UNIT-II

Cultivation, Collection, Processing and storage of drugs of natural origin:

Cultivation:

Cultivation of medicinal plants requires intensive care and management. The conditions and duration of cultivation required vary depending on the quality of medicinal plant materials required.

There are certain drugs which are obtained only from cultivated plants such as Indian hemp, isapghula, cardamom, saffron, peppermint, linseed, ginger etc.

It provides the medicinal plants or crude drugs of better quality and purity. The crude drugs obtained posses a good quality with regard to colour, odour, taste, shape and size. The drugs contain appropriate quantities of chemical constituents and therefore exerts a better therapeutic action.

There are two methods of cultivation:

1. Sexual method(Seed propagation)

- In case of sexual method, the plants are raised from seeds and such plants are known as seedlings. Seeds which are to be used propagation should be of standard quality and should be free from disease, insects and extraneous material.
- They should posses high germination rate. If the seeds are no to be germinated in near future they should be stored in cool and dry place.
- Long storage of all seeds decrease the percentage of germination. If the seeds have slow germination rate then special treatments can be given to the seeds which increases the germination rate.

The sexual method of propagation enjoys following advantages

1. Seedlings are comparatively cheaper and easy to raise.
2. It is a method of choice when any other vegetative method cannot be employed.
3. Seedlings are long- lived (in case of perennial drugs) and bear more heavily (in case of fruits).

- Disadvantages of Sexual Propagation
- Seeds take a long time to turn into mature plants i.e. time interval between sowing and flowering is longer.
- These plants are they yield less as compared to grafted plants.not uniform in the growth and
- These plants are less resistant against the disease as compared to grafted plants .
- Plants that do not have seeds can't be propagated through this process.
- There are many factors that can affect the viability of seeds, including moisture, air, temperature, and light.

2. asexual method (Veg. propagation)

In case of asexual method of vegetative propagation, the vegetative part of a plant, such as stem or root, is placed in such an environment that it develops into a new plant.

- Methods:

1. Natural Vegetative Propagation
2. Artificial vegetative Propagation

1. Natural Vegetative Propagation :

- Vegetative part of plant is taken for propagation. By Stem: for example Bulb- Onion, Garlic Tuber- Potato, Aconite Rhizome- ginger, Turmeric By root: eg. Asparagus

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- Vegetative propagation (Asexual propagation): Vegetative propagation can be defined as regeneration or formation of a new individual from any vegetative part of the plant body. The method of vegetative propagation involves separation of a part of plant body, which develops into a new plant.

2. Artificial vegetative Propagation :

- (Cutting: - part of plant developed into new, viz Stem cutting Eg. Sugercane, Rose Layering: Root induced into stem, Later this stem is detached and grown separately)

Other Vegetative propagation methods

1. Cutting: These are the parts of the plant (stem, root or leaf) which, if grown under suitable' conditions, develop new plants. Stem cutting are generally used to obtained new plants. Examples: Sugarcane and rose, etc.

2. Layering: Roots are induced on the stem while it is still attached to the parent plant. This part of stem is later detached from the parent plant and grown into a new plant. Examples: Jasmine plant

3. Grafting: New variety is produced by joining parts of two different plants. The rooted shoot of one plant, called stock, is joined with a piece of shoot of another plant known as scion.

Examples: Rose, citrus and rubber, etc.

Advantages of Asexual Propagation

- There is no variation between plant grown and the parent plant.
- As resultant species formed through asexual process are genetically identical, useful traits can be preserved among them.
- These plants are more resistant against the disease as compared to seedling plant.
- The seedless varieties of the fruits can be propagated. Eg. Lemon, grapes.
- The plants are uniform in size and yield more as compared to seedling plants.
- The process is faster than sexual propagation. This helps in rapid generation of crops which in turn balances the loss.

Disadvantages of Asexual Propagation

- Diversity is lost in asexual propagation which is the main reason behind occurrence of diseases in future plant species.
- As many crops are produced with this process, it leads to overcrowding & lack of nutrients.
- New varieties of crops cannot be developed in this type of propagation.
- Asexual propagation is an expensive process that requires special skills for successful cultivation of crops.
- Crops produced through this process have shorter life-span than those grown through sexual process.
- Species involved in this process are less likely to resist pests and diseases.

- ADVANTAGES OF CULTIVATION

1. It ensures quality and purity of medicinal plants.
2. Collection of crude drugs from cultivated plants gives a better yield and therapeutic quality.
3. Cultivation ensures regular supply of a crude drug.
4. The cultivation of medicinal and aromatic plants also leads to industrialisation to a greater extent.
5. Cultivation permits application of modern technological aspects such as mutation, polyploidy and hybridisation.

- DISADVANTAGES OF CULTIVATION

1. The high cost of cultivation drugs as compared to wild source and losses due to ecological imbalance such as storms, earthquakes, floods, droughts etc are major disadvantages of cultivation

FACTORS AFFECTING CULTIVATION OF MEDICINAL PLANT:

- Altitude,
- temp and
- humidity
- Rainfall or irrigation
- Soil and soil fertility
- Fertilizers Pests and pests control
- Plant hormones effects

Altitude, temperature and humidity

- **Altitude** :
- The bitter constituent of *Gentian lutea* increases with altitude, while alkaloids of *Aconite and lobelia* and the oil content of *peppermint* decreases with altitude
- Altitude is a very important factor in cultivation of medicinal plants. Tea, Cinchona and Eucalypts are cultivated at an altitude of 1000-2000 meters.
- Cinnamon and Cardamom are grown at a height 500-1000 meters while Senna can be cultivated at sea level. The following are the example of medicinal plants.

Plants	Altitude(meters)
1. Clove up to	900
2. Cardamom	600-1600
3. Camphor	1500-2000
4. Cinchona	1000-2000

- **Temperature:**
- Temperature is another major factor that enhance the cultivation of the medicinal plant. Excessive temperature, as well as, frost also affect quality of medicinal plants adversely.
- It plays important role in the production of phytoconstituents.
- The yield of the secondary metabolites could be increased or decreased by the effect of temperature. For instance, in general, the production of volatile oil appeared to be enhanced at higher temperature. Fixed oil produced at low temperature contains fatty acid with a higher content of double bond than those formed at higher temperature. Many enzymatic changes in plant secondary metabolites proceed more rapidly at the slightly temperature upto about 45°C.
- Camphor and coffee cannot withstand frost, whereas saffron needs only cold climate and pyrethrum requires dry weather for cultivation.
- The following are few examples of ranges of temperature necessary for luxuriant growth of certain medicinal plants.

Plant	Optimum temperature
Cinchona	60-75° C
Coffee	55-70° C
Tea	70-90° C

- Moisture/Humidity:
- Moisture present in drug depends largely upon the amount of moisture in the atmosphere which is usually in terms of humidity.
- Drug stored in non air tight container is termed as air dried drug and contains about 10-12% of water. This amount of water is sufficient to activate the enzyme present in some dried plants.
- Low moisture may be maintained it necessary by the use of desiccant in the container provided, but direct contact should be avoided.
- Rainfall: Variable results have been shown for the production of volatile oil under different conditions of rainfall.
- Continuous rain can lead to a loss of water soluble substance from leaves and roots by leaching. This could account for low yield of some active constituents in wet season.
- Excepts the xerophytic plants like aloe, acacia and few others, most of the plants need either proper arrangements for irrigation or sufficient rainfall for their favourable development. In few cases, well distributed rainfall throughout the year is desired.

Length of Day: Research has shown that light is a factor that determines the amount of glycoside or alkaloid produced. The crude drugs like Bellandona, Stramonium and Cinchona in full sunshine gives a higher content of alkaloid. In long day condition, Peppermint leaves contain menthone, menthol and menthofuran; but when grown undershort day condition, it contains menthofuran as a major component.

- **Soils and soil fertility:**
- Soil is the most important natural resource as it supports growth of all plants.
- Soil provide mechanical strength as well as and the essential plant food elements for plant.
- The capacity of soil to supply plant nutrients quantities and proportion required and to provide suitable medium for plant growth is known "soil fertility".
- Soil makes chemical make up and nutrients available to plants. Plant growth depends upon physical arrangement and nature of soil particles, organic matter content of soil and its living organisms.
- The commonly known soil is the shallow upper layer and is the friable material in which plants find foot-hold and nourishment.
- Clay is one of the highly weathered portion of the soil, consisting of finest particles. This provides the soil adhesive and cohesive properties and also holds plant nutrients with the result that nutrients are not lost through leaching.

- Soil consists of mineral matter, air, water and organic matter. It is the mineral matter which makes a lot of difference in various forms of soil. Mineral matter may be coarse gravel sand or in the form of finest particles of clay and slit.
- Depending upon size of mineral matter, following names are given to the soil.

Particle size (diameter)	Types of soil
1. Less than 0.002 mm	Fine clay
2. 0.002 to 0.02 mm	Coarse clay or slit
3. 0.02 to 0.2 mm	Fine sand
4. 0.2 mm to 200 mm	Course sand
5. 2.0 and more	Stone

- Depending upon the percentage covered by clay, soils are classified as given below

Types of Soil	Percentage covered
1. Clay	More than 50% of clay
2. Loamy	30 to 50% of clay
3. Slit loam	20 to 30% of clay
4. Sandy loam	10 to 20% of clay
5. Sandy soil	More than 70% sand
6. Calcarious soil	More than 20% lime

Any type of soil containing less than 0.5% of organic matter is describe as poor.

If more than 1.5 to 5% of organic matter is present, it is describe as rich soil. The soil with 0.5 to 1.5% of humus is termed as intermediate soil.

A soil good for plant should have half of the pore spaces filled with water and the rest with air, since good aeration is essential for root development.

The pH of soil decides favourable growth of plants and presence of micro-organisms. The maximum availability of plant nutrients is in between the pH range of 6.5 to 7.5.

- **Soil fertility**

It is the capacity of soil to provide nutrients in adequate amounts and in balanced proportion to plants. If cropping is done without fortification of soil with plant nutrients, soil fertility gets lost. It is also diminished through leaching and erosion.

Soil fertility can be maintained by addition of animal manures, nitrogen-fixing bacteria or by application of chemical fertilizers.

4. Fertilizers and Manures

Plant also need food for their growth and development. What plants need basically for their growth are the carbon-dioxide, sun-rays, water and mineral matter from soil.

- Fertilizer: Fertilizer are nutrients which are necessary for development & growth of the plant .

Plants need of 16 nutrients elements for growth and metabolism.

Depending upon the quantity needed the nutrients are classified into macronutrients and micronutrients.

- Macronutrients are needed in large quantities and micronutrients in traces.
- Carbon, hydrogen, nitrogen, sulphur, oxygen, calcium, phosphorous, potassium and magnesium are the macronutrients
- Copper, zinc, boron, molybdenum, iron, manganese, chlorine are the micronutrients .
- Carbon, hydrogen and oxygen are obtained from air and water.
- These elements are supplied to the plants through the soil. These elements can also be supplied to the plants through animal manures and chemical fertilizers.
- Each elements has its own role in growth and development of plants and their deficiency may cause disease.
- For example fertilizers containing nitrogen increase the size of plants and also influence the chemical constituents like alkaloids, glycosides, volatile oils etc.
- Commonly used chemical fertilizers are Urea, DAP(Di ammonium phosphate), NPK & SSP (Single super phosphate)

Mannures :

Manure is material which are mixed with soil. Theses supply almost all the nutrients required by the crop plants. This results in the increases in crop productivity.

Munnures are three types:

1. Farmyard manure :
2. Composited Manure:
3. Green manure:

5. Pests and pest control :

- A pest is an undesired animal or plant species and pesticides are chemicals derived from synthetic and natural sources effective in small concentrations against pest.

- The different types of pests infecting medicinal plant are fungi, viruses, weeds, insects and non insect pests.
- These pests directly affects the plant growth and development and produce disease which ultimately influence the quality and yields of crude drugs. Hence, control of pest is essential and it should be given importance.

Types of pests:

The various types of pests which infests the plants are like virus, fungi,weeds, insects and non-insects pests

a) Virus:

Various types of virus causes disease in medicinal plants.

Ascochyta atopae cause the formation of greyish- white irregular spots which further cause necrosis of leaves. The disease is called leaf necrosis.

Strains of *Cucumber mosaic* virus cause disease in hyoscyamus whereas Tobacco *mosaic virus, Tobacco ring spot virus, Cucumber mosaic virus are known to cause infection in digitalis.*

2) Fungi

Various types of virus causes disease in medicinal plants.

Cerscospora atropae cause leaf spot in which round brown spots are produced on the both sides of leaves.

Cerscospora dioscorea produces leaf-spot on dioscorea and Alternaria tennussima produces leaf spot on datura.

3)Weeds

A weed is an undesired plant growing in crop field. Weeds cause drastic damages to the plants and this problems is common in agriculture.

Majority it causes loss of nutrients and water in all the plants. It also causes loss of space, increases the attacks of fungi, bacteria, virus and insects which causes disease and ultimately it influence the quality and price of crude drugs.

Some weeds cause allergies e.g. medican tea and ragweed causes hay fever. Varnish tree and western poison oak causes dermatitis. Therefore weeds should be controlled properly.

4) Non-insects pests

Non-insects pests are classified into two groups: Vertebrates and invertebrates

Vertebrates includes rabbits, monkey, rat, pigs, squirrel and deer etc.

Invertebrates like snails, crabs, mites and nematodes etc. The rodents have sharp and gnawing incisor with which they causes severe damage to stored crude drugs.

The fecal materials of these animals causes contamination of crude drugs.

Methods of pest control:

The different types of methods used to control the pest are

1) Agricultural Methods:

It involves various types of methods. One of the method is that in which fields are deeply ploughed which removes the weeds and insects. Crop rotation can also be followed. In this method crops are grown alternatively.

Another method which is common now a days is **crop improvement**. It is achieved by a technique called as plant breeding. By this technique hybrid varieties of the plants are produced which are resistant to disease and pest

2) Mechanical Methods: It employs manual labour along with different devices for collection and destruction of pest.

- Examples include:

1. Hand picking to remove insects
2. Pruning
3. Burning
4. Trapping of pests

3) Chemical Methods : Pests are controlled by using chemical pesticides. Examples:

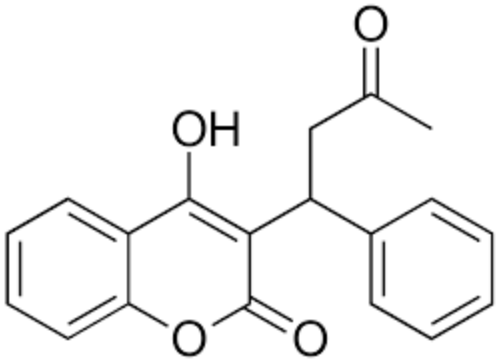
1.Insecticides: to control insect (DDT, gammaxine, parathione, malathione)

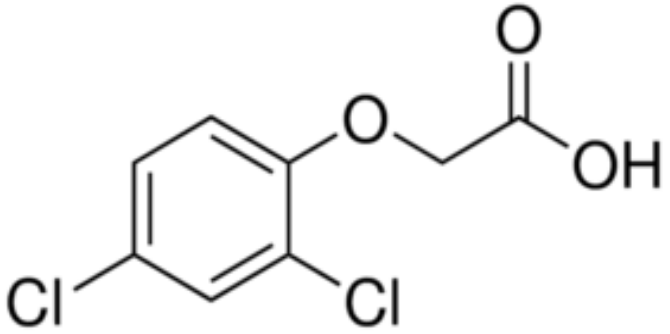
2.Fungicides: to control fungus diseases (Bordeaux mixture, chlorophenols, antibiotics)

3. Herbicides: to control weeds (2,4-di chlorophenoxy acetic acid, Sulphuric acid)

4.Rodenticides: to control rodents (Warfarin, Strychnine, Red squill)

- Warfarin 2,4-di chlorophenoxy acetic acid





- 4) Biological Control methods:
- Biological control brings about reduction in activity of pest mostly insect by another organism. This may be biocidal or biostatic. In biocidal biological control one organism kills the other while in biostatic the organism only inhibits the other.
- Biological control is defined by Garrett as "any condition under which or practice whereby sutvival or activity of a pathogen is reduced through the agency of any other living organism(except man) with the result that there is a reduction in the incidence of the disease caused by pathogen"
- Natural pest control agents:

Example of natural agents acting as pesticide:

1. Tobacco
2. Nux-vomica
3. Pyrethrum
4. Neem
5. Citronella

Neem : Biological sources: It consist of fresh or dried leaves of Azadirachta indica belonging to family Meliaceae Chemical Constituents: Azadirachtin , Nimbin , Nimbidin , Nimbidol , Sodium nimbinate , Gedunin , Salannin and Quercetin . Uses: Antiseptic, anti microbial, anti malarial.

- **Growth regulator:**
- Growth regulator It is a hormone like synthetic organic compound. In small amounts, it modifies the growth and development either by promoting or inhibiting the growth. General plant hormones: The phyto-hormones are broadly grouped under five major classes namely
- (1) Auxins (cell elongation)
- (2) Gibberellins (cell elongation + cell division - translated into growth)
- (3) Cytokinins (cell division + inhibits senescence)
- (4) Abscisic acid (abscission of leaves and fruits)
- (5) Ethylene (promotes senescence, epinasty , and fruit ripening)

1) Auxin:

- Auxin : Auxin increases the plasticity of plant cell walls and is involved in stem elongation. Occurs in very low concentrations. auxin enhanced cell elongation.

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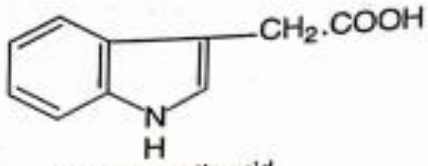
9.1.1 Auxins

Auxin is a general term used to indicate substances that promote elongation of coleoptile tissues. Natural auxins can be produced by plants themselves. Synthetic auxins have the same action as natural auxins. The examples of natural auxins are indole acetic acid, indole-3-acetonitrile (IAN), Phenyl acetic acid, 4-chloroindole-3-acetic acid. The synthetic auxins are indole-3-butyric acid (IBA), 2-Napthyl oxyacetic acid (NOA), α-Napthyl acetic acid (NAA).

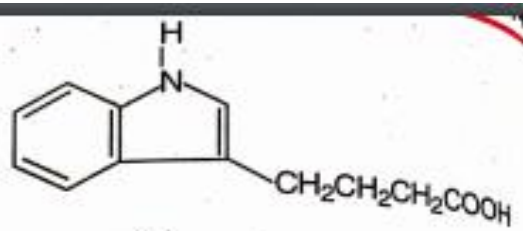
Role of auxins:

Auxins are involved in different growth processes:

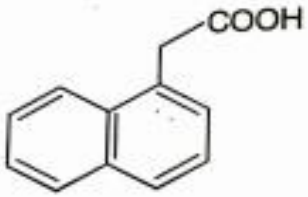
- Internode elongation.
- Leaf growth.
- Initiation of vascular tissue.
- Fruit setting in absence of pollination.
- Fruit growth.
- Inhibition of root growth.



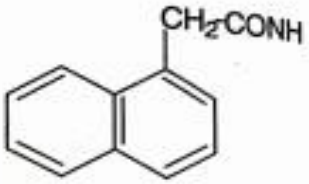
Indole-3-acetic acid



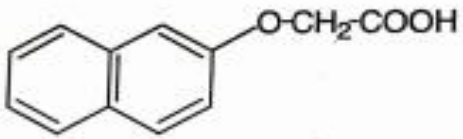
IBA



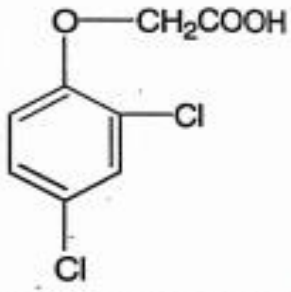
NAA



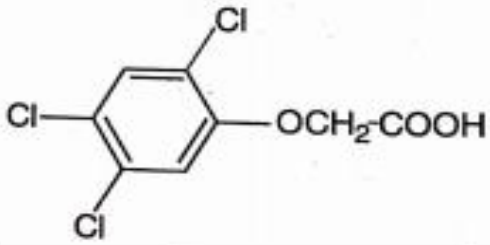
NOD



NOA



2,4-Dichlorophenoxyacetic acid

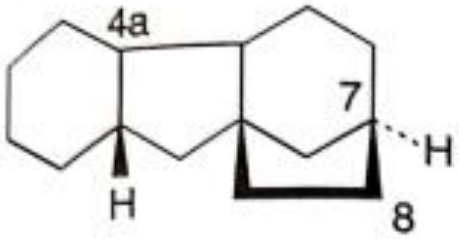


- Gibberellins

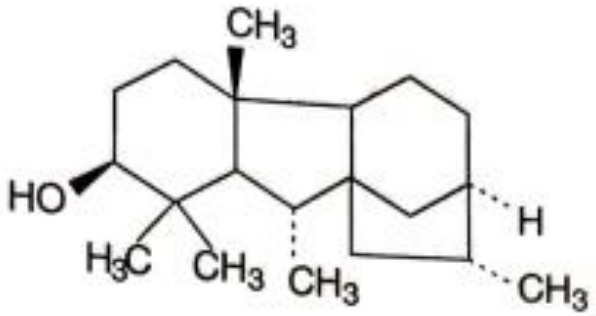
They are the class of endogenous plant growth regulators and at present 50 gibberelins are known in which 40 of them occur in green plant, while other are present in fungi. They are present in different organs and tissues like roots, shoot, buds, leaves etc.

- The commercial formulation of gibberellin is used currently for promoting vegetative and fruit growth, breaking dormancy, flower initiation and induction of parthenocarpy.
- A Japanese physiologist is credited for initiating the discovery of gibberelin from fungus Gibberela fujikuroi grown on rice. Gibberellins are compounds having gibbane skeleton and biological activity in stimulating cell division or cell elongation or both. Gibberellin A is actually a mixture of at least 6 gibberellins referred to as GA1, GA2. GA3, GA4, GA6 and GA7 and GA9. GA3 is termed as gibberellic acid.

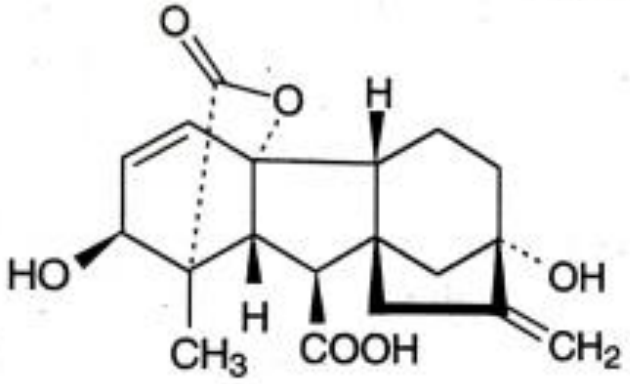
- GA has not yet been synthesized, but can be produced by large scale fermentation on commercial scale. Angiosperms, gymnosperms, algae, fungi and bacteria contain several forms of gibberellins, but no single plant contains all of them together.
- Many activities attributed to gibberellins are: Promotion of rapid expansion of plant cell. Stimulation of seed germination.
- Marked increase in stem elongation.
- Increase in the size of leaves
- Induction of flowering
- Showed higher production of digoxin (in root culture of digitalis).



Gibbane



Gibberellane



Gibberellic acid

3)Cytokinins

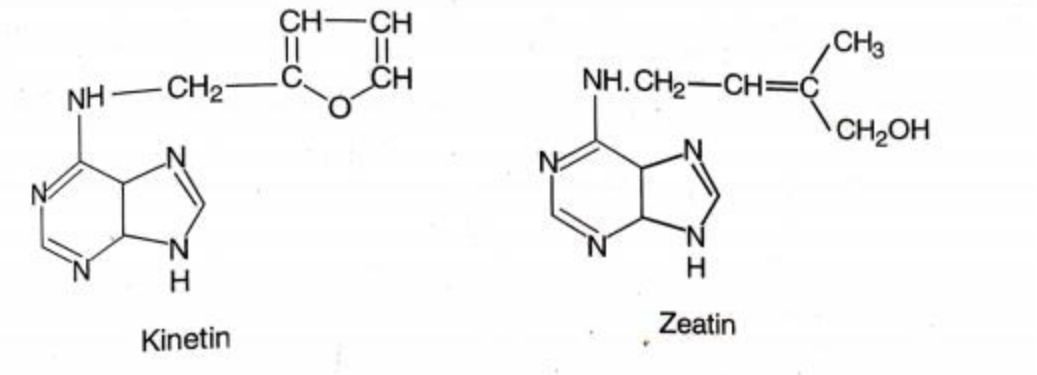
Cytokinins are the compounds with a structure resembling to adenine and promotes cell division(Cytokinins)

In this, Zeatin or kinetin compounds have significant growth regulating activity.

Zeatin is utilized to promote cell division and leaf senescence, whereas synthetic cytokinins have role in promoting lateral bud development and inhibition of senescence.

Cytokinins show promotion of cell division, orderly development of embryos during seed development, influencing the expansion of cells and cotyledons, delaying breakdown of chlorophyll and degradation of leaves in ageing leaves.

These substances are found in young and actively dividing tissues like embryos, seedlings. Kinetin reported to play the role in nucleic acid metabolism and protein synthesis. Some t-RNA contains cytokinin like property which acts on some enzymes responsible for formation of certain enzyme. Cytokinin in several leaves increase sennoside content in opium cause formation of elongated capsule.

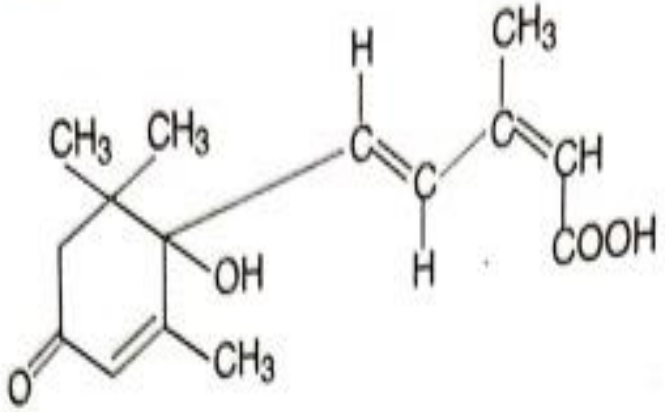


5) Abscisic acid:

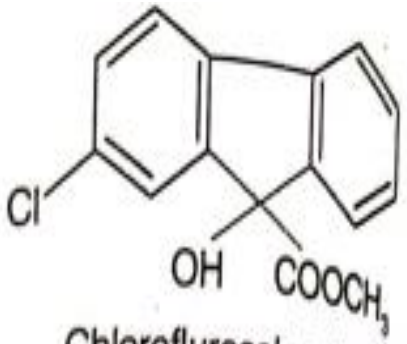
- Abscisic acid (ABA) was originally discovered for its role in regulating abscission and bud dormancy.
- They are naturally occuring plant growth inhibitors and have been isolated from the fungus, Cenospora rosicola . Abscisic acid inhibit seed germination. Carn et al. isolated several abscission accelerating substances from cotton plants and named them as Abscisin I & Abscisin II.

- Physiological effects of abscisic acid:
- Abscisic acid acts as growth inhibitor and induces bud dormancy in a variety of plants. ABA is a powerful growth inhibitor.
- As the name suggests abscisic acid is an hormone that stimulates abscission. ABA controls geotropic responses of roots. It stimulates positive geotropism in roots. Abscisic acid causes closure of stomata.

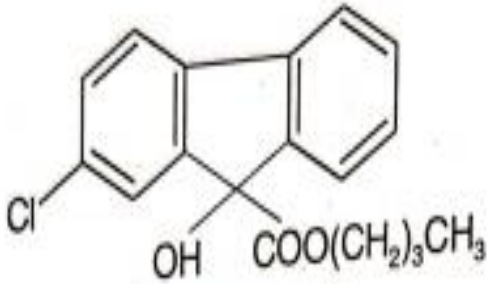
- Abscisic Acid (ABA)
- Several abscission accelerating substances are isolated from cotton plants and are named as abscisin I and abscisin II.
- MOA:
- In an inhibitory way ABA interacts with other plant growth substances.
- It inhibits the gibberellic acid induced synthesis of amylase and other hydrolytic enzymes.
- ABA accumulates in many seeds and helps in seed dormancy.
- ABA concentrations are found to be enhanced in stress condition like mineral deficiency, injury, drought, and flooding.
- ABA serves as important role as potential anti-transpirant by closing the stomata when applied to leaves.
- Other inhibitors are maleic hydrazides 2, 3, 5 tri-iodobenzoic acid.



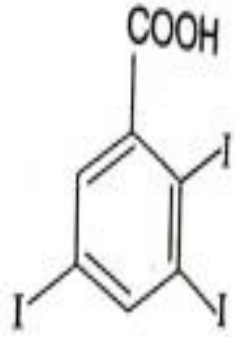
Abscisic acid (Abscisin II)



Chloroflurecol methyl



Flurecol-butyl



(2, 3, 5-tri-iodobeonzoic acid) Tri

- Ethylene
- It is a simple organic molecule present in the form of volatile oil and shows profound physiological effect.
- Ethylene is a simple gaseous hormone
- It is present in ripening fruits, flowers, stem, roots, tuber and seeds. It is present in very less quantity in plant normally about 0.1 ppm. A gas evolved from ripe apple which can also affect the ripening of green apples was ethylene.
- Ethylene shows a broad array of growth responses in plants which include fruit ripening; leaf abscission, stem swelling, leaf blending, flower petal discolouration and inhibition of stem and root growth.
- It is commercially used for promotion of flowering and fruit ripening, induction of fruit abscission, breaking dormancy and stimulation of latex flow in rubber trees.

- **Polyploidy:**
- The specific no. of chromosomes is a character of each species and is called genome which is observed in all types of organism. [Set of chromosomes = Genome].
- The term euploidy is a type of ploidy in which genome contain whole set of chromosomes and euploidy includes monoploidy , diploidy and polyploidy . When some plants contain more than two genomes it is called as polyploidy . For 3,4,5,6,7 genome no. = polyploidy may be triploid, tetraploid, pentaploidy, hexaploidy, heptaploidy , plants.

  POLYPLOIDY IS CAUSED BY ARTIFICIALLY INDUCED METHODS/ AGENTS : Physical agents like: X-rays centrifugation temperature chocks Specific chemical agents like: (a) Colchicine (b) V eratrine (C) Sulphanilamide and (d) Mercuric chloride.

- Significant Effects of polyploidy: Greater significance to medicinal plants. It may cause formation of new species. adoptability of medicinal plant to various habitat and mainly accumulation of vitamins. Usefull effects on Digitalis, opium & Mentha. And also Usefull effects on Commercial crops (wheat, oats, cotton).

POLYPLOID

- Polyploids are organisms with multiple sets of chromosomes in excess of the diploid number (Polyploidy refers to when an organism has more than two complete sets of chromosomes.)
- This condition is frequently found in plants. Polyploids can be divided into types based on the number of chromosomes they carry

1. Triploids (three sets)
2. Tetraploids (four sets)
3. Pentaploids (five sets)
4. Hexaploids (six sets)
5. Octoploids (eight sets)
6. Decaploids (ten sets)
7. Dodecaploids (twelve sets)

- POLYPLOIDY
- Plants and animals have in their somatic cells two sets of chromosomes (paternal and maternal) expressed as diploid or 2n, while in their reproductive cells they have one set of chromosomes expressed as haploid or n.
- An increase in the number of chromosomes in certain tissue or entire organism in multiples of the basic or haploid number is expressed as polyploidy.
- This is of wide occurrence in nature, particularly in plants and rarely in animals. There are organisms with more than two sets or less than two sets of chromosomes in their somatic cells.
- The term heteroploidy is sometimes used to include all types of variations found in chromosomes number.
- All variations can be studied under euploidy and aneuploidy. Euploidy-(eu-true or even ploid-unit): This represents cases where the somatic chromosome complements are exact multiples of the haploid number of a particular species.

- Aneuploidy: This represents cases where somatic chromosome complements are not exact multiples of basic number.
- There is addition or loss of usually 1 or 2 chromosomes. When the number is less, it is hypolpoidy and when the number is more, it is hyperploidy.

- Effect of Polyploidy: Polypoidy plant is usually healthier, stronger & larger than their diploid counterparts.
- Polyploidy contains larger flowers, pollen grains & stomata. The effect of polyploidy is not generally predictable.

- Induction of Polyploidy: Polyploidy is caused through cell generation, physical agents like X-Rays, centrifugation, temperature, shock and chemical agents. Colchicine (an alkaloid) is an important chemical which can induce polyploidy formation.
- Seeds are frequently soaked in an aqueous solution of colchicines (0.2 to 2.0% solution for 1-4 days) before planting.
- Alternatively the soil around the roots of young seedling can be moistened with the alkaloid solution. Young buds and shoots can be treated by immersion. Newly formed polyploidy usually require a number of generations to establish themselves

- **Mutation:**
- Sudden change in genotype causing qualitative or quantitative alteration of genetic material called MUTATION. OR Sudden heritable change in the structure of a gene or chromosomes or change the chromosome number.
- Mutation is the inheritable change in the characters of organisms.
- Mutation can occur spontaneously in nature or they may be induced experimentally.
- Mutation can be beneficial or harmful to an individual.

- Types of Mutations:

1. Spontaneous and induced mutations.
2. Recessive and dominant mutations
3. Somatic and germinal mutations.
4. Forward, back and suppressor mutation.
5. Chromosomal, genomic and point mutation.

Mutation can artificially produced by certain agents called mutagenes or mutagenic agent . Types of mutagenes:

(A) Physical Mutagenes: Ionizing radiations: x-rays, gamma radiation and cosmic rays. Non Ionizing radiations: UV radiation

(B) Chemical Mutagenes: Alkylating agents: Nitrogen and sulphur mustard. Acridines: Acridines and proflavins. Nitrous acid.

- MUTATION
  Mutation is the change in nucleotide sequence of a gene. This gives rise to a new genetic trait or changed genotype.
- A cell or an organism which shows the effect of mutation is called a mutant and the agent which causes mutation is known as mutagenic agent. Thus we occasionally see sudden changes in familiar plants and animals. Two common types of mutation are:
  (i) Point mutation
  (ii) Frameshift mutation
- (i) Point mutation occurs as a result of the substitution of one nucleotide for another in the specific nucleotide sequence of a gene. It is categorized as follows:
  Transition type: One purine to another purine or one pyrimidine to another pyrimidine. Transversion type: Purine converted into pyrimidine.
  (ii) Frameshift mutation results from an addition or loss of one or more nucleotides in a gene and is termed as insertion or deletion mutation respectively.
  Artificial mutation: The artificial mutations are those which are induced artificially in the living organism by exposing them to abnormal environment such as radiations, certain physical conditions like temperature and chemicals, all of which are called mutagens or mutagenic agents.

- Radiation mutation: The electromagnetic waves of short wavelength (UV light, y-rays and B-rays) are radiation mutagens. Chemical mutation: Some chemical mutagens like nitrogen mustard, formaldehyde and nitrous acid alter chemical constitution of DNA bases and cause transitional substitution in DNA.

Importance of Mutation

- Evolution of new and better food plants having good desirable characters such as higher yield and greater resistance.
- Production of new species.
- Agricultural revolution.
- Mutation breeding often provide solution for some specific plant breeding problems.
- Can produce improved verity of plants than the traditional verity.
- Less time consuming.
- Improvement in genetic characters so that the new species give desirable effects.
- To increase crop yield.

- HYBRIDIZATION

Hybridization is a method of union or crossing of genetically two dissimilar plants. As a result, individual produced by crossing of genetically two different parents is known as hybrid. Hybridization is a natural or artificial process to produce a hybrid and whole process . known as hybridization

Or

Hybridization is a process to produce a hybrid by crossing over of two plants of opposite genetical makeup. Hybridization is a vital technique to combine the characters of different plants. Hybridization does not modify genetic contents of organisms, however it produces new combination of genes.

Hybridization can be of following types:
(1) Intra-varietal hybridization: In this method, crossing should be done between the plants of the same variety.
(ii) Inter-varietal or Intraspecific hybridization: In this method, crossing should be done between the plants of two different varieties.
(iii) Interspecific hybridization or intrageneric hybridization: In this crosses are made between two different species of the same genus.

- Methods of Hybridization
  Following steps take place during hybridization:
  (a) Selection of parents.
  (b) Selfing of parents or artificial self-pollination.
  (c) Emasculation.
  (d) Bagging
  (e) Tagging
  (f) Crossing
  9) Harvesting and storing the F1 seeds
  (h) Raising the F1 generation.
- (a) Selection of parents:
- The parents are mainly selected upon the object of breeding. It should be selected from the local areas. The selected plants should be best suited to the existing conditions.
- (b) Selfing of parents or artificial self-pollination:
- It is necessary to induce homozygosity for elimination of undesirable or unwanted characters and obtain inbreeds.

- (c) Emasculation:
- Emasculation is the process to remove the stamens from female parent plants before they burst and shed their pollens. Removal of stamens/anthers or killing of the pollen grains of a flower should be done in such away which does not affect the female reproductive organs. Emasculation mainly occurs in bisexual or self-pollinated plants, it is not required in unisexual plants. This is the third step in hybridization. Inbreeds are developed under suitable conditions and emasculated.
- Various Methods Used for Emasculation:
- (i) Hand emasculation or Forceps or Scissor method: Hand emasculation method is mainly used in those plants which have large flowers. In this technique the corolla of the selected flowers are opened and anthers are carefully separated with the help of pointed forceps.
- **Following precautions should be taken while performing this method:**
- Selection of the flowers should be done at proper stage.
- All the anthers should brought out from the flowers without any damage.
- The reproductive organs of flower like ovary of the flower should not be damaged.

- (ii) Hot water treatment: Removal of stamens can also be done by hot water treatment because it is very difficult to separate the stamens with the help of forceps in minute flowers, In such case emasculation is done by dipping the flowers in hot water for certain duration of time, near about one to ten minutes, which mainly depends on plant species. In this treatment the gynaecium will withstand the hot temperature, whereas the anthers will be killed.
- (iii) Cold water treatment: Same as hot water, cold water also kills pollen grains without harming the gynoecium. This method is not so much effective than hot water treatment.
- (iv) Alcohol treatment method: This is not widely used method for emasculation because time duration of treatment is very important factor.
- A very short duration is required, otherwise gynoecium could be damaged. The flowers and inflorescence are dipped in alcohol of a suitable concentration for a brief period. For example, in Alfa alfa, a treatment with 57% alcohol for only 10 seconds is sufficient to kill the pollen grains.

- (v) Suction method: In this method, mechanical pressure is applied in such a way that only anthers are sucked out and other parts of the flower like gynoećium remain intact.
- (vi) Male sterility or Self-incompatibility method: Emasculation process can also be eliminated by the use of male-sterile plants. In some self-pollinated plants anthers are sterile and do not produce any viable pollens. Likewise self-incompatibility may also be used to avoid emasculation.
- (vii) Chemical Gametocides: Certain chemicals e.g., 2, 4-D, naphthalene acetic acid (NAA), maleic-hydrazide (MA), tribenzoic acid, etc. are capable of causing male sterility when sprayed before flowering.
- **(d) Bagging:** The emasculated flower or inflorescence is immediately bagged to avoid cross pollination.
- The bags may be made of paper, butter paper or fine cloth. This is the fourth step taken after emasculation. The bags are tied at the base of the flower or inflorescence with thread or wire. The bagging step is necessary for bisexual plants.
- The separate bagging is provided for both male and female flowers to prevent contamination in male flowers and cross-pollination in female flowers.

- (e) Tagging:
- The emasculated flowers are tagged just after bagging. Generally circular tags (about 3 cm) or rectangular tags (about 3 x 2 cm) are attached to the base of flower or inflorescence with the help of thread. The information on tags must be in brief and complete like:
- (a) Number referring to the field record, (b) Date of emasculation, (c) Date of crossing, (d) Name of the female parent and male parent (e.g. A x B indicates that A is the female parent and B is the male parent).
- (f) Crossing:
- This is the sixth step. Here artificial cross-pollination between the genetically dissimilar plants takes place. The mature, fertile and viable pollens from the male parent are placed on the receptive stigma of emasculated flowers and fertilization occurs.
- Pollen grains are collected in petridishes or in paper bags and applied to the receptive stigmas with the help of a brush, piece of paper, tooth pick or forceps. In some cases inflorescences of both the parents are packed in the same bag.

- (g) Harvesting and Storing the F_1 Seeds:
- Crossed plants are harvested and dried, Seeds are stored in airtight containers.
- (h) Raising the F1 generation:
- In the favourable season, the stored seeds are sown separately and raise the F_1 generation. The plants of F_1 generation are progenies of cross seeds or developed hybrids.
- Significance of Hybridization
- Hybridization forms a single variety by combining two desirable characters which are not found in parent plants and produces a new hybrid plant. The hybridization between two varieties of *Withania somnifera Israeli chemotype II* and *Withania somnifera South African chemotype* develops a new hybrid which contains 3 new withanolides.

- COLLECTION OF CRUDE DRUGS
- Crude drugs may be collected from cultivated or wild plants. The factors like season, time, age of plants etc. affects the collection of crude drugs.
- Season is an important factor which should be considered during collection because it influences the nature and amount of active constituents.
- There are certain drugs like wild cherry, podophyllum, aconite, rhubarb etc.in which active constituents is not constant through out the year.
- For example rhubarb does not contain anthraquinone derivatives in winter but in summer the anthranol is converted into anthraquinone by oxidation; the contents of C-glycosides, O-glycosides and free anthraquinones in the developing shoots and leaves of Rhamnus purshiana fluctuate throughout the year.
- Day and night affects the daily variation of proportion of secondary metabolites in some plants. For example there is daily variation in the alkaloid content of hemlock, ergot and poppy. Daily variation is also observed in glycosidal content of Digitalis lanata and Digitalis purpurea.

- The age of plant is an another factor which influences the collection of crude drugs Firstly controls the total quantity of active constituents produced and secondly it also controls the relative proportion of components of the active mixture.
- For example in Digitalis lanata highest level of total glycoside is observed in first year leaves but glycosides which are but which are medicinally important reaches to highest level in second year plants.
- Leaves are collected when flowers are just beginning to expand or the flowering is just arriving at its height. The collection should be done in dry weather since leaves collected in wet weather deteriorate in quality and may become discolored during drying.

- The time of collection is sometimes varied for special reasons ; for e.g. coca leaves are collected when they are nearly ready to fall from stem whereas bearberry leaves may be collected at any time of the year.
- Flowers are collected just before they are full expanded in dry weather because petals which are damp when gathered become badly discoloured during drying.
- For eg. red rose and clove are collected when in bud and kousso is collected after pollination and fertilization.
- The fruits are collected when they are fully grown but they may be either ripe or half ripe. For e.g. fennel, dill and ajowan are collected when they are fully ripe where as cardamom is collected just before they dehiscence.
- The Barks are collected in spring or early summer when the cambium is active as it is easy to separate them from wood in this season.

- Sometimes the bark like cinnamon is collected in rainy season and wild cherry bark is collected in autumn because during this season the content of active constituents is highest.
- Barks are collected by three methods namely
- (i) felling (ii) uprooting (iii) coppicing.

1) In felling method the tree is cut down near the ground level and bark is removed from branches and stem.
2) In uprooting method the trees from ten to fifteen years are cut down and root is dug up; the bark is then removed from trunk and branches and from root also. Cinchona bark may be collected by this method.
3) In coppicing method trees are allowed to grow for a definite period of time and then the stems are cut down at a short distance from the ground. The bark is removed from trunk and branches.

- The Underground Organs such as roots and rhizomes are generally collected in autumn as their tissues are fully stored with reserve foods; it is being assumed that chemical constituents will be most abundant in this season. Underground organs should be freed from soil and this can be achieved by shaking or brushing the drug.
- Large roots and rhizomes are generally sliced transversely or longitudinally or in both directions to facilitate drying.
- The Unorganised drugs such as latex, juices, gums, resins etc. are collected as soon as they exudes out of the plant.
- The juice of aloe is collected as it oozes out after giving incision to leaves.
- Tragacanth gum is collected after two days of the incision. Myrrh is collected from the wounded bark as soon as it oozes out.

- DRYING
- Drying is a process of removing the moisture content of crude drugs.
- Drying includes various treatments/operations which depends upon the chemical constituents and sources crude drugs. Drying increases the resistance against the growth of microorganism, enhances the quality and helps in size reduction of drugs. After drying it is easy to store the drugs for a longer time.
- The method of drying is selected on the basis of chemical constituents present in the crude drugs. There are two methods of drying viz. Natural drying (Sun drying) and Artificial drying. Artificial drying can be accomplished by
- (i) Tray dryers (Hot air oven) (ii) Spray dryers (ii) Vacuum dryers.

- After collection of drugs they are subjected to drying.
- The drugs like gentian root, vanilla pods and cocoa seeds where enzyme action is to encouraged should be subjected to slow drying at moderate temperature.
- The drugs where enzyme action is not desired should be dried as soon as possible.
- The drugs like cardamom, clove, cinnamon etc, which are subjected to open air drying largely depends upon weather. They require suitable weather for drying.
- The drugs which are cultivated in countries containing high humidity are subjected to an artificial drying
- The most important factor in the process of drying is the temperature. The temperature should be controlled on the basis of chemical constituents and physical nature of crude drugs.
- For example Digitalis leaves should be dried at a temperature below 60°C.
- Colchicum corm are dried at a temperature not exceeding 65°C. Generally speaking the flowers, leaves, herbs should be dried between 20 to 40°C and roots, rhizomes and barks between 30 to 60°C.
- Drugs containing volatile oil are liable to lose their aroma if not dried properly.

- GARBLING (DRESSING):

- The next step in preparation of crude drug for market after drying is garbling. This process is desired when sand, dirt and foreign organic parts of the same plant, not constituting drug are required to be removed. This foreign organic matter (extraneous matter) is removed by several ways and means available and practicable at the site of the preparation of the drugs.
- If the extraneous matter is permitted in crude drugs, the quality of drug suffers and at times, it does not pass pharmacopoeial limits.
- Excessive stems in case of lobelia and stramonium need to be removed, while the stalks in case of cloves are to be deleted. Drugs constituting rhizomes need to be separated carefully from roots and rootlets and also stem bases.
- The pieces of bark should be removed by peeling as in gum acacia. The dirt and sand from fennel, dill, coriander, caraway etc is removed by winnowing.

- PACKING
- During packing of crude drugs various factors such as morphological and chemical nature of drug, effect of climatic conditions during transportation and storage and their uses should be taken into consideration.
- Turkish opium is imported in rounded or conical cakes covered with poppy leaves. Persian opium occurs in brick shaped masses and is covered with red paper.
- Indian opium is in cubical pieces enclosed in tissue paper and its weight is also kept constant.
- Zanzibar aloe is packed in the skin of carnivorous animals and pieces of skin in the drug indicates its source.
- Colophony is packed in large pieces in kerosene tins to avoid auto -oxidation. The drugs which are sensitive to moisture and are costly such as digitalis, squill, ergot etc also needs special care during packing.
- If the moisture content of digitalis increases beyond 5% then it looses its potency due to decomposition of glycosides.

- Ergot becomes susceptible to microbial growth and squill becomes flexible if brought in contact with moisture during storage. Therefore these types of drugs should be packed along with suitable dehydrating agent.
- The packing of drugs containing volatile oil also requires special attention. Cinnamon bark which is available in the form of quills is packed one inside the other quill to facilitate the loss of volatile oil.
- Similarly fennel, clove, coriander, caraway, dill etc are also packed in well closed containers.
- Fixed oils such as cod liver oil, sesame oil, shark liver oil are sensitive to light therefore such drugs should be stored in those containers which are not affected by sunlight..
- Crude drugs like seeds, roots, rhizomes etc do not require attention and are packed in gunny bags. Sometimes these bags may be coated with polythene, internally.

- **STORAGE**
- Drugs can be maintained in good conditions for a longer time by adopting proper methods of storage. The factors which affects the storage of crude drugs or drug deterioration are moisture content, temperature, light and oxygen of the air.
- The Moisture content is the important factor which should be considered during storage of drugs. A number of drugs absorb moisture during storage which increase the bulk of drugs and are liable to the attack of microorganisms.
- For e.g. the moisture content more than 9% enhances the growth of fungi and bacteria in cotton wool.
- The drugs like digitalis and wild cherry bark when absorbs excessive moisture activates enzymatic reactions and it leads to decomposition of glycosides.
- These drugs should be stored in sealed containers with dehydrating agent. The powdered squill which contains mucilage if not properly stored absorb moisture and is converted into a sticky mass.

- Various types of bacteria, insects, mites, nematodes, worms, moths etc. are reported to attack the crude drugs if not stored properly.
- The effects produced by bacteria are not always very visible but in case of the chromogenic species their presence is recognized.
- For e.g. Bacillus (Chromobacterium) Prodigiosus produces red patches on potatoes, bread, paste and other starchy materials. For other bacteria the effect of their presence cannot be seen immediately.
- An increase in temperature along with moisture enhances the enzymatic activity. High temperature causes loss or volatile oil from the drugs like ginger,, asafoetida, chamomile flowers etc. Therefore majority of drugs should be stored in cool as well as dry place.

- Direct sunlight also influences the crude drugs. It causes bleaching of flowers and leaves. For example the rose petals changes their colour in direct sunlight. It also decomposes the chemical constituents of few drugs like cod liver oil and ergot.
- The ill-effects produced by direct sunlight can be prevented by using opaque or amber glass containers. Oxygen of the air causes rancidification of fixed oils and can deteriorate the drugs containing volatile oil.
- Hence oxygen of the container can be replaced by an inert gas like nitrogen. Therefore the crude drugs should be stored in well closed, moisture proof and light resistant containers such as cans, tins, opaque or amber glass containers in cool and dry place.
- Crude drugs should not be stored in paper bags and wooden boxes.