Crude Drugs: Cultivation, Collection, Processing and Storage

Cultivation of Crude Drugs:

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.

Methods of Propagation:

- **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.

Methods of vegetative propagation:

They are two types:

1. Methods of natural vegetative propagation:

2. Methods of artificial vegetative propagation.

A.) Methods of natural vegetative propagation:

Vegetative propagation by stem:

Examples: Runner: peppermint.

(i) Bulb:
    Allium, Squill.
(ii) Corms:
    Colchicum.
(iii) Tuber:
    Potato, aconite.
(iv) Offset:
    Valerian.
(v) Rhizome:
    Ginger and haldi.

Vegetative propagation by root:

Examples: Asparagus

B.) Methods of artificial vegetative propagation:

Various parts developed for natural vegetative propagation have also been used for artificial vegetative propagation.

Following methods are used:

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.

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.

4. Micro propagation:
This method consists of growing cell, tissue and organ in culture. Small pieces of plant organs or tissues are grown in a container with suitable nutrient medium, under sterilized conditions. The tissue grows into a mass of undifferentiated cells called callus which later differentiates into plantlets. These are then transferred into pots or nursery beds and allowed to grow into full plants.

Importance of asexual propagation:

1.) It is a cheaper, easier and rapid method of multiplication. Many fruit trees usually require 4-5 years to bear the fruits when developed from seeds. The plants developed by vegetative methods, take only a year to bear fruits.

2.) Plants like roses and chrysanthemum, etc do not form viable seeds. Thus, vegetative propagation is the only method of propagation is the only method of reproduction and continuation of species in such plants.

3.) All the plants developed by these methods will be generally similar to the parent plant.

4.) Micro propagation is useful in raising disease free plants, homozygous diploids, and those without viable seeds.

- **Seed Propagation (Sexual Propagation):**

The process of sexual propagation:

(i) Microsporogenesis:
Microspores are formed from microspore mother cells inside the anther.

(ii) Pollination:
This is the transfer of pollen grains from the anther to the stigma.

(iii) Micro gametogenesis:
This involves the formation of male gametes from microspore.

(iv) Mega sporogenesis:
This process leads to the formation of megaspores from megaspore mother cell, inside the ovule.

(v) Mega gametogenesis:
The events involving the formation of embryo sac from megaspore are included in this process.

(vi) Fertilization:
Fusion of male and female gametes takes place, resulting in the formation of zygote.

(vii) Embryogeny:
The process involves development of embryo from zygote.

- **Collection of drugs:**

Medicinal plant materials should be collected during the appropriate season or time period to ensure the best possible quality of both source materials and finished products. It is well known that the quantitative concentration of biologically active constituents varies with the stage of plant growth and development.

This also applies to non-targeted toxic or poisonous indigenous plant ingredients. The best time for collection (quality peak season or time of day) should be determined according to the quality and quantity of biologically active constituents rather than the total vegetative yield of the targeted medicinal plant parts.

In general, the collected raw medicinal plant materials should not come into direct contact with the soil. If underground parts (such as the roots) are used, any adhering soil should be removed from the plants as soon as they are collected.

Collected material should be placed in clean baskets, mesh bags, other well aerated containers or drop cloths that are free from foreign matter, including plant remnants from previous collecting activities. After collection, the raw medicinal plant materials may be subjected to appropriate preliminary processing, including elimination of undesirable materials and contaminants, washing (to remove excess soil), sorting and cutting.

The collected medicinal plant materials should be protected from insects, rodents, birds and other pests, and from livestock and domestic animals. If the collection site is located some distance from processing facilities, it may be necessary to air or sun-dry the raw medicinal plant materials prior to transport.

If more than one medicinal plant part is to be collected, the different plant species or plant materials should be gathered separately and transported in separate containers. Cross-contamination should be avoided at all times.

Collecting implements, such as machetes, shears, saws and mechanical tools, should be kept clean and maintained in proper condition. Those parts that come into direct contact with the collected medicinal plant materials should be free from excess oil and other contamination.

Time of collection:
The period of growth or development at which medicinal activity is highest has been carefully determined for many plants. The proportion, of alkaloid in the leaves of Hyocyamus Niger and of belladonna is largest at the beginning of flowering, whilst with Stramonium the peak coincides with full bloom.

Example:
Stramonium leaves, gathered in the morning, contain a higher proportion of alkaloids than those collected in the evening.

Harvesting:

The best time for harvest (quality peak season/time of day) should be determined according to the quality and quantity of biologically active constituents rather than the total vegetative yield of the targeted medicinal plant parts during harvest, care should be taken to ensure that no foreign matter, weeds or toxic plants are mixed with the harvested medicinal plant materials.

Medicinal plants should be harvested under the best possible conditions, avoiding dew, rain or exceptionally high humidity. If harvesting occurs in wet conditions, the harvested material should be transported immediately to an indoor drying facility to expedite drying so as to prevent any possible deleterious effects due to increased moisture levels, which promote microbial fermentation and mould.

Cutting devices, harvesters, and other machines should be kept clean and adjusted to reduce damage and contamination from soil and other materials. They should be stored in an uncontaminated, dry place or facility free from insects, rodents, birds and other pests, and inaccessible to livestock and domestic animals.

Contact with soil should be avoided to the extent possible so as to minimize the microbial load of harvested medicinal plant materials where necessary, large drop cloths, preferably made of clean muslin, may be used as an interface between the harvested plants and the soil.

If the underground parts (such as the roots) are used, any adhering soil should be removed from the medicinal plant materials as soon as they are harvested.

The harvested raw medicinal plant materials should be transported promptly in clean, dry conditions they may be placed in clean baskets, dry sacks, trailers, hoppers or other well-aerated containers and carried to a central point for transport to the processing facility.

When containers are not in use, they should be kept in dry conditions, in an area that is protected from insects, rodents, birds and other pests, and inaccessible to livestock and domestic animals. Any mechanical damage or compacting of the raw medicinal plant materials, as a consequence, for example, of overfilling or stacking of sacks or bags that may result in composting or otherwise diminish quality should be avoided. Decomposed medicinal plant materials should be identified and discarded during harvest, post-harvest inspections and processing, in order to avoid microbial contamination and loss of product quality.

As per WHO Guidelines:

1. Medicinal plants/herbal drugs should be harvested when they are at the best possible quality for the proposed use.

2. Damaged plants or parts plants need to be excluded.

3. Medicinal plants/herbal drugs should be harvested under the best possible conditions avoiding wet soil, dew, rain or exceptionally high air humidity. If harvesting occurs in wet conditions possible adverse effects on the medicinal plant/herbal drug due to increased moisture levels should be counteracted.

4. Cutting devices or harvesters must be adjusted such that contamination from soil particles is reduced to a minimum.

5. The harvested medicinal plant/herbal drug should not come into direct contact with the soil. It must be promptly collected and transported in dry, clean conditions.

6. During harvesting, care should be taken to ensure that no toxic weeds mix with harvested medicinal plants/herbal drugs.

7. All containers used during harvesting must be clean and free of contamination from previous harvests. When containers are not in use, they must be kept in dry conditions free of pests and inaccessible to mice/rodents, livestock and domestic animals.

8. Mechanical damage and compacting of the harvested medicinal plant/herbal drug that would result in undesirable quality changes must be avoided. In this respect, attention must be paid to

(a) overfilling of the sacks,

(b) Stacking up of sacks.

9. Freshly harvested medicinal plants/herbal drugs must be delivered as quickly as possible to the processing facility in order to prevent thermal degradation.

10. The harvested crop must be protected from pests, mice/rodents, livestock and domestic animals. Any pest control measures taken should be documented.

Primary processing:

Harvested or collected raw medicinal plant materials should be promptly unloaded and unpacked upon arrival at the processing facility. Prior to processing, the medicinal plant materials should be protected from rain, moisture and any other conditions that might cause deterioration. Medicinal plant materials should be exposed to direct sunlight only where there is a specific need for this mode of drying.

Medicinal plant materials that are to be used in the fresh state should be harvested/collected and delivered as quickly as possible to the processing facility in order to prevent microbial fermentation and thermal degradation.

The materials may be stored under refrigeration, in jars, in sandboxes, or using enzymatic and other appropriate conservation measures immediately following harvest/collection and during transit to the end-user. The use of preservatives should be avoided if used, they should conform to national and/or regional regulations for growers/collectors and end-users.

Medicinal plant materials that are to be employed fresh should be stored under refrigeration, in jars, in sandboxes, or using enzymatic or other appropriate conservation measures, and transported to the end-user in the most expeditious manner possible.

The use of preservatives should be avoided. If used, this should be documented and they should conform to national and/or regional regulatory requirements in both the source country and the end-user country.

All medicinal plant materials should be inspected during the primary-processing stages of production, and any substandard products or foreign matter should be eliminated mechanically or by hand.

For example, dried medicinal plant materials should be inspected, sieved or winnowed to remove discoloured, mouldy or damaged materials, as well as soil, stones and other foreign matter. Mechanical devices such as sieves should be regularly cleaned and maintained.

All processed medicinal plant materials should be protected from contamination and decomposition as well as from insects, rodents, birds and other pests, and from livestock and domestic animals.

Drying:

When medicinal plant materials are prepared for use in dry form, the moisture content of the material should be kept as low as possible in order to reduce damage from mould and other microbial infestation.

Medicinal plants can be dried in a number of ways:

1. In the open air (shaded from direct sunlight);

2. Placed in thin layers on drying frames, wire-screened rooms or buildings.

3. By direct sunlight, if appropriate.

4. In drying ovens/rooms and solar dryers.

5. By indirect fire; baking; lyophilization; microwave; or infrared devices.

6. Vacuum drying

7. Spray dryer: Examples: Papaya latex and pectin's, etc.

When possible, temperature and humidity should be controlled to avoid damage to the active chemical constituents. The method and temperature used for drying may have a considerable impact on the quality of the resulting medicinal plant materials.

For example, shade drying is preferred to maintain or minimize loss of colour of leaves and flowers; and lower temperatures should be employed in the case of medicinal plant materials containing volatile substances. The drying conditions should be recorded. In the case of natural drying in the open air, medicinal plant materials should be spread out in thin layers on drying frames and stirred or turned frequently.

In order to secure adequate air circulation, the drying frames should be located at a sufficient height above the ground. Efforts should be made to achieve uniform drying of medicinal plant materials and so avoid mould formation.

Drying medicinal plant material directly on bare ground should be avoided. If a concrete or cement surface is used, medicinal plant materials should be laid on a tarpaulin or other appropriate cloth or sheeting. Insects, rodents, birds and other pests, and livestock and domestic animals should be kept away from drying sites.

For indoor drying, the duration of drying, drying temperature, humidity and other conditions should be determined on the basis of the plant part concerned (root, leaf, stem, bark, flower, etc.) and any volatile natural constituents, such as essential oils.

If possible, the source of heat for direct drying (fire) should be limited to butane, propane or natural gas, and temperatures should be kept below 60°C. If other sources of fire are used, contact between those materials, smoke and medicinal plant material should be avoided.

Vacuum drying:
This is conducted in steam- heated ovens with perfect closure, and a pump is used to exhaust the air. The low pressure maintained within the oven ensures rapid and complete drying.

Example:
Digitalis

Advantages of vacuum drying:
(i) Rapid drying.
(ii) Relatively low temperature.
(iii) Cleanliness and freedom from odour and dust.
(iv) Independence of climate conditions.
(v) Control of temperature.
(vi) Elimination, of risk of fire.

- **Storage of crude drug:**

1. Storage facilities for medicinal material should be well aerated, dry and protected from light, and, when necessary, be supplied with air-conditioning and humidity control equipment as well as facilities to protect against rodents, insects and livestock.

2. The floor should be tidy, without cracks and easy to clean. Medicinal material should be stored on shelves which keep the material a sufficient distance from the walls; measures should be taken to prevent the occurrence of pest infestation, mould formation, rotting or loss of oil; and inspections should be carried out at regular intervals.

3. Continuous in-process quality control measures should be implemented to eliminate substandard materials, contaminants and foreign matter prior to and during the final stages of packaging. Processed medicinal plant materials should be packaged in clean, dry boxes, sacks, bags or other containers in accordance with standard operating procedures and national and/or regional regulations of the producer and the end-user countries.

4. Materials used for packaging should be non-polluting, clean, dry and in undamaged condition and should conform to the quality requirements for the medicinal plant materials concerned. Fragile medicinal plant materials should be packaged in rigid containers.

5. Dried medicinal plants/herbal drugs, including essential oils, should be stored in a dry, well-aerated building, in which daily temperature fluctuations are limited and good aeration is ensured

6. Fresh medicinal plant materials should be stored at appropriate low temperatures, ideally at 2-8°C; frozen products should be stored at less than -20°C.

7. Small quantity of crude drugs could be readily stored in air tight, moisture proof and light proof container such as tin, cans, covered metal tins or amber glass containers.

8. Wooden boxes and paper bags should not be used for storage of crude drugs.

Factors Influencing the Cultivation of Medicinal Plants

- **The following factors are influencing of cultivation:**

1. Light:
Light is the only external source of energy for the continuation of life of the plant. It influences photosynthesis, opening and closing of stomata, plant movements, seed germination, flowering and vegetative growth like tuber formation. Dry sunny weather increases the proportion of glycosides in digitalis and of alkaloids in belladonna.

2. Temperature:
Temperature is the major factor influencing the cultivation of the medicinal plant. The sudden decrease in temperature caused the formation of the ice crystals in intercellular spaces of the plant. As a result, water comes out of the cells and ultimately plants die due to drought and desiccation. The ice crystals also mechanical injury to the cells temperature stimulates the growth of seedlings. Water absorption decreases at low temperatures. The rate of photosynthesis is affected by change in temperature. The rate of respiration increases with increase in temperature. Examples; Cinchona- 58-73°F; Tea- 75-90°F and coffee- 55-70°F

3. Atmosphere humidity:
It is present in the form of water vapors. This is called atmospheric humidity. Clouds and fog are the visible forms of humidity. The major sources of water vapors in the atmosphere are evaporation of water from earth surface and transpiration from plants the major effect of humidity on plant life and climate. Evaporation of water, its condensation and precipitation depend upon relative humidity and humidity affects structure, form and transpiration in plants.

4. Altitude:

The altitude is the most important factor influencing of cultivation of medicinal plants. The increase the altitude, the temperature and atmospheric pressure decreases while the wind velocity, relative humidity and light intensity increases.

Thus, as the climatic conditions change with height, they also produce change in the vegetation pattern. The bitter constituents of Gentiana lutea increase with altitude, whereas the alkaloids of Aconitum nacelles and lobelia inflate and oil content of thyme and peppermint decrease. Pyrethrum gives the best yield and Pyrethrum at high altitude. Examples: Tea- 9500-1500 meters; cinnamon- 300-1000 meters and saffron- up to 1250 meters.

5. Rainfall:

The rainfalls are most important factor influencing of cultivation of medicinal plants. The main source of water for the soil is rain water. Rainfall and snowfall have a large effect the climate condition. The water from rainfall flows into the rivers and lakes percolates into the soil to form ground water and remaining is evaporated. The minerals in the soil get dissolved in water and are then absorbed by plants. Water influences morphological and physiology of plant. Examples: continuous rain can lead to a loss of water- soluble substance from leaves and root by leaching; this is known to apply to some plants producing glycoside and alkaloids.

- **Soil:**

Soil is defined as surface layer of the earth, formed by weathering of rocks. The soil is formed as a result of combined action of climate factors like plants and microorganisms. The soil should contain appropriate amounts of nutrients, organic matter and other elements to ensure optimal medicinal plant growth and quality. Optimal soil conditions, including soil type, drainage, moisture retention, fertility and pH, will be dictated by the selected medicinal plant species and/or target medicinal plant part.

The soil made of five components:

(i) Mineral matter.

(ii) Soil air.

(iii) Soil water.

(iv) Organic matter or humus.

(v) Soil organisms

Plants depend on soil for nutrients, water supply and anchorage. Soil influences seed germination, capacity of plant to remain erect, form, vigour and woodiness of the stem, depth of root system, number of flowers on a plant, drought, frost, etc.

Classification of soil particles:

S. No.	Type of Particle	Size (mm in diameter)
1.	Clay	Less than 0.002
2.	Silt	0.002-0.02
3.	Fine sand	0.02-0.2
4.	Coarse Sand	0.2-2.0
5.	Stone or Gravel	2.0 and more

1. Clay
2. Loamy.
3. Silt loam
4. Sandy loam
5. Sandy soil.
6. Calcareous soil.

1. Clay soil:

Clay particle are very small. These fit together very closely and therefore, leave very less pore space. These spaces get filled up with water very easily. Hence, the clay soil becomes quickly waterlogged. Such soil has practically no air, therefore, the plants growing in these soils are not able to absorb water. This soil known as physiologically dry soil clay soil is plastic and forms a colloid when moist. It cracks and shrinks when conditions are dry the soil rich in nutrient elements and therefore, acts as a negatively charged colloidal system.

2. Sandy soil:

Sand particles are large sized. These leave large pore spaces which do not have capillary action and therefore, water is not retained by them. Most of the water is quickly drained off and reaches deep into the soil. As a result, roots spread and also reach a great depth. The sandy soil is poor in nutrient elements; it is less fertile and plants growing in this soil have less dry weight.

3. Loam soil:

The mixture of clay, silt and sand is known as loam. Loam is very useful for growth. It is fertile soil because it contains available nutrient elements in sufficient amounts. It has a high water retention capacity and appropriate amount of soil air is also present. The plants growing in loam are vigorous and have very high weight.

4. Sandy loam:

The amount of sand particles is more than other types of loam.

Silt loam:

Silt loam is considered to be the most fertile as it contains more amount of organic substances than others.

➢ Fertilizer:

The fertilizers are two types:
1. Biological origin fertilizer.
2. Synthetic fertilizers
3. Chemical fertilizer

1. Biological origin fertilizer:
Soil is generally poor in organic matter and nitrogen. The substances of biological origin used as fertilizer are thus selected if these could provide the elements required. These are two types:

(i) Green manures:
Manure is material, which are mixed with soil. These supply almost all the nutrients required by the crop plants. This results in the increase in crop productivity.

Manures are three types:

Farmyard manure:
This is a mixture of cattle dung and remaining unused parts of straw and plants stalks fed to cattle.

Composited manure:
This consists of a mixture of rotted or decomposed and useless parts of plants and animals.

Green manure:
It is a herbaceous crop ploughed under and mixed with the soil while still green to enrich the soil. The plants used as green manure are often quick growing. These add both organic as well as nitrogen to the soil. It is also forms a protective soil cover that checks soil erosion and leaching. Thus, the crop yield increases by 30-50%.

(ii) Bio-fertilizer:
It can be defined as biologically active products or bacteria, algae and fungi which useful in bringing about soil nutrient enrichment. These mostly include nitrogen fixing microorganisms.

Some of the Bio-fertilizer are as follows:
(i) Legume- Rhizobium symbiosis
(ii) Azolla- Anabaena symbiosis.
(iii) Free- living bacteria.
(iv) Loose association of nitrogen fixing bacteria.
(v) Cyanobacteria (blue green algae).
(vi) Mycorrhiza.

1) Ectomycorrhizae. Increase the interface surface between plant root and soil. Mycorrhizae absorb and store nitrogen, phosphorous, potassium and calcium.
2) Endomycorrhizae

2. **Chemical fertilizers:**

(i) Macronutrients:

a) Nitrogen
b) Phosphorous
c) Potassium
d) Calcium
e) Magnesium
f) Sulpher.

(ii) Micronutrients:

a. Iron
b. Magnese
c. Zinc
d. Boron
e. Copper
f. Molybdenum

Carbon, oxygen, hydrogen and chorine are provided from water and air.

Examples:
Urea, Potash etc.

- **Polyploidy:**

Plants whose cells contain two sets of chromosomes, derived at fertilization from the union of one set from the pollen and one set from the egg cells, are described as diploids and denoted by "2n". The term polyploidy is applied to plants with more than two sets of chromosomes in the cells; when four sets are present the plants are described as tetraploids and denoted by "4n".

Tetraploidy is induced by treatment with colchicine, which inhibits spindle formation during cell division, so that the divided chromosomes are unable to separate and pass to the daughter cells. The two sets of chromosomes remain in one cell and this develops to give tetraploids plant.

Treatment with colchicine may be applied in various ways, but all depend on the effects produced in the meristem. The seeds may be soaked in a dilute solution of colchicine, or the seedlings, the soil around the seedling or the young shoot treated with colchicine solution. Fertile seed and robust, healthy tetraploid plants were obtained, the tetraploid condition being indicated by the increased size of the pollen grains and stomata; chromosome counts in root-tip preparations confirm the tetraploid condition.

The average increase in alkaloids content compared with diploid plants of Datura stromonium and Datura tatula was 68%, with a maximum increase of 211.6%. Similar results were obtained with Atropa belladonna and Hyoscyamus niger, the average increase in belladonna being 93%.

Increased Alkaloidal content of tetraploids plants has been confirmed for Datura stromonium and Datura tatula. The diploid of Acorus calamus is 2.1% of volatile oil content but they are converted into tetraploid, they produce 6.8% of volatile oil contents.

- **Mutation:**

Definition: Sudden heritable change in the structure of a gene on chromosome or change the chromosome number.

Type 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 mutations.

Mutations can be artificially produced by certain agents called mutagens or mutagenic agent. They are two types:

a.) Physical mutagens:

(i) Ionizing radiations:

X-rays, gamma radiation and cosmic rays.

(ii) Non-ionizing radiation:

U.V. radiation,

b.) Chemical mutagens:

1) Alkylating and hydroxylating agents:

Nitrogen and Sulphur mustard; methyl and ethylsulphonate, ethylethane sulphonates.

2) Nitrous acid:

3) Acridines:

Acridines and proflavines. Ionizing radiation cause breaks in the chromosome. These cells then show abnormal cell divisions. If these include gametes, they may be abnormal and even die prematurely. Non-ionizing radiation like Ultra Violet rays are easily absorbed by purine and pyrimidines. The changed bases are known as photoproducts. U.V. rays cause two changes in pyrimidine to produce pyrimidine hydrate and pyrimidine dimmers. Thymine dimer is a major mutagenic effect of U.V. rays that disturbs DNA double helix and thus DNA replication.

Example:
Penicillin, as an antibiotic was first obtained from Penicillium. However, the yield was very poor and the preparation was commercially expensive. Since then mutants with higher yield of penicillin have been selected and produced. Penicillium chrysogenum used in the production of penicillin yielded about 100 units of penicillin per ml of culture medium.

By single-spore isolation, strains were obtained which yielded up to 250 units per ml of medium, X-ray treatment of this strain gave mutants which produce 500 units per ml and ultraviolet mutants of latter gave strain which produced about 1000 unit per ml. Similarly, improvements have been obtained with other antibiotic- producing organism. Mutant strains of Capsicum annum with increasing yields (20-60%) of capsaicin have been isolated from M_3 and M_4 generations originating from seed treated with sodium azide and ethyl methane sulphonate.

- **Hybridization:**

It is mating or crossing of two genetically dissimilar plants having desired genes or genotypes and bringing them together into one individual called hybrid. The process through which hybrids are produced is called hybridization.

Hybridization particularly between homozygous strains, which have been inbred for a number of generations, introduces a degree of heterozygosis with resultant hybrid vigour often manifest in the dimensions and other characteristic of the plants. A hybrid is an organism which results from crossing of two species or varieties differing at least in one set of characters.

The following steps are involved in hybridization of plant:

1. Choice of parents:
The two parents to be selected, at least one should be as well adopted and proven variety in the area. The other variety should have the characters that are absent in the first chosen variety.

2. Emasculation:
Removal of stamens or anthers or killing the pollen grains of a flower without affecting the female reproductive organs is known as emasculation. Emasculation is essential in bisexual flowers.

3. Bagging:
Immediately after emasculation, the flowers or inflorescences are enclosed in bags of suitable sizes to prevent random cross-pollination.

4. Pollination:
In pollination, mature, fertile and viable pollens are placed on a receptive stigma. The procedure consists of collecting pollens from freshly dehisced anthers and dusting them on the stigmas of emasculated flowers.

5. Raising F_1 plants:

Pollination is naturally followed by fertilization. It results in the formation of seeds. Mature seeds of F_1 generation are harvested dried and stored these seeds are grown to produce F_1 hybrid. Hybrids of cinchona yield more amount of quinine. A hybrid developed by crossing Cinchona succirubra with Cinchona ledgering yields a bark, which contains 11.3% of alkaloids. The parent species produced 3.4% and 5.1% of alkaloids, respectively.

Pyrethrum hybrids have been used for Pyrethrum production; these hybrids are produced either by crossing two clones assumed to be self- sterile or planting a number of desirable clones together and bulking the seed. The hybridization of plant to increase the Pyrethrin contents.

- **Greenhouse effect:**

Normal conditions sun rays reach the earth and heat is radiated back into space. However, when carbon dioxide concentration increases in the atmosphere, it forms a thick cover and prevents the heat from being re-radiated. Consequently, the atmosphere gets heated and the temperature increases.

This is called greenhouse effect. In recent past, amount of carbon dioxide has increased from 290 ppm to 330 ppm due to cutting of forests and excessive burning of fossil fuels. The rate at which the amount of carbon dioxide in the atmosphere is increasing, it is expected to cause rise in global temperature.

The global warming by two or three degrees would cause polar ice caps to melt, floods in coastal areas, change in hydrologic cycle and islands would get submerged. The following gases produce greenhouse effect like carbon dioxide, sulphur dioxide, oxide of nitrogen, chlorofluorocarbons, etc.

- **Plant Growth Regulators (PGR):**

Plant Growth Regulators

Plant growth regulators (also called plant hormones) are numerous chemical substances that profoundly influence the growth and differentiation of plant cells, tissues and organs. Plant growth regulators function as chemical messengers for intercellular communication . There are currently five recognized groups of plant hormones: auxins, gibberellins, cytokinins, abscisic acid (ABA) and ethylene. They work together coordinating the growth and development of cells. Ethylene is mainly involved in abscission and flower secscence in plants and is rarely used in plant tissue culture. In addition to the five principal growth regulators, two other groups sometimes appear to be active in regulating plant growth, the brassinosteroids and polyamines.

Auxins:

Auxins stimulate cell elongation and influence a host of other developmental responses, such as root initiation, vascular differentiation, tropic responses, apical dominance and the development of auxiliary buds, flowers and fruits. Auxins are synthesized in the stem and root apices and transported through the plant axis. The principal auxin in plants is indole-3-acetic acid (IAA). Several other indole derivatives, all as precursors to IAA, are known to express auxin activity, probably by converting to IAA in the tissue. Auxins in plant tissue culture are used to induce callus from explants , and cause root and shoot morphogenesis . Auxins are often most effective in eliciting their effects when combined with cytokinins.

Cytokinins:

Cytokinins are able to stimulate cell division and induce shoot bud formation in tissue culture. They usually act as antagonists to auxins. (Cytokinins are N6 substituted derivatives of the nitrogenous purine base adenine.) Cytokinins most used in tissue culture include zeatin, adenine, 6-(g,g-dimethylallylamino)purine (2 iP) and kinetin. Cytoknins often inhibit embryogenesis and root induction.

Gibberellins:

The main effect of gibberellins in plants is to cause stem elongation and flowering. They are also prominently involved in mobilization of endosperm reserves during early embryo growth and seed germination. Gibberellins are an extensive chemical family based on the ent-gibberellane structure. There exit over 80 different gibberellin compounds in plants but only giberrellic acid (GA3) and GA4+7 are often used in plant tissue culture. In tissue culture, gibberellins are used to induce organogenesis, particularly adventitious root formation.

***Abscisic Acid*:**

Abscisic acid (ABA) in plants is a terpenoid involved primarily in regulating seed germination, inducing storage protein synthesis and modulating water stress. In plant tissue culture, it is used to help somatic embryogenesis, particularly during maturation and germination.

Ethylene:

Ethylene is a simple gaseous hydrocarbon with the chemical structure H2C=CH2. Ethylene is apparently not required for normal vegetative growth. However, it can have a significant impact on development of root and shoots. Usually, ethylene is not used in plant tissue culture.