UNIT I - ECONOMIC IMPORTANCE OF ALGAE-FRESH WATER AND MARINE ALGAE - MACRO AND MICRO ALGAE -OCCURRENCE– DISTRIBUTION-CULTIVATION-IMPORTANCE OF CULTIVATION

Objectives:

- To understand the economical important value of algae.
- To understand the distribution, availability and utilization of both fresh water and marine water algae.
- To trace the occurrence and distribution of micro-macro algae.

To familiarize technique of algal cultivation and their importance

1.1. General Characteristics of Algae:

The algae comprise a large heterogeneous group of plants. The term algae (Alga) mean different things to diverse people. Algae are plant like organism that contain chlorophyll and other pigments that trap light from sun. This light energy converted into food molecules in a process called photosynthesis. The general characters of algae may be listed briefly as follows:

- Algae range from single celled forms to aggregations of cells, to filaments or parenchymatous thalli.
- Many unicellular forms are motile, and integrate confusingly with protozoa.
- Even complex multicellular forms show a low level of differentiation compared with other groups of plants, with only most advanced posses elementary conducting tissues.
- Range of morphology is extremely varied; brown algae (Phaeophyceae) attain a size comparable to a small tree. However, relative simplicity of algae exhibit high degree of complexity at cellular level.

- Algae reveal high degree of variation in reproduction vegetative, asexual and sexual processes are involved and in many forms an alternation of generations occurs.
- In greater part of algae motile zoospores or gametes are formed.
- Various types of sexual processes (isogamy, anisogamy and oogamy) met with in different groups of algae have evolved independently. However, sexual reproduction is altogether absent in prokaryotic algae (Cyanophyceae). Sexual reproduction in Rhodophyceae is the most complex in the plant kingdom.

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- Biochemically and physiologically algae are similar in many respects to other plants. They possess basic biochemical pathways; all possess chlorophyll a, and have carbohydrates and protein as end products of photosynthesis as in higher plants. Therefore, many algae are ideal experimental organisms in that, due to their small size and easy manipulation, they can be studied under controlled conditions in the laboratory.
- Algae are ubiquitous, occur in all habitable environments.

1.1.1. Economic Importance of Algae

Algae are distributed over shallow as well as deep waters, while seaweed inhabits shallow waters. Fresh water algae thus refer to algae that grow in such fresh water ecosystem. They are microscopic, small unicellular organisms; some of them form colonies and thus reach sizes visible to naked eye. These organisms dispersed throughout the water and may cause considerable turbidity at high densities. The term fresh water usually is used for all water bodies that are non-marine. Seaweed is a term applied to multicellular marine algae which are large enough. Some can grow up to 60m in length. Seaweed includes red, brown and green algae. Algae are of importance in many fields including agriculture, pharmaceutical and industry as discussed below:

1.1.2. Role in Industry

Algae are useful as source of commercial products. Industrial utilization of algae, particularly sea weeds, dates back to many hundreds of years. Four major products derived commercially include agar-agar, carrageenan alginic acid, and diatomite.

1.1.3. Agar-agar

Macro-algae, mainly Gelidium sp. and Gracilaria sp., but also Gelidiella and Ahnfeltia sp., are used as a source of hydrocolloid agar, an unbranched polysaccharide. Its melting point is between 90 and 100°F.

At lower temperature it changes into a solid. It is insoluble in cold but soluble in hot water. The gelatinous agar is used as a standard medium in almost all microbiological, molecular biological or medical laboratories. Moreover, agar is used as food (ice creams, soups, jellies etc.), pharmaceuticals and feed as a gelling agent. It is also used as a vegetarian gelatin substitute, as a clarifying agent in brewing industry and fermentation industries and as a laxative.

1.1.4. Alginic acid

Alginates, salts of alginic acid and their derivatives, are extracted from cell walls of brown macro algae like Laminaria sp, Macrocystis pyrifera, Ecklonia spp., Lessonia sp., Durvillaea sp., and Ascophyllum nodosum. These carboxylated polysaccharides are used for a wide variety of applications in food production as thickeners, stabilizers, emulsifier, and gelling agents. Alginates are required for production of dyes for textile printing, latex paint, and welding rods. Water absorbing properties of alginates are utilized in slimming aids and in production of textiles and paper. Calcium alginate is used in burn dressings that promote healing and can be removed pain lesly. Alginates are of great value as emulsifiers, as gelling agents in confectionary, powders, paints and ice cream. In addition, alginates are widely used in prosthetics and dentistry for making molds, and, in addition, they are components of cosmetics.

1.1.5. Carrageenan

Carrageenan is cell wall polysaccharide extracted from red macro-algae including Kappaphycus sp., Eucheuma sp., Betaphycus gelatinum, Chondrus crispus, Gigartina spp., Mozzarella spp., and Sarcothalia sp. There are three basic types of carrageenan with somewhat different characteristics, kappa carrageenan, iota carrageenan and lambda carrageenan. Carrageenan is used as gelling agents, stabilizers, text rants, thickeners, and viscosities for a wide range of food products. It is also used to stabilize emulsions and as remedy for cough. Its use as a component of tooth pastes, deodorants cosmetics and paints is no less important. Carrageenan when cooked with milk and mixed with vanilla forms a tasty dish called blancmanges. Japan produces about 100 tons of iodine annually from Kelps. Chief genera employed for the purpose are Laminaria, Ecklonia, Fucus and Eugenia. Kelps are a source of soda and potash. Red algae Rhodomella and Polysphonia are source of bromine.

1.1.6. Glue manufacturing

Glue manufacturing is an important algal industry in Japan. Red alga Gloeopetis furcata is used for sizing paper and cloth. Funori and Funorin are used in textile industry as glue and sizing agent.

1.1.7. Diatomite (or) Kieslguhr

Fossil deposits of unicelled alga, diatoms are formed due to siliceous cell wall called Diatomite or diatomaceous earth. It is a rock-like deposit formed from indestructible, siliceous frustules (cell walls) of diatoms that had collected over millions of years on floors of seas to form oceanic sediment. It is also used in insulation of refrigerators, boilers, hot and cold pipes, hollow tile bricks for construction of constant temperature rooms, sound proof rooms, in packing corrosive chemical liquids, in polishes for metals, insulation for pipes and furnaces, in the manufacture of dynamite, a constituent of some tooth powders, bleaching powders, a reinforcing agent in both concrete and rubber. It is also used as a base on automobile and silver polishes. Its use in wine and paper industries

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1.1.8. Role in Agriculture

Blue-green algae are chief agents for nitrogen fixation in rice fields. They increase fertility of the soil by fixing atmospheric nitrogen. Some of important nitrogen fixing blue-green algae are Oscillatoria princeps, O. forrnosa, and species of Anabaena, Spirulina, Nostoc, and Cylindrospermum. Practical application of algae as fertilizers is the seeding of rice fields with nitrogen-fixing species to increase the nitrogen content of soil.

1.1.9. Algae used as fertilizers

Algae are rich in minerals and vitamins. So they also used as fertilizer which helps to restore the level of nitrogen present in the soil. Agricultural utilization of sea weeds as manure is confined to the farm land near the coastal regions. Large brown and red algae are used as organic fertilizers. They are richer in potassium but not as good as in nitrogen and phosphorous. Sea weed may be used direct and ploughed. The direct application of sea weeds is in vogue close to the sea in some countries such as France, Ireland and Sri Lanka, for earth vegetables such as potatoes, and turnips, and coffee plantations. In Japan, algae are used in the rice fields whereas, in China for groundnuts and sweet potatoes. In India Turbinaria is used as a fertilizer around palm trees. In Rajasthan Anabaena and Spindina are employed as green manure. Sea weeds are used as compost, they are burnt and ashes scattered over the farm lands. Sea weeds are processed into a sea weed meal for transport inland. Concentrated liquid extracts of sea weeds are sold as liquid fertilizers and also as insecticides. Sea weed products aid in binding sandy soils, help to break down clayey soils and thus promote a good crumb structure.

In some countries Lithothamnion and Lichophylhan are used it in place of lime. Similarly, Chara is encrusted with calcium carbonate. Chlorella helps to aerate water by removing carbon dioxide and restoring supply of

oxygen in photosynthesis therefore used in sewage disposal plants as they indirectly, help aerobic bacteria to decompose raw sewage.

1.1.10. Algae and Space Travel

Biologists are working on systems that would complete recycling of biological materials during space travel. In this regard, Chlorella and Scenedesmus have been selected as potential algae for providing high amount of oxygen to the astronauts. Organic waste and CO2 can be used by the algae. It has been considered beneficial to use microscopic, unicellular, algae such as Chlorella and Synechococcus as a possible food source in anticipated space flight. It multiplies rapidly and thus will synthesize a rich harvest of food utilizing carbon dioxide and liberating sufficient oxygen as a by-product for use. As a source of nitrogen for protein synthesis it will assist in decomposition of human wastes.

1.1. 11. Algae as Food

Algae are rich source of carbohydrates, fats, proteins, and vitamins A, B, C, and E as well as minerals like iron, potassium, magnesium, calcium, manganese, and zinc. Hence, people of countries like Ireland, Scotland, Sweden, Norway, North and South America, France, Germany, Japan, and China uses it as food ingredient. Algae are important as a source of food of fishes, aquatic amphibia, mammals and other animals. Man's dependence on fish and other aquatic animals to supplement this diet is a well known fact. Indirectly, therefore, algae are of great value to man.

Biologists however, suggest that algae might be raised by man as a source of food. In fact in some coastal parts of the world they are used directly as food. Spirogyra and Oedogonium are valuable genera in India and Ulva in Europe. Colonies of Nostoc are boiled and used as food in Brazil. Porphyra is considered a tasteful dish in England and is a common item of diet in Korea, and China. A red alga Rhodymenia paimata is used as food and also as a salty confection named DULSE. The algae are considered rich in proteins, fats and vitamins A. B, C and E. The vitamins A and D are commercially obtained from livers of shark and similar fish originally come from synthesis by the plankton algae particularly form the food of the fish.

Some countries have even developed industries for processing weeds such as Ascophylluem, Fucus, and Laminaria into suitable cattle feed. Macrocystis sp. is also used being rich in vitamins A and E. Red alga, Rhodymenia is used as a cattle fodder in France. Milk-yielding capacity of the cattle is enhanced when dried sea weeds such as Pelvetia forms an ingredient in cow feed. Similarly, increased butter and fat content of milk

is reported from cattle whose diet is supplemented with sea weed meal. In China Sargassum is used as fodder.

1.1.12. Role of Algae in Medicine

Many sea weeds contain a high percentage of iodine and thus are employed in pre unknown of various goiter medicines or are administered directly as a powdered weed. Laminaria sp such as L. Japonica and L. religiosa have a high value of iodine. Among the green algae Codium intricatun contains a considerable quantity of iodine. Red algae such as Gelidium and Grateloupia contain a medium amount of iodine. First algal antibiotic chlorellin was extracted from chlorella. Antibiotic substances have been also obtained from Rhodomella, Ascophyllum, Laminaria, Polysphonia, Cladophora etc. An effective vermifuge is obtained from extracts of Digenea, Codium, Alsidium and Durvillea. A few algae are a source of antibiotics which inhibit the growth of other bacteria. Cladophora and Lyngbya extracts possess antiviral properties, kills bacteria (Pseudomonas and Mycobacterium).

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1.1.13. Role of Algae in origin of petroleum and fuel gas

Fuels such as petroleum and natural gas have their origin in the organic matter in marine environment. Minute algae constituting the plankton trap sun's energy during photosynthesis. The organic compounds derived from the dead bodies of plants and animals constituting the plankton gradually accumulated at the bottom of the ocean and were buried in the course of time by sedentary action. In the environment free from oxygen these compounds were decomposed and convened into oil and gas. The natural gas associated with oil is largely methane which is produced by the action of methane producing bacteria upon organic compounds.

1.1.14. Algae and sewage disposal

Sewage is the foul domestic and industrial liquid waste which is deficient in oxygen but abounds in dissolved and suspended organic and inorganic materials. It harbors microorganisms for decay and decomposition. The use of small green algae such as Chlamydomonas, Chlorella and Euglena in large, shallow tanks of effluent has proved a rapid, cheap and effective means of converting dangerous and expensive waste into an odorless and expensive fertilizer. Tanks promote growth of the algae which increase at the expense of mineral nutrients present in sewage. Photosynthetic activity of the algae produces abundant oxygen which is used by the micro-organisms responsible for decomposition of remaining organic matter in sewage.

1.1.15. Algae and Pisciculture

In fish farming, an alga has a vital role in the production process. Fish used plankton and zooplankton as a food. It helps in maintaining the

health of the marine ecosystem because algae are naturally absorbent of carbon dioxide and also provide oxygen to the water.

1.1.16. Reclaiming Alkaline

Blue Green Algae helps in the reduction of high concentration of alkalinity in the soil. Besides, algae act as binding agents against natural processes such as erosion.

1.1.17. Biological indicator

Algae are very sensitive. If there is a slight change in the environment their pigments changes or might get died. The water pollution is checked with the help of algae like Euglena and Chlorella.

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1.1.18. Lime stone formation

Some red algae, a few green and blue green forms contain CaCO3 in their cell wall. They are responsible for the lime stone deposits around the hot water springs. The calcareous red algae play important role in the formation of coral reef as also for the formation of lime stone rocks in the sea.

1.1.19. Algae and water supplies

The algae are of negative value as well. In the rainy season and spring the blue-green algae, green algae, golden brown algae and the diatoms become so abundant that the water in the ponds, lakes and reservoirs becomes cloudy and assumes a yellowish or greenish tinge. Sometimes a floating yellowish green scum may develop on the surface of water popularly called as Water Blooms. Such concentrations of algae impart unpleasant odor, oily and fishy taste to the drinking water. Some of the blue green algae (Macrocystis, Aphanizomenon and others) produce toxic protein - poisonous to fish, cattle, sheep and other animals.

1.2.1. Fresh and marine water algae

Algae from diverse terrestrial water bodies, some of which are not entirely “fresh” are also however included as freshwater algae. Although, the oceanic forms are clearly saline (≈ 35 g salts L^{-1}) and most lakes are relatively dilute, there is enormous variation in chemical composition of the non marine habitats. Conditions in lakes and rivers vary not only in salinity, but also in size, depth, transparency, nutrient conditions, pH, pollution, and many other important factors. Ecologists use the term “inland algae” to encompass a wide range of aquatic ecosystems. However, this term may be unsatisfactory, because algae occupy other habitats such as snow, soil, cleft and crevices and symbiotic associations.

Organisms grouped as freshwater fall into large, but ecologically meaningful collection of environments: all habitats that are at least slightly wet, other than oceans and estuaries. One reason for such a broad scope is that inland saline lakes, snow and ice, damp soils, and wetlands are studied by phycologists and ecologists who also examine more traditional freshwater environments. Some genera with terrestrial species, such as Vaucheria, Nostoc, Chlorella, and Prasiola, also have species found principally in streams or lakes. In North America, variety of freshwater habitats colonized by algae is very rich, and offers an enormous and fascinating range of environments.

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Distinction between marine and freshwater habitats is revealed in a variety of algae that occur in these environments. As of now, there is no exclusively freshwater division of algae, but certain groups exhibit greater abundance and diversity within freshwater bodies, especially Cyanobacteria, Chlorophyta and Charophyta. Within green algae, conjugating greens and desmids comprise a very rich collection of species that almost exclusively occupy fresh water. Other groups, such as the diatoms and chrysophytes, are well represented in both spheres. Other groups, particularly the Phaeophytes, Pyrrophyta and Rhodophyta, exhibit greater diversity in marine waters. Most freshwater algae are best described as cosmopolitan, although, there are reports of endemic chrysophytes, green algae, red algae, and diatoms and at least some species of BGA. Many algal taxa have particular environmental tolerances or requirements, and are ecologically restricted, but still geographically widespread. The euglenophyte Colacium is almost exclusively epizooic on aquatic invertebrates, but is widely distributed throughout North America. The chrysophyte Hydrurus foetidus is an exclusive inhabitant of cold mountain streams, but is distributed worldwide. Even specialized taxa such as Basicladia chelonum is restricted mainly to the shells of turtles, has been collected from many habitats throughout eastern North America. The actual distribution of apparently disjunction freshwater species must therefore be viewed with some caution until detailed surveys have been conducted.

Inland waters represent only about 0.02% of all water in the biosphere, and nearly 90% of this total is contained within only about 250 of the world's largest lakes. Nonetheless, it is fresh water that is most important for human consumption and is most threatened by human activities. Algal ecologists play an important role in the understanding of aquatic ecosystems, their productivity, and water quality issues. This chapter examines the habitats of freshwater algae and how differences in these systems affect algal communities.

Marine Algae

Seaweed is a term applied to multicellular, marine algae which are large enough to be seen by the eye unaided. Some can grow to up to 60 meters in length. Seaweeds include members of the red, brown and green algae. They are members of kingdom Protista means they are not Plants. They do not have the vascular system (internal transport system) of plants and do not have roots, stems, leaves and flowers or cones. Like plants they use the pigment chlorophyll for photosynthesis but also contain other pigments which may be colored red, blue, brown or gold. They are divided into three groups: Brown Algae (Phaeophyta) Green Algae (Chlorophyta) Red Algae (Rhodophyta)

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Blue-green algae are not marine algae. They are in a group called cyanobacteria and are more closely related to bacteria. Some cyanobacteria form brown, green, red or purple tufts on coral reefs. The kelps form dense forests which support entire underwater communities providing both food and shelter. Intertidal seaweeds can be exposed to many environmental stresses including drying out when not under water, temperature and salinity changes and wave action.

Seaweeds play roles in many marine communities. They are a food source for many marine animals such as sea urchins and fishes, and form the base of some food webs. They also provide shelter and a home for numerous fishes, invertebrates, birds, and mammals.

1.3.1. OCCURRENCE AND DISTRIBUTION OF MICRO AND MACRO ALGAE

1.3.1. Occurrence

Algae are basically aquatic, growing in waters of low salinity as low as 10 ppm (Fresh water algae), and in marine waters where the solutes are usually 33-40%. Some algae occur in waters of very high salinity may rise to 100% in dry seasons. Algae are occurs in every feasible habitat. One can find algae in practically all places where photosynthesis can be conducted at a rate sufficient for net production, i.e. growth after losses due to metabolism and predation. They are found from polar region to hot deserts, from mountain tops to the limits of phonic zones in oceans and lakes. Some algae form associations with varying degrees of relationship with other organisms, and are found free floating, as plankton in all but the most temporary water bodies. Seaweeds occur attached to the sea-bed principally in the intertidal zones where adequate light can penetrate the water column for supporting their growth. Among the environmental factors light, temperature, salinity, water motion and nutrient availability are the major factors affecting their growth in the natural habitats.

Seaweeds grow in diverse light regimes. The water quality and flood of tides have profound effects on the quantity and quality of light that reaches the seaweeds.

1.3.2. Distribution

The phytoplankton is the most wide spread and extensively studied of all ecological groups of algae. Phytoplankton occurs in all bodies of water where they float freely and largely. While most are independent of shores and benthos, some shallow water species have adopted life history strategies involving benthic resting stages (Fryxell, 1983).All algal divisions except the Rhodophyceae, Charophyceae, and Phaeophyceae contribute species to the phytoplankton flora. In coastal zones the zoospores produced from benthic Phaeophytes and Chlorophytes may at least temporarily add to the plankton.

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Phytoplankton algae are mainly unicellular, though many colonial and filamentous forms occur, especially in fresh water. They range from small flagellates and cocci (< 5 µm in diameter) to colonial forms such as Volvox at 500 µm. Some algae have remarkable tolerance to variations in salinity. For example, Species of Enteromorpha live attached to ships that play both freshwater and oceans. Most algae inhabiting estuaries belong to this category. Some are sub aerial, meaning that they are exposed to the atmosphere rather than being submerged in water.

1.3.3. Habitats

Algae of unusual habitats, such as hot springs, snow, ice, extremely saline conditions etc., the most important feature that unify the vast majority of the algae are that they are the primary producers. They convert the physical energy of the sun's radiation into chemical energy by the process of photosynthesis. As primary producers they provide much of the basis for the food web in aquatic environment. The importance of this production in the aquatic system can be realized from the fact that over 70% of the earth's surface is covered by water. Many algal communities are extremely productive and rival many terrestrial systems.

1.3.4. Seaweeds - Diversity in Marine Ecosystem

The diversity of life in the marine environment is extraordinary; the greatest biodiversity is in the world's oceans, with 34 of the 36 phyla of life represented. The oceans cover more than 70% of the earth's surface and contain more than 300,000 described species of plants and animals. Algae are known to be comparatively sensitive to chemicals. Their ecological position at the base of the aquatic food chain and their essential roles in nitrogen and phosphorus cycling are critical to aquatic ecosystems. Moreover, the alternation of species composition in an

aquatic community as a result of toxic stress may affect the structure and function of the whole aquatic ecosystem. The marine environment represents a treasure of useful products awaiting discovery for the treatment of infectious diseases. Ecological pressures, including competition for space, the fouling of the surface, and predation have led to the evolution of unique secondary metabolites with various biological activities. The important role of these secondary metabolites in the control of infectious micro-organisms was for many years largely unnoticed.

1.4.1. Global Trade and Economic Importance of Algal cultivation

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1.4.1. Introduction

In round the world there are 46 countries where commercial seaweed activity were represent such as China, Japan, Philippines, North Korea, South Korea, Chile, Indonesia, Norway, USA and India. China leads the first place in cultivation of seaweed, especially Laminaria sp, Kappaphycus, Eucheuma, Porphyra, Undaria and Gracilaria. The most valuable crop is the red alga Nori (Porphyra species, mainly Porphyra yezzoensis), used as food and feed in Japan, China and Pacific region. Worldwide aquatic plant production is increased from 7.2 million tons to nearly 13.1 million tons (wet weight), upholding US $ 7 billion world trade in 2013, compared to US $ 350 million trade in 2014. The involvement of cultured seaweeds is 25% of total global aquaculture volume (45,715,559 tons) or nearly 5 % of total volume of world fisheries production (141,798,778 tons) for 2012. In most reported, brown algae with 4,906,280 tons (71 % of total production) followed by the red algae (1,927,917 tons) and a small amount of green algae (33,700 tons).

East and South-East Asian countries contribute almost 99% seaweeds production, with half of the production (3.5 million tons) supplied by China. The majority of amount produced is used locally for food, other than there is a growing international trade. Total EU imports of seaweed in 2012 amounted to 65,000 metric tons with the Philippines, Chile and Indonesia as the biggest suppliers. Significant quantities of Eucheuma are exported by the Philippines, Tanzania and Indonesia to USA, Denmark and Japan.

In several oriental countries like Japan, China, Korea, etc. seaweeds are a staple part of the diet. Agar is widely used in paper manufacturing, culture media, packaging material, photography, leather industry, plywood manufacturing, preservation of food stuffs, dairy industry, cosmetics industry and pharmaceutical industry. Carrageenan is employed in food industry. Its value in the manufacture of sausages,

corned beef, meat balls, and ham preparations of poultry and fish, chocolates, dessert gels, ice creams, juice concentrates, marmalade, sardine sauces is well known. It is also used in the manufacturing of non-food items like beer, air fresheners, textiles, toothpastes, hair shampoos, sanitary napkins, tissues, culture media, fungicides, etc. The applications of alginate find place in frozen foods, pastry fillings, syrups, bakery icings, dry mixes, meringues, frozen desserts, instant puddings, cooked puddings, chiffons, pie and pastry fillings, dessert gels, fabricated foods, salad dressings, and meat and flavor sauces.

1.4.2. Importance of algae cultivation:

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Algae are highly important as oxygen producers by their primary productivity. The primitive atmosphere of the primordial earth was totally different from that of the present day. It contained methane, carbon dioxide, and very little oxygen. In such a situation, when the first photosynthetic organisms namely the algae appeared, they consumed the carbon dioxide and released oxygen into the atmospheric air. The environment became suitable other aerobic organisms to evolve. Thus in way owe our origin and existence to these modest organisms namely algae.

In the aquatic environment, during daylight hours the algae oxygenate the water bad.The association of algae with corals, sponges, marine worms and certain protozoa, is an example for the dependence of the aquatic animals on algae as primary source of food. The carbon components of the exudates originate in the photosynthesis of algae that are symbiotic components of the corals.

Reasons for consumption of algae include food value, flavor, color and texture. The structural carbohydrates of seaweeds are largely indigestible. But some soluble carbohydrates of seaweeds are largely indigestible. But some soluble carbohydrates are metabolized. The protein content of many of the edible seaweeds is 20-25% dry weight. Seaweed are an excellent source of vitamins, including vitamin cat levels equivalent of citrus fruits and Vitamins A, D, B1, B2, Riboflavin,Niacin, Pantothenic acid and folic acid. Seaweeds also provide all the required trace elements for human nutrition.

In addition to seaweeds, micro algae also are used as human food. Chlorella was advocated as a regular part human diet in the early part of 20th century. But later with the advent of the concept of single cell protein (SCP) many micro algae such as Spirulina and Scenedesmus besides chlorella have been found to be ideal candidates for mass cultivation. Algae are also sources of many commercial products such as Alginate (alginic acid), Agar- agar, carrageenan, Iodine and other compounds of Funori and Funorin.

In the early period, mass cultivation of algae faced criticism as a result of high cost of alga production, acceptability problems of this unconventional source of protein, and safety criteria. However, later developments have established the potentiality of algae for the production of a variety of compounds such as polysaccharides, lipids, proteins, carotenoids, pigments, vitamins, sterols, enzymes, antibiotics, pharmaceuticals and several mother fine chemicals or their precursors, as well as hydrogen, hydrocarbons and other biofuels such as methane and alcohol.

At present however, commercial algal production is still restricted to very few plants producing high-value health food or pigments, most located in the Far East and the USA. Health food production systems mainly cultivate the filamentous blue green alga Spirulina and the unicellular green algae Chlorella and -to a lesser extent -the green alga, Scenedesmus. For production of pigments Dunaliella and Spirulina are preferred.

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Numerous attempts have been made to exploit micro algae on a technological scale as a source of the products listed in the table. This common interest has arisen as a result of the need for additional food supplies, increasing problems of waste disposal and the shortage of raw materials and energy resources. Algal cultivation is a special form of agriculture. It is also frequently stated that it is easier to handle and to manipulate the micro algae in such cultivation facility than to handle and manipulate the higher plants in conventional agriculture. At the first glance, this statement may appear correct. When it comes to practice in large cultivation facilities, controlling the factors involved such as fertilization, contamination, time of harvesting etc., becomes really difficult.

Algal cultivation may be considered an intermediate between that of agriculture and fermentation technology. Though it may not be true that micro algae have higher photosynthetic efficiency than conventional crops plants, the algal production units can be more productive in terms of product output than conventional agriculture.

The consideration that primarily dictates the design and construction of algal cultivation facilities is the "Nature of the end product":

Plants for the production of protein or food additives, which must be cheap to build and easy to maintain and therefore suitable for developing countries.

More sophisticated biotechnologies with a high input of equipment and energy for the growth of specialized algal strains intended for the

production of specific bio chemicals such as enzymes, pigments, therapeutic agents etc.,

Review questions:

1. Describe the economic importance of algae in various fields.
2. List out the importance of algal cultivation in our society.
3. Write about the distribution of micro and macro algae in India and abroad
4. Write short notes on the fresh and marine water algae

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UNIT II - MASS CULTIVATION TECHNIQUES OF MICROALGAE - UPSTREAM AND DOWNSTREAM PROCESSES OF ALGAL CULTIVATION - SPIRULINA, DUNALIELLA, HEMATOCOCCUS AND BOTRYOCOCCUS

Objectives

The overall objective of this unit is to understand the micro algae and familiarize with cultivation technique in Spirulina, Dunaliella, Hematococcus and Botryococcus.

2.1. Mass cultivation technique of Micro algae

2.1. Introduction

A culture can be defined as an artificial environment in which the algae grow. In theory, culture conditions should resemble the alga's natural environment as far as possible; in reality many significant differences exist, most of which are deliberately imposed. A culture has three distinct components: a) a culture medium contained in a suitable vessel; b) the algal cells growing in the medium; and c) air, to allow exchange of carbon dioxide between medium and atmosphere.

For an entirely autotrophic alga all that is needed for growth is light + CO2 + H2O + nutrients + trace elements. By means of photosynthesis the alga will be able to synthesize all the biochemical compounds necessary for growth. Only a minority of algal seems, however, to be entirely autotrophic; many are unable to synthesize certain biochemical compounds (certain vitamins, for example) and will require these to be present in the medium. This condition is known as auxotrophy.

Based on their growth characteristics, two kinds of cultures can be defined.

In limited volume (batch) cultures, resources are finite. When the resources present in the culture medium are abundant, growth occurs according to a sigmoid curve, but once the resources have been utilized by the cells, the cultures die unless supplied with new medium. In

practice this is done by sub culturing, i.e. transferring a small volume of existing culture to a large volume of fresh culture medium at regular intervals.

In continuous cultures, resources are potentially infinite: cultures are maintained at a chosen point on the growth curve by the regulated addition of fresh culture medium. In practice, a volume of fresh culture medium is added automatically at a rate proportional to the growth rate of the alga, while an equal volume of culture is removed.

Physical parameters

2.1.1. Temperature

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The temperature at which cultures are maintained should ideally be as close as possible to the temperature at which the organisms were collected; polar organisms (<10°C); temperate (10-25°C); tropical (>20°C). An intermediate value of 18-20°C is most often employed. Temperature controlled incubators usually use constant temperature (transfers to different temperatures should be conducted in steps of 2°C/week), although some models permit temperature cycling. In temperate regions ambient room temperature is generally acceptable for culturing purposes.

2.1.2. Light

Natural light is usually sufficient to maintain cultures in the laboratory. Cultures should never be exposed to direct sunlight (which may cause photo pigment damage), and should therefore be placed next to a north-facing window (in the northern hemisphere). Artificial lighting by fluorescent bulbs is often employed for culture maintenance and experimental purposes. Light intensity should range between 0.2-50% of full daylight (= 1660 µE/s/m2), with 5-10% (c. 80-160µE/s/m2) most often employed. Light quality (spectrum) depends on type of bulb used the most common types being 'cool white' or 'daylight' bulbs. Light intensity and quality can be manipulated with filters. Many microalgae species do not grow well under constant illumination, and hence a light/dark (LD) cycle is used (maximum 16:8 LD, usually 14:10 or 12:12).

2.1.3. Mixing

Mixing of micro-algal cultures may be necessary under certain circumstances: when cells must be kept in suspension in order to grow (particularly important for heterotrophic dinoflagellates); in concentrated cultures to prevent nutrient limitation effects due to stacking of cells and to increase gas diffusion. It should be noted that in the ocean cells seldom experience turbulence, and hence mixing should be gentle.

Soil extract:

Soil extract is prepared by heating, boiling, or autoclaving at 5 to 30% slurry of soil in fresh water or seawater and subsequently filtering out the soil. Soil extract has historically been an important component of culture media. The solution provides macronutrients, micronutrients, vitamins, and trace metal chelators in undefined quantities, each batch being different, and hence having unpredictable effects on microalgae. With increasing understanding of the importance of various constituents of culture media, soil extract is less frequently used. Soil extract should only be used on a non-experimental basis.

Buffers:

NOTES

The control of pH in culture media is important since certain algae grow only within narrowly defined pH ranges, and in order to prevent the formation of precipitates. Except under unusual conditions, the pH of natural seawater is around 8. Because of the large buffering capacity of natural seawater (due to a bicarbonate buffering system, HCO_3 being present at c. 2.2M) it is quite easy to maintain the pH of marine culture media. The buffer system is overwhelmed only during autoclaving, when high temperatures drive CO_2 out of solution and hence cause a shift in the bicarbonate buffer system and an increase in pH, or in very dense cultures of microalgae, when enough CO_2 is taken up to produce a similar effect. As culture medium cools after autoclaving, CO_2 reenters solution from the atmosphere, but certain measures must be taken if normal pH is not fully restored. The pH of seawater may be lowered prior to autoclaving (adjustment to pH 7-7.5 with 1M HCl) to compensate for subsequent increases.

Certain media recipes include additions of extra buffer, either as bicarbonate, Tris (Tris-hydroxymethyl-aminomethane), or Glyoxyglycine to supplement the natural buffering system. Tris may also act as a Cu buffer, but has occasionally been cited for its toxic properties to microalgae. Glycylglycine is rapidly metabolized by bacteria and hence can only be used with axenic cultures. These additions are generally not necessary if media are filter sterilized, unless very high cell densities are expected.

2.1.4. Types of culture vessel

Culture vessels should have the following properties: non toxic (chemically inert); reasonably transparent to light; easily cleaned and sterilized; provide a large surface to volume ratio (depending on organism).

Certain materials which could potentially be used for culture vessels may leach chemicals which have a deleterious effect on algal growth into the medium. The use of chemically inert materials is particularly important when culturing oceanic plankton and during isolation. A list of materials which should be safe, inhibitory, or toxic to algal cultures. Recommended materials for culture vessels and media preparation include:

Teflon (very expensive, used for media preparation);

Polycarbonate (expensive and becomes cloudy and cracks with repeated autoclaving);

NOTES

Polystyrene (cheaper alternative to Teflon and polycarbonate, not autoclavable);

Borosilicate glass (has been shown to inhibit growth of some species).

Materials which should generally be avoided during micro algal culturing include all types of rubber, and PVC. The following types of culture vessel are most commonly used for micro algal culturing (but may not prove suitable for all species):

Erlenmeyer flasks (glass or polycarbonate) with cotton, glass, polypropylene, or metal covers.

Tubes (glass or polycarbonate) with cotton, glass, polypropylene or metal caps.

Polystyrene tissue culture flasks, purchased as single-use sterile units.

2.1.5. Cleaning procedure:

scrub (abrasive brushes not appropriate for most plastics) and soak with warm detergent (not domestic detergents, which leave a residual film on culture ware – use laboratory detergent such as phosphate-free Decon);

rinse extensively with tap water;

soak in 10% HCl for 1 day-1 week (not routinely necessary, but particularly important for new glass and polycarbonate material);

rinse extensively with distilled and finally bidistilled water;

Leave inverted to dry in a clean, dust-free place.

2.1.6. Transfer protocol

The following procedures should always be used when preparing media or transferring cultures:

Work in clean place or preferably in a laminar flow cabinet (cabinet must be turned on at least 30 minutes before transfer; if equipped with UV lamps, leave on overnight prior to use).

Clean working surface with 70% alcohol (ethanol/isopropanol) prior to and after use.

Clean hands with disinfecting soap and rinse with 70% alcohol prior to all operations.

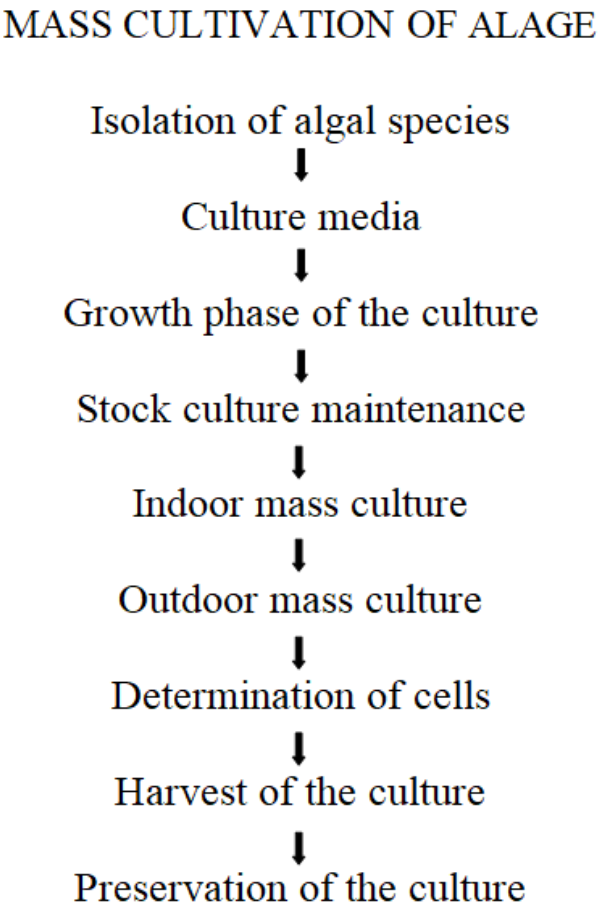
When not using a laminar flow cabinet (and to be safe even when using a cabinet), sterilize (flame) the neck of vessel of origin before and after transfer (not possible with some plastic vessels, which must, therefore, be opened in a laminar flow cabinet).

NOTES

Pipettes must be clean and sterile; use autoclavable tips for repeating pipettes (eg. Gilson), pre-wrapped sterile single use plastic/glass pipettes, or if using non-sterile glass pipettes (with cotton plugs), sterilize in the flame before use.

The frequency of culture transfer depends on species and culture conditions (it is advisable to follow growth for each unfamiliar species to get a feeling). Transfers should be conducted as population growth slows, preferably before population growth ceases (stationary phase). Culture transfers (usually 1-5 % of culture volume into fresh sterile medium) can be conducted directly (pouring, not used when vessel neck is flame sterilized), or more often with a pipette.The flow chart of Mass cultivation of algae are given below.

NOTES



2.1.7. Media preparation

The salinity of the seawater base should first be checked (30-35‰ for marine phytoplankton), and any necessary adjustments (addition of fresh water/evaporation) made before addition of enrichments.

Always use reagant grade chemicals and bidistilled (or purer) water to make stock solutions of enrichments. Gentle heating and/or magnetic stirring of stock solutions can be used to ensure complete dissolution. When preparing a stock solution containing a mixture of compounds, dissolve each individually in a minimal volume of water before mixing, then combine and dilute to volume.

Autoclaving is the most widely used technique for sterilizing culture media, and is the ultimate guarantee of sterility (including the destruction of viruses). A commercial autoclave is best, but pressure cookers of various sizes are also suitable. Sterility requires 15 minutes at 1-2 bar pressure and a temperature of 121°C in the entire volume of the liquid

(i.e. longer times for larger volumes of liquid; approximately 10 min for 100ml, 20 min for 2l, 35 min for 5 l).

Bottles containing media should be no more than full, and should be left partially open or plugged with cotton wool or covered with aluminium foil. Ensure the heating elements are covered with distilled water, and the escape valve should not be closed until a steady stream of steam is observed. After autoclaving, the pressure release valve should not be opened until the temperature has cooled to below 80°C.

Pasteurization

NOTES

Pasteurization (heating to 90-95°C for 30 minutes) of media in Teflon or polycarbonate bottles is a potential alternative, reducing the problems of trace metal contamination and alteration of organic molecules inherent with autoclaving. Pasteurization does not, however, completely sterilize the seawater; it kills all eukaryotes and most bacteria, but some bacterial spores probably survive.

Heating to 90-95°C for at least 30 minutes and cooling, repeated on two successive days ('tyndallisation') may improve sterilization efficiency; it is assumed that vegetative cells are killed by heat and heat resistant spores will germinate in the following cool periods and be killed by subsequent heating.

Ultraviolet radiation

UV can be used to sterilize seawater, but very high intensities are needed to kill everything in the seawater (1200 W lamp, 2-4h for culture media in quartz tubes). Such intense UV light necessarily alters and destroys the organic molecules in seawater and generates many long lived free radicals and other toxic reactive chemical species (Brand, 1986). Seawater exposed to intense UV light must, therefore, be stored for several days prior to use to allow the level of these highly reactive chemical species to decline.

Sterile filtration

Sterile filtration is probably the best method of sterilizing seawater without altering the chemistry of the seawater, as long as care is taken not to contaminate the seawater with dirty filter apparatus. Sterilization efficiency is, however, to some extent reduced compared with heat sterilization methods.

Membrane filters of 0.2µm porosity are generally considered to yield water free of bacteria, but not viruses. 0.1µm filters can be used, but the

time required for filtration of large volumes of culture media may be excessively long. The filtration unit must be sterile: for small volumes (<50ml) pre-sterilized single use filter units for syringe filtration (e.g. Millipore Millex GS) can be used; for volumes up to 1 liter reusable autoclavable self-assembly filter units (glass or polycarbonate) with 47mm cellulose ester membrane filters (e.g. Millipore HA) can be used with suction provided by a vacuum pump; for larger volumes an in-line system with peristaltic pump and cartridge filters may be the best option.

Mixing or agitation of the cultures is achieved by the following means:

Aerating medium

NOTES

In many culture devices, mixing or agitation of the culture is achieved by aerating the medium. A stream of sterile air/CO2 mixture is continuously bubbled into the culture.

Use of magnetic stirrer

Small volumes kept in flasks can be effectively agitated by using Teflon coated magnetic bars and a magnetic stirrer.

Rotary Shakers

When several culture vessels are to be shaken simultaneously, a rotary shaker is used. The shaker is electrically operated. Illuminated and thermally controlled shakers are used to maintain the cultures in controlled conditions. If continuous aeration is needed, Roux Flasks with sealed -in aeration tubes are ideal.

In today's aerobic ocean, iron is present in the oxidized form as various ferric hydroxides and thus is rather insoluble in seawater. While concentrations of nitrogen, phosphorus, zinc and manganese in deep water are similar to plankton elemental composition, there is proportionally 20 times less iron in deep water than is apparently needed, leading to the suggestion that iron may be the ultimate geochemically limiting nutrient to phytoplankton in the ocean. Very little is known about iron in seawater or phytoplankton uptake mechanisms due to the complex chemistry of the element. Iron availability for micro-algal uptake seems to be largely dependent on levels of chelation. It is highly recommended that iron be added as the chemically prepared chelated iron salt of EDTA rather than as iron chloride or other iron salts; the formation of iron chelates is relatively slow, and iron hydroxides will form first in seawater, leading to precipitation of much of the iron in the culture medium.

2.2 UPSTREAM PROCESS

2.2.1. Introduction

Upstream processes include selection of a microbial strain characterized by the ability to synthesize a specific product having the desired commercial value. This strain then is subjected to improvement protocols to maximize the ability of the strain to synthesize economical amounts of the product. Included in the upstream phase is the fermentation process itself which usually is carried out in large tanks known as fermenters or bioreactors. In addition to mechanical parts which provide proper conditions inside the tank such as aeration, cooling, agitation, etc., the tank is usually also equipped with complex sets of monitors and control devices in order to run the microbial growth and product synthesis under optimized conditions. The processing of the fermentation reactions inside the fermenter can be done using many modifications of engineering technologies. One of the most commonly used fermenter types is the stirred-tank fermenter which utilizes mechanical agitation principles, mainly using radial-flow impellers, during the fermentation process.

NOTES

2.2.2. Overview of Upstream Processing

Upstream processing normally deals with three important points. The first relates to fermentation media, especially the selection of suitable cost effective carbon and energy sources, along with other essential nutrients. The media optimization is a vital aspect of process development to ensure maximization of yield and profit.

The second aspect involves aspects associated with the producer microorganism. They include the strategy for initially obtaining a suitable microorganism, industrial stain improvement to enhance productivity and yield, maintenance of strain purity, preparation of a suitable inoculums and continuing development of selected strains to increase the economic efficiency of the process.

The third component relates to the fermentation which is usually performed under rigorously controlled conditions developed to optimize the growth of the organism or the production of a target microbial product.

2.2.3 Fermentation medium

The medium used for fermentation may be classified as defined, complex or technical medium. Defined medium consists only of precisely chemically defined substrates. Complex medium is composed of substrates with undefined composition, such as extracts or hydrolysates from waste products, which are cheap substrates commonly used in

industrial production. Relatively expensive substrates, such as yeast extract, brain heart infusion, peptone, and tryptone are often used for complex medium. Technical media are used on an industrial scale and are cheaper. The substrate sources can also be derived from industrial waste, and are often highly impure mixtures, requiring pretreatment before they could be used for a fermentation process. Examples are soy meal, whey, fishmeal, malt extract, and sulfite waste liquor. Wastewater from monosodium glutamate production, which contains high levels of chemical oxygen demand (COD), sulphate, and ammonia cal nitrogen at a low pH, has been used as the nitrogen and water source, with sugar beet pulp as the carbon source, for the production of pectinase.

NOTES

Media sterilization is necessary to ensure that only the desired microorganism is present to carry out the fermentation, that products are made of predicted quality, that the environment is protected from undesirable contamination, and that deterioration (microbial spoilage) of products is prevented. Sterilization by high temperature achieved by direct or indirect steam or electric heating, membrane filtration, microwave irradiation, high voltage pulses and photo semiconductor powders which involve the rupture of the cell membrane by increasing the transmembrane electric field strength beyond a certain threshold.

Inoculation is the transfer of seed material or inoculums into the fermenter. Inoculation of a laboratory fermenter is generally done using presterilized tubing and a peristaltic pump. However, on a larger scale, inoculums transfer is done by applying a positive pressure on the inoculum fermenter and connecting it aseptically to the production fermenter. The connecting lines are sterilized before being used for transfer of inoculum. Heat susceptible substances such as amino acids and some vitamins must be dissolved in small volumes of water, sterilized by filtration and added separately to the final medium aseptically.

2.2.4. Inoculums

Upstream processing of proteins using bioreactors and cells usually begins with the preparation of the inoculums which proceeds in scale-up steps until enough inoculums are made to aseptically inoculate the final, sterile, media-filled bioreactor. During the culture period samples are removed, aseptically, and various parameters are measured by fermentation technicians or operators including optical density (OD) and live cell count. Samples are also brought to quality control where other parameters may be measured such as the levels of glucose, lactate and ammonia, as well as the identity and concentration of the human protein that the cells are producing. Also part of upstream processing is the initial purification steps which could include centrifugation and/or filtration in order to separate cells from media. The cells or the media would be

discarded to the kill tank, depending on where the protein was located. In this course we are using glass bioreactors and representative of three types of cells used in upstream processing of human protein pharmaceuticals: bacterial, animal, and fungal cells. In bacteria, such as biotechnology's workhorse, Escherichia coli, Pichia pastoris, proteins are secreted into the media so the media is saved for later isolation and purification of the protein of interest in downstream processing. Proteins remain inside the cell so the cells are separated from the media and the media is discarded to the kill tank. In animal cells, such as Chinese Hamster Ovary (CHO) cells, and in fungal cells, such as the yeast

2.2.5. Fermentation systems

NOTES

A fermentation system is usually operated in one of the following modes: batch, fed batch, or continuous fermentation. The choice of the fermentation mode is dependent on the relation of consumption of substrate to biomass and products.

Today the most common type of upstream processing of proteins utilizes two tools: bioreactors and suspension (or attached) cells transformed with expression vectors genetically engineered to contain one (or more) human genes that produce copious amounts of their protein(s).

2.3.1. Overview of Downstream Process:

Downstream processing, the various stages that follow the fermentation process, involves suitable techniques and methods for recovery, purification, and characterization of the desired fermentation product. A vast array of methods for downstream processing, such as centrifugation, filtration, and chromatography, may be applied. These methods vary according to the chemical and physical nature, as well as the desired grade, of the final product.

Downstream processing encompasses all processes following the fermentation. It has the primary aim of efficiently, reproducibly and safely recovering the target product to the required specifications (biological activity, purity) while maximizing recovery yield and minimizing costs. The target product may be recovered by processing the cells or the spent medium depending upon whether it is in intracellular or extracellular product. The level of purity that must be achieved is usually determined by the specific use of the product.

Each stage in the overall recovery procedure is strongly dependent on the protocol of the preceding fermentation. Fermentation factors affecting downstream processing include the properties of microorganisms, particularly morphology, flocculation characteristics, size and cell wall rigidity. These factors have major influences on the filterability,

sedimentation and homogenization efficiency. The presence of fermentation by-products, media impurities and fermentations additives such as antifoams may interfere with downstream processing steps and accompanying product analysis.

The products of fermentation are usually found in complex mixtures of dilute solutions and must be concentrated and purified. The separation of the product of interest from the fermentation broth depends on the accumulation of the product, which may be intracellular or extracellular. The typical downstream operations and the unit operations involved in the processing of fermentation broth are:

Cell disruption (high pressure homogenization, wet milling, and lysis)

NOTES

Clarification of extract (centrifugation, extraction, dead end filtration, and cross flow filtration)

Enrichment (precipitation, batch adsorption, ultra filtration, and partition)

High resolution techniques (ion exchange, affinity, hydrophobic, gel filtration, adsorption chromatography, and electrophoresis)

2.3.2. High resolution techniques like chromatography techniques

The molecules of interest are adsorbed or stuck to beads packed in the column. The higher the affinity of the molecule (protein) for the bead the more will be bound to the column at any given time. Proteins with a high affinity travel slowly through the column because they are stuck a significant portion of the time. Molecules with a lower affinity will not stick as often and will elute more quickly. We can change the relative affinity of the protein for the column (retention time) and mobile phase by changing the mobile phase (the buffer).

The most common type of adsorption chromatography is ion exchange chromatography. The others used in commercial biopharmaceutical production are affinity, hydrophobic interaction and gel filtration. Column chromatography separates molecules by their chemical and physical differences. Most common types: Size exclusion (Gel filtration): separates by molecular weight, Ion exchange: separates by charge, Affinity chromatography: specific binding Hydrophobic Interaction: separates by hydrophobic/hydrophilic characteristics.

2.3.3. Ion exchange chromatography

Ion Exchange Chromatography relies on charge-charge interactions between the protein of interest and charges on a resin (bead). Ion exchange chromatography can be subdivided into cations exchange chromatography, in which a positively charged protein of interest binds to a negatively charged resin; and anion exchange chromatography in

which a negatively charged protein of interest binds to a positively charged resin.

2.3.4. Isoelectric focusing

If the pH of the buffer is less than the pI (iso-elcetrical focal point; the point at which the net change exerted on a protein is neutral), the protein of interest will become positively charged. If the pH of the buffer is greater than the pI, the protein of interest will become negatively charged.

2.3.4. Affinity chromatography

NOTES

Affinity chromatography separates the protein of interest on the basis of a reversible interaction between it and its antibody coupled to a chromatography bead (here labeled antigen). With high selectivity, high resolution, and high capacity for the protein of interest, purification levels in the order of several thousand-fold are achievable.

2.3.5. Hydrophobic interaction chromatography (HIC)

HIC is finding dramatically increased use in production chromatography. Antibodies are quite hydrophobic and therapeutic antibodies are the most important proteins in the biopharmaceutical pipeline. Usually HIC media have high capacity and are economical and stable. Adsorption takes place in high salt and elution in low salt concentrations.

2.4 MASS CULTIVATION OF ALGAL BIOMASS-SPIRULINA

2.4.1. Introduction

Algae (cyanobacteria and unicellular eukaryotes) grow autotrophically and synthesize their food by taking energy from sunlight or artificial light, carbon source from carbon dioxide, and nutrients from carbohydrates present in growth medium. In a few countries, cultivation of algae is carried out in large trenches i.e. particularly in sewage oxidation ponds by using sunlight or in an artificial illumination conditions for use in life supportive systems for extended space exploration.

Spirulina strains are being used for a variety of applications in biotechnology. Due to their very high protein contents, they serve to improve protein deficiency and can be used as feed for production of animal protein. In many countries strains of Spirulina and Chlorella are utilized for sewage oxidation and waste water treatment.

NOTES

For cultivation of algae on sewage wastes, oxidation ponds are prepared, where sewage is allowed to accumulate. It is awaited till mixed cultures of algae grow (or inoculated with a singly prepared algal culture). For example, in Japan mixed culture of Spirulina maxima and Scenedesmus obliguus was developed in open pond systems.

2.4.2. Mass cultivation : Spirulina

This method has been started in many countries such as Japan, West Germany (now Germany), Mexico, Czechoslovakia, India, etc. In India, National Environmental engineering Research Institute (NEERI), Nagpur has developed a technique of cultivates algae in sewage oxidation pond systems. This practice is also in use at NBRI (Lucknow), Hyderabad and other centers. Interestingly, experiments conducted at the CETRI, Mysore have shown that the microalgae e.g. Scenedesmus inns and Spirulina platens could be cultivated on a large scale and used as food and feed as they are rich in protein and their nutrient value is comparable to conventional foods. A flow diagram of use of different groups of algae at various stages in waste water ponds and possible application of algal biomass is shown in Table.1.

Table.Composition of muffin (i.e. Dried powder of Spintlina fusiformis) (constituents in per 100 of powder).

A. Major constituents (%)		C. Minerals (mg)	
lutai protein	64.6	Calcium	6.58
Fat	6.7	Phosphorus	977
Crude fibre	9.3	Iron	44.7
Carbohydrates	16.1	Sodium	796
Calories	346	Potassium	1.28
B. Vitamins		D. Essential amino acids (%)	
Beta-carotene	320,000113	Lysin	2.99
Biotin	0.22 mg	Cystine	0.474
Cyanocobalamin (B12)	65.7 mg	Mcthionine	138
Folk acid	17.6 mg	Phenylalanine	2.87
Riboflavin	1.78 mg	Threonine	3.04
Thiamin	0.118 mg.		
Tocophaol	0.773 IU		

2.4.3. Requirements for Growth of Spirulina:

Following are the requirements for growth of Spirulina:

Algal Tanks:

Generally, circular or rectangular cemented tanks are constructed. The circular tanks are more preferred over the rectangular one because of case in handling. Size may be according to convenience and yield needed. Depth should be about 25cm. Open tanks arc suitable for tropical and sub-tropical regions.

Light:

Low light intensity is required at the beginning to avoid photolysis. Sperm/um exposed to high light intensity is photolysis.

Temperature:

Temperature for optimum growth should be between 35-40°C.

pH:

Spirulina grows at high pH ranging from 8.5 to 10.5. Initially, culture should be maintained at pH 8.5 which automatically is elevated to 10.5.

Agitation:

Agitation of culture is very necessary to get good quality and better yield. The culture is agitated by brush, paddle power, pipe pumps, wind power, rotators, etc.

Harvesting:

The filaments of Spirulina float on surface of water forming thick mat Therefore, it can be harvested by fine mesh steel screens, nylon or cotton cloths, etc.

Drying:

As it has a thin wall, sun drying is the most suitable and economical. Various trials done at CFTRI, Mysore and MCRC, Madras with sun drying have given good results.

Yield:

An average yield of 8-12 g Spirulina powder/m2/day has been obtained in India and other countries. This is equivalents to20 tonnes/ha/annum. In winter climate, the yield can increase to about 20 g/m2/day.

Avoiding contamination:

NOTES

Although there is the least chance for contamination, yet regular monitoring of algal culture is necessary, because the microbial load is likely to affect the quality and safety of the product. At MCRC and CFTRI the cultures of Spirulina were found either within or very close to safety limits of Indian Standard Institute (ISI) for baby food, to about 5x105 propagates per gram. Dried Spirulina powder is packed in aluminium bags or sealed in bottles and sent to market.

2.4.5. Harvesting the Algal Biomass

NOTES

Harvesting of algal culture becomes problematic because of settling down of collie's bottom and mixing of the algal cultures. The cells are recovered by concentration. Dewater in drying. Sometimes flocculants e.g. aluminium sulfate and calcium hydroxide and cationic polymers are added to the medium but they cannot be separated from the harvested cells. Therefore, this method warrants the application of SCP products in food and feed Methods of separation and concentration also follow centrifugation, flocculation and centrifugation plus flocculation. But it is not economically feasible so far.

Harvesting the cyanobacteria, for example, Spirulina sp. is less troublesome as their spinal filaments float on the surface of water because of gas filled vacuoles in their cells which result in floating algal mats. Cells are able to fix atmospheric nitrogen. Algal mats are filtered and suspension of Spirulina is dried with hot air to get fine powder. Algal yield from stabilization pond is around114 tonnes/ha/year. In California 70 tonnes/ha/year of Scenedesmus was obtained from sewage. From Bangkok much high yield of about 170 tonnes /ha /year has been reported.

(a) Benefits from Spiraling SCP: Mass Cultivation of Spirulina offers several advantages over Chlorella and Scenedemus as given below:

Being a filamentous alga, Spirulina can be harvested by simple and less expensive methods such as nylon or cotton cloth filter.

Filaments of Spiraling float on water surface due to presence of gas vacuoles. Hence, there is no problem of harvesting unlike Chlorella and Scenedesmus.

There is least chance of contamination in growth tanks of Spiraling as it grows at high alkaline pH 8-11.

Heat drying is sufficient for Spirulina as it has thin cell wall, whereas spray drying is required for Chlorella and Scenedesmus which is expensive.

Spirulina is highly digestive (85-95%) due to thin wall and low nucleic acid contents (4%). It contains high percentage of digestible proteins (62.72%), vitamins, amino acids and other nutrients.

2.5 MASS CULTIVATION OF DUNALIELLA sp

2.5.1. Introduction:

Dunaliella is a unicellular, bi-flagellate, naked green alga (Chlorophytes, Chlorophyceae).Its morphologically similar to Chlamydomonas, with the main difference being the absence of a cell wall in Dunaliella. Dunaliella has two flagella of equal length and a single, cup-shaped chloroplast, which in the marine and halophilic species has a central pyrenoid. In D. salina and D. parva the chloroplast accumulates large quantities of B-carotene so that the cells appear orange-red rather than green. The carotenoid are in the form of droplets (plastoglobuli) located at the chloroplast periphery and consist of a mixture of the Cis- and Trans isomers of β-carotene.

NOTES

The green unicellular flagellate Dunaliella salina is the richest natural source of the carotenoid β-carotene. The halophilic species of Dunaliella also accumulate very high concentrations of glycerol. Dunaliella salina was used as a commercial source of B-carotene and later as a source of glycerol. β-carotene from Dunaliella is now being produced on a commercial scale in Australia, China, Chile, Spain and Kuwait. Dunaliella species are commonly observed in salt lakes in all parts of the world from tropical to temperate to Polar Regions where they often impart an orange-red colour to the water. Marine Dunaliella species can generally be isolated from seawater, although they are not very abundant in nature.

2.5.2. Mass culture

The most commonly used medium for culture of Dunaliella is Modified Johnsons Medium (Table 2), however, these algae can also be grown in a wide range of other media including Guillard's f/2 medium (Guillard & Ryther, 1962), modified ASP medium (McLachlan & Yentsch, 1959) and enriched seawater (Rao & Chauhan, 1984).

2.5.3. Nutrient requirements

Carbon source

All Dunaliella spp. are strict photoautotrophs. Dunaliella appears to be able to take up CO_2 and HCO_3 for photosynthesis.

The supply of inorganic carbon is particularly important to the culture of D. salina since, at the very high salinities at which this alga grows, the solubility of inorganic C is low; i.e. at 25% NaCl the solubility of inorganic carbon is <50% of that at seawater salinity (3% NaCl).

Furthermore, at the high temperature and pH usually found in the natural brines in which D. salina grows, the bulk of the inorganic carbon (>99%) is in the form of CO32- and is thus unavailable for uptake by the algal. The presence of an extracellular carbonic anhydrate which catalyses the conversion of HCO3- to CO2 means that the alga can utilize HCO3- under these Conditions.

NOTES

Table-2. Modified Johnsons Medium (J/l) (Borowitzka, M.A., 1988).

To 980 ml of distilled water add:	
NaCl	as needed to obtain desired salinity
MgCl2·6H2O	1.5 g
MgSO4·7H2O	0.5 g
KCl	0.2 g
CaCl2·2H2O	0.2 g
KNO3	1.0 g
NaHCO3	0.043 g
KH2PO4	0.035 g
Fe-solution	10 ml
Trace-element solution	10 ml
Fe solution (for 1 litre)	
Na2EDTA	189 mg
FeCl3·6H2O	244 mg
Trace-element solution (for 1 litre)	
H3BO3	61.0 mg
(NH4)6Mo7O24·4H2O	38.0 mg
CuSO4·5H2O	6.0 mg

$CoCl_2 \cdot 6H_2O$	5.1 mg
$ZnCl_2$	4.1 mg
$MnCl_2 \cdot 4H_2O$	4.1 mg
Adjust pH to 7.5 with HCl	

2.5.4. Nitrogen source. The best source of nitrogen for Dunaliella is nitrate. Ammonium salts such as ammonium acetate, ammonium nitrate and ammonium chloride are generally less effective N sources and at high concentrations and high temperatures can be lethal. Urea can be used as a nitrogen source, especially in well buffered media. However, in large-scale outdoor cultures urea can lead to mass mortality of the culture due to the high concentrations of ammonium being released upon metabolism of the urea.

NOTES

Phosphorous: Phosphate is the best source of P and the optimal concentration is about 0.02 to 0.025 g1-1 K_2HPO_4. High concentrations may actually inhibit growth.

Magnesium and calcium: Both of these cations are required for growth and Dunaliella can tolerate a wide Mg^{2+}:Ca^{2+} ratio ranging from 0.8 to 20.0.

Sodium: All marine and halophytic species of Dunaliella require sodium.

Chloride and sulphate: The optimum Chloride and sulphate ratio for growth in D. salina is 3.2, whereas the optimum ratio for β-carotene formation is 8.6. There also appear to be some interaction between the anions and the cations and their effect on the alga, however, this is as yet little understood.

Iron: Low concentrations of iron in a form that can be assimilated are essential for the growth of Dunaliella and in some hyper saline brines Fe may be limiting to the growth of Dunaliella. The optimum concentration for iron in D.salina and D.viridis lies between 1.25 to 3.75 mg.1-1 and should be supplied in a chelated form such as iron citrate or Fe-EDTA (Table 2). High concentrations of Fe inhibit growth.

2.5.5. Trace elements and vitamins. Various trace elements such as Zn, Co, Cu, Mo and Mn are usually added to Dunaliella media, however little is known of the actual requirements of the alga. Dunaliella does not require any exogenous vitamins for growth.

2.5.6. pH. The optimum pH for growth for the marine Dunaliella is pH 6, whereas for the halophilic D. salina and D. viridis it is about pH 9.

2.5.7. Temperature

The optimum growth temperature for D.salina is in the range of 20 to 40°C (Borowitzka, LJ, 1981a) depending on the strain. Dunaliella salina can tolerate extremely low temperatures to below freezing (Siegel et al., 1984), but temperatures greater than 40°C are usually lethal.

Large-scale closed algal culture systems are still in the early stages of development, and it is likely that in the future better and more economic designs will be developed, however, until then open-air culture will remain the preferred option for large-scale commercial algal culture.

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2.5.8. Choice of site

Based on the observations above, a Dunaliella salina culture facility should be based at a site where:

There is ample flat land available;

There are cheap sources of high salinity brines, and also of lower salinity water (i.e. seawater) for salinity control and to provide the water for making up evaporative losses. The requirement for evaporative make-up water should not be underestimated. For example, a 5 ha pond at 20 cm depth contains 2 × 106 liters of water. At an evaporation rate of 1 cm per day, 100,000 liters of make-up water are required a day;

There are few cloudy days in the year and the mean daily temperature is >30°C for most of the year;

Rainfall is as low as possible, and falls only during a small part of the year;

The site is as far away as possible from any source of pollution which might affect algal growth or contaminate the algal product which will be used as a high quality food additive. This means that the plant should not be near agricultural activities where pesticides or herbicides are used, nor should it be near industrial activities which may emit heavy metals.

2.5.9. Harvesting

Harvesting and extraction represent the major cost areas in most algal processes. Harvesting D. salina is more difficult and costly compared to most other commercially produced algae, and therefore the methods used by the various commercial producers are closely guarded.

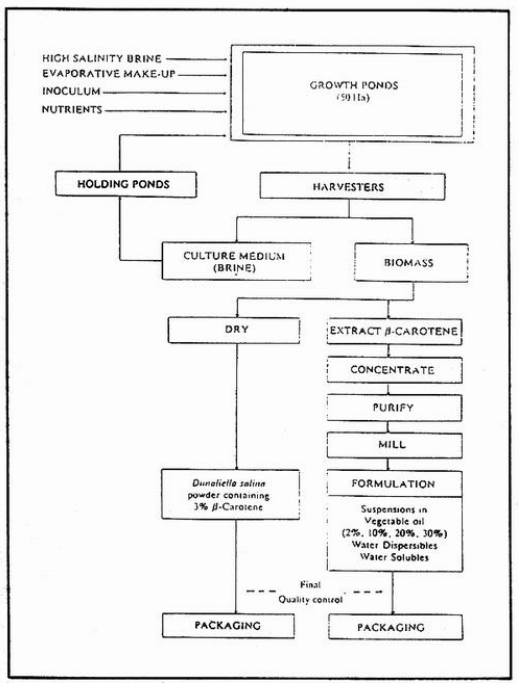

Figure-2. Schematic flow diagramed of the D. salina β-carotene production process.

The difficulty in harvesting D. salina is because this alga is a single cell with no protective cell wall, approximately 20 × 10 um in size and neutrally buoyant in a high specific gravity, high viscosity brine. Cell densities in large-scale cultures tend to be about 1 g. 1-1 and therefore very large volumes have to be processed. Centrifugation and filtration generally tend to shear-damage the cells, leading to loss of the β-carotene by oxidation. The cells also distort and pass through filters with pore sizes less than 10 um. Corrosion of all metal equipment by the brine is also a major problem.

These problems have led to development and patenting of several biological and chemical methods for harvesting the cells prior to the use of more conventional harvesting methods. Considering the low cell density of Dunaliella cultures it is likely that a harvesting process will have several steps. Any process which pre concentrates the alga easily may be of benefit. In this respect the observation that Dunaliella salina sometimes aggregates on the pond surface is important. When rain results in a layer of lower-salinity or even freshwater on the surface of the pond D. salina cells accumulate at the low salinity/high salinity interface. Furthermore, it is those cells with the highest β-carotene content which tend to stratify most readily. The algal cells also exhibit diurnal movement in the pond. It may be possible to utilize this phenomenon as part of a preliminary concentration step in a harvesting

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process. Finally, it must also be recognized that different products such as dry Dunaliella powder or extracted β-carotene may require different harvesting strategies.

2.5.10. Extraction and drying

Another critically important aspect of Dunaliella β-carotene production is extraction of the β-carotene. The process used for extraction depends, in part, on the harvesting procedure used and on market requirements. Extraction using conventional organic solvents is efficient but may not be acceptable to customers seeking a 'natural' product. More acceptable alternative extraction methods use hot vegetable oil or liquid or supercritical gas solvents.

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Harvested biomass may also be dried (i.e. spray-drying) rather than extracted and can be marketed as a β-carotene-rich supplement for human health or animal feed.

Dunaliella β-carotene production can also produce several useful by-products such as glycerol which can make up 30% of the biomass dry weight and high quality protein meal remaining after extraction.

2.5.11. Conclusion

Although β-carotene can be synthesized or extracted from other natural sources such as carrots, D. salina is still the richest and best 'natural' source of this carotenoid. The extreme environment in which D. salina grows also makes the open-air large-scale culture of this alga much easier compared to other unicellular algae. The low cell densities achieved by the algae and their small cell size however, make harvesting more difficult and costly. Furthermore, the use of the β-carotene as a food or feed additive and a nutritional supplement means that a high quality product is required. This means that great care must be taken in the extraction and formulation steps.

Despite these difficulties there are now several commercial producers of D. salina β-carotene and a number of others are expected to come on stream in the near future. The high value of the product also means that, even with the technology available today algal β-carotene production is an economic reality.

2.6 MASS CULTURE OF HAEMATOCOCCUS

2.6.1. Introduction:

Mass culture of Haematococcus is commercially available and astaxanthin derived from Haematococcus is used as a nutraceutical for human health and as a coloring agent. However, the Haematococcus industry has achieved only moderate success due to low astaxanthin

productivity and the high production cost associated with the current mass culture systems and processes.

Several major biological and environmental factors that affect growth and astaxanthin production are identified, of which fungal contamination is the most detrimental factor that is largely responsible for low astaxanthin production and frequent culture crashes. Future expansion of the Haematococcus industry will depend on significant improvement in our knowledge about the biology of Haematococcus and predator–prey interaction, and transformation of the knowledge into a next-generation mass culture system and process including an advanced Haematococcus crop protection program.

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2.6.2. Mass Cultivation of Haematococcus

Photoautotrophic culture Mass culture of Haematococcus is commonly carried out in open raceway ponds or closed photo bioreactors in which the cells grow photoautotrophically. Both culture systems have been adopted by the industry, though individual companies have their own preference in selecting a particular one or both. The Bold Basal Medium (BBM) or a modified BBM (enriched with three times more nitrate) is a culture medium commonly used for Haematococcus culture, although the BG-11 culture medium is also used for Haematococcus culture. Effects of various macro and micronutrients in culture media on growth and astaxanthin productivity of Haematococcus have been studied aiming at developing optimal culture media for enhancement of growth and astaxanthin production.

The chemical compositions of the commonly used culture media for Haematococcus are provided in Table 1. Regardless of the culture systems, mass culture of Haematococcus is often operated in a so-called two-stage culture mode where a “green stage” provides the optimal light intensity and nutrient-replete culture medium to promote vegetative growth. When the cell density reaches the maximal level, the culture is moved to a “red stage” at which green vegetative cells are subjected to photo oxidative stress, leading to the transformation of green vegetative cells into red aplanospores.

The “two-stage” culture strategy provides the flexibility for the combination of multiple technologies to achieve high productivity. For example, Haematococcus can be cultivated under heterotrophic conditions for enhanced biomass production, and the culture is then transferred to photoautotrophic conditions for astaxanthin induction under high light intensity or high light in combination with nutrient starvation. The “two-stage” culture mode will also enable different types

of photo bioreactors to be used in combination in order to meet the specific requirements of each stage of cultivation.

A single-stage culture process in which a Haematococcus strain was maintained in a chemostat where actively dividing motile cells were simultaneously producing astaxanthin at a constant light irradiance of 1220 μE m−2 s−1, a dilution rate of 0.9 per day, and a constant nitrate concentration of 2.7 mM in the feed medium. As a result, a maximum astaxanthin content of 0.8% of dry biomass and an astaxanthin productivity of 5.6 mg L−1 d−1 were obtained.

The technical feasibility of one-stage production at a pilot scale was demonstrated in an outdoor tubular photo bioreactor consisting of a horizontal loop made of 2.5-cm-internaldiameter tubes of a total length of 95 m and a culture volume of 50 L, which resulted in biomass and astaxanthin productivities of 0.7 g L−1 d−1 and 8 mg L−1 d−1, respectively. Astaxanthin-rich biomass composed of thin-walled motile flagellates obtained from the one-stage culture process may improve the bioavailability of astaxanthin compared to thick, rigid-walled aplanospores produced from the two-stage cultures, if cell biomass were not pretreated to disrupt cell walls. However, this perception will have to be verified by animal feeding trials. As motile flagellates are more susceptible than non motile palmelloids to photo oxidative stress, to maintain selected conditions to minimize potential ill-effects of strong solar irradiation can be a challenge to one-stage culture of H. pluvialis.

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Chemical compositions of culture media for Haematococcus

Component (mM)	BBM (3N BBM) (Bischoff & Bold, 1963)	BG-11 (Rippka et al., 1979)	OHM (Fabregas et al., 2000)	Kobayashi's basal medium (Kobayashi et al., 1992)
$NaNO_3$	2.94 (8.82)	17.6		
KNO_3			4.055	
$MgSO_4 \cdot 7H_2O$	3.04×10^{-1}	3.0×10^{-2}	2.46×10^{-1}	
NaCl	4.28×10^{-1}			
K_2HPO_4	4.31×10^{-1}	2.2×10^{-1}		
KH_2PO_4	1.29			
NaH_2PO_4			2.11×10^{-1}	
Na_2HPO_4				
$CaCl_2 \cdot 2H_2O$	1.70×10^{-1}	2.0×10^{-1}	7.48×10^{-1}	1.35×10^{-1}
$FeSO_4 \cdot 7H_2O$	1.79×10^{-2}			3.60×10^{-2}
$MgCl_2$				9.85×10^{-1}
Citric acid		3.0×10^{-2}		
Ammonium ferric citrate		2.0×10^{-2}		
Na_2CO_3		1.8×10^{-1}		
H_3BO_3	1.85×10^{-1}	4.6×10^{-2}		
$EDTA \cdot Na_2$	1.71×10^{-1}	2.0×10^{-3}		
H_2SO_4 (98%) (mL)	1×10^{-3}			
KOH	5.53×10^{-1}			
$ZnSO_4 \cdot 7H_2O$	3.07×10^{-2}	7.7×10^{-4}		
$MnCl_2 \cdot 4H_2O$	7.28×10^{-3}	9.0×10^{-3}	4.95×10^{-3}	
MoO_3	4.93×10^{-3}			
$CuSO_4 \cdot 5H_2O$	6.29×10^{-3}	3.0×10^{-4}	4.81×10^{-5}	
$Co(NO_3)_2 \cdot 6H_2O$	1.86×10^{-3}	1.7×10^{-4}		
$NaMoO_4 \cdot 2H_2O$		1.6×10^{-3}	2.58×10^{-4}	
$CoCl_2 \cdot 2H_2O$			4.62×10^{-5}	
Fe(III) citrate·H_2O			0.010	
SeO_2			4.51×10^{-5}	
Cr_2O_3			4.93×10^{-4}	
Biotin			1.02×10^{-4}	
Thiamine			5.82×10^{-5}	
Vitamin B12			1.11×10^{-5}	
Sodium acetate				14.6
L-Asparagine				2.7
Yeast extract (g L^{-1})				2

2.7.1. Mass cultivation of botrrycoccus:

Mass cultivation of B. braunii in open raceway pond this experiment was conducted in a concrete raceway pond (Length 6.1 m; Width 1.52 m; Height 0.3 m) lined with porcelain tiles having a total working volume of 2000 L. The seed culture (200 L) of B. braunii AP103 grown in the mini open raceway pond with a biomass concentration of 0.085 g L_1 was transferred to the 2000 L capacity raceway pond containing 1800 L of modified CHU 13 medium. CHU 13 medium was prepared by using the filtered ground water and the culture height in the pond was maintained at 15 cm level. The algal culture was mixed manually once in every 30 min during day time to prevent settling and enhance dissolved CO2 concentration. This experiment was conducted for a period of 15 days in batch mode. At every 3 days interval, pH, biomass, pigments, total carbohydrates, proteins and lipids were analyzed and recorded. Microscopic analysis was carried out daily to check the purity of the

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culture. The average temperature, humidity and solar radiation recorded during the experiment were 29oC, 71% and 5 kWh m_2 d_1, respectively. At the end of the study period the algal biomass was harvested and analyzed for different parameters.

2.7.2 Harvesting Of Algal Biomass

The algal cells are settled at the bottom in the open raceway pond was harvested after 12 h through auto flocculation on 15th day. The biomass in the open raceway pond was washed with ground water and the cells were allowed to settle. This process was repeated for 3 times in order to remove the excess salt in the algal biomass. The washed algal cells were spread on white plastic sheet and dried in the sun light for 3 h followed by oven drying at 60o C for 8 h.

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2.7.3. Biomass Estimation

The biomass (g L_1) was determined by filtering 20 mL of algal culture using pre-weighed 4.7 cm Whattman GF/C glass fiber filter. The filter with algal biomass was dried at 65o C for 2 h, cooled to room temperature in vacuum desiccators and weighed gravimetrically.

Review questions:

1. Write an essay on the upstream and downstream process of Spirulina cultivation.

2. Explain the mass cultivation technique of Dunaliella algae.

3. List out the importance of downstream process in algal cultivation.

4. Discuss about the mass cultivation of Hematococcus and Botryococcus and their importance

UNIT-3: SINGLE CELL PROTEIN– BIOACTIVECOMPOUNDS, INDUSTRIAL ENZYMES, BIOFUEL AND BIOPRODUCTS FROM ALGAE

Objectives:

- To know the single cell protein and their biological value.
- To understand the microalgae as the source bioactive compound.
- To have the knowledge microalgae used as industrial enzymes, biofuel, and their bio-products.

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3.1 SINGLE CELL PROTEIN (SCP)

3.1.1. Definition

The dried cells of micro-organisms (algae, bacteria, actinomycetes and fungi) used as food or feed are collectively known as 'microbial protein' since the ancient times a number of micro-organisms have been used as a part of diet. The term 'microbial protein' was replaced by a new term "single cell protein" (SCP) during the First International Conference on microbial protein held in 1967 at the Massachusetts Institute of technology (MIT), Cambridge, U.S.A. Criteria for coning this term was the single celled habit of micro-organisms as food and feed.

3.1.2. Introduction

In 1973, when Second International Conference was convened at MIT, some actinomycetes and filamentous fungi were reported to produce protein from various substrates. Since then many filamentous fungi have been reported to produce protein. Therefore, the term SCP is not logical, if an organism produces filaments.

Since the 1920s, filamentous fungi have been used for the production of protein. For such fungi, the term 'fungal protein' has been used by many workers. Recently, the term 'mycoprotein' has been introduced by Ranks novas McDougall (RHM) in the United Kingdom for protein produced on glucose or starch substrates. Importance of mass production of micro-organisms as a direct source of microbial protein was realized during World War I in Germany and consequently, baker's yeast *(S. mystic's')was* produced in an aerated

molasses medium supplemented with ammonium salts. During World War II (1939.1945) the aerobic yeasts *(e.g. Candida units)* were produced for food and feed in Germany. Since World War II considerable effort has been made to develop technologies for mass cultivation of SCP by formulating different types of growth media and improved cult of micro-organisms. In the late 1950s, British Petroleum started producing the SCP from hydrocarbons since the crude oil contains 10-25% (Paraffin's) and established a first large scale plant in Sardinia the end of 1975. It had a capacity of 1, 00,000 tones SCP per annum. Large scale production has been envisaged in England and Rumania with the annual production of 60,000 tones bacterial mass in England. The erstwhile U.S.S.R. was the World's largest producer of SCP in 1980. The production was estimated to 1.1 million tones of SCP per annum.

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Microorganisms like algae, fungi, yeast, and bacteria have very high protein content in their biomass. These microbes can be grown using inexpensive substrates like agricultural waste viz. wood shavings, sawdust, corn cobs etc. and even human and animal waste. The microorganisms utilize the carbon and nitrogen present in these materials and convert them into high-quality proteins which can be used as a supplement in both human and animal feed.

The average compositions of the different microorganisms present in the % dry weight of Single-cell protein.

Composition	**Fungi**	**Algae**	**Yeast**	**Bacteria**
Protein	30-45	40-60	45-55	50-65
Fat	2-8	7-20	2-6	1-3
Ash	9-14	8-10	5-10	3-7
Nucleic Acid	7-10	3-8	6-12	8-12

In India, little attention has been paid on the production of SCP, though mushroom cultivation started in the early 1950s. However, work on

mushroom culture at Solan (Himachal Pradesh from 1970 onward has brought satisfactory results. Recently, National Botanical Research Institute (NBRI),Lucknow and Central Food Technological Research Institute (CFTRI), Mysore, have established Centers for mass production of SCP from Cyanobacteria. At the NBRI, SCP is produced on sewage which is Anther utilized as animal feed.

Therefore, in the light of protein shortage, micro-organisms offer many possibilities for protein production. They can be used to replace totally or partially the valuable amount of conventional vegetable and animal protein feed. For this, development of technologies to utilize the waste products would play a major role for the production of SCR

3.1.3. Micro Algae as Source of Human Food

The micro algal market is dominated by *Chlorella* and *Spirulina* mainly because of their high protein content, nutritive value, and not least, because they are easy to grow. The biomass of these algae is marketed as tablets, capsules, and liquids. Macro algae are utilized as food in China, Japan, Korea, the Philippines, and several other Asian countries. The largest producer is China, which harvests about5 million wet tones/year. For example, "Nori" actually *Porphyra* spp., which is used for making sushi, currently provides an industry in Asia with a yearly turnover of ~US$ 1 x 109. Other species used as human food are *Monostroma* spp., *Ulva* spp., *Laminaria* spp., *Undaria* spp., *Hizikia fusiformis*, *Chondrus crispus*, *Caulerpa* spp., *Alaria esculenta*, *Palmaria palmata*, *Callophyllis variegata*, *Gracilaria* spp. and *Cladosiphon okamuranus*. Algae provide a large profile of natural vitamins, minerals, and essential fatty acids and thereby positively affect human health.

3.1.4. Substrates used for production of SCP

A variety of substrates arc used for SCP production. However, availability of necessary substrates is of considerable biological and economic importance for the production of SCP. Algae which contain chlorophylls do not require organic wastes. They use free energy from sunlight and carbon-dioxide from air, while bacteria (except photo autotrophs) and fungi require organic wastes, as they do not contain chlorophylls.

The major components of substrates ate the raw materials which contain sugars (sugarcane Sugar beet and their processed products), Starch (grains, tapioca, potato, and there by-products). Lignocelluloses from woody plants and herbs having

residues with nitrogen and phosphen$_e$ contents and other raw materials (whey and refuses from processed food). Organic wastes arc also generated by certain industries and are rich in aromatic compounds or hydrocarbons. Recent price-increase in petroleum and refined petroleum products has made hydrocarbons a chemicals derived from them (such as methanol and ethanol) less attractive as raw materials f SCP production than renewable sources such as agricultural wastes or by-products.

3.1.5. Nutritional Value of SCP

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Nutritional values of SCP product which indicate that protein digestibility range from good to very good and are trite for bacteria and yeasts growth on un-conventional substrates. However, there are certain problems which warrant the use of SCP products as human foods such as: (i) high content of nucleic acid leading to development of kidney stone and gout if consumed in high quantity, (ii) possibility for the presence of toxic secondary metabolites and (iii) poor digestibility and stimulation of gastrointestinal and skin reactions.

At present, production of SCP by mass culture of micro-organisms is in its infancy. It needs much boost to solve the problem of starvation in the coming decades. One of the ways to enhance productivity and quality of SCP product is the genetic improvement of micro-organisms. At Son Texcoco, Mexico, researches are in progress on production of genetically engineered cells, which can grow in alkaline environment even up to pH 8.0-10.0 and under the artificial conditions as well. Moreover, transfer and expression of beneficial genes in the micro-organisms have opened a new era for the production of algal proteins and other compounds to be used in food and feed.

3.1.6. The Production Is Carried Out In the Following Steps

1. Selection of suitable strain.
2. Fermentation.
3. Harvesting.
4. Post-harvest treatment.
5. SCP processing for food.

Similar to any other microbial culture, production of pure microbial cultures for preferred protein products requires sources of

nitrogen carbohydrates and other nutrients like phosphorus to support optimal growth of the culture. Contamination is prevented by maintaining strict sterile conditions throughout the process. The components of the culture media are either heat sterilized or filtered through micro porous membranes. The selected microorganism is then inoculated in pure conditions. Most of the processes are highly aerobic, except algal fermentation; hence a good supply of oxygen is necessary requirement. After the multiplication of the biomass, it is recovered from the medium and purified further for enhanced usefulness and or storability.

Single cell protein of *Spirulina* is used as below:

As protein supplemented food. Since *Spirulina* is a rich source of protein (60-72%), vitamins, amino acids, minerals, crude fibers, etc., it is used as supplemented food in diets of under-nourished poor children in developing countries The United Nations, Mexican National Institute of Nutrition. French Petroleum Institute and National Institute of Nutrition, Hyderabad has formulated four algal recipes as a weaning substitute for infants.

As health food: Spirulina is very popular as health food. Most of Sosa Texcoco products are exported to U.S.A., Europe and France where it is sold in health and food stores. It is the pan of the diet of the U.S. Olympic team. Jaggers take Spindina tablets for instant energy. Since it provides all the essential nutrients without excess calories and fats, it is taken by those who want to control obesity.

In therapeutic and natural medicine: Spirulina possesses many medicinal properties. Therefore it is used as social and preventive medicine also. It has been recommended by medicinal experts for reducing body weight, cholesterol and pre-menstrual stress and for better health. It lowers sugar level in blood of diabetics due to the presence of gamma-linolenic acid and prevents the accumulation of cholesterol in human body. It is a good source of II-carotene (a precursor of vitamin A) and, therefore, helps in monitoring healthy eyes and skin.

In cosmetics:

Spirulina contains high quality of proteins and vitamin A.These play a key role in maintaining healthy hair. Many herbal cosmeticians are making efforts to develop a variety of beauty products. Phycocyanin pigment has helped in formulating biolipstics and herbal face cream in Japan. These products can replace the present coaltar-dye based cosmetics which are known as carcinogenic.

3.1.7. Advantages of Single-Cell Protein

Large-scale Single-Cell Protein production has multiple advantages over conventional food production practices such as:

- Microorganisms have a high rate of multiplication which means a large quantity of biomass can be produced in a comparatively shorter duration.
- The microbes can be easily genetically modified to vary the amino acid composition.
- A broad variety of raw materials, including waste materials, can be used as a substrate. This also helps in decreasing the number of pollutants.
- Production is independent of climatic conditions.
- Provides instant energy.
- It is extremely good for healthy eyes and skin.
- Provides the best protein supplemented food for undernourished children.
- Serves as a good source of vitamins, amino acids, minerals, crude fibers, etc.
- Controlling obesity.
- Lowers blood sugar level in diabetic patients.
- Reducing body weight, cholesterol and stress.
- Prevents accumulation of cholesterol in the body.

3.1.8. Disadvantages of Single-Cell Protein

Single-Cell Protein has not been widely accepted for human consumption owing to certain problems as follows:

- High level of nucleic acid in biomass makes it difficult for consumption as it may lead to gastrointestinal problems.
- The biomass may trigger an allergic reaction if the digestive system recognizes it as a foreign product.
- The presence of nucleic acids in high content leads to elevated levels of uric acid.
- In certain cases, the development of kidney stone and gout if consumed in high quality.
- Possibility for the presence of secondary toxic metabolites which results in Hypersensitivity and other skin reactions.
- The capital cost of production is high as sophisticated machinery is required.

3.2.1. Bioactive Compound - Definition

The term bioactive compound is an expression in common use and includes compounds, which at low concentrations may be either beneficial or harmful to living organisms. Generally the terms refers to secondary metabolites that have attracted the attention of both scientists and industrialists.

3.2.2. Introduction

Seaweed have been identified as a rich source of bioactive compound.Microalgae ranges from 0.2 to 2 μm (picoplankton) up to filamentous forms with sizes of 100 μm or higher. These organisms have the potential for co-production of other molecules such as pigments, proteins, polyunsaturated fatty acids, antioxidants and are gaining attention as therapeutic agents for numerous health disorders and other applications in food, cosmetic, energy and pharmaceutical industries. Some microalgae are being studied and screened for the detection of active agents like anticancer, anti-inflammatory, antifungal, antibiotics and another pharmaceutical. The secondary metabolites from microalgae referred as "High-Value Molecules (HMV)".

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Micro and macro-algae, such as diatoms and seaweeds, are enriched with metabolites to fight bacteria and other microbes. Antimicrobial agents from the marine environment have been studied with a particular focus to struggle recent antimicrobial resistance issues. In addition introducing enzymes from other organisms via biotechnological means has succeeded in obtaining novel compounds. Due to their potential as a source of industrial, economic and medical/pharmaceutical attention microalgae have captured the interest of the scientific community.

3.2.4. Microalgae as Source Of Bioactive Compounds

The importance of marine organisms as a rich source of structurally diverse and novel bioactive compounds is growing rapidly, due to the presence of valuable compounds like fatty acids, pigments, and other biochemical's. Microalgae are considered the potential source for human and animal nutrition. However, the low level of these compounds in native microalgae and the difficulty in isolation of pure compounds have limited their production except in few cases such as astaxanthin, and β-carotene, which have been produced at large scale.

Microalgae have a broad range of application, like in animal feed and nutritional supplements, in cosmetics, pharmaceuticals, bioremediation

and water treatment, renewable energy and others. They also comprise a wide array of biomolecules such as proteins, lipids, vitamins, pigments, that can be harnessed for commercial use in food, cosmetic and pharmaceutical industry. In addition the pharmacological activities of various bioactive compounds of microalgae origin such as antioxidant, antitumor, Antiangiogenic, hem agglutinating and antiviral have been studied.

3.2 BIOLOGICAL ACTIVITY OF COMPOUNDS FROM MICROALGAE

3.2.5. Antimicrobial Activity

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In spite of the fact that microalgae are potential sources of high value molecules their application as antimicrobials are still in its early life.There is certainly an urgent need for new drugs to treat disease, in particular, new classes of antibiotics to overcome the growing problem of antibiotic resistance in many bacterial pathogens.

Seaweeds and diatoms have evolved to produce an endogenous system to counter pathogenic bacteria and other microbes, ubiquitous to their environment. Treatment for the multi-drug-resistant *S. aureus* has become a challenge except for vancomycin, although the possibility that vancomycin resistance might transfer from vancomycin-resistant *enterococci* to multi-drug resistant *S. aureus* has been extremely worrying.

3.2.6. Anti-Inflammatory Activity

Inflammation is a vital process in acute diseases, and it is essential to identify and destroy invading pathogens in the host. However, if it occurs as chronic and subclinical condition and if the process is not regulated over a long period, the activated immune system can damage host tissues and up-regulate chronic disease states like cardiovascular disease, Alzheimer's disease, inflammatory bowl, disease and obesity.

Many bioactive compounds isolated from marine life have shown potent and mechanistically intriguing anti-inflammatory activities, and contribution of marine cyanobacteria to this class of compounds is recognizable (for instance, anti-inflammatory bis-bromoindoles from *Rivularia sp.*)A microalga such as *Phaeodactylum*, *Porphyridium*, and *C. stigmatophora* produces polysaccharides with pharmacological activity, such as anti-inflammatory and immunomodulating activities. Recently, several metabolites from marine cyanobacteria were tested by nitric oxide (NO) inhibition assay in a mouse raw macrophage cell line.

3.2.7. Antioxidant

The demand for a safe and powerful antioxidant from a natural product is growing worldwide. This is because the need to minimize oxidative damage to living cells and prevent deterioration in commercialized products such as food, pharmaceuticals or cosmetics is increasing. Oxidative damage due to ROS such as hydroxyl radical (HO•), superoxide anion and hydrogen peroxide (H_2O_2) could induce atherosclerosis, cataracts, muscular dystrophy, rheumatoid arthritis, neurological damage, cancer and aging. Antioxidant molecules from microalgae, especially carotenoids, phenolic compounds, fatty acids, tocopherol, flavonoids, and alkaloids play a major role in the control of the oxidative process.

3.2.8. Anticancer activity

Micro algal pigments protect normal cells from genetic damages and exert ant proliferative, cytotoxic and pro-apoptotic activities in cancer cells, suggesting their possible use for cancer prevention or chemotherapy.

Algae not only contain chlorophylls, the photosynthetic pigments, but also contain a number of other pigments which are mainly used to improve the efficiency of light energy utilization and for protection from damage by the sunlight. From a commercial point of view, the Carotenoids and the phycobiliproteins seem to be the most important. Carotenoids are a class of widespread fat-soluble pigments that form a polyene chain that is sometimes terminated by rings. In addition to their role in coloration, Carotenoids act as provitamin A and as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen. The phycobiliproteins phycoerythrin and Phycocyanin are isolated from *Spirulina* and *Porphyridium* and utilized for health food, pharmaceuticals, and cosmetics. Phycobiliproteins is not only used as pigments, but have also been shown to have health-promoting properties. They are also used in research laboratories as labels for biomolecules.

Potential bioactive molecules such as microtubule polymerization inhibitors, curacin A-38, and dolostatin10-39, are in preclinical and clinical trials as anticancer drugs. Various derivatives of these molecules have been synthesized for drug development. The antitumor activity of a synthetic derivative of dolostatin 10, TZT-1027 (solbidotin), was reported to be superior to existing anticancer drugs, including vincristin and paclitaxel for treating solid tumors. A class of cyclic lipopeptide

cyanobacterial metabolites, apratoxins, has excellent cancer cell cytotoxicity.

3.3.1. MICROALGAE AS SOURCE OF ENZYMES

Introduction

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From the enzymes view point, microalgae proteases have demonstrated superior activities in the natural and alkaline ranges as compared to proteases of higher plant and other organisms. Biocatalytic potential of microorganisms have been employed for centuries to produce bread, wine, vinegar and other common products without understanding the biochemical basis of their ingredients. Microbial enzymes have gained interest for their widespread uses in industries and medicine owing to their stability, catalytic activity, and ease of production and optimization than plant and animal enzymes.

The use of enzymes in various industries (e.g., food, agriculture, chemicals, and pharmaceuticals) is increasing rapidly due to reduced processing time, low energy input; cost effectiveness, nontoxic and eco-friendly characteristics. Microbial enzymes are capable of degrading toxic chemical compounds of industrial and domestic wastes (phenolic compounds, nitrides, amines etc.) either via degradation or conversion. Here in this review, we highlight and discuss current technical and scientific involvement of microorganisms in enzyme production and their present status in worldwide enzyme market.

Microbes have been utilized since ancient human civilization with first reported commercial application of yeast to produce alcoholic beverages from barley by the Babylonians and Sumerians as early as 6000 BC. The microbial enzymes have gained recognition globally for their widespread uses in various sectors of industries, e.g., food, agriculture, chemicals, medicine, and energy. Enzyme mediated processes are rapidly gaining interest because of reduced process time, intake of low energy input, cost effective, nontoxic and eco-friendly characteristics. Moreover, with the advent of recombinant DNA technology and protein engineering a microbe can be manipulated and cultured in large quantities to meet increased demand. Associated driving factors that motivate the use of microbial enzymes in industrial applications are increasing demand of consumer goods, need of cost reduction, natural resources depletion, and environmental safety .

Enzymes are biological molecules, proteinaceous in nature with the exception of catalytic RNA molecules (ribozymes), and act as catalyst to support almost all of the chemical reactions required to sustain life. Enzymes are highly specific; only accelerate the rate of particular

reaction by lowering the activation energy without undergoing any permanent change in them, and therefore, are vital biomolecules that support life. They require typically milder condition of temperature and pressure for catalyzing reactions, and are used as an alternative to hazardous chemical pollutant owing to their biodegradable and nontoxic nature.

Enzymes are large macromolecules composed of polymers of amino acids connected by amide bonds, ranging from kilodalton (insulin) to megadalton (ribosome) in molecular mass. Catalytic site of these macromolecules is often buried deep within hydrophobic pockets, which determines the specificity for their substrate. This specificity of enzyme to catalyze reactions between one types of chemical compound over the other provides the basis of its classification and name. With the great advancement achieved in the area of biochemistry after 1940, a large number of enzymes were isolated and characterized, and therefore, it was necessary to regulate the enzyme nomenclature.

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Microorganisms are favored sources for industrial enzymes due to easy availability, and fast growth rate. Genetic changes using recombinant DNA technology can easily be done on microbial cells for elevated enzyme production and scientific development. Production of microbial enzymes is a necessary event in the industrial sectors, due to the high and superior performances of enzymes from different microbes, which work well under a wide range of varied physical and chemical conditions. Further, microbial enzymes are used in the treatment of health disorders associated with deficiency of human enzymes caused by genetic problems.

The extensive application of microbes in different bioprocess is used to deliver a variety of products in applied industries.

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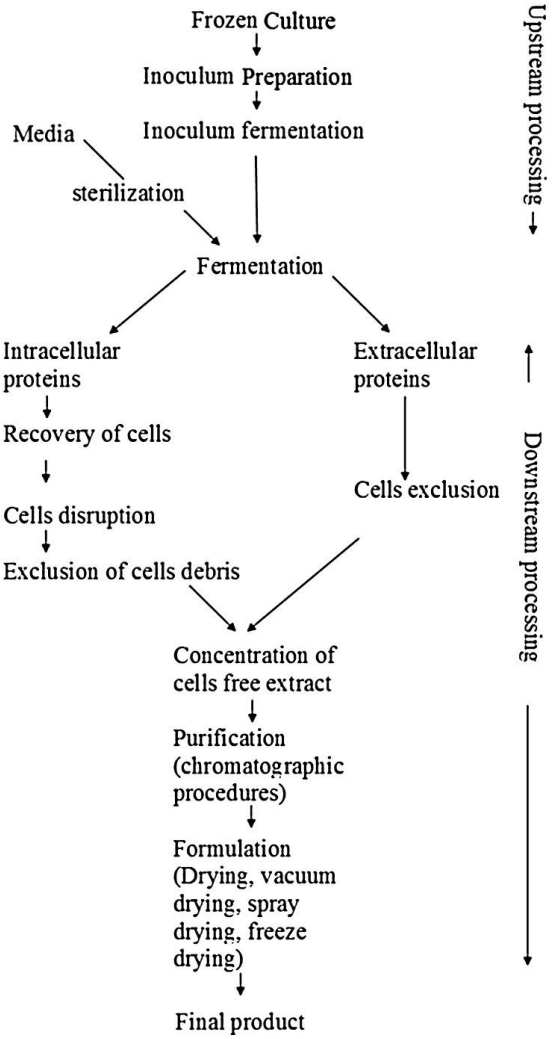

The extensive utilization of enzymes for scientific and analytical purposes is used to estimate the concentration of substrates and to determine the catalytic activity of enzymes present in biological samples. Advances in the enzyme technology have replaced or minimized the use to harmful radioactive elements in different immunoassays, which are used for the determination of a variety of proteins and hormones.

Furthermore, enzymes are used in clinical diagnostic for the quantitative determination of diabetes and other health disorders, for example, glucose oxidase (EC 1.1.3.4) for glucose; urease (EC 3.5.1.5) and glutamate dehydrogenase (EC 1.4.1.2) for urea; lipase, carboxyl esterase, and glycerol kinase for triglycerides; urate oxidase (EC 1.7.3.3) for uric acid; creatinase (EC 3.5.3.3) and sarcosine oxidases (EC 1.5.3.1) for creatinine. Cholesterol oxidase (EC 1.1.3.6) has also been reported for useful biotechnological applications in the detection and conversion of cholesterol. Putrescine oxidase (EC 1.4.3.10) is used to detect biogenic amines, such as putrescine, a marker for food spoilage.

Food industry

Quality food supply issue can be addressed by the application of enzymes in the food industry. These biomolecules are efficiently involved in improving food production and components, such as flavor, aroma, color, texture, appearance and nutritive value. The profound understanding of the role of enzymes in the food manufacturing and ingredients industry have improved the basic processes to provide better markets with safer and higher quality products. Furthermore, the enzymes gained interest in new areas such as fat modification and sweetener technology.

Currently, the food and beverage segment dominated the industrial enzyme market and it is projected to reach a value of $2.3 billion by 2020. In beverage and food industry, enzymes are added to control the brewing process and produce consistent, high-quality beer; to enhance the functional and nutritional properties of animal and vegetables proteins by the enzymatic hydrolysis of proteins, for higher juice yield with improved color and aroma.

The application of enzymes in food industry is segmented into different sectors, such as baking, dairy, juice production and brewing. Worldwide, microbial enzymes are efficiently utilized in bakery—the principal application market in food industry—to improve dough stability, crumb softness and structure, and shelf life of products. Increased uses of microbial enzymes in cheese processing are largely responsible for the use of enzymes in dairy industry, which is the next largest application industry followed by the beverages industry.

Baking industry

Baking enzymes are used for providing flour enhancement, dough stability, improving texture, volume and color, prolonging crumb softness, uniform crumb structure and prolonging freshness of bread. Bread making is one of the most common food processing techniques globally. The use of enzymes in bread manufacturing shows their value in quality control and efficiency of production. Amylase, alone or in combination with other enzymes, is added to the bread flour for retaining the moisture more efficiently to increase softness, freshness and shelf life.

Additionally, lipase and xylanase (EC 3.2.1.8) are used for dough stability and conditioning while glucose oxidase and lipoxygenase added to improve dough strengthening and whiteness. Transglutaminase (EC 2.3.2.13) is used in baking industry to enhance the quality of flour, the

amount and texture of bread and the texture of cooked pasta. Lipases are also used to improve the flavor content of bakery products by liberating short-chain fatty acids through esterification and to prolong the shelf life of the bakery products.

Dairy industry

Dairy enzymes, an important segment of food enzyme industry, are used for the development and enhancing organoleptic characteristics (aroma, flavor and color) and higher yield of milk products. The use of enzymes (proteases, lipases, esterases, lactase, aminopeptidase, lysozyme, lactoperoxidase, transglutaminase, catalase, etc.) in dairy market is well recognized and varies from coagulant to bio-protective enzyme to enhance the shelf life and safety of dairy products. Dairy enzymes are used for the production of cheese, yogurt and other milk products.

Rennet, a combination of chymosin and pepsin, is used for coagulation of milk into solid curds for cheese production and liquid whey. Currently, approximately 33 % of global demand of cheese produced using microbial rennet. Other proteases find applications for accelerated cheese processing and in reduction of allergenic properties of milk products. Currently, lipases are involved in flavor improvement, faster cheese preparation, production of customized milk products, and lipolysis of milk fat.

Lactose intolerance is the lack of ability of human being to digest lactose due to deficiency of lactase enzyme. Lactase (β-galactosidase, EC 3.2.1.23) catalyzes hydrolysis of lactose to glucose and galactose, and therefore, is used as a digestive aid and to enhance the solubility and sweetness in milk products. It is required to minimize or removal of lactose content of milk products for lactose-intolerant people to prevent severe tissue dehydration, diarrhea, and sometimes fatal consequences.

Beverages industry

The beverage industry is divided into two major groups and eight sub-groups. The nonalcoholic group contains soft drink and syrup, packaged water, fruit juices along with tea and coffee industry. Alcoholic group comprised distilled spirits, wine and beer (Encyclopedia of Occupational health and safety). Industrial enzymes are used in breweries as processing aids and to produce consistent and high-quality products. In the brewing industries, microbial enzymes are used to digest cell wall during extraction of plant material to provide improved yield, color, and aroma and clearer products.

The enzyme applications are an integrating ingredient of the current fruit and vegetable juice industry. Enzymes are used in fruit and vegetable juice industry as processing aids to increase the efficiency of operation, for instance, peeling, juicing, clarification, extraction and improve the product quality.

Application of cellulases, amylases, and pectinases during fruit juice processing for maceration, liquefaction, and clarification, improve yield and cost effectiveness. The quality and stability of juices manufactured are enhanced by the addition of enzymes. Enzymes digest pectin, starch, proteins and cellulose of fruits and vegetables and facilitate improved yields, shortening of processing time and enhancing sensory characteristics. Amylases are used for clarification of juices to maximize the production of clear or cloudy juice.

Cellulases and pectinases are used to improve extraction, yield, cloud stability and texture in juices. Naringinase (EC 3.2.1.40) and limoninase, debittering enzymes, hydrolyze bitter components and improves the quality attributes of citrus juices.

Pectin, a structural heteropolysaccharide, present in nearly all fruits is required to be maintained to regulate cloudiness of juices by polygalacturonase (EC 3.2.1.15), pectin esterases (EC 3.1.1.11), pectin lyase (EC 4.2.2.10) and various arabanases.

Microbial amylases may be utilized in the distilled alcoholic beverages to hydrolyze starch to sugars prior to fermentation and to minimize or remove turbidities due to starch. The application of enzymes to hydrolyze unmalted barley and other starchy adjuncts facilitate in cost reduction of beer brewing. In brewing, development of chill-hazes in beer may be control by the addition of proteases.

Feed industry

To meet the continuously increasing worldwide demand of milk and meat consumption, growth of feed enzymes occurred steadily. The use of enzymes in animal diets initiated in the 1980s and exploded in the 1990s. Feed enzymes are gaining importance as they can increase the digestibility of nutrients and higher feed utilization by animals. The global market for feed enzymes was estimated $899.19 million in 2014 and expected to reach nearly $1.3 billion by 2020, at a CAGR of 7.3 % from 2015 to 2020.

Feed enzymes may be used in animal diet formulation. For instance, these are added to degrade specific feed components which are otherwise

harmful or no nutritional value. In addition, the protein dietary value of feeds available for poultry may also be enhanced by the application of feed enzymes. Feed enzymes mainly used for poultry are phytases, proteases, α-galactosidases, glucanases, xylanases, α-amylases, and polygalacturonases. The phytase, largest enzyme segment in the feed industry, is used to utilize natural phosphorous bound in phytic acid in cereal-based feed.

Monogastric animals are unable to digest plant based feeds containing high amount of cellulose and hemicelluloses. Xylanase and β-glucanase are added to their feeds as these enzymes fully degrade and digest high amount of starch. Proteases are also used in animal feeds to overcome anti-nutritional factors by degrading proteins into their constituent amino acids. Apart from improving the nutritional value of feed for better feed conversion by the animals, these feed enzymes are gaining importance for their role in feed cost reduction and meat quality improvement.

Polymer industry

To meet the increased consumption of polymers and the growing concern for human health and environmental safety has led to the utilization of microbial enzymes for synthesis of biodegradable polymer. In vitro enzyme catalyzed synthesis of polymer is an environmental safe process having several advantages over conventional chemical methods. Biopolymers are environmentally friendly materials as these are synthesized from renewable carbon sources via biological processes, degrade biologically after use and return to the natural environment as renewable resources, such as CO_2 and biomass.

Paper and Pulp industry

With increasing awareness of sustainability issues, uses of microbial enzymes in paper and pulp industry have grown steadily to reduce adverse effect on ecosystem. The utilization of enzymes reduce processing time, energy consumption and amount of chemicals in processing. Enzymes are also used to enhance deinking, and bleach in paper and pulp industry and waste treatment by increasing biological oxygen demand (BOD) and chemical oxygen demand (COD).

Xylanases and ligninases are used in paper and pulp industries to augment the value of the pulp by removing lignin and hemicelluloses. In these industries, amylases uses include starch coating, deinking, improving paper cleanliness and drainage improvement.

Lipases are employed for deinking and enhancing pitch control while cellulases are used for deinking, improving softness and drainage improvement.

Cellulase has also been used for the development of the bioprocess for recycling of used printed papers. The application of laccase is an alternative to usage and requirement of large amount of chlorine in chemical pulping process; subsequently, reduce the waste quantity that causes ozone depletion and acidification. Moreover, mannases are used for degrading glucomannan to improve brightness in paper industry.

Leather industry

The leather industry is more customary, and therefore, discharges and waste disposed from different stages of leather processing are causing severe health hazards and environmental problems. The biodegradable enzymes are efficient alternative to improve the quality of leather and help to shrink waste. The initial attempt for application of enzyme in leather industry was made for dehairing process, the largest process in leather preparation which require bulk amount of enzymes like proteases, lipases and amylases. Enzymatic dehairing applications are attractive because it can preserve the hair and contribute to fall in the organic load released into the effluent. Enzymatic dehairing processes minimize or eliminate the dependence on harmful chemicals, such as sulfide, lime and amines.

Enzymes are required for facilitating procedure and enhancing leather quality during different stages in leather processing, such as, curing, soaking, liming, dehairing, bating, picking, degreasing and tanning. The enzymes used in leather industries are alkaline proteases, neutral proteases, and lipases. Alkaline proteases are used to remove non fibrillar proteins during soaking, in bating to make leather soft, supple and pliable. Neutral and alkaline proteases, both are used in dehairing to reduce water wastage. In addition to this, lipases are used during degreasing to remove fats. The advantages of using enzymes instead of chemicals in liming are stainless pelt, reduced odor, low BOD and COD in effluents, and improved hair recovery.

Textile industry

The textile industry is responsible for vast generation of waste from desizing of fabrics, bleaching chemicals and dye is one of the largest contributors to environmental pollution. In such industries, enzymes are used to allow the development of environmentally friendly technologies

in fiber processing and strategies to improve the final product quality. The main classes of enzymes involved in cotton pre-treatment and finishing processes are hydrolase and oxidoreductase. The group of hydrolase includes amylase, cellulase, cutinase, protease, pectinase and lipase/esterase, which are involved in the biopolishing and bioscouring of fabric, anti-felting of wool, cotton softening, denim finishing, desizing, wool finishing, modification of synthetic fibers, etc. Oxidoreductase, other group of enzyme, includes catalase, laccase, peroxidase, and ligninase, which are involved in bio-bleaching, bleach termination, dye decolorization, fabric, wool finishing, etc.

3.4.1. Microalgae as source of Fuel

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Algae are photosynthetic organisms that grow in a range of aquatic habitats, including lakes, ponds, rivers, oceans, and even wastewater. They can tolerate a wide range of temperatures, salinities, and pH values; different light intensities; and conditions in reservoirs or deserts and can grow alone or in symbiosis with other organisms.

Microalgae can be a rich source of carbon compounds, which can be utilized in biofuels, health supplements, pharmaceuticals, and cosmetics. They also have applications in wastewater treatment and atmospheric CO_2 mitigation. Microalgae produce a wide range of bioproducts, including polysaccharides, lipids, pigments, proteins, vitamins, bioactive compounds, and antioxidants. The interest in microalgae as a renewable and sustainable feedstock for biofuels production has inspired a new focus in biorefinery. Growth enhancement techniques and genetic engineering may be used to improve their potential as a future source of renewable bioproducts.

The rapid growing population of the world continuously increases the global demand for fuel energy. The intensive use of fossil fuels worldwide leads to its depletion and will bring them close to the point of exhaustion due to unsustainable and nonrenewable nature. Thus, biofuels are now a growing opportunity throughout the world as alternative to fossil fuels. Some developed countries are already producing biofuels at the commercial level. Biofuels such as biodiesel and bioethanol are proving to be excellent alternative fuels and can be produced from several resources of biomass, such as food crops, crop wastes or fruits, woody parts of plants, garbage, and algae. The advantageous features of biofuels produced from biomass are renewability and a significantly smaller contribution to environmental pollution and global warming. The emission of greenhouse gases mainly CO_2 from burning of fossil fuels are the main cause of global warming. Fossil fuels are responsible for 29 giga tons/year release of CO_2 with a total of 35.3 billion tons CO_2 till now.

Biofuels including algal fuels have oxygen levels of 10–45% and very low levels of sulphur emission while petroleum-based fuels have no oxygen levels with high sulphur emission. Biofuels are non-polluting, locally available, accessible, sustainable and reliable fuel obtained from renewable sources. Microalgae algae-based fuels are eco friendly, nontoxic and with strong potential of fixing global CO_2. It has been reported that 1 kg of algal biomass is can fix 1.83 kg of CO_2 furthermore some species use SO_2 and NO_2 as nutrient flow along with CO_2. CO_2 constitutes 50% of dry weight of algal biomass. The selection and development of biomass is a crucial, cost-limiting phase in biofuels generation for adjusting and optimizing energy structure and cost. Selection of biomass for biofuels production is also directly related to greenhouse gas emissions, environmental and economic sustainability. The current focus is on microalgae as a feedstock for bioenergy production as the most promising raw material to compensate and balance the ever-increasing demands for biofuels, food, feed and valuable chemicals production. Many countries in Asia, Europe, and America have started industrialization of bioenergy from microalgae biomass.

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Microalgae are rapidly growing photosynthetic organisms having potential of transforming 9–10% of solar energy (average sunlight irradiance) into biomass with a theoretical yield of about 77 g/biomass/m^2/day which is about 280 ton/ha/year. At lager scale cultivation this yield is lower both in outdoor and indoor culture system. In Photobioreactors the actual yield is lower due to loss of absorbed active radiation, proper shaking and mixing of the culture in the bioreactor is necessary for uniform distribution of light energy to avail the same strength to all the cells to convert maximum light energy to biomass.

In several aspects, microalgae feedstock is competent and preferable to produce biofuels for examples microalgae do not require cultivable land and fresh water for cultivation, they are not edible therefore no effect on human and animal's food chain, can be grown to several folds irrespective to seasonal conditions, mitigation of atmospheric CO_2 and treatment of waste water. Absence of lignocellulosic materials in microalgae cell wall facilitates the pretreatment process and reduces overall cost of production. Microalgae can feed on industrial wastes and the processing energy is less than the energy produced by the algae. Second generation biofuels involve terrestrial plants, especially food crops as feedstocks, a highly controversial issue, since biofuels production from food crops can only occur at the expense of their use as food and feed. Additionally, crop foods require arable land and large amounts of water, which makes their use for fuel production

unsustainable and thus, incomputable as alternative liquid fuels. The algal fuels technology is still incipient, and much improvement is required to make it commercially attractive to both, investors and consumers.

Most of the microalgae species are favorable for biodiesel production due to high lipids contents 50–70% and may reach to 80% such as in case of the microlaga *B. braunii* which accumulate up to 80% of oil in it biomass. Microalgae are capable of producing algal oil 58,700 L/hac which can produce 121,104 L/hac biodiesels. The infeasibility of algal biodiesel is due to the associated high operational, maintenance, harvesting and conversion cost.

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Bioethanol is one of the major and clean biofuel used as transportation fuel. Bioethanol has many advantages over fossil fuels, such as

(i) High octane number in bioethanol prevents knocking of cylinders in engines

(ii) Due to the presence of higher oxygen contents, bioethanol burning produces much less greenhouse-effect gasses

(iii) Bioethanol is the only biofuel that can be used directly in the current automotive industry without any modifications

(iv) Bioethanol can be mixed with oil.

Global production of biofuels has increased from 4.8 to 16.0 billion of gallons from 2000 to 2007. Currently, the USA and Brazil are the world leaders in the production of bioethanol. Their contribution is approximately 75–80% of the world total bioethanol production. The USA has 187 commercial bioethanol plants, which mainly produce bioethanol from corn grain. In 2013, Brazil produced 37 billion l of bioethanol using sugar cane as the main feedstock. The European Union (EU), uses wheat and sugar beet as the preferred feedstock for bioethanol production and produces 2.0 billion gallons annually.

Biofuels from renewable and sustainable feedstock are the future permissive energy sources in place of fossil liquid fuels. Today, bioethanol is the most common biofuel, mainly produced from sugars of corn and sugarcane, but the technology is now shifting towards algal carbohydrates as potential raw materials for bioethanol production. Global bioethanol production has vigorously increased from 1 billion to 39 billion l within a few years and will reach 100 billion l soon. Microalgae possess high contents of different carbohydrates, such as glycogen, starch, agar and cellulose, etc. which can be easily converted to fermentable sugars for bioethanol production.

3.5.1. Use of Algae for Other Products

Microalgae are an excellent source of food and other important bioproducts, such as natural antibiotics. The world energy crisis in the 1970s led to the identification of algae as renewable and sustainable sources for biofuels production, prompting the exploration of microalgae as a new field of research for fuels and other valuable products. The first large-scale culture of the microalgae *Chlorella* for commercial purposes was reported in Japan, in the 1960s. Over the last few decades, algae culturing expanded to new fields, such as food and feed, biofuels, and biopharmaceuticals. Natural products in algal extracts are used in cosmetics and medicinal products. According to one estimate, about 5000 metric tons of dry algal biomass processed for bioproducts generates US$ 1.25 × 109 each year.

Microalgae produce a wide range of other commercially important and valuable products. They produce vitamins, which elevates their importance as a nutritional food for people and animals. They also produce different types of medicinally important polysaccharides. Various species produce bioactive and commercially important pigments, such as chlorophyll, β-carotene and other Carotenoids, phycobiliproteins, and astaxanthin. These pigments are crucial in therapies for tumorigenesis, neuronal disorders, and optical diseases. Microalgae are also rich sources of protein. Their production of essential amino acids increases their potential for use as protein-rich foods. Microalgae synthesize starch, cellulose, hemicelluloses, and other polysaccharides from simple monomeric sugars: basically, glucose. The higher amounts of carbohydrates in algal cells make them an important food source. Microalgae also produce and accumulate large amounts of lipids, which vary among species and are affected by various factors. Lipids in algal cells are present mainly in the form of glycerol, esterified sugars to different types of fatty acids (12–22 carbon atoms). Algal fatty acids have nutritional and medicinal applications. Most of the substances produced by microalgae have therapeutic effects. Therefore, a new area of research is extracting and identifying substances from microalgae and determining their biological and medicinal activities. Microalgae are becoming economical sources of natural substances for use as food and in cosmetics.

Pharmaceuticals on the market mainly consist of tablets or liquid forms of health-promoting substances, but several microalgae species are available as a supplement of various active substances in extract form, a new trend in the market. The microalgae market is growing due to the increasing demand for beneficial algal food and health products.

Polyunsaturated fatty acids produced by microalgae are important commercial products of high therapeutic value for cardiac diseases, asthma, and arthritis. Many important microalgae products, such as eicosapentaenoic acid and docosahexaenoic acid (DHA), have been marketed by various biotechnological companies. Some species of microalgae produce protective substances against free radicals to prevent oxidative stress. These compounds are utilized as antioxidants in neutraceutical and foods.

Researchers are taking a keen interest in algal substances with antioxidant properties that may be used in beverages and functional foods. These natural substances are highly important in pharmaceutical formulations for the treatment of free radicals and oxidative stress–associated diseases and complications. Blue-green microalgae (Cyanobacteria) are rich in various pigment compounds that enhance the efficiency of light energy utilization (phycobiliproteins) and protect photosynthetic pigments from photo-oxidation (carotenoids). Currently, microalgae products with high nutritional value are available both in pure form as extracts, tablets, or capsules and as additives to several food products, such as candy bars, gums, pastas, and beverages. These products are either used as nutrients or food coloring agents. Many microalgae strains, such as *Aphanizomenon flos-aquae*, *Chlorella, and Arthrospira*, are being cultured at a commercial scale for their high protein content and other health-promoting substances. These products reportedly have important biological effects, such as anti-hyperglycemia and anti-hyperlipidemia, which are helpful in diabetes and obesity control because they affect the elevated serum glucose level 6.

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3.5.2. Conclusion

Microalgae are a potential producer of unique natural compounds with significant biological activities. In the face of the fact that much research work has been published on high-value compounds of microalgae origin, the natural product research in the area is still in its infancy. There is a growing demand for high-value compounds of microalgae due to their importance in industrial applications as pharmaceutical, nutraceutical, cosmeceutical, animal feed, biological waste treatment,industrial enzymes,biofuel etc. Microalgae as prolific sources of bioactive molecules such as carotenoids, proteins, polysaccharides, glycolipids, which have been used or being trialed extensively to treat cancer, inflammation, malaria, TB, HIV, and others. In this regard, there has been a success in obtaining potent drugs and lead compounds like tasidotin and soblidotin, which are on clinical phase study as anticancer agents. Moreover, involving traditional biotechnology in the marine biotechnology could be a great leap towards finding high-value compounds with unique and multifunctional activities.

REVIEW QUESTIONS:

1. Write an essay on the single cell protein algae. Add their advantages and its disadvantages.
2. Define bioactive compound. Discuss about an algae as source of bioactive compound-Justify
3. Write short notes algal producing enzymes and their importance.
4. Write an account on the microalgae biofuels and its uses.

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BLOCK-2: MICRO AND MACRO ALGAE

UNIT-4 Micro algae used as biofertilizers – nitrogen fixing forms – free living and symbiotic Nitrogen fixers - Azolla- Mass cultivation of blue green algae in field – Importance and selection of carrier materials - Immobilization technique.

Objectives:

- To understand the Micro algae used as biofertilizers
- To study the free living and symbiotic Nitrogen fixers for nitrogen fixation.
- To know the Mass cultivation of blue green algae in agriculture and its – Importance
- To study the selection of different carrier materials for immobilization technique.

4.1.1. MICROALGAE AS BIOFERTILIZERS

Biofertilizers:

Biofertilizers are the substance that contains microbes, which helps in promoting the growth of plants, trees by increasing the supply of essential nutrients to the plants. It comprises of living organisms which include Mycorrhizal fungi, blue-green algae, and bacteria. Mycorrhizal fungi preferentially withdraw minerals from organic matter for the plant whereas cyanobacteria are characterized by the property of nitrogen fixation.A **biofertilizer** (also **bio-fertilizer**) is a substance which contains living microorganisms which, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances.

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Biofertilizer can be expected to reduce the use of synthetic fertilizers and pesticides. The microorganisms in Biofertilizer restore the soil's natural nutrient cycle and build soil organic matter. Through the use of Biofertilizer, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their byproducts. Hence, Biofertilizer do not contain any chemicals which are harmful to the living soil.

Biofertilizer provide "eco-friendly" organic agro-input. Biofertilizer such as *Rhizobium*, *Azotobacter*, *Azospirillum* and blue green algae (BGA) have been in use a long time. *Rhizobium* inoculants are used for leguminous crops. Blue green algae belonging to a general cyanobacteria genus, *Nostoc* or *Anabaena* or *Tolypothrix* or *Aulosira*, fix atmospheric nitrogen and are used as inoculations for paddy crop grown both under upland and low-land conditions. *Anabaena* in association with water fern *Azolla* contributes nitrogen up to 60 kg/ha/season and also enriches soils with organic matter.Nitrogen fixation is defined as a process of converting the di-nitrogen molecules into nitrogen compounds. For instance, some bacteria convert insoluble forms of soil phosphorus into soluble forms. As a result, phosphorus will be available for plants.

Azolla is an aquatic heterosporous fern which contains an endophytic cyanobacteria *Anabaena, Azolla*, in its leaf cavity. The significance of *Azolla* as biofettilizer in rice field was realized in Vietnam. Recently, it has become very popular in China, Indonesia, Philippines, India and Bangladesh.A total of six species of *Azollas* are known so far viz., *A. camlinian A.filiculoides.A.mericana, A. microphylla, A. nilotica, A. pinata* and *A. rubra*. Out of these *A. pinata* is commonly found in India.

4.1.2. Cyanobacteria-As Potential Biofertilizer

Cyanobacteria are one of the major components of the nitrogen fixing biomass in paddy fields and provide a potential source of nitrogen fixation at no cost. Due to the important characteristic of nitrogen fixation, cyanobacteria have a unique potential to contribute to enhance productivity in a variety of agricultural and ecological situations .Cyanobacteria play an important role to build-up soil fertility consequently increasing the yield. Biofertilizer being essential components of organic farming play vital role in maintaining long term soil fertility and sustainability by fixing atmospheric dinitrogen (N=N), mobilizing fixed macro and micro nutrients or convert insoluble phosphorus in the soil into forms available to plants, thereby increases their efficiency and availability.

The blue green algae (cyanobacteria) are capable of fixing the atmospheric nitrogen and convert it into an available form of ammonium required for plant growth. Dominant nitrogen-fixer blue-green algae are Anabaena, Nostoc, Aulosira, Calothrix, Plectonema etc. Blue-green algae have the abilities of photosynthesis as well as biological nitrogen fixation. Cyanobacteria are one of the major components of the nitrogen fixing biomass in paddy fields. The agricultural importance of cyanobacteria in rice cultivation is directly related with their ability to fix nitrogen and other positive effects for plants and soil. Bio-fertilizers are eco-friendly and have been proved to be effective and economical alternate of chemical fertilizers with lesser input of capital and energy.

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4.1.3. Microalgae as Biofertilizers

Biofertilizer is defined as a substance, contains living microorganisms which colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrient and/or growth stimulus to the target crop, when applied to seed, plant surfaces, or soil. The Biofertilizer are natural fertilizes which are Living microbial inoculants of bacteria, algae, fungi alone or in combination and they augment the availability of nutrients to the plants. Bio-fertilizers containing beneficial bacteria and fungi improve soil chemical and biological characteristics, phosphate solutions and agricultural production. The use of biofertilizer, in preference to chemical fertilizers, offers economic and ecological benefits by way of soil health and fertility to farmers.

Biofertilizers add nutrients through the natural processes of Nitrogen fixation, solubilizing phosphorus and stimulating plant growth through the synthesis of growth promoting substances. Biofertilizer can be expected to reduce the use of chemical fertilizers and pesticides. The microorganisms (*Azotobacter, Blue green algae, Rhizobium Azospirillum* in biofertilizer restore the soil's natural nutrient cycle and build soil organic matter. Biofertilizer contains microorganisms which promote the adequate supply of nutrients to the host plants and ensure their proper development of growth and regulation in their physiology. Living microorganisms are used in the preparation of Biofertilizer, only those microorganisms a reused which have specific functions to enhance plant growth and reproduction. Microorganism converts complex nutrients into simple nutrients for the availability of the plants. Crop yield can be increased by 20-30% if biofertilizers are used properly.

Biofertilizer can also protect plants from soil born diseases to a certain degree. The need for the use of Biofertilizer has arisen, primarily for two reasons. First, because increase in the use of fertilizers leads to increased crop Productivity, second, because increased usage of chemical fertilizer

leads to damage in soil texture and raises other environmental problems. Therefore, the use of biofertilizers is both economical and environment friendly. Organisms Used as BiofertilizerMicrobiological fertilizers are important to environment friendly sustainable agricultural practices.

The Biofertilizer includes mainly the nitrogen fixing, phosphate solubilizing and plant growth promoting microorganisms.Among biofertilizer benefiting the crop production are *Azotobacter, Azospirillium*, Blue Green Algae, *Azolla*, P-solubilizing micro organisms, *Mycorrhizae* and *Sinorhizobium* .There are different types of microorganisms which are used as the biofertilizer. Some are capable of nitrogen fixation such as *Azotobacter*, Blue green algae,*Rhizobium* and *Azospirillum. Rhizobium* is used to increase the capacity of nitrogen fixation in the leguminous plants. *Azotobacter* is used as biofertilizers for the development of various vegetable plants such as mustard, maize, wheat, cotton etc. *Azospirillum* is applied in the millets, sorghum, sugarcane, and maize and wheat field

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Blue green algae such as *Nostoc, Tolypothrix, Anabaena,and Aulosira* fix atmospheric Nitrogen and enrich the soil fertility.BGA or cyanobacteria) are diverse groups of photoautotrophic microorganisms comprising of a large, heterogeneous, and polyphyletic assemblage of relatively simple plants. Most microalgae usually occur in water, be it freshwater, marine, or brackish. They can also be found in extreme environments e.g. hot springs (Anderson, 2005).Cyanobacteria or Blue green algae (BGA) are a group of microorganism that can fix the atmospheric nitrogen.

BGA can adapt to various soil types and environment which has made it cosmopolitan in distribution. Efficient nitrogen fixing strain like *Nostoc linkia,Anabaena variabilis, Aulosira fertilisima, Calothrix sp.,Tolypothrix* sp., and *Scytonema sp.* were identified from various agro-ecological regions and utilized for rice production.After water, nitrogen is the second limiting factor for plant growth in many fields and deficiency of this element is met by fertilizers. Cyanobacteria play an important role in maintenance and build -up of soil fertility, Consequent ly increasing rice growth and yield as a natural biofertilizer.

Blue green algae (BGA) are photosynthetic nitrogen fixers and are free living. They are found in abundance in India. They too add growth promoting substances including vitamin B12, improve the soil's aeration and water holding capacity and add to biomass when decomposed after life cycle. *Azolla* is an aquatic fern found in small and shallow water bodies and in rice fields. It has symbiotic relation with BGA and can help

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rice or other crops through dual cropping or green manuring of soil. They manufacture their food by photosynthesis, as they have chloroplasts. Hence, they can live independently.Heterocystous nitrogen-fixing blue-green algae consist of filaments containing two types of cells: the heterocysts, responsible for ammonia synthesis, and vegetative cells, which exhibit normal photosynthesis and reproductive growth. Cyanobacteria are capable of abating various kinds of pollutants and have advantages as potential biodegrading organisms. As these organisms have simple growth requirements, they could be attractive host for production of valuable organic products. *Anabaena* and *azolla* are being used as a biofertilizers exhibited lignolysis and released phenolic compounds which induced profuse porulation of the organism. This report gives the usefulness of coir waste as carrier for cyanobacterial biofertilizer with supporting enzyme studies on lignin degrading ability of cyanobacteria and use of lignocellulosic coir waste as an excellent and inexpensive carrier for cyanobacterial biofertilizer.

4.1.4. Methods of Application of BGA Biofertilizer

One packet (500 g) of ready to use multani mitti based BGA biofertilizer is recommended for one acre of rice growing area. The packet is opened and mixed with 4 kg dried and sieved farm soil. The mixture is broadcast on standing water 3-6 days after transplantation. Use of excess algal material is not harmful; instead it accelerates the multiplication and establishment in the field. The field should be kept waterlogged for about 10-12 days after inoculation to allow good growth of BGA.When nitrogenous fertilizers are used, reduce the dose by one-third and supplement with BGA. Normal pest control measures and other management practices do not interfere with the establishment and activity of BGA in the field. Apply BGA for at least four consecutive seasons to have cumulative effect. One may not need to apply BGA further as these will establish in the field and reappear as and when the condition becomes favorable.

Precautions

When fertilizer or pesticides (e.g. weedicides.) are applied in the field; the algal application should be followed after a gap of 3-4 days. Application of a small dose of phosphate fertilizer after BGA inoculation accelerates BGA multiplication. However, this quantity should be considered in the total application dose for rice corp.

4.1.5. Advantages of Using Biofertilizers

Biofertilizers are becoming a rage, considering the irreparable damage that the chemical fertilizers are causing to the soil. Some of the advantages associated with biofertilizer include:

- The first and the most important advantage of using biofertilizer is that they are environment Friendly, unlike chemical fertilizers that damages the environment.
- They are comparatively low on cost inputs and are light on the pockets of the farmers
- Their use leads to soil enrichment and the quality of the soil improves with time
- Though they do not show immediate results, but the results shown over time are extremely spectacular.

Importance of Biofertilizer

Biofertilizer are important for the following reasons:

- Biofertilizer improve soil texture and yield of plants.
- They do not allow pathogens to flourish.
- They are eco-friendly and cost-effective.
- Biofertilizer protect the environment from pollutants since they are natural fertilizers.
- They destroy many harmful substances present in the soil that can cause plant diseases.
- Biofertilizer are proved to be effective even under semi-arid conditions.

Loose Association of Nitrogen-Fixing Bacteria

Azospirillum is a nitrogen-fixing bacterium that lives around the roots of higher plants but do not develop an intimate relationship with plants. It is often termed as rhizosphere association as these bacteria collect plant exudates and the same is used as a food by them. This process is termed as associative mutualism.

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4.2.1. Free Living Nitrogen Fixation

They are free-living soil bacteria which perform nitrogen fixation. They are saprotrophic anaerobes such as Clostridium, Beijerinckii, Azotobacter and Bacillus polymyxin.Among all the types of Biofertilizer, Rhizobium and Azospirillum are most widely used.Microorganisms which pass independent life and fix atmospheric nitrogen are known as free living diazotrophs. Free living bacteria and Cyanobacteria prefer a variety of habitats with varying degree of nutrients, H, oxygen, etc. Photosynthetic nitrogen fixing bacteria are divided into non-sulfur purple, purple sulfur and green bacteria.Environmental factors which influence number, community size, vegetative growth and activity of microorganisms are temperature, organic matter, pH, inorganic fertilizers, light, oxygen, season, soil and depth. In water-logging fields (anaerobic condition) such as flooded soils, lakes, ponds, rice fields, etc. non-sulfur purple bacteria grow luxuriantly.

The azotobacters are the most intensively heterotrophic group. They are the aerobic bacteria possessing the highest respiratory rates. Members of these genera are mesophilic, which require optimum temperature of about 30°C for their, growth. Density of Azotobacters ranges from l02 to 106 per gram soil. Other dominant N2 fixing aerobic bacteria present in soil are Beijerinckia and Derxia. Beijerinckia grows luxuriantly in acid soil in tropical region. However, Derxia can tolerate a pH range of 5.0 to 9.0. The studies on facultative anaerobes have not been given due consideration. The presence of 20 to 18 x 103 Klebsiella cells and less than 103 Enterobacter and Bacillus cells per gram soil would be significant to utilize an adequate amount of N2.
In water-logging conditions the number of clostridia (eg.Clostridium censbutylicum.C.butyricum.C.pasteurianum) increases in soil in a range from 102 to 106 cells per gram. Cyanobacteria are found commonly in well drained paddy and other crop fields. Some of them possess heterocyst's (e.g. Anabaena, Nostoc, Tolypothrix, etc).

4.3.1. Symbiotic N_2 -Fixing Cyanobacteria

Blue-green algae or cyanobacteria from the symbiotic association with several plants Liverworts, cycad roots, fern, and lichens are some of the Nitrogen-fixing cyanobacteria. Anabaena is found at the leaf cavities of the fern. It is responsible for nitrogen fixation. The fern plants decay and release the same for utilization of the rice plants. *Azolla* pinnate is a fern that resides in rice plants but they do not regulate the growth of the plant.

Rhizobium is one of the vital symbiotic nitrogen-fixing bacteria. Here bacteria seek shelter and obtain food from plants. In return, they help by

providing fixed nitrogen to the plants.There are some microorganisms which establish symbiotic relationships with different pots of plants and may develop (or may not) genial structures as the site of nitrogen fixation.*Azospirillum* is Gram-negative aerobes it is curved and rod shaped, and has polar flagellum. Bacteria are associated with the grass roots in such a way that a gentle washing do not dislodge the nitrogen metabolizing activity. Based on acetylene reduction it has been calculated that *A. paspali* contributes 15-93 kg Nibs/annum on *P notatum* roots, and Beijerinckia assimilates about 50 kg N/ha/annum on sugarcane root. Other bacteria on corn roots may fix about 2.4kg.N/h/day.*Azospirillum* increases yield of cereals amounting to a saving of fertilizer nitrogen equivalent to 20-40 Kg/ha.

In addition to these bacteria *Frankia, Rhizobisats* sp and Cyanobacteria undergo symbiosis by getting established inside the plant tissues and may or may not develop special symbiotic structures.The classical examples of symbiotic association developed by *Rhizobium* sp. are found in about 13,000 or more leguminous plants, both cultivated and non-cultivated herbs, shrub and trees. Among leguminosac, the largest number of plants is in papilionoidae. Moreover species of *Anabaena, Nostoc, and Tolypothrix* etc. develop symbiotic association with fungi (symbiotic structure is lichen), bryophytes, pteridophytes, gymnosperms and angiosperms and fulfill the requirement of nitrogen deficiency. However, actinorrhizic nodules are developed by *Frankia* a member of actinomycetes, on roots of about 170 species of woody dicot non-leguminous plants like Alma, Africa *Casuarinas* etc. Nodules an of two types: (1) Alma type where nodules show dichotomous branching to form a coralloid root, and (ii) Myrica/Casuarinas type in which case the apex of each nodule produces a normal but negative geotropic root. The function of nodules is to facilitate gas diffusion to the nitrogen fixing entophyte in the nodule under low oxygen tension.

4.4.1. Mass Cultivation of Azolla

Introduction

In the recent past agriculture as a profession is losing its charm among the farmers. This has been attributed to several reasons; important

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among them are spiraling cost of inputs coupled with uncertainty in the price of the product. This has been aggravated by non-availability of assured irrigation due to depletion in ground water. This has in turn manifested as distress among the farmers in substantial areas in Andhra Pradesh, Maharashtra, Karnataka and Kerala, which are otherwise considered as agriculturally developed areas. A couple of committees have gone into the root cause of distress and suggested that alternate income generating opportunities can be a major remedy for such disappointment among the farming community. Animal husbandry is one such alternative available to such distressed farmers. Again, availability of quality fodder to the animals is the major impediment in scientific management of animals because India, having only 2.4% of theworld's geographical area sustains 11% of the world's livestock population. It accounts for 55% of the world's buffalo population, 20% of the goat population and 16% of the cattle population. This has put unbearable burden on our natural vegetation.*Azolla*, hitherto used mainly as a green manure in paddy has tremendous potential to meet the growing demand for fodder among the small farmers taking up animal husbandry.

4.4.2. Azolla

Azolla is an aquatic floating fern, found in temperate climate suitable for paddy cultivation. The fern appears as a green mat over water. The Blue Green Algae cyanobacteria (Anabaena azollae) present as a symbiont with this fern in the lower cavities actually fixes atmospheric nitrogen. The rate of nitrogen fixed is around 25 kg/ha. As green manure, Azolla is grown alone for two to three weeks in flooded fields. Afterwards, water is drained out and Azolla fern is incorporated in the field before transplanting of paddy. Otherwise, 4-5 q of fresh Azolla is applied in standing water one week after planting of paddy. Dry Azolla flakes can be used as poultry feed and green Azolla is also a good feed for fish. It can be used as a bio-fertilizer, a mosquito repellent, in the preparation of salads and above all as a bio-scavenger as it takes away all heavy metals.

4.4.3. Advantages of Azolla

1. It easily grows in wild and can grow under controlled condition also.

2. It can easily be produced in large quantity required as green manure in both the seasons –Kharif and Rabi.

3. It can fix atmospheric CO2 and nitrogen to form carbohydrates and ammonia respectively and after decomposition it adds available nitrogen for crop uptake and organic carbon content to the soil.

4. The oxygen released due to oxygenic photosynthesis, helps the respiration of root system of the crops as well as other soil microorganisms.

5. It solubulises Zn, Fe and Mn and make them available to the rice.

6. Azolla suppresses tender weeds such as Chara and Nitella

in a paddy field.

7. Azolla releases plant growth regulators and vitamins which enhance the growth of the rice plant.

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8. Azolla can be a substitute for chemical nitrogenous fertilizers to a certain extent (20 kg/ha) and it increases the crop yield and quality.

9. It increases the utilization efficiency of chemical fertilizers.

10. It reduces evaporation rate from the irrigated rice field.

4.4.4. Nutrition value in Azolla

Azolla is very rich in protein (25-35%), Calcium (67 mg/100g) and Iron (7.3 mg/100g). A sustainable feed substitute for livestock", Spice India.In addition to their farming activity, small and marginal farmers are generally capable of rearing 2 to 3 units of cow/ buffaloes. For traditional methods of rearing, the feed requirements are met out from agriculture residues and very rarely the farmers can afford to provide green fodder and oil cakes. In rare cases, green fodder is provided to the animals in the form of grass collected from the field or in few cases fodder is grown in the backyard. Even then the supply of green fodder is restricted to 5 to 6 months when water is a available. Azolla fodder plot, if set up by these small farmers can cater to the fodder requirements of remaining part of the year. Azolla can be supplemented with regular feed of the animal @ 2-2.5 kg of azolla per animal. Azolla, if grown for fodder is essentially required to be grown in hygienic environment and there should be regular supply throughout the year. The fodder plots should preferably be near the homestead, where the female member of the family can attend to nurturing and maintenance.

4.4.5. Cultivation process

The biomass production under natural condition i.e. in rice field is only 50 g/sq.m/day as against optimum production of 400 g/sq.m/day. The production efficiency can be increased by reducing contamination and

competition with other algae. This can be achieved by growing *Azolla* in pits lined with synthetic polythene sheet in courtyard /back yard preferably in open space or on terrace where availability of sunlight is adequate. Although production of azolla is good in nursery plots, production of azolla as green manure in paddy fields; 10% area of the paddy field is cordoned off and azolla is grown. The land should be puddle and leveled so that standing water is uniform throughout the field.

4.4.6. Preparation of azolla inoculum

Azolla inoculum is sprinkled in the plot and 45kg of single upper phosphate per acre is applied in the field. The land used for cultivation of azolla is not wasted because after broadcasting azolla in the transplanted paddy crop (four days after transplantation) the plot itself may be used for cultivation of paddy. Even the water bodies, ditches in the vicinity can also be used for production of *azolla. Azolla* cultivated for fish feed, is grown in situ in the pond. A part of the pond is earmarked and is cordoned off by rope made up of straw. Once the mat is formed azolla is released slowly to the pond by lifting the rope. Setting up of Azolla fodder plot does not require expertise and farmers themselves can handle it with ease. If set up in backyard, the area should be leveled and lined with bricks. The side of the plots should be raised to enable the water to stand.

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4.4.7. Cultivaton process

Alternatively, the fodder plot can be in a pit with depth of 0.2 m. A polythene sheet is spread over the bed in such a way that 10 cm of standing water can be maintained. Width of the bed is maintained at 1.5 m to enable the Cultural operation from both sides. Length may be varied depending upon the fodder requirement of the unit. For two cows, two units of beds of length 2.5 m each with an area of around 8 sqm can meet 50% of the green fodder requirements. Once the bed of size 2.5 m x 1.5 m is ready, about 15 kg of fine sieved soil is spread over the bed, which will provide nutrient to the azolla plant. About 5 kg of pre-decomposed (2 days) cow dung is mixed with the water, which provides carbon source for the azolla. About 40 g of nutrient mix (made by mixing 10 kg Rock phosphate, 1.5 kg Magnesium salt and 500 g of murate of potash) is added to the azolla bed. The solution is fortified with micronutrient of desired quantity. This not only takes care of the micronutrient requirement of azolla but also the cattle when it is fed with the azolla. Sufficient water is added to make the water level of the bed to 10 cm. Production of azolla scientifically and on a continuous basis requires cement concrete tanks of size 2 m long, 1 m wide and 0.5 m deep. Due care should be taken so that water can stand in the tank. Ten or more tanks can be constructed in one place covering an area of 25 sq. m.

Arrangements for water to each tank should be made by laying pipe and tap from the over head tank.

Soil is distributed evenly across the bottom of the tank. The depth of soil layer should be about 10 cm. Cow dung is to be added at the rate of 1 to 1.5 kg per sqm of the tank area (2 to 3 kg of cow dung per tank). Single Super Phosphate (SSP) is to be added at the rate of 5 g per sqm of the tank area every week. (10 g SSP per tank). Fill the tank with water till the water collects to a height of 10 to 15 cm above the soil. Allow the soil particle to settle down. Prepare the fresh *Azolla* inoculum by adding 2 g of carbofuran to prevent pest infestation. Remove the layer of foam and scum that forms on the surface of the water. The foam impedes the growth and root penetration of *azolla*. Allow the tank to stand Overnight. On the following day, spread around 200 g of fresh *Azolla* inoculum over the surface of the water. It takes about 2 weeks for *Azolla* to form a mat over the water surface. Water level in the tank should be maintained especially during summer months. To reduce excessive ambient light, a shade made out of coconut leaves may be laid above the tank. This also prevents dew formation on the growing *Azolla* during winter. About 1.5 kg of mother culture of azolla seed material brought from *azolla* mother nursery is spread uniformly over the bed after stirring the water in the azolla bed. Care should be taken about the source of the azolla seed. Initially, *azolla* will spread over the entire bed and will take the shape of thick mat within seven days. Ideally it will give 10 kg of azolla within seven days. During the initial seven days azolla is not harvested. Water level is maintained by applying water every day. After the seventh day, 1.5 kg of azolla can be harvested every day.

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4.4.8. Harvesting process

Azolla should be harvested in plastic trays with sieve. Harvested azolla should be washed in fresh water before it is fed to the cattle. Washing is necessary to remove the smell of cow dung. The azolla wash can be used as bio-manure for plants grown nearby. Azolla harvested can be mixed with the commercial feed in 1:1 ratio. Cow dung and mineral mixture removed by *azolla* mass has to be supplemented at least once in seven days after harvest. A mixture made of cow dung, mineral mixture, soil and water should be added once in seven days. After every 60 days, soil is removed from the bed and another 15 kg of fresh fertile soil is added into the bed to avoid nitrogen build up and also provide nutrient to the *azolla*. Fresh inoculation of *azolla* after removing soil and water should be made at least once in six months repeating the whole process afresh.

4.5.1. Immobilization techniques for microalgal cells

Definition

Immobilization of enzymes (or cells) refers to the technique of confining/anchoring the enzymes (or cells) in or on an inert support for their stability and functional reuse. By employing this technique, enzymes are made more efficient and cost-effective for their industrial use. Some workers regard immobilization as a goose with a golden egg in enzyme technology. Immobilized enzymes retain their structural conformation necessary for catalysis.

4.5.2. Advantages of immobilized enzymes

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a. Stable and more efficient in function.

b. Can be reused again and again.

c. Products are enzyme-free.

d. Ideal for multi-enzyme reaction systems.

e. Control of enzyme function is easy.

f. Suitable for industrial and medical use.

g. Minimize effluent disposal problems.

4.5.3. Disadvantages of immobilization

a. The possibility of loss of biological activity of an enzyme during immobilization or while it is in use.

b. Immobilization is an expensive affair often requiring sophisticated equipment.

Immobilized enzymes are generally preferred over immobilized cells due to specificity to yield the products in pure form. However, there are several advantages of using immobilized multi-enzyme systems such as organelles and whole cells over immobilized enzymes. The immobilized cells possess the natural environment with cofactor availability (and also its regeneration capability) and are particularly suitable for multiple enzymatic reactions.

4.5.4. Methods of Immobilization:

The commonly employed techniques for immobilization of enzymes are adsorption, entrapment, covalent binding and cross-linking.

4.5.4. Adsorption

Adsorption involves the physical binding of enzymes (or cells) on the surface of an inert support. The support materials may be inorganic (e.g. alumina, silica gel, calcium phosphate gel, glass) or organic (starch, carboxymethyl cellulose, DEAE-cellulose, DEAE-sephadex).

Adsorption of enzyme molecules (on the inert support) involves weak forces such as van der Waals forces and hydrogen bonds. Therefore, the adsorbed enzymes can be easily removed by minor changes in pH, ionic strength or temperature. This is a disadvantage for industrial use of enzymes.

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4.5.5. Entrapment

Enzymes can be immobilized by physical entrapment inside a polymer or a gel matrix. The size of the matrix pores is such that the enzyme is retained while the substrate and product molecules pass through. In this technique, commonly referred to as lattice entrapment, the enzyme (or cell) is not subjected to strong binding forces and structural distortions.

Some deactivation may however, occur during immobilization process due to changes in pH or temperature or addition of solvents. The matrices used for entrapping of enzymes include polyacrylamide gel, collagen, gelatin, starch, cellulose, silicone and rubber. Enzymes can be entrapped by several ways

4.5.6. Enzyme inclusion in microcapsules

In this case, the enzymes are trapped inside a microcapsule matrix. The hydrophobic and hydrophilic forms of the matrix polymerise to form a microcapsule containing enzyme molecules inside. The major limitation for entrapment of enzymes is their leakage from the matrix. Most workers prefer to use the technique of entrapment for immobilization of whole cells. Entrapped cells are in use for industrial production of amino acids (L-isoleucine, L-aspartic acid), L-malic acid and hydroquinone.

4.5.7. Microencapsulation

Microencapsulation is a type of entrapment. It refers to the process of spherical particle formation wherein a liquid or suspension is enclosed in a semi permeable membrane. The membrane may be polymeric, lipoidal,

lipoprotein-based or non-ionic in nature. There are three distinct ways of microencapsulation.

1. Building of special membrane reactors.

2. Formation of emulsions.

3. Stabilization of emulsions to form microcapsules.

Microencapsulation is recently being used for immobilization of enzymes and mammalian cells. For instance, pancreatic cells grown in cultures can be immobilized by microencapsulation .

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4.5.8. Covalent Binding

Immobilization of the enzymes can be achieved by creation of covalent bonds between the chemical groups of enzymes and the chemical groups of the support. This technique is widely used. However, covalent binding is often associated with loss of some enzyme activity. The inert support usually requires pretreatment (to form pre-activated support) before it binds to enzyme. The following are the common methods of covalent binding.

1. Cyanogen bromide activation

The inert support materials (cellulose, sepharose, sephadex) containing glycol groups are activated by CNBr, which then bind to enzymes and immobilize them.

2. Diazotation

Some of the support materials (amino benzyl cellulose, amino derivatives of polystyrene, aminosilanized porous glass) are subjected to diazotation on treatment with $NaNO_2$ and HCI. They, in turn, bind covalently to tyrosyl or histidyl groups of enzymes.

3. Peptide bond formation

Enzyme immobilization can also be achieved by the formation of peptide bonds between the amino (or carboxyl) groups of the support and the carboxyl (or amino) groups of enzymes. The support material is first chemically treated to form active functional groups.

4. Activation by bi- or poly-functional reagents

Some of the reagents such as glutaraldehyde can be used to create bonds between amino groups of enzymes and amino groups of support (e.g. aminoethylcellulose, albumin, amino alkylated porous glass).

4.5.9. Cross-Linking

The absence of a solid support is a characteristic feature of immobilization of enzymes by cross- linking. The enzyme molecules are immobilized by creating cross-links between them, through the involvement of poly-functional reagents. These reagents in fact react with the enzyme molecules and create bridges which form the backbone to hold enzyme molecules. There are several reagents in use for cross-linking. These include glutaraldehyde, diazobenzidine, hexamethylene diisocyanate and toluene di- isothiocyanate.

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4.5.10. Choice of Immobilization Technique

The selection of a particular method for immobilization of enzymes is based on a trial and error approach to choose the ideal one. Among the factors that decide a technique, the enzyme catalytic activity, stability, regenerability and cost factor are important.

Stabilization of Soluble Enzymes

Some of the enzymes cannot be immobilized and they have to be used in soluble form e.g. enzymes used in liquid detergents, some diagnostic reagents and food additives. Such enzymes can be stabilized by using certain additives or by chemical modifications. The stabilized enzymes have longer half-lives, although they cannot be recycled. Some important methods of enzyme stabilization are briefly described.

Solvent Stabilization

Certain solvents at low concentrations stabilize the enzymes, while at high concentrations the enzymes get denatured e.g. acetone (5%) and ethanol (5%) can stabilize benzyl alcohol dehydrogenase.

Substrate Stabilization

The active site of an enzyme can be stabilized by adding substrates e.g. starch stabilizes a-amylase; glucose stabilizes glucose isomerase.

Stabilization by Polymers

Enzymes can be stabilized, particularly against increased temperature, by addition of polymers such as gelatin, albumin and polyethylene glycol.

Stabilization by Salts

Stability of metalloenzymes can be achieved by adding salts such as Ca, Fe, Mn, Cu and Zn e.g. proteases can be stabilized by adding calcium.

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Stabilization by Chemical Modifications

Enzymes can be stabilized by suitable chemical modifications without loss of biological activity. There are several types of chemical modifications.

a. Addition of poly-amino side chains e.g. polytyrosine, polyglycine.

b. Acylation of enzymes by adding groups such as acetyl, propionyl and succinyl.

Stabilization by Rebuilding

Theoretically, the stability of the enzymes is due to hydrophobic interactions in the core of the enzyme. It is therefore, proposed that enzymes can be stabilized by enhancing hydrophobic interactions. For this purpose, the enzyme is first unfold and then rebuilt in one of the following ways.

1. The enzyme can be chemically treated (e.g. urea and a disulfide) and then refolded.

2. The refolding can be done in the presence of low molecular weight ligands.

3. For certain enzymes, refolding at higher temperatures (around 50°C) stabilize them.

Immobilization of Cells

Immobilized individual enzymes can be successfully used for single-step reactions. They are, however, not suitable for multi-enzyme reactions and for the reactions requiring cofactors. The whole cells or cellular

organelles can be immobilized to serve as multi-enzyme systems. In addition, immobilized cells rather than enzymes are sometimes preferred even for single reactions, due to cost factor in isolating enzymes. For the enzymes which depend on the special arrangement of the membrane, cell immobilization is preferred.

Immobilized cells have been traditionally used for the treatment of sewage. The techniques employed for immobilization of cells are almost the same as that used for immobilization of enzymes with appropriate modifications. Entrapment and surface attachment techniques are commonly used. Gels, and to some extent membranes, are also employed.

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Immobilized Viable Cells

The viability of the cells can be preserved by mild immobilization. Such immobilized cells are particularly useful for fermentations. Sometimes mammalian cell cultures are made to function as immobilized viable cells.

Immobilized Non-viable Cells

In many instances, immobilized non-viable cells are preferred over the enzymes or even the viable cells. This is mainly because of the costly isolation and purification processes. The best example is the immobilization of cells containing glucose isomerase for the industrial production of high fructose syrup.

Limitations of Immobilizing Eukaryotic Cells

Prokaryotic cells (particularly bacterial) are mainly used for immobilization. It is also possible to immobilize eukaryotic plant and animal cells. Due to the presence of cellular organelles, the metabolism of eukaryotic cells is slow. Thus, for the industrial production of biochemical, prokaryotic cells are preferred. However, for the production of complex proteins (e.g. immunoglobulin's) and for the proteins that undergo post- translational modifications, eukaryotic cells may be used.

Review questions

1. Microalgae used as biofertilizers-justify Add their importance.

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2. Write short notes on symbiotic nitrogen fixing cyanobacteria.
3. Define immobilization. Explain the different method of immobilization technique

UNIT - V

MASS CULTIVATION OF MACRO ALGAE: ROPE CULTIVATION IN THE LABORATORY-APPLICATIONS OF SEAWEEDS IN BIOTECHNOLOGY

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Objectives:

- To know the Mass cultivation technique of macro algae.
- To study the various applications of seaweeds in biotechnology.

5.1.1. MASS CULTIVATION OF MACRO ALGAE

5.1.1. Introduction of test plants

Seedlings (propagules) used for test planting should come from within the area to avoid stress on the plant.

1. Weigh individual test seedlings before test planting.
2. Undertake actual planting with numbered tag sandals for identification purpose.
3. Clear daily the test plant.
4. Treat test plant individually and avoid direct exposure to sunlight or rain.
5. Weigh plants to get growth rate from the test plant with a duration of at least 6 weeks or more for more reliable data.
6. Weigh test plants weekly or every 15 days to avoid stress on the plants.
7. Check test plants, missing plants should be replaced immediately.
8. Take note of the seedlings growing points for it is one indicator of good growth.
9. Use triple beam balance/string balance or actual calculation in determining the incremental weight.
10. Formula for getting growth rate.

$$\frac{TW - OW}{\text{No. of culture days}} = \text{percent daily growth rate}$$

Where:

TW = Total weight of plant after test planting
OW = Original weight of the plant before test planting
No. of culture days = Period of test planting

The data gathered on the proposed site should be carefully analyzed and the result of the test planting will serve as basis in determining the suitability of the area and the desired methods of farming to be used.

NOTES

A good seaweed farmer must know first his farm, available resources, the environmental condition as well as its location, boundaries and shape of the area. A knowledge of all these will guide the farmer on the proper construction of the farm.

5.1.2. Site Selection:

The search for a suitable area is the most difficult task encountered in the industry due to the very delicate nature of the plant. The following are some guides in selecting and prospecting areas for cultivation of macro algae.

1. Choose a location where there is a good water movement or where there is a rapid water turnover, but not heavy enough to damage the farm. Water current speed should be between 20 to 40 meters per minute.
2. Area should be sheltered from very strong wave action, current and winds.
3. Avoid areas that are near the mouth of rivers or where there is a heavy freshwater runoff. *Eucheuma* is a purely marine alga. Salinity of the farm area should be from 27 to 35 parts per thousand.
4. The area should have a water temperature range between 25°C and 30°C.
5. Water depth in the farm should not be less than 2 feet during the lowest tide and more than 7 feet during high tide.
6. The ground should be stable enough to permit easy installation of stakes or bamboos.
7. Farm bottom composition should be sandy and rocky depending upon the variety of *Eucheuma*.

8. If possible, choose areas where *Eucheuma* is endemic. However, the absence of such is not necessarily a negative sign.
9. Take note of the other marine plants and animals that are associated with *Eucheuma*, for they are good indicators of possible site for *Eucheuma* farming.
10. Consider also the availability of labor, materials, accessibility to transportation and communication as well.

Generally, there is no way of determining the appropriateness of the area unless actual testing is done. The level of productivity of the selected area or locality cannot be accurately ascertained until a history of growth rate has been established. Once the site is pinpointed, the task of production will be relatively easier.

NOTES

5.1.3. Line/ Rope cultivation method:

The use of loop or knotted monofilament line or polyethelene rope or any twine (durable enough to last for the duration of the test planting activities) tied end to end of two mangrove stakes installed 12 meters apart from each other, into which test plants are tied to the loop with corresponding numbered rubber sandal tag for identification (Figure 1).

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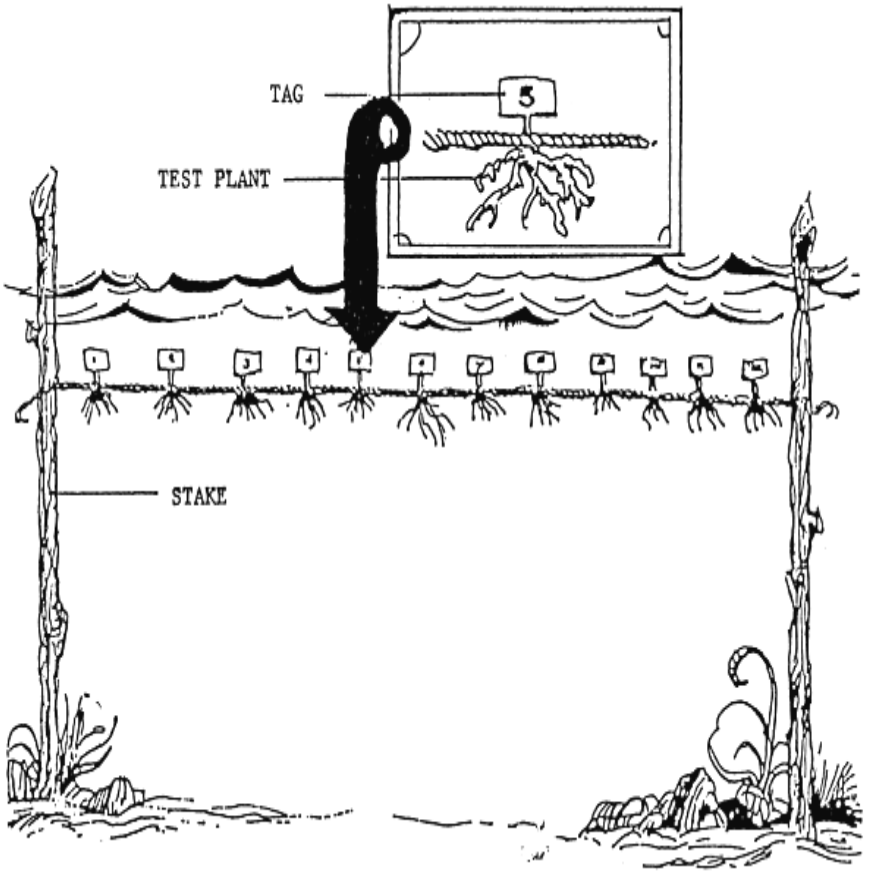

Figure 1. Line/rope method

5.1.4. Preparation of The Farm Site And Other Culture Activities

When a site suitable for Eucheuma farming has been selected, secure a permit from the nearest Bureau of Fisheries and Aquatic Resources office which has a direct jurisdiction over the area for legal acquisition. The following are the steps to follow

1. Cut all grasses and remove all obstacles from the area.

2. Remove the rocks, stones, starfishes, sea urchins and other predators.

3. Construct a farm house with a drying platform on the selected site.

4. Prepare rattan/buri baskets or seed-bin for holding seed stocks.

5. Prepare also other culture materials such as nylon monolines, nylon nets, mangrove stakes, boats dug-out/banca, digging bars, plastic twines, gas lamps, knives, hammer/mallet, etc..

5.1.5. Bottom mono line method

This method consists of modules which are units of planting in a hectare. A module has 28 monolines (single line) each measuring 30 ft (9.8 m) in length. About 36 plants can be tied to a monoline. A hectare of 35 modules consequently contains 35 000 plants with about 1 000 plants per module (Genu Manual).

Here are the planting details in bottom monoline method:

Seedling size	- 50–150 g
Total weight or seedling hectare	- 3–5 tons
Plastic tie length	- 6 inches (15.3 cm)
Plastic tying allowance	- 1 inch (2.5 cm)
Mangrove stake length	- 29 inches (74 cm)
Monoline distance from bottom	- 8–10 inches (20–25 cm)
Module dimension	- 20 × 70 ft (10 × 20 m)
Space between modules	- 5 ft (1.6 m)
Nylon line size	- 160 lbs
Plastic tie quality	- soft, flexible, not easily shredded, medium-sized, 7 mm width

Procedures in the construction of monoline

1. Using a mallet, drive wooden posts to the bottom one meter apart in rows and 10 meters between rows.
2. Tie nylon monolines at both ends of the posts, parallel to each other.
3. The distance of the line from the bottom should be about 20–25 cm (8–10 inches).

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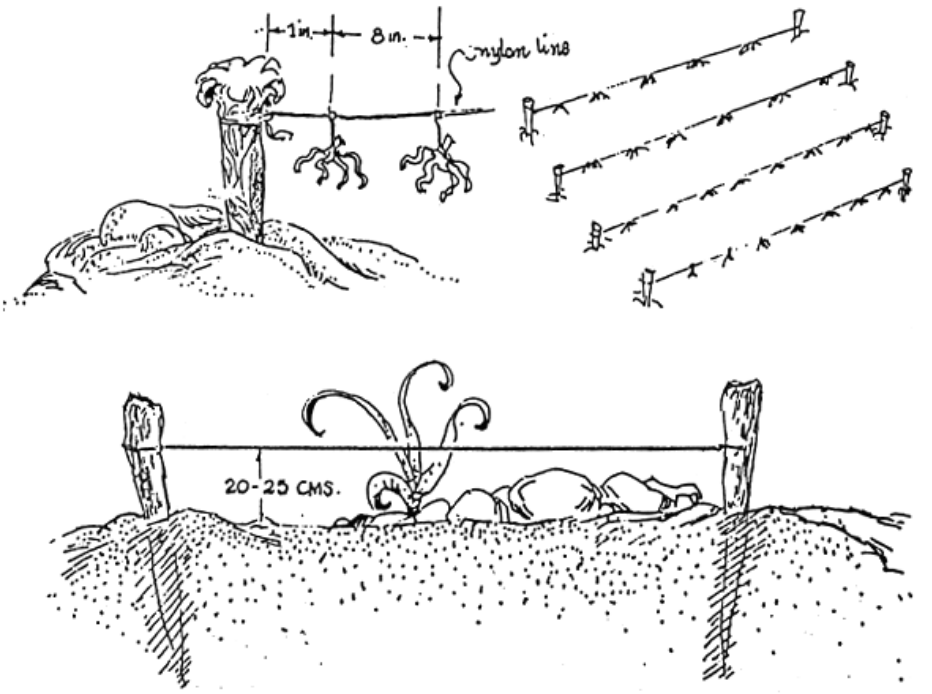

Fig. 2. Bottom mono line method

5.1.6. Seed selection and preparation

Careful selection of seedlings is a must. Healthy strong branches should be chosen. Good seedlings are usually found at the center and near the tip of a healthy plant. The ways to prepare the seedlings are:

a. Use a clean and sharp stainless knife to cut the branches in order to leave a smooth surface.
b. Never cut the branch in a slant position.
c. Do not produce seedlings with any cuts at its branches.

Tying of seedlings

Use of soft, flexible, not easily shredded and medium-sized plastic. Tying length will be 6 inches (15.3 cm). The following are the ways to tie seedlings:

a. Seedlings should be tied at the strongest point where they are well-balanced for free movement. Avoid breakage of the branches.
b. Tie the seedlings properly with enough allowance for growth.

c. Do not tie two or more seedlings together.

5.1.7. Planting

Before planting, clean the seedlings thoroughly by removing dirt, epiphytes and other clinging materials and rinse with sea water. Plant prepared seedlings promptly. In the net method, seedlings, (80–100 g) are tied to each inter section of the net. Original net can hold about 150 plants (BFAR Handouts).In bottom mono line method, the seedlings (50–150 g) are planted by tying to the mono line with an allowance of one inch (2.5 cm) of the tied portion of the nylon line.

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Harvesting

Harvesting can also be made a part of the maintenance procedure by pruning the harvestable plants and allowing them to regenerate.

Pre-harvest activities

a. Prepare all the necessary harvesting materials such as baskets, sacks, knife and goggles.
b. Prepare the drying area.

5.1.8. Harvesting procedures

a. Go row by row through the farm, harvest each plant by pruning. Remove branches from each plant using a sharp stainless knife. Leave about 200 g on each plant for regeneration.
b. In total harvesting, just cut the allowance portion of the tie in-between the plant and the nylon line.
c. Using scoop nets, scoop harvested plants.
d. Collect all harvested plants in the banca.
e. Paddle the banca to the drying area when it is already full.
f. Unload, weigh and keep a record of all the harvests before spreading them to dry.

5.1.9. Drying

Recommended practices in drying the harvested plants:

1. Spread the harvested seaweed thinly over the prepared drying site of platform.
2. Use coconut palm as flooring when drying in land. Never dry the sea weed directly on and or soil to avoid contamination.

3. In a drying platform, the approximate amount of wet seaweed should be about 2 1bs/sq ft.
4. Turn the seaweeds at regular intervals under the sun for three days and at the same time removing foreign materials. During summer months, seaweeds can be dried for only 1–½ days.
5. Always cover the harvest to protect from the rain.
6. Separate dried harvest according to the day it was harvested.
7. Seaweeds are considered dry when there are no moist and particles of sand on the surface. They are crispy and have a rubbery touch.
8. Remove salt particles from the dried seaweeds by screening them with meshed screens.
9. Pack, clean dried seaweeds free of salt particles in large sacks and lace them up.
10. Sell the seaweeds and keep a record of all its weight.

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5.1.10 Maintenance of The Farm

Assurance for good farm production largely depends upon farm management, procedures and practices coupled with the farmer's own initiative and creativity. Some ways to maintain a Eucheuma farm:

1. Remove sea urchins, starfishes, rocks, dead corals and other obstacles found inside the farm every day.
2. A boat (with or without engine) would be required depending upon the distance of the residence to the farm site.
3. Replace missing plants.
4. Never allow the plant to grow more than 5 kgs for it will be destroyed by the water current.
5. Harvest completely all unhealthy and loose plants.
6. Tighten any loose nets and repair any broken lines and destroyed stakes.
7. Always keep the record of the farm such as weekly test plant record, daily harvest record (wet and dry), record of receipts and expenses, etc.
8. When a farm is a hectare or more, it is advisable to install nets in the lower position of the farm to collect seaweeds washed out by the tidal current. The mesh size of net to be used should not be more than 4 inches made of No. 100 nylon line.

5.2.1. APPLICATION OF SEAWEEDS IN BIOTECHNOLOGY

Introduction

Seaweeds are aquatic plants like kelp and algae that thrive in saline ocean waters. Seaweed has been touted as a weapon in the war on aging skin, a replacement for seafood, a way to improve the qualities and texture of foods, and is now the next big hope for biofuel production.

The idea of using seaweed to make biofuel is getting the most attention today since seaweed has high concentrations of the right kinds of sugars for making fuel. According to Market watch, a team of scientists found a way to use microbes that eat the alginate in seaweed. The microbes then produce sugars that would convert to fuel and other chemicals.

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About 60 percent of the dry biomass of seaweed are sugars, and more than half of those are locked in a single sugar – alginate, Scientists have developed a pathway to metabolize the alginate, allowing us to unlock all the sugars in seaweed, which therefore makes macro algae an economical alternative feedstock for the production of renewable fuels and chemicals.

One estimate is that only 3 percent of the world's coastal waters would produce more than 60 billion gallons of much cleaner burning seaweed biofuel. The beauty of seaweed is that it is globally available and does not require dry land or precious freshwater to grow. Seaweed farming is the biggest aquaculture industry in the world. According to Seafood Source, over 17.3 million metric tons of seaweeds are farmed, mostly for food supplements, with China as the lead grower. The next decade, however, will see explosive growth in seaweed farms throughout the world if the bio fuel process and infrastructure becomes viable.

5.2.2. Food technology

Seaweed contains Alginate, carrageenan, and agar. These substances help ice cream to freeze in a creamy fashion by controlling the way that ice crystals form. Beer foam becomes more stable and lasting. Many foods, including mayonnaise, yogurt, syrups and sauces are thickened and stabilized by these substances.

5.2.3. Nutrition

Seaweed contains omega-3, proteins, vitamins and minerals, and is in increasing demand where seafood supplies are becoming scarcer. Processed seaweed supplements are in big demand.

5.2.4. Cosmetics

Seaweed stabilizes such cosmetic items as toothpaste and helps to gel various substances together. With no scientific proof yet, seaweed is claimed to have anti aging properties, but has always been known to be good for the skin.

5.2.5. Sexually transmitted disease

Seaweed is found to be somewhat effective in fighting Human Papillomavirus (HPV), Herpes and HIV and is an ingredient in sex lubricants.

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5.2.6. Fertilizer

Seaweed is an old natural fertilizer that has been effectively used in coastal areas of the world. Seaweed has a lot of nitrogen and potassium, plus the kind of carbohydrates that help to condition the soil. The first seaweed was easy to harvest because it could be easily found cast ashore. The weed was too heavy to carry inland, but today's new technologies process the seaweed so that it can be transported and used anywhere.

5.2.7. Water treatment

After the alginate has been removed, seaweed may be capable of lowering the amount of phosphorous, nitrogen and ammonium in waste water. These chemicals are responsible for causing too much algae and other aquatic plants to grow in wastewater.

5.2.8. Industrial Utilization of Seaweeds in India

Approximately 7.5-8 million tons of wet seaweeds are harvested worldwide per year. In India, seaweeds are utilized by the industries, mainly for commercial production of agar and alginate. These have been used as food for human beings, feed for animals, fertilizers for plants and source of various chemicals. Carrageenan industries are least developed due to non-availability of sufficient raw materials for carrageenan production. Agar production in India started in 1940 on a cottage industry-scale, using *G. edulis* as raw material. Subsequently, a viable cottage industry method for the manufacture of agar from *G. lichenoides* was developed.

Later a process for industrial manufacture of agar was developed by some researcher using *Gelidium micropterum* as raw material. With the development of this industrial method, a few industries started agar production using either *G. acerosa* or *G. edulis* as raw material. Previously some reports says that there are 46 seaweed based industries – 21 agar and 25 alginate – but not functioning up to their rated capacity, as there has been a short supply of raw materials.

In the recent years, seaweed products are used in our daily lives in one or the other way.For example, some seaweed polysaccharides are employed in the manufacture of toothpastes, soaps, shampoos, cosmetics, milk, ice creams, meat, processed food, air fresheners and a host of other items.

5.2.9. Seaweed Bio Filter

Aquaculture has been supporting human demands for fish products for centuries and is an important industry worldwide. Fish aquaculture production on an intensive scale has caused many environmental problems to reduce the nutrient burden of the fish farm effluents; the integration of seaweed cultivation with fish aquaculture has been proposed.

Various strategies for integrating seaweed cultivation with fish culture have been successful. Several species of *Gracilaria*, *Ulva* and *Laminaria* and other macro algae have been considered in the integrated bio filter system and showed reasonably high efficiency in the removal of waste inorganic nutrients. Recently, *Porphyra* (known as "Gim" in Korean) has been recommended as an attractive candidate for the integrated aquaculture with salmonids. Seaweeds were used in treatment of sewage and some agricultural wastes to decrease the total nitrogen and phosphorus and it might removal of toxic metals from industrial wastewater.

5.2.10. Plant Growth Hormones

The plant growth stimulating Plant growth hormones (PGH) were found most of the seaweed. Especially auxins, cytokinins and gibberellins. Cytokinins have been detected in fresh seaweed. Cytokinins stimulate rapid cell division and the production of new cell walls, so cytokinins are particularly important for new growth. Other plant hormones present in seaweed extracts auxins, were shown to initiate root formation and inhibit its elongation. Plants are able to synthesize these compounds from tryptophan or indole. The indole-3-acetic acids (IAA) were found in different species of seaweeds such as *Ascophyllum nodosum, Porphyra*

perforata, *Botryocladia* spp, *Enteromorpha* sp. and in cyanobacteria. Gibberellins are best known for their affects on stem elongation and flower development, but they are also important for breaking seed dormancy. The gibberellins stimulate the dormant seeds to produce enzymes that break down the stored starches into energy molecules needed for respiration. The seed germinating hormones gibberellins was identified in *Fucus vesiculosus* and *Fucus spiralis*. Generally in this plant growth hormones extracted from seaweeds it can increase the crop productivity in commercial world. An additional role of this plant regulator is to enhance chlorophyll content in leaves by decreasing its degradation.

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5.2.11. Seaweeds as Food and Feeds

Food and feed has long been used to improve health, our knowledge of the relationship between food components and health is now being used to improve food. Food components that have been demonstrated to provide specific health benefits beyond basic nutrition. Food components and nutrition science has moved from identifying and correcting nutritional deficiencies to designing foods that promote optimal health and reduce the risk of disease. Today's science and technology can be used to provide many additional functional foods components, and future scientific and technological advances promise an even greater range of health benefits for consumers. Foods and feeds can provide health benefits by reducing the risk of chronic diseases and enhancing the ability to manage chronic diseases, thus improving the quality of life. Seaweeds are recognized for their enriches in polysaccharides, minerals and certain Vitamins, but they also contain bioactive substances like polysaccharides, proteins, lipids and polyphenols, with antibacterial, antiviral and antifungal properties, as well as many others.

Seaweed great potential as a supplement in functional food or for the extraction of compounds. Physiologically active compounds in marine algae are classified into two types based on the difference in the mechanisms: Non-absorbed high-molecular materials like dietary fibers' and low-molecular materials, which are absorbed and which affect the maintenance of human homeostasis directly. Generally near coastal lived animals like sheep, cattle and horses have been eaten seaweeds in olden days. Nowadays various advanced technologies were applied and produced the animal feeds, the seaweeds were collected and it was washed and dried that has been crushed to a fine powder after that commercialized.

This seaweed feed contains useful amounts of minerals (potassium, phosphorus, magnesium, calcium, sodium, chlorine and sulphur), trace

elements and Vitamins. Trace elements are essential elements needed by humans and other mammals in smaller quantities than iron (approximately 50mg/kg body weight), and include zinc, cobalt, chromium, molybdenum, nickel, tin, vanadium, fluorine and iodine. Because most of the carbohydrates and proteins are not digestible, the nutritional value of seaweed has traditionally been assumed to be in its contribution of minerals, trace elements and Vitamins to the diet of animals. Currently the aquatic organisms were cultivation was more in worldwide it might increase economic level of the country, that's the reason to origin, the governmental and non-governmental aquaculture industries were developed. The aquatic organisms especially in fish cultivation were applied in seaweed feeds.

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5.2.12. Pharmaceutical and Cosmaceutical Applications of Seaweeds

To date, researchers have isolated approximately 7000 marine natural products, 25 percent of which are from algae. The antimicrobial properties of seaweed extracts have been well documented over the years. The seaweed extracts were used as a therapeutic and protective agent for various diseases and its ability were viewed such as antibiotics, antihelminthics, cough remediates, antihypertensive, anti tumor and anti diarrhea drugs or compounds. Most of the seaweeds have the bioactive components which inhibit the growth of Gram-positive bacteria as well as the Gram-negative bacteria pathogens. *Bryopsis* sp. was noted for its *in vitro* activity against *Mycobacterium tuberculosis, Delisea pulchra*.

Effects are seen on the swarming of *Serratia liquefaciens* and the bioluminescence and virulence in several pathogenic *Vibrio* species. It also inhibits carbapenem antibiotic synthesis and exoenzyme virulence factor production in the phytopathogen *Erwinia carotovora*. Recently, many researchers have embarked on chemical investigations of marine algae with a special account on their bioactive properties. Several investigations have proved that crude seaweeds and their organic extracts have anti-proliferative activity on human cancer cell lines *in vitro*, as well as inhibiting activity on tumors' growing in mice *in vivo*. "Extract of seaweed" is often found on the list of ingredients on cosmetic packages, particularly in face, hand and body creams or lotions. This usually refers to the use of alginate or carrageenan in the product some algae are also potential skin irritants. For example, the phycocyanin present in blue-green algae has been suspected of allergenicity and of causing dermatitis on the basis of patch tests.

Extracts of 25 seaweeds from the Indian coast have been put through a broad biological screen which includes tests for antiviral, antibacterial,

antifungal, antiprotozoal, anti-fertility activities, and a wide range of pharmacological activities. Significant activity is found in 13 seaweeds. Most promising activity being 100% ant fertility (anti-implantation) activity observed in three species. The antiviral activity observed in *Codium elongatum* and the two species of *Hypnea* was attributed to the poly-saccharides to date, in world wild researchers are achieved some kind of pharmaceutical valuable activities from seaweeds. Cosmetic products, such as creams and lotions, sometimes show on their labels that the contents include "marine extract", "extract of alga", "seaweed extract" or similar. This means that one of the hydrocolloids extracted from seaweed has been added. Alginate or carrageenan could improve the skin moisture retention properties of the product. Pastes of seaweed, made by cold grinding or freeze crushing, are used in thalassic therapy, where they are applied to the person's body and then warmed under infrared radiation. This treatment, in conjunction with seawater hydrotherapy, is said to provide relief for rheumatism and osteoporosis.

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5.2.13. Seaweed as Bio-fuels

Bio-fuel from seaweed is produced by converting alginate, mannitol and fiber contained in seaweed into ethanol, butanol, etc. Seaweed is a known potential carbon-dioxide (CO_2) neutral source of second generation bio-fuels. Energy is stored inside the cell as lipids and carbohydrates, and can be converted into fuels such as biodiesel (in the presence of oils) and ethanol (in the presence of carbohydrates). Its high protein content implies that waste from the feedstock conversion process may yield a saleable waste stream as well. Fuels derived from algae generally fall into two groups; oils which are extracted from algae by a mechanical or chemical process; and ethanol resulting from the fermentation of algae in the presence of a yeast, and isolating the ethanol produced. Its use can reduce green house gas emission up to 40%.

The ethanol production already reported from *Laminaria hyperborean* extracts was evaluated with yeast *Pichia angophorae* and its possibility of utilizing both mannitol and laminaran as substrates. Seaweed extract showed that *Pichia angophorae* was able to utilize both mannitol and laminaran for ethanol production. Higher yield of ethonal was recorded while using *Saccharina latissima* as a substract. Many researchers reported that seaweeds might better for higher biodiesel production, in this way algae be capable of used as renewable energy.

5.2.14. Pigments

The significance of marine algae as sources of natural pigments has been well documented due to their important beneficial effects in food, feed

and pharmaceuticals. The colour in crate of green seaweeds is due to the presence of chlorophyll a and b in the same proportions as the 'higher' plants; beta-carotene (a yellow pigment) and various characteristic xanthophylls (yellowish or brownish pigments). The ability of the xanthophylls pigment, fucoxanthin, is responsible for the colour of brown seaweeds. Recently, many potential antioxidant compounds were identified as some pigments (*e.g.* fucoxanthin, astaxanthin, carotenoids) and poly-phenols (*e.g.* phenolic acid, flavonoid, tannins). Recently, a pigment, carotenoids (fucoxanthin) reported that it could even provide a new functional food and cosmetic ingredient with anti-metabolic syndrome activity (anti-obesity, anti-diabetes).

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Among polyphenols phenolic acids, flavonoid, isoflavones, cinnamic acid, benzoic acid, quercetin and lignin can be mentioned. Seaweed extracts contain appreciable amounts of polyphenols, but their content is strongly dependent on the extraction method. *Ascophyllum* spp. has significantly more polyphenols than other sea-weeds, while *Ulva* spp. has the lowest content of these compounds.

5.2.15. Hydrocolloids and Foods

Hydrocolloids based gums are a diverse group of long chain polymers characterized by their property of forming viscous dispersions and/or gels when dispersed in water. Most important seaweed hydrocolloids are agars, carrageenan and alginates, which are produced in form of colour less powders. Agar was the first hydrocolloid used as an additive into food in Asian countries 300 years ago. About 90 percent of the agar produced is for food applications, the remaining 10 percent being for microbiological and biotechnology uses. Most of the agars are extracted from the species of *Gelidium gracilaria.*

Agar can be divided into two principal components: agarose and agaropectin. Agarose is the gelling component; agaropectin has only a low gelling ability. High quality agarose mainly has biotechnology applications. Alginate, sometimes present in the cell walls of brown seaweeds, and it is partly responsible for the flexibility of the seaweed. Most carrageenan is extracted from *Kappaphycus alvarezii*. Carrageenan or carrageenan is a family of linear sulphate based polysaccharides that are extracted from red edible seaweeds. They are commonly used in the food industry, for gelling, thickening, and stabilizing properties. Fucoidin is the naturally available marine product of brown algae.

It is applied in medicinal and therapeutics fields. Especially fucoidin from marine algae have been reported to exhibit outstanding biological activities that aid human health fucoidans were extracted from different seaweeds such as *Ascophyllum nodosum, Sargassum stenophyllum, and Laminaria japonica* and were report to have pharmaceutical potentials. There are several carrageenan, differing in their basic chemical structure and properties, and therefore in their uses. The carrageenans of commercial interest are called iota, kappa and lambda, and it's susceptible to depolymerisation through acid catalyzed hydrolysis. At high temperatures and low pH this may rapidly lead to complete loss of functionality.

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CONCLUSION

The marine environment has great potential for the discovery of lead compounds that could be used. Particularly in seaweeds populations of the aquatic environments provide a vast genetic resource and biodiversity. Scientists have opined that seaweeds can be utilized in a completely different manner in the drug industry. The therapeutic drugs prepared from seaweeds recently, the polysaccharides and peptides, isolated from seaweeds have become a matter of great interest for cancer therapy. The mechanisms of their anticancer activity are related to their ability to suppress the growth of cancer cells. The applications of Seaweed and their usage have not been fully established. The research on seaweeds needs to be conducted for getting more information about their unknown benefits

REVIEW QUESTIONS

1. Explain the mass cultivation methods of macro algae and their importance

2. Illustrate the application of seaweeds in biotechnology

UNIT-6: SEAWEED LIQUID FERTILIZERS PREPARATION AND THEIR POTENTIAL IN AGRICULTURE AND HORTICULTURE

Objectives:

- To know the preparation of seaweed liquid fertilizers.
- To knowledge the seaweed liquid fertilizers potential in agriculture and horticulture.

6.1.1. SEAWEED AS A FERTILIZER

Soil is a vital importance as a worldwide resource and it has approximately 50% of its volume filled with solids and the other 50% with water and air. Ninety percent of the solids should be minerals, basically eroded rocks, and 10% should be organic matter such as decaying leaves. The spaces between the solids contain water and allow air to reach plant roots, a vital step in plant growth. Healthy soil is the most biologically productive environment on Earth. A single gram of soil can contain up to a billion organisms, representing over a thousand species.

Soil contains approximately 70 different minerals. Thirteen of these are known to be essential for plant growth: nitrogen, phosphorous, potassium, sulfur, calcium, magnesium, iron, boron, manganese, copper, zinc, molybdenum, and chlorine. The other 50 or so, including things like cobalt, iodine and selenium, often referred to as micronutrients, are likely to be important to plant growth even if the mechanisms are not fully understood. For plants to be able to utilize these minerals efficiently, the soil environment must have proper moisture, pH, and organic content. In particular, when soils become deficient in organic matter, the ability of plants to absorb minerals from the soil drops precipitously.

In a similar fashion to plants, humans need a wide array of minerals in our diet to maintain our health. With the exception of taking vitamin supplements, a practice which is less effective than you might think, we get the vast majority of our minerals from the soil, either by eating plants that have extracted them from the soil for us, or by eating animals that have eaten plants. Seafood provides another important source of minerals.

As farmers harvest plants, minerals which had formerly been in the soil are removed. Unless these minerals are replaced, the field will quickly

lose productivity. Replenishment of minerals can be accomplished by the application of fertilizer.

Generally speaking, the industrial agriculture systems used worldwide are not particularly effective at replacing soil minerals. Most fertilizers applied on large agricultural operations include only nitrogen, phosphorous, potassium and, at best, a small handful of other minerals. To make matters worse, over-tilling and insufficient application of compost result in a reduction of the soil's organic content. Therefore, the absorption of even the small subset of minerals applied to the fields is inefficient. To overcome this inefficiency, farmers increase the amount of fertilizer that they apply, which then results in the run-off of excess nitrogen and phosphorous into the surrounding watershed, which creates a number of additional problems.

6.2. SEAWEED LIQUID FERTILIZER AND ITS ROLE IN AGRICULTURE AND HORTICULTURE PRODUCTIVITY

6.2.1. Seaweed as Bio-fertilizer

Seaweed as a fertilizer is suitable in organic agriculture. Marine algae consist of macro and micro nutrient amino acid, Vitamins, cytokinins, gibberellins, auxins, auxins-like and other growth-promoting compounds.More over seaweeds are used in soil amendment, pests control and plant diseases management. Liquid extracts obtained from seaweeds have gained importance as foliar sprays and soil drench for several crops including various grasses, cereals, flowers and vegetable species.

For example, aqueous extracts of *Sargassum johnstonii* at particular concentration increased the rooting of *Vigna mungo* enhanced vegetative growth (plant height, shoot length, root length, and number of branches) and reproductive parameters (flower number, fruit number, and fresh weight) of tomato. The effect of the extracts of *Sargassum wightii* gave 11% increase in seed germination, a 63 % enhancement in number of lateral roots formation and 46% increase in shoots length of *Triticum aestivun* compared to control. Aqueous extract of *Sargassum wightii* when applied as a foliar spray on *Zizyphus mauritiana* showed an increased yield and quality of fruits. Growth promoting effect of seaweed liquid fertilizer (SLF) (*Enteromorpha intestinalis*) on the sesame crop plant has also been reported.

Previously many researches prove that the seaweed liquid fertilizer (SLF) treatment improved the growth parameters significantly when compared to the control on green gram. Researchers reported that lower

concentration of SLF from *Stoechospermum marginatum* promoted the growth of brinjal. The recent research to introduce new methods the different seaweed preparation as mixed consortium for the application to the agricultural field and for the gainful yield.

The macro-algae inhabiting the intertidal zones of estuaries, lagoons and in the seas across the world play an important role in the marine ecosystems. The saline nature of the environment favours the growth of certain macro-algae known as the seaweeds. These organisms are important renewable bio-resources of the seas. Many of them are used as food, preparation of several industrial products and also as a raw material for fertilizer for amendment in the crop fields as liquid fertilizer or compost for increasing productivity.

6.2.2. Compost used as fertilizer

On the open ground, for growing wheat, corn, soybeans, and similar crops, compost can be broadcast across the top of the soil using spreader trucks or spreaders pulled behind a tractor. It is expected that the spread layer is very thin (approximately 6 mm (0.25 in.)) and worked into the soil prior to planting. However, application rates of 25 mm (one in.) or more are not unusual when trying to rebuild poor soils or control erosion. Due to the extremely high cost of compost per unit of nutrients in the western world (such as the United States) on-farm use is relatively rare since rates over 4 tons/acre cannot be afforded. This is unfortunate and results from over-emphasis on "recycling organic matter" than on "sustainable nutrients". In other countries such as Germany, where compost distribution and spreading are partially subsidized in the original waste fees, compost is used more frequently on open ground, but only on the premise of nutrient "sustainability"

In plasticulture, strawberries, tomatoes, peppers, melons, and other fruits and vegetables are often grown under plastic to control temperature, retain moisture and control weeds. Compost may be banded (applied in strips along rows) and worked into the soil prior to bedding and planting, be applied at the same time the beds are constructed and plastic lay down, or used as a "top dressing".

Many crops are not seeded directly in the field but are started in seed trays in a greenhouse. When the seedlings reach a certain stage of growth, they are transplanted in the field. Compost can be used as an ingredient in the mix used to grow the seedlings, but is not normally used as the only planting substrate. The crop to be grown and the seeds' sensitivity to nutrients, salts, etc. dictate the ratio of the blend, and

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maturity is important to insure that oxygen deprivation will not occur or that no lingering phyto-toxins remain.

Seaweed Liquid Fertilizer and Its Role in Horticulture

A comparison of the growth rate of two carob seedlings planted in a compost-sand mixes (right) and in sand alone (left).

Compost is used in horticulture in a wide range of contexts. In raised bed gardening, compost can be mixed with sand, clay, aged sawdust, and other materials to create an enriched mix for landscape beds or raised-bed gardens. Compost should be no more than 30 percent of the total mix. Use a high quality mature compost to avoid nutrient and oxygen competition with plants.

In a container garden, as in bedding mixes, compost may be a beneficial ingredient in potting media, used up to 30 percent of the total mix, depending on salinity and maturity. It is considered a partial substitute for peat moss, but generally lacks the porosity and water-holding capacity of peat so must be used in limited percentages. The nutrient content of compost can also reduce the need for supplemental chemical fertilizers, although this has to be determined in each situation.

Excavated areas around the foundation of new buildings are backfilled when construction is complete, but these planting zones may contain rubble, residues of toxic chemicals, and other undesirable substances. Removing the backfill and replacing it with a soil/compost mix will improve soil structure and give foundation plantings a healthier start.

Two or more inches of compost can be used alone or in conjunction with conventional mulch products to keep root zones cool, conserve moisture, and act as a slow-release fertilizer, provided the product is coarsely textured and mature. For a weed barrier, double or triple the depth of compost can be used, placed on top of a thick layer of newspapers, to replace geo membrane weed barriers. This is obviously only true if the compost is weed free; many are not.

For trees and shrubs, mixes of *well aged* compost with the native soils can be used as backfill. Immature composts may cause settling and young root disturbance due to oxygen deprivation. Seasonally, top dress with compost to the drip line and rake into the soil.

To establish new turf areas (lawns, recreation fields, golf courses), compost can be applied prior to seeding and work into the soil. Compost can seasonally be used to top dress and may also be raked into the soil. Some turf farms also use compost, growing grass in a couple of inches of the material to prevent topsoil loss.

6.2.3. Erosion control

Topsoil loss is a serious ecological issue. The use of compost to control sediment run-off and fight erosion is a relatively new technology, now being adopted by local authorities, developers, farmers, and other major disturbers of soil as another tool to reduce topsoil loss.

A layer of compost spread over a disturbed area of soil is called a compost blanket. With a high water-holding capacity, compost is not tilled into the soil but remains on the surface to temper the impact of rainfall. Even small amounts can help, but typical recommendations call for a 5 cm (2 in.) layer to insure adequate surface coverage. The blanket can also be directly planted into.

Compost berms and socks are used alone or in conjunction with compost blankets to mitigate the impact of high volume water discharges and flows. Compost berms are more aesthetically pleasing than silt fences and eliminate the need to remove the berms when the project is complete. Over time, a compost berms simply biodegrades and returns to the earth. As the name implies, a compost sock is a mesh tube stuffed with compost. Socks stand up better to heavy equipment, can be anchored in place, and are easily removed / reused. If a biodegradable fiber is used for the sock, it can also be left in place to biodegrade. This is rarely if ever practiced, however, since it defeats the idea of the sock.

Special uses

Additional special uses for compost include use as a planting media for constructed or artificial wetlands, as a cap for a landfill cell when it is closed to encourage vegetation and reduce erosion, and as erosion control along stream banks to restore functionality and beauty to riparian zones while possibly mitigating future damage.

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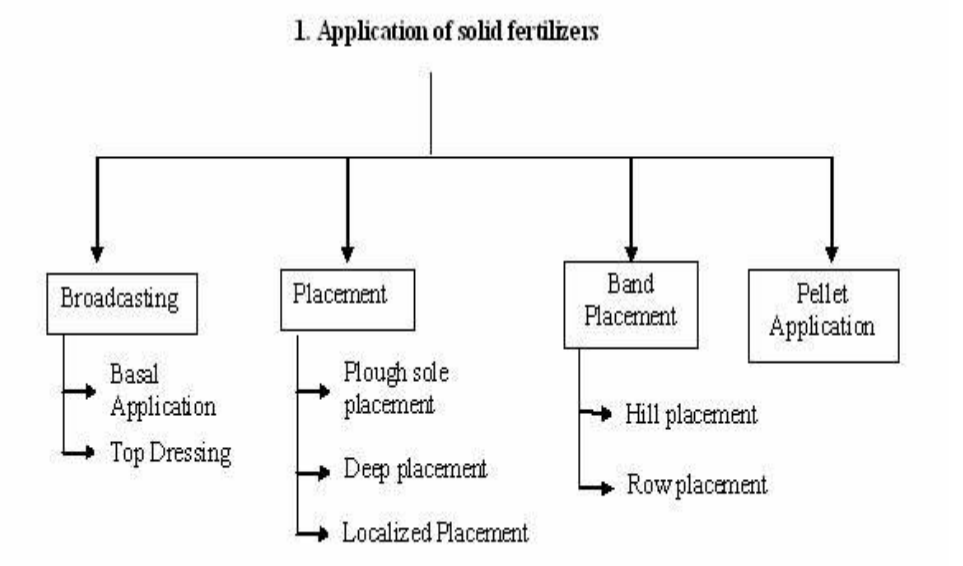
6.2.4. METHODS OF FERTILIZER APPLICATION FOR PLANTS

The different methods of fertilizer application are as follows:



a) Broadcasting

1. It refers to spreading fertilizers uniformly all over the field.
2. Suitable for crops with dense stand, the plant roots permeate the whole volume of the soil, large doses of fertilizers are applied and insoluble phosphatic fertilizers such as rock phosphate are used.

Broadcasting of fertilizers is of two types.

i) Broadcasting at sowing or planting (Basal application)

The main objectives of broadcasting the fertilizers at sowing time are to uniformly distribute the fertilizer over the entire field and to mix it with soil.

ii) Top dressing

It is the broadcasting of fertilizers particularly nitrogenous fertilizers in closely sown crops like paddy and wheat, with the objective of supplying nitrogen in readily available form to growing plants.

Disadvantages of broadcasting

I) Nutrients cannot be fully utilized by plant roots as they move laterally over long distances.
Ii) The weed growth is stimulated all over the field.

iii) Nutrients are fixed in the soil as they come in contact with a large mass of soil.

b) Placement

1. It refers to the placement of fertilizers in soil at a specific place with or without reference to the position of the seed.

2. Placement of fertilizers is normally recommended when the quantity of fertilizers to apply is small, development of the root system is poor, soil has a low level of fertility and to apply phosphatic and potassic fertilizer.

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The most common methods of placement are as follows:

I) Plough sole placement

1. In this method, fertilizer is placed at the bottom of the plough furrow in a continuous band during the process of ploughing.

2. Every band is covered as the next furrow is turned.

3. This method is suitable for areas where soil becomes quite dry up to few cm below the soil surface and soils having a heavy clay pan just below the plough sole layer.

ii) Deep placement

It is the placement of ammonia cal nitrogenous fertilizers in the reduction zone of soil particularly in paddy fields, where ammonia cal nitrogen remains available to the crop. This method ensures better distribution of fertilizer in the root zone soil and prevents loss of nutrients by run-off.

iii) Localized placement

It refers to the application of fertilizers into the soil close to the seed or plant in order to supply the nutrients in adequate amounts to the roots of growing plants. The common methods to place fertilizers close to the seed or plant are as follows:

a) Drilling

In this method, the fertilizer is applied at the time of sowing by means of a seed-cum-fertilizer drill. This places fertilizer and the seed in the same row but at different depths. Although this method has been found suitable for the application of phosphatic and potassic fertilizers in cereal crops,

but sometimes germination of seeds and young plants may get damaged due to higher concentration of soluble salts.

b) Side dressing

It refers to the spread of fertilizer in between the rows and around the plants. The common methods of side-dressing are

1. Placement of nitrogenous fertilizers by hand in between the rows of crops like maize, sugarcane, cotton etc., to apply additional doses of nitrogen to the growing crops and

2. Placement of fertilizers around the trees like mango, apple, grapes, papaya etc.

c) Band placement

If refers to the placement of fertilizer in bands.

Band placement is of two types.

i) Hill placement

It is practiced for the application of fertilizers in orchards. In this method, fertilizers are placed close to the plant in bands on one or both sides of the plant. The length and depth of the band varies with the nature of the crop.

ii) Row placement

When the crops like sugarcane, potato, maize, cereals etc., are sown close together in rows, the fertilizer is applied in continuous bands on one or both sides of the row, which is known as row placement.

Row placement

d) Pellet application

1. It refers to the placement of nitrogenous fertilizer in the form of pellets 2.5 to 5 cm deep between the rows of the paddy crop.

2. The fertilizer is mixed with the soil in the ratio of 1:10 and made small pellets of convenient size to deposit in the mud of paddy fields.

Advantages of placement of fertilizers

The main advantages are as follows:

i. When the fertilizer is placed, there is minimum contact between the soil and the fertilizer, and thus fixation of nutrients is greatly reduced.

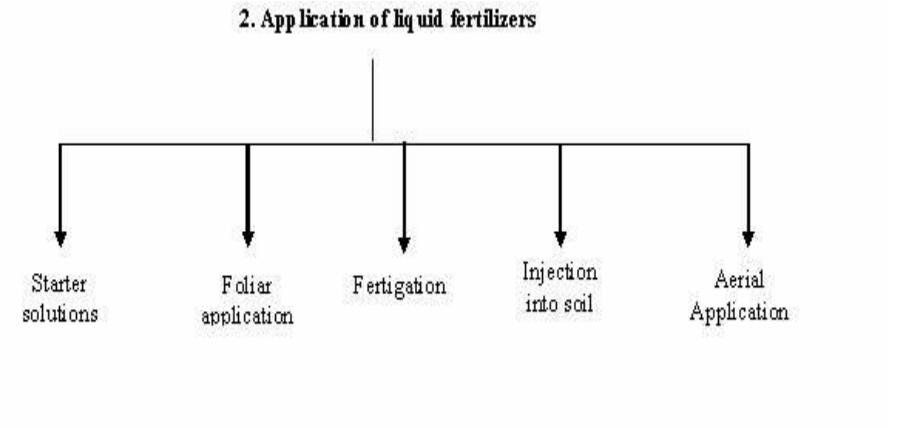
ii. The weeds all over the field cannot make use of the fertilizers.

iii. Residual response of fertilizers is usually higher.

iv. Utilization of fertilizers by the plants is higher.

v. Loss of nitrogen by leaching is reduced.

vi. Being immobile, phosphates are better utilized when placed.



Following are the common methods of applying liquid fertilizers

a) Starter solutions

It refers to the application of solution of N, P_2O_5 and K_2O in the ratio of 1:2:1 and 1:1:2 to young plants at the time of transplanting, particularly or vegetables. Starter solution helps in rapid establishment and quick rowth of seedlings.

The disadvantages of starter solutions are

i. Extra labours are required, and

ii. The fixation of phosphate is higher

b) Foliar application

1. It refers to the spraying of fertilizer solutions containing one or more nutrients on the foliage of growing plants.

2. Several nutrient elements are readily absorbed by leaves when they are dissolved in water and sprayed on them.

3. The concentration of the spray solution has to be controlled; otherwise serious damage may result due to scorching of the leaves.

4. Foliar application is effective for the application of minor nutrients like iron, copper, boron, zinc and manganese. Sometimes insecticides are also applied along with fertilizers.

c) Application through irrigation water (Fertigation)

1. It refers to the application of water soluble fertilizers through irrigation water.

2. The nutrients are thus carried into the soil in solution.

3. Generally nitrogenous fertilizers are applied through irrigation water.

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d) Injection into soil

1. Liquid fertilizers for injection into the soil may be of either pressure or non-pressure types.

2. Non-pressure solutions may be applied either on the surface or in furrows without appreciable loss of plant nutrients under most conditions.

3. Anhydrous ammonia must be placed in narrow furrows at a depth of 12-15 cm and covered immediately to prevent loss of ammonia.

e) Aerial application. Formerly it refers to as crop dusting, involves spraying crops with crop protection products from an agricultural aircraft. Planting certain type of seed are also included in aerial

application. The specific spreading of fertilizers is also known as aerial topdressing in some countries.

6.2.5. The benefits of using seaweed extracts in agriculture

Seaweed extracts have been reported to assist plants in many ways. The benefits are enhanced crop yield; improved root structures, improved plant development like flowering and leaf development and fruit set, and enhanced ability to tolerate plant disease and climatic stresses such as cold or drought. There are also benefits that relate to improved soil structure, soil water holding capacity and improved soil microbiology. However, the modes of action for these benefits are not well understood, despite increasingly sophisticated research reported in quality scientific publications. Common research findings have focused on:

1.Many types of plant growth regulators that have been identified in seaweed extracts, such as auxins, cytokinins, ethylene, gibberellins, abscisic acid and more.

2. In early work, liquid seaweed extracts were characterized by plant callus-inducing assays, and the responses were equated to growth regulators (i.e. specific plant hormone) equivalents. In seaweeds, the first definitive discovery of cytokinins (zeatin, zeatin-riboside, dihydro-zeatin, and dihydro-zeatin-riboside) was found in *Durvillaea potatorum*, using the Seasol liquid seaweed extract.

3. Recently, Stirk et al. (2014) reported the presence of brassinosteroids (along with gibberellins and abscisic acid) in a South African kelp extract (Kelpak™—manufactured from *E. maxima*).

4. Quaternary ammonium molecules, such as betaines and proline, that buffer against major osmotic changes were reported by Karabudak et al 2014. These osmo protectants have an important role in plant stress and importantly have been observed to accumulate during increased stress tolerance. Betaines have been reported in several brown algae genera such as *Ascophyllum, Fucus, and Laminaria.*

5. Alginate and diverse polysaccharides, some sulphated, have been characterized that (i) stimulate root growth both directly and indirectly in association with microbes (ii) trigger the plant's defense mechanisms and (iii) induce plant genes involved in pathogenesis-related defense.

6. Minerals and trace elements that enhance nutrition or have a critical role in plant development, along with lipid-based molecules such as sterols.

In addition, seaweed extracts have many other molecules that are typically found in plants, which are not characterized, but might also contribute to the efficacy of various seaweed extracts. This is consistent with genomic and cell biology bioassay studies using seaweed extracts. Bioinformatics studies have uncovered hundreds of plant genes that respond when plants are treated with seaweed extracts.

The seaweed extracts are likely to attract increased scientific attention in the agricultural field, especially as they develop upon their proven usage in all countries. However, for agriculture to fully exploit the biological benefits in seaweed extracts, a major cross-disciplined research effort will be needed to elucidate their complex modes of action and applications on diverse crops and in different production environments. In addition, we need to recognize that seaweed extracts are inherently different as they are derived from different sources and extraction processes and have particular extract stability properties. Furthermore, their capacity to elicit plant responses also depends in part upon the application usage rates, application frequency and the timing of applications in relation to plant development life cycle. Therefore, new translational research is needed for determining the appropriate times and plant stages for their application and for defining the optimal dosages required to maximize both farm productivity and economic benefits.

6.2.6. CONCLUSION

Though seaweed and its derived product are increasingly used in production of agricultural crops, the mechanism of action of seaweed extract on enhancement of productivity is still unknown. The recent challenge in sustainable food production is due to the increasing occurrence of biotic and abiotic stress as due to climate change, which may lead to reduction of agricultural productivity globally. Under this situation sea weed liquid fertilizers may work as a good inducer for sustainability in agricultural production coupled with maintenance of soil health. In India seaweeds are not used extensively except for production of phycocolloids. But being a rich source of vitamins, minerals and growth promoters, they can be of immense help to the coastal farmers for their use as a source of organic fertilizer. Hence there is a need for popularizing the use of seaweed as health food and liquid organic fertilizer through mass scale field trials and organization of public awareness programmes. In this regard, research institutes / agencies and private entrepreneurs should come forward with scientific and technical knowledge and marketing expertise. The farmer of the area where seaweed cultivation is feasible should be educated about the significance and the benefits from multifaceted use of seaweeds.

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REVIEW QUESTIONS

1) How to prepare seaweed liquid fertilizers and their role in agricultural field

UNIT-7: GENETICS OF ALGAE – NIF GENES – STRAIN IMPROVEMENT – TRANSFORMATION – PROTOPLAST FUSION TECHNIQUE FOR MACRO ALGAE

Objectives

- To study the Genetics of nif genes in macro algae.
- To understand the knowledge of nif gene strain improvement technique for macro algae.

7.1.1. Genetics of algae

Nodule formation and nitrogen fixation are the two biological processes which are controlled by genes of diazotrophs. From rhizoidal genome numerous symbiotic genes (nod genes) encoding for nodulation, and nitrogen fixing genes (nif genes) have been identified. In free living and symbiotic nitrogen fixers nodule-forming and non-nodule forming nif genes are present on genome or mega plasmid in their cells.

7.2.1. Nif Genes

The fixation of atmospheric nitrogen (N_2) is a very energy intensive endeavor. If there is no need for N_2 fixation, the production of proteins needed for fixation is tightly controlled. The nif genes are responsible for the coding of proteins related and associated with the fixation of atmospheric nitrogen into a form of nitrogen available to plants. These genes are found in nitrogen fixing bacteria and cyanobacteria. The nif genes are found in both free living nitrogen fixing bacteria and in symbiotic bacteria in various plants.

The nif genes are genes encoding enzymes involved in the fixation of atmospheric nitrogen. The primary enzyme encoded by the nif genes is the nitrogenase complex which is in charge of converting atmospheric nitrogen to other nitrogen forms such as ammonia, which plants can use for various purposes. Besides the nitrogenase enzyme, the nif genes also encode a number of regulatory proteins involved in nitrogen fixation. The expression of the nif genes is induced as a response to low concentrations of fixed nitrogen and oxygen concentrations (the low oxygen concentrations are actively maintained in the root environment). Nitrogen fixation is regulated by nif regulon, which is a set of seven operons which includes 17 nif genes. Nif genes

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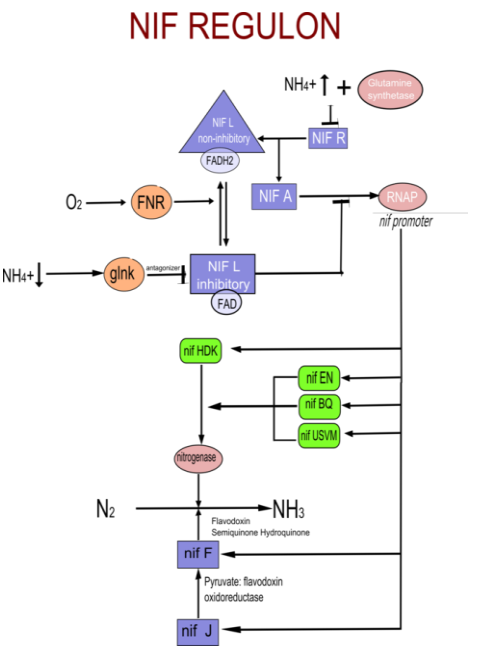
have both positive and negative regulators. Some of nif genes are: Nif A, D, L,K, F,H S,U,Y,W,Z.

*Figure: **Nif Regulon**: This is a schematic representing many of the proteins in the nif regulon and where they act in the pathway needed for nitrogen*



Activation of nif genes transcription is done by the nitrogen sensitive Nif A protein. When there isn't enough fixed nitrogen factor available for the plant's use, Ntr C, which is a RNA polymerase, triggers NifA's expression. Nif A then activates the rest of the transcription for the nif genes. If there is a sufficient amount of reduced nitrogen or oxygen is present, another protein is activated, Nif L. In turn, Nif L inhibits Nif A activity, which results in the inhibition of nitrogenase formation. Nif L is then regulated by other proteins that are sensors for the levels of O_2 and ammonium in the surrounding environment. The nif genes can be found on bacteria's chromosomes, but many times they are found on bacteria's plasmids with other genes related to nitrogen fixation, such as the genes needed for the bacteria to communicate with the plant host.

Key Points

- Nitrogen fixation takes a great deal of energy. If the conditions are not favorable for nitrogen fixation or there is enough ammonia around, nitrogen-fixing bacteria turn off the production of proteins needed for nitrogen fixation.

- Nitrogen fixing protein production is regulated by the nif regulon.
- The nif regulon contains factors which both turn on and off the production of proteins needed for nitrogen-fixation.

Key Terms

- **Regulon**: A group of genes that is regulated by the same regulatory molecule. The genes of a regulon share a common regulatory element binding site or promoter. The genes comprising a regulon may be located non-contiguously in the genome.
- **Operon**: A unit of genetic material that functions in a coordinated manner by means of an operator, a promoter, and structural genes that are transcribed together.

7.3 Strain improvement

7.3.1. Cloning of nif Genes

In many countries researches on nif gene transfer into higher plants, especially in monocots, and gene expression in them are in progress. However, success has been made in nif gene cloning into *E. coli*. Since nif genes are prokaryotic in origin, the best strategy of their transfer into non-leguminous crops would be to transfer nif genes into chloroplast. The transcriptional and translational machinery of chloroplast bears several prokaryotic features. These attempts would be successful because the chloroplasts are geared to the production of ATP and reducing power, as both of which are required for nitrogen fixation. But the major problems for doing so are (i) lack of chloroplast transferring techniques, and protection of nitrogenase from 0_2 evolved during photosynthesis.

7.3.2. Direct Transfer of *nif* Genes

Transformation of virtually every major crop species is now possible, so this is no longer a limitation for transfer of *nif* genes. When considering the direct transfer to non nodulating plants such as cereal crops, the crucial question is how to achieve sufficient expression of nitrogenase without exposure to damaging levels of oxygen. Secondly, a sufficiently large supply of ATP and reducing power must be available, in addition to a mechanism for assimilation of the product, NH_3. At first glance, plant chloroplasts may seem an odd choice as target for *nif* gene expression, because of their photosynthetic oxygen production. However, the *nif* genes could be engineered for nocturnal expression, as is the case in

many diazotrophic cyanobacteria. It is widely accepted that plant chloroplasts are derived from endosymbiotic Cyanobacteria, possibly even diazotrophs. Many free-living cyanobacterial species have developed mechanisms to reconcile photosynthesis with N_2 fixation. Furthermore, chloroplast photosynthetic products provide a potential energy source for nitrogenase, and enzymes for ammonium assimilation, such as glutamine synthetase, are present in chloroplasts. Tobacco (*Nicotiana tabacum*) plants have been transformed with *nif H* (the Fe-protein of nitrogenase) and *nif M* (essential for activation of the Fe-protein). They expressed both genes in the chloroplast, but no nitrogenase enzyme activity was detected, probably due to poor translation or degradation of the protein.

Recently, three chloroplast genes encoding enzymes involved in light-independent chlorophyll biosynthesis were discovered. They have a high degree of similarity to the *nif HDK* genes in diazotrophs, and catalyze analogous chemical reactions. This supports the theory that chloroplasts are derived from diazotrophic cyanobacteria. One of these genes, *chl L* was replaced by its "homolog" *nif H* from *Klebsiella pneumoniae* by direct chloroplast transformation of the unicellular green alga *Chlamydomonas reinhardtii*. The *nifH* gene product was able to partially complement the *chl L* phenotype, indicating that the oxygen-sensitive *nifH* gene product can function in chloroplasts. Despite these interesting results, there are still formidable obstacles to overcome, including the introduction of the remaining necessary *nif* genes and regulatory sequences. It is also not certain that sufficient levels of ATP and reductants or sufficient respiratory capacity to remove oxygen are available to support N_2 fixation in the chloroplast.

The recent discovery of an oxygen-tolerant nitrogenase from *Streptomyces thermoautotrophicus* raise the possibility of introducing this nitrogenase complex to chloroplasts or mitochondria to ensure sufficient levels of reducing power and ATP to support N_2 fixation. However, the plant has to be able to tolerate the superoxide radical required by this enzyme. This nitrogenase may only be expressed in plants exposed to photo oxidative stress, unless an alternative electron donor can be found.

7.4. PROTOPLAST FUSION CULTURE TECHNIQUE FOR MACRO ALGAE

7.4.1. Introduction

Protoplasts are the cells of which cell walls are removed and cytoplasmic membrane is the outermost layer in such cells. Protoplast can be obtained by specific lytic enzymes to remove cell wall. Protoplast fusion is a

physical phenomenon. During fusion, two or more protoplasts come in contact and adhere with one another either spontaneously or in presence of fusion inducing chemicals. After adhesion, membranes of protoplasts fuse in some localized areas and, ventually, the cytoplasm of the two protoplasts intermingles.

7.4.2. Methods of Protoplast Fusion:

Protoplast fusion can be classified into two categories:

1. Spontaneous fusion;

2. Induced fusion.

In somatic hybridization, spontaneous fusion is of little significance. The methods used for induced fusion can again be sub-categorized.

I. Spontaneous Fusion:

Protoplasts, during isolation, often fuse spontaneously and this phenomenon is called spontaneous fusion. Simple physical contact is sufficient to bring about the spontaneous fusion among the similar parental protoplasts. During the enzyme treatment for the isolation of protoplasts, it is found that protoplasts from adjoining cells fuse through their plasmadesmata to form a multinucleate protoplast.

The protoplasts, once they are freely isolated, do not fuse spontaneously with each other. An exception is the protoplast from micro sporocytes of some plants of lily family where the freely isolated protoplasts fuse spontaneously. This type of spontaneous fusion has been used to produce inter-generic fusion, e.g., the spontaneous fusion of microsporocyte protoplast of *Lolium longiflorum* and *Trillium* spp.

II. Induced Fusion:

Fusion of freely isolated protoplasts from different sources with the help of fusion inducing chemical agents is known as induced fusion. Normally, isolated protoplasts do not fuse with each other because the surface of the isolated protoplast carries negative charge (-10 to -30 mV) around the outside of plasma membrane and, thus, there is a strong tendency for protoplasts to repel one another due to their same charges. So this type of fusion needs to fusions inducing chemical agent or system which actually reduces the electro negativity of the isolated protoplasts and allow them to fuse with each other.

Actually, induced fusion is a highly important and a valuable technique because the protoplast from widely different and sexually incompatible plants can be used by this procedure. This technique has the possibility and ability to combine different genotypes beyond the limits imposed by sexual process. The fundamental objectives of somatic hybridization are mainly based on induced protoplast fusion.

7.4.3. The isolated plant protoplasts can be induced to fuse by three ways:

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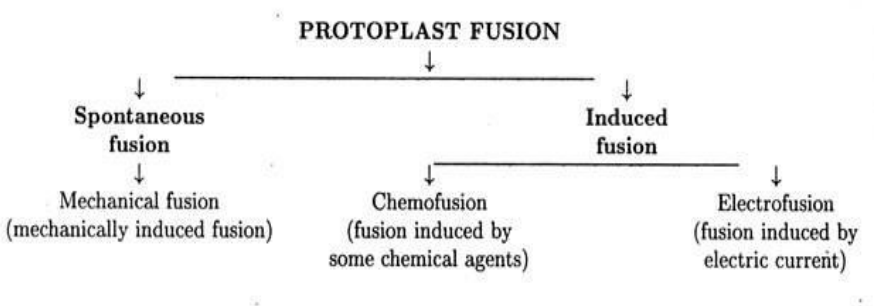
(i) Mechanical Fusion:

In this process, the isolated protoplasts are brought into intimate physical contact mechanically under microscope using micromanipulator and perfusion micropipette. This micropipette is partially blocked within 1 mm of the tip by a sealed glass rod. In this way the protoplasts are retained and compressed by the flow of liquid. By this technique occasional fusion of protoplast has been observed.

(ii) Chemo-Fusion:



Spontaneous fusion of two or more adjoining several chemicals have been used to induce somatic protoplasts is of no practical use, but protoplast fusion. Sodium nitrate ($NaN0_3$), this may be important in studies of the nature polyethylene glycol (PEG), Calcium ions and function of plasmodesmata, the physiology (Ca^{2+}), Polyvinyl alcohol etc. are the most and control of mitosis in multinucleated cells commonly used protoplast fusion inducing and nuclear fusion.

Perhaps spontaneous fusion agents which are commonly known as chemical has some practical importance for chromosome Fusogens. Generally, chemo fusion techniques doubling are followed in most of induced fusion experiments.

Chemical fusogens cause the isolated protoplasts to adhere to one another and leads to tight agglutination followed by fusion of protoplast (Fig.1). The adhesion of isolated protoplast takes place either due to reduction of

negative charges of protoplast or due to attraction of protoplast by electrostatic forces caused by chemical fusogens.

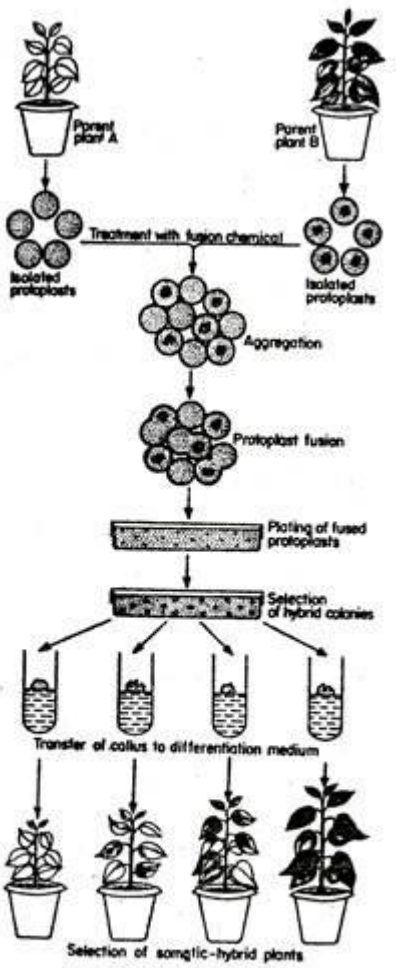


Fig. 6.14: Diagrammatic illustration for the fusion of protoplasts from two different plant species and later plating and selection of hybrid colonies and the regeneration of "Somatic-Hybrids". (after Reinert and Bajaj 1977)

Chemo Fusion Procedures:

Several chemo fusion procedures have been proposed time to time to improve the fusion frequency and reproducibility of the fused product. Each and every method has its own merits and limitations.

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7.4.4. Some chemo fusion methods are described below:

(a) Fusion Induced by Sodium or Potassium Nitrate:

Fusion of isolated onion sub-protoplasts plasmolysed with sodium salts was achieved for the first time by Kiister (1909). Subsequently, Michel (1937) demonstrated fusion between protoplasts using potassium nitrate as plasmolyticum. By this method, equal densities of protoplasts from two different sources are mixed and then centrifuged at 100 g for 5 minutes to get a dense pellet. This is followed by addition of 4 ml of 5.5% sodium nitrate in 10.2% sucrose solution to re-suspend the protoplast pellet. The suspended protoplasts are kept in water-bath at 35° C for 5 minutes and again centrifuged at 200 g for 5 minutes. The pellet is once again kept in water-bath at 30°C for 30 minutes.

Fusion of protoplast takes place at the time of incubation. The, pellet is again suspended by 0.1% sodium nitrate for 5-10 minutes. The protoplasts are washed twice with liquid culture medium by repeated centrifugation. Finally, the protoplasts are plated in semisolid culture medium.

Using the above principle, intra-and interspecific fusions have been achieved by several workers. However, sodium nitrate is toxic to cell at fusogenic concentration. The frequency of fusion is not very high in this method. Yet it is useful only for the protoplasts derived from meristematic cells.

(b) Fusion Induced by Calcium Ions at High pH:

In 1973, Keller and Melcher (from Germany) developed a method to effectively induce, fusion of tobacco protoplast at high temperature (37° C) in media containing high concentration of Ca^{2+} ions, (i.e., calcium chloride) at a highly alkaline condition (pH 10.5).

Equal densities of protoplasts are taken in a centrifuge tube and the protoplasts are spun at 100 g for 5 minutes. The pellet is suspended in 0.5 ml of medium. 4ml of 0.05M $CaCl_2 2H_20$ in 0.4M Mannitol at pH 10.5 is mixed to the protoplast suspension.

The centrifuge tube containing protoplasts at high pH/Ca^{2+} is placed in the water bath at 30° C for 10 minutes and is spun at 50 g for 3-4 minutes. This is followed by keeping the tubes in water bath (37°C) for 40-50 minutes. About 20-30% protoplasts are involved in this fusion experiment.

(c) Fusion Induced by PEG:

In 1974, Kao and Michayluk from Canada discovered another fusion inducing chemical polyethylene glycol (PEG) which is the most effective agent discovered so far. Many fusion experiments are performed by a polyethylene glycol.

PEG induces protoplast aggregation and subsequent fusion. But the concentration and molecular weight of PEG are important with respect to fusion. A solution of 37.5% w/v PEG of molecular weight 1,540 or 6,000 aggregates mesophyll and cultured cell protoplasts during a 45 minutes incubation period at room temperature.

Fusion of protoplast takes place during slow elution of PEG with liquid culture medium. Carrot protoplast can be used by 28% PEG 1540 and the fusion can be promoted by Ca^{2+} ion at the concentration of 3.5 mM. But higher concentration of Ca^{2+} ion (10 or 50 mM) has been considered beneficial.

In some studies, high pH/Ca^{2+} and PEG method have been combined. By this method, the agglutination of protoplasts can be brought about using sufficient quantities (0.1-5 ml) of protoplast in centrifuge tube or micro densities (150 µ l) of protoplast on a cover slip. The PEG method has been modified slightly to fuse higher plant protoplast.

The modifications are given below:

1. PEG is more effective when it is mixed with 10-15% dimethyl sulfoxide (DMSO).

2. Addition of concanavalin A (Con A) to PEG increases protoplast fusion frequency.

3. Sea water has been used alone or in combination with PEG to fuse protoplasts.

(d) Fusion Induced by Other Chemicals:

Some other chemicals have also been observed to promote protoplast fusion:

1. 15% solution of polyvinyl alcohol (PVP) in combination with 0.05 $CaCl_2$ and 0.3 M Mannitol are used to fuse plant protoplasts.

2. Lectins are also known to agglutinate protoplasts.

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3. Various proteins are also used for agglutination of protoplast.

Some important explanations are:

1. When the protoplasts are brought into close proximity, this is followed by an induction phase whereby changes induced in electrostatic potential of the membrane result in fusion. After fusion, the membrane stabilizes and the surface potential returns to their former state.

2. When the protoplasts are closely adhered, the external fusogens cause disturbance in the intramembranous proteins and glycoprotein. This increases membrane fluidity and creates a region where lipid molecule intermix, allowing coalescence of adjacent membranes.

3. The negative charge carried by protoplasts is mainly due to intramembranous phosphate groups. The addition of Ca^{2+} ions causes the zeta-potential of plasma membrane to be reduced and under that condition the protoplasts aggregate.

4. The high alkaline solution used in chemo fusion induces the intramembranous production of lysophospholipid which may be linked with membranous fusion.

5. The high molecular weight (1,000-6,000) polymer of PEG acts as a molecular bridge connecting the protoplasts and Ca^{2+} ions link the negatively charged PEG and membrane surface. One elution of the PEG, the surface potential is disturbed, leading to inter-membrane contact and subsequent fusion. Besides this, the strong affinity of PEG for water may cause local dehydration of the membrane and increase fluidity, thus inducing fusion.

6. PEG itself induces aggregation, but a-tocopherol present as an impurity in commercial grade PEG actually promotes membrane fusion.

BLOCK-3: MUSHROOM TECHNOLOGY

UNIT-8 INTRODUCTION TO MUSHROOM CULTIVATION HISTORY - SCOPE OF EDIBLE MUSHROOM – TYPES OF EDIBLE MUSHROOM AVAILABLE IN INDIA – MEDICINAL AND OTHER USES – POISONOUS MUSHROOM.

Objectives

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- To study introduction of mushroom cultivation.
- To study edible mushroom historical background and their scope.
- To study various types of edible mushroom in India and its medicinal uses.
- To study different types of poisonous mushrooms.

8.1.1. INTRODUCTION TO MUSHROOM CULTIVATION

India is the second populous country of the world with a population of over 120 corers. Increase in population is creating an alarming situation in the food problem in India. Malnutrition in terms of protein deficiency is one of the major factors responsible for high mortality and morbidity in this country and other developing countries in world. The use of mushrooms and its cultivation has a long history in human health and the development of their food style. For millennia, mushrooms have been valued as edible and medical provisions for humankind. Humans used mushrooms as food even before they understood the use of other microorganisms that has been discovered later.

Mushroom farming is becoming successful because of its very low inputs. It is estimated that about 300 million tonnes of fresh mushrooms can be produced from just one forth of world's annual yield of straw. Mushrooms are a natural product with many nutritional advantages. They are cholesterol free and contain virtually no fat or sodium, all of which dieticians' say need to be reduced in Australian diets. Mushrooms supply dietary fibre and are a good source of several important B group vitamins, especially niacin and riboflavin. The sophistication of today's mushroom farms is in marked contrast to the first commercial attempts to grow this crop in Australia in 1933.

Mushroom is a saprophytic fungus that grows on dead and decaying organic matter. Due to the absence of chlorophyll, it is unable to

synthesize its own food and hence is dependent upon the organic matter/substrate for food.

Annual mushroom production has increased to 80,000 ton in 2006 from a mere 1,000 ton in 1981. Fifty percent of this is produced by marginal and small production units and the rest by industrial establishments. Mushroom husbandry is now one of the major sources of income for farmers of many states like Haryana, Uttar Pradesh, Punjab, Uttarakhand and Himanchal Pradesh. The major producers of mushrooms are Punjab (35,000 MT) Tamilnadu (15,000MT), and Andhra Pradesh (5000MT). Mushroom production of Uttarakhand alone increased from 2,640MT in 2000 to 5340MT in 2006, with Dehradun, Nainital, Haridwar and Udham Singh Nagar the major production centres. Button mushroom (*Agaricus bisporus*) constitutes about 90% of total production in India where that of other cultivated mushrooms viz. *Pleurotus, Lentinula, Auricularia* and *Calocybe* are very marginal.

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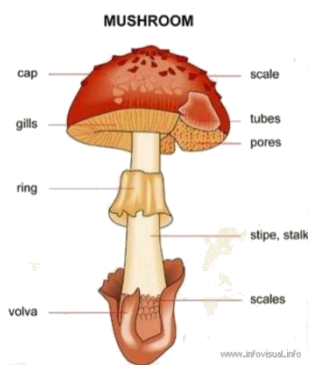
8.1.2. DEFINITION

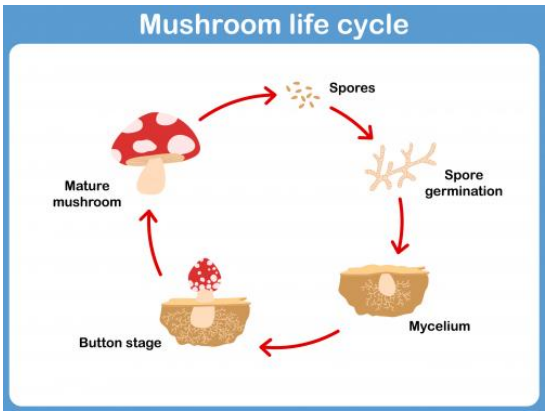
The definition of mushroom given by Chang and Miles as "the mushroom is a macro-fungus with a distinctive fruiting body which can be either epigeous or hypogenous and large enough to be seen with the naked eye and to be picked by hand. Mushroom are the fleshly fungi which constitute a major group of lower plant kingdom. The mushroom is a common fungal fruit body that produces basidiospores at the tip of club like structure called basidia which arranged along the gills of mushroom.



8.1.3. STAGES OF DEVELOPMENT

For different distinctive types of mushrooms, it is very difficult to generalize different stages of development in their life-cycle. Mushroom developmental stages represent the nature of vegetative mycelial growth (spawn run or mycelial run), pinning (production of mushroom primordia or the pin stage), button stage (membrane closed and stipe still short and condensed) and cap formation with well-developed membranes and

slightly longer stipe. For a researcher studying about mushroom development which is more precise to define, so that other mushroom researchers can apply the same criteria to their studies.



8.2.1. HISTORICAL BACKGROUND

For the first time, cultivation of white button mushroom (*A. bisporus*) started in France around 1630. In the beginning, it was grown in open conditions. Around 1810, a French gardener (Chambery) cultivated them in underground queries in Paris: The possibility of continuous production was demonstrated when he cultivated *A. bisporus* in a cropping house in England. He was able to produce about 1.5 lb/sq. The U.S.A. took up this work in the late 19th century. After the Second World War mushroom cultivation spread in about 80 countries. Nowadays, edible mushrooms are eaten in Africa, Australia, Switzerland, Italy, France, Germany, Japan, Europe, India, Bhutan, Pakistan, Afghanistan, Tibet and China.

Table 1 Distribution of edible mushrooms in India

Agasieus baporus	**Distribution**
A. compestrer	Solon (Himachal Pradesh), Punjab
Psalhoto eompestro	Punjab, W.Bengal, Bihar, Jammi

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Amanita stigmata	Uttar Pradesh, Deoban
Cantharellus cibarius	W.Bengal, Kashmir, Solan
Heterobasidium annostum	U.P. Punjab, H.P. Assam
Laccaria laccata	Sikkim, Missouri, Assam
Lycopenion perlaturn	Himalayas, Punjab,Darjeeling, H.P., Sikkim
Morrchella esculenta	Punjab, Kashmir, H.P., Kumaon hills(U.P)
M.conica	Dehradun, siwalik hils
M.deliciosa	Kashmir, Kumaon hills, H.P.
Pleurotus sojar- caju	W.Bengal, foot hills of Himalaya

Distribution of edible mushrooms in India is given in Table 1. The common mushroom *A. bisporus* is abundant in cattle fields in Punjab. It is used by many people. The morel (*M. esculents*) is found in Kashmir and hills of Kumaun region in U.P. Bhutan's consume *Hypoxylon vernicosum*. Kashmiri guchhi (*Morchella* spp.) is very popular which is sold even at the rate of Rs. 1000/kg dries mushroom.

In India, mushroom cultivation started long before a century, as the *Volvariella valvocea* was cultivated on paddy straw. Therefore, this mushroom is also known as the paddy straw mushroom. In 1950s, an attempt was made to cultivate mushroom in Coimbatore. In 1962, *Pleurotus flabellatus* (*Dhingri coroyester*) was successfully cultivated in Mysore. Besides many attempts, its cultivation could not be popularized up to the late 1960s. For the first time an attempt was made for artificial cultivation of *A.bisporus* at Solan (Himachal Pradesh) where synthetic compost preparation technology was developed, by using horse dung and wheat straw.

8.2.2. Present status of mushroom cultivation in India

Since 1983, a large number of growers started mushroom cultivation during winter around Delhi, Chandigarh and some districts of Haryana (e.g. Sonepat, Rohtak, Karnal) and Punjab (e.g. Ferozpur, Patiala, Ludhiana and Jalandhar). In Bhiwani district of Haryana, mushroom cultivation is gaining much popularity. Under the guidance of specialists of Krishi Gyan Kendra and Scientists of Haryana Agriculture University (Ilissar) the farmers in villages Tagrana and Bamla have undertaken cultivation of mushrooms. These villages have been declared as mushroom villages.

Since mushrooms have a very short life, it should reach to consumers within a short time or immediately canned. This will lead to proper marketing of mushrooms. In India, over-production and improper care for marketing have resulted in the increase in price. The cost of production in India at present is comparatively higher than other countries (Rs. 10/kg in North Indian plains and Rs. 15/kg in hills) and hence cannot compete in international market. The yield obtained so far is low due to: (improper infrastructure of preparation of pasteurized compost, (ii) use of ordinary buildings lacking proper temperature control as cropping rooms, (in) use of low yielding strains, (iv) inadequate supply of quality spawn, (v) lack of trained manpower, and (vi) inadequate research support. The technology for cultivation and processing of mushroom has been developed at CFTRI (Hyderabad),RRL (Jammu) and NBR1 (Lucknow). RRL and NBRI are distributing mushroom spawns in new areas for mass cultivation. CFTRI has developed technique for processing and drying mushrooms.

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8.3.1. SCOPE OF EDIBLE MUSHROOM

Today, the popularity of mushrooms is due not only to their culinary value but also to their potential as a source of protein that can enrich human diets especially in some developing countries where meat may be rare and expensive. Mushrooms contain more protein than either fruits or vegetables. They can be eaten, as they are cooked or raw, unlike other protein sources such as soya. Mushrooms are also low in cholesterol. Besides their protein content, mushrooms are also high in certain vitamins such as B, C, D, riboflavin, thiamine, and nicotinic acid. Mushrooms are also a good source of iron, potassium and phosphorus in addition to folic acid, an ingredient known for enriching the bloodstream and prevention deficiencies.

8.3.1. Medicinal Importance Of Mushrooms

The invention of the so called "wonder drug" penicillin was a landmark in the field of medicinal uses of fungi. Since then several fungi have been well recognized for their antifungal, antibacterial, antiviral, anti tumour and many others such properties of pharmacological values.

Mushrooms are regarded as an ultimate health food, low in calories due to presence of good amount of quality protein, iron, zinc, vitamins, minerals and dietary fibres which protects from digestive ailments and strengthening of the human immune system. Medicinal mushrooms are the golden medicinal fungi and are yet to be exploited commercially. The extractable bioactive compounds from medicinal mushrooms enhance human's immune systems and improve their quality of life. Mushrooms as medicine were used since long but the full extent of their therapeutic properties was unknown to us. Modern scientific studies on medicinal mushrooms have now expanded exponentially during the last two decades in Japan, Korea, China and USA.

Oyster mushrooms are best known medically for their cardiovascular and cholesterol-controlling benefits. In addition, they have been shown to have antitumor, immune response, anti-inflammatory, antiviral and antibiotic activities.

Volvariella volvacea (Chinese or straw mushroom) are edible fungi with medicinal properties widely diffused and cultivated. Other species, such as *Pleurotus* (oyster), *Auriculata*, *Flammulina Tremella* and *Grifola* all have varying degrees of immune system boosting, lipid lowering, anti-tumour, microbial and viral properties, blood pressure regulating, and other therapeutic effects.

Mushroom substrate can be prepared from any clean agricultural waste material, and produced in temporary clean shelters. They can be cultivated on a part-time basis, and require little maintenance. It provides an opportunity for improving the sustainability of small farming systems through the recycling of organic matter, used as a growing substrate, and then returned to the land as fertilizer. Through the provision of income and improved nutrition, successful cultivation and trade in mushrooms can strengthen livelihood assets, which can not only reduce vulnerability to shocks, but enhance an individuals and a community's capacity to act upon other economic opportunities.

1. It is long been used as a source of food.

2. Unique taste and flavour.

3. Traditional medicine in ancient times.

4. Without fungi, we would not have bread, beer, wine or antibiotics.

5. But more importantly, the nutrient recycling and plant nutrition provided by fungi.

6. It is estimated that there are about 140,000 species of mushrooms.

7. There are 700 mushrooms has pharmacological properties.

8. Drugs are effective against cancer, asthma, allergies, diabetes and cholesterol reduction

9. Rich in protein can be used to bridge the protein malnutrition gap.

10. Rich source of natural bioactive compounds.

8.4.1. Types of Edible Mushroom Available In India

Mushrooms have been recognized internationally as food, contributing to ameliorate the protein malnutrition of countries which are normally cereal dependant. Mushrooms used as food for nutritive value and medicinal values as dietary supplements which produces high quality and economic value to the world mushroom market. Generally edible mushrooms possess three essential values for good food - nutrition, taste and physiological functions.

In India commercial production of all type edible and medicinal mushrooms is maintained by WTO. Though India is not the leading producer of mushroom in the world scenario, it cultivates mushrooms and has great potential as an important producer in near future. India produces near about 306.6 million tonnes of crop residue per annum and about 70% of these crop residues are burnt in the fields (The Hindu Survey of Indian Agriculture). The excessive production of crop residues and cheap manpower sources are the main reason to have an efficient mushroom production programme under government and private sector. However, the increased Indian population requires more vegetable food in their daily diet and thus mushroom production is increased side-by-side as one of the main reasons lagging behind. But now-a-days, this scenario has changed a lot in the society and people have realized the importance of various mushrooms in nutritional and medicinal filed, and

their multifaceted applications such as having good percentage of protein, minerals, carbohydrate, immunological attributes etc. and high efficiency in lingo cellulosic waste management. So it is a good sign that both mushroom cultivation and consumption is increasing steadily in India as well as other mushroom producing countries.

8.4.2. Button mushrooms (*Agaricus* sp.)

They are popular strains grown during winter season in northern India (Jammu and Kashmir, H.P, Punjab, Haryana, Uttaranchal and Bihar) where temperature remains below 20°C during winter. However, its cultivation requires highly stable infrastructural facilities for different operations like composting; spawn making, cropping and post-harvest practices with great efficiency.

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8.4.3. Oyster mushroom (*Pleurotus* sp.)

It is much popular species that are commercially grown over a wide temperature range due to their temperate, sub-tropical and tropical nature. Unlike other mushrooms they have much diversity in their adaptation to varying agro-climatic conditions along with low substrate-specificity for a wide range of ligno cellulase activity. The flexible nature of this genus is mainly due to their rapid mycelial growth, high saprophytic colonizing ability and simple cultivation technology. Now there are only two varieties which are also tropical in nature viz; the paddy straw mushroom (*Volvariella* sp.) and the milky mushroom (*Calocybe* sp.). The paddy straw mushroom is popular for its taste and flavour and having very short cropping cycle. But this variety is losing ground due to its low yield (due to weak substrate colonization) and poor shelf life. Cultivation of this mushroom is restricted to few places of Orissa and West Bengal where it shows good growth at a temperature range of 25-40°C on pasteurized paddy straw. The milky mushroom as a new introduction to the edible mushroom world, no doubt India has a greater prospects to exploit its cultivation and having greatest advantage for being Indian origin. The simple production techniques, and sustainable yield, increased shelf-life, attractive colour and shape have all attracted many oyster mushroom growers in India to switch over the cultivation of this variety. Best temperature optimum for cultivation of this type is 30-35°C.

8.4.4. *Lentinula edodes* (Berk.)

Pegler is the third most important edible mushroom in the world in terms of total production which develops as sporophytes on tree logs and form fruit bodies at low temperature (15-20°C). Mushroom cultivation in India has witnessed tremendous revolution in recent years with respect to the types and strains of cultivated mushrooms. Cultivation of different edible

mushroom strains involves various environmental conditions. Maintenance of mother strain, multiplication and spawn run etc. are the important event to commercialize a new strain in the mushroom world. Based on the agro-climate changes, resources and availability of manpower, the mushroom production varies country to country in different years. Large scale mushroom production is now playing a pivotal role in solving the main problems facing mankind in the 21st century to feed an ever increasing population.

8.5.1. Mushrooms availability in india

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The most popular cultivated mushroom species are *Agaricus bisporus, Agaricus bitorquis, Lentinula edodes, Pleurotus spp., Auricularia spp., Volvariella volvacea, Flammulina velutipes, Tremella fuciformis, Hypsizygus marmoreus, and Grifola frondosa.* In recent years, few new species of edible mushrooms namely, *Dictyophora indusiata, Strophariaruguso-anulata, Agrocybe aegirita* etc. have also been successfully cultivated. Introduction to date, more than 60 mushroom species have been artificially cultivated in the East Asian countries and China where it has been cultivated more than 30 species of these „exotic mushrooms" on a commercial scale. Thus, it would be worth mentioning that China has now become the biggest mushroom producer, consumer and export country in the world.

The world production of mushrooms increased steadily during the last 2 decades in China, as follows: 1.2 million tonnes in 1981; 2.2 million tonnes in 1986; 3.8 million tonnes in 1990; 4.9 million tonnes in 1994; 6.2 million tonnes in 1997. The mushroom industry in UK and in some other Western countries is focused on only one mushroom genus, *Agaricus bisporus* and these industries are nearly 100% dominated by this mushroom.

Commercial mushroom production has been started all over India from the year 1960- 61, when only button mushroom cultivation and production initiated in Himachal Pradesh. From the year 1990 onwards, the seasonal production of button mushroom began in the northern states of India like, Haryana, Punjab, Uttar Pradesh and Uttaranchal and the total production increased from 4000 tonnes (1985) to 30,000 tonnes (1995) and its estimated to be about 50,000 tonnes/annum. But 85% of this production is dominated by the button mushrooms.

The oyster mushroom production and its market in India is meagre and only an estimated 2000 tonnes, but the potential for the future is rated as high for several reasons . The low cost and easy availability of different kinds of substrate material required for mushroom production, such as

agro-wastage, wheat and paddy straw, bagasse, chicken manure, gypsum, tea wastes, de-oiled cakes etc. are reasons for the tremendous potential of our country in this field. Moreover, India has a large num.ber of agro-climatic regions like tropical, sub-tropical with varied temperature and humidity that is suitable for different types of mushroom cultivation. Our country has a good combination of both technical and non-technical manpower needed to operate and manage the mushroom growing operations

8.6.1 Medicinal and other uses of Mushrooms

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Mushroom is considered to be a complete, health food and suitable for all age groups, child to aged people as it contains all nutrient element required for human in desired proportion. The nutritional value of mushroom is affected by numerous factors such as species, variety, stage of development and environmental conditions. Mushrooms are rich in protein, dietary fiber, vitamins and minerals. The major proportion of carbohydrate is occupied by dietary and fermentable fibers and it do not contain starch with insignificant proportion of sugars. Edible mushrooms contain rich proteins that are composed of theronine and valine but deficient in sulphur containing amino acids (Methionine and cysteine). The low lipid level with no cholesterol and higher proportion of polyunsaturated fatty acids is further advantage. The ergosterol present in mushrooms is the precursor for Vitamin D synthesis in human body. Meeting the food demand for the increasing population from the limited land resource is a big challenge for our Indian democracy in this vulnerable climate change era. In addition to this, wide spread malnutrition and associated diseases are more common among the economically poor population. This compels us to search for cheap alternative quality nutritional sources for our huge population. Non green revolution otherwise referred as mushroom farming is one among the appropriate ways to meet this challenge because mushroom grow on wastes without requiring additional land besides its exceptional nutritional and medicinal properties.

8.6.2. Medicinal Values

Since thousands of years, edible fungi have been revered for their immense health benefits and extensively used in folk medicine. Specific biochemical compounds in mushrooms are responsible for improving human health in many ways. These bioactive compounds include polysaccharides, tri-terpenoids, low molecular weight proteins, glycoproteins and immune modulating compounds. Hence mushrooms have been shown to promote immune function; boost health; lower the risk of cancer; inhibit tumor growth; help balancing blood sugar; ward off viruses, bacteria, and fungi; reduce inflammation; and support the

body's detoxification mechanisms. Increasing recognition of mushrooms in complementing conventional medicines is also well known for fighting many diseases.

Medicinal values of the some important mushroom are given in Table 2. Hence mushrooms have been shown to promote immune function; boost health; lower the risk of cancer; inhibit tumor growth; help balancing blood sugar; ward off viruses, bacteria, and fungi; reduce inflammation; and support the body's detoxification mechanisms. Mushroom diet is good for heart due to its low fat content, higher proportion of unsaturated fatty acids and absence of cholesterol. Minimal sodium with rich potassium in mushroom enhances salt balance and facilitates blood circulation in human and found suitable for high blood pressure patients. The diabetic and obese patients choose mushroom as an ideal food owing to its low calorific value, no starch, and less sugars. The fermentable dietary fibre in mushrooms augments healthy functioning of bowel system as fiber serves as a food for beneficial microbes in human digestion system. Compounds restricting tumor activity such as Kresin is widely used as a leading cancer drug in pharmaceutical industries. Ergothioneine is a specific antioxidant found in Flammulina velutipes and Agaricus bisporus, which is necessary for healthy eyes, kidney, bone marrow, liver and skin and consequently reducing the aging process. Antioxidants present in mushroom scavenge the free radicals present in body system and reduce cell maturity. Thus by it acts as an anti-aging agent. A diverse collection of polysaccharides (beta-glucans) and minerals, isolated from mushroom is responsible for regulation and strengthening the human immune system.

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8.6.3. Good for Heart

The edible mushrooms have little fat with higher proportion of unsaturated fatty acids and absence of cholesterol and consequently it is the relevant choice for heart patients and treating cardiovascular diseases. Minimal sodium with rich potassium in mushroom enhances salt balance and maintaining blood circulation in human. Hence, mushrooms are suitable for people suffering from high blood pressure. Regular consumption of mushrooms like Lentinula, Pleurotus spp were severe to decrease cholesterol levels.

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Table 2 Medicinal values of some important mushrooms

Mushroom	Compounds	Medicinal properties	Source(s)
Ganoderma lucidum	Ganoderic acid Beta-glucan	Augments immune system Liver protection Antibiotic properties Inhibits cholesterol synthesis	Lin and Zhang, 2004 Wang *et al.,* 2007 Moradali *et al.*, 2006 Komoda *et al.*, 1989
Lentinula edodes	Eritadenine Lentinan	Lower cholesterol Anti-cancer agent	Enman *et al.*, 2007
A. bisporous	Lectins	Enhance insulin secretion	Ahmad, 1984
P. sajor-caju	Lovastatin	Lower cholesterol	Gunde and Cimerman, 1995
G. frondosa	Polysaccharide Lectins	Increases insulin secretion decrease blood glucose	Horio and Ohtsuru, 2001
Auricularia auricula	Acidic polysaccharides	Decrease blood glucose	Yuan *et al.*, 1998
Flammulina velutipes	Ergothioneine Proflamin	Antioxidant Anti cancer activity	Bao (2008) Ikekawa *et al.*, 1985
Trametes versicolor	Polysaccharide-K (Kresin)	Decrease immune system depression	Coles and Toth, 2005

Cordyceps sinensis	Cordycepin	Cure lung infections Hypoglycemic activity Anti-depressant activity	Li *et al.*, 2006 Ko *et al.*, 2009 Nishizawa *et al.*, 2007

8.6.4. Low calorie food

The diabetic patients choose mushroom as an ideal food due to its low calorific value, no starch, and little fat and sugars. The lean proteins present in mushrooms help to burn cholesterol in the body. Thus it is most preferable food for people striving to shed their extra weight.

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8.6.5. Prevents cancer

Compounds restricting tumor activity are found in some mushrooms but only a limited number have undergone clinical trials. All forms of edible mushrooms, and white button mushrooms in particular, can prevent prostate and breast cancer. Fresh mushrooms are capable of arresting the action of 5-alpha-reductase and aromatase, chemicals responsible for growth of cancerous tumors. The drug known as Polysaccharide-K (Kresin), is isolated from Trametes versicolor (Coriolus versicolor), which is used as a leading cancer drug. Some mushroom-derived polysaccharides have ability to reduce the side effects of radiotherapy and chemotherapy too. Such effects have been clinically validated in mushrooms like Lentinul aedodes, Tramtes versicolor, Agaricus bisporous and others.

8.6.6. Anti-aging property

The polysaccharides from mushrooms are potent scavengers of super oxide free radicals. These antioxidants prevent the action of free radicals in the body, consequently reducing the aging process. Ergothioneine is a specific antioxidant found in Flammulina velutipes and Agaricus bisporus which is necessary for healthy eyes, kidney, bone marrow, liver and skin.

8.6.7. Regulates digestive system

The fermentable fibre as well as oligosaccharide from mushrooms acts as a prebiotics in intestine and therefore they anchor useful bacteria in the colon. This dietary fibre assists the digestion process and healthy functioning of bowel system.

8.6.8. Strengthens immunity

Mushrooms are capable of strengthening the immune system. A diverse collection of polysaccharides (beta-glucans) and minerals, isolated from mushroom is responsible for up-regulating the immune system. These compounds potentiate the host's innate (non-specific) and acquired (specific) immune responses and activate all kinds of immune cells.

Mushrooms, akin to plants, have a great potential for the production quality food. These are the source of bioactive metabolites and are a prolific resource for drugs. Knowledge advancement in biochemistry, biotechnology and molecular biology boosts application of mushrooms in medical sciences. From a holistic consideration, the edible mushrooms and its by-products may offer highly palatable, nutritious and healthy food besides its pharmacological benefits.

Still there are enough challenges ahead. Until now, how these products works is elusive and vast number of potential wild mushrooms are not explored. The utility of mycelia is paid little attention but it has tremendous potential, as it can be produced year around with defined standard. Knowledge on dose requirement, route and timing of administration, mechanism of action and site of activity is also lacking. Work is under progress in various laboratories across the world to validate these medicinal properties and isolation of new compounds. If these challenges are meeting out in the coming days, mushroom industries will play a lead role in neutraceutical and pharmaceutical industries.

The increasing awareness about high nutritional value accompanied by medicinal properties means that mushrooms are going to be important food item in coming days and at places may emerge as an alternate to non-vegetarian foods. Growing mushroom is economically and ecologically beneficial. Consuming mushroom is beneficial in every respect.

8.7.1. POISONOUS MUSHROOM

The order Agaricales, commonly called gill fungi contains over 270 genera. These fungi incapable of causing infectious disease but it produce toxic substance that poison a person who ingests them .These poisonous substances are collectively known as Mycotoxins .

Some species of mushrooms are known as toxic and in some countries many cases of mushroom poisoning are reported every year. In the year 1998 in France 1,675 cases of intoxications by mushrooms were reported

and in this country alone it is estimated that 8- 10.000 cases are expected to be registered every year. Most of these accidents are due to incorrect identification of species that is often made by empirical and traditional knowledge.

8.7.2. Characteristics of Poisonous Mushrooms

1. Two species of mushrooms namely *Venenarius muscarius* and *Venenarius phalloides,* which owing to their abundance, wide distribution, conspicuous appearance and deadly qualities are known to be poisonous. They had been the chief cause of death from mushroom eating all over the world years earlier.

2. The poisons occurring in flowering plants belong chiefly to two classes of substances known as alkaloids and glucosides. The former, are known bases such as aconitine from aconite, atropine from belladona, nicotine from tobacco and morphine from poppy plant. Glycosides' are sugar derivatives of complex, unstable and often unknown composition like poisons found in digitalis, hellebore, wisteria and several other plants.

3. The more important poisons of mushrooms also belong to the same classes-one represented by the alkaloid as found in *Venenarius muscarinus* and the other, the glycoside which is the deadly principle found in *Venenarius phalloides,* is known mainly through its effects.

4. There are various other minor poisons, which usually manifest its toxic effects to the taste or smell that cause local irritation. Such poisons are more or less damaging to the health. They usually function adversely according to the body constitution of the individual.

5. In some cases in olden days, poisonous species were used in committing murder. The annual number of deaths due to mistaking poisonous species for edible ones was probably as more as many hundreds.

6. The tests used to distinguish poisonous mushrooms are most varied and curious. They are usually mixed with queer traditions and superstitions. Therefore, the only safe rule is to know each species accurately before eating it. Mushrooms grown by the reputed mushroom farms should only be taken for safety in all respects.

7. The chief poisonous species listed by ancient mushroom eaters were - *Venenarius phalloides, V. muscarius, V. cothurnatus, V solitarius,*

Clitocybe illudens, Inocybe infida, Panaeolus venenosus, Panus stypticus and *chlorophyllum molybdites.*

8.7.3. Identification of Poisonous Mushrooms

There are no clear cut visible signs on any mushroom that can differentiate edible from poisonous species. Similarly, there are no thumb rules to distinguish edible and poisonous species. These species may be differentiated among them on the basis of experience as there are certain characteristics of poisonous species. However, no generalized characteristics are possible.

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8.7.4. Nature of Poisonous Mushrooms

1. Some people have common belief that wild mushroom species, which have been nibbled by insects, squirrels and rabbits, are edible. Such kind of belief does not hold any scientific explanation and is not sure to be edible in actual practice.
2. Some mushrooms are poisonous when raw but becomes harmless when cooked. *Example--Clitocybe nuda.*
3. Some mushrooms are poisonous before they are boiled. After boiling, they are safe to eat after cooking properly. Example *Gyromitra esenlenta.*
4. Some mushrooms are poisonous regardless to the manner of preparation. *Example—Amanita phalloides.*
5. Some mushrooms are poisonous only under certain conditions such as when consumed with alcoholic beverages, such as *Coprinus atramentarius.*
6. Some mushrooms are poisonous only when eaten in large quantities, such as *Verpa bohemica.*
7. Some mushrooms for reasons unknown are deadly poisonous in some geographical areas but edible in others, such as *Paxillus involutus.*
8. Some mushrooms are poisonous when they are old, decayed or damaged by frost and are edible when fresh and young such as *Armillariella mellea.*

8.7.5. Kinds of mushroom poisoning

There are about 24 genera splitted into many species and out of that more than 100 cultivated species/forms are presently domesticated either on a small or large scale. The fruit bodies of hundreds of other species of edible mushrooms cannot be easily grown under controlled conditions because of their biological and ecological speciality. Besides these edible species, there are so many fleshy fungi, which are deadly poisonous, are called as toadstools. Most of the people ignorantly use to

say mushroom to these poisonous toadstools. Due to lack of knowledge about such poisonous species or almost frequent carelessness on the part of mushroom collector, mushroom poisoning is usually observed. With adequate knowledge and familiarity with these fungi and with due care during collection, troubles resulted from eating of such poisonous mushrooms can easily be avoided. One should not eat any mushroom unknown to him and must be sure that the collected specimens are safe to eat.

8.7.6. Different types of mushroom poisoning

There are different kinds of mushroom poisoning, which may be classified under five major types as under:

Gastro-intestinal type of poisoning may be characterized by the symptoms of nausea, vomiting and diarrhoea. The symptoms terminate rapidly and usually spontaneously. The patient recovers to normal health in a day or two. This type of poisoning may be suspected to be caused by *Russula emetica, Lactarius torminosus* and *Entoloma levidum.*

Nerve affecting type of poisoning is usually caused by eating of *Amanita muscaria, Inocybe infelix* and *Clitocybe illudens.* The symptoms produced in this type of poisoning include convulsions, coma and often death. The active principle of such poisoning is `muscarin'. Its antidote is atropine sulphate.

Blood-dissolving type of poisoning is manifested by the abdominal stress with jaundice developing in 4 to 5 days. It may cause death in some cases. Transfusion of blood may be desirable in such type of poisoning. Causative toadstools include *Gyromitra esculenta*.

Choleriform type of poisoning usually develops in 10 to 15 hours after eating of poisonous mushroom species followed by rapid loss of strength and weight. The causative toadstools include *Amanita plzalloides* and *A. virosa*.

Cerebral type of poisoning manifests symptoms like exhilaration and disturbance of vision. The patients recover the health after some time. The causative toadstools are species of Panaeolus**.**

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8.7.7. Categories of Mushroom Poisoning

There are not general rules or methods by which deadly poisonous mushrooms may be recognised. One of the most important precautions is not to tough any mushroom for consumption that has a volva (cup) at the base. Generally, the poisonous varieties are attractive to the eyes. The most deadly ones are the death cap-*Amanita phalloides,* destroying *angels-Amanita virosa,* the fool's *cap-Amanita Verna* and the fly agaric-Amanita *muscaria.* The toxic principle in these species is the mixture of *amanitin* and *phalloidin.* Both of these are complex cyclic polypeptides containing sulphur. Cooking does not destroy toxins nor are these affected by the human digestive juices. Symptoms of poisoning appears only after 8-24 hours of ingestion and by that time the toxin is absorbed by the body and neither vomiting nor a stomach pump can help.

There are more than 7 species of poisonous Amanita which are responsible for poisoning. Poisoning based on organs affected may be of different categories as given below:

Cyclopeptide poisoning

This is the most dangerous type of poisoning and is responsible for most deaths caused by mushroom poisoning especially in Europe and America. Symptoms may obscure liver and kidney damage.

Haemolytic poisoning:

The patient of such kind of poisoning shows the symptoms of anaemia after eating raw or under cooked mushrooms especially belonging to *Amanita rubescens* and *Amanita vaginata.*

Muscarine poisoning:

This type of poisoning is known to be caused by the eating of Amanita muscaria and Amanita pantherina. The symptoms include increased perspiration, salivation, nausea, vomiting, abdominal pain, thirst and mucous with bloody stool. Death usually results from respiratory arrestation.

Coprine (Ant abuse-like) poisoning:

This type of poisoning is usually caused by the eating of Coprinus attramentarius. The nervous system is adversely affected in this kind of poisoning.

Gastero-enteric irritants:

Mushrooms responsible for such kinds of poisoning are Agaricus xanthodermus, Boletus satanus, Paxillus involutus and some of the species of Tricholoma, Lactarius and Russula. In this poisoning; gastro-intestinal irritation is the most important symptom to be experienced. The symptoms appears usually after 30 minutes to 3 hours after ingestion.

Psychotropic poisoning:

Poisonous mushrooms affect nervous system in such a way that the man perceives nonexistent sights and sounds or has hallucinations and delirism 2 to 4 hours after ingestion of the poisonous mushroom. The symptoms include sleep, torpidity or coma in extreme cases. Isoxazole derivatives and indole group derivatives are the important poisons responsible for this kind of poisoning. Psillcybe mexicana, P. strichfer, Amanita muscaria and species of Panaeolus and Stropharia are the mushrooms responsible for such kind of poisoning.

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8.7.8. Common poisonous species of mushrooms with their distinguishing

Genus: *Amanita*

The family Amanitaceae (genus Amanita) is well known as having many toxic species. Amatoxins are present in species of Amanita genus such as: Amanita phalloides, A. virosa, A. Verna, A. ocreata, A. bisporigera, A. suballiacea, A. tenuifolia and A. hygroscopica.

The specie A. phalloides is responsible for the majority of the fatalities caused by mushroom poisoning. The toxic effects are caused by phallotoxin and amatoxin. Phallotoxin causes alterations of enterocytes cellular membrane, while amatoxin inhibits protein synthesis at a transcriptional level within enterocytes, hepatocytes and proximal renal tubular cells. After ingestion of A. phalloides, amatoxin causes necrosis of liver cells with mortality rates ranging from about 10% to 20%. Only a minority of patients need emergency liver transplantation

A. muscaria and *A. pantherina* grow in North America, Europe, Africa and Japan, in recent years it has been reported that young people in several countries have intentionally eaten *A. muscaria* to suggest hallucinations. The most common symptoms of intoxication are motor depression, ataxia, changes in mood, perception and feelings, dizziness, euphoria, drowsiness, gastrointestinal disturbances and muscle twitches.

The pantherina-muscaria syndrome is atropine-like and in the number and severity of poisoning cases fatality is rare. In most cases recovery is complete after 24 hours. The treatment is mainly symptomatic cholinesterase inhibitors may be recommended as it counteracts the effects of poisoning, benzodiazepinics or phenobarbitone can be used in case of seizures. The treatment of patients intoxicated with species containing amatoxin includes detoxification, careful monitoring and sometimes liver transplantation is necessary.

Genus: *Clitocybe and Inocybe*

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A particular syndrome that affected five people in the region of Savoie in France was later identified as intoxication caused by the mushroom *Clitocybe amoenolens*. First symptoms appeared 24 hours after ingestion. Patients presented paresthesia of the toes and fingers followed by paroxysmal burning pain lasting 2-3 hours, notably at night. A sensation of heat, numbness, oedema and local erythema are associated with crises. Symptoms are partially relieved with cold water, acetylsalicylic acid, morphine and clomipramine. Recovery is completely after 1-4 months.

The administration of high dose of *C. amoenolens* in rats caused weight loss, locomotors disability and erythema of the toes. Examination of the sciatic nerves showed decreased axon density and neuronal fibre degeneration.

Genus: *Cortinarius*

The genus *Cortinarius* comprises between 2,000- 3,000 species of mushrooms that were considered as non-toxic until 1950. One hundred-and-thirty-five cases of intoxication caused by *C. orellanus* were described from 1953-1962 in Poland. Poisoning syndrome is characterized by a delayed acute tubulopathy that can progress to chronic renal insufficiency. In several case reports it was demonstrated that the mushrooms *C. speciosissimus* and *C. orellanus* are nephrotoxic due to the presence of the cyclopeptide orellanine whose metabolites are supposed to be most active.

The symptoms of orellanine intoxication may appear between 2-20 days after ingestion. Initially people can experience nausea, vomiting and abdominal pain. This is followed by intense thirst, chills, polyuria or oliguria and possibly anuria. Haemodialysis may be necessary until renal function gradually improves. Some species of genus *Cortinarius* can be confused with members of *Psilocybe* genus which is known as magic because the hallucino genic properties. This fact has led to several cases of accidental intoxication because *Psilocybe* mushrooms are used for some people for recreational purposes.

Genus *Gyromitra*

Species of genus *Gyromitra*, family *Helvellaceae* are really attractive to hunters and gourmets because of their taste. However, some species of *Gyromitra* contain a well known toxin named gyromitrin, whereas other species are non-toxic. This is one of the reasons why intoxications occur, toxic and non-toxic species are sometimes difficult to distinguish because they are mixed-up. The other reason is that the toxin is water soluble and volatile, boiling for long time and drying allows ingestion without risk of poisoning, but if these procedures are not done properly intoxication may occur. The third reason for intoxication is the confusion with species that are consumed frequently. The specie *G. esculenta* is known as false morels and is commonly confused with morels such as *Morchella esculenta* and *M. elata*. The toxin gyromitrin is the responsible for the effects of this species. Intoxications have occurred not only by eating fresh false morels but also by the inhalation of vapours from cooking.

Genus *Psilocybe*

The use of psychoactive substances of fungal origin for recreational purposes has become an increasing problem in many countries all over the world. Species of genus *Psilocybe* are known due to their psychedelic effects caused by psilocybin. Common psylocibin containing mushrooms are: *P. semilanceata, P. Mexicana, P. bohemica, P. cubensis* and *P. baeocistis*. The symptoms of intoxication occur 30 minutes after ingestion of fresh or dried mushroom and start with anxiety, nausea, vertigo and asthenia, neurosensorial symptoms consists of visual problems, disorientation, motor in coordination and sympathomimetic symptoms consist of mydriasis, tachycardia and hypertension. Recovery is completely 4 to 12 hours after ingestion. The need of hospitalization is rare and in exceptional cases myocardial infarction may occur in adult patients while children may present hyperthermia, seizures and comma.

REVIEW QUESTIONS

1. Describe the historical background and scope of edible mushroom.
2. Explain the various types of edible mushroom in India.
3. Illustrate the medicinal value and other uses of Mushrooms.
4. Write an account of poisonous mushroom.

UNIT – 9: PURE CULTURE-PREPARATION OF MEDIUM (PDA AND OATMEAL AGAR MEDIUM) STERILIZATION– PREPARATION OF TEST TUBE SLANTS

NOTES

Objectives:

- To understand the isolation and maintenance of mushrooms cultures.
- To know the sterilization technique and media preparation.

9.1.1. INTRODUCTION

Fungi are multicellular heterotrophic members of the plant kingdom that lack roots and stems and are referred to as thallophytes. They are larger than the bacteria and more complex in their morphology. The form of sporulation and the type of spore are important criteria in the identification of various fungi. Fungi are extremely successful organisms, as evidenced by their ubiquity in nature. Identification and classification of fungi is primarily based on the morphologic differences in their reproductive structures. Fungi reproduce sexually or asexually or by both means. Sexual reproduction is associated with the formation of specialized structures that facilitate fertilization and nuclear fission, resulting in the production of specialized spores. Large, multi celled spores are called macro conidia, macro aleuriospores or macrospores and are produced by aerial sporulation.

Culture media are mixtures of nutrients or substances used in the laboratory for cultivation of many microorganisms. Fungi may be cultured on a variety of media types. These media can be either solid or liquid depending on the experiment to be performed. Most fungi grow in medium containing a high carbohydrate source, nitrogen source, a pH of 5-6, and a temperature range from 15-37°C. Since no medium will sustain growth of every fungal species, the type of medium on which a fungus grows will vary from species to species. Some fungi, such as the downy mildews, cannot be cultured in vitro, and survive only on their plant host.

There are two general types of culture media: natural and synthetic.

9.1.2. Natural media

It is composed of natural substrates such as herbaceous or woody stems, seeds, leaves, corn meal, wheat germ, and oat meal. The exact natures of these substrates vary from time to time and cannot be duplicated exactly. Natural media are usually easy to prepare but they have the disadvantage that their composition is largely unknown. Some examples include corn meal agar, potato dextrose agar, and dung agar.

9.1.3. Synthetic media

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On the other hand, contains ingredients of known composition. These types of media can be duplicated with exactness each time they are made and contain defined amounts of carbohydrates, nitrogen, and vitamin sources. Czapek-Dox medium, glucose-asparagines and *Neurospora crassa* minimal medium would fall in this category.

All media used in culturing fungi must be sterilized before use. Steam sterilization by autoclaving is the customary method of sterilizing most culture media, but it cannot be used with heat labile compounds. Generally, materials are autoclaved for 15-20 min at 15-17 psi, and at temperature of 121^{o}C or 25^{o} F.

9.2.1. Pure Culture- Preparation of Medium

Potato Dextrose Agar (PDA) Medium:

Potato dextrose agar (PDA) contains dextrose as a carbohydrate source which serves as a growth stimulant and potato infusion that provides a nutrient base for luxuriant growth of most fungi. Agar is added as the solidifying agent. A specified amount of sterile tartaric acid (10%) may be incorporated to lower the pH of the medium to 3.5 so that bacterial growth is inhibited.

Care should be taken not to reheat the acidified medium; heating in the acid state will hydrolyze the agar which can render the agar unable to solidify.

Peeled, diced potatoes- 200 g
Dextrose- 20 g
Agar- 15 g
Distilled water- 1000 ml

(OR)

Dextrose - 20 g

Potato Extract - 4 g

Agar - 15 g

4.0gm of potato extract is equivalent to 200gm of potato infusion

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If supplement added: tartaric acid – 1.4 gm (pH-3.5 +/- 0.3 at 25°C)

Chloramphenicol – 25 mg (pH-5.6 +/- 0.2 at 25°C)

Chlortetracycline – 40 mg

9.2.2. Procedure for Preparation of (PDA) media

1. Suspend 39 grams of dehydrated media (supplied by commercial suppliers) in 1000 ml distilled water. Heat to boiling to dissolve the medium completely.

2. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before dispensing.

3. In specific work, when pH 3.5 is required, the medium should be acidified with sterile 10% tartaric acid. The amount of acid required for 100 ml of sterile, cooled medium is approximately 1 ml. Do not heat the medium after addition of the acid.

4. For processing of specimen, streak the specimen onto the medium with a sterile inoculating loop in order to obtain isolated colonies.

5. Incubate the plates at 25 – 30°C in an inverted position (agar side up) with increased humidity.

6. Cultures should be examined at least weekly for fungal growth and should be held for 4 – 6 weeks before being reported as negative.

7. Count the number of colonies and consider the dilution factor (if the test sample was diluted) in determining the yeast and/or mold counts per gram or millilitre of material.

9.3.1. Oatmeal Agar Medium:

Oat meal is a source of nitrogen, carbon, protein and nutrients necessary for the cultivation of fungi, particularly for macrospore formation.

MATERIALS

Agar, 4 g

Oatmeal, 5 g

Water, distilled or deionised

Balance, 0.1-g precision

Beaker, 400-mL

Culture dishes, sterile

Gauze, 6″ × 6″, folded

Graduated cylinder, 250-mL

Hot plate

Mortar and pestle

9.3.2. Procedure:

1. Mass 5 g of oatmeal, Place the oatmeal in mortar and grind with the pestle.

2. Using a 250-mL graduated cylinder measure 200 mL of distilled water.

3. Transfer the water and oatmeal to a 400-mL beaker.

4. Place the beaker on a hot plate and bring the solution to a boil for 20 minutes.

5. Pour off the supernatant fluid through gauze to remove the oat residue.

6. Add 4.0 g of agar to the fluid and boil until dissolved.

7. Allow the agar to cool to 50–55 °C and pour into sterile culture dishes.

Yeasts will grow as creamy to white colonies. Moulds will grow as filamentous colonies of various color in table 1.

Table 1 Typical morphological colony characteristics of some fungi

Fungi	**Colony Characteristics**				
	Texture	Surface colour	Reverse colour	Zonation	Sporulation
A.candidus	Velvety thick	Creamish white	Slightly creamish	Radially furrowed on the reverse	Moderate
A.niger	Velvety	White with typical black spores	Yellow	Heavily furrowed on the reverse	Heavy
A.sulphureus	Velvety	Dirty white with yellow spores at the centre	Orange to chocolate colour	Slightly radially furrowed	Moderate
A. versicolor	Floccose	White to orange-cream with green spores at the centre	Bright orange	Heavily wrinkled on reverse	Moderate
Penicillium corylophilum	Velvety	Dark green	Colourless to Creamish	With shallow centre and radially furrowed raised margin	Moderate
P. expansum	Velvety	Dark green with clear exudates and distinct sterile	Yellow	Radially furrowed	Heavy

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		white margin			
Penicillium spp	Powdery	Olivaceous green with sterile white margin	Orange to red, wrinkled	Radially furrowed	Heavy
Fusarium oxysporum	Floccose	Magenta pink	Magenta-red turning violet	With concentric zones of dark and light reddish colouration	Poor

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9.4.1. Preparation of Test tube slants:

Pure culture of fleshy fungi/mushrooms can be prepared either by multi-spore culture or tissue culture. Multi-spore culture is made from spore print that can be obtained by having a fresh fruit body after alcohol sterilization above a petriplate/sterilized paper. Millions of spores are collected within 48 hours. Serially diluted loop full of spores are then transferred to sterile Potato-dextrose-agar (PDA) or Malt-extract-agar culture slants. The Petri-plates /slants are incubated at $25^{o}C \pm 2^{o}C$ in BOD incubator for one week. Mycelium from growing edges is carefully transferred to MEA/PDA slants and again incubated for 2-3 weeks to obtain pure cultures .Basic materials and equipment required for pure culture is given below in Fig.1.

REVIEW QUESTIONS

1. Explain PDA and OMA media preparation methods in mushroom cultivation.

2. Write an essay on the sterilization technique.

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UNIT - 10: SPAWN PREPARATION: SPAWN SUBSTRATE, MOTHER SPAWN IN SALINE BOTTLE - INOCULATION, INCUBATION, STORAGE AND TRANSPORTATION OF SPAWN – QUALITY OF SPAWN AND CONTAMINANTS

Objectives:

- To have knowledge on spawn preparation and development of mother spawn in saline bottle.
- To have knowledge on the sterilization, inoculation, incubation and storage methods.
- To know the qualities of spawn and its transportation method.
- To study the various contaminants of microbes in spawn.

10.1.1. Introduction - Spawn preparation:

Mushrooms either isolated from nature, purified and characterized in laboratory before their use or procured from national or international mushroom culture centres. When they are isolated from the nature the method of isolation is totally microbiological one for which aseptic conditions are essential.

Sterilized and cooled potato dextrose agar (PDA) or malt extract medium is poured into sterile Petri dishes and when solidified they are inoculated by a piece of tissue or spore(s) of mushroom. In tissue culture method fresh mushroom is removed from the bed (or Stipe is collected after cropping), washed in running water to remove adhering soil particles, dried with blotting paper, gently washed with 70 per cent ethyl alcohol and finally cut from centre into two halves. A small portion of pseudo parenchymtous tissue from the centre of stipe is transferred onto Petri dishes. Petri dishes are incubated at suitable temperature for the growth of hyphae.

10.1.2. Preparation of Spawn

Spawn is a fungal growth medium impregnated with mycelial fragments of mushroom which serves as inoculums for mushroom cultivation.

There is a great problem in preparing pure spawn of a particular strain of a mushroom because of fungal, bacterial or viral contamination.

Many substrates are used for spawn making either alone or in combinations, for example, rice straw cuttings, cotton waste, hulls of cotton seed and rice, and grains of sorghum and rye. For the selection of substrates to be used in making spawn, care is taken for cost and availability of raw materials and mycelial growth on it as well. The steps of grain spawn (e.g. rye/sorghum/wheat grains) or straw spawn (paddy/wheat straw) preparations follow (Fig.1)

1) Cooking the grains in water until they swell/cutting of straw into 5 cm long pieces and soaking in water for 5-10 minutes,

2) Decantation of water, mixing of 2 per cent lime (calcium carbonate),

3) Transferring into glass tuber/flasks.

4) Plugging with cotton,

5) Autoclaving at 121°C for 30 minutes and cooling down to 30-40°C,

6) Inoculating the substrate with pure culture of mushroom as described earlier, and

7) Incubation at suitable temperature for proper infestation of mycelium for their use as spawn.

Spawn Preparation (Fig.1)

Wheat grains

↓

Boiled with water (1:1.5 w/v)

↓

Drain off excess water

↓

12g/kg grains $CaSO_4$ and 3g/kg grains $CaCO_3$

↓

Filled in bottle

↓

Sterilized at 22psi for 90 minutes

↓

Inoculation with pure culture

Incubation at suitable temperature

↓

Mycelium used as Spawn

10.2.1. Substrate Preparation

Mushroom spawn can be prepared on any kind of cereal grains like wheat, jowar,bajra or rye and agricultural waste like corn cobs, wooden sticks, rice straw, saw dust and used tea leaves, etc. Spawn substrate i.e. cereal grains should be free from diseases and cereal grains should not be broken, old, and insect damaged. Most of the cereal grains are good substrate for spawn production of white button mushroom (Agaricus bisporus and A.bitorquis), oyster mushroom (Pleurotus spp.) and paddy straw mushroom (Volvariella volvacea, but wood rotting fungi like shiitake (Lentinula edodes) and black ear mushroom(Auricularia spp.) grow better on saw dust based substrates over cereal grains. The grains are thoroughly washed in sufficient water three to four times to remove soil debris, straw particles and undesirable seed of grasses, weeds, etc. Washed grains are then soaked in sufficient water for 20-30 minutes and boiled in a container for 20-25 minutes. Normally for soaking and boiling 20 kg of wheat grain, 35 litres of water is required. Excess water from the boiled grains is removed by spreading on sieve made of fine wire mesh or muslin cloth. The grains are left as such for few hours on the sieve so that the water on surface is evaporated. Now the grains are mixed with Gypsum (Calcium sulphate) and chalk powder (Calcium carbonate) so that the pH of the grains is around 7 to 7.8 and they do not form clumps. Different people have given different ratios for mixing Gypsum and Calcium carbonate. The best results have been obtained by using 200 g Gypsum and 50 g chalk powder for 10 kg grains (dry weight basis). First Gypsum and chalk powder are separately mixed and then they are thoroughly mixed with the grains. This mixing should be done on a smooth surface after wearing gloves.

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10.3.1. Mother Spawn/ Master Spawn

The commonly followed method in India is as given below:

Ten kg of wheat or sorghum grains are boiled in 15 litres water. Water is drained off over a wire netting to dry slightly. 120 g gypsum and 30 g lime ($CaCO_3$) are mixed with 10 kg of boiled grains. The gypsum revents

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The sticking of grains together as clump and lime adjusts the pH. The grains are then filled into a half litre milk or glucose bottle container up to three-fourth the capacity. Bottles are plugged with non-absorbent cotton plug and are to be sterilized at 20-22 lb. psi (126° C) for 1 ½ to 2 hours. Sterilized bottles are taken out from the autoclave while still hot and are shaken to avoid clump formation. The bottles are immediately transferred to inoculating room or chamber and allowed to cool down overnight. On the following day, bottles are inoculated with two bits of agar medium colonized with the mycelium of pure cultures raised either by tissue or spore by putting the culture bits just opposite to each other in the inner side of glass surface in the middle of the bottle. About 7-10 days after inoculation, bottles are to be shaken vigorously so that mycelial threads are broken and mixed with grains evenly. Three weeks after incubation, the stock culture becomes ready for further multiplication of spawn. One bottle of stock culture is sufficient to multiply in 30-40 polypropylene bags or bottles. Inoculated bottles are incubated at ambient temperature.

About 350 g prepared substrate is filled in glucose/milk bottles up to 2/3 volume and Plugged with non-absorbent cotton. The plugs are covered with aluminium foil. These bottles are then autoclaved at 22 lbp si pressure at 126oC for 1.5 to 2 hr. These autoclaved bottles are left in the room for 24 hours for cooling and are kept on laminar flow under UV tube for 20-30 minutes before inoculation. A piece of mycelium (pure culture) grown in Petri plates is aseptically transferred to these bottles and inoculated bottles are incubated at 25oC. Inoculated bottles are gently shaked on 5th and 10th day. This spawn prepared using pure culture mycelium on agar medium in Petri plates as inoculants' is referred as mother spawn. Fully colonized mother spawn bottles can be used for inoculating commercial spawn bags after two to three weeks. Incubated bottles are incubated at 22-250C for Agaricus bisporus, Pleurotus spp. and Lentinula edodes but at 300C for Volvariella spp.

Preparation of Mother Spawn

Step-1 Healthy and cleaned cereal grains

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Step-2 Boil Grains in water (15-20 min.)

↓

Step-3 Remove excess water on sieve

↓

Step-4 Dry grains in shade (4 hrs.)

↓

Step-5 Mix $CaCO_3$ (0.5%) and $CaSO_4$ (2%)

↓

Step-6 Fill 300g grains in glucose/milk bottle

Step-7 Plug cotton and autoclave at 22 psi for 1.5 to 2 hrs.

↓

Step-8 Inoculate growing mycelium of desired strain using laminar flow

↓

Step-9 Incubate in BOD at 23+20C for 20-25 days

↓

Step-10 Master spawn is ready

10.3.2. Commercial Spawn

The technique for raising commercial spawn is essentially the same as for master spawn except that instead of glass bottles, polypropylene bags can be used as the containers for filling grains. Inoculated bottles or polypropylene bags are incubated at ambient temperature. In two to three weeks after inoculation, spawn becomes ready for seeding the compost.

Commercial spawn can be prepared in polypropylene bags (heat resistant). Normally for half and one kg spawn the bags should be of 35 x 17.5cm and 40 x 20cm size, respectively. Polypropylene bags should have double sealing at the bottom and after filling the grains they are plugged with the help of a PP neck and non absorbent cotton. The bags are then sterilized at 22 lb psi pressure for 1.5 to 2 hours. Autoclaved bags are shaked well before inoculation so that the water droplets accumulated inside the bags is being absorbed by the grains. The sterilized bags are kept on the laminar flow under U.V. tube for 20-30 minutes. Ten to fifteen g of grains from master spawn bottle is inoculated per bottle under aseptic condition or one bottle of master spawn is sufficient for inoculating 25 to 30 commercial spawn bags. Inoculated

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bags are again shaked so that the inoculum is well mixed with other grains. Then the bags are kept in incubation room for mycelium spread.

During incubation the bags are regularly examined for mould infestation. Contaminated bags should be immediately removed before discarding the bags to avoid build-up of contamination in the vicinity. Normally it takes 15-20 days for complete spread of mycelium on the grains. Fully colonized bags should be kept in cold room (+4^{0}C) for future use. The spawn of button mushroom, *Pleurotus* can be stored at this temperature. However neither the culture nor spawn of *Volvariella*, *Ganoderma* and *Calocybe* is stored below 15^{0}C.

Preparation of Commercial Spawn

Step-1 Use polypropylene bags instead of bottle

↓

Step-2 Up to autoclaving (Step 1 to 7) is same as of mother spawn

↓

Step-8 Inoculate 15-20 grains from mother Spawn to polypropylene bags containing grains.

↓

Step-9 Shake bags after 7-8 days

↓

Step-10 Incubate at 23+20C in incubation room

↓

Step-11 Commercial spawn is ready in 20-22 days.

10.3.3. Liquid Spawn

Mycelium cultured on liquid medium followed by maceration/ homogenizing can also be used for spawning. This is commonly referred as liquid spawn. It can be used for mechanizing inoculation process of spawn multiplication or can be used for inoculating substrates. In one of our experiments shiitake (*Lentinula edodes***)** was cultured continuously in liquid medium and used as a liquid spawn. Shiitake mushroom was cultivated on synthetic sawdust substrate. Normal fruit-bodies were harvested from the colonized substrate blocks after 120 days incubation with a solid spawn. The incubation time was reduced to 90 days with the liquid spawn. Strandy cultures showing good growth and not showing fluffy growth, sectoring or slow growth are desirable. During cropping bare patches on bed, deformed fruit bodies with no or few gills, weeping mushrooms indicate degeneration. Multispore cultures degenerate faster than single spore cultures

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10.4.1. Spawn storage and its transport

Freshly prepared spawn should be used because the mycelium is in the state of active growth. The spawn bag after completion of growth maintained of 2-3 months. Earlier spawn was prepared in milk or glucose bottles, which was difficult to transport from one place to another. Heat resistant polypropylene bags have revolutionized the spawn industry. High-tech multinational spawn labs now use polypropylene bags with microfilm windows for aeration. Though polypropylene translucent bottles of 5-10 litres capacity are also used in Europe and USA for spawn production, but it has not been introduced in India due to high cost of the material ready spawn in polypropylene bags should be packed in well ventilated cardboard cartons and maintained at 2-4 ^{o} C in storage. The spawn is transported from one place to another in refrigerated vans or during night when temperature does not rise above 32^{o}C.

During transportation, the spawn should not be exposed to temperatures higher than 35°C. The spawn bottles or bags can be packed in thermocol boxes containing ice. Alternatively, spawn can be transported during night hours. Immediately after arriving at the destination, the spawn bottles or boxes should be taken out of the thermocol boxes for spawning. Spawn should be used fresh. If not used at once, it can be stored at 5- 10°C for a maximum period of one month.

Volvariella spawn should never be refrigerated. Once the container is opened, spawn should be used in its entirely. The source of contamination in a spawn making plant is the grain used to prepare the substrate. Modern equipment and facilities for sterilization and maintenance of sterile conditions reduce fungal and bacterial contamination to a very great extent. Quality control measures, such as, inspection of to eliminate spawn bottles or bags visible contaminated or exhibiting unacceptable differences in appearance, growth, colour or odour help in checking contamination.

10.5.1. Qualities of a good spawn and Contaminants

The spawn selected should satisfy the following criteria:

• The spawn should be fast growing in the compost

• It should give early cropping after casing

• It should be high yielding

• It should produce better quality of mushroom

The following are guidelines for spawn grower to follow:

1. Select sporophore from high yielding, early producing and better quality strain.

2. Select unbroken and good quality grain for spawn production.

3. Boiling of grains should be done according to the procedure recommended to maintain about 48¬50% moisture in the grains.

4. The pH of the boiled grains should be adjusted to 6.5-7.5 by mixing appropriate quantity of calcium carbonate and Gypsum.

5. Prepare spawn from mother culture only. Do not multiply from spawn to spawn.

6. The inoculation should be done in a double chambered closed air-tight inoculation room or under laminar-flow.

7. Shake the inoculated bottles thoroughly and incubate at 24-26°C for the growth of the mycelium.

8. Sort out and remove the contaminated bottles from spawn room regularly.

9. Store the fully grown spawn at 3-5°C in cold store or refrigerator.

10. Recommended storage time even at this cold temperature is not more than 2 months.

It is a general experience of many a spawn grower and mushroom cultivator, that spawn produced on jowar (*Sorghum bicolor*) or wheat grains gives a higher yield over the spawn produced on bajra,or barley grains.

The following are criteria for good spawn, which both the spawn grower and buyer should ensure:

- There should be proper coating of mycelium around the grain used as a substrate for spawn production. No loose grains should be seen in the bottle or packet. The grains left without mycelial coating invite contamination in the compost during spawn running.
- The growth of the mycelium in the spawn should be silky or Strandy type. It should not be cottony because, it will lead to stoma formation in the casing layer. It interferes with gaseous

exchange and absorption of water in the casing resulting in lower yields,

- Fresh spawn is white in colour. Brown colouration develops as the spawn gets older. Fresh spawn gives higher yield than the older one.
- There should be no greenish or blackish spots in the spawn. Presence of such spots indicates contamination with moulds.
- Presence of slimy liquid in the spawn indicates bacterial contamination.
- Avoid using older spawn as the vigour may have decreased.

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REVIEW QUESTION

1. Explain the Preparation of mushroom Spawn
2. Explain the mother spawn and commercial spawn preparation.
3. What are the important qualities of a good spawn
4. List out the contaminants in mushroom spawn.

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UNIT - 11: PREPARATION OF COMPOST AND CULTIVATION OF WHITE BUTTON MUSHROOM(*AGARICUS BISPORUS*)- CULTIVATION OF PADDY STRAW MUSHROOM (*VOLVARIELLA VOLVACEA*) AND OYSTER MUSHROOM (*PLEUROTUS* SPP.) – LOW COST MUSHROOM FARM DESIGN OF PRODUCTION

Objectives:

- To have a clear idea for the Preparation of compost and involving substrates.
- To study the cultivation of white button mushroom (*Volvariella volvacea*) and oyster mushroom (*Pleurotus* spp)
- To study the design for low cost mushroom production.

Preparation of composts

11.1.1. Introduction

A method of preparation of composts for mushroom cultivation is known as composting. Initially, composting was restricted to industrial levels by using horse manure, but now can easily be applied to other substrates as the methods of formulation and preparation of composts are the same. *Agaricus bisporus*, the white button mushroom is one of the most popular mushrooms in the world and contributes around thirty per cent of world production of mushrooms. It is cultivated on a specially prepared substrate known as compost, which is a product of fermentation by a number of thermophilic organisms that decompose plant residues and other organic and inorganic matters. The main purpose of composting is to release the nutrients in the straw and supplements and to transform them in such a way that they are suitable for the nutrition of this

mushroom. During composting various chemical and biological processes help in achieving this. The nitrogen available in the ingredients is converted into proteins of the microorganisms and further a lignin humus complex is also formed during composting both of which later on are utilized by the mushroom mycelium as food. Compost if properly prepared is very selective in nature and only *A.bisporus* mycelium can grow successfully on it at the practical exclusion of other competing organisms.

11.1.2. Raw Materials and Formulations

White button mushroom requires a well-composted substrate for its growth. It is a saprophytic fungus and requires carbon compounds, which generally come through the agricultural waste materials. Besides carbon, it requires nitrogen and other essential elements, such as phosphorous, sulphur, potassium and iron, vitamins such as thiamine, biotin, etc. All the raw materials that contain these compounds are mixed in a fixed proportion and fermented in a set pattern to form a substrate, which is known as compost. Raw Material and Ingredients required for Composting

11.1.3. Base materials

Base materials are the bulk component of the compost. Various crop residues can be used for this purpose though the wheat straw is favoured all over the world. However, quality compost can be prepared using variety of other materials including paddy straw, hay, barley, oat, maize stalks and leaves, sugarcane bagasse, sugarcane trashes and leaves, soybean stalks, mustard stalks, etc. These materials should preferably be freshly harvested / procured and should be around 5-8 cm long. These base materials act as a reservoir of cellulose, hemi-cellulose and lignin which is utilized by *A.bisporus* during its growth as a carbon source. They also provide a little quantity of nitrogen. Besides acting as a nutrient source, they also add bulk to the compost and proper physical structure to the substrate and ensure adequate aeration during composting for the build up of microflora essential for the composting process and also for the nutrition of mushroom. Rice and barley straw are very soft and degrade quickly during composting. These materials also absorb more water as compared to wheat straw. While using these materials care must be taken on quantity of water used for wetting, schedule of turnings and adjustment to the rate and type of supplements.

11.1.4. Supplements

The compounding mixture is supplemented with other materials having nitrogen and carbohydrate sources to achieve the proper CN ratio. These materials can be classified as follows.

a. Animal manure

Horse manure undoubtedly is the best material for compost preparation. However, due to difficulties encountered in procuring good quality horse manure, use of this material has been restricted to few farms only. More and more farms are switching over to easily accessible materials. Chicken manure has proved to be the best alternative to horse manure. Other manures viz., pig cattle and sheep have also been tried for compost preparation but with limited success. All these manures provide nitrogen to the compounding mixture, little of carbohydrate is also provided. These materials are highly variable in composition and their N-content may vary from 1-4 percent and it is released slowly during composting process.

In addition to providing nutrients, they greatly increase bulk of compost, which is very important factor under Indian conditions considering the high cost of wheat straw in relation to chicken manure. If horse manure is used in composting then it should be used along with bedding and urine, as it may not require any further supplementation. If it is not having enough bedding and urine when collected from a clean stable, supplementation with inorganic nitrogen along with some wheat straw may prove useful. Chicken manure if used, should preferable be deep litter chicken manure having nitrogen contents above 3%. If such manure is not available then the manure from cages can also be tried.

b. Carbohydrate sources

These materials are essentially required to hasten the composting process, to balance the C/N ratio and also for the establishment of the bacterial flora in the compost. Molasses, wet brewer's grains, malt sprouts, potato wastes, apple and grape pumice can be employed as carbohydrate source, since these materials provide readily available nutrients to microorganisms.

11.1.5. Nitrogen fertilizers

In this category of fertilizers, urea, calcium ammonium nitrate, ammonium sulphate can be kept. Nitrogen content of these fertilizers is

very high (24-46%) which is released quickly, resulting in quick establishment of microflora.

11.1.6. Concentrate meals

Animal feeds are generally kept in this category, which include wheat or rice bran, dried brewer's grain, meal/cakes of soybean, cotton seed, castor, sunflower, etc. These materials supply both nitrogen and carbohydrates, which as in case of animal manures are released slowly. Nitrogen content may vary from 3-8% depending upon the source.

11.1.7. Supplements to rectify mineral deficiencies:

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In addition to carbon and nitrogen, *A. bisporus* also requires little quantities of potash, phosphorous, calcium and magnesium for its growth. Gypsum also has stabilizing effect on ammonium content. An increased ammonium concentration is obtained with gypsum, which is an indicator of productive compost. Furthermore, gypsum serves as a calcium source for the mushroom. It converts the oxalic acid produced by the mushroom mycelium into calcium oxalate. Requirement of phosphorous, potassium, and magnesium is generally met by chicken manure or horse manure when compost is produced by short or by indoor method.

Table1: Moisture and nitrogen content (dry wt. basis) of compost raw materials

	% Moisture	**% N**
Wheat straw	15	0.6
Horse manure – light	30	0.8
Horse manure - Heavy	50	1.0
Deep litter chicken manure	30	3.0
Wheat bran	10	2.0
Brewer's grain	40	2.0
Soybean meal	10	6.5

Cotton seed meal	10	7.0

Composting platform

It is advisable to have spacious platform consisting of two parts-one at lower level with slop and medium sized tank to collect seepage water and another part about 3' above the ground level connected with small tank for collection of seepage water. It should be provided with shed. Platform must have road approach for delivery of raw materials. For 10 tons compost, 50' x 25' sized platform can be utilized. For big projects, big platforms should be used.

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Equipments and facilities

During pasteurization, airflow of 150-200 m3/hour per ton of fresh compost is required. Compost filling should be 900-1000 kg/m2 floor area with depth of 2.0-2.2 m. There will be a loss of about 25-30% dry matter. Filling in tunnel should always be loose and uniform. There may be about 10% loss in moisture content. In India, small chamber measuring 22' x 8' x 10' with iron door measuring 4' x 6' has been used after providing proper insulation. The boiler of an usual capacity and coal/wood/oil fired will be enough. Oil fired to get temperature should be preferred. The blower is used to introduce and re-circulate hot air. The blower has 24" fan with an opening fresh air from one side with provision to control air by adjusting shutter. The blower is run by 1440 rpm motor and pulling 6" x 3" is used to run blower.

Pasteurisation room

In India, many farms have conventional pasteurisation rooms where compost is filled in trays and stacks in centre of the room. Perforated steam pipe is provided on one side at about 4' (feet) height from the ground. On this side, only fan is provided at about 75 cm away from the vent at ceiling level. The fan should be kept covered with a duct having capsized holes at 30 cm gap at right angle to the wall, so that it may introduce and re-circulate hot air. Trays are stacked in centre leaving 3' gap from ceiling to provide free movement of air from the duct. At 1-2' above the ground level on other end of the duct ventilator with wire mesh is provided for the escape of gases. The size of pasteurisation room should be same as for spawn running and cropping rooms or a little smaller than that. It should be insulated or should be made of hollow cement brick wall.

Growing rooms

Growing rooms should be insulated properly. Generally, ground is not insulated. In Europe, cropping rooms are kept quite big in size but, in India different sizes of such rooms are constructed. These rooms usually measure 15' x 17' x 10-12' or 25' x 15' x 10-12' or 35' x 20' x 12' and 35' x 25' x 12' in size. The racks/shelves in the room should preferably be made of iron with wooden base and sides. The side support of bed should be movable for periodic inspection of the compost texture and quality. If cultivation is to be done in bags, racks can be made of steel with net floors for keeping the compost filled polythene bags. Practically 10-12" deep compost bags are used for optimum utilization of space and compost. The working distance between two shelves should be at about 26-28", in case of bag cultivation as against 18-20" for shelved beds. The growing room should be made in such a way where air exchange in and out of room should be at the will of the grower. The floor of the growing room should have a slight slope to remove runoff water and facilitate regular cleaning after various operations. Forced air circulation system should also be arranged.

The Casing pasteurisation

The casing pasteurisation chamber is also insulated (both walls and ceiling) as in bulk chamber. The walls can also have simple air space for insulation. The stream pipes are placed in plenum below grated floor. The casing soil is placed in trays and pasteurised for 4-6 hours at 65C°.

Low cost bulk chamber and growing rooms

Low cast bulk chamber and growing mushroom rooms can be built with high density polythene sheeting supported by skeleton of iron pipes. In this case, the thickness of insulation has to be increased to 15 cm.

11.2.1. Cultivation of white button Mushroom (*Agaricus bisporus*)

11.2.1. Introduction

Mushroom production is a growing business in India as the demand is increasing from last few years. Mushroom cultivation is a matter of practice and technical knowledge rather than labor intensive farming with a high value of return in short time. Mushroom production has some key raw materials to get good yields. Spawn, Compost and Casing Soil are three raw materials are used in it.

11.2.2. Cultivation of White Button Mushroom

The following steps arc adopted for cultivation of the white button mushroom:

i. Formulation of compost and methods of composting;
ii. Spawn preparation,
iii. Spawning of compost,
iv. Casing of compost, and
v. Harvesting the crops.

It requires a temperature of 15-18°C during cropping. Therefore, its cultivation is gaining much popularity in hilly regions in our country.

11.2.3. Spawn Preparation

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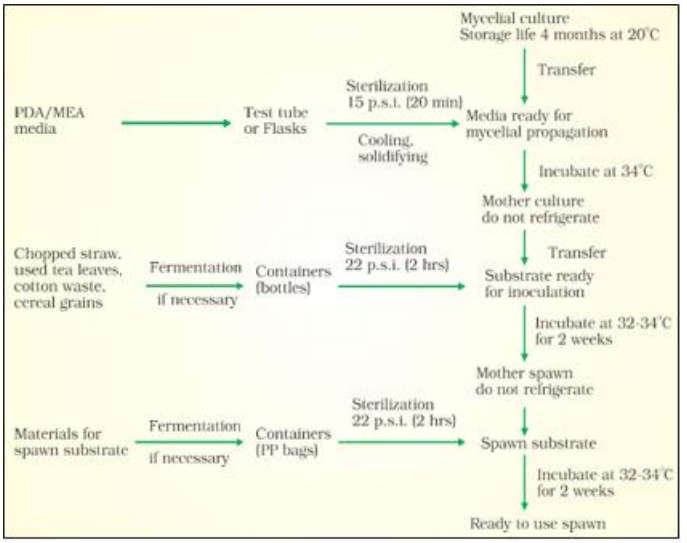
Spawn is just equivalent to the seed of a plant, although, it is only pure mushroom mycelium (vegetative part of fungus) growing on a sterilized grain medium (in case of solid spawn). The grain medium is prepared by boiled grains of cereal or millet like wheat, bajra, jowar and rye mixed with calcium source (chalk-powder and gypsum). The medium is sterilized after in heat resistant glass bottles or polypropylene bags at 121°C and 15 lb psi pressure or for 2 hours at 100°C and inoculated with pure primary culture of *A. bisporus*. The medium is incubated at 25°C and soon gets impregnated with mushroom mycelium. This spawn would be ready for use in 2–3 weeks. The spawn production flow chart is given below (Fig.1).

SPAWN PRODUCTION FLOW CHART (Fig.1)



11.2.4. Spawning Of Compost

The substrate used for button mushroom is a prepared after partial digestion of organic matter prepared under aerobic conditions and is generally termed as Champost (dutch style) or compost. In India, straw of wheat or paddy have been generally used. It is known as synthetic compost. Out of several formulations of compost, the most commonly used are:

• Long method compost (unpasteurized)

• Short Method Compost (Pasteurized)

Any of the formulations can be used for preparing the compost (LMC) according to the available materials. It is completed outdoors in about 28 days. The constituents include in the formulations, ensure the initial Nitrogen levels at 1.5–1.75 and final level at 1.25%. The Carbon: Nitrogen lay in between 25 and 30 at beginning and 16–18 in the end. The straw is thoroughly wet for 24 hr and mixed with the bran and fertilizer mixture prepared separately with 2/3 quantities of ammonium sulphate and urea and the entire quantity of SSP and SOP added to 75 kg of well moistened wheat bran and left for 16 hrs (overnight) covered with wet gunny sheets. The substrate so prepared is formed into a large heap of 5-6 feet width and 5-7 feet height to encourage intense microbial activities causing the generation of heat reaching up to 75°C–80°C. This heap is broken on the 6th day and remade after adding the bran and fertilizer mixture made the previous night with the remaining ingredients and mixture slurry made with molasses, nematicide and insecticides in 50 litters of water. This breaking and remaking process is called turning. Turing has to be done every 3–4 days after adding water to maintain around 75% moisture and allowing passing the air and getting the aerobic conditions. Normally 7–8 turnings are necessary. Gypsum is added at 3rd and BHC or Lindane at the last or second last turning. If free from ammonia the compost is ready for spawning after 7th or 8th turning, otherwise more turnings are necessary until it is completely free form ammonia. This is very much necessary to get rid of ammonia at this stage. This short method composting needs equipment and can be completed in 18–20 days in outdoor and indoor phases.

The phase-I composting

In outdoor phase, the straw is pre-wetted and entire quantities of chicken manure and Brewer's grains are added in layers. Plenty of water and trampling makes the loose stack which is almost anaerobic. A turning is given after 2–3 days. An aerobic heap is prepared 2 days later

after adding the full quota of urea. About 3–4 turnings on each 2nd day complete the outdoor phase.

The phase-II composting

The phase-II composting is done indoors either in a bulk chamber, pasteurization tunnel or in a pasteurization room. These indoor chambers are especially designed for phase-II composting and are fitted with boiler-fed steam-pipes and a blower. The phase-I compost is filled into the chamber up to a height of 6–7 feet, or if it is filled in trays or shelves to a depth of 15–20 cm for spawning in a pasteurization room. In either case, the temperature is rise first to 48°–50°C for 6-8hrs and then it is raised by steam injection to strictly 57°C–59°C for effective pasteurization of the compost. This temperature of air and compost is maintained at this range for 4–6hr, and allowed to fresh filtered air introduction slowly to lower down the compost temperature to 50°C–52°C for conditioning, which takes 3–4 days when the compost gets free of ammonia further air is introduced for bringing down the temperature of compost to 25°C–28°C. After cooling down, it is ready for seeding.

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11.2.5. Spawning and Spawn Run

The compost made by long or short method (Phase-II compost) is

Nemagon (60%)	0.200 litre
Furadan 3 G	0.750 kg
Lindane or BHC 5% dust	1.250 kg
Potassium sulphate or muriate of potash	15.0 kg
Urea (46% N)	18 kg
Molasses	25 kg
Calcium ammonium nitrate or ammonium sulphate (20.6% N)	45 kg
Wheat bran or	150 kg
Gypsum	150 kg
Spent brewer’s grains	200 kg
Wheat straw	1500 kg

Long Method Compost (Unpasteurized): Sola

Temik	0.200 kg
Kelthane or Ecalux	0.200 litre
BHC 5%	1.250 kg
Sulphate of potash	15 kg
Single super phosphate	15 kg

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Urea	18 kg
Molasses	25 kg
Ammonium sulphate	45 kg
Wheat bran (or flour)	150 kg
Gypsum	150 kg
Paddy straw	2000 kg
Nemagon (60%)	0.200 litre
Furadan 3 G	0.750 kg
Lindane or BHC 5% dust	1.250 kg
Potassium sulphate or muriate of potash	15 kg
Single Super Phosphate	15 kg
Urea (46% N)	18 kg
Molasses or Sheera	25 kg
Calcium ammonium nitrate or ammonium sulphate (20.6% N)	45 kg
Wheat bran	150 kg
Gypsum	150 kg
Wheat straw	1500 kg
Nemagon (60%)	0.200 litre

Long Method Compost (Unpasteurized):

Gypsum	37.5 kg
Wheat Bran	125 kg
Chicken manure	500 kg
Wheat straw	1250 kg

Short Method Compost

After mixing the spawn through compost @ 0.5%- 0.75% compost is filled in trays or shelves or in poly bags up to 15-20 cm. The spawned compost are kept covered with 2% formalin dipped unprinted newspaper sheets or polythene or by closing the mouth of the bags. Temperature are then maintained at 24°C in the culture room, with relative humidity maintained between 80–85%, Mushroom mycelia start impregnation within 24-48 hrs after spawning. The spawn-run is completed in 2 weeks approximately at appropriate conditions (If temperature is lower than 24°C spawn run would take longer time). Identification of spawn is done when the compost colour turns light brown from initial dark brown. Now this is ready for casing and called phase-III compost.

11.2.6. Casing and case Run

Casing is a 1–2" thick layer of casing material used to covered the spawn run bed. Without casing material mushrooms don't start fruiting or gives very low yield. Casing is necessary to initiate fruiting, although its role in

fruiting is only partially understood. Casing material should not have any nutrient and should possess good water-holding capacity but a texture permitting good aeration and a pH range of 7–7.5. The most commonly used casing material is the peat-moss, and is directly used for casing after adjusting the pH with lime or chalk and pasteurization. However, but Peat moss is unavailable in India, so one of the following mixtures is being used in our country.

- 1–2years old cow manure + clay loam soil (1:1).
- 1–2 years rotten cow dung + clay loam soil + 2 year's old spent compost (1:1:2).
- 2 years old spent compost + sand (4:1)
- Garden loam soil + sand (4:1)
- 1-2 year's old cow manure + Ashes (1:4)
- Fired Brick Chips

It works very well and even easier to use brick chips than any other

They must be from fired bricks, not mud bricks. All casing needs water and lime. The good thing about peat is that it holds water. Coir does not wet easily, brick chips do, but none of them hold water well, so less water, more often is what is needed. Actually, the chips can be reused, after they are washed and more lime added. They shake off easily and leave the mushrooms clean. One important thing, Do not mix different kinds of casing on the same bed. It is a good idea, to try several kinds on different beds in the same room. In my experience, adding peat to soil might make the soil a little better for casing, but it would still be a much poorer casing than either the peat or the brick chips. The casing material is pasteurized either by steaming (60°C–65°C for 6 hr) or by adding 5% formalin solution, covering with a polythene sheet for 48-72 hrs. Before casing, the material is allowed to cool or become completely free from formaldehyde traces. This is a very important step because heat and formalin both would harm the mycelia.

For mushroom mycelium growth in the casing soil, temperature is in the room is maintained around 24°C for next 7–10 days. When the casing soil is infiltrated with mushroom mycelia, room temperature is brought down to 14°C–18°C and ample ventilation is provided to reduce CO2 level, preferably below 1,000 ppm. These conditions initiate the fruiting in mushrooms. The relative humidity of the room is maintained between

85 and 90% all the time. The casing layer is given light spray of water to prevent its drying and for evaporation.

11.2.7. Casing and spraying

When casing, growers must decide which type of casing soil suits the growing methods best, and what the market is currently asking for in terms of quality and size. If large mushrooms are popular, then a heavier casing soil is the best option. If the market for small mushrooms is good and picking is no problem, growers can use a finer casing soil. Remember that the structure will become even finer during casing if this is done mechanically. The more intensive the treatment of casing soil, the finer the structure will be. Manual casing retains the structure better, but this method is virtually impossible with heavy casing soil.

Directly after casing growers can start spraying the casing soil to reach a good moisture level. Don't wait until the mycelium in the compost starts penetrating the casing soil, otherwise there's a risk of a layer of thin, grey mycelium forming in the lower layer of the casing soil, which can cause problems later. On the first day (the casing day) spraying can last until the compost is reached. With dry compost it's often beneficial to keep spraying until water flows into the compost. This is however, only possible with phase 3 compost that is not wetter than 65%. This is unnecessary if the compost is wetter to start with. If the compost is only moderately incubated, then caution is required. This type of compost can absorb water and that's not the idea here. Apply caution with watering on the second day so the mycelium has a chance to recover. From day three spraying can start again, but reduce the amounts as blow down approaches. Spray using a decreasing pattern. This means the casing soil has the right moisture content from the start and in the final days spraying is only necessary to replace the moisture lost through evaporation.

When spraying, be careful the right pressure is used. Too high pressure forces the fine peat particles out of the casing soil and creates an obstructive layer in the upper part of the casing soil. Later on this will cause problems with pin heading and mushroom growth.

The increasing competition and lower prices for mushrooms mean the pressure is on to perform well. In most countries, good phase 3 compost is currently available and the expertise of growers is good enough to enable good production. But frequently, the casing soil quality is a barrier to achieving the goal of good production one often heard argument is that there's nothing else available, or that better casing soil is too expensive. But bearing in mind that good casing soil gives higher production, better quality and a higher picking performance, then it's logical that good

casing soil costs a bit more. For many growers it's high time they critically examined this aspect of growing. Many farms are otherwise missing out on the chance to improve their profits.

11.2.8. Harvesting and Postharvest Management:

Mushroom pin-heads start appearing after another 7–10 days. They appear in flushes every 7–10 days and harvested accordingly. About 2/3 of the total crop can be harvested within first two flushes. The beds retain up to 3rd flush for fruiting. The growing rooms are then cooked out to kill pests/pathogens and emptied. This practice protects the subsequent crop-cycle from infection.

Generally mushrooms are harvested on the basis of maturity rather than size. At buttons stage, it is best to harvest (Grade-A) which if allowed to grow further ruptures 'veil' to reveal pink gills and are known as 'cup' (Grade-B). If they further grow in size and become fully 'open' or 'flat' (Grade-C) exposing dark gills. If the harvesting is not done even after, it deteriorates and dies soon.

Mushrooms have a very short shelf-life. It is better to sold them immediately after harvesting. If it has to be stored they should be stored dry in paper envelopes kept in plastic bags to prevent moisture loss and are stored in a refrigerator (lower shelves) for less than a week or after giving immediate cool temperature with vacuum refrigerator.

Cultivation of paddy straw mushroom (*Volvariella volvacea*):

11.3.1. Introduction:

Paddy straw mushroom (Volvariella volvacea) commonly known as the straw mushroom, or the Chinese mushroom, belongs to the family Pluteaceae of the basidiomycetes. It is an edible mushroom of tropics and subtropics, and first cultivated in China in 1822 .Initially this mushroom was known as "Nanhua mushroom" after the name of Nanhua Temple in Northern Guangdong Province in China. In the beginning, paddy straw mushroom was cultivated by Buddhist monks for their own table however by1875it was sent as attribute to the royal family. It is presumed that cultivation of this mushroom begun before the 18th Century almost 300 years ago. Around 1932 to 1935, this mushroom was introduced into the Philippines, Malaysia and other South Asian countries by Chinese.

Paddy straw mushroom is also known as "warm mushroom" as it grows at relatively high temperature. It is a fast growing mushroom and under

favorable growing conditions total crop cycle is completed with in4-5 weeks time. This mushroom can use wide range of cellulosic materials and the C: N ratio needed is 40 to 60, quite high in comparison to other cultivated mushrooms. It can be grown quite quickly and easily on uncomposted substrates such as paddy straw and cotton waste or other cellulosic organic waste materials. It has been considered as one of the easiest mushrooms to cultivate. Paddy straw mushroom was first cultivated in India in 1940, however, its systematic cultivation was first attempted in 1943. Presently this mushroom is more popular in coastal states like Orissa, Andhra Pradesh, Tamil Nadu, Kerala and West Bengal, however, it can also be cultivated in most of the states, where agro climatic conditions suit and agro waste is available in plenty.

The common methods employed for paddy straw mushroom cultivation are given below:

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11.3.2. Conventional method

Preparation of paddy straw bundles of 0.75 – 1.0 kg (80-95cm long & 12.16cm wide) preferably from hand threshed paddy.

The different steps involved in this method are as follows in Fig 1.

- Immersing of bundles in clean water for 12-18 hours in a cemented water tank.
- Draining out of excess water by placing bundles on raised bamboo platform.

  Making bed by placing 4 bundles side by side and another four bundles, similarly but from the opposite side, forming one layer of eight bundles. The open ends of bundles from opposite sides should overlap in the middle.
- Forming of second, third &fourth layer by intermittent spawning between first and second, second and third and third and fourth layers.
- Spawning on entire surface of the layers of the beds at a space of 5cm apart leaving margin of 12-15cm from edges.
- Sprinkling of red gram powder over the spawned surface.
- Using 500 gm spawn and150 gm of red gram powder for a bed of 30-40 kg of dried paddy straw.
- Pressing of bed from the top and covering with clean plastic sheet

for maintain in g required humidity (80-85%) and temperature (30-35 °C).

- Removing of plastic sheet after 7-8 days of spawning and maintaining temperature of 28-32°C and relative humidity about 80%.
- Mushroom will start appearing after4-5days of sheet removal and will continue for next 20 days.
- After crop harvest the substrate can be used for manure in the field.

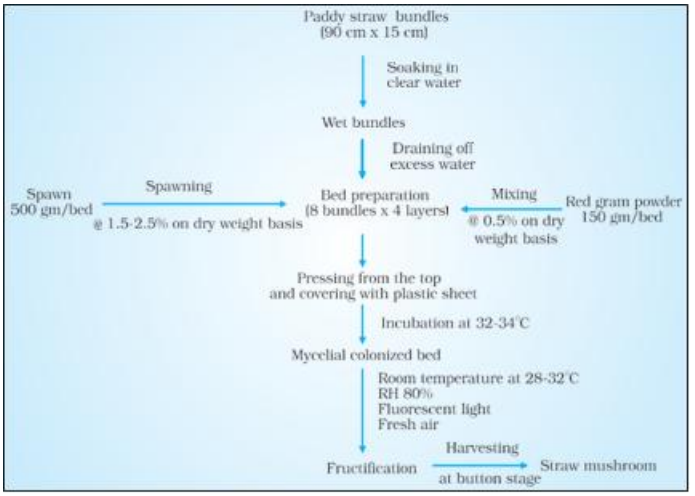

Fig 1.Conventional method of paddy straw mushroom cultivation

Material required

1. Paddy straw 60/Cage bundles
2. Spawn bottle 2/Cage
3. Wooden cage 1No.(1mx50cm X 25cm)
4. Drum 1 No. (100 litre capacity)
5. Polythene sheet 4meters
6. Binding thread 3meters
7. Sprayer/Rose cans 1No.

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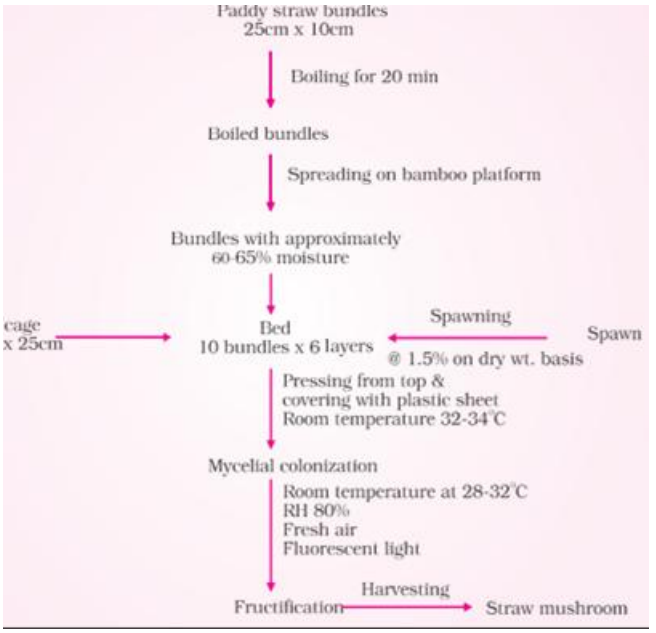
8.	DithaneZ-78/	1 Pkt. (200gm) Bavistin
9.	Malathion	1 bottle (250ml)
10.	Dettol/Formalin	1bottle(1/2liter)
11.	Dao(Hand	1 No. chopper)
12.	Thermometer	1No.

11.3.3. Methodology

- Select dry, fresh and hand threshed paddy straw free from moulds and leafy portion. Make 25 cm long and 10 cm thick bundles @60bundles for each cage (Bed).
- Soak the bundles in boiling water for 20-30 minutes and allow cooling and draining of excess water.
- Spray solutions of 0.1% Malathion and 0.2% DithaneZ-78 all over the bed. Cover the whole bed with polythene sheet and bind securely with a binding thread.

  Keep the spawned cage in a room orashed for mycelia run (Fig.2). A warm place with temperature around30^{0}C is helpful for better mycelial growth.
- Remove the polythene sheet after the mycelial run is completed. Maintain high humidity in and around the bed till pinheads appear.
- Continue spraying water for the next flesh of mushroom to appear within a week.

Cage method of paddy straw mushroom cultivation (Fi.2)



Cultivation of Oyster Mushroom (*pleurotus ostreatus*) on sawdust:

11.4.1. Introduction:

Oyster mushroom (*Pleurotus* spp.) cultivation has increased tremendously throughout the world during the last few decades Oyster mushroom accounted for 14.2 % of the total world production of edible mushroom in 1997 Oyster mushroom cultivation can play an important role in managing organic wastes whose disposal has become a problem. Oyster mushroom can be cultivated in any type of lignocellulose material like straw, sawdust, rice hull, etc. studied the oyster mushroom cultivation on sawdust of different woods and found that *P.ostreatus* gave the maximum yield. Presently sawdust is commonly used and is the preferred medium at commercial scale. reported that *P.ostreatus* gave maximum biological efficiency on sawdust. Of the sawdust types, softwood sawdust like mango and cashew are known to be more suitable than hardwood sawdust. Malnutrition is a problem in developing third world countries. Mushrooms with their flavour, texture, nutritional value and high productivity per unit area have been identified as an excellent food source to alleviate malnutrition in developing countries among the reasons for the quick acceptance of mushroom is its nutritive content. Mushrooms are eaten as meat substitutes and flavouring. In general edible mushrooms are low in fat and calories, rich in vitamin B and C,

contain more protein than any other food of plant origin and are also a good source of mineral nutrients Currently, high biofuels prices have caused an increase in food prices and food scarcity in many countries To alleviate hunger and malnutrition in a world of rising food prices, cultivation of mushrooms is a very reliable and profitable option. The objectives of this study were to evaluate selected grain media for spawn production and find out the most suitable media for spawn production.

11.4.2. Spawn Production:

The grains, maize, sorghum and paddy were cleaned manually to remove inert matter, stubble and debris. The cleaned grains were soaked in 0.5% CuSO4 for 10 min and the soaked grains were thoroughly washed and soaked in tap water for 2 hours. Thereafter, the soaked grains were drained and the excess water removed and the following additives added. Rice bran at the rate of 10%, chalk (CaCO3) at the rate of 2%, and Epsom (MgSO4) at the rate of 0.2% on dry weight basis of the grains. The additives were thoroughly and evenly mixed with the grains. The grain medium was filled in to polypropylene bags (200 gauges, 37.5 cm long and 17.5 cm wide). About 200 g of medium was packed in each bag. The bags were sealed using cotton wool plugged conduit/ poly vinyl chloride pipe rings, and covered by a piece of paper by tying a rubber band around the neck. The bags were autoclaved at 121o C, 15 psi, for 30 min and the sterilized bags were allowed to cool for 24 hours. The bags were immediately inoculated with mycelial culture of *P. ostreatus* maintained on PDA.

11.4.3. Media Preparation:

A medium was prepared using sawdust of mango, rice bran (at the rate of 10%), chalk (at the rate of 2%), and Epsom (at the rate of 0.2%) on the dry weight basis of the substrate and were mixed thoroughly with water. The correct water content was checked by pressing the medium by hand. The medium was filled into polypropylene bags. About 800 g of medium was packed into each bag. The bags were then sealed, autoclaved and inoculated with the spawn using surface spawning technique under laminar flow and incubated in a dark chamber.

11.4.4. Cultivation of Dhingri (*Pleurotus sajor -caju*)

Pleurotus is also one of the important edible mushrooms gaining popularity in recent years. It is found growing naturally on dead organic materials rich in cellulose. Its several species are edible such as *P.sajor-caju, P. sapidus, P flabellatus. P ostriatus, P corticatus, P florida* etc.

These species can be cultured successfully on various agricultural, domestic, industrial and forestry waste materials. It is very versatile in nature as far as substrate preference and growth are concerned. However, it can be grown on paddy straw, gunny bags, rice husk, copped Parthenium stem, etc.

The steps for cultivation of dhingri start with preparation of substrate for growth.

Paddy is cut into 2.5 cm long pieces and soaked in hot water at 60°C for about 30 minutes.

Excess water is drained off from straw. About 4 kg of wet straw is transferred into the large sized polythene bags.

NOTES

About 5 grams of Bengal-gram powder with half bottle of spawn of fungus are mixed with straw. This mixture is filled in large-sized polythene bags.

Mouth of the bags is tied and kept on a raised platform in well ventilated cropping room or in open when properly protected for about 15 days.

At this time when mycelia are visible inside the polythene bags over the surface of paddy straw, the polythene bass are cut and gently removed.

Now paddy straw forms a compact mass and does not lose its makeup. This composite mixture is watered daily just to maintain moisture.

The temperature where the compost has been kept should be between 20°C and 25°C with relative humidity of 75 per cent.

After 15 days first flash of dhingri becomes apparent. These are harvested when become young.

11.5 LOW COST MUSHROOM FARM DESIGN OF PRODUCTION

11.5.1. Introduction:

A mushroom house is a wooden 6m x 8m roofed structure that has racks stored within. The racks hold rolled rice straw which is the bulk substrate for mushroom growth. There is a layer of plastic around the racks, sealing them off from the outside, as well as an external layer of fabric that protects the mushrooms from sunlight. Currently, mushroom house designs vary widely in materials, size, and construction processes, but all include the same basic features. These features are: posts, a roof, protective plastic, protective fabric, rice straw racks, and a steaming system.

Mushroom houses all follow a basic design and are altered depending on the resources of the farmers and each farmer's personal preferences. New farmers follow a relatively similar design because they are unwilling to take large risks and are unfamiliar with the growing process. Farmers building their second mushroom house are more likely to make changes and almost always decide to increase size. The construction schedule varies greatly from farmer to farmer, depending on the resources and skills available to them. From start to finish, the building process can take from 5 days to a month. If one farmer is building a mushroom house by himself, it can take a month, depending on his time availability. These farmers still need help from their family or neighbours to install the exterior posts, but the rest of the mushroom house can be built by a single person. Many farmers choose to hire workers for a few days. Farmers may choose to hire workers to expedite the process or because they do not personally have the knowledge and capabilities to build a mushroom house. Mushroom houses usually take 5 days to build when hiring 3 workers per day

11.5.2. Low cost mushroom farm design production

Selection of site and pre-requisites before selection of site, the following points have to be taken into consideration for greater operational efficiency and cost effective production of mushrooms at the farm: Chosen site should preferably be away from the municipal limits and entrepreneur should purchase sufficient land in one go looking to the future expansion.

1. The site should be serviced by a motorable road, or nearer to a road head to reduce costs on transportation of raw materials to the farm/finished product to the market.

2. Plentiful availability of water at the site either through a perennial source or should have sufficient underground water.

3. Easy availability of raw materials especially straw and poultry manure around the chosen site at cheaper costs in the area.

4. Availability of cheap labour in abundance.

5. Uninterrupted Proper power supply at the chosen site.

6. Nearness to the market for the proper disposal of the produce.

11.5.3. Components of a mushroom farm:

NOTES

For round the year cultivation of this mushroom employing environment controlled conditions a medium size plant would require under mentioned components.

Spawn unit: This will have under mentioned major components.

1. **Cooking/autoclaving room:** For boiling the grains and sterilization of the bottles/pp bags

2. **Inoculation room:** For inoculation of the sterilized bottles/ pp bags

3. **Incubation room:** For incubating the inoculated bottles. Insulated and provided with AC.

4. **Cold Store:** For storage of prepared spawn for its further disposal.

Composting Unit

This will have under mentioned main components for production of compost

1. **Pre wetting area:** For dumping of raw materials and their pre wetting (uncovered).

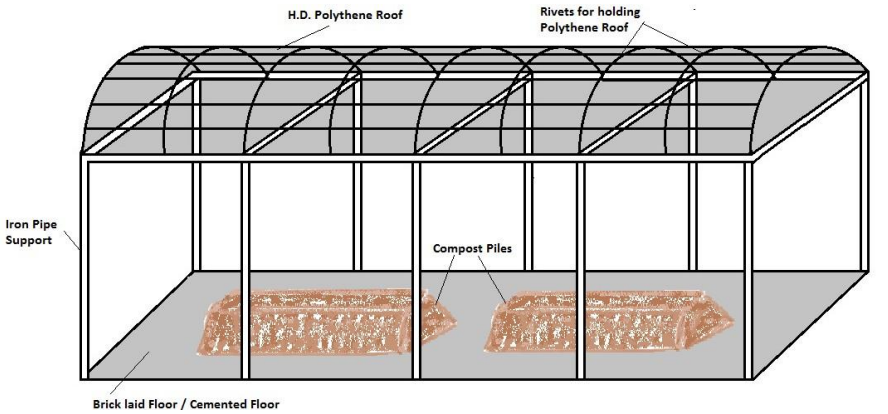
2. **Composting yard:** For making piles out of the wetted materials (covered)

3. **Phase-I bunker:** For phase -I phase of composting (in case indoor composting is employed).

4. **Phase -II tunnels**: For performing pasteurization and conditioning of the compost.

5. **Casing soil chambers:** For pasteurization of the casing soil.

6. **Spawning area:** For spawning of the prepared compost



Low cost composting yard

11.5.4. Principles Of Mushroom Farm Lay Out:

Mushroom cultivation is usually an indoor horticultural activity and a wide variety of designs and constructional materials have been used for mushroom houses. Mushroom farms comprise a set of buildings constructed in such a way that various operations related to mushroom cultivation may be done continuously and efficiently to get mushroom production all the year around. Mushroom farm should not be constructed near the other mushroom farms or business points because it may adversely affect the consumption of the produce in the market. It is one of the most important conditions for obtaining good mushroom yields at a low cost with profitable marketing. Mushroom farm should be property designed and well equipped with modern facilities. For obtaining profit, one should start with at least three growing rooms and one-bulk chambers each of about 20 tons capacity with scope of future extension.

Location of the building plot

While selecting plot, one must keep in mind the following precautions for profitable mushroom cultivation:

1. Plot for constructing building should be next to or close to public road / high way, so that raw materials/removing materials and spent compost etc. may be easily delivered.
2. There should be facilities for water, electricity and disposal of sewage etc.

3. The building must be located on the plot in such a way that the compost filling area can be reached in a direct-line from the road. The access road must be about 5 meters broad so that vehicles/trucks may be turned easily.
4. Farm layout should be prepared after drawing plan of the farm and should preferably be constructed in phased manner. For example, if farm is proposed to be of 12 rooms, only three or four rooms should be constructed initially.

An ideal commercial mushroom farm should have about 12 rooms each of 200-m^2 area having capacity of 20 tons of compost in bags with two bulk chambers or tunnels each of 20 tons capacity. The composting yard with dimensions of 100' x 45' for holding phase I stacks with scope for future extension. The composting yard should be covered and cemented with facilities for water and proper drainage.

NOTES

Design of farm

There are different types of mushroom farm designs adopted in different parts of the world suiting to the local conditions. The Dutch farm design and construction method is being widely adopted all over the world with some modifications to suit local conditions. There are two systems, which are usually used in mushroom growing farms. These are as under:

1. Single zone system: In single zone system, peak heading, spawn run and cropping is done in one room. Each room is insulated properly and equipped in such a way that the highest temperature required can be maintained. In this system, growing is almost done in fixed beds called shelf bed

II. Second zone system: In second zone system, separate rooms for peak healing, spawn run and cropping are maintained. In this system, temperature in the cropping rooms will not rise higher than 16-20 C° and there is no need of heavy insulation. Rooms for peak heating and spawn run are heavily insulated to maintain desired temperature.

In the Netherlands, cropping rooms with 200 m2 cultivation surfaces are used. There are two rows of shelves each having five beds one above the other. The room size is kept 6.00m x 17.75m x 3.80m. In the front of room 4m wide passage is provided and in backside of the room, cemented area to facilitate compost filling is provided.

NOTES

11.5.5. Bulk chamber:

Situation of a bulk chamber in a mushroom farm is extremely important. The size of chamber depends on the compost to be loaded. For 20 tons capacity, bulk chamber with dimensions of
36' (Long) x 9' (Wide) x 12' (Height) is best suited under Indian conditions. The wall should be well insulated. The plenum should be 3' deep at lower end and 6' deep on upper end, thus giving a desired slop for run off sufficient space of steam to penetrate the compost mass about 25-30% of the total floor area should be left in the form of gaps for efficient steam circulation. The walls as well as ceiling of the tunnel should be effectively insulated. Walls are provided with 5.0 cm thick insulating materials between brick wall (9" wide) and inside plaster. The roof should also be insulated and surface be sprayed with specific paint to serve as a vapour barrier. Two vents should be kept, one connected to main re-circulating duct and the other to outside to exhaust extra air/ gases from the chamber. The doors of the chamber should be made airtight by using a rubber gasket on inner side of the door and the door insulated with 5-6 cm thick insulation material should be covered with GI sheets on both inner and outside.

REVIEW QUESTION

1. How do you prepare compost for mushroom cultivation?
2. What are the characteristic features of paddy straw mushroom?
3. What are popular mushrooms cultivated in India. Explin any one mushroom.
4. Describe the cultivation of oyster mushroom.
5. Explain the design of low cost mushroom farm.

BLOCK-4: STORAGE AND VALUES MUSHROOM

Unit-12: Factor affecting mushroom cultivation (Temperature, pH, Air and Water management)-Insects and pest attacking mushroom – Fungal, Bacterial Viral diseases.

Objectives:

- To know about the factor affecting mushroom cultivation.
- To study a detailed account on the insects and pest attacking mushroom.
- To study the Fungal, bacterial and viral diseases in Mushroom.

12.1.1. Introduction

Like any organism, growth and development of mushrooms are also subject to impact of the environmental factors such as, temperature, pH, Air, water, carbon dioxide etc., adequate Knowledge of the impact of the environmental factors on mushrooms is essential for setting up a mushroom farm.

12.1.2. Temperature

Temperature influences morphology of mushrooms. The stipes of *Lentinula edodes* and *Pholeota nameko* may elongate and the Pileus diameter may be reduced at temperatures above 60°C. In *Flammulina velutipus*, production of fruit bodies at optimal vegetative growth temperature (22-25°C) results in small slender mushrooms. In commercial cultivation, mushrooms should be produced near their optimum temperature range to obtain products of higher quality.

Heat treatment Mushroom production techniques may involve previously composted and/or steam pasteurized natural substrates or may use axenic cultivation, which consists of using a sterile substrate. A number of different methods for substrate pasteurization or sterilization have been proposed autoclaving (axenic), axenic and inoculation with thermophilic microorganisms, rapid substrate steam treatment between 80 and 100° C for several hours, pasteurization at 72° C for four or five days, and

pasteurization by substrate steam treatment for several days (60° C) in a tunnel. The most common pasteurization process uses vapour injected into chambers or tunnels, where the substrate is packaged and pasteurization time varies as a function of the temperature. In the non-axenic culture (steam pasteurization), the substrate is packaged and subjected to heat treatment at 75–100°C for 4–10 h. In this technique, only a fraction of the microorganisms is eliminated.

The objective is to destroy the microorganisms that are in the vegetative form, forcing the rest to stay in spore form. The rapid cooling causes the microorganisms remain static, disadvantaging the optimal conditions that stimulate spore germination. As these temperatures are easily reached, pasteurization can be done even in containers less resistant to high temperatures, such as those polyethylene bags.

Substrates also can be saturated in water for 24 h, pasteurized for 2 h, drained from excess of water, mixed as their combination and cooled for ready to inoculate considered that the immersion of substrate in water can have different consequences according to the type of raw material. They pointed out that there is a ‘‘nutrient washing” effect that can be negative when old raw material is used, but useful in new raw material because there is a decrease in soluble sugars that can prevent the development of antagonistic microorganisms related that the hot water immersion treatment of substrate reduces yields in at least 20% when compared to other straw treatments, such as steam, chemical or untreated wheat straw.

Compounds which are hydro-soluble are lost during wheat straw immersion in hot water. The loss of these nutrients would be the cause of yield decrease. Although this method is inexpensive and easy to implement, crop reduction is very important, causing significant loss, especially when the majority of *pleurotus* farmers in Latin America, India or Africa use this methodology to treat the substrate. Additionally, another important factor to take into account is that this method uses a high amount of water, which could be a negative factor due to scarcity of this resource in some areas. Although there are a lot of alternative procedures for substrate preparation, most of the papers published by the scientific community report a preference for axenic cultivation, cultivation in a substrate previously sterilized in an autoclave, with some variations.

According to the technique of axenic cultivation is unfeasible in a commercial scale due to the required investment in equipment. However, in developed countries this is the technique that presents best results. Sterilization is an important step for mushroom cultivation. Several studies have reported the use of heat treatment such as sterilization,

wherein the cultivation packages were sterilized in an autoclave for 1, 1.40 and 2 h, respectively, at 121 C 1.2 psi of pressure tested different sterilization techniques including hot water, autoclave, formalin and bavistin. Sterilizing with formalin and bavistin and autoclave found to have better spawn running, pin head and fruiting body formation, and yield.

To prevent the development of competing microorganisms and subsequent economic loss, it is also important to thoroughly clean the vessels, often applying heat treatment.

12.1.3. pH

Each mushroom has its optimal pH range for development, and it is variable; for example, pH between 4.0 and 7.0 for the mycelium and 3.5 to 5.0 for formation of basidiocarps. The optimum pH for mycelial growth and subsequent fruiting body development is obtained at between 6.5 and 7.0 with fungal colonization; the substrate pH is reduced to values close to 4.0 for the reduction of organic acids, primarily oxalic acid in step preceding the cutting of the package fruiting crop of solid-state fermentation.

12.1.4. Light

The quality and quantity of light are important for the formation of fruit bodies and their maturation. The duration of light and its intensity should be carefully considered for individual species, line or fruit body is known for the mushroom *Shitake*. For this mushroom, the exposure to light intensities greater than 50 lux during spawn run may inhibit primordial formation.

Positive phototropism in *Pleurotus* spp has been observed. A light intensity of 10 lux is sufficient to induce the response. At least 15 minutes in full sunlight are required for fruit body initiation in *Volvariella volvacea*. Fruiting in the mushroom has been obtained under a 12 hour dark cycle and under continued light with an intensity of 500 lux.

Sufficient light for fruit body initiation and maturation in most *shiitake* and *Pleurotus* spp is provided by cool white fluorescent bulbs for 2 to 5 hour per day. Further, sufficient light is usually provided by the normal picking periods.

12.1.5. Water relations

Some mushrooms have specific substrate moisture optima that mat depend on the type of substrate used. A moisture content of 55-68% is optimal for nutrient supplemented sawdust used to produce *Lentinula edodes*. The optimal moisture content of 70% is needed for spawn run of *L. edodes*, on natural logs. The optimum moisture content of traditional rice Paddy straw substrate used to produce *Volvariella volvacea* should be in the range of 65-70% While that of cotton waste substrate should be about 70%.

12.1.6. Carbon Dioxide

CO_2 levels as low as 0.1% may delay sporophore formation and reduce sporophore initials in *Agaricus bisporus*. In this mushroom stipe growth increase at CO_2 levels of 1% in *Lentinula edodes* restricting by capping cultures with polky propylene membranes does not prevent the formation of pigmented buried primordial or exudation, but reduces and delays the number of primordia formed. Elevated levels of CO_2 cause stipe elongation and Pileus expansion in this mushroom.

In *Flammulina velutipus*, pileus diameter decreases with increasing concentrations of CO_2 (0.06 to 4.9%).Stipe elongation is less sensitive to CO_2 than is pileus expansion. In case of *Pleurotus*, high concentrations of CO_2 cause elongation and branching of stipes.

12.1.7. Air

Gaseous environment control in aerobic solid-state fermentation is an important factor in the development of microorganisms, dependent on oxygen flow speed through the substrate and the speed of O_2 consumption by microorganisms. Aeration has different functions, being O_2 provision for aerobic growth and metabolism; moisture regulation; temperature adjustment; water vapour, CO_2 and some volatile metabolite elimination. Aerobic mushrooms require oxygen for their survival and development.. During the darkened spawn-running, it is important to keep CO_2 concentration at 2000–2500 mg L. After the completion of spawn-running and mycelia stimulation, fruit bodies were allowed to develop at CO_2 concentration 1500–2000 mg L. Since air contains high CO_2 levels, it will produce mushrooms with thick and short stipe pileus. Therefore, during the fruiting stage is a reduction in CO_2 concentration is required, as well as an increase in O_2. This is possible by opening packages of cultivation and ambient air change through ventilation (rational room cubic capacity / cultivation area in cubic meter ration should not be lower than 1.85:1).

The maximum number and size of holes (air entrance) can be made, provided that there is no contamination by being careful not to damage the mycelium. An increased number of holes in the cultivation of packets results in smaller mushroom. It is expected that the level of O_2 required for solid-state fermentation is lower than the submerged fermentation mycelia., the problem with O_2 diffusion, in solid-state fermentation, comes down to the transfer of gas among the particles. An ideal situation would be to increase the ability of a microorganism to achieve directly the atmospheric O_2 gas .whatever the form of O_2 transport is, it is noted that transfer speed in solid-state fermentation is higher than in submerged fermentation mycelia

12.1.8. Moisture

Water is one of the main factors that influence the success in mushroom growth. Nutrients are transported from the mycelium to the fruiting bodies by a steady moisture flow High moisture content in the substrate will result in difficult breathing for the mycelium, inhibiting perspiration, rendering the development of fruiting body impossible, even with elevated inoculum amounts or number of holes in mushroom cultivation packages, resulting in the development of non-desired organisms such as bacteria and nematodes. Low moisture content will result in the death of the fruiting body. The optimum moisture content for growth and substrate utilization depends upon the organism and the substrate used for cultivation. Increasing moisture level is believed to reduce the porosity of the substrate, thus limiting oxygen transfer. For this reason, the use of high moisture content limited the growth within the whole substrate, resulting in surface growth. The appropriate moisture in the substrate should encompass a range between 50% and 75% in the substrate, enabling the satisfactory growth of *Pleurotus* spp. Similarly, cultivated *P. eryngii* where the moisture was maintained at 65–68%. Moisture above 70% makes the development of diseases and competing moulds possible.

12.2 INSECTS AND PEST ATTACKING MUSHROOM

12.2.1. Insect-pests and Nematodes

Like the field crops, mushrooms are also attacked by several pests. Insect-pests, mites and nematodes usually cause damage to the mushrooms right from spawning to harvesting of the crop Sciarid flies. Phorid flies, cecid flies, springtails and mites are the important arthropod posts of cultivated mushrooms in India. Mushrooms being the indoor

crop, provide a very suitable habitat for the insect-pests. Moreover, maintenance of optimum temperature and humidity in the cropping room provides idea condition for the development and survival of the pests. Therefore measures should be taken to prevent the entry of insect posts into the cropping room. However chemical method of control should be taken as the last step rather than the first.

Common Insect and Pests of Mushrooms

12.2.2. Sciarid flies (Diptera: Sciaridae) Morphology

Sciarid flies are also called as mushroom flies. Mushroom Sciarid are small flies, measuring 3-4 mm in length. They are black gnat like flies with long thin antennae which are held characteristically erect.

Characteristics

Females usually trend to rest on the surface of the walls and trays while males often remain on the surface of the casing. A single female lays about 110 eggs in dusters or single or in chains. Different species of sciatic) flies are known to be found throughout the year in and around the mushroom farms. Most important species which cause much harm to mushroom crops are *Lycotiolla soiani, Lycoriella mall. Lyoariella aunpila, Bradysia pauper* and *Bradysia tritici.*

Economic importance

The damaging stage of the pest is larva which is white and legless maggot, ranging from 1 mm to 8 mm in length. The larva boar is shiny black head. Larvae cause damage by making tunnels in the stipes. They cause most serious injury to pin heads and buttons of the mushrooms. Mycelial attachment can he damaged, causing the pinheads to become brown and leathery. Pinheads may become hollow or may even be consumed entirely. Adults act as vectors of *Verticilliurn fungicola.*

12.2 3.Phorid flies (Diptera: Phorldae)

Morphology

Phorid flies have small hump on the back having inconspicuous antennae. They resemble house flies and are brown black in color. Generally, they are stouter than so arid flies. The larvae arc creamy white legless maggots with a pointed head, whirl) is not black.

Characteristics

Female lays up to 50 eggs in close proximity to the growing hyphae lips of the mushroom mycelium. Flies are attracted to mushroom houses due to the odors resulted from fermentation of the compost during the cool down period of peak heat. Alter the laying of eggs. New generation of adults emerge from the compost within 2-3 weeks. By this time crop has been cased and it is the casing layer in which the subsequent generations of those flies develop.

Economic importance

Larvae feed on mushroom tissue and moves upward to the cap forming tunnels. Larvae hatching from the eggs laid on mushroom gills directly bore into the mushroom cap. Larvae hatching from the eggs and laid in spawned compost or casing material make tunnels through the button of mushroom stalks. The color of the infested mushroom becomes yellow brown when attacked during the pin head stage. Maximum infestation of 11-74% occurs during March-April. The various species of phorid flies are *Megaselia Itinerate, Megaselia nigra* and *Megaselia agarica*

12.2.4. Cecil fly (Diptera: Cecidomyiidae)

Morphology

Geoids are rarely identified from the fly stage because they are so minute to be seen. Larvae are legless maggots either white or orange. They have no discernible heads but there are Iwo eyes-spots at the head ends which together give the appearance of IC. Six species of cecids have been recorded on mushroom throughout the world. Only three namely, *Heteropeza pygmaea, Mycophila spoyori* and *Mycophila barnesi* are common in occurrence.

Characteristics

Larvae, being sticky are usually carried out by trays. Reproduction in cecid is through pseudo genesis. Each cecid larva becomes mother larva in due course. It can give birth to 12-20 daughter larvae within a week of its own birth without being any adult cock' present.

Economic importance

Occurrence of a cecid fly species- *Hereropenzina calhistes* has been reported on oyster mushroom from Jind and Chandigarh. Initial Infestation probably arises from the Infested casing soil. Larvae feed on the mycelium. The white or orange larvae are first noticed. During waterlog, they move on to the mushroom where they feed on the outside of stipes or at the junction of stipe and gills. Bacteria presort tin the skin of the larvae usually results in brownish stripes on gills. Gill tissues break down to produce tiny pustules with black fluid. They usually cause spoilage of mushrooms upto 50%.

12.2.6. Spring Tails As Insect-Pests

Springtails mostly cause more serious damage to oyster mushroom because of its preferred host; Seven species of springlails namely, *Lopidocyrlus cyaneus, Lopidocyrtus lanuginosus, Xenylla muemnata, Achomtes ormolus, Proisotoma minute, Proisotorna simplex* and *Seim irk-dor* have been recorded in different parts of the country

including Delhi Rajasthan and Punjab. They move by jumping and rarely by walking.

Morphology

Springlails are wingless pests and have ground colour with light violet coloured band along the sides of the body. Dark and round scales are observed to be characteristically present all over the body of springtails. Body length including appendage measures about 2.85 millimetres.

Economic importance

Both the adults and nymphs of springlails, usually toed on mycelium by scraping It from the spawn grains and cutting the mycelia' strands thereafter. They produce shallow pits on the sporophore. Springtails arc usually found at the base of the stem, resulting in arrested growth of young primordial showing withered look and small pits.

12.2.7. Beetles As Insect-Pests

Three species of beetles have been reported to cause damage to oyster mushrooms. The most common beetles reported are *Staphytinld* beetles from Kerala, cucuieic1 beetle from Chandigarh and *Aiphitobius laovigatus* beetle from Solan.

Morphology

Damaging stage of the beetle is grub which is whitish and long in appearance with tubular terminal segments.

Characteristics

The adults are attracted to the small newly developed mushrooms. They lay eggs in the over-matured pileus and discarded mushroom debris. Grubs feed on the soft gills and crawl over the beds. The life cycle is completed within 3 weeks.

Economic importance

Grubs feed on the softer tissues of the stipes, gills and pileus. At initial stage, grubs make small irregular holes in the hymenium and stipos. In severe cases, adults bore windows In the pileus. Eaten margins of gills and edges of the fruit bodies give a fringed look to the damaged mushroom.

Management

Removal of wastes and debris of the mushrooms from the mushroom house and surrounding areas should be done. II prevents the adults from lying of eggs and thus checking further built up of the population. Over-matured mushroom should be harvested immediately. Bleaching powder usually repels the adults. Its application should be done in mushroom houses and premises to repel the adults.

12.2.8. Fly Pests

Commercial mushroom fly pests include three dipteran families: *Sciaridae, Phoridae,* and *Cecidomyiidae* .Each family can cause significant yield or quality loss and vector mites, nematodes, and diseases on commercial farms. The dominant problematic species display regional, annual, and seasonal variations. Dark- Winged Fungus Gnat Sciarids are commonly known as the dark- winged fungus gnats, sciarid flies, big flies, or mushroom flies. The predominate fly species in North America is *Lycoriella ingenua* (Dufour). while in the UK both *L. castanescens* (Lengersdorf) (syn. *L. auripila*) and *L. ingenua* are mushroom pests with *L. castanescens* being the more serious pest there. *Bradysia* spp. are also pests of mushrooms in other parts of the world . Only *Agaricus bisporus* is subject to severe attack by this gnat but it also will breed on oyster and shiitake mushrooms. In general, the dark- winged fungus gnat can be found in greenhouses in both soilless and soil mixtures, in composting debris such as leaves, and outdoors in wild mushrooms.

Damage

Dark- winged fungus gnats can be found on any mushroom farm, but direct yield losses occur only when the gnats go unchecked. Larvae of this fly are general feeders, consuming mushroom compost, mycelia, spawn grains, mushroom primordia (pins), and carpophores. When mushroom primordia are small, up to about 1.5 cm diameter, the larvae can consume the entire internal contents. The mushrooms will appear glossy and light brown, and the small carpophores may be completely perforated and, when picked, the tissues crumble. Carpophores that are larger when attacked show black necrotic areas in the stipe where the larvae made feeding galleries. Some larvae do not tunnel into the stipe but consume the mycelia at the base of the stipe, in which case the mushroom does not develop normally. Generally, little direct damage from this gnat will be evident on first- flush mushrooms because the fly population has not developed sufficiently. However, second- and

subsequent- flushes may show some damage from larval feeding. Perhaps its greatest impact is as a vector of mushroom pathogens, such as dry bubble disease (*Lecanicillium fungicola* (Preuss) Zare and Gams [syn.*Verticillium fungicola* (Preuss) Hassebrauk, *Verticillium malthousei* (Preuss) Ware or green mould disease (*Trichoderma aggressivum* Samuel and Gams *f. aggressivum* and *F. europaeum*) (syn. *Trichoderma harzianum* biotype Thz or Th2, respectively), from diseased to clean areas in the same production room or to clean crops on the farm.

Identification Eggs of this fly (family Sciaridae) measure 0.25 by 0.15 mm and are smooth, oval, white, and translucent. Mature larvae are about 7 mm in length, have a white, translucent body and a black head capsule Pupae are about 2.0–2.5 mm in length, are white at first but turn black prior to eclosion. Adult males and females measure between 2 and 3 mm in length; most often they can be found near a light source. The adult wing has a distinctive forked and cross vein.

Gall Midges

They are economic pests of agricultural and food crops, forest trees, and ornamentals. Damage Cecid larvae feed on the outside of the stipe or at the junction of the stipe and gills of both *Agaricus* and *Pleurotus* species. Their presence can result in direct yield loss or in a loss of quantity of fresh or processed marketable product. The actual presence of the orange or white larvae on the stem and gills make the product unmarketable. And, as the larvae migrate from the casing and across the mushroom tissue, their presence induces moisture accumulation and they spread the bacteria that induce browning. Identification Gall midges (family *Cecidomyiidae*) are small, rarely seen flies, about 1.5 mm in length. Several species are associated with commercial mushroom production: *Mycophila speyeri* Barnes, *Heteropeza pygmaea* Winnertz, and *Mycophila barnesi* Edwards. Midge larvae are white (*Heteropeza* spp.) or orange (*Mycophila* spp.); mature larvae are about 2 mm in length. Adult *Mycophila* can be distinguished from Heteropeza adults by the former have wing venation and the latter none.

Phorid Flies in this family of insects are world- wide and are a pest of mushrooms in many countries. They are commonly known as humpbacked flies or scuttle flies. Six species have been reported 228 Edible and Medicinal Mushrooms from commercial mushroom facilities in the US The predominant species in the recent literature is *Megaselia halterata* (Wood).

Damage

Megaselia halterata larvae feed at the growing hyphae tips of the mushroom mycelium. This species, unlike *Megaselia nigra* and agarica that were pests in the 1940s in the United States, does not consume the sporophore. Thus, direct yield loss correlates to the number of larvae grazing on the mushroom mycelium. More than 12,000 females per square meter of production surface are necessary before significant yield loss occurs, which is 12 times more than the number required for *Lycoriella ingenua*. Although direct yield loss can be a problem, the greater threat is the transmission of Lecanicillium fungicola.

Management And Control Of Flies

The flies should be controlled so as to get better crop of mushroom. Both prophylactic and curative measures should be taken at proper limo as described below:

Prophylatic Measures

1. Maintenance of hygiene and sanitation

Hygiene is very important at every stop of mushroom cultivation. The first step in the compost preparation is to clean the composting yard properly and thereafter. the composting yard should be sprayed with 2% lurmalin about 24 hours earlier old compost preparation. Hygiene and sanitation will check the prevalence of insect pests in the composting yard. Pasteurization of compost kills insect pests at its all stages.

2. Treatment of compost and casing material

About 20 ml of Lindane 20 EC alter diluting it in water should be mixed into the compost at the time of last turning. If the mushroom flies are present in the mushroom house before casing, then mix thoroughly 15 mole Undone 20 EC after dilution in 3 -4 litres of water in 100 kg of ready to use casing material before use. Incorporation of any other suitable insecticide in compost and in casing soil may also serve the purpose of controlling the larvae of flies.

3. Screening of doors and other inlets of mushroom house

Mushroom flies are attracted to spawn and mushroom compost due to their odor. During cropping period, the flies enter the mushroom house and breed in the spawned compost and mushroom beds. Since the body sire of mushroom flies is too small, they can pass through an

ordinary wire screen. Therefore, it becomes imperative to prevent the entry of the flies into the cropping rooms by screening of doors and ventilators with nylon or wire nets of 14-16 mesh/cm. It is effective in checking the entry of the flies into the mushroom house.

4. Poison baiting

It flies are present during the cropping period; various other methods can be employed for the control of adult pests. Poison baiting with baygon diluted in water al Ono ratio of 1:10 should be used with the addition of little sugar into it. It is also an effective method for controlling adult flies.

5. Light traps

Polythene sheets coated with sticky material and attached with florescent strip tight may be used continuously in each cropping room. II also helps in controlling the adult flies to a great extent.

Curative Measures

Following curative measures should be taken into account for the management of flies:

1. Spraying of non persistent and safe chemicals

Presence of mushroom flies in the mushroom house helps in further built up of the fly population resulting In high infestation of mushrooms. Under such circumstances, the flies should be killed by using ion-persistent and comparatively safe chemicals. This can be done by the methods as given below:

After 7^{th} day of spawning, Malathion (0.01%) should be sprayed on beds. Flies can also be controlled by spraying 30 ml of Nuvan 76 EC in fine droplets. After spraying, the mushroom house should be closed for 2 hours. Direct spraying on beds should be avoided. An interval of 48 hours between splaying and picking of mushrooms must be observed.

Judicious and need based application of only safer insecticide should be done. However, care should be taken that same insecticide should not be used again and again. Continuous application ol same insecticide helps in building of resistance in insects. Therefore, rotation of insecticides should be done at each application.

2. Disposal of spent compost

The spent compost and casing material contains the immature stages of pest, nematodes and mites. Damping of spent compost and casing material in moist and shady places become ideal substratum for fly breeding. Putting spent compost and casing material in manure pit and covering it with 10 centimetres layer of manure helps in checking the fly breeding.

3. Other precautions to be taken

Sticky traps should be used to monitor the number of flies in the spawn running room. Freckles, effective screening of doors and ventilators should be arranged. During cropping, pyrethrums may be used to kill the flies. At the end of cropping, suitable cook out temperature should be maintained and all the spent compost should be removed from the farm. In addition to these, strict hygienic Conditions should be maintained at the farm and casing ingredients must always be stored and mixed in a clean area.

Management

Springlails are managed by the measures given below:

- Clean properly the surroundings and inside of the mushroom house.
- Proper disposal of spent compost and casing material should be done immediately.
- Rising of the crop above the floor level should be preferred.
- Efficient pasteurization of the compost and timely spraying of the infested place with any suitable insecticide must be done.
- In case the compost is infested by springlails, mixing of 15 ml of diazinon 20 EC after dilution in water should be mixed in 100 kg compost at the time of filling.

12.3.1. FUNGAL DISEASES

Introduction

Like all other crops, mushrooms are also affected adversely by a large number of biotic and non-biotic diseases causing agents. Among the biotic agents, fungi, bacteria, viruses, nematodes and insect-pests are of paramount importance, as they cause considerable damage to mushrooms either directly or indirectly. The abiotic agents include high or low temperature, air pollutants, extreme light and excess moisture which initiate different kinds of abnormalities in mushrooms at different stages of development.

12.3.2. Diseases of Button Mushrooms

Disease may be defined as "a physiological or structural abnormality incited by a biotic or abiotic pathogen that is harmful to the plant or to any of its part. or products that reduce the economic value". Although a number of harmful fungi, bacteria and viruses encounter to cause diseases in button mushroom but the most common and economically' important diseases known to be of great economic importance may be summarized according to their occurrence at different stages of cultivation.

1. **Compost moulds:** Competitor moulds and pathogenic fungi occurring mainly in compost are olive green moulds (*Chaetomium olivaceum*), ink caps (*Coprinus species*), green moulds (*Aspergillus* species, *Penicillium* species and *Trichoderma* species) , black moulds (*Mucor* species and *Rhizopus* species) and some others which include species of *Sporotrichum, Sepedonium Fusarium* and *Cephalosporium*.

2. **Compost and casing soil moulds:** Fungi occurring in compost and in casing soil mostly include white plaster moulds (*Scopulariopsis fimicola*), Brown plaster mould (*Papulospora byssina*), lipstick mould (*Sporendonema purpurescens*), false truffle (*Diehliornyces microsporus*) and green moulds (*Aspergillus species, Penicfifium species* and *Trichoderma species*).

3. **Casing soil and growing mushroom moulds:** Such moulds include cinnamon mould (*Peziza ostracoderma*), wet bubble (*Mycogone perniciosa*), dry bubble (*Verticillium fungicola*), cobweb (*Cladobotryum dendroides*) pink mould (*Trichothecium roseum*) and green moulds (*Aspergillus* species, *Penicillium* species and *Trichoderma* species).

4. **Fungi attacking fruit bodies:** Such fungi include species of *Fusarium* causing Fusarium rot of mushroom fruit bodies.

In intensive and continual cropping, the growing conditions sometimes trend to be unfavourable and temperature above 20C° coupled with excess moisture in the beds usually encourage the development of many diseases and competitor moulds. These diseases may be incited by both pathogenic and non pathogenic agents. Pathogenic diseases are incited by fungi, bacteria, viruses and nematodes

12.3.3. DRY BUBBLE DISEASES

Casual organism: ***Verticillium fungicola***

Common Name: Verticillium disease, brown spot, fungus spot, dry bubble,

The fungus produces numerous one celled thin walled, oblong to cylindrical, hyaline conidia. Conidiophores are relatively slender and tall Conidia accumulate in clusters surrounded by sticky mucilage.The fungus abounds in soil.This is the most common and serious fungal disease of mushroom crop. If it is left uncontrolled, disease can totally destroy a crop in 2-3 weeks .*Verticillium fungicola* was major pathogen responsible for considerable yield losses of cultivated mushrooms.

Symptomatology

Whitish mycelial growth is initially noticed on the casing soil which has a tendency to turn grayish yellow. If infection takes place in an early stage, typical onion shaped mushrooms are produced. Sometimes they appear as small- undifferentiated masses of tissue up to 2cm in diameter. When affected at later stage, crooked and deformed mushrooms with distorted stipes and with tilted cap can be seen. When a part of the cap is affected hare lip symptom is noticed. Affected mushrooms are grayish in color. If the infection occurs at later stage, grey moldy down can be seen on the mushrooms.

Sometimes little pustules or lumps appear on the cap. On fully developed sporophore, it produces localized light brown depressed spots. Adjacent spots coalesce and form irregular brown blotches. Diseased caps shrink in blotched area, turn leathery, dry and show cracks. Infected fruit bodies are malformed; onion shaped and become irregular and swollen mass of dry leathery tissue.

Management

Proper sterilization of casing soil is significantly important. As soon as the disease spots are located, complete soil related with infected spots should be removed and destroyed. Other hygienic conditions should be maintained to prevent the spread of the disease.

12.3.3. Wet Bubble

Casual organisms: *Mycogone perniciosa*

Common Name: Wet bubble, La mole, w h i t e mould, b u bble, *Mycogone* disease.

Wet bubble in white button mushroom incited by Mycogone perniciosa Magn.This is one of the serious diseases from almost all the major mushroom growing countries of the world. Bubbles or mole (M. perniciosa), first described from Paris in 1888, is stated to be responsible for the heaviest losses in mushroom beds in France, England and United States. In India, this disease was reported for the first time in1978 from some mushroom farms in Jammu and Kashmir Later, this disease has been reported from the States of Himachal Pradesh, Haryana and Maharashtra.

Symptomatology

Many workers have described Symptoms of wet bubble at different stages of mushroom development. it recognized two main symptom types, infected sporophores and scleradermoid masses, which he considered to be the result of infection by M.perniciosa at different stages in the development of the sporophores.when infection took place before the differentiation of stipe and pileus the selerodermoid form resulted, infection after differentiation resulted in the production of thickened stipe with deformation of the gill. The symptoms in the form of white moldy growth on the mushrooms, leading to their putrefactions (giving foul odor) with a golden brown liquid exudate.That the infected sporophore may be recognized by two symptoms, one is tumorous form

Infected from pinheads and other is malformation, infected at later stage. Both types of infections may exude water drops on the surface of infected sporophore. This water drops later change into amber color. Cross section of deformed sporophore without cottony growth showed black circular area just beneath the upper layer. Infection when young (up to 6mm) pin heads were infected.

Etiology

Mycelium of the pathogen is white, compact and felt-like. Hyphae are branched, interwoven, septate, and hyaline and measure 3-5 nm broad. Conidiophores are short, slender, branched, and hyaline and measure 200 x 3.5 nm in size. Conidiophores bear conidia that are thin walled,

single celled and measure 5-10 x 4-5 nm in size. Large two celled chlamydospores are also observed.

Management

(1) Benomyl sprays at the rate of 0.5-4g per square meter immediately after casing has been reported to be very effective for protecting the crop.

(2) Aerated steam at 54.4C° for 15 minutes can eliminate the pathogen from casing soil.

(3) Use of plastic pots to cover mushroom showing wet bubble symptoms durinthe cropping season should be done to prevent spread of disease.

12.3.4. Cobweb

Casual Organism

Cladobotryum dendroides (Dactylium dendroides) imperfect state of Hypomyces rosellus. Sterile hyphae form a turf and are prostrate, branched, septate and hyaline with approximately opposite branches, which divide above into usually three pointed branchlets. Conidiophores are erect, similar or branched in many whorls.

Common Name: Mildew, Soft decay, Hypomyces mildew disease, Dactylium disease.

This disease renders extensive damage either by causing soft rot or decay of fruiting body. This disease as Botrytis dendroides and transferred it in to the genus Cladobotryum by making a combination C.dendroides.

Symptomatology

Cobweb appears first as small white patches on the casing soil which then spreads to the nearest mushroom by a fine grey white mycelium. A floccose white mycelium covers the stipe, pileus and gills, eventually resulting in decomposition of entire fruit body. As the infection develops, mycelium becomes pigmented eventually turning a delicate pink cover.

12.3.5.Weed Fungi and Competitor Moulds

Moulds acting both as competitors and pathogenic fungi are undesirable for proper growth and development of mushroom. They adversely affect the quality and yield to such a great extent that sometimes complete failure of the crop may be observed. Competitor moulds, also known as weed moulds or indicator moulds are those fungi which adversely affect the growth of the mushroom mycelium during the spawn rum (colonization) on the substrate/compost/easing soil. They compete with the mushroom for nutrition, oxygen, water and space. As a result of such competition, the mushroom growth is checked and in some cases there is no growth at all. A partial list of important competitor moulds along with the symptoms and methods of management have been given as under.

Etiology

The fungus is known to be found in soil. It produces one celled, thin walled, oblong to cylindrical hyaline conidia, measuring 3.5 - 15.9 M x 1.5 - 5min size. Conidiophores are slender and tall. Conidia accumulate in clusters and are surrounded by sticky mucilage.

Management

1. Maintain proper temperature, ventilation and humidity as this disease is favoured- by temperature above 23 C°, poor ventilation and high humidity.

2. The diseased mushrooms should be picked carefully and destroyed so as to prevent the diseases to spread.

3. Perfect sanitary conditions should be maintained in growing houses and when the disease appears, the temperature should be lowered to below 28 C°.

12.3.5. False Truffle

The causal agent

This disease is caused by the fungal species *Diehliomyces microspores*. Sometimes, it is considered as competitor and not the pathogenic fungus to the mushroom. It was first reported by Lambert (1930) from Ohio, USA.

Symptomatology

The disease is characterized by the appearance of cottony-weft of mycelium on the bed surface. These wefts soon become denser and develop into small, reddish brown and wrinkled somatic bodies resembling a truffle. The infected beds usually have a peculiar disagreeable odour. The yield of the mushroom crop is reduced significantly.

Etiology

Ascocarps are formed from the dense tangled hyphae knots. These are fleshy, first white then brownish and finally reddish brown with 3-8 ascosporos. Chlamydospores may also be formed in the hyphal web of ascocarp.

Management

Soil used should be well sterilized. After casing, excessive moisture in bed, temperature above 20°C poor ventilation and high humidity should be avoided. Proper temperature, moisture, ventilation and humidity should be maintained in cropping houses.

12.4.1. BACTERIAL DISEASES

In India, three mushrooms namely white button mushroom (Agaricus bisporus), dhingri or oyster mushroom (Pleurotus species) and paddy straw mushroom (Volvariella volvacea) are being exploited for commercial cultivation. In addition to this, recently Calocybe indica which is commonly known as milky mushroom is also gaining popularity in some part s of the country and is suited for cultivation in warmer areas where A. bisporus cannot be cultivated. These mushrooms like any other living organism are attacked by several pathogens .The bacterial pathogens which produce recognizable symptoms and cause significant crop losses. he expression of disease symptoms in mushroom depends upon the stage of development of the fruit body at the time of infection and cause of the disease/inoculums potential present.

The bacterial diseases have been reported from all over the world on fruit bodies of A. bisporus, A. bitorqu is, Pleurotus species, Volvariella species, Lentinus edodes,

The bacterial pathogens induced varieties of symptoms like blotch, mummy, pit, drippy gill, soft rot, yellowing and immature browning but

in India, bacterial diseases has been reported only on fruit bodies of A. bisporus and species of Pleurotus and Auricularia .

Mushroom	Disease	Causal organism	Distribution	Reference
Agaricus bisporus	Bacterial blotch	*Pseudomonas tolaasii*	Worldwide	Fletcher *et al.*
		P. fluorescens		(1986)
	Ginger blotch	*P. gingeri***	UK, Netherlands	Fletcher *et al.*
				(1986)
	Drippy gill**	*P. agarici*	UK, Netherlands	Fletcher *et al.*
				(1986)
	Mummy	*P. aeruginosa*	UK	Wuest and
				Zarkower
				(1991)
A.bitorquis	Bacterialblotch	*P. tolaasii*	Worldwide	Fletcher *et al.*
				(1986)
	Soft rot	*Bukholdria gladioli*	Worldwide	Guleria *et al.*
		pv. Agaricicola		(1987)
Oyster mushroom	Bacterial rot	*P. alcaligens* **	India	Biswas *et al.*
(*Pleurotus* spp.)				(1983)
	Brown blotch	*P. tolaasii*	Japan,	Fermor (1986)
			Australia	Ferri (1985)

Bacterial Diseases of Oyster Mushroom

Four bacterial pathogens namely Pseudomonas alcaligens, P. tolaasii, P. agarici and P. fluorescens have been reported parasitizing Pleurotus fruit bodies and causing considerable economic losses to the growers.

12.4.2. Bacterial Diseases Of Mushrooms

Bacteria are among those micro-organisms which are having the size in the range of microns and could not be seen by naked eyes. First time in 1876, Koch proved that bacteria can cause diseases as observed by him through a series of experiments. T.J. Burrill in 1878, first time proved the association of a bacterium with a plant disease. Bacteria have boon defined as being extremely microscopic, unicellular organisms usually rigid in structure without chlorophyll but rarely containing other photosynthetic pigments. They multiply mostly by fission although a few may reproduce sexually. They are characterized by not having a readily demonstrable nucleus'. There are four important diseases in winter button mushroom which are induced by bacteria. Only one disease is known to occur in summer button mushroom which is suspected to be of bacterial in nature and etiology.

Bacterial diseases of Button Mushroom

There are five important bacterial diseases of button mushroom. These are as under:

1. Bacterial blotch or brown blotch (*Pseudomonas tolaasii*).
2. Ginger blotch (*Pseuclomonas gingen*).
3. Mummy disease (*Pseudomonas* species)
4. Drippy gall (*Pseudomonas agarici*.
5. Pit disease.

12.4.3.. Bacterial Blotch

Bacterial blotch in mushroom was described for the first time by Tolaas from America in 1915. Paine (1919) in England named the bacterium after Tolaas together with other Pseudomonas. This disease has also been reported from India in 1976. An extra cellular toxin a tolaasii in was found responsible for this disease. The toxin is an ion channel farming lipopeptide having bio-surfactant property.

Symptoms

1. When mushrooms remain moist for longer time, yellowish-brown patches or blotches occur initially which in serious cases usually turn dark brown in color.
2. Gradually, these patches spread over the whole surface of the mushroom cap.

3. Brown pits can also sometimes be observed in such mushrooms.
4. Mushrooms with bacterial blotch seem sticky.
5. In serious attacks, the pin heads can turn completely brown and they do not develop further.

The causal pathogen

The disease is caused by *Pseudomonas tolaasii*. It has boon observed that persistent moisture on sporophore for more than three hours alter watering usually encourage the symptoms development. High relative humidity with low evaporation rate also favors' the development of the symptoms.

The disease cycle

The pathogen usually seems to be endemic at many mushroom farms. It survives probably between crops on surfaces, in debris, in tools and on various materials of the farms.

Management

1. Store all casing materials in an area tree from contamination before and alter mixing it. Also avoid surface condensation on developing mushroom.
2. When the disease has established, removal of all affected mushrooms is desirable. Also ensure preventive measures to check disease spread. Pasteurization of casing soils by steam/ air mixture and short wave length radiation have been reported to be effective. However, over heating should be avoided.
3. Spray suitable bactericide such as streptomycin for the control of the disease. Application by tetracycline 9 mg per square feet, streptomycin (200 ppm), Oxytetracycline (300 ppm) have been found effective in managing the disease.
4. Spraying of Lime salt has been found to be effective in controlling the disease.
5. Adjust the conditions within the cropping house so that whenever possible, evaporation may take place from the surface of the developing mushrooms.
6. Biological control should be explored with antagonists like *Pseudomonas fluorescens* and bactoriophages.
7. Management of *Aphelenchoides composticola* (nematode) infection is also important as it helps in spreading the disease.
8. In some countries, a software system has been developed to manage the disease.

12.4.4. Ginger Blotch

This disease is closely related with bacterial blotch caused by *Pseudomonas tolaas*ii. This disease is most commonly observed in England and Netherlands (Fletcher et al., 1986).

Symptoms

The ginger colour of blotches which do not change with the advancement of age, distinguishes this disease from bacterial brown blotch. The blotches are not deeper than 1-2 millimeters. Unlike *Pseudamonas tolaasii*, Pseudomonas ginger; does not give a positive reaction in the white line test.

The causal pathogen

The disease is caused by the bacterium *Pseudomonas gingeri*. The ecology and epidemiology of the disease is similar to that of bacterial brown blotch.

Disease cycle

The contaminated casing material is probably tho most important primary source of the disease.

Management

Control measures are similar to those recommended for bacterial blotch.

12.4.5. Mummy Disease

Royse (1980) using what he called the 'mummy bacterium' demonstrated the intracellular growth of the bacterium in artificially infected mushroom by electron microscopy. it has been found very difficult to induce artificial infection producing same symptoms with bacterium isolated from mummy-diseased tissues.

Symptoms

1. During spawn run no symptoms of infection are noticeable. But after casing, the spread of spawn and development of pin heads are observed in patches.
2. The pin heads which have not emerged may remain stuck in the casing soil.
3. The fruit bodies can turn grayish in color and open prematurely.
4. In second flush, the stipe becomes crooked and caps tilt.
5. The stem is often thickened at the base and the base is usually surrounded by a fluffy edge of mycelium.
6. The mushrooms become tough and spongy or leathery dry (mummy) and they change in color from grayish while to brown.
7. An Al picking, a cracking sound is heard.

8. When the stem is cut through, cut surface seems reddish brown.
9. Under some situations, very fast spread of the disease has been reported.

Control

The causal pathogen: The disease is caused by *Pseudomonas aenvinosa.*

Disease cycle

The spread of the disease is through infected mushroom mycelium and not through spores. The disease has been reported by placing of compost or casing material from diseased beds to healthy compost at the time of spawn running.

Observe and select affected boxes or areas of beds by digging 20 centimetres wide channel and treat them with 0.5% formalin. Strict hygienic measures should be followed.

124.6. Drippy Gall Disease

The disease is observed to be most severe in autumn and winter months. It is known to occur in U.K. and Netherland in severe form.

Symptoms

Gills are attacked by the bacterium before the veil of the mushroom is open. Affected gills are often under developed. Underdeveloped affected gills exhibit small, brown and decaying areas with creamy white bacterial ooze on them. Hence the name drippy gall has been given.

The causal pathogen

This disease is reported to be caused by *Pseudomonas agaricii* and *Pseudomonas cichorii.*

Disease cycle

Probably flies, pickers and water splashes are helpful for spreading the disease within the crop. As the gills are attacked before veil breaks, it seems that the bacteria are systemic.

Management

Spray suitable insecticide to control the flies and follow strict hygienic precautions to avoid infection and spread of the disease.

12.4.7. Pit Disease

This disease is fairly common but rarely causes considerable crop losses. The cause of the disease Is not yet established but is suspected to be a bacterium. Mites and nematodes have also been implicated.

Symptoms

Small dark (often black) and slimy pits appear on the cap surface. The depth of pit may be few millimetres deep and their numbers may range from 1 to 10 per cap.

Management

Same control measures as recommended for bacterial blotch should be used to reduce the incidence and spread of the disease.

12.4.8. BACTERIAL DISEASES OF OYSTER MUSHROOM

Several bacterial diseases have been reported on *Pleurotus ostreatus, Pleurotus Saror-caju* and *Pleurotus evyngii* Such bacterial diseases are known to be caused by *Pseudomones egarici*, *Pseudomonas fluorescens* including some unidentified bacteria and some other species of *Pseudomonas*. Different symptoms induced by the bacterial pathogens are brown blotch, black spotting, yellowing and mottling of fruit bodies. However very little work has been done on bacterial diseases of oyster mushroom in our country. Some very common diseases reported in India are as under:

Four bacterial pathogens, namely, *Pseudomonas alcaligens, P. tolaasii, P. agnbi and P. fluorescens* have been reported to parasitize *Pleurotus* fruiting bodies. Among these, *P. agarici* and *P. alcaligens* have been reported from India.

12.4.9.Bacterial Rot Disease

This disease was first reported from West Bengal on *Pleurotus sajor-caju* in India.

Symptoms

It induces appearance of water-soaked areas and yellow brown discoloration on young sporophores. Rotting of grown up fruit bodies, starts from the centre towards periphery. The gills on the lower surface usually turn yellow and caps get crinkled and rolled upward.

The causal pathogen

The bacterium *Pseudomonas alcaligens* causes this disease. It produces whitish effused growth on PDA medium.

Management

Follow strict hygienic precautions and spray suitable bactericide well in time for the management of the disease.

12.4.10. Brown Spot Disease

A common substrate competitor bacterium - *Pseudomonas stutzeri* has been reported from Karnataka on paddy straw substrate used for cultivating *Pleurotus sajor-caju*. The bacterium, being a common soil saprophyte, induces brown spots in the substrate. It causes a reduction in the yield of *Pleurotus sajor-caju*. Streptocycline dip beyond 100 ppm and formalin dip beyond 25 ppm controlled the competitor bacterium as reported.

12.4.11. Yellow Blotch disease

This disease has recently been reported on *Pleurotus sajor-caju*. The incidence of the disease has been reported from 42 to 89% during 1989-93. In and around Solan, it causes complete crop failure in some cases.

Symptoms

The disease appears on pileus as blotches of varying sizes which are sometimes depressed and yellow, hazel-brown or orange in colour. When disease appears during primordial formation, entire flush may be affected. The infected fruit bodies rot and emit foul smell under high temperature and humid conditions. The slimy appearance on infected fruit bodies is the characteristic symptom of the disease.

The casual pathogen

The pathogen has been identified as *Pseudomonas agarici.*

Management

Oxytetracycline, Streptocycline and sodium hypochlorite (400 ppm each) have been reported to be effective for the management of the disease.

12.5.1. Virus Diseases Of Cultivated Mushrooms

A virus disease in cultivated mushroom (*Agaricus bisporus*) later having various names Including 'La France disease' and 'die-back disease', was first reported in 1948 in the United States of America. In 1957, devastating crop losses occurred in Britain due to a similar disease. It was demonstrated by the experiments that three types of virus particles were associated with the disease under investigation. The disease was named as 'Die-back disease' to distinguish it from any other possible disorder. Disorders of this type were not reported in the Netherlands until 1994. After few years a heavy outbreak occur causing significant losses in yield; therefore, serious attempts were made to investigate these diseases incited by viruses.

Viruses are obligatory parasitic pathogens with dimension of less than 200 millimicrons. Being ultramicroscopic, they are infective entities that multiply only intracellulary in the form of nucleic acid and are potentially pathogenic. Viruses act as living organism in the host plant tissue and behave as non living molecule/chemical outside the host plant.

In Australia, mushroom diseases of viral nature, probably dated back to the early days old mushroom growing were reported. From India, spherical virus particles associated with *Agaricus bisporus*, measuring 25 nm and 35 nm in diameter have also been reported years back.

12.5.2. Viruses And Virus Like Particles (Vlp)

Virus and VLPs have been commonly observed in *Agaricus bisporus*. There are few reports of virus particles found associated with Pleurotus species and *Lentinus edodes*. Different shapes and sizes of the viruses and VLPs have been reported from different pads of the world.

12.5.3. Detection And Management Of Viruses

Viruses produce different kinds of symptoms on different parts of the mushroom, resulting in deterioration of the mushroom quality and yield of the produce. The wide variations in the symptomalology of diseases caused by viruses usually reflect variations in the economic impact of mushrooms. General symptoms produced by the viruses may be summarized as under.

Symptoms

The general symptoms produced by the viruses on different parts of the mushrooms have been given below:

1. Mushrooms appear only in dense clusters. Mycelium does not develop properly or develops hardly in the casing layer and sometimes disappears alter normal spread.
2. Mycelium isolated from diseased sporophore in agar media show a slow and degenerated growth.
3. Delayed appearance of the pinheads dunes.] the first flush or formation of fruiting primordial below the surface of casing layer may bo an important indication of the disease incited by viruses.
4. Symptoms on sporophore are highly variable. These variations may show following abnormalities as listed below:
    - Off-white color of the caps and early maturity.
    - Slow development of pin heads and dwarfing.
    - Elongated and slightly bent stipes is formed. This stipes is thin.
    - Mushrooms are loosely attached to the substrate and at the slightest touch they are pushed over.
    - Watery stipes and streaking in the stipes are observed. Sipes are spongy and they quickly lum brown on welting. Thickened and barrel shaped stipes may be formed. Pileus may remain small and under developed.
    - Brown and slimy caps may occur owing to a secondary bacterial rot.
    - Abnormal or absent veils with hard gills may be observed
    - A specific and musty smell in diseased mushrooms may be lound.

12.5.4. Epidemiology of Virus Diseases

Various factors like time of infection, cultural conditions and strain of the spawn used, greatly affect the incidence and loss in yield. The viruses usually infect the mushroom spores and the mycelium. The spread of viruses through infected spores or mycelium can spread the diseases in a number of ways such as via air flow through ventilation, through flies, through dirty containers, by means of transport which have not been disinfected and also by persons with dirty clothing and footwear etc. Disease spread may also be due to poor disinfection of wooden furniture, floor, walls and/or side planks after harvesting. Infected spores of mushrooms on germination produce infected mycelium which by means

of anastomosis transmits the virus to healthy spawn/mycelium. Viruses are also known to be transmitted through mites and fungi.

Difficulties In detection of viruses:

Diagnosis of virus infection in mushrooms is difficult because of the following reasons.

1. Symptoms induced by viruses are similar to the symptoms produced by the different biotic and abiotic agents. Such as elongation of slipe incited by virus infection may be similar to the symptoms incited by concentration of high carbon dioxide. Water-logging of the stripe tissue is usually incited by virus infection; however, it may also be induced by higher moisture conditions. Loss in yield and bare patches may be induced by a number of factors.
2. The second difficulty: in detection of virus is due lo its low concentration in host mushroom.

12.5.6.Detection methods

1. Electron microscopy through ultra-thin sectioning, dip method as well as through purified preparations.
2. Serologically specific election microscopy (SSEM).
3. Poly-acryl amide gel electrophoresis of viral ds RNA (PAGE).
4. Enzyme linked immuno-sorbent assay (ELISA).

REVIEW QUESTIONS

1. What are the environmental factors affecting mushroom cultivation. Explain.

2. Write an essay on the insect and pest attacking mushroom.

3. Explain the casual organisms, symptoms and control management of bacterial diseases in oyster mushroom.

4. Explain the casual organisms, symptoms and control management of fungal diseases of mushroom.

BLOCK-4: STORAGE AND VALUES MUSHROOM

UNIT-13: PACKING AND PRESERVATION TECHNIQUES FOR MUSHROOM - STORAGE -SHORT-TERM STORAGES, LONG TERM STORAGES, DRYING, STORAGES IN SALT SOLUTION

NOTES

Objectives:

- To know the packing and preservative processing methods of mushrooms
- To study the different storage methods for mushroom processing.

13.1.1. Processing Methods Of Mushrooms

Although the major part of cultivated mushrooms is consumed in the fresh condition, trading mushrooms totally at fresh status seems unfeasible for every point of the chain and for all year around. Practices and aspects for mushroom post harvest care include proper storage/packaging and/or minimal processing of fresh mushrooms for their short-term maintenance, as well as various processing techniques for their long-term preservation. However, traditional eating patterns have been changed in more selective and individual preference eating behaviors, along with a corresponding abatement of formal eating times and occasions. This has increased the demand for individual portion packs and convenience formats in every day diet and has lead to reduction of the demand for whole processed frozen or canned mushrooms. Also, increase of out-of-home consumption (hotels, restaurants, pubs, etc.) and institutional catering (workplace canteens, universities, schools, etc.) has led in increased requirement for fresh, prepared, and processed mushrooms from these sectors. Another aspect of modern mushroom processing concerns techniques aiming at the value-addition of the product as well as environmental aspects such as waste disposal and utilization of waste and off-grade mushrooms

13.1.2. Packaging Of Mushrooms

Packaging plays an important role in marketing and exports of fruits and vegetables including mushrooms. Improper packaging often results in rejections and under-valuation ol the produce affecting price realization. Therefore, following precautions should be taken In packaging of the produce.

1. With a view to compete in the highly sophisticated and discriminating markets especially abroad, the packages should be attractive and distinctive.
2. Packaging of mushrooms in India is primitive as they are unlabeled and simple in appearance. Polyethylene or polypropylene pouches are preferred for retail sale. Bulk Packaging is not in existence.
3. In developed countries, packaging is given due importance. Modified atmosphere packaging (MAP) and controlled atmosphere packaging (CAP) are in vogue there.
4. Retail packs are mostly made of polystyrene or card board punnets over wrapped with differentially permeable films which create a modified atmosphere of CO, and 02 within the punnets extending shell-life. Such packaging increases the consumers' confidence in the product.
5. Bulk packaging led super market includes polystyrene containers and easy-to-carry card board boxes of different sizes.
6. Besides the packaging for fresh mushrooms. Attention should be paid to the packaging of processed mushrooms. Dried oyster mushrooms are being packed in poorly printed poly pouches which besides giving shabby appearance result in breaking up of the dried fruit bodies.
7. Laminates can also be used for packing mushroom pickles. But future is going to be ruled by 'environment-friendly' recyclable and biodegradable packaging materials.
8. A combination of rigid a flexible packaging is expected to find significant place in future.

NOTES

13.2.1. Preservation technique for mushroom culture:

Proper maintenance of pure cultures of cultivate mushroom is necessary to maintain vigour and productivity. There is no satisfactory way to check and evaluate the qualities of spawn by any rapid on the spot examination. The strains of cultivated mushroom must suitable be preserved and carefully tested from time to time for vigour and productivity.

13.4.1. Short term storage

13.4.1. Storage for few days

Fresh mushrooms are usually packed in poly packs of less than 100 gauge thickness. Generally, packs measuring 200 g and 400 g should be made for retail sale. Poly packs should be properly sealed by sealing instrument. In few regions of Northern India, some growers wash the mushrooms in low concentration of potassium meta-bi-sulphite (0.25%) because few buyers insist on such washing of mushrooms before packing them in packs. Although, this practice should not be recommended and if so desired, the concentration of potassium metabisulphito should be reduced to 0.05%. Poly packs so made, should be stored at low temperature (below 5 Cr) till they are consumed or sold. These poly packs should not be stored even at this temperature for more than 3 days. Higher storage temperature usually results in blackening, cap opening, weight loss and microbial spoilage of mushrooms.

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13.4.2. Storage for a month

Stooping preservation is a method useful for short period preservation usually for a month or so. The practice is useful in canneries to accumulate the desired quantity needed for operational efficiency. Cleaning of mushroom is done through washing it in water or in chemical of suitable concentration. Usually low concentration of potassium metabisulphite (0.05%) solution should be used. After such treatment, mushrooms are lined in largo plastic containers and brine (10-12%) is added into the cans completely steeping the produce. Some preservatives are also added in the brine by few processors.

13.5.1. Long term storage

In event of the difficulties in the sale of fresh mushroom, long term preservation methods have been evolved in order to meet the demand of preserved mushrooms. Canning is the most popular method of preserving the mushrooms. Canned produce may be traded in the international market. Asian countries like China, Taiwan and Korea usually export their produce to the American and European countries in the form of canned mushrooms. Besides canning, freeze drying and pickling are also practiced in different countries including India for long term storage of mushrooms. Thus, there are following 5 methods of mushroom preservation. These methods are canning, freeze drying, pickling, drying and vacuum cooling.

13.5.2. Canning of Mushrooms

Canning is the most popular method of preservation of white button mushrooms. Mushrooms are graded according to their size and stems are cut to less than 1 centimetre in size. These are canned as whole mushroom or in the term of sliced mushroom parts. Mushrooms are washed several times and are blanched for 45 minutes in a solution made of 1% brine + 0.1% citric acid. After cooling the blanched mushrooms in running water these are filled In cans. Cans are then filled with hot brine (1.5 or 2% with citric acid or ascorbic acid as per the customer's requirement) and then passed through steam jacket exhaust box.

Normally cans at the exit of exhaust box have the desired temperature of 85°C in the centre and there after those are sealed properly. Sealed cans are then sterilized either on 'sterilize' or in autoclaves at proper temperature and pressure for recommended time. Crates of hot cans are then immersed in running water cooling tanks, these are then taken out, wiped dry, labelled and stored in a cool and dry place.

Canning units of various sizes ranging from 200 cans to 3 tons/ shift is commercially available. Such firms usually provide complete services including installation, demonstration and maintenance.

13.5.3. Reezing Methods Of Preserving Mushroom Cultures

Freeze-Drying Method

This is another method of preservation of mushrooms. It is costly and energy-intensive processes in which special machines called as freeze-dryers or lyophilize are used. First of all, mushrooms are cleaned, washed well and there after mushroom is sublimed by slowly raising the temperature under a very low vacuum for 10-12 hours. Freeze-dried mushrooms usually appear very similar to that of fresh mushrooms. These are packed in sturdy containers under inert gas atmosphere. Though the process has failed to maintain its economic viability but recently there is renewed interest in the process.

The most effective methods are Freeze Drying (Lyophilisation) and Freezing and storing in Liquid Nitrogen:

a) Freeze drying

This is the most economical and effective method of long-term preservation of speculating fungi. The spores are frozen and at the same time dried under low pressure in vacuum to remove water from spores. Under this condition the spores remain dormant and viable for a long period.

For freeze drying, the strains are grown on 'plates containing suitable agar medium. Three plugs of the advancing edge of the culture are removed with the 755 help of a 5 mm sterile cork borer and transferred in heavy -walled borosilicate glass ampoules for freeze-drying and storing in liquid nitrogen.

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b) Cryogenic freezing

Freeze drying and freezing are compounds called Cryogenic agents that protect living cells and organisms against damage due to freezing and thawing. The cryopreservative agents prevent the formation of intracellular crystals; reduce electrolyte concentration and cell dehydration due to cooling process. The cryogenic agents are of the following two types:

1. Penetrating cryogenic agents

These agents penetrate the cell membranes and exercise their preserving act in the intracellular and extra cellular environments. Eg.Glycerol and Dimethyl sulfoxide.

2. Non penetrating agents

These do not cross cell membranes. They exercise their protective action by remaining in the extra cellular environment. Eg. Sucrose, Lactose, Glucose, Mannitol, Sorbitol, Dextran and Polyvinyl pyroliodone etc.,

13.5.4. STEPS INVOLVED IN CRYOGENIC PRESERVATION:

1. Preparation of cultures:

The culture is raised on agar slants. At optimum production, the mycelium, slants are flooded with sterilized 10% glycerol or5% Dimethyl sulfoxide (DMSO) and gently scraped to obtain a suspension for freezing.

Glycerol solution is sterilized by autoclaving and DMSO by filtration.

2. Filling and sealing of ampoules:

Suspensions of mushroom cultures are filled in heavy walled borosilicate glass ampoules and pre cooled to 5°C for 30 minutes and then sealed with a semi-automatic sealer.

3. Freezing of cultures:

Ampoules can be frozen by dipping directly into the liquid nitrogen or by controlled freezing procedures. Best results are obtained by slow cooling. The ampoules, placed onto aluminium cans in boxes, are placed in the chamber of the programmed freezer.

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The freezing rate is programmed to cool at 1°C minute to -35°C at which time the temperature is lowered to levels below -100°C. After the ampoules have been frozen, they are immediately transferred to storage in liquid nitrogen at -196°C or liquid nitrogen vapour storage at -150°C to -180°C.

4. Maintenance of culture tubes and mite control:

Mushroom cultures are maintained on a suitable agar medium in glass test tube. The tubes are plugged with cotton wool which allows for satisfactory gas exchange.

Mites are a nuisance in mushroom cultures. They move from one culture to another, carrying with them various fungal spores and bacteria. Acaricides such as Para dichlorobenzene cannot be used to keep mites out because they have adverse effects on the fungal culture. One method of keeping them out is to cover the cotton plugs of test tubes with cigarette paper that allows passage of air but not mite.

Addition of pre sterilized mineral oil (petrol) of technical grade with a specific gravity 0.865-0.890 to the fully grown culture is another way of preventing mite infestation. It should be sterilized in autoclave or pressure cooker for at least half an hour. The cooled oil should be added uptown a depth of 1 cm above the top of the agar slant of fully grown cultures. If oil is added, screw-capped test tubes or bottles should be used instead of test tubes plugged with cotton wool.

Cultures stored in mineral oil can be kept either at room temperature or in a refrigerator. Mushroom cultures can be kept in oil for 1 to 3 years. Viability and original characteristics may be checked after 2 years of storage. Retrieval is carried out by cutting a mycelial plug, draining away as much oil as possible, and transferring the plug to another agar tube.

Addition of mineral oil to the culture also heirs preventing desiccation of agar slants. Alternatively, desiccation can be prevented by sealing the culture tubes with molten paraffin wax or wrapping the cotton wool plug with paraffin

Volvariella cultures do not survive in oil or at temperatures below 10°C. Hence they are kept only at room temperature or in an incubator at 15-20°C. The only long storage method for this mushroom is liquid nitrogen storage. If this is not available, *Volvariella* cultures, stored at room temperatures are simply transferred to new agar media at least once in every 3 - 5 months (sub cultured) or when the agar starts to desiccate. Alternatively, the culture can be re-isolated from selected freshly harvested fruiting bodies of the same line, especially after the original cultures have undergone at least 5 to 8 transfers or show signs of degeneration.

NOTES

Mushroom after harvesting. Is usually consumed, sold or Preserved for consumption or marketing in future. Mushrooms have very short shell life, therefore. efforts should be made to consume or sell them in fresh state as soon as be possible. The produce should not be exposed to the temperature of more than 10^0 C for longer time. Keeping in view the trend of market, produce should be preserved for using and consuming it in various ways. Various methods of preservation of mushroom have boon evolved.

13.5.6. Pickling of Mushrooms

Mushroom pickling is another method of mushroom preservation. This method is gaining popularity as It is the simplest and cheapest method of preservation. This method can easily be practiced by small growers also. Generally, the mushrooms are blanched or fried before normal pickling procedures.Vinegar and other preservatives are generally essential in making the mushroom pickles. Mushrooms preserved as pickles can be stored up to 6-12 months.

13.6.1. Drying of Mushrooms

Drying is the most common method for preservation of the oyster, shiitake and paddy straw mushrooms. These mushrooms are traded mostly in dried form. Drying procedure varies with the scale of operation, financial inputs and customer's requirement. Oyster mushrooms can easily be sun-dried. The sun-dried product should be oven dried at 55 C"-60 for 6 hours before packing in sealed containers. Mechanical driers (electric as well as steam operated) are available in

the market for drying fruits and vegetables and the same driers could be used for drying mushrooms as well.

13.6.2. Vacuum Cooling Method

Principles

Vacuum cooling or evaporative cooling is based on the principle that as liquids evaporate, they absorb heat from their surroundings and evaporation can be increased by vacuum.

Method of vacuum cooling

In order to ensure high quality mushrooms in the market place with enhanced shelf-life, vacuum cooling method is considered to be a safe and suitable method of preservation. In this method, mushrooms must be cooled as quickly as possible alter picking and washing. Thereafter, it is kept cool throughout. Rapid cooling can conserve the quality of mushrooms better than just leaving them in cold storage. Largo stacks of fresh mushrooms can be loaded directly into a cold storage, with little consideration for the time it takes to cool the mushroom at par to the temperature of the store.

13.6.3. Factors affecting vacuum cooling

Following factors are responsible in cooling and maintenance of quality:

1. To maintain proper quality, water in mushrooms should be consented.
2. The rate at which the product is cooled is very important in mushrooms. Therefore, packs of mushrooms should be placed in very cool atmosphere.
3. Packaging and over wrapping usually retard the heat exchange. In the absence of ventilation for heat removal, the mushrooms in the centre of the stack may gel heated.
4. After releasing the vacuum, the mushrooms are kept in conventional stores till its 'marketing. But there is loss of fresh weight during vacuum cooling.
5. Cooling with positive ventilation is another modification where cold air is directed over or through boxed produce. For this, perforated boxes containing the produce are placed. The air is drawn to promote rapid heat exchange.

6. In conventional cooling systems, drying of mushrooms is done and the air is generally dehumidified during refrigeration. Ono method of humidifying air is to pass it through ice-cold water which cools and saturates it with water vapor. Ice is made by conventional refrigeration. Since the produce is coded by water saturated air, there is little loss of moisture. Ice bank cooling with forced ventilation is now becoming common.

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BLOCK-4: STORAGE AND VALUES MUSHROOM

Unit-14: Nutrition – Nutritive value –amino acids, Mineral Elements – Carbohydrates, crude fibre – vitamins – Cost benefit ratio – Marketing in India and Abroad, Export value.

Objectives:

- To study the nutritional values of mushrooms and their cost benefit ratio.
- To know the mushroom marketing value in India and Abroad.
- To know the future scope of mushroom industry in India and its export value.

14.1.1. Nutritional Values Of Mushrooms

Indian diet is primarily based on cereals (wheat, rice and maize), which is deficient in protein. Supplementation of mushroom recipe in Indian diet will bridge protein gap and improve the general health of socio-economically backward communities. Earlier mushrooms were considered as an expensive vegetable and were preferred by affluent peoples for culinary purposes. Currently common populace also considers mushroom as a quality food due to its health benefits.

Mushroom is considered to be a complete, health food and suitable for all age groups, child to aged people. The nutritional value of mushroom is affected by numerous factors such as species, stage of development and environmental conditions. Mushrooms are rich in protein, dietary fiber, vitamins and minerals. The digestible carbohydrate profile of mushroom includes starches, pentose's, hexoses, disaccharides, amino sugars, sugar alcohols and sugar acids. The total carbohydrate content in mushroom varied from 26-82% on dry weight basis in different mushrooms. The crude fibre composition of the mushroom consists of partially digestible polysaccharides and chitin.

Edible mushrooms commonly have insignificant lipid level with higher proportion of polyunsaturated fatty acids. All these resulted in low calorific yield from mushroom foods. Mushrooms do not have cholesterol. Instead, they have ergosterol that acts as a precursor for Vit-

D synthesis in human body. The protein content of edible mushrooms is usually high, but varies greatly. The crude protein content of mushrooms varied from 12 – 35% depending upon the species. The free amino acids composition differs widely but in general they are rich in theronine and valine but deficient in sulphur containing aminoacids (Methionine and cysteine). Mushrooms comprise about eighty to ninety per cent of water, and eight to ten per cent of fiber. In addition to these, mushroom is an excellent source of vitamins especially C and B (Folic acid, Thiamine, Riboflavine and Niacin).Minerals viz potassium, sodium and phosphorous are higher in fruit bodies of the mushroom. It also contains other essential minerals (Cu, Zn, Mg) in traces but deficient in iron and calcium.

14.1.2. Carbohydrates

Coprinus atramentarius contain 24% of carbohydrate on dry weight basis. The mannitol, also called as mushroom sugar constitutes about 80% of the total free sugars. A fresh mushroom contains 0.9% Mannitol, 0.28% reducing sugar, 0.59% glycogen and 0.91% hemicellose. Water soluble polysaccharides of mushrooms are antitumor.

14.1.3. Proteins

Mushrooms are good source of high quality protein. It contains 20-35% protein (dry wt. basis) which is higher than vegetables and fruits and is of superior quality. It is rich in lysine and tryptophan, the two essential amino acids that are in deficient in cereals. It is also called white vegetables or "boneless vegetarian meat". Protein is an important constituent of dry matter of mushrooms. Protein content of mushrooms depend on the composition of the substratum, size of pileus, harvest time and species of mushrooms indicated that protein in *A. bisporus* mycelium rang

ed from to 42% on the dry weight basis. In terms of the amount of crude protein, mushrooms rank below animal meats but well above most other foods including milk. It is reported that mushrooms are very useful for vegetarian because they contain some essential amino acids which are found in animal proteins observed decrease in the protein content of mushroom on storage. Mushrooms in general have higher protein content than most other vegetables and most of the wild plants. Mushrooms contain all the essential amino acids required by an adult.

14.1.4. Fats

Mushrooms are low caloric food with very little fat (4- 6%) and without cholesterol. observed that mushrooms are rich in linolenic acid which is an essential fatty acid. Total fat content in *A. bisporus* was reported to be 1.66 to 2.2/100 g on dry weight basis and it is reported that fat fraction in mushrooms is mainly composed of unsaturated fatty acids.

14.1.5. Vitamins

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Mushrooms are one of the best sources of vitamins especially Vitamin B3, a comprehensive data of vitamin content of mushrooms and some vegetables. Mushrooms also contain vitamin C in small amounts11,42 and which are poor in vitamins A, D, and E3 .

Species	Initial Moisture	Crude protein Nx4.38	Fat	Carbohydrate Fiber		Ash	Energy	Value (K.cal)
				Total	N-free			
Agaricus bisporus (fresh)	89.5	26.3	1.8	59.96	49.5	10.4	12.0	328
Volvariella volvacea (fresh)	88.0	29.5	5.7	60.0	49.6	10.4	4.8	374
Il'olvariella diplosia (fresh)	90.4	28.5	2.6	57.4	40.0	17.4	11.5	304
Lentinula Modes (fresh)	90.0	17.5	8.0	67.5	59.5	8.0	7.0	387
Pleurotusostreatus (fresh)	73.7	10.5	1.6	81.8	74.3	7.5	6.1	367
Auricularia polytricha (fresh)	87.1	7.7	0.8	87.6	73.6	14.0	3.9	347
Flaninnilbia velutipes (fresh)	89.2	17.6	1.9	73.1	69.4	3.7	7.4	378
Pholiota naineko (fresh)	95.2	20.8	4.2	66.7	60.4	6.3	8.3	372
Trcinclla fuciforinis (dried)	19.7	4.6	0.2	94.8	93.4	1.4	0.4	412

14.1.6. Mineral Constituents

The fruiting bodies of mushrooms are characterized by a high level of well assimilated mineral elements. Major mineral constituents in mushrooms are K, P, Na, Ca, Mg and trace elements like Cu, Zn, Fe, Mo, Cd as minor constituents44. K, P, Na and Mg constitute about 56 to 70% of the total ash content of the mushrooms38 while potassium alone forms 45% of the total ash. Mushrooms have been found to accumulate heavy metals like cadmium, lead, arsenic, copper, nickel, silver, chromium and mercury. The mineral proportions vary according to the species, age and the diameter of the fruiting body. It also depends upon the type of the substratum.

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Ess. Amino Acid	***Agaricus bisporus***	***Agaricus edodes***	***Pleurotus florida***	***Pleurotus ostreatus***	***Pleurotus sajorcaju***	***Volvereilla volvacea***
Leucine	7.5	7.9	7.5	6.8	7.0	4.5
Isoleucine	4.5	4.9	5.2	4.2	4.4	3.4
Valine	2.5	3.7	6.9	5.1	5.3	5.4
Tryptophan	2.0	-	1.1	1.3	1.2	1.5
Lysine	9.1	3.9	9.9	4.5	5.7	7.1
Threonine	5.5	5.9	6.1	4.6	5.0	3.5
Phenylalanine	4.2	5.9	3.5	3.7	5.0	2.6
Methionine	0.9	1.9	3.0	1.5	1.8	1.1
Histidine	2.7	1.9	2.8	1.7	2.2	3.8
Total Essential	38.9	36.0	46.0	33.4	37.6	32.9

All edible mushrooms are good sources of selenium. Selenium may also be an anti-cancer substance since it has been proven to reduce the risk of prostate cancer.

Potassium: An extremely important mineral that regulates blood pressure and keeps cells functioning properly. Mushrooms are a good source of potassium.

Major vitamin & Minerals	Daily requirement	Mushroom content

Thiamine (B- 1)	1.4 mg	4.8 - 8.9 mg
Riboflavin (B-2)	1.5 mg	3.7-4.7 mg
Niacin	18.2 mg	42-108 mg
Phosphorus	450 mg	708-1348 mg
Iron	9 mg	15-17 mg
Calcium	450 mg	33-199 mg
Copper	2 mg	12-22 mg

14.1.7. Fiber

Mushroom contains good quality fibre. It helps in lowering the cholesterol. Fresh mushrooms contain both soluble and insoluble fiber. The soluble fiber is mainly beta-glucans and chitosans, which are components of the cell walls. Soluble fiber has been shown to help prevent and manage cardiovascular disease by lowering total and LDL cholesterol levels. It also helps regulate blood sugar levels. So, mushrooms are good for health because it contains zero Fat, low Calories, low Carbohydrates, low Sodium and no Cholesterol.

14.2. Cost Benefit Ratio

Understanding of economics of mushroom cultivation depending on the availability of man power, infrastructure, raw materials and market, planning for a small, medium or large unit maybe chalked out, yet it is crucial to determine the optimum output level which makes a farm viable. The economics of mushroom cultivation vary across regions and also have a bearing on the benefit-cost ratio. Keeping in view all these aspects, with the following objectives:

- To analyze the cost, returns and break-even point of mushroom production on different categories of farms and
- To study the existing marketing system along with marketing cost, margins and marketing efficiency.
- The returns were calculated based on the actual prices received by the growers. The return over variable cost, return over material cost and net returns were calculated by deducting the

respective cost from the gross returns.

- Break-even point (BEP) of output was calculated by using the following formula(1)

- BEP = TFC/ (ASP–AVC) …(1)

Where,

- TFC = Total fixed cost,
- ASP = Average sale price of mushroom (Rs/kg), and AVC = Average variable cost (Rs/kg)
- For estimating marketing cost and margins through major channels in the study area, the data collected from different marketing function arise were analyzed.

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The important channel so for mushroom marketing in the study area were

(1) Mushroomgrower/Wholesaler/Commission agent/Retailer/Consumer,

(2) Mushroomgrower/Wholesaler/Commission agent/Consumer,

(3) Mushroom grower/Retailer/Consumer, and

(4) Mushroom grower/Consumer.

Marketing efficiency was calculated by using formula (2):

$$\text{Marketing efficiency} = \frac{\text{Value of produce per kg}}{\text{Marketing cost per kg}} - 1 \quad \ldots (2)$$

Higher the ratio, higher is the efficiency and vice versa.

14.3MUSHROOM MARKETING

14.3.1. Introduction

Mushrooms have been recognized by Food and Agriculture Organization (FAO) as food item contributing to the protein nutrient to the diet of developing countries like India, where there is heavy dependence on

cereal diets. The significant feature of mushroom is that this nutritious and tasteful food is cultivated entirely from waste products and converts a wide spectrum of agricultural and industrial waste into substrate on which the growth of mushroom is supported. After harvesting the mushroom, the solid residual left is organic compost with natural nutrients to further enrich the soil. In addition to converting the waste into valuable product, it enhances the income and provides additional gainful employment to the producers. Keeping in view the increasing demand of mushroom due to globalization and opening of the economy. Thus, this is to analyze the current scenario of the mushroom industry.

14.3.2. Global Scenario

Mushroom industry globally has expanded both horizontally and vertically, meaning that the expansion has been in production and addition of newer types of mushrooms for commercial cultivation, both edible and non-edible mushrooms. Today China is leading in global mushroom production both in cultivation of edible and non-edible types. China produces approximately 70 percent of world mushroom production and mushroom is their sixth economically important crop as far as country's revenue generation is concerned. The second highest mushroom producing country is USA, followed by some European countries. European production is confined to France, Germany, Holland, Italy and other countries in western-Europe. There is a matching contribution in mushroom production in Eastern European countries like Hungary and Poland where mushroom production has received a boost as can be seen from the production figures available and mushroom activity in these countries.

14.3.3. Mushroom Marketing In India

Though mushroom cultivation, both in east and west started many centuries ago, yet its cultivation in India is of recent origin. Paddy straw mushroom cultivation was first attempted in India at Coimbatore in 1943 by Thomas and his associates. However, first systematic attempt in cultivating button mushroom was made in 1961, when a scheme entitles "Development of Mushroom Cultivation in Himachal Pradesh" was started at Solan by H.P. Government in collaboration with ICAR, New Delhi. In the late sixties, few progressive growers in H.P. and Jammu and Kashmir started growing button mushroom on commercial scale viz Teg's Mushroom (Chail, H.P.), Saigal Mushroom farm (Kasauli, H.P.), Harco's (Srinagar, Kashmir) and Col. Kak's mushroom Farm (Srinagar, Kashmir) (Dhar, 1997) . In early seventies mushroom cultivation started spreading to other hilly regions of Uttar Pradesh and Tamil Nadu. By late

seventies and early eighties, mushroom cultivation was adopted by farmers in Haryana, Punjab and Uttar Pradesh in areas around Delhi, as a seasonal crop in a big way Delhi and Bombay together formed the big market, for fresh mushroom and could utilize more than 10 to 12 tons of fresh mushrooms per day. In the significant development in mushroom cultivation one of the biggest units in the country is located at Madras, a coastal city in Southern Tropics. Now mushroom cultivation is also picking up in the states of Maharashtra, Tamil Nadu, Karnataka and Andhra Pradesh as a result of adoption of technology of mushroom production under controlled conditions. The present status of mushroom production in various regions of the country is very result oriented with encouraging figures. Many export-oriented units are being put up by corporate houses/industrialists, throughout the country with use of advanced technology and machinery for mushroom growing. In the last 10 to 12 years, the mushroom production in India has increased many folds and present mushroom production stands at 50,000 tons

14.3.4. Scenario of Mushroom Cultivation In Different States

Till 1980, cultivation of white button mushroom was confined to the Northern Hill States of Jammu and Kashmir and Himachal Pradesh. However, there has been a remarkable change in its scenario and it has now spread its wing, all over the country from Jammu and Kashmir in north to west Bengal and North East to East Mushroom production in H.P. alone has crossed 8000 tons mark since the establishment of two Commercial/Export Oriented Unit at Paonta Sahib and Nalagarh, each producing more than 3000-3500 tons per annum. Seasonal mushroom cultivation is confined to Solan, Shimla, Kanga and other cooler region in the state. In Punjab,Several cold storage is reported to have been converted in to mushroom growing units. One such large Export oriented Unit is located at lalru with annual production of more than 75% of total mushroom production comes from seasonal growers concentrated near Sonepat. Medium sized commercial units are located near Gurgaon, Panipat, and Kalka and Hisser districts with total production of about more than 8000 tons of fresh mushrooms. With the creation of Uttaranchal, from Uttar Pradesh, the mushroom activity is now confined to seasonal growing in the areas, particularly Saharanpur, Agra, Ghaziabad, Aligarh, Lucknow and some other places. The mushroom growing in centralU.P.is confined to a few units at Allahabad and Kanpur with no activity in eastern part of state. The present total productions units at 2000-2500 tons only in the states of Uttaranchal, oyster mushroom cultivation is emerging as one of the leading cottage industry in and around Dehradun.

In Madhyapradesh, besides, some commercial units in Bhopal and Indore, M.P. Agro Industries Corporation are endeavoring to popularize mushroom cultivation in the state. With the introduction of new state of Chhattisgarh, Oyster mushroom cultivation is being taken up in the tribal area particularly in and around Raipur, with total annual production of more than 1500 tons estimated. In Rajasthan and Gujarat, button mushroom cultivation is still confined to experimental level; however oyster mushroom is being cultivated by some mushroom growers. The mushroom cultivation activity in the state of Maharashtra is confined to Mumbai and Pune, with total annual production estimated around 8000 tons, while annual production of mushroom in Goa is around 12-15 tons produced mainly by one big Export oriented Units, based at Panaji Darjeeling hill is main centre of mushroom production in West Bengal.

Industrialists and entrepreneurs of Southern and Western States of Tamil Nadu, Karnataka, Kerala, Andhra Pradesh and Mah arashtra are adopting mushroom cultivation on large scale. The main mushroom producing centers are Bangalore, Hyderabad, Pune, Chennai, Munnar, Ooty and Coimbatore With the concentrated efforts of research workers and interested growers, there has been a considerable increase in mushroom production particularly in the last one decade.

Mushroom production, which was estimated at 100 tons in 1970, 400 tons in1975, 1000 tons in1986, 7000tons in 1990, 12000 tons in 1992, 25,000 tons in 1993 and more than 50,000 tons at present. However this production is negligible, considering the world mushroom production of over 5 million tons mostly contributed by Europe (55%), North America (27%) and Eastern Asia (14%). At present, white button mushroom is very popular among growers in India, contributing more than 85% of total production closely followed by Oyster mushroom which is being cultivated in tropical and sub-tropical regions. Paddy straw has not made any further progress because of poor and unpredictable yield.

14.3.5. Reason For Slow Progress Of Mushroom Industry In India

The retardation of progress is due to the following reasons:

1. Non-availability of funds.
2. Poor harvest management and marketing
3. No serious efforts have been made in popularizing other edible mushrooms in spite of abundant availability of raw materials, cheap labour force and suitability of

agro- climatic conditions.

4. No serious efforts have been made for collection and subsequent evaluation of locally available germplasm suitable for various agro-climatic conditions.

5. Use of unpasteurized compost widely prevalent with small growers.

6. Utilizing locally available substrates for compost preparation has not been fully explored.

7. Pasteurization technique for compost and casing need to be refined.

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8. Technology for successful cultivation of Oyster and Paddy straw Mushroom needs to be properly standardized.

9. Serious efforts need to be made to evolve cheap production technology for other edible mushrooms.

10. Research is required with respect to the right stage of picking, grading and preservation.

11. Practical training is necessary to create right environment as well as awareness to properly learn the art of mushroom growing.

14.3.6. Mushroom Marketing In Abroad

Fresh and processed button mushrooms and fresh specialty mushrooms are produced and consumed in many countries. Fresh mushrooms are perishable, so their global movement often has been restricted to transactions mainly between neighboring countries. The movement of fresh mushrooms on a global scale increased lately but canned mushrooms are shelf stable, with a shelf life of two to three years and thus, are the major mushroom product traded globally. Global mushroom production amounted to 3.4 million tons in 2007; trending steadilyupwardfrom2003.China remained the leading global producer of mushrooms for all uses and has been for the past five years. Since 2005-06, the Chinese national government increasingly encouraged to shift their agricultural production out of traditional crops to value-added crops like mushrooms for processing. The United States and the EU countries

were the second and third largest global producers, respectively, in 2007, other important global producers included Canada, Japan, India, Australia, and Indonesia. Countries showing noticeable increases in production included China, Spain, Poland, and Ireland. The production in most of the remaining countries decreased slightly or remained almost the same.

14.7. Export Value

Introduction

In the face of all major constraints, face mushroom industry, the current Indian set-up is quite encouraging with an overall increase in production by 5 to 6 times. During the last one decade, estimated production is likely to cross 50,000 tons of all types of mushrooms. However, this is very small quantity if the vast market potential of this large country is to be fully exploited. Mushroom industry has a bright future in India, chiefly because of large quantity of agro- byproducts and agro-waste generated, as well as availability of large and cheap labour force. This will not only provide a gainful employment to our rural youths, but cost of mushroom production per unit area will be greatly reduced. The educated unemployed will be tempted to adopt mushroom cultivation as their profession, by creating awareness about this'healthfood'. With the establishment of mother compost unit and spawn laboratories in different agro-climatic zones of our country, the techniques required for cultivation of mushroom are readily available now.

In 2007, global consumption amounted to 3.3 million tons and china, the euro countries and the United States were the leading global consumers of mushrooms. Other major consumers included Canada, Japan, Russia, Australia and india.Virtually all consumption in China, and India was supplied from domestic production. On the other hand, practically all Russian consumption was supplied by imports. Finally, consumption in the United States, Canada, Japan, and Australia met mostly by domestic production but also by significant quantity of imports.

Global exports of canned mushrooms amounted to 458,137 tons in 2008,up by 25 per cent from 365,967 tons in 2004,with China accounting for 87percent of total export volume in 2008 and for nearly all the rise in global exports during the report period. The increase in exports from China through 2008 resulted from a fall in freight rates from China to most global markets in 2007–08. Other major global exporters in 2008 included Indonesia and India, although export levels from Indonesia remained almost the same throughout the 2004–08 period and exports

from India during the same period were down because of intense competition from Chinese exports. Global exports of fresh mushrooms averaged around 43,730 tons during 2004–07 before falling to 34802 tons in 2008. Canada and the United States were the largest global exporters of fresh mushrooms in 2008, together accounting for nearly 80 per cent of the total, with most exports from both countries shipped to each other. Other major exporters in 2008 were Malaysia and Mexico. Most of the fall in exports of fresh mushrooms from 2007 to 2008 was accounted for by a drop in exports from China, where a greater share of fresh mushroom production was processed and mushroom growers in China switched into production of other crops.

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Exports from traditional supplier Canada also fell following a decision by Canadian shippers to concentrate in their home market as a result of an unfavorable change in the US-Canada exchange rate. Global imports of canned mushrooms amounted to 292,267 tons in 2008, up by 12 per cent from 260,944 tons in 2004, with the United States and Russia accounting for the largest individual shares of total import volume in 2008.Global imports of fresh mushrooms amounted to 90,879 tons in 2008, up by 42 per cent from 63,618 tons in 2004. Russia and the United States together were the most important global import markets in 2008 Canada,Norway,Malaysia and Ukraine were other major markets. The rise in imports from 2004 to 2008 was due to arise in Russian imports with Russia becoming the primary market for Chinese mushrooms in 2007 and2008.