

**MAHATMA GANDHI UNIVERSITY**  
**PhD course work in Botany**  
**Course II - Biological techniques**

**Unit 1: Principles of Plant Biological and physical chemistry**

Buffers, pH, pH electrodes, Solutions and methods of expression of concentration of solutions. Henderson- Hasselbalch equation and Iso-electric point of enzymes and proteins. Enzymes- Different types of enzymes- enzyme regulation, enzyme catalysis, isolation, purifications and storage of enzymes. Water relations, Osmosis and membrane transport principles in plants. Photosynthesis and photo-bioreactors. Allelopathy, Phyto allexins and quorum sensing in plants. Types of induced plant regulations- Vernalization, flower induction, fruit modifications and fruit ripening. Plant derived medicines- general methods of isolation, purification, identification and estimation of phyto-constituents.

**Unit 2: Analytical and botanical separation techniques**

Plant secondary metabolites, analysis, isolation and purifications. Plant Lipids, proteins, amino acids, nucleic acids and pigments. Separation of Biomolecules and secondary metabolites by cold and heat methods, precipitation, salting out, dialysis and by using organic solvents. Separation by Chromatographic techniques- Paper, TLC, Column, Gel filtration, affinity, ion exchange, HPLC, HPTLC, Gas chromatography, Gas Liquid Chromatography, GCMS, LCMS, LC-TOF-MS (Liquid chromatography time-of-flight mass spectrometry). Separation by centrifugation methods- Differential centrifugation, relative centrifugation forces, density gradient centrifugation, low speed and high speed cooling ultracentrifuges. Structural elucidation, quantitative and qualitative analysis of Biomolecules by light and UV-spectroscopic analysis, BOMB Calorimeter, FTIR, ATR, AAS, ICPMS, EDS, NMR, X-Ray crystallography, Multi plate reader, ELIZA reader, Amino acid analyzer and Flow cytometry. Analysis and isolation of Biomolecules by electrophoresis techniques- Agarose, AGE, 2D gel electrophoresis, 3D gel electrophoresis, SDS and native PAGE. Lyophilization of samples.

**Unit 3: Molecular and Microscopic techniques**

Plant DNA and RNA isolations, mitochondrial and chloroplast genome isolations, competent cell preparations, transformations, restriction digestion, ligation and expression of plant gens. PCR techniques and types, sequencing of DNA, RNA and amino acids. Molecular markers- Plant based molecular markers, RFLP, RAPD, AFLP, SCAR, SNP and SSR. Blotting techniques- Western, southern and Northern blotting. Microscopy- Microscopes, Numerical aperture, magnifications, bright field microscopy, Phase contrast, fluorescence, confocal, interference and polarization microscopes. Electron microscopes and specimen preparations. Microscopic specimens, plant anatomical specimen preparations by microtome techniques- taxonomic

techniques- chemotaxonomy, numerical taxonomy, molecular taxonomy and DNA bar-coding. Molecular characterization and identification of new organisms.

#### **Unit 4: Environmental analysis**

Soil and water sampling techniques- Soil chemical analysis- Extraction of metals, phyto remediation techniques- Microwave digestion- soil microbiological studies- Endophytic fungi, AM fungi types, plant relationship of AM fungi and spore counting. Multi parameter analysis of water samples. Water chemical analysis- Aquatic biological techniques. Environmental radioactivity analysis, radioisotopes, radio labeling of samples, detection methods, half life periods and safety guidelines. GIS and remote sensing application in plant diversity.

#### **Unit 5: Microbial, Plant and Animal Tissue culture techniques**

Plant and soil microbes interactions, isolation of microorganism from the soil, water and environment. Identification of organism by staining techniques and phylogenetic analysis, Cultivation of soil microorganisms, AM fungi, Edible mushrooms and algae. Storage and transportation of samples for experiments, Safety guidelines and ethics. Aseptic culture and sterilization techniques. Antibiotic, antifungal and anti bacterial tests of plant samples. Diffusion and dilution techniques. Immunological tests: Agglutination and precipitation reactions, immunomodulatory and suppressive effects, radio immunoassay and immunofluorescence. Plant tissue culture techniques: culture units, green house technology, aseptic conditions and culture media. Different types of plant tissue culture methods- protoplasm culture, embryo, leaf, anther and root culture methods. Plant transformations and identifications. Animal cell culture techniques: Culture conditions, sterilizations, media types and cultivation protocols. Histological techniques: nitric oxide scavenging assay, *in vitro* study of antioxidants, free radical scavenging, super oxide scavenging, peroxide scavenging assays. Transgenic animals and CPCSEA guidelines.

### **Exercises related to Course- II**

**Seminar:** Each student must present a seminar on biological techniques which he/she will be using in his/her research

**Assignment:** Each student must submit an assignment on a biological technique as per the directions of the course coordinator

### **References**

1. APHA, 1998. Standard methods for the examination of water and waste water. American Public Health Association, 1015 fifteenth Street, NW, Washington, DC.
2. Sharma BK (2000) Instrumental Methods of Chemical analyses.

3. Khandpur RS (2004) Handbook of Biomedical Instrumentation, Tata Mc Graw Hil
4. Glick BR and Psternak JJ (1998) Molecular Biotechnology: Principles and applications of recombinant DNA, ASM Press
5. Brigal L W (1998) A Biologist Guide to Principles and Techniques of Practical Biochemistry
6. Trivedy, R.K and P.K. Goel (1986) Chemical Biological methods for water pollution studies, Environmental publications, Karad 415110, India
7. Monica Cheesbrough (2005) Medical Laboratory Manual for tropical countries, Vol 2, LBS
8. Mackie and Mc Cartney (2002) Practical Medical Microbiology, Churchil- Livingstone
9. Dubey RC and Maheswary DK (2002) S Chand and Company Ltd., New Delhi
10. Sambrook J and Russel D (2001) Molecular Cloning, A Laboratory Manual, 3<sup>rd</sup> Edition, Cold Spring Harbor, New York
11. Widmaier Raff and strang (2005) Vander's Human Physiology- the mechanism of body function, McGraw- Hill, New York
12. Ronald Danley Gibbs (1974) Chemotaxonomy of flowering plants. Volume 1 and 2, Betterworldbooks, New York
13. Peter H. A. Sneath (1973) Numerical Taxonomy: The principles and practice of Numerical Classification, W H Freeman & Co
14. Z. Deyl (1984) Separation methods, Vol8. Elsevier pbl. Amsterdam- New York- Oxford