS**

Excessive use of chemical fertilizers and other agro chemicals, which are the important inputs in modern farming creates depletion in soil fertility and pollution in surface water bodies. 1. Water soluble fertilizers when applied to soil, a good portion of the added nutrients does not become available to the crop plants and lost either to the atmosphere up to the hydrosphere due to non stimulation of the activities of heterotrophic soil organisms but facilitate that of the autotrophic nitrifying organisms, thereby hindering the immobilization of nutrients. 2. As a matter of fact, it results in rapid rate of nutrients loss in different forms

and increases the soil acidity with nitrification. 3. Emission of ammonia, methane, nitrous oxide and elemental nitrogen from the soil system as a result of denitrification. 4. Depletion of secondary and micronutrients especially Sulphur and Zinc. 5. Deficiency of these nutrients (S & Zn) along with that of Mg, Mn, Fe, Mo, B and Cu limits productivity of many field crops especially in rice. 6. Dhar (1962) cautioned that by adding large doses of N-fertilizers in modern agriculture without the use of organic manures, there is always the danger of humus depletion and fall in crop production, which can be avoided only by adding additional amounts of organic residues and manures. 7. When high levels of N-fertilizers especially nitrate forms are applied to soil, nitrate pollution of drinking water is a serious health hazard found in extensively irrigated coarse textured highly percolating soils of central Punjab, where 40-50% of applied nitrogen is lost in leaching and the mean concentrations of nitrate nitrogen was 3.88 ppm during 1982 (Rainy season) ans

1.02 ppm in 1975. In 10% of the ground water samples nitrate concentration was 10 ppm which was the upper tolerance limit in drinking water against nil in 1975 (Singh et al, 1987). 6 8. Alarming issue to human health is regular use of phosphatic fertilizer in large quantities often causes the build up of trace metal contamination such as arsenic, fluoride, cadmium etc. in soil and plants. Cadmium in single super phosphate is available to plants as the Cd in cadmium chloride. Similarly, chloride contained in MOP and NH4Cl creates toxicity to many crops like beans, citrus, grapes lettuce, potatoes etc. These trace metal toxic contaminants reach the human body, through food chain and cause health problems. 9. The water soluble nutrients when carried to lakes and stream through leaching and surface run off cause eutrophication as manifested by the luxurient growth of algae and other water weeds on the water surface leading to oxygen deficient condition. This situation is not conducive to healthy aquatic life.

**ADVANTAGES OF ORGANIC MANURES**

1. Organic manure provides all the nutrients that are required by plants but in limited quantities. 2. It helps in maintaining C:N ratio in the soil and also increases the fertility and productivity of the soil. 3. It improves the physical, chemical and biological properties of the soil. 4. It improves both the structure and texture of the soils. 5. It increases the water holding capacity of the soil. 6. Due to increase in the biological activity,

the nutrients that are in the lower depths are made available to the plants. 7. It acts as much, thereby minimizing the evaporation losses of moisture .from the soil. MAJOR ORGANIC SOURCES AND TRANSFORMATIONS Carbon present in soil is in the form of organic matter. The organic materials most commonly used to improve soil conditions and fertility include farm yard manure (FYM), animal wastes, crop residues, urban organic wastes (either as such or composted), green manures, bio-gas spent slurry, microbial preparations, vermicompost and biodynamic preparations. Sewage sludge and some of the industrial wastes also find application in agriculture. For all organic matter, atmospheric carbon dioxide serves as the main source of carbon. Carbon dioxide is converted to organic carbon largely by the action of photoautotrophic organisms; the higher green plants on land and algae in aquatic habitats. Carbon is being contentiously fixed into organic form through the process of photosynthesis and once bound; the carbon becomes unavailable for use in the generation of new plant life. Carbon fixation involves a reduction of carbon dioxide by hydrogen donor NADPH (reduced form of the co-enzyme nicotinamide adenine dinucleotide phosphate, NADP) and the synthesis of carbohydrate from reduced carbon through complex cyclic mechanism called the 7 Calvin cycle. Carbon dioxide constitutes only 0.03 percent by volume of the earths atmosphere. It has been estimated that the vegetation of the earth's surface consumes some 90 billion kg carbon dioxide per annum, about one twenty - fifth of the total supply of the atmosphere and that the total supply of carbon dioxide would be completely exhausted in twenty years at the present rate of photosynthesis , if not replenished by decomposition of organic materials. As the availability of carbon dioxide on the earth's surface is very limited, it must be recycled. Upon the death of the plants and animals, microbiological metabolism assumes the dominant role in cyclic sequence. The dead tissues added to soil undergo decay and are transformed into microbial cells and a vast heterogeneous body of carbonaceous compounds. According to the different stages of decomposition, the soil organic matter becomes available in distinct fractions. Farm yard manure made from cattle dung, excreta of other animals, animal tissues and excretory products, and compost from rural and urban wastes, crop residues and green-manure are collectively designated as bulky organic manures because of their low contents of major nutrients, while materials like oil cakes, fish meal, animal meal, poultry manures, slaughter house wastes containing

comparatively higher contents of plant nutrients are grouped under concentrated organic manures. In general organic manures containing upto two percent nitrogen are included in bulky category and those with more than two percent nitrogen are treated as concentrated. Irrespective of source and composition, organic matter when added into the soil undergoes microbial decay and becomes the food for micro flora and fauna. Even the microbial cells serve as a source of carbon for succeeding generations of microscopic populations. A great variety of microorganisms live in soil which include bacteria, actinomycetes, fungi, algae and protozoa. In general the number per gram of soil is bacteria > actinomycetes > fungi > algae > protozoa.

**HUMIFICATION AND RESIDUAL EFFECT**

All organic residues incorporated into the soil undergo decomposition from the original residues, a series of products are formed. As the original material and the initial products undergo further decomposition, they become a brown black organic complex known by the name humus. Humus remain in dynamic yet fairly stable state. It is under continual attack by soil microorganisms. Decomposition and synthesis by microbial processes occur simultaneously, the rate depending on the nature and abundance of microorganisms involved, the moisture content, temperature, pH, aeration, quantity of freshly added organic matter and the extent of availability of carbon, nitrogen, phosphorus and potassium. Chemically humus has been characterized as ligno-protein or ligno-acid complex containing approximately 45 percent lignin compounds, 35 percent amino acids, 11 percent carbohydrates (4 percent cellulose, 7 percent hemicelluloses) 3 percent fats, waxes and resins and 6 percent other miscellaneous substances. (Finch et al., 1971). Martin et al. (1974) have shown that some fungal mycelium and lignin, and lignin like substances of plant origin are the main building blocks for synthesis of humic substances. The humus is a finally synthesized stable product in natural or agricultural conditions. During humification the nitrogen compounds react in most cases as organic amino compounds and to a lesser extent, as ammonia. Some losses of nitrogen occur in gaseous form as nitrogen or nitrous oxide. Sulphur is bound in the orginal organic compounds as sulphide very rarely as sulphate and transformed in the soil to sulphate, Phosphorus occurs in the organic compounds as

phosphate esters, which are hydrolyzed. Potassium, calcium, magnesium and the heavy metals present in plant residues as complexes are liberated during humification into ions. Very often they form other complexes with newly formed organic compounds during humifiction and interact with the soil colloids (salycilic acids, sesquio-oxide and clay) depending on soil properties and environment.The mechanism leading to an increase in the nitrogen content in lignin residue of plants has not been clearly explained. It has been clarified to certain extent that amino acid, protein and amino sugar nitrogen and the products are stabilised against microbial decomposition by their entry during polymerization of humic materials (Verma et al., 1975). The nitrogenous substances formed in this way, serve as slow release nitrogen source for plant growth and have a residual effect because they are only gradually subjected to microbial attack. The isotopic labeled studies have shown that there is a loss of 70, 80 and 90-92 percent of incorporated organic matter into soil after 1, 2 and 8 - 10 years respectively.

**GREEN MANURES** Green manuring can be defined as a practice of ploughing or turning into the soil undecomposed green plant tissues for improving physical structure as well as soil fertility. Green manuring, wherever feasible, is the principal supplementary means of adding organic matter to the soil. The green- manure crop supplies organic matter as well as additional nitrogen, particularly if it is a legume crop, due to its ability to fix nitrogen from the air with the help of its root nodule bacteria. The green-manure crops also exercise a protective action against erosion and leaching. Green manure to be incorporated in soil before flowering stage because they are grown for their green leafy material, which is high in nutrients and protects the soil. Green manures will not break down in to the soil so quickly, but gradually, add some nutrients to the soil for the next crop. The nutritional potentials and nutritional contents of some important green manures are given in the Table 5 and 6 respectively.

**ADVANTAGES OF GREEN MANURES:** Usage of green leaf manure is advantageous both for crops and soil. The advantages are: 1. As they decompose rapidly, it is easy to retain the organic matter in the soil. 2.

Green manures improve both physical and chemical properties of the soil. 3. They provide energy to microbes. 4. They provide nutrients to the standing crop and also to the next crop. 5. Addition of green manure crops to the soil, acts as much and prevent soil erosion. 6. Leaching of nutrients in light soils can be prevented by addition of green manure. 7. Cultivating green manure crops can control weeds. 8. Majority of green manure crops being legumes, use of nitrogenous fertilizers can be minimized. There are different green leaf manure crops that can be cultivated and they are:

1. **COWPEA :** Cowpea is one of the important leguminous green leaf manure crops. As this plant is easily decomposable and very well suited for green manure purpose. June-July months are best suited for sowing of this manure. Even though it is being cultivated in summer months ( March to April). Use of effective Rhizobium bacteria increase the fixation of nitrogen up to 40 kg/ha.

2. **DHAINCHA (SESBANIA ACULEATE)** : Dhaincha is suitable for loamy and clayey soils. It is fairly resistant to drought as well as stagnation of water. It grows well even in alkaline soils and corrects alkalinity if grown repeatedly for 4-5 years. The roots have plenty of nodules. It yields about 10-15 tonnes of green manure per ha and requires a seed rate of 30-40 kg/ha. Use of effective Rhizobium strain with seeds fixes the Nitrogen 1 kg / day.

3. **SESBANIA SPECIOSA :** It is a valuable green manure for wetlands and can be grown in a wide range of soils. Seed production is prolific however, pods are frequently attacked by insects. This green manure can be raised on the field 15 borders. Sesbania seedling (21days) can be planted in a single line at 5-10 cm apart in the borders of the fields. In about 90 days it produces about 2-4 tonnes of green manure per ha. It does not affect the rice yield by shading or root effect. If second rice crop is planted immediately after the first crop, the manure can be incorporated into the field. About 300-400g of seeds are sufficient to raise nursery and plant the seedlings around the boundary of one hectare. To control insects Verticillium lacanii ( Liquid ) fungi is useful.

4. **SUNNHEMP (CROTALARIA JUNCEA)** : It is a quick growing green manure crop and gets ready for incorporation in about 45 days after sowing. It does not withstand heavy irrigation leading to flooding. The

crop is at times subject to complete damage by leaf eating caterpillars. The crop can produce about 8-12 tonnes of green biomass per ha. The seed requirement is 30 kg/ha.

**5. SESBANIA ROSTRATA** : One of the important features of this green manure is that in addition to the root nodules, it produces nodules in the stem. The stem nodulation is an adaptation for waterlogged situation since flooding limits growth of green manures and may reduce root nodulation. Under normal condition, both root and stem nodules are effective in N fixation. It has higher N content of 3.56% on dry weight basis. Biomass production is higher during summer (April – June) than in winter (Dec. – Jan.) season. This green manure can also be produced by raising seedlings (30 days old) and planted in the paddy field along the bunds or as intercrop with rice. Use of Rhizobium bacteria increase the nitrogen fixation about 60-100 kg/ha/year.

**6. WILD INDIGO (TEPHROSIA PURPUREA) :** This is a slow growing green manure crop and cattle do not prefer to graze it . The green manure is suitable for light textured soils, particularly in single crop wetlands. It establishes itself as a self sown crop and the seeds remain viable till the harvest of rice. On an average about 3-4 tonnes of green manure is obtained in one ha. The seed rate is 30 kg/ha. The seeds have a waxy impermeable seed coat and hence scarification is required to induce germination. Soaking seeds in boiling water for 2-3 minutes is also equally effective in promoting germination.

**7. INDIGO (INDIGOFERA TINCTORIA) :** It resembles wild indigo and is along duration crop with more leafy growth. It comes up well in clayey soils with one or two irrigations

**8.PILLIPESARA (PHESEOLUS TRILOBUS) :** This is a dual purpose crop yielding good fodder for the cattle and green manure. Pillipesara comes up well in hot season with sufficient soil moisture. Loamy or clayey soils are best suited. After taking one or two cuttings for fodder or light grazing by animals, the crop can be incorporated into the soil. About 5-8 tonnes of manure can be obtained from one ha. 16

**9. GLYRICIDIA (GLYRICIDIA MACULEATA)** : This is a shrubby plant that comes up well in moist situations. Under favourable conditions, it grows well like a tree. It can be easily grown in waste lands, farm road sides, field bunds, etc. The crop can be established by stem cuttings or seedlings planted in the field borders. It can be pruned for its tender loppings and compound leaves for green leaf manuring at the time

of puddling rice. On an average, a well established plant yields 12-15 kg green matter. About 400 plants on the peripheral bunds yields 5-6 tonnes green manure/ha.

**10.KARANJ (PONGAMIA GLABRA) :** It is a leguminous tree grown in wastelands. On an average, a tree can yield 100-120kg of green matter. The leaves contain about 3.7% N (on dry weight basis).

**11. CALATROPIS (CALOTROPIS GIGANTCA) :** On roadsides and fallow lands, the plant grows wild under different soil and climatic conditions. The leaves are more succulent and a plant can produce about 4-5 kg of green matter. Besides it also helps in controlling soil born pests like termite.

**Pile method**

The pile method is the easiest and most commonly used by farmers. • Select an area in your farm that is protected from strong wind and sun, for instance, under the shade of a tree. • Mark the area you intend to locate the compost (the minimum area is 1.25m x 1.25m). • Dig a shallow trench, same size as the compost heap 20cm deep. Smear the sides of the trench with water or a mixture of water and cow dung to prevent moisture and nutrients from leaking from the compost heap. The shallow trench will become the foundation of the compost heap. The trench also helps to hold moisture especially during the dry season. Foundation layer Put the dry plants material such as small tree branches, maize stalks or sorghum stalks. Cut the plant material into small pieces. Spread the dry material evenly over the bottom of the trench to make a layer of 15-25cm. Sprinkle with water using a watering can or basin to ensure all material is moist but not wet.

Layer 1: In this layer, put dry plant material such as grass, dry leaves mixed with top soil, manure and ashes. The layer should be about 20-25cm thick (as thick as the palm of your hand). Mix the material with soil, manure and ashes and sprinkle water to make it moist. Know if news is factual and true.

Layer 2: Make another layer of moist (green) material which is fresh or wilted such as weeds or grass cuttings, stems and vegetable leaves, tree branch leaves, damaged fruits, or vegetables or even kitchen waste. Do not sprinkle water in this layer. But you can spread it to remain even or flat.

Layer 3: This layer should be composed of animal manure collected from fresh or dried cow dung, chicken waste, donkey manure and sheep or goat droppings. The animal manure can be mixed with soil, old compost and some ashes to make a layer that is 5 -10 cm thick. If the manure is not adequate, make a watery mixture and spread it over as a thin layer about 1-2cm thick. Covering layer The finished heap has to be protected from the sun or animals or anything that might interrupt with the mix. The farmer can prepare wet mud mixed with grass or straw, or with wide pumpkin leaves, banana leaves or plastic polyethylene sheets. The cover should be sealed with only the ventilation stick (also called thermometer stick). Turning the compost After three weeks, you can open up the compost heap mixing all the layers while sprinkling water to make it moist but not wet. A little EM1 can be mixed with water to hasten the decomposing process. Check the decomposition progress Using the ventilation or temperature stick, you can keep on checking the decomposition process of your compost every week by pulling out the stick. If it has a white substance on it and has a bad smell, it means the decomposition is not going on well. You can turn the compost further and sprinkle some more water to make it moist. Check if compost is ready A mature compost heap is about the half the size of the original heap. Check to ensure the compost has a dark brown colour or black soil, which has a nice smell. All the original material should not be seen if the decomposition process went on well.

## Environmental Benefits of Manure Application

For centuries, animal manure has been recognized as a soil -builderll because of its contributions to improving soil quality. Environmental benefits are possible from manure application if manure and manure nutrients are applied and timing and placement follows best management practices. When compared to more conventional fertilizer, manure properly applied to land has the potential to provide environmental benefits including:

- Increased soil carbon and reduced atmospheric carbon levels
- Reduced soil erosion and runoff
- Reduced nitrate leaching

- Reduced energy demands for natural gas-intensive nitrogen(N) fertilizers

**Manure Effects on Soil Organic Matter**

Manure contains most elements required for plant growth including N, P, potassium, and micronutrients (Manure as a Source of Crop Nutrients and Soil Amendment). However, it is manure's organic carbon that provides its potential environmental value. Soil organic matter is considered nature's signature of a productive soil. Organic carbon from manure provides the energy source for the active, healthy soil microbial environment that both stabilizes nutrient sources and makes those nutrients available to crops.

Manure is comparable to commercial fertilizer as a plant food and, if applied according to a sound nutrient plan, has environmental benefits over commercial fertilizer.

The ability of manure to maintain or build soil organic matter levels has a direct impact on enhancing the amount of carbon sequestration in cropped soils.

Manure organic matter contributes to improved soil structure, resulting in improved water infiltration and greater water-holding capacity leading to decreased crop water stress, soil erosion, and increased nutrient retention. An extensive literature review of historical soil conservation experiment station data from 70 plot years at 7 locations around the United States suggested that manure produced substantial reductions in soil erosion (13%-77%) and runoff (1%-68%). Increased manure application rates produced greater reductions in soil erosion and runoff. Additional studies during years following manure application suggest a residual benefit of past manure application.

**Manure Effects on Soil Erosion**

Similarly to crop residue. Crop residue significantly decreases soil erosion by reducing raindrop impact which detaches soil particles and allows them to move offsite with water runoff. Data has been published showing how manure can coat the soil surface and reduce raindrop impact in the same way as crop residue. Therefore, in the short-term, surface manure applications have the ability to decrease soil erosion leading to a positive impact on environmental protection.

**Organic Nitrogen**

In addition, organic N (manure N tied to organic compounds) is more stable than N applied as commercial fertilizer. A significant fraction of manure N is stored in an organic form that is slowly released as soils warm and as crops require N. Commercial fertilizer N is applied as either nitrate or an ammonium (easily converted to nitrate). Nitrate-N is soluble in water and mobile. These forms contribute to leaching during excess precipitation (e.g., spring rains prior to or early in growing season) or irrigation. Manure N's slow transformation to nitrate is better timed to crop N needs, resulting in less leaching potential. In fact, manure N is a natural slow-release form of N.

**Energy Benefits**

Recycling of manure nutrients in a cropping system as opposed to manufacturing or mining of a new nutrient resource also provides energy benefits. Commercial nitrogen fertilizers consume significant energy as a feedstock and for processing resulting in greenhouse gas emissions. More… Anhydrous ammonia requires the equivalent of 3300 cubic feet of natural gas to supply the nitrogen requirements of an acre of corn (assuming 200 lb of N application). Phosphorus and potassium fertilizers also have energy requirements for mining and processing. Substituting manure for commercial fertilizers significantly reduces crop production energy costs

It is important to remember that the environmental benefits of manure outlined in this article are only beneficial when best management practices for reducing soil erosion are implemented in concert with proper levels of manure nutrient application and use.

## Factors Impacting Nutrient & Organic Matter Benefits

Manure composition varies with animal type, age, feed ration and the environment.

- **Ruminants** usually have forage based diets, while monogastrics (i.e. hogs) are fed grain-based rations. Beef and dairy manure contain undigested forages and often contains bedding materials. These materials are high in cellulose and lignin and take longer to decompose in soil when compared to the less complex sugars from undigested corn.

- **Animal stage / age** will influence the amount of feed consumed, but also dictate the protein and mineral content.
- **Ration** formulations vary. High production phases require more concentrated diets, including phosphorus, potassium and trace elements such as calcium. Regular and high application rates of manure will build up phosphorus and potassium levels, pH (in acidic soils). Micro-nutrients deficiencies, including zinc and sulphur, are rare with regular applications.
- The animal **environment** (housing) determines the amount and type of bedding. The **storage method** and additional materials or wastewater determine the manure dry matter content. The carbon-to-nitrogen (C:N) ratio of wood chips (200+) will be much higher than straw (50 - 80).
- The **C:N ratio** is the proportion of organic carbon to total nitrogen of manure or organic material. The nitrogen is a food source for the soil micro-organisms while they break down the carbon material. When that process is complete, the soil microbes die and decompose. The microbial nitrogen is then returned to the soil and becomes available to the plants. This is considered the "organic nitrogen" component. How long this process takes depends on the ratio of carbon to nitrogen in the material.
- Manure or organic material with C:N ratio under 20:1 is considered ideal for crop production. When there is not enough nitrogen in the organic material to break down the carbon, the micro-organisms utilize nitrogen from the soil. When C:N ratios are higher than 25 to 30:1, it could result in a nitrogen deficiency of a crop that relies on soil nitrogen, such as corn.

Manure will add organic matter but also adds nutrients. Over-application of nutrients could lead to crop damage or nutrient losses into the environment. In addition, any benefits from soil organic matter are easily negated if soil compaction results from application on unfit soils.

**Building Soil Organic Matter With Manure**

Applying manure to the soil will provide other benefits, such as a greater diversity and activity of organisms and better soil structure. Table 1, *Effects of 11 Years of Manure Additions on Organic Matter Levels*, shows the increase in soil organic matter (SOM) over time. This suggests that at an application rate of 20 tons / acre / year, the SOM level was maintained, while at lower rates and without other additions such as residue or cover crops, the organic matter level gradually decreased.

**Manure**

**Composition**

**Nitrogen in Manure**

Nitrogen in manure is found in the organic and inorganic forms. The organic form (slow release) slowly mineralizes providing plant-available N, while inorganic forms (fast release) consist primarily of NH4-N and are immediately plant available. However, inorganic forms are also susceptible to loss through ammonia volatilization during storage and field application. Promptly incorporating the manure into the soil can reduce these N losses. Due to the slow release organic form and potential losses of the inorganic form, not all of the N is available to the crops during the year of application. Nitrogen that is expected to be available to the plant has value as a fertilizer. The N which is lost to the environment or which is not available to the crop in the year it is needed or subsequent years does not have value. The guide -Fertilizer Nutrients in Animal Manure provides information on the amount of N expected to be available in the 1st year and subsequent years from various manure sources:

**Phosphorus and Potassium in Manure**

Phosphorus and Potassium in manure are mostly present in the inorganic form. This means that P and K are similar to commercial fertilizer in that they are readily available for plant uptake. Most nutrient management plans are based on a P-Index or P-threshold which may limit manure application on some fields. Therefore, the value of these nutrients is based on crop nutrient needs as determined by a soil test and yield goal.

**Micronutrients in Manure**

Other nutrients such as calcium (Ca), magnesium (Mg) and sulfur (S) may be found in manure and are beneficial to the soil if a deficiency exists. Both Ca and Mg create an added value by producing a liming effect when added to the soil.

**Organic Matter**

Organic matter, primarily undigested feed and bacteria in the feces, increases infiltration of water, increases water holding capacity, enhances retention of nutrients, reduces wind and water erosion and promotes the growth of beneficial organisms when added to the soil. Although the value of organic matter is hard to quantify, higher quality soils are associated with increased yields and higher economic returns.

**Manure As a Plant Fertilizer**

Because manure is not a balanced fertilizer, some plant nutrient needs may be met while other nutrients may be under- or over-supplied. Any nutrient that is undersupplied by a manure application could incur a subsequent fertilizer application cost which would, in effect, lower the net value of the manure. Any nutrient that is oversupplied by a manure application would not have immediate value because it was not needed by the crop.

## Biogas:

Biogas is a type of biofuel that is naturally produced from the decomposition of organic waste. When organic matter, such as food scraps and animal waste, break down in an anaerobic environment (an environment absent of oxygen) they release a blend of gases, primarily methane and carbon dioxide. Because this decomposition happens in an anaerobic environment, the process of producing biogas is also known as anaerobic digestion.

Anaerobic digestion is a natural form of waste-to-energy that uses the process of fermentation to breakdown organic matter. Animal manure, food scraps, wastewater, and sewage are all examples of organic matter that can produce biogas by anaerobic digestion. Due to the high content of biogas (typically

50-75%) biogas is combustible, and therefore produces a deep blue flame, and can be used as an energy source.

Biogas Digesters

As opposed to letting methane gas release to the atmosphere, biogas digesters are the systems that process waste into biogas, and then channel that biogas so that the energy can be productively used. There are several types of biogas systems and plants that have been designed to make efficient use of biogas. While each model differs depending on input, output, size, and type, the biological process that converts organic waste into biogas is uniform. Biogas digesters receive organic matter, which decompose in a digestion chamber. The digestion chamber is fully submerged in water, making it an anaerobic (oxygen-free) environment. The anaerobic environment allows for microorganisms to break down the organic material, and convert it into biogas.

All-Natural Fertilizer

Because the organic material decomposes in a liquid environment, nutrients present in the waste dissolve into the water, and create a nutrient-rich sludge, typically used as fertilizer for plants. This fertilizer output is generated on a daily basis, and therefore is a highly productive by-product of anaerobic digestion.

Biological breakdown

To produce biogas, organic matter ferments with the help of bacterial communities. Four stages of fermentation move the organic material from their initial composition into their biogas state.

1. The first stage of the digestion process is the hydrolysis stage. In the hydrolysis stage insoluble organic polymers (such as carbohydrates) are broken down, making it accessible to the next stage of bacteria called acidogenic bacteria.
2. The acideogenic bacteria convert sugars and amino acids into carbon dioxide, hydrogen, ammonia, and organic acids.
3. At the third stage the acetogenic bacteria convert the organic acids into acetic acid, hydrogen, ammonia, and carbon dioxide, allowing for the final stage- the methanogens.

4. The methanogens convert these final components into methane and carbon dioxide- which can then be used as a flammable, green energy.

Many Uses of Biogas:

Biogas can be produced with various types of organic matter, and therefore there are several types of models for biogas digesters. Some industrial systems are designed to treat: municipal wastewater, industrial wastewater, municipal solid waste, and agricultural waste.

Small-scale systems are typically used for digesting animal waste. And newer family-size systems are designed to digest food waste. The resulting biogas can be used in several ways including: gas, electricity, heat, and transportation fuels.

For example, in Sweden hundreds of cars and buses run on refined biogas. The biogas in Sweden is produced primarily from sewage treatment plants and landfills.

Traditional systems typically found in India and China focus on animal waste. Due to a lack of energy in rural areas combined with a surplus of animal manure, biogas digesters are very popular, useful, and even life-changing. In many developing countries, biogas digesters are even subsidized and advocated by the government and local ministries, who see the variety of benefits produced from using biogas. In addition to having a clean renewable energy provide gas in the kitchen, many families make extensive use of the fertilizer by-product that biogas digesters provide.

In African countries, some biogas users even turn a profit by selling the bio-slurry by-product produced by biogas systems. This bio-slurry is different from the liquid fertilizer that is produced daily. Bio-slurry refers to the most decomposed stage of the organic matter, after it has been broken down in the system. Bio-slurry sinks to the bottom of the biogas system, and with the help of modern units like HomeBiogas, is easily emptied out once accrued (usually an annual process). This bio-slurry is in fact a nutrient-dense sludge that provides lots of benefits to soil, and can increase productivity of vegetable gardens.

Biogas is a technology that mimics nature's ability to give back. Both industrial-size and family-size biogas units are becoming incredibly popular and relevant in today's world. As the application and efficiency grows, biogas can make a significant impact on reducing greenhouse gases. As a clean source of energy and a renewable means of treating organic waste, biogas is applicable both in under-developed and industrialized countries.

Biofuel:

**Biofuel**, any fuel that is derived from biomass—that is, plant or algae material or animal waste. Since such feedstock material can be replenished readily, biofuel is considered to be a source of renewable energy, unlike fossil fuels such as petroleum, coal, and natural gas. Biofuel is commonly advocated as a cost-effective and environmentally benign alternative to petroleum and other fossil fuels, particularly within the context of rising petroleum prices and increased concern over the contributions made by fossil fuels to global warming. Many critics express concerns about the scope of the expansion of certain biofuels because of the economic and environmental costs associated with the refining process and the potential removal of vast areas of arable land from food production. Biofuels are grouped by categories - first generation, second generation, and third generation – based on the type of feedstock (the input material) used to produce them.

- First generation biofuels are produced from food crops. For ethanol, feedstocks include sugar cane, corn, maize, etc. For biodiesel, feedstocks are naturally occurring vegetable oils such as soybean and canola[2].
- Second generation biofuels are produced from cellulosic material such as wood, grasses, and inedible parts of plants. This material is more difficult to break down through fermentation and therefore requires pre-treatment before it can be processed[2].
- Third generation biofuels are produced using the lipid production from algae.

In addition, the term -Advanced BiofuelsII is used to describe the relatively new technological field of biofuel production that uses waste such as garbage, animal fats, and spent cooking oil to produce liquid fuels.

Biofuels are not as energy dense as conventional transportation fuels. 1 gallon of biodiesel has 93% of the energy of 1 gallon of diesel and 1 gallon of ethanol (E85) has 73% of the energy of 1 gallon of gasoline

## Bt crops:

BT Crops are defined as crops which are genetically transformed to prompt one or more proteins from the bacterium. In this process, BT genes are inserted which helps the plant to produce proteins to fight against the insects or pest which results in destroying the yield. The proteins produced by the plants are known as cry protein. BT stands for Bacillus thuringiensis.

Bacillus thuringiensis is a gram-positive, spore-forming bacterium which is mainly found in the soil and hence it is also known as soil dwelling bacterium. This bacterium produces a protein which acts as a toxin for those insects destroying the yield. This bacterium is mainly used in the sprays for commercial agriculture and for organic farming. The use of this spray on crops are safe for the environment and causes no harm to the consumers.

The practice of using BT started in the year 1996 and began with using small quantities of genes from BT. With the help of this genetic transformation, plants used to create the necessary proteins to protect the crop from pests. All over the globe, in a land spanning 29 million acres, BT corn, BT potato, and BT cotton were grown in the year 1999. Relying on this technology alone, approximately 92 million dollars was saved by the United States.

Here is the list of few pests which destroy the yield like:

- European and southwestern corn borer.
- Tobacco and cotton budworm.
- Pink bollworm and the Colorado potato beetle.

**List of BT crops available in the market.**

These crops include corn, potato, cotton, cottonwood, brinjal, rice, soy. These crops are called as genetically modified crops as they are transformed and modified through genetic engineering.

**How does the cry protein work?**

When an insect feeds on the plants, the cry protein present in the plants crystallizes the digestive system of insects and it starves to death since the cry protein is toxic to organism's digestive tract.Remember that it affects insect's digestive system and has no effect on human's digestive system.

**Advantages of BT Crops.**

- They help in controlling the soil pollution as the use of synthetic pesticides are reduced when the plants begin to produce the toxins by themselves in own tissues.
- BT Crops help in protecting the beneficial insects.
- Reduced manpower and labor charges.
- The pests hiding inside plant parts are controlled effectively.
- It is cost effective as multiple sprays are not needed.

**Disadvantages of BT Crops.**

- The BT crops are more costly than the normally grown crops.
- There is a possibility for allergic reactions while using these crops.
- BT Crops are not effective for certain pests including spider mites, seed corn, etc.

**Summary:** *Bacillus thuringiensis* (*Bt*) is a common bacteria that has played a very uncommon role in agriculture and the development of genetically modified foods. The natural insecticidal abilities of these bacteria have made them an important pest control tool for nearly a century. While their use as a natural biopesticide is widely accepted and approved for organic applications, the engineering of Bt genes into major crops has been more controversial.

- Protecting our food from pests has been an ongoing battle ever since humans began cultivating food. The U.S. Food and Agriculture Organization estimates that some 20-40% of global crop totals are lostannually due to disease and pests, and the Environmental Protection Agency estimates that around 5 billion pounds of pesticides are used globally each year, costing more than 35 billion dollars [1-2].
- However, with the advent of genetic engineering, new highly targeted strategies for pest control have become available in the form of transgenic plants that are designed to have insecticidal traits.

Specifically, these bug-fighting plants were developed by moving some of the genes from the bacterium *Bacillus thuringiensis* (*Bt*) into corn and cotton. So called *Bt* crops are highly effective at combating pests such as European corn borer, rootworm, corn earworm, tobacco budworm, and bollworm [3-4].

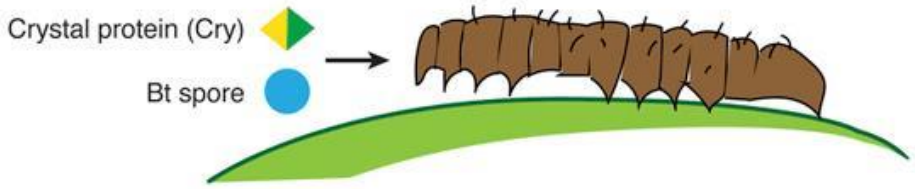
- Since the 1990s, corn and cotton with *Bt* genes have become the predominant varieties planted in North America [3]. Yet despite their long-term usage and widespread presence in the U.S. food supply, there continues to be heated debate and rampant consumer misinformation about the safety of *Bt* and other genetically modified (GM) crops.
- So what exactly do we know about the safety of *Bt* crops?
- *Bacillus thuringiensis* (*Bt*) is a very common bacterium found in a variety of distinct environments, from soil, to dessert, to tundra. It was first isolated in 1901 by Japanese biologist Ishiwata Shigetane as he studied the causes of a disease afflicting silkworms. Then in 1911, the German scientist Ernst Berliner re-isolated *Bt* from flour moth caterpillars that had been collected from Thuringia, Germany (hence the species name). Soon Berliner determined that the *Bt* bacterium was specifically toxic to certain insect larva and not others. However, it wasn't until 1928 that anyone attempted to harness *Bt* as a tool for pest control [4].
- Bacillus thuringiensis *has been used to control pests for almost a century, with its first agricultural application dating back to 1928 and first commercialization a decade later.*
- In this first instance, the bacteria were used to fend off European corn borer (*Ostrinia nubilalis),* which historically has been a common and very damaging corn pest. This initiated the development of the first commercial *Bt* based biopesticide, Sporine, which was introduced in 1938 in France [4]. Since then, *Bt*-based biopesticides have been a significant pest control strategy, and are actually a common pest control strategy in organic agriculture. By the 1990s, tens of thousands of *Bt* strains had been isolated, with toxicity to a broad range of insect species [5].

Still, it was a game changer when the first GM corn engineered with genes from *Bt* became available in 1995. Since then, crops with *Bt* genes have come to dominate the majority of varieties planted in the U.S., representing 81% of total corn and 84% of total cotton acreage [5].

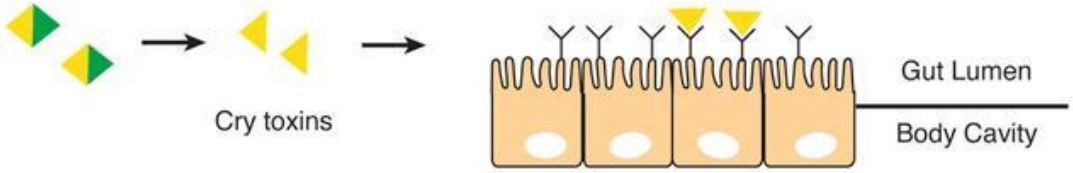
### The mechanism of *Bt* toxicity

After *Bt* was first discovered, the mechanism of its toxicity still remained a mystery for many years. But in the 1950s, scientists discovered that the crystalline proteins that formed in *Bt* spores, previously observed by Berliner, were responsible for *Bt* toxicity [4]. These crystal proteins, called Cry proteins, exhibit such a high degree of target specificity because of their mode of action within insect larvae.

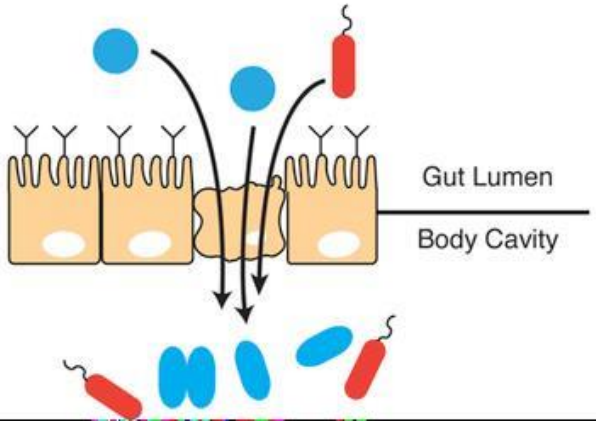
(A) Larvae ingest Bt spores and Cry proteins




(B) In larval midgut, proteolytic digestion of proteins release Cry toxins, which bind to epithelial receptors




(C) Toxin binding causes cell lysis destroying barrier to body cavity




*The production of* Bt *toxins is coupled to the organism's sporulation, and the multi-stage toxic mechanism by which* Bt *kills insects directly benefits the proliferation of the bacteria.*

When the Cry protein reaches the gut, it is partially degraded, releasing a smaller and potentially toxic part of the protein [6]. But this toxin will only be active if it finds the right matching protein receptor

sticking off the cells lining the gut of a larval insect. This is the most important aspect of the Cry toxin mechanism. Much in the same way that a certain key will only open a certain lock, the Cry toxin can only exert its toxic effect on a particular cell receptor. Consequently, the toxin tends to only impact insects within a particular taxonomic order.

- Once the toxin is bound, the process is fairly straightforward. The toxin recruits other Cry toxins to the same cell and together they form a hole in cell's membrane that ultimately causes the cell to burst . The cumulative effect of this happening to many cells is the irreversible destruction to the midgut membrane, compromising the barrier between the body cavity and gut. Without this barrier, *Bt* spores and other native gut bacteria can infiltrate and grow within the nutrient-rich body of the insect.
- What makes *Bt* such a great candidate for pesticide and GM applications is that while these Cry toxins are highly effective against insects, they have been shown to be safe for consumption by mammals. Tests by the EPA have demonstrated that Cry proteins, like any other benign dietary protein, are very unstable in the acidic stomach environment. Furthermore, an oral toxicity test, which involves giving mice exceptionally high doses of purified toxic *Bt* proteins, showed no significant health impacts. In their 2001 reassessment of several *Bt* Cry proteins, the EPA concluded from these findings that -there is reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the Cry1AB and Cry1F proteins and the genetic material necessary for their production.‖ Similar conclusions were drawn about the Cry1Ac protein of *Bt* cotton . Other mouse studies on have shown that even high doses of truncated Cry proteins, such that only the toxic region is conserved, have no deleterious effects [8]. A paper in Annual Review of Entomology from 2002 also makes the strong point that, in addition to no demonstrated toxicity of *Bt* toxins, their use provides important health benefits to livestock and humans by preventing certain insect-caused crop diseases that produce toxic and carcinogenic compounds .

**The environmental safety of *Bt***

Two major questions about the environmental impacts of *Bt* crops must be addressed. First, to what extent does the use of *Bt* crops reduce the application of more harmful pesticides? Second, do Cry proteins have significant off-target effects on other organisms?

*Bt* crops have enormous potential to reduce the use of both synthetic and organic pesticides. By relying on their *Bt* corn or cotton, farmers can decrease pest control-related costs and increase their yield. The USDA reports that -generally, *Bt* adoption is associated with lower insecticide use,‖ based on acollection of surveys from1998 to 2007 [9].

In 2001, *Bt* adopters were using approximately 36% less insecticide than non-adopters. The major caveat of this data, though, is that over the following decade the use of pesticides, on both *Bt* and non-*Bt* crops, has dramatically decreased overall. According to the USDA, between 1995 and 2010, the amount of pesticide used per acre of corn decreased by 99%, while insecticide use on cotton crops decreased by about 95% [9].What is interesting about these numbers, though, is that some studies have found evidence that the use of *Bt* corn and cotton is associated with a broad suppression of the overall population of damaging pests like corn borer, bollworm, and aphids.

One notable example of potential concerns for off-target effects occurred in the late 1990s, when it was widely published that high levels of pollen from *Bt* crops were toxic to the larvae of Monarch butterflies, commented on by David S. Pimentel and Peter H. Raven in 2000 [11]. While this initially raised concern, it has since been shown that the conditions under which this toxicity was observed do not exist in real-world applications of *Bt*. Specifically, butterfly larvae are not likely to be exposed to levels of *Bt*pollen that would be toxic, and are less likely to directly ingest toxic Cry proteins, as they do not feed on corn or cotton. The comparison must be made between plants that have been engineered to produce *Bt* toxins and the application of *Bt*-based pesticides. The more targeted and localized action of GM *Bt* crops appears by all accounts to have less of an ecological impact than *non-Bt* methods.

*Bacillus thuringiensis* has a long agricultural history dating back nearly one hundred years. Even within the relatively recent age of genetic engineering, *Bt* has been one of the longest-running applications and successes of GM foods in the United States. The targeted mechanism of the *Bt* Cry toxin

makes it an excellent pesticide since it has been shown to be safe for human consumption, reduces the use of insecticide application, improves crop yield, and reduces the amount of management crops require [9]. The engineering of *Bt* insecticidal traits into crops like corn, cotton, and potatoes demonstrates the

potential benefits and possibilities that advances in biotechnology are now providing. Perhaps most importantly, the story of *Bt* spotlights the thorough regulatory oversight that governs the development and application of these GM foods, ensuring their safety and sensible use.

**Golden rice:**

Golden Rice is conventional rice that has been genetically engineered to have high levels of beta-carotene, the precursor to vitamin A. Beta-carotene is found in a variety of fruits and vegetables (it's what makes carrots orange), but rice, which can make up to 80 percent of the daily diet in Asia, contains few micronutrients. The Golden Rice prototype was developed in the 1990s by European scientists Ingo Potrykus and Peter Beyer without any direct corporate involvement, and was greeted with much enthusiasm. Potrykus appeared on the cover of Time Magazine in 2000 along with the headline -This Rice could save a million kids a year.‖ However, the prototype didn't contain high enough levels of beta-carotene to be an effective source of vitamin A.

Recognizing the need to improve upon their breakthrough discovery, the scientists licensed their intellectual property to Syngenta on the condition that it would be made available to farmers in the developing world for free. The company developed an improved Golden Rice variety with much higher levels of beta-carotene in 2005 and decided not to commercialize it in the developed world as there was no market for it. Syngenta continues to support the project with advice and scientific know-how, but has no commercial control over it.

The current version of Golden Rice has two transgenes, or genes from another species. One is from corn and the other comes from a commonly-ingested soil bacterium. These two genes activate rice's metabolic carotenoid pathway, which produces beta-carotene.

The first generation of Golden Rice showed that it was possible to produce provitamin A in rice grains, but it was recognised that to combat vitamin A deficiency more higher β-carotene levels would be required. As only two biosynthetic transgenes are required in the process, the logical approach was to identify the bottleneck of the biosynthetic pathway and fine-tune the enzymatic activities of the two gene products involved, phytoene-synthase (PSY) and carotene-desaturase (CRTI).

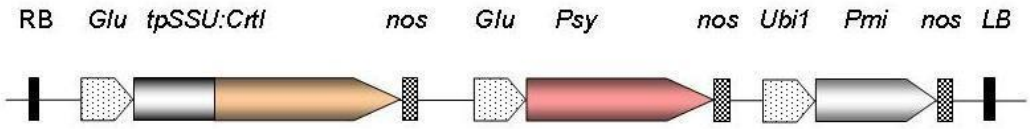



Fig 3: Gene construct used to generate *Golden Rice*. RB, T-DNA right border sequence; Glu, rice endosperm-specific glutelin promoter; tpSSU, pea ribulose bis-phosphate carboxylase small subunit transit peptide for chloroplast localisation; nos, nopaline synthase terminator; Psy, phytoene synthase gene from *Narcissus pseudonarcissus* (GR1) or Zea mays (GR2); Ubi1, maize polyubiquitin promoter; Pmi, phosphomannose isomerase gene from *E. coli* for positive selection (GR2); LB, T-DNA left border sequence.

In multi-step biosynthetic pathways there is generally a rate-limiting step that controls the flux through the whole pathway. This can be overcome by either increasing the amount of the rate-limiting enzyme or by using one that is more active. It was established that in this case was PSY and not CRTI (Al-Babili et al., 2006). Experimentation with PSY genes from different sources identified the maize and rice genes as the most efficient in rice grains (Paine et al., 2005), a result that has was confirmed later at the enzyme level

(Welsch et al. 2010). This led to the second generation of Golden Rice lines, often referred to as GR2, capable of accumulating up to 37 µg/g carotenoids, of which 31 µg/g was β-carotene, as compared to the first generation, where only 1.6 βg/g were obtained. (see also Al-Babili and Beyer, 2005).

Given that bioconversion of β-carotene from *Golden Rice* is a very efficient process, as highlighted on the homepage of this website, a typical diet containing GR2 has a great potential to help alleviate vitamin A deficiency-induced diseases.


**Transgenic Animals**

A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome. The foreign gene is constructed using recombinant DNA methodology. In addition to the gene itself, the DNA usually includes other sequences to enable it

- to be incorporated into the DNA of the host and
- to be expressed correctly by the cells of the host.
- Transgenic sheep and goats have been produced that express foreign proteins in their milk.
- Transgenic chickens are now able to synthesize human proteins in the "white" of their

eggs. These animals should eventually prove to be valuable sources of proteins for human therapy. Transgenic **mice** have provided the tools for exploring many biological questions.

**An example:**

Normal mice cannot be infected with polio virus. They lack the cell-surface molecule that, in humans, serves as the receptor for the virus. So normal mice cannot serve as an inexpensive, easily-manipulated model for studying the disease. However, transgenic mice expressing the human gene for the polio virus receptor

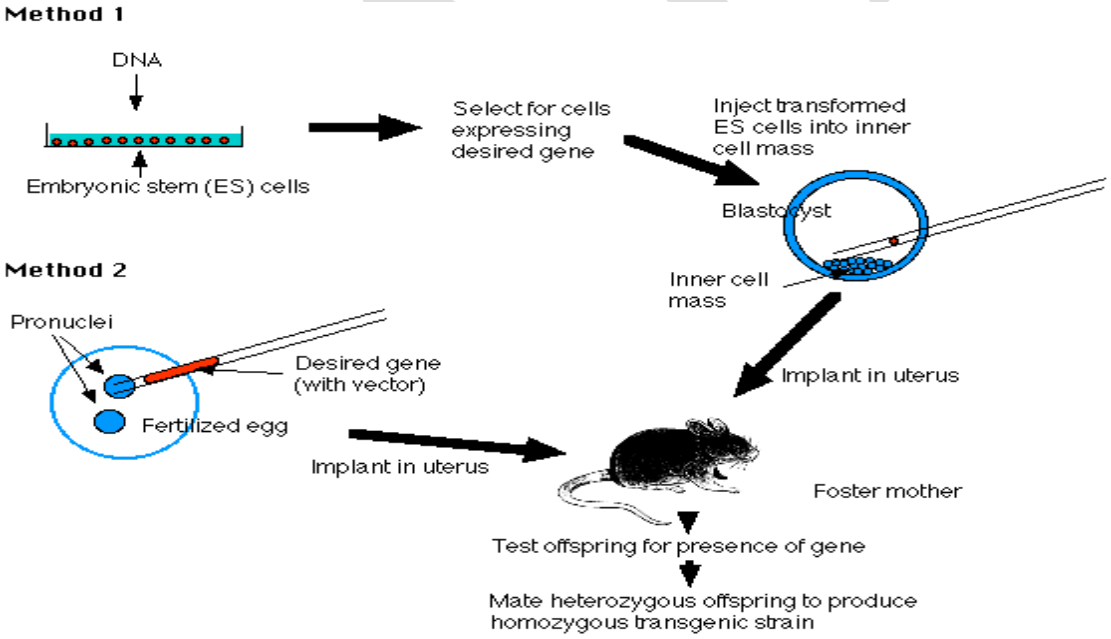
- can be infected by polio virus and even
- develop paralysis and other pathological changes characteristic of the disease in humans.

**Two methods of producing transgenic mice are widely used:**

- transforming **embryonic stem cells** (**ES** cells) growing in tissue culture with the desired DNA;
- injecting the desired gene into the **pronucleus** of a fertilized mouse egg.

**The Embryonic Stem Cell Method (Method "1")**

Embryonic stem cells (**ES** cells) are harvested from the **inner cell mass** (ICM) of mouse blastocysts. They can be grown in culture and retain their full potential to produce all the cells of the mature animal.



**1. Make your DNA**

Using recombinant DNA methods, build molecules of DNA containing

- the gene you desire (e.g., the insulin gene);
- **vector** DNA to enable the molecules to be inserted into host DNA molecules;
- **promoter and enhancer sequences** to enable the gene to be expressed by host cells.

**2. Transform ES cells in culture**

Expose the cultured cells to the DNA so that some will incorporate it.

**3. Select for successfully transformed cells. [Method]**

**4. Inject these cells into the inner cell mass (ICM) of mouse blastocysts.**

**5. Embryo transfer**

- Prepare a **pseudopregnant** mouse (by mating a female mouse with a vasectomized male). The stimulus of mating elicits the hormonal changes needed to make her uterus receptive.
- Transfer the embryos into her uterus.
- Hope that they **implant** successfully and develop into healthy pups (no more than one-third will).

**6. Test her offspring**

- Remove a small piece of tissue from the tail and examine its DNA for the desired gene. No more than 10–20% will have it, and they will be heterozygous for the gene.

**7. Establish a transgenic strain**

- Mate two heterozygous mice and screen their offspring for the 1 in 4 that will be **homozygous** for the transgene.
- Mating these will found the transgenic strain.

**The Pronucleus Method (Method "2")**

**1. Prepare your DNA as in Method 1**

**2. Transform fertilized eggs**

- Harvest freshly fertilized eggs before the sperm head has become a pronucleus.

- Inject the male pronucleus with your DNA.
- When the pronuclei have fused to form the diploid zygote nucleus, allow the zygote to divide by mitosis to form a 2-cell embryo.

**3. Implant the embryos in a pseudopregnant foster mother and proceed as in Method**

**1. An Example**


This image (courtesy of R. L. Brinster and R. E. Hammer) shows a transgenic mouse (right) with a normal littermate (left). The giant mouse developed from a fertilized egg transformed with a recombinant DNA molecule containing:

- the gene for **human growth hormone**
- a strong mouse gene **promoter**

The levels of growth hormone in the serum of some of the transgenic mice were several hundred times higher than in control mice.

**Random vs. Targeted Gene Insertion**

The early vectors used for gene insertion could, and did, place the gene (from one to 200 copies of it) anywhere in the genome. However, if you know some of the DNA sequence flanking a particular gene, it is possible to design vectors that replace that gene. The replacement gene can be one that

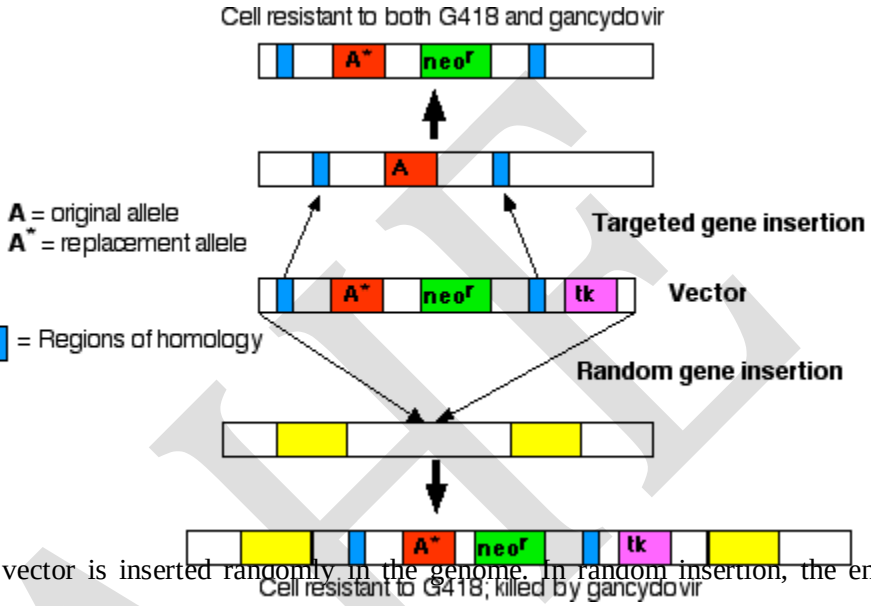
- restores function in a mutant animal or
- knocks out the function of a particular

locus. In either case, targeted gene insertion requires

- the desired gene
- ***neo*$^{r}$**, a gene that encodes an enzyme that inactivates the antibiotic neomycin and its relatives, like the drug G418, which is lethal to mammalian cells;
- ***tk***, a gene that encodes **thymidine kinase**, an enzyme that phosphorylates the nucleoside analog **ganciclovir**. **DNA polymerase** fails to discriminate against the resulting nucleotide and

inserts this nonfunctional nucleotide into freshly-replicating DNA. So ganciclovir kills cells that contain

the *tk* gene.




**Step 1**

Treat culture of ES cells with preparation of vector DNA.

Results:

- **Most cells** fail to take up the vector; these cells will be killed if exposed to G418.
- In a **few cells**: the vector is inserted randomly in the genome. In random insertion, the entire vector, including the ***tk***gene, is inserted into host DNA. These cells are resistant to G418 but killed by gancyclovir.
- In **still fewer cells**: **homologous recombination** occurs. Stretches of DNA sequence in the vector find the homologous sequences in the host genome, and the region between these homologous sequences replaces the equivalent region in the host DNA.

**Step 2**

Culture the mixture of cells in medium containing both G418 and ganciclovir.

- The cells (the majority) that failed to take up the vector are killed by G418.
- The cells in which the vector was inserted randomly are killed by gancyclovir (because they contain the *tk* gene).
- This leaves a population of cells transformed by homologous recombination (enriched several thousand fold).

**Step 3**

Inject these into the inner cell mass of mouse blastocysts.

**Knockout Mice: What do they teach us?**

If the replacement gene (**A*** in the diagram) is nonfunctional (a "null" allele), mating of the heterozygous transgenic mice will produce a strain of "**knockout mice**" homozygous for the nonfunctional gene (both copies of the gene at that locus have been "knocked out").

Knockout mice are valuable tools for discovering the function(s) of genes for which mutant strains were not previously available. Two generalizations have emerged from examining knockout mice:

- Knockout mice are often surprisingly unaffected by their deficiency. Many genes turn out not to be indispensable. The mouse genome appears to have sufficient redundancy to compensate for a single missing pair of alleles.
- Most genes are **pleiotropic**. They are expressed in different tissues in different ways and at different times in development.

**Tissue-Specific Knockout Mice**

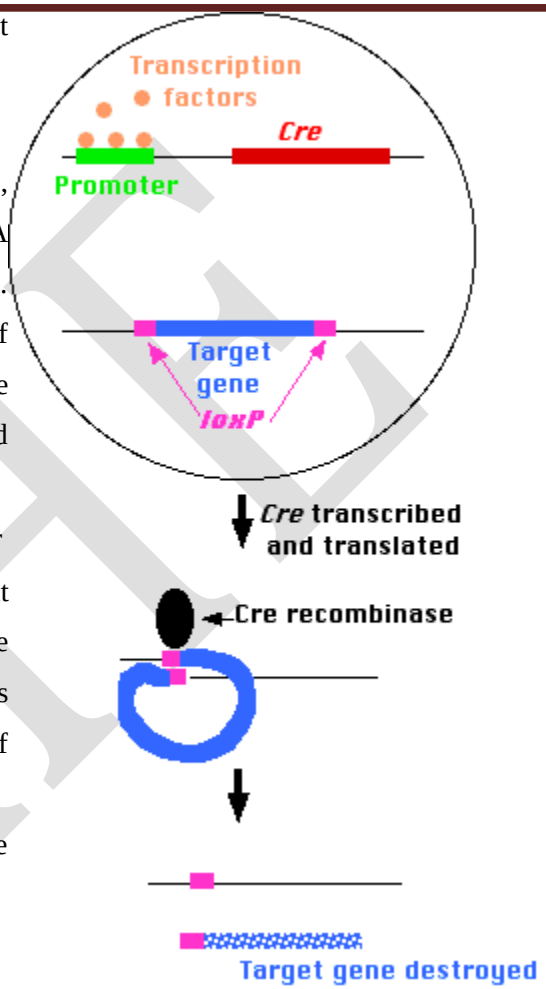
While "housekeeping" genes are expressed in all types of cells at all stages of development, other genes are normally expressed in only certain types of cells when turned on by the appropriate signals (e.g. the arrival of a hormone).

To study such genes, one might expect that the methods described above would work. However, it turns out that genes that are only expressed in certain adult tissues may nonetheless be vital during embryonic development. In such cases, the animals do not survive long enough for their knockout gene to be studied.

Fortunately, there are now techniques with which transgenic mice can be made where a particular gene gets knocked out in only one type of cell.

**The Cre/*loxP* System**

One of the bacteriophages that infects E. coli, called P1, produces an enzyme — designated Cre — that cuts its DNA into lengths suitable for packaging into fresh virus particles. Cre cuts the viral DNA wherever it encounters a pair of sequences designated *loxP*. All the DNA between the two *loxP* sites is removed, and the remaining DNA ligated together again (so the enzyme is a recombinase).




Using "Method 1" (above), mice can be made transgenic for

- the gene encoding Cre attached to a promoter that will be activated only when it is bound by the same transcription factors that turn on the other genes required for the unique function(s) of that type of cell;
- a "target" gene, the one whose function is to be studied, flanked by *loxP* sequences.

In the adult animal,

- those cells that
    - receive signals (e.g., the arrival of a hormone or cytokine)
    - to turn on production of the transcription factors needed
    - to activate the promoters of the genes whose products are needed by that particular kind of cell

  will also turn on transcription of the Cre gene. Its protein will then remove the "target" gene under study.

- All other cells will lack the transcription factors needed to bind to the Cre promoter (and/or any enhancers) so the target gene remains intact.

The result: a mouse with a particular gene knocked out in only certain cells.

**Knock-in Mice**

The Cre/*loxP* system can also be used to

- remove DNA sequences that block gene transcription. The "target" gene can then be turned **on** in certain cells or at certain times as the experimenter wishes.
- replace one of the mouse's own genes with a new gene that the investigator wishes to study.

Such transgenic mice are called "knock-in" mice.

**Transgenic Sheep and Goats**

Until recently, the transgenes introduced into sheep inserted randomly in the genome and often worked poorly. However, in July 2000, success at inserting a transgene into a specific gene locus was reported. The gene was the human gene for **alpha1-antitrypsin**, and two of the animals expressed large quantities of the human protein in their milk.

This is how it was done.

Sheep fibroblasts (connective tissue cells) growing in tissue culture were treated with a vector that contained these segments of DNA:

1. 2 regions homologous to the sheep *COL1A1* gene. This gene encodes Type 1 collagen. (Its absence in humans causes the inherited disease osteogenesis imperfecta.)
   This locus was chosen because fibroblasts secrete large amounts of collagen and thus one would expect the gene to be easily accessible in the chromatin.
2. A neomycin-resistance gene to aid in isolating those cells that successfully incorporated the vector. [Link to technique]
3. The human gene encoding alpha1-antitrypsin.

Some people inherit two non- or poorly-functioning genes for this protein. Its resulting low level or absence produces the disease **Alpha1-Antitrypsin Deficiency** (**A1AD** or **Alpha1**). The main symptoms are damage to the lungs (and sometimes to the liver).

4. Promoter sites from the **beta-lactoglobulin** gene. These promote hormone-driven gene expression in milk-producing cells.
5. Binding sites for ribosomes for efficient translation of the beta-lactoglobulin

mRNAs. Successfully-transformed cells were then

- fused with enucleated sheep eggs [Link to description of the method] and
- implanted in the uterus of a ewe (female sheep).
- Several embryos survived until their birth, and two young lambs lived over a year.
- When treated with hormones, these two lambs secreted milk containing large amounts of alpha1-antitrypsin (650 µg/ml; 50 times higher than previous results using random insertion of the transgene).

On June 18, 2003, the company doing this work abandoned it because of the great expense of building a facility for purifying the protein from sheep's milk. Purification is important because even when 99.9% pure, human patients can develop antibodies against the tiny amounts of sheep proteins that remain.

However, another company, GTC Biotherapeutics, has persevered and in June of 2006 won preliminary approval to market a human protein, antithrombin, in Europe. Their protein — the first made in a transgenic animal to receive regulatory approval for human therapy — was secreted in the milk of transgenic goats.

**Transgenic Chickens**

Chickens

- grow faster than sheep and goats and large numbers can be grown in close quarters;
- synthesize several grams of protein in the "white" of their eggs.

Two methods have succeeded in producing chickens carrying and expressing foreign genes.

- Infecting embryos with a viral vector carrying
    - the human gene for a therapeutic protein

promoter sequences that will respond to the signals for making proteins (e.g. lysozyme) in egg white.

- Transforming rooster sperm with a human gene and the appropriate promoters and checking for any transgenic offspring.

Preliminary results from both methods indicate that it may be possible for chickens to produce as much as g of human protein in each egg that they lay.

Not only should this cost less than producing therapeutic proteins in culture vessels, but chickens will probably add the correct sugars to glycosylated proteins — something that E. coli cannot do.

**Transgenic Pigs**

Transgenic pigs have also been produced by fertilizing normal eggs with sperm cells that have incorporated foreign DNA. This procedure, called sperm-mediated gene transfer (SMGT) may someday be able to produce transgenic pigs that can serve as a source of transplanted organs for humans.

**Transgenic Primates**

In the 28 May 2009 issue of **Nature**, Japanese scientists reported success in creating transgenic marmosets. Marmosets are primates and thus our closest relatives (so far) to be genetically engineered. In some cases, the transgene (for green fluorescent protein) was incorporated into the germline and passed on to the animal's offspring. The hope is that these transgenic animals will provide the best model yet for studying human disease and possible therapies.

**Possible questions**

**Part B (2 Mark questions)**

1. Define silage
2. Write the ethical issue of the Bt toxin
3. Write the advantages of golden rice
4. Add note on transgenic cattle
5. What is biomanure?
6. Write the any one methodology adopted for developing transgenic animals
7. Write the benefits of biomanure
8. How the biogas generated with the support of microbes
9. Write the efficiency of biomanure
10. How does cry protein work on biocontol
11. Write about transgenic chickens
12. Write nutrient value of golden rice

**Part B (8 Mark questions)**

1. Describe the advantages of Biofuel, Biogas and biomanure
2. Describe in detail about the advantages of silage and biotech feed
3. Discuss the environmental benefits of biomanure
4. Add note on biofuel and biogas production and environmental benefits
5. Give a brief note on transgenic animals and its benefits
6. Describe the Production process of silage and biotech feed
7. General account on transgenic animals and golden rice
8. Briefly describe the development and advantages of Bt crops.
9. Discuss in detail about the biofuel production.
10. Brief note on biotech feed and its advantages

# KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: III B.Sc MB COURSE NAME: Microbes in Sustainable Agriculture and Development

COURSE CODE: 17MBU602B UNIT: V BATCH-2017-2020

| Sl. No. | Question | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1. | The fermentation in _____ reduces harmful nitrates accumulated in plants. | Silage | Hay | Whey | Maize straw | Silage |
| 2. | Maize silage has _____ higher nutritive value. | 50% | 30% | 30-50 % | 70% | 30-50 % |
| 3. | Silage is produced in _____ weather. | Hot | Cold | Optimum | Too hot | Cold |
| 4. | Silage is stable for _____. | 1 yr | 2 yrs | 5 yrs | 3 yrs | 5 yrs |
| 5. | The first transgenic plant to be produced _____. | Maize | Wheat | Tobacco | Barley | Tobacco |
| 6. | The hydrolysis of polysaccharides to soluble sugars is called _____. | Solubilisation | Gellation | Liquefaction | Saccharification | Saccharification |
| 7. | Linolic acid content are increased in the egg yolk by _____. | Silage | Low phytate corn | High phytate corn | Soybean | Low phytate corn |
| 8. | Silage is high _____ content fodder. | Phosphate | Manganese | Moisture | Mineral | Moisture |
| 9. | Genetically modified soybean promote the _____. | Body weight | Protein | Fat | Increased digestability | Increased digestability |
| 10. | The enzymes help in digesting phytic acid, which are present in cereals and oil seeds. | Amylase | Lecithinase | Pectinase | Phytase | Phytase |
| 11. | The _____decreases the antinutritive factors. | Crops | GM crops | Improved crops | Aged crops | GM crops |
| 12. | Tripsin inhibitor is a _____. | Anti-nutritive factor | Nutritive factor | Digestive factor | Indigestive factor | Anti-nutritive factor |
| 13. | Golden rice consist of _____. | Vit A | Vit C | Vit D | Vit E | Vit A |
| 14. | Genes responsible for golden rice are _____. | Bt | Tra | Leg | Vir | Psy & crt i |
| 15. | Golden rice help to prevent the _____. | Vomiting | Clotting | Infection | Anaemia | Blindness |
| 16. | A green house gas is _____. | $H_2$ | CO | $CO_2$ | $N_2$ | $CO_2$ |
| 17. | Which is not a green house gas? | CFC | $CH_4$ | CO | $H_2$ | CFC |
| 18. | Main function of biofertilizer is _____. | To increase chemical process | To increase physiological process | To increase biological process | To increase photosynthesis process | To increase biological process |
| 19. | Seed treatment is done to control _____. | Air-borne disease | Soil-borne disease | Seed-borne disease | Viral disease | Seed-borne disease |

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| 20. | Composted manure is produced from _____. | Farmyard manure and green manure | Farm refuse and household refuse | Organic remains of biogas plants | Rotten vegetables and animal refuse | Rotten vegetables and animal refuse |
| 21. | The process by which nutrient chemicals or contaminants are dissolved and carried away by water or moved into a lower layer of soil | Mulching | Desertification | Incineration | Leaching | Leaching |
| 22. | Kitchen waste material such as fruits/vegetable peels can be used to generate _____. | Biogas | Fertilizer | Solar energy | Bioproduct | Biogas |
| 23. | The _____ is not the composition of biogas. | Methane | $CO_2$ | $N_2$ | $O_3$ | $O_3$ |
| 24. | The term biomass most often refers to _____. | Inorganic matter | Organic matter | Chemicals | Ammonium compounds | Organic matter |
| 25. | Which of the following forestry materials can be used as biomass? | Logging residues | Tallow | Fish oil | manure | Logging residues |
| 26. | The aerobic digestion of sewage is used to produce _____. | Logging residues | Tallow | Fish oil | Manure | Logging residues |
| 27. | Bio ethanol is denatured alcohol that is also called as _____. | Biomass | Bio fuels | Synthetic fuels | Metal articles | Bio fuels |
| 28. | The production of bio ethanol is by fermenting the _____ and starch components. | Ethylene | Methylated spirit | Ethylene glycol | Methylene | Methylated spirit |
| 29. | The bio ethanol is subjected to rectification to remove _____. | Acid | Milk | Sugar | Alcohol | Sugar |
| 30. | Broiler chicken weight increased by _____. | Biotech feed | Providing more protein | More fat | More fibre | Biotech feed |
| 31. | Biotech feed improve the _____ of the animal. | Enzyme activity | Fermentation activity | Digestability | Intestinal activity | Digestability |
| 32. | Linoleic acid enhance the _____ of the broiler chicks. | Weight | Enzyme activity | Fermentation | Immune function | Immune function |
| 33. | Properly _____ is essential to obtain larger egg. | Formulated feed | Induced enzyme | Optimized medium | Formulated food | Formulated feed |
| 34. | Raffinose in the soybean act as _____. | Antinutritive factor | Growth factor | Nutritive factor | Digestive factor | Antinutritive factor |
| 35. | Using genetically modified crops as feed decreased the amount | Sugar | Nitrogen | Carbon | Protein | Nitrogen |

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| | of _____ excretion in poultry. | | | | | |
| 36. | Adding specific and efficient additives to the animal feed drastically improves the _____ of the animals | Enzyme activity | Fermentation activity | Digestability | Intestinal activity | Digestability |
| 37. | Daily consumption of a very modest amount of golden rice supply _____ of the recommended daily allowance of vitamin a for an adult. | 20% | 50% | 70% | 100% | 50% |
| 38. | Most advanced version of golden rice gr2r, showed_____ yield compared to its conventional equivalent. | Lower | Higher | Not good | Average | Lower |
| 39. | _____ of rice plant enabled the production of provitamin a in rice grains. | Type | DNA | Variety | Plant epidermis | DNA |
| 40. | Gene of interest for golden rice from _____. | *Pseudomonas* | *Erwinia uredovora* | *Agrobacterium* | *Rhizobium* | *Erwinia uredovora* |
| 41. | crti is a _____. | Pesticide gene | Herbicide resistant gene | Beta carotene biosynthesis gene | Stress tolerance gene | Beta carotene biosynthesis gene |
| 42. | Psy gene is from _____ for golden rice. | *Erwinia uredovora* | *Pseudomonas* | *Streptomyces* | *Narcissus pseudonarcissus* | *Narcissus pseudonarcissus* |
| 43. | A japonica variety of rice to produce _____. | Niacin | Riboflavin | beta-carotene | Biotin | beta-carotene |
| 44. | DNA virus _____. | Baculo virus | Mosaic virus | Simian virus | Satellite virus | Baculo virus |
| 45. | Baculo virus particle consist of cylindrical _____ that surrounds viral DNA. | Nucleocapsid | Nucleus | Nucleosome | Proteosome | Nucleocapsid |
| 46. | The microinjected transgene construct is in _____form and free of vector DNA sequences. | Linear and prokaryotic | Circle and prokaryotic | Circle and eukaryotic | Linear and eukaryotic | Linear and prokaryotic |
| 47. | Low phytate corn is a used as _____. | Food | Feed | Digester | Composer | Feed |
| 48. | Low phytate corn will improve the _____in the egg yolk. | Linolic acid | Amniotic fluid | Calcium | Magnesium | Linolic acid |
| 49. | The bio ethanol obtained in the fermentation process has _____ purity. | 99% | 99.20% | 99.40% | 99.70% | 99.70% |
| 50. | The by-products that are produced during rectification of bio ethanol is used as_____ | Feed | Food | Manure | Fertilizer | Feed |

| | | | | | | |
|---|---|---|---|---|---|---|
| 51. | _____ is called as the bio gas. | Bio methane | Bio ethanol | Bio diesel | Bio butanol | Bio methane |
| 52. | The percentage of carbondioxide in the bio methane is _____. | 30-40 | 32-43 | 35-45 | 55-60 | 32-43 |
| 53. | Green manuring increases the crop yield by _____. | 30-90 % | 30-80 % | 20-30 % | 50-60 % | 30-50 % |
| 54. | The _____ is green manure. | Maize | Oat | Rice | Sesbania | Sesbania |
| 55. | The first crop plant genome sequenced | Maize | Wheat | Rice | Barley | Rice |
| 56. | Adding urea to corn silage that is fed to dairy cows is generally done to _____. | Inhibit the overgrowth of bacteria | Increase the nitrogen available to the bacteria | Prevent the growth of molds and fungi | Add high-quality carbohydrates to the silage | Increase the nitrogen available to the bacteria |
| 57. | Which of the following classes of pesticides are responsible for most acute cases of pesticide poisoning | Organophosphates | Carbamates | Chlorinated hydrocarbons | Pyrethrins | Organophosphates |
| 58. | In general, fungal diseases of crops such as fusarium wilt in tomatoes, corn smut in corn, and stem rust of wheat are best controlled by _____. | Spraying with insecticides to control vectors. | Rotating the susceptible crops with crops that are not susceptible. | Planting resistant varieties. | Spraying with fungicides as soon as symptoms appear. | Planting resistant varieties. |
| 59. | The golden rice prototype was developed in the _____by european scientists ingo potrykus and peter beyer | 1990s | 1880 | 2000 | 1900 | 1990s |
| 60. | Green manuring increases the crop yield by _____. | 30-90 % | 30-80 % | 20-30 % | 50-60 % | 30-50 % |