

BHARATHIAR UNIVERSITY::COIMBATORE – 46
M.Phil / Ph.D BIOTECHNOLGOY
From October 2011 batch onwards
PAPER-I RESEARCH METHODOLOGY

UNIT I: INTRODUCTION TO RESEARCH METHODOLOGY

What is research? Basic and applied research, Essential steps in research, Defining the research problem, Research/Experimental design, Literature collection, Literature citation, Research report: components, Format of thesis and dissertation, Manuscript/research article, Review monographs, Bibliography and Reference, Significance of research.

UNIT II: BIOPHYSICAL METHODS

Analysis of biomolecules using UV/Visible, fluorescence, circular dichroism, NMR and ESR spectroscopy, structure determination using x-ray diffraction and NMR; Analysis using light scattering, different type of mass spectrometry and surface plasma resonance methods.

Radiolabeling techniques: Properties of different types of radioisotopes normally used in biology, their detection and measurement; incorporation of radioisotopes in biological tissues and cells, molecular imaging of radioactive material safety guidelines.

UNIT III: MICROSCOPIC TECHNIQUES

Visualization cells and subcellular components by light microscopy, resolving powers of different microscopes, microscopy of living cells, scanning and transmission microscopes, different fixation and staining techniques for EM, freeze-etch and freeze-fracture methods for EM, image processing methods in microscopy.

Histochemical and Immunotechniques: Antibody generation, detection of molecules using ELISA, RIA, western blot, immunoprecipitation, flowcytometry and immunofluorescence microscopy, detection of molecules in living cells, *in situ* localization such as FISH and GISH.

UNIT IV: ELECTROPHYSIOLOGICAL METHODS

Single neuron recording, patch-clamp recording, ECG, Brain activity recording, lesion and stimulation of brain, pharmacological testing, PET, MRI, fMRI, CAT.

Computational methods: Nucleic acid and protein sequence database; data mining methods for sequence analysis, web-based tools for sequence searches motif analysis and presentation.

UNIT V: STATISTICAL METHODS

Measures of central tendency and dispersal; probability distributions (Binomial, Poisson and normal); sampling distribution; difference between parametric and non-parametric statistics; confidence interval; errors; levels of significance; regression and correlation; t-test; analysis of variance; X^2 test; basic introduction to Multivariate statistics, etc.,

Reference

1. C.R. Kothari, 2nd edition (2004) Research methodology, Methods and techniques, New Age International (P) Ltd, Publishers, New Delhi.
2. Jerrod H. Zar (1999) Biostatistical analysis by, Prentice Hall International, Inc. Press, London.
3. Attwood. T.K and Parry-Smith D.J. (2002) Introduction to Bioinformatics, Pearson education Singapore.
4. Sharma.BK. Instrumental methods of chemical analysis.
5. Upadhyay, Upadhyay and Nath, Biophysical chemistry.
6. Khandpur R.S. Handbook of biomedical instrumentation, Tata Mc Graw Hill.
7. Bragal.L. Williams, A biologist guide to principle and techniques of practical biochemistry.

PAPER-II RECENT TRENDS IN BIOTECHNOLOGY

UNIT I: BASIC TECHNIQUES IN MOLECULAR BIOLOGY

Isolation and purification of RNA , DNA (genomic and plasmid). Analysis methods for RNA, DNA. Enzymes in recombinant DNA technology. Genomic and cDNA library construction and screening. DNA sequencing methods, strategies for genome sequencing.

UNIT II: CLONING IN MICROORGANISMS AND HIGHER ORGANISMS

Vectors in Molecular Biology. Molecular cloning of DNA or RNA fragments in Prokaryotic (E.coli, Bacillus) and eukaryotic systems - plasmid, phage, cosmid, BAC and YAC vectors. Direct and indirect gene delivery techniques. Gene transfer in plants, Expression of recombinant proteins using bacterial, animal and plant vectors. Fusion proteins.

UNIT III: GENETIC MARKERS IN MOLECULAR BIOLOGY

DNA fingerprinting, RFLP, RAPD and AFLP techniques. Somatic cell nuclear transfer, Polymerases chain reaction types and applications, DNA footprinting, Site directed mutagenesis.

UNIT IV: APPLICATIONS OF MODERN TECHNIQUES

New drugs/ Therapies for genetic diseases- combating infectious disease. Metabolic engineering, DNA vaccines. Protein sequencing methods, detection of post translation modification of proteins. Identification of protein by MALDI-TOF, yeast two and three hybrid system-phage display

UNIT V: ADVANCED TECHNIQUES IN BIOTECHNOLOGY

Microarray, Chromosome Engineering, Targeted gene replacement. Current status of stem cell research. Gene Knockout, Gene Silencing techniques (Antisense technology & RNAi).

Reference:

1. Bowtell, D and Sambrook, J. DNA Microarrays: A Molecular cloning manual. CSHL press
2. Glick, BR., Pasternak, JJ (1998) Molecular Biotechnology: Principles and Applications of recombinant DNA, ASM Press.
3. Grandi ,G (2004) Genomics, Proteomics and Vaccines. Wiley press.
4. Hannon, GJ, RNAi: A guide to gene silencing. CSHL Press
5. Kirby, LT (1990) DNA finger printing: An introduction, Stockton press.
6. Lewin, B (2004). Genes VIII. Pearson P- rentice Hall Press
7. Principles of genetic manipulation; Ed. Old and Primrose, 6th Edition. Blackwell Science publication

PAPER-III: ELECTIVE
I. ANIMAL BIOTECHNOLOGY

UNIT I: BASICS OF ANIMAL CELL CULTURE

Introduction to animal tissue culture: culture media; Serum and protein free defined media and their applications. Balanced salt solutions and simple growth medium: Physical, Chemical and Metabolic functions of different constituents of culture medium; Role of carbon dioxide, serum, growth factors, glutamine in cell culture; Types of cell culture: primary and established culture; organ culture and three dimensional culture feeder layers; cell synchronzation; cryopreservation.

UNIT II: MOLECULAR TECHNIQUES IN ANIMAL CELL CULTURE

Biology and characterization of cultured cells: tissue typing; cell-cell interaction; measuring parameters of growth; measurement of cell death; Apoptosis and its determination; cytotoxicity assays. Cell transformation: physical, chemical and biological methods; Cell cloning and micromanipulation. Production and application of animal cell culture products.

UNIT III: STEM CELL BIOLOGY AND TISSUE ENGINEERING

Stem cells: characteristics and classification; stem cell niche; stem cell culture; stem cell markers. ES cells, EG cells; Adult stem cells: HSC, MSC, NSC, UCBS cells, iPS cells. Stem cell banking. Therapeutic applications: Neurodegenerative disorders, diabetes, cardiac disorders. **Tissue Engineering:** Principles of tissue engineering; biomaterials in tissue engineering; tissue engineering bioreactors. Applications: bioartificial organs-regeneration of bone, liver, epidermis and bladder. Ethical issues in stem cell research and tissue engineering.

UNIT IV: HUMAN GENETICS

Pedigree analysis; Inheritance pattern: Autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive, Y-linked inheritance. Genetic diseases due to aneuploidy: Klinefelter's syndrome, Turner's syndrome, Down's syndrome, Edward's syndrome, Patau's syndrome; Biochemical genetics: Disorders of purine and pyrimidine metabolisms, galactosemia, hyperphenylalaninemia, lysosomal storage disorders; Monogenic disorders: cystic fibrosis, Haemophilia A & B, sickle cell anaemia; Multifactorial diseases: Diabetes, Rheumatoid arthritis; Cancer genetics: tumor suppressor genes, oncogenes, DNA repair genes. Gene mapping; Gene therapy; Human genome project.

UNIT V: IMMUNITY IN HEALTHCARE AND TRANSGENICS

Immunity in healthcare: production of interferons, interleukins and vaccines through mammalian cell cultures. Hybridoma technology and its applications. Tumor immunology; transplantation immunology; autoimmune disorders; immunity to infectious agents. Transgenics: Transgenic animal production and applications- transgenic animals as models for human disease; transgenic animals in live- stock improvement; transgenics in industry; Ethical issues in animal biotechnology.

References:

1. Animal cell culture; A practical approach, 4th Edition by Freshney, R.I. John, Wiley Publications
2. Methods in cell biology; Volume 57, Animal cell culture methods, d. Jennie P, Mather, David Barnes, Academic press
3. Mammalian cell biotechnology; A practical approach, Ed, M. Butler Oxford University press
4. Human genetics by A. Gardner, R.T. Howell and T.Davies, Scion publishing ltd. UK
5. Applied Genetics Recent Trends and Techniques, C.Emmanuel, Rev. Fr. S. Ignacimuthu S.J, S. Vincent, MJP publishers, Chennai.
6. Animal cell culture, a practical approach; Ed. John R.W. Masters, Third edition, Oxford University Press.
7. In Vitro cultivation of animal cells; Published on behalf of Open University Alkenburgerrweg 167, 6401DL Heerlen Nederland and University of Green wich (Formerly Thales Polytechnic) Averu Jo; Rpad. E,tja.' pmdpm SE9 zHB, United Kingdom
8. Immunology by Janus Kuby, 4th edition (Freeman).
9. Fundamentals of Immunology edited by William Paul. 4th edition (Lippincott Rave)
10. Essentials of stem biology-Robert Lanza, John Gearhart, Brigid Hogan
11. Principles of Tissue Engineering – Robert Lanza

PAPER III: ELECTIVE
II. PROTEIN AND ENZYME BIOTECHNOLOGY

UNIT I: STRUCTURE AND FUNCTION OF PROTEINS AND ENZYMES

An overview on amino acids and proteins. Protein structure –Primary, secondary, tertiary and quaternary structure. Naming and classification of enzymes. Amino acids - the building blocks of proteins, the basis of protein structure. Types of specificity. The active site, Fischer 'lock-and-key' hypothesis, Koshland induced-fit hypothesis, hypotheses involving strain or transition- state stabilization. Monomeric enzymes and oligomeric enzymes. Isoenzymes, ribozymes and abzymes. The identification of binding sites and catalytic sites. The investigation of the three dimensional structures of active sites.

UNIT II: KINETICS AND MECHANISMS OF ENZYME CATALYSED REACTIONS

Some concepts of bioenergetics, Denaturation and renaturation of proteins, factors affecting the rates of chemical reactions, kinetics of single-substrate and multi-substrate enzymes catalysed reactions. Allosteric enzymes and sigmoidal kinetics. Inhibition – reversible and irreversible inhibition. Mechanisms of catalysis - enzymes without cofactors and metalloenzymes. The involvement of coenzymes in enzyme catalysed reactions.

UNIT III: PURIFICATION, CHARACTERIZATION AND APPLICATION OF PROTEINS AND ENZYMES

Extraction and purification of enzymes (Chromatography, Zymogram and 2D Electrophoresis). Identification and determination of molecular weights of enzymes - MALDI-TOF-MS. Prediction of 3D structure of proteins – X-ray crystallography, Investigation of sub-cellular compartmentation of enzymes. Immobilized enzymes - application in industry and medicine. Protein engineering – increasing the biological activity. Drug development - Prodrug delivery using enzymes. Studying Protein-DNA Interaction - EMSA, DNA microcircles.

UNIT IV: COMPUTATIONAL TOOLS AND TECHNIQUES

Predicting protein structure from sequences - Homology Model Building, Secondary Structure Prediction, Fold Recognition, *ab initio* method. Energy minimizations and optimization Validation of models (WHATIF, PROSA, PROCHECK, VERIFY 3D); Protein Structure Alignment – Modeler; Protein Folding; Structure based Drug Design -Molecular Docking, De Novo Ligand Design, Virtual Screening; Ligand based Drug Design - Pharmacophore Identification, QSAR; Molecular Dynamics with Gromacs; Binding Site Identification; ADMET: basic principles. Protein – Ligand binding energy prediction.

UNIT V: Structural Bioinformatics

Protein sequence and structure databases; The Structure Classification of Proteins (SCOP); Class, Architecture, Topology, Homology (CATH) Classification; BLAST algorithm & BLAST online tools; Multiple Sequence Alignments with ClustalW & Protein Analysis (Pfam, Prosite, Prints, Blocks); Protein arrays - Computational methods for identification of polypeptides from mass spectrometry; Bioinformatics-based tools for analysis of proteomics data (Tools available at ExPASy Proteomics server); InterPro database and analysis tools; Protein-protein interaction tools: DIP, PPI server.

References:

1. Trevor Palmer and Philip Bonner, Enzymes: Biochemistry, Biotechnology, Clinical chemistry, East-West Press, New Delhi.
2. L. Lehninger, Principles of Biochemistry, 4th Edition.
3. A.Travers & M. Buckle (2004), DNA- Protein interaction, Oxford University Press, India edition.
4. A.J. Kirby and F. Hollfelder (2009), From Enzyme Models to Model Enzymes, RSC Publishing, UK
5. Phil Bourne and Helge Weissigon (2009), Structural Bioinformatics, John Wiley and sons, New Jersey
6. Andreas D. Baxevanis B. F. Francis Ouellette (2009), Bioinformatics - A Practical Guide to the Analysis of Genes and Proteins, Second Edition , John Wiley & Sons, Inc, New Jersey.

PAPER-III: ELECTIVE
III. MICROBES IN BIOTECHNOLOGY

UNIT I: MICROBIAL DIVERSITY

Classification: Bacteria, Actinomycetes, Archaea, Fungi, Viruses - Bergey's system of classification - Molecular techniques for classification - Biochemical, microbiological, Chemotaxonomic parameters (Peptidoglycan, Isoprenoid quinones, Fatty acids), 16S rRNA gene sequencing, construction of phylogenetic tree, G +C analysis, DNA-DNA hybridization. Recent trends in Diversity studies: Rapid identification systems (MALDI) - Culture independent approach: 16S rRNA gene libraries - Microbial Community analysis (DGGE, TGGE, SSCP, T-RFLP, FISH)

UNIT II: BIOPROCESS TECHNOLOGY

Introduction to Bioreactors - Types: Batch, continuous and fed batch; Solid-state and submerged; Aerobic and anaerobic; Specialized reactors (fluidized, immobilized cell air-lift). Bioreactor operations: Sterilization - Aeration - Sensors - Instrumentation - Analysis of mixed microbial populations. Optimisation of conditions: Screening of factors- Plackett Burman design. Fractional factorial design, Pareto chart; Optimization of factors - Response Surface methodology.

UNIT III: DOWN STREAM PROCESSING

Separation of cells - flocculation, filtration (plate filters, cross flow filtration, rotary vacuum filters) - Sedimentation - Centrifugation (tubular bowl, disc-bowl, basket bowl) - Disintegration: mechanical and non-mechanical - Filtration: membrane filtration, ultra filtration, reverse osmosis - Extraction: two phase, organic solvents, salts - Chromatography: adsorption, ion-exchange, gel affinity - Drying: Cabinet, vacuum shelf, spray driers, drum driers, freeze driers - Crystallization (Principles and Equipment).

UNIT IV: MICROBES IN PHARMACEUTICAL AND FOOD INDUSTRIES

Microbial production of: Enzymes, Antibiotics, Vitamins (B12, B2), Organic acids (citric acid), Alcohol (ethanol), Organic solvents (acetone, butanol), Amino acids, beverages (brandy, whisky, rum), as medicine, biopolymer. Milk products (Cheese, yoghurt) - Edible mushrooms (Oyster, paddy-straw, button), SCP (Spirulina), microbial supplements (Lactic acid bacteria).

UNIT V: MICROBES IN AGRICULTURE AND BIOREMEDIATION

Biofertilizers-Types: Nitrogenous (Symbiotic, Non-symbiotic), Phosphate solubilizers - Biopesticides: *Bacillus thuringiensis*, *Pseudomonas*, Viruses. Xenobiotics- microbial mechanism, microbial mining, ore leaching - Solid waste management (composting)- vermicomposting- biofuel (Algae)- oil spill remediation- Wastewater treatment: primary, secondary and tertiary (Biological), heavy metal removal- Steroid biotransformation.

REFERENCES:

1. Microbial Biotechnology – Fundamentals of applied Microbiology by A.N. Glazer and H. Nikaido. W.H. Freeman and company.
2. Principles of Fermentation Technology, P. F. Stanbury & A. Whitaker, Pergamon Press.
3. Microbial process Development H.W Woelle, World Scientific.
4. Biotechnology Text book of Industrial Microbiology by W. Creuger and A. Creuger. Industrial Microbiology by Casida, John Wiley and Sons Inc.
5. Industrial Microbiology by Prescott, AVI Pub. Co.
6. Biochemical Engineering Fundamentals (2nd Ed) by J.E Bailey and D. Ollis. Mc Graw Hill Book Company.
7. Biochemical Engineering Fundamentals Baily, J.E and Ollis, D.F. McGraw-Hill Book Co. New York.
8. Bioprocess Technology: Fundamentals and Applications, KTH, Stockholm.
9. Bioprocess Engineering Basic Concepts, Shuler, M .L. and Kargi, P., Prentice Hall Engelwood Cliffs.
10. Principles of Fermentation Technology, Stanbury, P .F. and Whitaker. A. Pergamon Press, Oxford.
11. Bioreaction Engineering Principles Neilson, J . and Villadsefl. J, Plenum Press.

PAPER-III: ELECTIVE
IV. PLANT MOLECULAR BIOLOGY AND BIOTECHNOLOGY

UNIT I: GENOME ORGANIZATION AND CHARACTERIZATION

Nuclear Genome, Organellar Genomes, Status of Plant Genome Sequencing and its applications, Structure of Plant Gene and Gene Families. Molecular Markers: RFLP, RAPD, AFLP, SSR and SCAR. Genome mapping and applications. EST's and cDNA libraries.

UNIT II: CELL AND TISSUE CULTURE

Laboratory Organization, Media Preparations and Sterilization Techniques. Protoplast Culture, Suspension culture, Tissue Culture: Somatic Embryogenesis, Haploid Culture, Embryo Culture and Meristem Culture. Micropropagation, Somaclonal variation, Somatic hybridization, Cryopreservation and Synthetic seeds. Secondary metabolites production and purification.

UNIT III: PLANT GENETIC ENGINEERING

Plant Expression Vectors: Binary and Viral Vectors. Promoters, Selection markers and Reporter genes, Construction of recombinant vectors. Genetic Transformation Methods: Agrobacterium mediated and direct gene delivery systems. Chloroplast Transformation, Transient Expression systems, Gene silencing and RNAi technology.

UNIT IV: GENETICALLY MODIFIED PLANTS

Application of Transgenic Plants: - Bt cotton and Pest Resistance, Herbicidal Resistance, Viral Resistance, Disease Resistance, Abiotic stress resistance. Increased shelf life of flowers and fruits, Seed storage Proteins, Nitrogen fixation, Metabolic Engineering, Molecular Pharming/Farming.

UNIT V: PLANT BIOTECHNOLOGY INDUSTRIES AND REGULATIONS

Pharmaceutical, Horticultural, Food Industries and GM Foods. IPR and Ethical Issues. Biosafety, RCGM and GEAC- Guidelines by Department of Biotechnology (DBT), India.

References:

1. Maarten J. Chrispeels and David E. Sadava. Jones (2002) Plants, genes and agriculture Bartlett Publishers, 1 Exeter Plaza, Boston, USA.
2. <http://dbtbiosafety.nic.in>
3. Harvinder Singh Chawla (1998) Biotechnology in Crop Improvement IBD Publishers.
4. Donal Grierson and Convey S. V. (1988). Plant Molecular Biology, Routledge Publishers.

5. J Hammond (2000) Plant Biotechnology: New Products And Applications, IK International Publishers.
6. Razdan MK (2003). Introduction to Plant Tissue Culture, Oxford-IBH Publishers
7. Slater, A, Scott, N. and Fowler, M. (2003). Plant Biotechnology: The Genetic Manipulation of Plants. Oxford Press.
8. Monica A. Hughes (1999). Plant Molecular Genetics, Pearson Education Publishers.
9. Mantel, Mathews and Mickee (1985). An introduction to genetic engineering in plants. Blackwell Publishers.
10. Eds. Rainer Fischer and Stefan Schillberg (2004). Molecular Farming: Plant made pharmaceuticals and technical proteins.

**PAPER-III – ELECTIVE
V. FOOD BIOTECHNOLOGY**

UNIT- I :**STRUCTURE, FUNCTION AND CLASSIFICATION OF FOOD BIOMOLECULES:**

Food ingredient challenges, carbohydrates, proteins, lipids, energy, minerals, vitamins-sources, biofunctional properties, physiological role, bioavailability and nutritional significances. Antinutritional factors: enzyme inhibitors, phytohemagglutinin/lectins, glucosinolates, cyanogenic glucosides/cyanogens, tannins, gossypol, saponins, phytates, toxic non-protein amino acids, allergens, oxalic acid, oligosaccharides, nitrate and nitrite and antivitamin-nature, occurrence and health effects, toxic factors induced by processing. Nutrient evaluation of proteins and carbohydrates of legumes and grains.

UNIT-II : FOOD PREPARATION, PROCESSING AND PRESERVATION

Biotechnology in relation to the food industry. Cooking of Foods: Objectives of cooking foods, methods of cooking, cooking media, microwave cooking, nutritional changes by cooking. Food processing: Milling, soaking, cooking, germination, microwave heating, thermal inactivation of microorganisms, thermal process, freezing and thawing of foods, biotechnological approaches, nutritive value of processed foods, packing, canning, labelling. Food preservation: preservation by heat, cold, chill storage, deep freezing, drying, concentration, fermentation, radiation and dehydration. Food quality: sensory evaluation, objective methods, nutritional constituents and food safety, food adulteration, control of food quality.

UNIT-III : FOOD AND FOOD PRODUCTS

Origin, scope and development. Fruits: tropical and subtropical fruits, dry fruits, vegetables, cereals, pulses, nuts, oils and fats in foods. Beverages: Coffee, tea, cocoa, soft drinks, fruit juices. Fermented products: Dairy products. Fermented vegetable products- miso, sufu, natto, tempeh, idli, dosa, sour kraut, pickles, soy sauce, fermented fish products, organic acids, amino acids, vitamins. Regulatory and social aspects of biotechnology of foods, safety assessment of nutritionally improved foods and feeds developed through the application of modern biotechnology.

UNIT-IV : FOOD SPOILAGE AND FOOD ADDITIVES

Food borne illness, quality control, case studies on biotechnology in the evaluation of food quality, contamination and spoilage of fruits, vegetables, cereals, legumes, milk products, egg, meat, poultry, sea foods. Food additives: Introduction, chelating agents, coloring agents, curing agents, emulsions, flavours and flavouring enhancers. Flour ingredients, humectants, anticaking agents, leavening agents, nutritional supplements and non nutritive sweeteners, pH control agents, preservatives, stabilizers, and thickeners. Additives and food safety – Evaluation of safety, safety versus hazards, unintentional additives.

UNIT-V : FUNCTIONAL FOODS

Dietary fiber-physicochemical properties, functional ingredients, significance and role in health and disease, Resistant starches-Fermentation, large bowel health and lipid metabolism; natural food antioxidants-nature and occurrence in vegetables, grains, fruits, non-alcoholic beverages, biofunctional and health effects, probiotics and prebiotics-introduction, probiotic food ingredients, source, human health; Nutraceuticals-historical and technological aspects, sources, properties, nutraceutical potential. Nutraceutical proteins and peptides in health and disease. Microbes as direct food-single cell proteins, baker's yeast, Antimicrobial in foods-introduction, role of natural and synthetic bioactive molecules.

REFERENCES

1. Food Biotechnology, 2005. K. Shetty, G. Paliyath, A. Pometto and R. E. Levin (Eds.), CRC Press, Florida, USA.
2. Foods-Facts and Principles, 2005. Shakuntala Manay and M. Shdaksharaswamy, IInd edition, New Age International Pvt Ltd, New Delhi, India.
3. Handbook of Nutraceuticals and Functional Foods, 2006. R. E. C. Wildman (Ed.), CRC Press, Florida, USA.
4. Hand book of Dietary Fiber, 2001. S. S. Cho and M. L. Dreher (Eds.), Marcel Dekker, Inc. New York, USA.
5. Probiotics in Food Safety and Human Health, 2005. I. Goktepe, V. K. Juneia and Mohamed Ahmedna (Eds.), CRC Press, Florida, USA.
6. Food Science, 2007. B. Srilakshimi, IVth Edition, New age International Pvt Ltd, New Delhi, India.
7. Functional Foods and Biotechnology, 2006. K. Shetty, G. Paliyath, A. Pometto and R. E. Levin (Eds.), CRC Press, Florida, USA.
8. Post Harvest Biotechnology of Food Legumes, 1985. D. K. Salunkhe, S. S. Kadam and J. K. Chavan, CRC Press, Florida, USA.
9. Food and Feed from Legumes and Oilseeds, 1996. E. Nwokolo and J. Smartt (Eds.), Chapman & Hall, London, UK.
10. Toxic Constituents of Plant Foodstuffs, 1980. I. E. Liener, IInd Edition, Academic Press, New York, USA.
11. Antimicrobial in Food, 2005. M. Davidson, J.N. Sofos and A. L. Branen (Eds.), CRC Press, Florida, USA.

12. Toxic Substances in Crop Plants, 1991. J. P. F. DMello, C. M. Duffs and J. H. Duffs (Eds.), Royal Society of Chemistry, UK.
13. Plant Nonprotein Amino and Imino Acids-Biological, Biochemical and Toxicological properties, 1982. G.A. Rosenthal, Academic Press, New York, USA.
14. Food Antioxidants-Technological, Toxicological, and Health Perspectives, 1996. D. L. Madhavi, S. S. Deshpande and D. K. Salunkhe (Eds.), Marcel Dekker, Inc, New York, USA.
