SYLLABUS for M. Sc. BIOTECHNOLOGY
Semester Pattern
Kakatiya University, Warangal

Effective from 2014-2015

The syllabus is divided into four semesters. The first three semesters carry four theory papers and two practical papers and a seminar. In the IVth semester only two theory papers and a practical paper is included. An internal project work is required to be completed in the fourth semester. Apart from the project, the student will also have to present a seminar in the fourth semester. Each theory paper is divided into four units and all the units carry equal weightage. All theory and practical papers are compulsory. Each theory and practical papers carries 100 marks. 100 marks are allotted to the project work to be presented at the end of the fourth semester and the projects are compulsory. 25 marks are allotted to the Seminar.

1) **Number of theory and practical periods:** The syllabus is based on 18 theory periods and 16 practical periods per week. Candidates are required to pass separately in theory and practical examination.
2) **Seminars:** In all the semesters every student has to give at least one seminar and submit a written summary of the same.
3) **Project work:** The student will undergo training in any Biotechnology Industry/Institute for 45 days after completion of II semester. An internal group project work is also required to be completed in the fourth semester. The reports will be submitted at the end of the IVth semester. The project reports will be evaluated by the External and Internal (Chairperson, BOS, Biotechnology) examiners at end of the fourth semester, 100 marks are allotted to the project work. The project is compulsory.
4) **Study tour:** Students of M. Sc. Biotechnology are encouraged to visit some research institutes of national and international repute during the two-year course.
5) **Distribution of theory/practical/seminar/project marks:**

M. Sc. Biotechnology
Semester I

SUBJECTS	PAPER	Internal Marks	Examination		CREDITS
			Max Marks	Pass Marks	
BT-101: **Biomolecules**	I	20	80	32	04
BT-102: **Microbiology and Biodiversity**	II	20	80	32	04
BT-103**: Cell Biology and Genetics**	III	20	80	32	04
BT-104: **Biophysical and Biochemical Techniques**	IV	20	80	32	04
BTP-101:Biomolecules, Microbiology and Biodiversity	I	--	100	40	04
BTP-102: Cell Biology, Genetics, Biophysical and Biochemical Techniques	II	--	100	40	04
SEMINAR/TUTORIALS	--	--	25	--	01

M. Sc. Biotechnology
Semester II

SUBJECTS	PAPER	Internal Marks	Examination		CREDITS
			Max Marks	Pass Marks	
BT-201: Biochemistry and Enzymology	V	20	80	32	04
BT-202: Immunology and Immunotechnology	VI	20	80	32	04
BT-203: Molecular Biology	VII	20	80	32	04
BT-204: Bioinformatics and Biostatistics	VIII	20	80	32	04
BTP-201: Biochemistry, Enzymology and Immunotechnology	III	--	100	40	04
BTP-202:Molecular Biology, Bioinformatics and Biostatistics	IV	--	100	40	04
SEMINAR/TUTORIALS	--	--	25	--	01

MASTER OF SCIENCE (BIOTECHNOLOGY)
TWO YEAR (FOUR SEMESTER) DEGREE COURSE

GENERAL RULES & REGULATIONS

A) Pattern of Question Paper

1. There will be four units in each paper.
2. Question paper will consist of five questions.
3. First question will be compulsory with questions from each of the four units having equal weightage and there will be no internal choice.
4. Four questions will be on four units with internal choice (One question on each unit).
5. Maximum marks of each paper will be 80.
6. Each paper will be of 3 hours duration.
7. Projects shall be evaluated by both internal and external examiners.
8. Practical/laboratory examination of 100 marks.
9. Minimum passing marks in each head (theory& practical) will be 40%.

B) Grade Point Average (GPA) and Cumulative Grade Point Average (CGPA)

1. On clearing a paper, based on the cumulative score (out of 100) in that paper a student will be given GRADE POINT AVERAGE (maximum of 10, and minimum of 4) for that paper on the following basis. The description for each of the grades is as follows:

Grade Proposed Norms:

SCORE (out of 100)	Grade	Grade Point Average (Out of 10)
100-85	O: Out Standing	10
84-70	A: Very good	9
69-60	B: Good	8
59-55	C: Average	7
54-50	D: Satisfactory	6
49-40	E: Pass	5
Below 40	F: Fail	4 or Fail

2. On clearing all the papers in a semester, a student will be allotted a **Semester Grade Point Average (SGPA)** for that particular semester. As the pattern given above does not have differential weights for papers, the SGPA of a student for a particular semester will be calculated as per the following computation.

$$\text{SGPA} = \frac{C1xG1+C2xG2----------CnxGn}{C1+C2----------Cn}$$

Where C1 = Credit of individual Theory / Practical G1 = Corresponding Grade Point obtained in the Respective Theory/ Practical

3. A student will be allotted a **Cumulative Grade Point Average (CGPA)** after clearing all the four semesters. Again as there is no differential weight system for semesters, the CGPA of a student will be the average of the four SGPA's of that student. The CGPA would be as follows:

CGPA	Final Grade	Equivalent Class/Division
9.00-10.00	O	First Division with Distinction (Outstanding)
8.00-8.99	A	First Division with Distinction (Excellent)
7.00-7.99	B	First Division with distinction
6.00-6.99	C	First Division
5.00-5.99	D	Second Division
4.00-4.99	E	Pass Division
Below 4.00	F	Fail

4.The computation of Semester Grade Point Average (SGPA) and Cumulative Grade Point Average (CGPA) of an examinee shall be given below:

a. The marks will be given in all examinations which will include the internal assessment marks, and the total marks for each Theory/ Practical shall be converted into Grades as per above table. SGPA shall be calculated based on Grade Points corresponding to Grade as given in above table and the credits allotted to respective Theory / Practical shown in the scheme for respective semester.
b. SGPA shall be computed for every semester and CGPA shall be computed only after IV semester. The CGPA will be calculated based on SGPA of all four semesters as per following computation:

$$\textbf{CGPA} = \frac{(SGPA)\ I \times (Cr)\ I + (SGPA)\ II \times (Cr)\ II + (SGPA)\ III \times (Cr)\ I\ II + (SGPA)\ IV \times (Cr)\ IV}{(Cr)\ I + (Cr)\ II + (Cr)\ III + (Cr)\ IV}$$

Where, (SGPA) I = SGPA of I Semester; (Cr) I = Total Credits for I Semester;
(SGPA) II = SGPA of II Semester; (Cr) II = Total Credits for II Semester;
(SGPA) III = SGPA of III Semester; (Cr) III = Total Credits for III Semester;
(SGPA) IV = SGPA of IV Semester; (Cr) IV = Total Credits for IV Semester

Kakatiya University, Warangal

M. Sc. BIOTECHNOLOGY

(SEMESTER SYSTEM)

(Effective from 2014 -2015)

SYLLABUS

SEMESTER – I

BT-101: BIOCHEMISTRY

Unit I

1. Buffers and measurement of pH.
2. Hydrodynamic properties of biomolecules: viscosity, diffusion, osmosis, partial specific volume and Donnan's effect.
3. Carbohydrates – Classification and properties of carbohydrates, mono (glucose, galactose, fructose), di (lactose, maltose, sucrose) and poly (starch, glycogen, cellulose) sacharides. Chemical and enzymatic methods for structural elucidation of starch and mucopolysaccharides.
4. Amino acids – classification, Structure and physico-chemical properties, Peptides – Peptide bonds.

Unit II

1. Naturally occurring peptides (glutathione, bradykinin, kallikrien, tyrocidin). Peptide synthesis by solid-phase technique.
2. Proteins – Classification, Isolation and purification of proteins, criteria of homogeneity.
3. Primary structure of proteins and its sequence determination.
4. Lipids – Classification. Structure and biological functions of fatty acids, triacylglycerols, steroids. Physico-chemical properties and analysis of fats and oils. Structure and functions of prostaglandins, leukotrienes, thromoboxanes.

Unit III

1. Secondary (Ramachandran plot), tertiary and quaternary structural features of proteins, Protein folding (Alfensen's experiment on ribonuclease).
2. Forces responsible for protein stability. Structural organization: globular (myoglobin, Hemoglobin), fibrous proteins (collagen, Keratins, silk fibroin).
3. Denaturation and renaturation of proteins
4. Structure and functions of glycoproteins and lipoproteins.

Unit IV

1. Nucleic Acids – Structure of purines, pyrimidine, nucleosides, and nucleotides.
2. Structure, Properties and functions of nucleic acids (DNA, RNA). Different forms of DNA and RNA. Three dimensional structure of RNA.
3. Isolation of nucleic acids, Denaturation and renaturation of nucleic acids,
4. Chemical synthesis of DNA. DNA constancy & C-Value paradox

BT-102: MICROBIOLOGY AND BIODIVERSITY

Unit-I

1. Systematic position of microorganisms in living world, classification of microorganisms: Hackle's three kingdom concept, Whittaker's five kingdom concept, three domain concept of Cral Woese
2. Historical account of bacterial classification, detail account of bacterial classification according to the 1st edition of Bergy's manual of systematic bacteriology (up to sections). Detail account of bacterial classification according to the 2nd edition of Bergy's manual of systematic bacteriology (up to orders)
3. General characters, thallus organization, cell structure, reproduction and classification of fungi, nutrition, reproduction and parasexuality, structure, reproduction and molecular and biotechnological aspects of yeasts.
4. History, general properties and structure of viruses: Viruses related agents (viroids & prions), nomenclature and classification of viruses, auto virus infection, and persistent viruses. General features of virus reproduction, replication of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) viruses, bacteriophages, transmission of viruses, and management of viruses.

Unit-II

1. Microbial Evolution: Evolution of earth and earliest life-forms; primitive organisms and their metabolic strategies.
2. Microbial Diversity: Bacteria, Archea and their broad classification eukaryotic microbes, Yeast, Fungi, moulds and Protozoa; Viruses and their diversity.
3. Metabolic Diversity-I: Photosynthesis in microorganisms-Role of chlorophylls, carotenoids and phycobilins.
4. Metabolic Diversity-II: Calvin cycle, chemolitho-trophy; Hydrogen-iron, nitrateoxidizing bactetria. Nitrate and sulphate reduction; Methanogenesis and acetogenesis.

Unit-III

1. *Biodiversity*: Definition, levels, organization , uses, and valuing biodiversity
2. *Genetic Diversity*: Nature and origin of genetic variation, measuring genetic diversity variation. Wild relatives of cultivated/domesticated/cultured organisms (plants/animals/microbes). *Species Diversity:* Concept of species, measurement of species diversity, global distribution of species riches. *Ecosystem diversity:* Terrestrial and aquatic ecosystems. Centers of mega diversity and hotspots.
3. *Biodiversity vs. Biotechnology and* Bioprospecting, biosafety, biopiracy and Intellectual Property Rights (IPR).
4. *Biodiversity for Sustainable Development:* Sustainable management of biodiversity: International and regional policies. Biodiversity Act, National Biodiversity Board and Andhra Pradesh State Biodiversity Board.

Unit-IV

1. Global Animal Diversity: A bird's view of animal kingdom. Domesticated animal diversity and wild animal resources of India. A brief account of diversity in aquatic life. A case study of over-fishing resulting in ecological disaster.
2. Concept and characteristics of plant community. Methods of studying vegetation. Raunkier's life forms. Biological spectrum. Plant succession. Concept of climax and climax communities.
3. IUCN categories. Rare and endangered categories and extinct animals of India. Trends of extinction rates. Wildlife Act of India and CITES.

4. *Biodiversity Conservation* : Principles and rationale. *Ex situ* and *In situ* conservation strategies (Incl. sperm/seed banks, cryopreservation, embryo collection and freezing creation of parks, wildlife sanctuaries, botanical gardens, etc.)

BT-103: CELL BIOLOGY AND GENETICS

Unit I

1. Nucleus -Ultra structure of nucleus and nuclear envelope.
2. Organization of eukaryotic chromosome - structure of nucleosome and extent of chromatin condensation in metaphase chromosome. Euchromatin and heterochromatin (constitutive and facultative).Special Types of Chromosomes: Polytene and Lampbrush chromosomes, Nomenclature of chromosome, dosage compensation.
3. Cell cycle - Overview of eukaryotic cell cycle, regulation of cell cycle by cell growth and extra cellular signals, Cell cycle check points, Regulators of cell cycle progression -MPF, cyclins and cyclin-dependent kinases.
4. Cell differentiation. Cell death and proliferation-Apoptosis: definition, morphological and biochemical differences between apoptosis and necrosis, mechanism (internal and external signals) and significance. Brief account of biology of cancer.

Unit II

1. Cell Communication - General principles, Cell surface receptors (ion channel linked, G-protein linked and enzyme-linked receptors) and intracellular receptors,
2. Forms of intracellular signaling – Autocrine, paracrine, contact dependent, synaptic and endocrine signaling. Response of cell to signals. Intracellular signaling proteins: Different types and their role. Second messengers - cAMP pathway and role of calcium. Cellular interactions -Mocrovilli, tight junctions, belt and spot
3. Desmosomes, gap junctions-Electrical coupling, The connexon, factor mediating cell-self recognition (aggregation factor).
4. Cytoskeleton - Structure and functions of actin, microfilaments and intermediary filaments.

Unit III

1. Introduction to genetics: Mendel's principles, Gene interaction & Modified ratios,
2. Multiple alleles, multiple factor inheritance, Extra chromosomal inheritance
3. Linkage and crossing over and genetic mapping: sex-linked inheritance, cytological evidence of crossing over in maize, crossing over frequency and map distances, recombination models: maize, yeast and Neurospora.
4. Population genetics: Hardy –Weinberg's law, factors influencing the equilibrium

Unit IV

1. Bacterial Genetics: Conjugation, Transformation, Transduction, recombination and gene mapping.
2. Phage Genetics: Gene fine structure, concepts of cistron, muton & recon, r II locus
3. Molecular mechanisms of mutations, Ames test for mutagenesis, DNA damage and repair,
4. Mutations: Chromosome variations in number and structure, Role of mutations in crop improvement

BT-104: BIOPHYSICAL AND BIOCHEMICAL TECHNIQUES

Unit I

1. Microscopy: Principles and application of light, phase contrast, fluorescence, scanning and transmission electron microscopy.
2. cytophotometry and flow cytometry, fixation and staining.
3. Centrifugation: Basic principles of sedimentation, types of centrifuges and rotors. Preparative ultracentrifugation-differential centrifugation, Density-gradient, analytical ultracentrifugation.
4. Applications in determination of molecular weight, purity and detection of conformational changes in macromolecules.

Unit II

1. Separation methods - General principles and definitions, Paper chromatography, adsorption chromatography (thin-layer chromatography), gas-liquid chromatography,
2. Methods based on size: Principle of Gel filtration, methodology and applications. Dialysis, ultra filtration
3. Methods based on affinity: Principle of Affinity chromatography; methodology and applications. Ion-exchange chromatography: Principle & methodology
4. High-performance liquid chromatography: Principle, instrumentation, practical procedure and applications.

Unit III

1. Electrophoresis: General principles and definitions. PAGE-Native-PAGE, SDS-PAGE,
2. Iso-electricfocussing, 2D electrophoresis, identification of novel proteins in 2D gels, capillary electrophoresis.
3. Agarose gel electrophoresis : Preparation, separation and determination of molecular size of DNA, denaturing agarose gel electrophoresis and their applications, recovery of DNA from agarose gels.
4. Pulse-field gel electrophoresis : principle, methodology and applications in separation of large DNA fragments.

Unit IV

1. Spectroscopy: Electromagnetic spectrum of light, simple theory of absorption of light molecules, Beer-Lambert law, absorbance, transmittance, extinction, coefficient, light sources, monochromatic, type of detection, UV, visible spectrophotometer, infra red spectroscopy.
2. Raman spectroscopy, flame photometer, atomic absorption, plasma emission, mass ESR and NMR spectrometry, MALDI – TOF, ESI MS.
3. Radioisotope Techniques : Types of isotopes, radioactive decay. Detection and measurement of radioactivity-GM counter, scintillation counter, autoradiography.
4. Preparation of label compounds: Pulse chase studies and tracer techniques, isotopes used in biology, safety methods in handling radioisotopes.

SEMESTER – II

BT-201:ENZYMOLOGY AND PLANT BIOCHEMISTRY

Unit I

1. Enzymes: Definitions and nomenclature (EC recommended).
2. Mechanisms of enzyme action, active site and its location, binding site, chymotrypsin, ribonuclease, carboxyl peptidase as models.
3. Enzymes kinetics, derivation of Michaelis-Menten constant, determination of V-max and Km, enzyme inhibition: competitive and non-competitive inhibition.
4. Regulation of enzyme activity: allosteric enzymes, models explaining allosteric behaviour-KMF, MWC models, feed back inhibition in metabolism.

Unit II

1. Concept of free energy: Energy metabolism, Thermodynamic principles in biology, Energy rich bonds, weak interactions,
2. Coupled reactions and oxidative phosphorylations, group transfer, biological energy transducers, bioenergetics. Glycolysis and TCA cycle, HMP shunt, Gluconeogenesis, Energy derivations in fermentation, aerobic and anaerobic respirations.
3. Glyoxylate cycle, Components and organization of mitochondrial electron transport system

Unit III

1. Chloroplast as an energy transducing organelle.
2. Photosynthetic pigments and photosynthesis in bacteria and higher plants. Organization of thaylakoid membrane protein complex involved in photosynthesis Cyclic and non-cyclic photophosphorylation, Mechanism of photophosphorylation.
3. Pathways of CO_2 fixation by C3, C4, and CAM pathways.
4. Photorespiration. Fatty acid oxidation (ß-oxidation). Biosynthesis of fatty acids, triglycerides and cholesterol, ketone bodies synthesis.

Unit IV

1. Nitrogen fixation: Diazotrophic microorganisms, nitrogen fixation genes. Transfer of *nif* genes to non-diazotrophic microorganisms.
2. Organization, regulation and expression of Nif genes,
    1. *Nod* genes, principles of food preservation
3. role in nodulation,
4. Photoreceptor phytochrome- Phytochrome regulated gene expression

BT-202: IMMUNOLOGY AND IMMUNOTECHNOLOGY

Unit I

1. Phylogeny of immune system. Types of immunity – innate and acquired.
2. Cells of the immune system – B-cells, T-cells, phagocytes, inflammatory cells, antigen presenting cells.
3. Organs – primary, secondary and tertiary lymphoid organs. Antigens – nature, types, factors influencing antigenicity, haptens, adjuvants and super antigens.
4. Antibodies – structure, types, classes and functions. Antibody diversity – theories of antibody diversity, mechanism of diversification, allelic exclusion.

Unit II

1. T-cell receptor – structure and diversity.
2. MHC – Types, structure, distribution, self-restriction, T-and B-cell activation. Maturation of lymphocytes – positive and negative selection, process of maturation.
3. Antigen processing and presentation – cytosolic and endosomal pathways, T and NK cell – mediated lysis of cells, ADCC.
4. Complement system – components, cascades, MAC, outcomes. Cytokines – classification, properties and role as immunomodulators.

Unit III

1. Hypersensitivity – classification, mediators, mechanism, consequences of hypersensitive reaction.
2. Autoimmunity - concept of tolerance of autoimmune disorders, basis and therapy for autoimmune disorders.
3. Transplantation – transplantation antigens, mechanism of graft rejection, graft versus host reaction, immunosupressors. Tumor immunity – tumors of immune system, immune responses against tumors.
4. Immunodeficiency – primary and secondary immunodeficiency, combined immunodeficiency, complement deficiency, AIDS.

Unit IV

1. Antigen- antibody interactions – principle, lattice hypothesis.Precipitation reaction – radial immunodiffusion, Ouchyterlony technique, immunoelectrophoresis, counter current and rocket electrophoresis.
2. Agglutination reactions – bacterial and hemeagglutination, passive agglutination, agglutination inhibitions assay. RIA and ELISA – principle, methodology and application. Immunofluorescence, FAACS, immunoblotting.

3. Hybridoma technology – polyclonals, monoclonals, selection, HAT medium, production of monoclonal antibodies and applications.
4. Vaccines – concept of immunization, routes of vaccination. Types of vaccines – whole organism (attenuated and inactivated) and component vaccines (synthetic peptides, DNA vaccines, recombinant vaccines, subunit vaccines, idiotypic based vaccines, deletion vaccines, glycoconjugate vaccines), Vaccine delivery systems.

BT-203: MOLECULAR BIOLOGY

Unit I

1. DNA Replication and repair: Modes of replication. Experimental evidences for semi-conservative mode of replication - Meselson–Stahl, and Cairns experiments.
2. Replication fork, continuous and discontinuous DNA synthesis.
3. Enzymes and proteins in replication - Single strand DNA binding proteins (SSB), Helicases, Topoisomerases, DNA ligases. Priming by RNA polymerase and primase. DNApolymerases - E.coli DNA polymerase I, II and III, and Eukaryotic DNA polymerases.
4. Replication of E. coli chromosome and M13 genome. Rolling circle replication in bacteriophage. Eukaryotic DNA replication. Autonomous replication sequences (ARS). Regulation of ColE1 plasmid DNA replication. Termination and fidelity of DNA replication. Nearest neighbour base pair analysis. Inhibitors of DNA replication.

Unit II

1. Promoters and their characterization. Enhancer sequences.
2. Transcription (RNA Biosynthesis): Initiation, elongation and termination of RNA synthesis. Monocistronic and polycistronic RNAs. Polynucleotide phosphorylase. RNA polymerases - structure of E. coli RNA polymerase, and nature of eukaryotic RNA polymerases.
3. RNA splicing and splicing mechanisms. Splicing of nuclear pre-tRNA, group I and group II introns, and pre-mRNA splicing. Excision of multiple introns. Role of catalytic RNA. Inhibitors of transcription.
4. Posttranscriptional modifications of eukaryotic hnRNA – capping, methylation and polyadenylation,

Unit III

1. Translation (Protein synthesis): Elucidation of the genetic code - experimental studies of Nirenburg and Khorona.
2. General features of genetic code, codon degeneracy and universality. Mitochondrial genetic code, tRNA role in protein synthesis. Amino acyl-tRNA synthetases, wobble hypothesis.
3. Mechanism of initiation, elongation and termination of protein synthesis. Translational factors.
4. Inhibitors of protein synthesis – antibiotics and other inhibitors. Post-translational modifications.

Unit IV

1. Regulation of gene expression: House-keeping genes, constitutive genes, and regulatory genes. Induction and repression. Regulatory proteins- DNA-binding motif of regulatory proteins. Role of zinc fingers, leucine zippers, helix-turn-helix.
2. Regulation of gene expression in prokaryotic operons. Negative regulation and positive regulation. Fine structure of lac operon. Repressor and the catabolite activator proteins in gene regulation of lac operon. Dual functions of the repressor in ara operon.
3. Transcriptional control by attenuation in trp-operon. Regulation of gene expression in eukaryotes.
4. Hormones and environmental factors affecting gene expression. Homeotic genes and their regulation.

BT-204: BIOINFORMATICS AND BIOSTATISTICS

UNIT-I

1. Introduction to Computers Overview of computer organization and historical perspective, computer applications in various fields of science and management, Data representation: Number systems, character representation codes
2. Binary, hex, octal codes and their interconversions. Binary, arithmetic, floating point arithmetic, signed and unsigned numbers. Data storage: Primary and Secondary storage.
3. Introduction to various computer devices such as keyboard, mouse, printers, disk files, floppies etc. Concept of computing, Operating Systems such as Windows NT, UNIX etc. (only brief user-level description).
4. Introduction to organization and architecture of mainframe, mini and micro systems. Introduction to E-mail, ftp, login and other network services, world wide web, MS-Office.

UNIT-II

1. Biological databases: Basic concepts of databases, bioinformatics and importance of databases, integration of databases and its need.
2. DNA databases, protein-sequencing databases, functional motifs databases, protein-structure databases.
3. Sequence analysis: Concepts of DNA/protein-sequence alignment and their importance, sequence alignments and alignment programs.
4. Comparative-sequence analysis: Pair-wise sequence alignment, multiple-sequence alignments, methods like BLAST, FASTA. Tools like CLUSTAL. Dynamic programming, similarity algorithms, affina gap penalty

UNIT-III

1. Molecular modeling, Proteomics: functional and structural proteomics.
2. Genomic studies, 3D structure, and domain structure – DNA binding domains. Molecular modeling.
3. Determination of structure of proteins. Predicting protein structure – secondary structure, Methods of protein modeling
4. Microarray technology, human genome project and applications.

Unit IV

1. Introduction to biostatistics: Variables, random variables, discrete and continuous variables, population and sample estimate, mean, median, mode, frequency distribution, frequency curve, frequency polygon and histogram.
2. Measures of dispersion: Range, variance, coefficient of variance, standard deviation (SD) and standard error (SE).
3. Probability distribution: Normal, binomial and poison.
4. Test of hypotheses: Students t-test, X^2 distribution (Chi-square), correlation coefficient and analysis of variance (ANOVA)