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## ANTIFUNGAL POTENTIAL OF PLANT EXTRACTS FOR THE MANAGEMENT OF ANTHRACNOSE OF CHILI CAUSED BY *COLLETOTRICHUM CAPSICI*

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### ABSTRACT

Chili anthracnose damages chili fruits extensively at pre- and postharvest stages causing anthracnose lesions. Even very small lesion of anthracnose on fruits of chili reduces the market value of chili crop. Fungitoxic effects of methanolic plant extracts were tested in vitro through poisoned food technique. There was a significant decrease in mycelial growth of the fungus with an increase in methanolic plant extracts concentration in all the tested methanolic plant extracts over the control. The fungitoxicity of methanolic plant extracts varied greatly among each other and their concentrations. In general, there was a decrease in mycelial growth *Colletotrichum capsici* with an increase in concentration of methanolic plant extracts. However, when growth of the fungus in response to various methanolic plant extract concentrations after an incubation period of nine days at  $28 \pm 2^\circ\text{C}$  was compared, neem leaf extract proved to be the best as it had given the maximum control (64 percent) followed by datura leaf extract (63 percent) on 1000  $\mu\text{g/ml}$  concentration. While eucalyptus leaf extract was proved to be the least effective (45 percent) at highest concentration (1000  $\mu\text{g/ml}$ ). None of the tested methanolic plant extracts has completely checked the mycelial growth of *Colletotrichum capsici* at any of their concentrations. Although there was an overall trend of reduction in mycelial growth of *Colletotrichum capsici* with increase in the concentration of methanolic plant extracts. These plant extracts can be used to control anthracnose of chilies to reduce the use of fungicides, as plant extracts are safer than synthetic fungicides.

**Keywords:** methanolic plant extracts, Chili, anthracnose, *Colletotrichum capsici*

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## A NOVEL TOOL FOR EFFICIENT AND RAPID EXTRACTION OF PHENOLICS FROM BARK OF *CASSIA OCCIDENTALIS* L.

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### ABSTRACT

Microwave assisted extraction is a modern and new approach for rapid and efficient extraction of plant material which are rich in number of secondary metabolites of prime importance. In present study a comparison was made between conventional method of extraction i.e., Soxhlet extraction, and modern method of extraction i.e., Microwave assisted extraction for the rapid and efficient extraction of *Cassia occidentalis* Linn bark. Microwave assisted extraction was proved to be more rapid and more efficient for the extraction of bark of *Cassia occidentalis* which yielded 0.19mg of extract/g of bark powder in 70 sec of microwave irradiation of 700W as compared to 0.16mg of extract/g of bark powder in 14 hours of continuous heating of the soxhlet extraction. It was also observed that a maximum of 89  $\mu\text{g}$  equivalent of Gallic acid of phenolics were obtained from extract of Microwave heating (900W) for 30 seconds. While 45  $\mu\text{g}$  equivalent of Catechin of flavonoids were obtained from a microwave assisted extract at 60 seconds of 700W of irradiation. In contrast, soxhlet extraction produced only 47.6 equivalent of gallic acid and 27.3  $\mu\text{g}$  equivalent of Catechin after 12 hours of extraction. So it was proved that microwave assisted extraction was efficient as well as cost effective in terms of lesser use of energy, time and solvent.

**Keywords:**

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## ANTIBIOTIC RESISTANCE OF BACTERIAL BIOFILMS

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### ABSTRACT

Biofilms are microorganisms' communities that get attached to a surface. It is evident through various researches that cells grown in a biofilm express distinct properties from that of planktonic cells, one of such property which is of high significance is an increased resistance to antimicrobial/ antibiotic agents. Bacterial biofilms are a cause of chronic infections worldwide because of their increased antibiotic tolerance. Their resistance to the antibiotics is due to mutation, resistant phenotypes, adaptations to stress, quorum sensing, stratified activity, nutrient gradients, oxidative stress, failure of antibiotic penetration and heterogeneity. Thus in order to control the bacterial infections caused by these biofilms, there is a need of novel drug delivery approaches and enhanced therapeutic use of quorum sensing inhibitors.

**Keywords:** Biofilms, antibiotic resistance, quorum sensing, quorum sensing inhibitors

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## BIOENERGY A STEP TOWARDS SUSTAINABILITY

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### ABSTRACT

Extraction of energy from the biological sources is the most environmental friendly way to conserve the natural resources. The biomass used for the production of energy comprises of the crops (corn, sugarcane and many others) and other way is the waste (e.g., wastewater from food industries, wood waste, straw, manure, sludge from sewerage etc). Bio energy extraction tends to shift from nonrenewable resources to the renewable resources of energy. Microbe's serves as directly in the extraction of bio energy as the algae the best source of bio fuel, and indirectly microbes are taking part in decomposition process for the formation of biogas. Geo bacter species are the naturally electric current generating species and playing an important role in the microbial fuel cell technology. Bio gas and bio diesel are the cheapest ones and least pollution generating as well as neutral to CO<sub>2</sub> emissions. Rather it is appropriate to say it is the greener technology and a step towards sustainability. Bio gas plants are installed in Pakistan for extraction of bio gas. This extraction is limited only to the research level and there is not any use of algal bio fuel. Pakistan is rich in the resources for the production of bio energy but unfortunately lack of technologies and financial assistance is the main hindrance behind its implication. Bio energy is increasing in response to concerns about energy security, energy independence and environmental and climate impacts associated with use of non-renewable energy resources.

**Keywords:** Bio energy, Bio fuel, Environmental friendly, Greener technology.

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## POOR SOLID WASTE MANAGEMENT AND PROBLEMS RELATED TO WASTE COLLECTORS IN PAKISTAN

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### ABSTRACT

The solid waste management is really the critical issue in Pakistan and trash pickers are in very crucial conditions. They are facing the condition of low status, very miserable living conditions, and poverty. All of them are surrounded by large number of problems from risks in domestic waste to the chemical waste which they collect on daily basis. Number of waste collectors is increasing day by day because the poor people who are illiterate have no any other option to earn for their families. So all family members get their self involved in this profession which does not require any education or any experience. Most of them are not aware of safe work practices, which causes large number of work hazards. Pakistan has very poor occupational safety and health laws and implementation is very low.

**Keywords:** Waste Collectors, Occupational Health & Safety, Solid Waste Management

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## PROSTATE CANCER AND GLUTATHIONE S TRANSFERASE DELETIONS

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### ABSTRACT

*GSTM1* and *GSTT1* gene polymorphisms have been studied in many populations to evaluate their association with prostate cancer risk with contrasting results. The current study was aimed to find out the association of *GSTM1* and *GSTT1* gene polymorphisms with prostate cancer in Pakistani men. This case control study included pathologically confirmed prostate cancer patients and age matched male controls. Epidemiological data was collected by a standard questionnaire and presence or absence of *GSTM1* and *GSTT1* gene was observed by multiplex PCR using CYP1A1 as housekeeping gene. Prostate cancer was more prevalent in age of >60 years and most of the patients were at stage IV (70%) and have undergone surgery. Family history of cancer, smoking, metastasis and surgery were found to be significant ( $P \leq 0.05$ ) risk factors in prostate cancer development. Gleason score 7 was most prevalent (40.5%) in prostate cancer patients. Source of drinking water, residential area, occupation, eating habits and number of family members had no association ( $P \geq 0.05$ ) with prostate cancer risk. No significant association was found when comparing *GSTM1* (OR=0.78) and *GSTT1* (OR=0.8) gene deletions with prostate cancer risk. Smoking and TNM staging were also not associated with deletion of *GSTM1* and *GSTT1* genes. Comparison of dual null deletion of both genes with prostate cancer also showed non-significant associations. Deletion of *GSTM1* gene at stage IV prostate cancer patients was significantly higher compared with other stages of cancer while no significance was shown by *GSTT1* gene deletion. *GSTM1*, *GSTT1* and deletion of both *GSTM1* and *GSTT1* genes do not contribute towards increased risk of prostate cancer in Pakistani population.

**Keywords:** *GSTM1*, *GSTT1*, polymerase chain reaction (PCR), prostate cancer.

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## CYTOCHROME P450 1A1 AND GLUTATHIONE S-TRANSFERASE PI 1 MUTATIONS IN PHARYNGEAL AND LARYNGEAL CARCINOMA.

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### ABSTRACT

In the current case control study, 94 pharyngeal, 67 laryngeal cancer cases and 150 cancer free controls were screened via PCR-SSCP assay. Mean ages of pharyngeal, laryngeal cancer patients and control was 48.14 (+16.7), 48.56 (+17.4) and 46 (+17.69) years respectively. Results revealed two novel mutations in CYP1A1 gene, a substitution mutation of A2842C resulting in missense tyrosine to serine formation and frameshift mutation due to insertion of thymidine at nucleotide 2842 resulting in 495 nucleotide sequences to alter. It was found that 3.2% pharyngeal and 2.98% laryngeal cancer patients had these mutations in CYP1A1. In GSTP1 gene exon 7, an A2848T substitution causes a leucine to leucine formation whereas G2849A substitution causes alanine to threonine formation at amino acid 166 and 167 respectively. These exonic mutations were found in 7.4% pharyngeal cancer and 9% laryngeal cancer patients. Two intronic deletions of C at nucleotide 1074 and 1466 were found in 1% pharyngeal and laryngeal cancer patients. Accumulation of mutations in CYP1A1 and GSTP1 genes seem to be associated with increased risk of pharyngeal and laryngeal cancer development.

### Keywords:

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## IDENTIFICATION OF TRAITS FOR IMPROVEMENT OF GRAIN YIELD IN BREAD WHEAT UNDER NITROGEN STRESS AND NON-STRESS CONDITIONS

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### ABSTRACT

Multiple statistical procedure including correlation, path and step wise regression analysis were used to identify crucial traits that effect grain yield in bread wheat. Thirty bread wheat varieties were field tested under with (N<sup>+</sup>) and without nitrogen (N<sup>0</sup>) conditions at New Developmental Research Farm, The University of Agriculture, Peshawar-Pakistan during 2013-14. The experimental material was planted in randomized complete block design with three replications. Correlation analysis revealed significant positive association of grain yield under both production system with tillers m<sup>-2</sup>, biological yield, harvest index, whereas, with 1000-grain weight under non-stress condition only. Furthermore, path coefficient analysis indicated that harvest index, biological yield, tillers m<sup>-2</sup>, and 1000-grain weight reflects true relationship with grain yield. Similarly, step wise regression analysis identified tillers m<sup>-2</sup>, grains spike<sup>-1</sup> and 1000 grain weight as important traits effecting grain yield in wheat. Overall, on the basis of above statistical procedure, tillers m<sup>-2</sup> and 1000-grain weight need due weightage in breeding program for further improvement in grain yield of bread wheat.

**Keywords:** Wheat, Path analysis, Step wise linear regression, Selection criterion

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## EVALUATION OF EXTRACTION METHOD FOR HOUSEHOLD HERBAL PRODUCTS

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### ABSTRACT

Microwave assisted extraction is a modern and novel approach for rapid and efficient extraction of the plant material which are of prime importance in herbal medicinal industry. In present study a comparison was made between conventional method of extraction (stove heating) and the modern method of extraction (Microwave assisted extraction) for analyzing the extraction efficiency of both commonly used methods for household herbal products (i.e., Green tea, Vital tea, Supreme tea, Danedar tea, Tez Dam tea, Tetley and Lipton tea), Qehwa (Peshawari Qehwa), Coffee (Nescafe Classic) and Joshanda (Qarshi industries). It was found that microwave assisted extraction gave the maximum amount of extracts than the stove heating extraction. Qualitative tests were performed for all the extracts for the presence of alkaloids, phenolics, flavonoids and terpenoids, while quantitative tests were applied to check the quantity of phenolics and flavonoids in the extracts. Results showed that there was a significant difference of amount of Phenolics and flavonoids from microwave assisted extract and stove heated extract of each sample. A maximum of 88.33 gram equivalent of gallic acid (standard phenolics) were observed from microwave assisted extract of Tapal Tez dam Tea. Stove extracts produced a maximum of quantity of phenolics 70 gram equivalent of gallic acid from Lipton tea. In the same way a maximum of 92 gram equivalent of Catechin (Standard of flavonoids) were observed from microwave assisted extract of Johar Joshanda. Stove extracts produced a maximum of quantity of phenolics 69 gram equivalent of Catechin from Coffee sample.

**Keywords:** Microwave assisted extraction, stove heating, herbal products, phenolics

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## BIOLOGICAL AND FUNCTIONAL SCREENING OF SYNTHETIC PROPANAMIDE DERIVATIVES FOR DRUG DISCOVERY

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### ABSTRACT

The foremost purpose of the current study was to biologically screen out some novel synthetic propanamide derivatives for antitumor, antimicrobial, antioxidant and insecticidal activity. Propanamide derivatives showed noteworthy results for tumor inhibition using Potato Disc Antitumor assay. Highest percentage of tumor inhibition (81.76, 100, 87.84, 100, 100) was observed with compound no. 10 at a concentration of 10000, 1000, 100, 10 and 1ppm respectively and compound 05 showed lowest percentage of tumor inhibition (45.28, 57.44, 57.44, 63.5, 69.90) at selected concentrations. Antibacterial potential of selected propanamide was checked by means of agar well diffusion assay. Some compounds showed reasonable antibacterial potential against the bacterial strains. Highest antibacterial potential  $14.66 \pm 2.51$ mm was given by compound 10 at a concentration of 2mg/mL against *Agrobacterium tumefaciens* (KF 875446) while lowest antibacterial activity  $1.66 \text{mm} \pm 2.8$  was given by compound 10 at a concentration of 0.5mg/mL against *Bacillus subtilis*. These compounds also showed low antiradical activity using 1, 1-diphenyl 2-picrylhydrazyl (DPPH) antioxidant test at different concentrations. Compound 03 showed highest antiradical activity (34%) at the concentration of 100µg/mL while compound 10 showed least antioxidant activity at all the concentration tested. Propanamide derivatives showed mild insecticidal activity in the range of 20-30% against *Tribolium castaneum*. This study suggests the beneficial effects of propanamide and their usage in pharmaceutical industries as a potent anticancerous and antimicrobial agent.

**Keywords:** Propanamide derivatives, antitumor activity, antioxidant potential, antimicrobial, synthetic compounds.

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## ADHERENCE TO LIFESTYLE ADVICE AND TREATMENTS IN PAKISTANI PATIENTS WITH TYPE 2 DIABETES MELLITUS

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### ABSTRACT

Type 2 diabetes mellitus (T2DM) is a chronic disease that has become a major health care concern, especially in developing countries like Pakistan where prevalence is increasing rapidly. Lifestyle modification and appropriate pharmacotherapy are shown to improve blood glucose levels, lipid abnormalities and blood pressure. It is not known how many patients adhere to advice and drugs prescribed. This study aimed to determine adherence to lifestyle, self-management and drugs, with specific evaluation of tobacco use, exercise, diet, glucose monitoring, foot care and medication use. A cross sectional hospital based study was conducted among patients attending the diabetic clinic at the Aga Khan University Hospital, using a structured questionnaire. Adult patients with T2DM, and with at least one year duration of diabetes were included in the study. Participants were aged between 32 and 92 years with a mean age of 55.7 years (SD=10.7). Mean duration of diabetes was 10.7 years (SD=7.7). Majority (94%) of the patients were literate. Around half (47.3%) of the patient have had achieved glycemic target (HbA1c <7%). Above target glycemic control was more common among patients with ischemic heart disease (68.1%), neuropathy (64.8%), those on insulin (62.5%). Self-Reported adherence for blood sugar monitoring (39.8%), dietary advice (75%), oral agents (91.1%), insulin (95.6%), physical activity (23.5%), tobacco use (56.6%) and foot care (56.1%) was noted. Partial adherence reported for: Self Monitoring of Blood Glucose (SMBG) (50.7%), diet (22%), oral agents (8.9%), insulin (4.3%) and physical activity (14.8%). Good adherence to physical activity was found in males with college degree. The highest percentage of tobacco use (33.3%) was reported among businessmen. We noted low adherence to advice for physical activity, tobacco use and SMBG. This was a selected group visiting a teaching hospital. This will need to be studied further in the community and efforts are required to motivate patients.

**Keywords:** Type 2 Diabetes Mellitus, HbA1c, Adherence, Lifestyle factors

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## WEEDS AND THEIR IMPACT ON BIODIVERSITY AND SOCIO-ECONOMIC STATUS OF THE PEOPLE IN PAKISTAN

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### ABSTRACT

Weeds are becoming a threat to the biodiversity all over the world and specially in Pakistan. Two plant viz., *Silybum marianum* and *Parthenium hysterophorus* are naturally grown in many parts of the country. Due to higher rainfall in the past few years, the growth and reproduction of *Silybum marianum* has much more increased. Due to bigger vegetative growth, the plants are collected by the poor people to use as biogas. Thus their seeds are disseminated to the far off areas hence this plant has taken the shape of invasive weed in Pakistan. Similarly, *Parthenium hysterophorus* is used in bouquets by the poor farmers and thus is a source of income. This might be reason that many people that are susceptible to the *P. hysterophorus* pollens are passing through severe allergic problems. Both these plants are invasive and deprive millions of people of their food due to reduction of crop yields. Field studies were conducted to decipher the competitive ability of *S. marianum*. It was found the environmental conditions favoured this plant against wheat and thus has proved more competitive with high seed production. These plants are used for different purposes but on the other hand it is a serious threat to biodiversity as it leads towards monoculture. Hence the spread of these plants should be prevented and other alternatives should be searched for the inhabitants of the localities.

**Keywords:**

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## ASSESSMENT OF THE DEMAND OF COAL IN BRICK KILN INDUSTRIES OF BALOCHISTAN AND THE POSSIBLE ALTERNATIVES OF COAL BY USING LEAP MODEL

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### ABSTRACT

The present study analyses the demand of coal energy in the brick kilns of Balochistan province in Pakistan. The LEAP (Long Range Energy Alternative Planning System) was used to evaluate the coal energy demand and its emissions calculated from the base year of 2004 till 2030. This paper basically covers the energy demand and emissions from the base year to the end year. Use of Solar energy and wind energy are the suggested alternatives sources of energy for coal energy conservation in LEAP. Alternatives solar energy and wind energy having potential of 2.5% and 5% respectively. Therefore by using these cost effective alternative energy resources in Balochistan, the problems like energy crisis, emissions and pollution can be reduced.

**Keywords:** Emissions, Solar energy, Wind energy, Leap Model, Alternative scenarios

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## USE OF NEEM LEAVES AS SOIL AMENDMENT FOR CONTROL OF COLLAR ROT DISEASE OF CHICKPEA

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### ABSTRACT

Collar rot disease of chickpea (*Cicer arietinum* L.) is caused by *Sclerotium rolfsii* and results in significant yield losses annually. The present study was carried out to manage this disease by extracts and soil amendment by leaves of neem (*Azadirachta indica*). In a laboratory bioassay, the effect of seven concentrations (0.5 to 3.5%) of methanolic leaf extract of *A. indica* was studied against *in vitro* growth of the target fungal pathogen. All the concentrations of methanolic extract significantly reduced fungal biomass by 86-90% over control. In a pot trial, *S. rolfsii* inoculated soil was amended with 1, 2 and 3% dry leaves of *A. indica* and their effect was studied on mortality and growth of chickpea plants. A commercial fungicide mencozeb was used for reference. The highest plant mortality (56%) after 30 days growth was recorded in positive control (only inoculated with *S. rolfsii*) as compared to negative control. There were 49%, 38% and 38% plant mortality in 1%, 2% and 3% *A. indica* leaf amendment treatments, respectively. The lowest plant mortality (28%) was recorded in mencozeb treatment. Mencozeb as well as 1 and 2% leaf amendments significantly enhanced shoot and root dry biomass over positive control. The present study concludes that 2% *A. indica* leaf amendment is useful for management of collar rot disease of chickpea.

**Keywords:**

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## PHYTOMEDICINAL POTENTIAL OF SELECTED PLANT SPECIES FROM KUND, VILLAGE, KAHUTA PAKISTAN

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### ABSTRACT

Therapeutic properties of plants are due to production of secondary metabolites which are produced in response to different environmental conditions or as defense mechanisms. Different biotic and abiotic factors influence and regulate the quality and quantity of phytochemicals in plants. Particular areas are responsible for providing necessary conditions which results in the sustainable production of bio-active agents in medicinal plants. Therefore it is important to consider the area and environment of medicinal plant while characterizing its pharmacological potential. Three different plant species namely *Adiantum incisum*, *Alternanthera pungens* and *Trichodesma indicum* with well-known folk medicinal uses were selected for assessment of phytomedicinal potential from an unexplored locality Kund village, Pakistan. Plant extracts, with varying polarity index of solvents including aqueous (9.0), methanol (5.1) and n-hexane, (0.0) were prepared. Phytochemical analysis confirmed presence of alkaloids, flavonoids, saponins, phenolics, tannins and terpenoids. Significant antitumor potential ( $P < 0.005$ ) was revealed by potato disc antitumor assay and *Trichodesma indicum* n-hexane extract was found to be the most effective (85% tumor inhibition). Insecticidal assay revealed methanol and hexane extracts of *Adiantum incisum* effective against *Callosobruchus chinensis* (100% mortality in 24 hours) and *Sitophilus oryzae* (100% mortality in 48 hours) respectively. Aqueous extract of *Alternanthera pungens* was determined to be most effective against *Tribolium castaneum* (100% mortality after 10 days). All the plants showed no antifungal activity against *Aspergillus flavus*, while moderate (50.73% inhibition) to significant activity (78.3%) was shown by *Adiantum incisum* aqueous and methanolic extracts against *Aspergillus niger*. Hexane extract of *Trichodesma indicum* revealed significant antifungal activity (98.9 % inhibition) against *Mucor* specie only. Significant DPPH radical scavenging potential (96.72% - 60.33% inhibition activity) and Ferric power reducing ability (absorbance values very close to the standard ascorbic acid) was displayed by methanolic extracts of all plants. Present study supports the extensive use of these plants in folk medicines and elucidates elaborative investigations for determining the antimicrobial, antioxidant and anti-cancer potential of these plants in vivo and also promotes isolation and characterization of important secondary metabolites in these plants for production of pure therapeutic compounds.

**Keywords:** Kund village, *Adiantum incisum*, *Alternanthera pungens*, *Trichodesma indicum*, antitumor potential, phytoconstituents

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## GENETIC ASSOCIATION OF GNB-3 PROTEIN C825T POLYMORPHISM WITH CARDIOVASCULAR DISORDERS IN PAKISTANI POPULATION

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### ABSTRACT

**Introduction:** Cardiovascular disease (CVD) is a leading cause of morbidity and mortality all over the world and Pakistan is no exception. Annually about 100,000 deaths occurred due to CVD and 12% of total morbidity in Pakistan is attributed to CVD. Cardiovascular disease susceptibility is not only associated with several environmental and biochemical risk factors but also customized by genetic predisposition. A common C825T polymorphism in exon 10 of the gene for the beta-3 subunit of heterotrimeric G-proteins, GNB3, has been associated in some studies with traits of the metabolic syndrome as well as cardiovascular diseases (CVD). The main role of G-protein is to translate signals from the cell surface into a cellular response. Polymorphisms of the G-protein  $\beta_3$  subunit gene have received considerable attention as a candidate gene for essential CVD relative to hypertension. Therefore, the association of the GNB3 polymorphism with CDV may be plausible and of potential clinical and scientific relevance.

**Objective:** The aim of the present study is to investigate the distribution and association of the C825T polymorphism of the GNB3 gene with cardiovascular diseases in Pakistani population.

**Methodology:** For this study 5ml venous blood sample from 100 patients and age and sex matched control individuals were collected randomly with informed consents from a primary health care hospital. All the patients were clinically evaluated for CVD, Diabetes mellitus, hypertension, and obesity. DNA was isolated from the collected blood samples. The C825T polymorphism of the GNB3 gene in the patient and control groups was determined by PCR-RFLP. Primers were designed by using Primer-3. The PCR product was then digested by Restriction Enzyme, BseDI. The results were statistically evaluated by SPSS ver. 20.

**Results:** The results strongly suggest that the C allele of the GNB3 C825T polymorphism of the G protein beta3-subunit is associated with an increased risk for the development of CVD in hypertensive patients. This polymorphism may thus be considered as a (co)factor favoring the development of cardiovascular diseases in Hypertensive patients, although the biological mechanism(s) underlying this association remain obscure.

**Conclusion:** Our findings show a significant association between the GNB3 C825T gene polymorphism and hypertension, with the CC genotype exhibiting higher blood pressure, BMI, and vascular remodeling markers.

**Keywords:** Cardiovascular disease (CVD); GNB3 Proteins; C825T Polymorphism; Hypertension

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## DEGRADATION OF POLY (E-CAPROLACTONE) (PCL) BY A NEWLY ISOLATED *BREVUNDIMONAS* SP. STRAIN MRL-AN1 FROM SOIL

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### ABSTRACT

A poly (-caprolactone) (PCL)-degrading bacterium designated as strain MRL-AN1 was isolated from soil. The bacterium was identified as *Brevundimonas* sp. strain MRL-AN1 through biochemical tests and 16S rRNA gene sequencing. Scanning electron microscopy and Fourier transform infrared spectroscopy results confirmed the degradation of PCL by strain MRL-AN1. An extracellular PCL depolymerase was purified to homogeneity by column chromatography and molecular weight was estimated to be approximately 63.49 kDa by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. PCL depolymerase could degrade not only PCL but also other aliphatic polyesters. The enzyme was stable at wide range of temperature (20–45°C) and pH (5–9) as well as stable in the presence of various metal ions, surfactants and organic solvents.

Phenylmethylsulfonyl fluoride inhibited enzyme activity that indicates this enzyme belongs to the serine hydrolase family. It is concluded from the results that the enzymes from strain MRL-AN1 might be applied in the process of biochemical monomer recycling in the polyester-contaminated environments.

**Keywords:** PCL; *Brevundimonas* sp.; biodegradation; PCL depolymerase; column chromatography

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## SERUM ADIPONECTIN AND LEPTIN LEVELS IN OBESE AND NON-OBESE POSTMENOPAUSAL HYPERTENSIVE WOMEN

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### ABSTRACT

The incidence of cardiovascular diseases is higher in postmenopausal obese women and hypertension being the most prevalent amongst them. The mechanisms responsible for the development of arterial hypertension in postmenopausal women are multifactorial and interrelated. Adipose tissue secretes a group of hormones known as adipokines, which affect the metabolism of lipids and glucose, development of atherosclerotic processes, and is involved in the development of hypertension and cardiovascular diseases. The two most significant adipokines are leptin and adiponectin. Adiponectin possesses anti-inflammatory and antioxidant properties. It prevents the development of hypertension owing to stimulation of the production of nitric oxide in endothelial cells and attenuates smooth muscle cell proliferation and migration. Moreover, it inhibits the production of tumor necrosis factor and suppresses the generation of reactive oxygen species. In females with postmenopausal hypertension, adiponectin secretion is decreased due to increased adipose tissue making them more prone to hypertension. Leptin effects regulation of body weight and metabolism. Chronic hyperleptinemia leads to hypertension due to oxidative stress, NO deficiency, enhanced renal Na reabsorption and overproduction of endothelin. This contributes to pathogenesis of postmenopausal hypertension. Postmenopausal hypertensive obese women have higher circulating levels of leptin in contrast to lowered levels of adiponectin. Hence it was important to evaluate the dynamic role between adiponectin and leptin in these women. The objective of this study was to find out whether there was any correlation between adiponectin and leptin level among different groups, and also to find any correlation between the obese postmenopausal status and hypertension. This study was a comparative study and it was conducted at Lahore General Hospital, in collaboration with PGMI. It included 92 females, divided into four groups such as normal normotensive, normal hypertensive, obese normotensive, obese hypertensive, with diagnosed hypertension whose BMI, BP and serum leptin and adiponectin were measured using ELISA. The data was analyzed using SPSS 20. In our study the mean Adiponectin level of obese hypertensive group was significantly lower as compared to obese normotensive group, normal hypertensive and obese hypertensive group. There was negative correlation between Adiponectin and serum Leptin level among all groups but it was only significant in normal normotensive group. A negative correlation was observed between blood pressure and Adiponectin in normal group. Systolic and diastolic blood pressure was significantly positively correlated with Leptin level in obese and normal groups.

**Keywords:**

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## EFFECT OF HOT EXTRUSION ON GOSSYPOL CONTENTS IN COTTONSEED MEAL

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### ABSTRACT

Cottonseed (CS) and its derived products e.g., cottonseed cakes and cottonseed hulls, have been attractive protein supplements for animal feeds and dairy animals' rations to increase fat and milk production in these animals. As a cheaper but rich source of high quality protein for supplementing animal diets, the use of cottonseed products have been continued over the last many decades. However, the presence of antinutritional compounds indiscriminate use of cottonseed or their byproducts and adversely affects the reproductive performance of dairy animals. Gossypol is a yellow pigment found in various parts of cotton plants, including seeds, of the Genus *Gossypium*. The free gossypol is biologically active and all the gossypol present in the whole cottonseeds is in this form. Processing whole cottonseeds into meal converts varying amounts of free gossypol to the bound form by binding of varying amounts of free gossypol to the proteins present in the seed. This results in a reduction of the biological activity of the compound. Gossypol is known to cause toxicity in monogastric as well as ruminant animals. The average concentration of gossypol ranged between 0.75% - 1.90%. The present study was undertaken to determine the effect of hot extrusion parameters on gossypol detoxification in cottonseed meal. The ranges of processing variables selected using Box-Behnken design were: barrel exit temperature (BET) of 80-140 °C; screw speed (SS) of 60-180 rpm and feed rate (FR) of 30-120 kg/h. The amount of gossypol reduction in extruded cottonseed meal samples ranged from 50-70%. Response variables were mainly dependent on BET, whereas significant reduction of gossypol was achieved in studied BET and SS combination of process parameters. The results of this study demonstrated that the hot extrusion can be successfully explored to reduce toxic compounds in cottonseed meal for feed purposes.

**Keywords:** Hot extrusion; Single-screw; Cottonseed meal; Gossypol; RSM

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## TYPE A AND TYPE B PERSONALITY AMONG UNDERGRADUATE MEDICAL STUDENTS: NEED FOR PSYCHOSOCIAL REHABILITATION

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### ABSTRACT

**Objectives:** To find out the frequency of Type A and Type B personality among the students of Undergraduate Medical College. To find association between student year and personality type.

**Methods:** A descriptive cross sectional study was conducted at Undergraduate Medical College, Rawalpindi from Sept. 2012 till Feb. 2013. Among 500 sample size, 100 students from each MBBS year were inducted by probability systematic sampling technique. After taking consent from the institute and students, data was collected on BECK anxiety inventory (BAI) questionnaire. According to BAI scale, students were identified as Type A or B personality. Data was analyzed using SPSS version 20. To find association between student year and personality type, Chi-square test of significance with 95% confidence level was used.

**Results:** First, second, third, fourth and final year students had 5 (1%), 6 (1.2%), 11 (2.2%), 13 (2.6%) and 19 (3.8%) type A personality respectively. Among all the study participants (n=500), total number of type A was 54 (10.8%) and type B personality students were 446 (89.2%). Type A personality was 29 (11.6%) in female students (n=250) and 25 (10%) in male students (n=250). Association between student year and personality type was significant (p=0.010) at 95% confidence level.

**Conclusion:** Type A personality students existed in every class and there was a gradual increase in the number of type A personality students from 1st year to final year in an undergraduate medical college of Rawalpindi. Significant association was observed in student year and type a personality.

**Keywords:** Medical, Students, Personality disorder.

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## FREQUENCY OF CO-EXISTENCE OF DENGUE AND MALARIA IN PATIENTS PRESENTING WITH ACUTE FEBRILE ILLNESS

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### ABSTRACT

**Objective:** To find out the frequency of co-existence of malaria and dengue fever in patients presenting with acute febrile illness.

**Methods:** The descriptive cross-sectional study was conducted at the Military Hospital Rawalpindi from June to November 2012. A total of 500 patients with complaint of acute febrile illness were selected after applying the inclusion and exclusion criteria. Preliminary data was collected on a pretested proforma. Blood samples of patients were tested for dengue serology and malaria parasite. Results were entered in respective proforma. Co-existence was considered present when a patient had both dengue serology and malaria parasite slide positive. SPSS 20 was used for data analysis.

**Result:** Of the total, 349 (69.8%) were males and 151 (30.2%) females. Dengue serology was positive in 16 (3.2%); 81(16.2%) had malaria parasite slide positive; 403 (80.4%) had none of the two findings. Co-existence of both dengue and malaria was nil among the whole sample. In males, 67 (13.4%) had malaria, while 11 (2.2%) had dengue. In females, 14 (2.8%) had malaria, while 5 (1%) suffered from dengue fever.

**Conclusion:** Co-existence of dengue and malaria was zero per cent in 500 patients visiting Military Hospital Rawalpindi. More studies shall be conducted to find out whether the reason of having zero percent co-existence is that dengue or/and malaria epidemic did not occur in 2012 or whether there are some other factors involved.

**Keywords:** Co-existence, Dengue, Malaria, Acute febrile illness.

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## KNOWLEDGE, ATTITUDE AND PRACTICE REGARDING FOLIC ACID DEFICIENCY; A HIDDEN HUNGER

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### ABSTRACT

**Objectives:** To find the Knowledge Attitude and Practice regarding Folic Acid Deficiency among Women of Child Bearing Age (WPCBA). To find out the Association of Education Level with Practice of Folic Acid in WPCBA.

**Methods:** A Descriptive cross sectional study (Knowledge Practice and Attitude) was conducted at Military Hospital and Combined Hospital Rawalpindi from September 2012 to February 2013. About 400 married females of age group 21-42 years were included by convenient sampling technique. After taking informed verbal consent, a closed ended interviewer administered questionnaire was filled. Data was entered and analyzed using SPSS version 20.

**Results:** Mean age of the respondents was 30.31 +5.280 years. Illiterate and literate were 165 (41.25%) and 235 (58.75%) respectively. The knowledge regarding folic acid need was 172 (43%). Only 161 (40.25%) thought that folic acid deficiency in pregnant women results in abnormality in newborn. In pregnancy, 205 (51.25%) had received folic acid supplementation. Association between education level and practice of folic acid was significant ( $p= 0.009$ ) at 95% confidence level.

**Conclusion:** Knowledge regarding folic acid deficiency among WOCBA was low along with the poor attitude. Practice was also not satisfactory. Education status plays important role in preventing micronutrient deficiency.

**Keywords:** Attitude, Folic acid Deficiency, Knowledge, Practice.

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## MICROBIOLOGICAL CONTAMINATION IN WATER FILTRATION PLANTS IN ISLAMABAD

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### ABSTRACT

**Objective:** To determine the frequency of microbiological contamination of water in different water filtration plants in Islamabad.

**Study Design:** Descriptive cross-sectional study.

**Place and Duration of Study:** Water Filtration Plants (WFP) in different sectors of Islamabad, from April to September 2012.

**Methodology:** Water samples were collected in sterilized bottles according to the standard water sampling protocol from site and transported to Pakistan Council for Research in Water Resources (PCRWR) for analysis. Microbiological quality of water was determined in terms of total coliforms (< 2.0 MPN/100 ml) and Escherichia coli (< 2.0 MPN/100 ml). Microbiological contaminated water was defined the sample which had more than 2.0 MPN per 100 ml of either total coliforms or Escherichia (E.) coli.

**Results:** Thirty two WFP were analyzed for microbiological contamination. E. coli was present in 8 (25.0%) water samples, while 24 (75.0%) water samples were free from it. Total coliforms were present in 13 (40.6%) of the samples of WFP, while 19 (59.3%) samples were free from total coliform. Faecal coliforms were present in 8 (25.0%) and absent in 24 (75.0%) samples. Both E. coli and total coliform were present in 8 (25.0%) samples. Nine (59.3%) WFP were free from E. coli, total coliform and faecal coliform. Statistically, no significant association was found ( $p > 0.05$ ) between microbiological contamination and the sectors.

**Conclusion:** Less than half of the water samples of the WFP were contaminated while certain sectors showed more frequent contamination than others.

**Keywords:** Microbiological contamination. Water filtration plants. Islamabad. Total coliform. Escherichia coli.

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## EFFECTS OF EMS ON FOOT DROP ASSOCIATED WITH GRADE III WOUNDS CASE REPORT

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### ABSTRACT

A 51 year old lady; known case of diabetes mellitus, post wound debridement i.e. 4 open wounds of grade III presented to us with foot drop, with prominent sensory deficit over right lower leg/foot i.e. 0 on Nottingham scale for impaired sensation, marked pedal edema and 5/10 – 6/10 pain on VAS during day and night respectively, Wounds were poorly granulated and foul smelling. Physiotherapy sessions were planned including twice a day electrical muscle stimulation sessions, strategies to decrease edema and improve muscle action which resulted in noticeable improvement in motor and sensory ability, pain levels, edema and psychological status of patient. Thus, this study gives evidence of the effect of Electrical muscle stimulation in grade III open wounds associated with motor/sensory weakness post-surgery.

**Keywords:**

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## LIPOLYSIS STIMULATED RECEPTOR BEYOND THE LIPOPROTEIN RECEPTOR

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### ABSTRACT

The lipolysis stimulated receptor (LSR) after free fatty acid triggered functional activation recognizes apolipoprotein B/E-containing lipoproteins and involves in the clearance of triglyceride-rich lipoproteins (TRL). The LSR expresses abundantly in major body organs and tissues like liver, lung, intestine, kidney, ovaries and testes and less abundant in muscle and heart. Homozygote LSR knockout mice were not viable and their liver was much smaller than that of their littermates, indicating that the expression of LSR is critical for liver and embryonic development. At hepatic level, the LSR contributes significantly in clearance of foodborne lipids. Indeed, it has an increased affinity for the lipid particles containing apoB and apo E-rich in TRL including CM. The reduced expression of the LSR in mice leads to a prolongation of the life of the lipoprotein in the post prandial phase and therefore to a higher post-prandial lipemia. Expression and activity are directly related to lipid and energy homeostasis and reducing the LSR activity results in increased lipids in the circulation. Colocalization studies revealed that LSR<sup>+/+</sup> exhibited mice was significantly different as compared to control LSR<sup>+/+</sup> with cholesterol content accumulation accompanied by significantly altered distribution of LSR in the membrane, and decreased intracellular lipid droplets in the cerebellum and hippocampus of old LSR<sup>+/+</sup> mice, as compared LSR<sup>+/+</sup>. The potential role of LSR in brain cholesterol distribution is particularly important in preserving neuronal integrity and thereby cognitive functions. LSR is specifically expressed at tricellular junctions and its expression correlates with the onset of blood barrier membrane formation during embryogenesis. Blood barrier membrane does not seal during embryogenesis in LSR<sup>-/-</sup> knockout mice with multiple sclerosis and a middle cerebral artery occlusion. Moreover; LSR altered gene expression of pathways involving in transformation and tumorigenesis as well as enhanced proliferation and survival in anchorage independent conditions, highlighting that reestablishment of LSR signaling promotes aggressive/tumor initiating cell behaviors. The present work shows that LSR is multifunctional receptor in different organs. The organ specific function of LSR is unmet to explore further.

**Keywords:** LSR, receptor, lipoprotein, lipid homeostasis, cell behaviours

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## COMPARATIVE GENOTOXIC STUDY OF MAJOR INDIAN CARP *LABEO ROHITA* AT RAVI, HEAD BULLOKI (PATTOKI) AND SATLUJ, HEAD SULEIMANKI (PAKPATTAN SHAREEF).

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### ABSTRACT

In relevance with environmental toxicity, expression of toxicity symptoms in the form of genetic damage and nuclear abnormalities like micronuclei under stress of heavy metals in fish is quite supportive. Comet assay and Micronucleus test are advantageous biomonitoring tools for detecting damage on level of individual cell. Present research work was conducted to estimate comparative genotoxicity in *Labeo rohita* by emphasizing on DNA damage (Tail Length) and nuclear abnormalities by Comet assay and Micronucleus Assay in research laboratory of department of Zoology, GCU, Faisalabad. Fresh fish samples weighing 1kg, 1.5kg and 2kg were captured from four sites i.e. BU, BD, SU, SD, blood was drawn into EDTA vials and kept in chilled ice box. Later blood samples were refrigerated at 4°C in laboratory. Comet Assay and Micronucleus assay were applied with slight modifications to all blood samples, slides were examined, comet tail length was scored and cells with micronuclei were counted. Heavy metals i.e. Cu, Cr, Zn, Ni, Pb, Mn and Fe in water and sediment of sites were analyzed by atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan). Mean weight of *Labeo rohita*, BU: 1391.90±279.8, BD: 1442.93±363.71, SU: 1593.66±337.24 and SD: 1653.26±436.56. Mean length measured BU: 40.3±2.63, BD: 42.0±2.72, SU: 44.7±4.60, SD: 44±4.7. Mean comet tail length BU: 8.7±1.11, BD: 7.56±0.86, SU: 13.67±3.32, SD: 12.31±2.82. Micronuclei counted BU: 3.12±1.24, BD: 4±1.60, SU: 3.75±1.98, SD: 3.87±1.45. Heavy metal concentration in water samples BU: Fe> Zn> Mn> Pb> Cr> Cu> Ni, BD: Fe> Pb> Mn> Zn> Ni> Cr> Cu, SU: Fe> Pb> Mn> Zn> Cr> Ni> Cu, SD: Fe> Mn> Zn> Ni> Cr> Cu> Pb> Ni, SD: Mn> Zn> Pb> Cu> Fe> Cr> Ni. Conclusively, Relative genotoxicity between Satluj and Ravi indicates high genotoxic potential in *Labeo rohita* of Suleimanki region as it receives polluted water from the Ravi on one side and from India on the other side. Natural and anthropogenic sources introduce heavy metals in soil and in fresh water thus disturbing the water quality and polluting the natural aquatic ecosystem creating danger for the fish.

**Keywords:** Bulloki upstream, BU: Bulloki downstream, BD: Suleimanki Upstream, SU: Suleimanki downstream, SD: Environmental Toxicity, DNA damage, *Labeo rohita*, Heavy metals.

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## ENVIRONMENTAL IMPACTS OF A 50MW WIND FARM AT ORMARA, BALOCHISTAN

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### ABSTRACT

Currently in Pakistan, dependency on the fossil fuels is much higher (64.2%) and renewable energy sources have not yet penetrated enough in the energy mix with hydro accounting for 29 % and nuclear contributing only 5.8% in the total energy mix. Using fossil fuels is causing immense harm to the environment by emitting greenhouse gases and other pollutants upon burning. Because of which, the country spends 6% of GDP on environmental impacts which has affected its economics adversely. Knowing the adverse health and fiscal impacts of greenhouse gases, most countries have therefore shifted to alternative renewable energy sources to reduce dependency on fossil fuels and minimize environmental degradation. Pakistan is still emerging in the renewable energy technologies and has not yet exploited its natural energy sources. This study aims to determine the environmental benefits of utilizing wind energy at Ormara, Baluchistan when compared to fossil fuels using RETScreen software. This study also calculated the carbon credits that the investor will earn by wind power project at Ormara Balochistan.

### Keywords:

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## DETERMINATION OF SERUM COPPER AND BODY MASS INDEX IN MYCOBACTERIUM PULMONARY TUBERCULOSIS PATIENTS BY ATOMIC ABSORPTION SPECTROSCOPY

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### ABSTRACT

**Background:** Mycobacterium pulmonary tuberculosis (MTB) is one of the leading causes of deaths in the globe. Though it is treatable, but still a most common health problem of developed nation. Alterations in trace elements have been consequential of clinical syndrome. The aim of study was to determine the concentration of serum copper in active pulmonary tuberculosis patients and compared with healthy volunteers. Copper was analyzed by means of Atomic Absorption Spectroscopy (AAS).

**Material & Method:** A total of 100 active pulmonary tuberculosis patients were nominated from Liaquat University of Medical & Health Sciences, Jamshoro/Hyderabad, Rajputana Hospital, Hyderabad and Institute of Chest Disease, Kotri, Sindh. The subjects were enlisted from both genders. The age group was in between >15-70 years. Blood samples of active pulmonary tuberculosis patients were collected and stored immediately before analysis. The healthy volunteers were recruited with non-symptoms of pulmonary tuberculosis. Before collection of data, approval was taken from each hospital. Consent was also taken from patients and healthy participants and was informed about the study. Researcher was used face mask and surgical gloves prior to collection. Serum copper was analyzed by Flame Atomic Absorption Spectrophotometer.

**Results:** Out of 100 PTB patients, 49% was male while 51% was females. Most of the patients were illiterate (66%) and were belonged to poor socioeconomic status. In our study, 85% patients were found underweight and only 15 were normal. These observations were made according to the criteria of WHO. Serum copper concentration in PTB patients was elevated ( $166.2 \pm 29.6$  mg/dl) as compared to healthy volunteers ( $127.8 \pm 26.4$  mg/dl).

**Conclusion:** It is concluded that besides the other clinical findings, the trace elements analysis must also be observed which may be helpful in the diagnosis and prognosis of infectious diseases like pulmonary tuberculosis.

**Keywords:** Mycobacterium Pulmonary Tuberculosis, Active TB, Blood, Copper, BMI

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## DREB GENE FROM *GOSSYPIMUM ARBOREUM* L. CULTIVAR FDH-786: IDENTIFICATION, CHARACTERIZATION, HOMOLGY MODELING AND PROTEIN-PROTEIN INTERACTIONS

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### ABSTRACT

Drought is one of the most yield depressing factors of major crop plants including cotton. For mitigating the drought stress, a number of genes identified and characterized, are up-regulated or down-regulated. Among these, genes conferring dehydration-responsive element binding (DREB) proteins play a major role in overcoming the stress. Cotton, an important natural fiber producing crop, sustains textile industry worldwide, is under investigation to improve its genome against biotic and abiotic stresses. The genome of *Gossypium arboreum* L. a diploid cotton species, contains many important genes combating drought, that can be explored to identify these genes. In this study, a DREB gene was identified in *G. arboreum* (GaDREB), cloned and sequenced. Phylogenetic analysis of GaDREB with twenty previously reported DREB gene sequences showed its highest homology with *Salicornia bigelovii* DREB gene. The Gene promoter region was found and protein 3D models were determined. The results indicated that 98% of the residues were found in the favored region. DREB was interacted with the NAC, MYB, AREB, ABRE, and DRE/CRT genes which are involved in various drought stress pathways. Findings of the present studies validated the involvement of gene in conferring the drought tolerance and its interaction with the other drought responsive genes. The findings of the study will facilitate the utilization of *G. arboreum* DREB gene for improving the drought tolerance in tetraploid cultivated cotton species.

**Keywords:** DREB, *G. arboreum*, ABA pathway, Drought, Protein-protein interaction, Homology modelling

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## MP3D: MEDICINAL PLANTS DATABASE FOR DRUG DESIGNING

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### ABSTRACT

In the era of computer aided drug designing (CADD), medicinal plants are the main natural resources for the discovery and development of new drugs. The information about the attributes of medicinal plants is continually increasing on the online web resources such as Google Scholar and PubMed. This prompts the need to construct and design database management system that allows proper data storage, retrieval and management with user-friendly interface. An extensive database having information about classification, activity and ready-to-dock library of medicinal plant's phytochemicals is therefore required to assist the researchers in the field of CADD. Medicinal Plants Database for Drug Designing (MP3D) is an open access database of phytochemicals derived from medicinal plants that contains extensive information regarding the classification, genus, therapeutic activities, targets and literature references of various phytochemicals. At present, MP3D contains information about more than 5,000 phytochemicals from around 1,000 medicinal plants with 80 different activities, more than 900 literature references and 200 plus targets in online and downloadable database. Both web-based and downloadable databases will save the time of researchers from searching and organizing the required information for docking of these phytochemicals. MP3D is publically available at: <http://bioinform.info>

**Keywords:** Medicinal plants, Phytochemicals, Database management system, Computer aided drug designing, ready to dock phytochemical library

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## MICROBIAL POTENTIAL OF LIPASE PRODUCTION FROM DIFFERENT INDUSTRIAL EFFLUENTS

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### ABSTRACT

Lipases hold significant position in the field of biotechnology due to its ability to catalyze esterification, interesterification, and transesterification reaction in nonaqueous media. In the present study four different lipase producing bacteria were isolated from effluents of leather, marble and textile industry. All the strains (L9, L15, L16 and L20) isolated were gram negative and subjected to both biochemical and molecular characterization. BLAST results for 16S rDNA sequences revealed that L9 (KJ748674) strain had 99% similarity with *Acinetobacter lwoffii*. L15 (KJ748675) and L16 (KJ864927) strains showed 98% and 99% sequence homology with *Acinetobacter junni* and strain L20 (KJ873872) belongs to *Alishewanella agri* sp. For enzyme production studies, pH and temperature were optimized on two different enrichment media. Comparison of two different enrichment media showed that medium containing peptone, yeast extract, NaCl and olive oil gave optimum production for all the strains. *Alishewanella agri* L20 (isolated from textile industry effluent) had growth at all temperatures (30, 37, 42 and 45°C) in medium containing yeast extract, NaCl and olive oil. In comparison to all the reported strains of the present study, *Alishewanella agri* (L20) had maximum enzyme production of 17 U/ml at elevated temperature of 45°C. Partial purification and SDS-PAGE analysis showed that protein bands of lipases were ranged between 35- 66.2 kDa. It is evident that high cost of lipase has generally reduced its exploitation at industrial level. Therefore, screening and identification of these lipolytic strains will act as a significant addition to the database on lipase research and its application at industrial level.

**Key Words:** Lipase, Industrial effluents, *Acinetobacter junni*, *Acinetobacter lwoffii*, *Alishewanella agri*, SDS-PAGE

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## USE OF SMS REMINDERS FOR IMPROVING FOLLOW-UP RATES AT A MEDICAL OUTDOOR CLINIC

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### ABSTRACT

**Background/ Objectives:** Patients often fail to follow up with doctors for repeat consultations. This study was done to evaluate the impact of short message service (SMS) reminders on rates of follow up visits at a medical outdoor clinic.

**Methods:** This prospective observational study was carried out at 1 Mountain Medical Battalion from February to March 2015. Adult patients visiting the medical outdoor clinic of the institute were included in this study if they were to follow up at the same clinic again in future. Their demographic data including age, gender, level of education and mobile telephone number were recorded. At the end of consultation, all of them were verbally told about the day and date of next hospital visit. The same information was also written down on their prescription records in Urdu language. Each day, half of the patients expected to follow up on next day were picked up randomly and SMS messages were sent to them during working hours. Receipt of messages was confirmed indirectly through cellular network based delivery reports. The attendance of all patients was marked next day.

**Results:** There were a total of 366 patients (178 males and 188 females), having a mean age of  $52.08 \pm 15.06$  years. Follow up rate on the day of appointment was 57.38% (210 patients). SMS reminders were sent to 181 patients, 119 (65.75%) of whom followed up the next day. Of the 185 patients not sent SMS reminders, 91 (49.19%) followed up. This difference in follow up rates amongst the two groups was statistically significant ( $P=0.002$ ).

**Conclusion:** SMS reminders should be sent to all patients as they improve follow up rates in medical outdoor clinic.

**Keywords:** office visits, reminder systems, no- shows, attendance

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## MODULATION OF METHYLPHENIDATE-INDUCED PLACE-PREFERENCE, SENSITIZATION, MEMORY AND 5HT-1A EXPRESSION BY BUSPIRONE IN RATS

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### ABSTRACT

Methylphenidate, a psychostimulant, is a drug of choice for treating symptoms of Attention deficit hyperactivity disorder (ADHD). Evidence suggests that the drug is addictive and abused by patients treated for ADHD. Previously, a study reported from our lab that co-administration of buspirone at 1mg/kg but not higher doses, can inhibit apomorphine-induced addiction in rat models. So, we hypothesized that co-administration of buspirone at doses of 1 mg/kg and 2 mg/kg can also modulate methylphenidate-induced addiction. We therefore investigate effect of methylphenidate, buspirone and their co-administration on conditioned place preference and behavioural sensitization. The effect of repeated administration is also monitored on spatial memory and 5HT-1A receptor expression in the nucleus accumbens (NAcc) and prefrontal cortex (PFC). With reference to our previous finding, we have selected a dose of methylphenidate (2.5 mg/kg) which produced modest and significant increase in learning acquisition and memory retention in Water maze test on single administration. Thirty six albino female Wistar rats were randomly assigned to six equal groups: (i) Water + Saline, (ii) Water + Buspirone (1mg/kg), (iii) Water + Buspirone (2mg/kg), (iv) Methylphenidate + Saline, (v) Methylphenidate + Buspirone (1mg/kg), (vi) Methylphenidate + Buspirone (2mg/kg). Potential addictive effects of drugs were monitored in conditioned place preference paradigm and drug-induced sensitization was also determined. Water maze test was used to monitor memory acquisition (2 h after training), retention (day next to training). Later, qRT-PCR was done to check expression of 5HT-1A receptor in the two brain regions. We find that methylphenidate at a dose of 2.5 mg/kg do not produces significant place preference but elicited motor activity which is prevented in rats co-injected with buspirone (1.0mg/kg and 2.0 mg/kg). Repeated administration of methylphenidate do not produces significant increase in memory but inhibits buspirone-induced impairment of spatial memory. We also report that 5HT-1A receptor mRNA expression is decreased in methylphenidate, buspirone as well as co-injected animals. The findings may help to improve therapeutics in ADHD.

**Key words:** Methylphenidate, Buspirone, Place-preference, Sensitization, Memory, 5HT-1A receptor, qRT-PCR.

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## PRODUCTION OF MONOCLONAL ANTIBODY AGAINST DOMOIC ACID (DOM) BY MURINE HYBRIDOMA USING CONDITIONED CELL CULTURE MEDIUM *IN VITRO*

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### ABSTRACT

Domoic acid (DOM) is a highly potent marine toxin which is produced by *Pseudo nitzschia*. When heavily intoxicated, it causes amnesic shellfish poisoning (ASP) globally in human patients living at coastlines. A murine hybridoma (C4H5) cell line was developed *in vitro* by fusion of Sp2/0 myeloma with BALB/c splenocytes. Cell culture medium supplemented with specific nutritional elements and optimal controlled environmental conditions have significant role in growth of hybridoma *in vitro*. In present study, the influence of carbohydrates on the growth viability of hybridoma (C4H5) have been investigated. Carbohydrates are important nutrient organic elements which considerably enhance the development of cells and the production of monoclonal antibodies (MAb) respectively. Brief research has been done on their effects on potentially essential cell growth issues, such as production of MAb and viability of the hybridoma. Supplementation of basic cell culture medium was conditioned with modification of glucose, maltose, galactose, fructose concentrations and with the addition of fetal calf serum have been studied. This amendment contributed an improvement in the cell growth and protein production. Significant increase in cell growth and yields by factor of 1.7, 2.0 and 2.2 were observed relative to control (>40% glucose).

**Keywords:** Domoic acid, Amnesic shellfish poisoning, Cell culture, Hybridoma, Monoclonal antibody, Fetal calf medium, Carbohydrates

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## INCREASE IN SEROTONIN AND DOPAMINE METABOLISM FOLLOWING SUBCHRONIC ADMINISTRATION OF NIGELLA SATIVA L. AND OLEA EUROPAEA L. OIL: RELATIONSHIP WITH INDUCED COGNITIVE AND ANOREXIGENIC EFFECTS

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### ABSTRACT

**Introduction:** Recent studies indicate that the prevalence of overweight and obesity in Pakistani population is 25%. A requirement for memory enhancing agent is also substantially increasing in the modern life style. The control of appetite is thought to be mediated by a number of hormones as well as the monoamines, including serotonin (5-hydroxytryptamine, 5-HT), dopamine, and noradrenaline. Phytodrugs (extracted from plants) have been used extensively in the past decades as a component of traditional & complementary/alternative medicine to treat various diseases. Both *Nigella sativa* L. (Black seed or *Kalonji*) (Family: Ranunculaceae) and *Olea europaea* L. (olive or *Zaitoun*) (Family: Oleaceae) have been traditionally used for a number of therapeutic purposes while recent studies show a potential neuroprotective effect of these oils in rats.

**Objective:** The aim of present study is to further investigate monoaminergic responses to subchronic administration of *Nigella sativa* L. and *Olea europaea* L. oil and to relate it with the behavioral effects in rat models.

**Methods:** Skinner's box activity, open field, and Morris water maze tests were carried out for behavioral studies. Brain samples were analyzed for serotonin, dopamine, noradrenaline, and their metabolites via HPLC-EC (High-Performance Liquid Chromatography with Electrochemical detection). For statistical analysis of data, version 16.0 of the SPSS computer program was employed.

**Results:** We find that subchronic oral administration of Black seed oil but not olive oil in doses of 10 and 25  $\mu\text{L } 100\text{g}^{-1} \text{ day}^{-1}$  significantly ( $p < 0.01$ ) decreases food consumption but none of the oil decreases body weight. Low dose ( $10 \mu\text{L } 100\text{g}^{-1} \text{ day}^{-1}$ ) of olive oil increases daily water intake and motor activity in Skinner box as well as open field. Both Black seed and olive oil ( $25 \mu\text{L } 100\text{g}^{-1} \text{ day}^{-1}$  p.o) significantly ( $p < 0.01$ ) improve long term memory in Morris water maze test. 5-hydroxyindole acetic acid (5-HIAA), the predominant metabolite of 5-HT and homovanillic acid (HVA), the metabolite of dopamine, increased in rats treated with Black seed oil. 5-HT levels increased only in rats treated with high dose of Black seed oil. No effects were observed on noradrenaline levels.

**Conclusion:** Memory enhancing effects of both oils are largely explainable in terms of an increase in dopamine metabolism. If an increase in brain 5-HT in Black seed oil treated animals is involved in its anorectic effects, it is not clear why anorexia is not produced in olive oil treated animals. Moreover, motor activity enhancing effects of olive oil are explainable in terms of an increase in dopamine neurotransmission. But it is not clear why high dose of olive oil and both doses of Black seed oil do not produce an increase in motor activity. The present study supports the notion that both of these oils may help to improve cognitive function. More conclusive studies on other neurotransmitters and hormones involved in motor activity and control of appetite will help to develop herbal remedies for the treatment of obesity and Attention-Deficit/Hyperactivity Disorder (ADHD).

**Keywords:** Rats; Serotonin; Dopamine; Anorexigenic; Memory; Olive; Black seed

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## ANALYSIS OF TUMORS THROUGH IMAGE PROCESSING TECHNIQUES

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### ABSTRACT

This paper represents and discusses the need of in depth research in DIP of Medical imaging processing. The problem faced by an engineer is, study of anatomical and physiological structure of human body. This paper will provide a guideline for engineers in their research pursuing in biomedical engineering. It is a result of personal experience faced by authors during their thesis. This paper will definitely help and provide an engineer engaged in research in this area. It consists of 4 sections; (a) discusses the literature review in detail. Review itself is divided into two major sections. (i) Medical Imaging and (ii) Digital Image Processing. Digital image processing is the fast evolving area. It basically involves examination and processing the images obtained from digital equipment. Analog processing of image is not as accurate as digital. It has various advantages over analog. Starting from lesser immunity to noise, less information can be sent over narrower bandwidth; only two levels are required as compare to various levels in analog signal etc. Digital processing is preferred even analog images are converted to digital for effective results. D.I.P. (Digital Image Processing) is applicable for both types of images. Many user-friendly softwares have led to enormous increase in the skills to enhance features, extract statistical information, and to transform digital image properties easily. Basically, D.I.P applications are not limited to any particular area. Its escalation has a great influence in Medical Field. Specially, the emerging necessity of growth and advancements in clinical diagnostics, prognostics and therapeutics. A new field of science Bio-medical Engineering has revolutionized medical discipline by providing assistance and tremendous support boundless. There are many imaging modalities are present to inspect patients tissues and organ throughout the therapy. Medical imaging has provided great assistance to medical practice. It dynamically advances and continues to-do so. Magnetic Resonance Imaging, Ultrasound, Computed Tomography etc are recommended test by medical professional. Modern medical imaging provides intensive information about human body. Cancer is a revolting disease all over the world. As world is on edge of economical shift, this is the great area to explore and research, to provide easy, cheap and effective solutions. DIP plays a key role in Cancer treatment. The death rates are increasing each year. It has become a major health problem in Pakistan. About 300,000 individuals acquire cancer every year. Due to unavailability of timely and effective treatment, majority cancer patients remained untreated. Resulting in increase in death rate. There is no statistical recorded data available for Pakistan's death rate and mortality rate due to cancer.

**Keywords:** Image processing, Digital imaging techniques, Medical Imaging, Tumor, Cancer treatment

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**G (ALPHA) 12 IS REQUIRED FOR THROMBOXANE A<sub>2</sub> TO REGULATE TUMOR CELL MOTILITY****Babar Malik, Xuejing Zhang, and Daotai Nie***Department of Medical Microbiology, Immunology and Cell Biology, Southern Illinois University School of Medicine, Springfield, IL***ABSTRACT**

Thromboxane (TX) A<sub>2</sub> is a prostaglandin produced by metabolism of arachidonic acid through cyclooxygenases and thromboxane synthase. TXA<sub>2</sub> is biologically active, mainly through activating its cognate, seven transmembrane, G protein coupled receptor. It was previously reported that thromboxane A<sub>2</sub> receptor (TP) was expressed in prostate cancer, and further activation of this receptor elicited cell contraction and modulated tumor cell motility through regulating small GTPase RhoA[1]. This study aims to identify G alpha protein(s) involved in thromboxane A<sub>2</sub> signaling in tumor cell motility.

**Methods:** The expression of G alpha proteins in the PC3MM cells was studied by real time PCR. Cell contraction assay was performed by staining cells with TRITC phalloidin. Tumor cell motility and invasion were evaluated using wound healing assay and transwell Boyden Chamber assay. To determine the roles of G alpha protein in thromboxane A<sub>2</sub> signaling, we expressed different alleles of G alpha proteins (wild type, constitutive active) in PC3MM cells and then the subsequent effects on cell contractions was determined. We also depleted G alpha protein expression by short hairpin RNA and examined subsequent changes in cell contraction and migration after activation of TP. G (alpha) 12 has been reported to play critical role in cell proliferation in vitro and tumor growth in vivo. These findings were validated by performing BrdU Incorporation assay, Cell proliferation assay and cell cycle analysis. Quantitative analysis of the epithelial to mesenchymal transition associated genes was performed by real time PCR. In vivo experiments were performed in order to validate in vitro findings.

**Results:** PC3 MM cell line had the endogenous level of all the G alpha protein (G alpha 12, G alpha i1, G alpha 11, G alpha 13), but not G alpha q. Overexpression of G alpha 12/13 increased cell contraction after activation of thromboxane A<sub>2</sub> receptor with U46619. Expression of constitutively active G alpha 12 by itself was sufficient to cause cell contraction, regardless whether TP is activated or not. We have identified two potent shRNAs that can silence the G Alpha 12. Depletion of G alpha 12 via shRNAs reduced cell contraction after TP activation. Tumor cells with depleted G alpha 12 had shown decreased tumor cell motility, invasiveness and cell proliferation. Cell cycle analysis showed that G alpha 12 depleted cells exhibit G1/G0 arrest. Quantitative expression of EMT associated genes was reduced in G alpha 12 depleted cells exhibiting increase in the E cadherin / Vimentin ratio. G alpha 12 depleted cells in comparison to scramble control show reduced rate of cell proliferation reminiscent of in vitro findings.

**Conclusion:** This study presents compelling evidence that G alpha 12 plays a major role in thromboxane A<sub>2</sub> regulation of prostate cancer invasion and metastasis. Silencing of G alpha 12 with shRNA may therefore provide a promising therapeutic strategy for prostate cancer patients.

**Keywords:**

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**EFFECT OF TEMPERATURE AND SOLVENTS ON BISBENZYLISOQUINOLINE ALKALOIDS ISOLATED FROM BERBERIS BREVISSIMA JAFRI ROOTS.**

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Valerie A. Ferro<sup>2</sup>, Alexander I. Gray<sup>2</sup> and M. Rafiullah Khan<sup>5</sup>**

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**ABSTRACT**

Bisbenzylisoquinoline alkaloids are very important class of alkaloids. *Berberis* is rich in isoquinoline and bisbenzylisoquinoline alkaloids. Three bisbenzylisoquinoline alkaloids have been isolated from the roots of *Berberis brevissima* Jafri. <sup>1</sup>H NMR spectra of two of the compounds were showing temperature effect. The <sup>1</sup>H NMR spectra were first recorded in CDCl<sub>3</sub> at 25 °C which looks like spectra of compounds mixture. Then its spectra were taken in MeOD at 25 °C, 40 °C, 60 °C and 80 °C. We have taken <sup>13</sup>C NMR, HSQC, HMBC and NOESY spectra of the compounds at 80 °C. Then we were able to characterize the structures of both bisbenzylisoquinoline alkaloids. One of the isolated alkaloids was a new nitro bisbenzylisoquinoline alkaloid while the other two were reported. These alkaloids were also tested for its anti-diabetic activities and were highly active against Protein Tyrosine Phosphatase 1B (PTP 1B), a negative insulin regulator.

**Keywords:** Bisbenzylisoquinoline alkaloids, *Berberis brevissima* Jafri, temperature, solvents

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## DECISION SUPPORT SYSTEM FOR TEA PLANT PRODUCTION USING DATA MINING

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### ABSTRACT

Pakistan is agricultural country but it is importing black tea from nineteen different countries of the world to fulfil its needs. Government of Pakistan has taken a lot steps to increase the growth and quality of tea. But such advancements still lack. "Decision Support System for Tea Plant Production using Data Mining" is an effort to help tea-farmers better understand the tea production environment to get maximum tea production. This project uses the state of the art technology of data mining to build and train model on historical data to discover relationship between tea production quantity and different parameters that affect tea production. This model uses knowledge of atmospheric temperature, soil PH level, humidity level and rainfall level recorded at black tea forms located in Shinkyari, Mansehra. Data mining model is tested on unseen testing data and high accuracy is achieved. Such model can result in significant improvement in crop production.

**Keywords:**

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## EVALUATION OF ANTIOXIDANT POTENTIAL OF RICE BRAN OIL IN REVERSING HALOPERIDOL-INDUCED TARDIVE DYSKINESIA AND SUPERSENSITIVITY OF 5-HT-1A RECEPTORS IN RATS

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### ABSTRACT

Tardive dyskinesia (TD) is a major disorder of the orofacial region resulting from chronic neuroleptic treatment. A high incidence and irreversibility of this hyperkinetic disorder has been considered a major clinical issue in the treatment of schizophrenia. Vacuous chewing movements (VCMs) in rats are widely accepted as an animal model of TD. Various studies have suggested that role of 5-hydroxy tryptamine (serotonin; 5-HT) 1A receptor in the pathophysiology and therapy of TD because repeated administration of haloperidol resulted in an increase in the effectiveness of 5-HT-1A receptor while drug with agonist activity at 5-HT1A receptors could reverse haloperidol-induced VCMs. The present study was designed to test the hypothesis that co-administration of rice bran oil (RBO) with their possible antioxidant mechanism could decrease in the effectiveness of somatodendritic 5-HT-1A receptors and attenuate the induction of VCMs and supersensitivity at 5-HT-1A receptors by haloperidol. Rats treated with haloperidol orally at a dose of 0.2 mg/kg/day for a period of 5 weeks developed VCMs which increased progressively as the treatment continued for 5 weeks. Motor coordination impairment started after the 1<sup>st</sup> week and was maximally impaired after 3 weeks and gradually returned to the 1<sup>st</sup> week value. Co-administration of RBO by oral tubes at a dose of 0.4 ml/day prevented the induction of haloperidol induced VCMs as well impairment of motor coordination. The intensity of 8-hydroxy dipropylamine tetraline (8-OH-DPAT)-induced locomotion was greater in water+haloperidol treated animals but not in RBO+haloperidol treated animals. 8-OH-DPAT-induced decreases of 5-HT metabolism were greater in water+haloperidol treated animals but not in RBO+haloperidol treated animals. The finding of the present study suggested that involvement of free radical in the development of neuroleptic-induced TD and point to RBO as a possible therapeutic option to treat this hyperkinetic motor disorder.

**Keywords:** TD; VCMs, motor coordination; Haloperidol, RBO, 8-OH-DPAT; somatodendritic 5-HT-1A receptors

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## INTEGRATION OF BASIC SCIENCES/LIFE SCIENCES AND CLINICAL MEDICINE: AN INNOVATION IN CLINICAL CURRICULUM

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### ABSTRACT

**Background:** Medical schools in the advanced and developing world are modifying Flexner's pre-clinical curriculum to display relevance of Basic Medical Sciences (BMS) with clinical medicine to convey the benefits of research from the lab and bench to the bedside.

**Objective:** To develop Integrated Life Science (ILS) courses for senior medical students who have greater clinical reasoning and analytical skills. This is an important approach towards integrated learning of ever expanding academic medicine (AM).

**Methods:** ILS courses foster learning through interactive didactic sessions, journal clubs, experiential learning, performance of laboratory procedures and critical appraisal of relevant scientific knowledge. **RESULTS:** In an attempt to integrate life sciences into clinical curriculum; it was found that 17% of US and 24% of Canadian Medical Schools offer ILS courses in clinical curricula. The University of Pittsburgh School of Medicine (UPSOM) has stressed the need of selective ILS courses in 4<sup>th</sup> year of clinical training of Medical School and developed speciality-oriented ILS courses for producing high quality medical graduates. In 2009, American Association of Medical Colleges (AAMC) and Howard Hughes Medical Institute (HHMI) published a report to determine the core scientific foundations for the physicians of 21<sup>st</sup> Century. The report described the core scientific competence required for the present day physicians.

**Summary and Conclusion:** Development of ILS courses are needed to meet the demands of medical students and to integrate advancing biomedical knowledge with AM. ILS courses can inspire motivated students to become scientists and life-long learners.

**Keywords:** Basic Medical Sciences (BMS), Integrated Life Science (ILS) Courses, Academic Medicine (AM).

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## STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF A UNIQUE HYPOTHETICAL PROTEIN OF *MYCOBACTERIUM TUBERCULOSIS*: A BIOINFORMATICS APPROACH

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### ABSTRACT

*Mycobacterium tuberculosis* (MTB), the causative agent of the tuberculosis (TB), has been a serious threat for the public health. Currently it was estimated that roughly one-third of the world's population has been infected with MTB. The recent emergence of the extreme drug resistant TB further deteriorated the situation and require more serious efforts to find a new cure against TB. The availability of the genomics data of various MTB strains provided a great initiative of exploiting them to propose new therapeutic targets against MTB. In the present study a hypothetical, non-homologous protein was identified by performing an *in silico* comparative genomics of various available MTB strains. The identified hypothetical protein was further characterized for the druggability potential considering multiple criteria including, physicochemical properties, subcellular localization, enzyme classification family, phylogeny, and etc. The bioinformatics approach revealed the hypothetical protein may belong to the transferase superfamily. We further used the structure-based methods (i.e. Homology Modeling and Docking) to build the 3D-structure of the hypothetical protein and predicted the putative binding site of the protein. The study is interesting and we proposed the identified unique, hypothetical protein as a novel drug target against which new drug candidates could be proposed in near future.

**Keywords:** Hypothetical, Tuberculosis, Bioinformatics, Therapeutics

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## ARTEMISININ PRODUCTION IN *ARTEMISIA ANNUA* AND *ARTEMISIA DUBIA* FOLLOWING TRANSFORMATION WITH THE *ROL ABC* GENES AND ELUCIDATION OF THE SITES OF ITS SYNTHESIS

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### ABSTRACT

The *rol ABC* genes have been shown to enhance production of secondary metabolites in plants, possibly through stimulation of the defense pathway. This report examines the effect of transformation of *A. annua* and *A. dubia* with the *rol ABC* genes expressing in *A. tumefaciens* and *A. rhizogenes*. The artemisinin content, trichome density and expression of key genes in the biosynthetic pathway of artemisinin were measured. Artemisinin content was significantly increased in transformed material of both *Artemisia* species when compared to un-transformed plants. The artemisinin content within leaves of transformed lines was increased by a factor of ten, indicating that the plant is capable of synthesizing much higher amounts than has been achieved so far through traditional breeding. Expression of all artemisinin biosynthesis genes was significantly increased, although variation between the genes was observed. Cytochrome P450 (CYP71AV1) and aldehyde dehydrogenase 1 (ALDH1) expression levels were higher than that of amorpha-4, 11 diene synthase (ADS). Levels of the trichome development and sesquiterpenoid biosynthetic gene (TFAR1) expression were also increased in all transgenic lines. Trichome density was also significantly increased in the leaves of transformed plants, but no trichomes were found in control roots or transformed roots. The detection of significantly raised levels of expression of the genes involved in artemisinin biosynthesis in transformed roots correlated with the production of significant amounts of artemisinin in these tissues. This suggests that synthesis is occurring in tissues other than the trichomes which contradict previous theories. This elucidation will help to increase production to meet the increasing demand of artemisinin because of its pharmacological importance.

**Keywords:** *Artemisia annua*, *rol ABC* genes, *Artemisia dubia*, *Agrobacterium tumefaciens*, *Agrobacterium rhizogenes*, Artemisinin.

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## RMA SPECIES AGAINST PHYTOPATHOGEN: *SCLTEROTIUMROLFSII*

**Amna Shoaib, Arooj Shezad and Arshad Javaid**

### ABSTRACT

Antifungal potential of three species of *Trichoderma* viz. *T. harzianum*, *T. viride* and *T. hamantum* was assess against well-notorious pathogen of over 500 plants i.e. *Sclerotiumrolfsii*. Laboratory bioassays were conducted in using solid as well as liquid malt extract. Experiments that wereperformed in solid medium (2% mat extract agar) through dual-culture method revealed that all the three species of *Trichoderma* significantly suppressed the growth of *S. rolfsii* by 80-85% over control. The effect of culture filtrate each of three *Trichoderma* spp. in liquid medium (2% malt extract) was performed using 8 different concentrations (5-40%) of the fungal metabolites. Results revealed that the growth of pathogenic fungus was significantly decreased with increasing concentrations of metabolites. All three *Trichoderm* spp. showed 80-100% inhibition in growth of the fungus at 35-40% concentrations of the metabolite. There was a linear relationship ( $R^2 > 0.95$ ) between concentration of metabolites of *Trichoderma* spp. and growth of *S. rolfsii* on solid as well as on liquid medium.

**Keywords:** *Trichoderma* spp., Biocontrol, Phytopathogen: *Sclterotiumrolfsii*

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## MODULATION OF ADDICTIVE AND THERAPEUTIC EFFECTS OF REPEATEDLY ADMINISTERED MORPHINE BY BUSPIRONE

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### ABSTRACT

Morphine, the main pharmacologically active alkaloid of opium poppy, is a potent analgesic and is widely used to treat chronic pain; however its utility is hindered by the development of tolerance, dependence and addiction to its pharmacological effects when the drug is used repeatedly. Serotonin has been shown to have a role in the rewarding/reinforcing effects of drug of abuse. 5-HT agonist/antagonists with selectivity towards various receptors can modulate reinforcing effects and sensitization by drug of abuse. It may be expected that addictive potential of morphine can be modulated by serotonin compounds. Conditioned place preference (CPP) test is used to evaluate motivational properties such as rewarding or aversive effects of drugs. Previous studies from our laboratory show that apomorphine-induced sensitization and reinforcement is inhibited in rats treated with 1.0 mg/kg buspirone. In the present study the effects of buspirone are determined on anti-nociceptive and addictive effects of morphine. We report that repeated administration of morphine at a dose of 7.5 mg/kg elicit CPP, hyperalgesia and tolerance in motor depressant effects of morphine. Co-treatment with buspirone prevented all these adverse effects of repeated morphine administration. The findings suggest that buspirone an anxiolytic agent if taken with morphine can attenuate morphine-induced addiction while improving pain relieving efficacy of morphine on repeated use.

**Keywords:** Morphine, Addiction, Buspirone, Analgesia, Serotonin

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## SPATIAL VARIABILITY & DISTRIBUTION OF SOIL AVAILABLE MICRONUTRIENTS: A REVIEW

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### ABSTRACT

Globally the imbalance fertilization practices have commonly been observed which eventually results in the excess or deficiency of soil available micronutrients. Variability of micronutrients in soil is caused by the pedogenesis and hydrological processes. Besides that the physical and chemical properties of soil (pH, O.M, CaCO<sub>3</sub>, CEC, soluble salts and soil texture) can also effect on the spatial variability of soil micronutrients. Spatial or temporal variability exists in the soil due to the occurrence of soil behavior changes. Hence, to understand the spatial variability of soil available micronutrients which persist on meters of distance in the field this study has planned to review the possible approach to estimate the difference to improve the soil fertility. Geostatistics approach describes the spatial continuity of natural occurrences and specifies an adaptation of standard regression methods to assess these contentions. Geostatistics is the strategic techniques that can evaluate the spatial variability of soil nutrients and it can predict the value of un-sampled location via kriging. Thereby this paper aims to highlight: the importance of spatial variability and factors affecting on micronutrient's availability, successful application of geostatistics to determine the spatial variability and to provide guidelines for future consequences.

**Keywords.** Micronutrients; Spatial variability; geostatistics; kriging

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## A COMPARISON OF CONVENTIONAL AND COATED UREA ON ALKALINE SOILS: TO MITIGATE N-LOSSES

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### ABSTRACT

Conventional urea is the primary source of Nitrogen (N) in an agro-ecosystem. Approximately 70% of applied urea may be lost into the atmosphere in different forms; among them ammonia (NH<sub>3</sub>) volatilization is the influential one. Conventional urea can be simulated by coated urea, which reduces the rate of release in urea and increases its efficiency for N-uptake and minimizes environmental pollution. A slow-release urea (coated with micronutrients) was produced in the laboratory. The aim of the study was; to prevent N-losses and enhance N-uptake efficiency. A laboratory experiment was conducted on alkaline soil of Pakistan by using a force draft technique for trapping the ammonia loss. The experiment was designed in a Completely Randomized Design (CRD) with the application of micronutrient coated urea with zinc (Zn) and copper (Cu) to assess the NH<sub>3</sub> loss for the six weeks of observation. The rate of NH<sub>3</sub> volatilization is 9% in the first two weeks from coated urea, which was recorded as double of that (17%) from un-coated urea. It was implied that the rate of hydrolysis in coated urea was slower than of the conventional urea and hence coated urea can improve N-use efficiency. From the six weeks of observation the maximum NH<sub>3</sub> volatilization was in the first two weeks of the experiment. It was perceived that the ammonia loss gradually decreased with time. The mean comparison (Tukey's Standardized Range test) showed the positive response of coated urea with both (Cu and Zn) micronutrients.

**Keywords:** urea release; micronutrient coated urea; NH<sub>3</sub> volatilization; N-uptake;

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## LACTOBACILLUS: A BIOTHERAPEUTIC AGENT AGAINST VAGINAL CANDIDIASIS

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### ABSTRACT

Vulvovaginal candidiasis (VVC) that is caused by *Candida species*, is the most common vaginal infection occurring worldwide. About 75% of women are affected by this type of vaginal infection at least once in their lives. The treatment typically involves use of antifungal agents yet there is an overgrowing concern about the resistant strains that are usually more difficult to eradicate. Lactobacillus is the predominant vaginal flora in healthy women that produces antimicrobial compounds such as H<sub>2</sub>O<sub>2</sub>, organic acids and bacteriocin like substances which inhibit the *Candida* growth. Use of these probiotic bacteria, either as a stand-alone therapy or in combination with antimicrobial drugs, for the treatment of vaginal infections has been extensively studied. The aim of the present study was to assess the biotherapeutic potential of Lactobacillus sp. against clinical isolates of *Candida* sp. For this purpose 12 *Candida* sp. were isolated from 17 hospital samples of females with vaginal infection. Antagonistic activity of Lactobacillus sp. was investigated by using agar well diffusion and agar spot diffusion assay. *Lactobacillus species* were found to inhibit the growth of all *Candida* strains in agar well diffusion method while no zone of inhibition was observed in agar spot diffusion assay. The antagonistic effects of these bacteria as well as their own survival at a given pH, however, vary considerably depending upon the species and strains. Nevertheless, antimicrobial – producing Lactobacillus is found to be a good barrier in vagina against *Candida*. Thus instead of using antifungal agents Lactobacillus is a good alternate in the treatment of vaginal infections. Moreover, by the use of Lactobacillus for inhibiting the growth of *Candida* we can prevent resistance from antifungal agents.

**Keywords:** Agar well diffusion, Antagonistic activity, Antifungal agent, Candidiasis, Probiotic.

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## BUGS EMPOWERING BATTERIES: ELECTRICITY GENERATION BY WASTE WATER

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### ABSTRACT

Microbial Fuel Cell (MFC) technology is becoming popular among microbiologists due to its ability to convert chemical energy into electrical energy by utilizing microorganism's ability to deliver electrons outside the cell. Electrons produced by the bacteria from these substrates are transferred to the anode (negative terminal) and flow to the cathode (positive terminal) linked by a conductive material containing a resistor, or operated under a load. This can be a promising technology for sorting energy crisis issues and might offer a good waste water treatment system. On the other hand, microbes play role in hydrogen production, bio-sensing and synthesizing valuable products. In the undertaken study, mediator-less microbial fuel cells were employed for the electricity generation. To grasp this approach a low cost Dual-chamber MFC was constructed; where salt agar-bridge used to connect chambers, carbon rods as electrodes. MFC operated in batch mode using waste water as substrate (anolyte) and 0.1M potassium dichromate in 0.1M potassium phosphate buffer (PBS) as catholyte without addition of any mediator. Micro-ammeter was also connected to MFC. Five setups of MFC were operated with different samples of waste water out of which four samples gave power output. A maximum power output of 0.25mW was obtained. Voltage yielded was 0.1-0.5V; 0.1V generated from domestic waste water and 0.38V-0.5V from industrial waste water. Waste water potential to generate electricity as waste water harbors a diverse group of microorganisms and has a multitude of substrates that serve as nutrients and electron acceptors for native microbes. Therefore, microbial fuel cell might be a good eco-friendly alternate to electricity generation as well as waste water treatment. Moreover, it may also help in saving money which makes waste water treatment and electricity production more affordable for developed and developing nations.

**Keywords:** Anolyte, Catholyte, Electricity, Microbial fuel cell, Salt agar-bridge, Waste water treatment.

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## ANTIMICROBIAL ACTIVITY OF SALVADORA PERSICA (MISWAK) ON ISOLATES OF CARIOUS TEETH

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### ABSTRACT

Oral diseases are major health problems with dental caries among the most important preventable global infectious disease. Dental plaque, biofilms of microorganisms on tooth surface, plays an important role in the development of caries and periodontal disease. The increase in incidence of oral diseases and increased resistance by bacteria to antibiotics is common now days. Miswak is a natural tooth cleaning tool which is being used in many parts of the world since ancient times. It is known to be useful in prevention of dental caries. But still it is not used as frequently as other oral hygiene tools. The aim of the present study was to test invitro antibacterial activity of *Salvadora persica* (miswak) stem and its extract against isolates of oral pathogens. Oral swabs and extracted carious teeth were taken and bacterial species were isolated and identified by standard methods. Stem of miswaks and extract were tested against isolates of oral pathogens by disc diffusion and well diffusion method. Different bacterial species (*Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa* and *Actinomyces sp.*) were isolated from oral swabs and extracted carious teeth. Stem of packed miswak showed inhibitory activity against *Staphylococcus aureus* and *Actinomyces sp* while rests of the oral isolates were found to be resistant. The aqueous extract of *S. persica* was found to be active against *Staphylococcus aureus*. Incessant novel developments in science have led most of the people to perceive use of miswak as an old custom. However, its use should be encouraged with scientific reasoning. Moreover, additional research work should be undertaken to understand the basic mechanism of the miswak activity against different oral pathogens.

**Keywords:** Agar well diffusion, Biofilm, Carious teeth, Dental plaque, Miswak.

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## VIRULENCE FACTORS OF CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS*

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### ABSTRACT

*Staphylococcus aureus* is a common causative agent of hospital acquired infection and community acquired infection as the cell possesses a wide armamentarium of virulence factor that include different exoenzyme and toxins. The main aim of this study was to evaluate the virulence factors from different clinical isolates of *Staphylococcus aureus*. In this study total ten isolates were used for the screening of different virulence factors such as catalase, coagulase, lipase, thermonuclease, beta-lactamase, Dnase and Haemolysin. The present study showed that 100% strains demonstrate  $\beta$ -haemolysis, catalase enzyme, coagulase enzyme, beta-lactamase. Out of 10 strains only 2 (20%) strains produced Dnase and thermonuclease enzyme while only 30% strains showed positive result for lipase production which is detected by phenol red test. Based on the result of this study almost all the strains showed positive result for various enzymatic tests but 3 strains considered to be more virulent as they showed the production of Dnase, lipase and thermonuclease. These enzymes make *S. aureus* more pathogenic and give rise to diverse spectrum of diseases ranging from minor to life threatening infections. Phenotypic tests offers an alternative method for simultaneous detection of the clinically important virulence factors of *S. aureus* strains for diagnostic purposes as well as research studies.

**Keywords:** Exoenzymes, Dnase, Lipase, *Staphylococcus aureus*, Thermonuclease.

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## MICROBIAL DEGRADATION OF PETROLEUM HYDROCARBON CONTAMINANTS

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### ABSTRACT

Bioremediation of oily waste water is treatment technology that uses microorganisms or their enzymes to reduce the concentration or toxicity of hydrocarbon contaminants into less toxic forms. Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment. The objective of the present study was to determine the ability of bacteria that carry oil degradation. Samples from areas (like petrol pumps, garages) which were heavily contaminated with oil were collected to access their degradation ability. These microorganisms help in the process of bioremediation (removal of wastes from the environment). Bioremediation is a method employed in restoration of oil-polluted environments in which microorganisms with biodegradative ability are used. For this study the soil samples were collected from 4 different areas of Karachi and were placed in beakers (with oil) under variable conditions and the biodegradation capability oil petroleum soil microorganisms is observed. The amount of oil biodegradation was observed by Grease Spot Test i.e. by measuring the size of zones. The results showed that the microorganisms in the petroleum soil have degradation ability as they showed biodegradation of oil. This study proves that microorganisms are capable of oil biodegradation and they can be used in the process of bioremediation to clean the oil spills without any hazardous impact on the environment. A better understanding of the mechanism of biodegradation has a high ecological significance that depends on the indigenous microorganisms to transform or mineralize the organic contaminants.

**Keywords:** Bioremediation, Contaminants, Grease Spot test, Hydrocarbon, Petroleum.

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## EFFECT OF FRESH POLLEN PELLETS AND POLLEN PATTIES COATED WITH AND WITHOUT BEESWAX ON THE LIFE HISTORY PARAMETER OF BUMBLEBEE *BOMBUS TERRESTRIS* (HYMENOPTERA: APIDAE)

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### ABSTRACT

We investigate the possible effect of fresh pollen pellets and pollen patties coated and without coated with beeswax on the life history parameter of bumblebee and their consumption rate. At colony initiation stage we found that mother queen start egg laying earlier ( $6.1 \pm 0.82$  days) at pollen patties without beeswax coated as compare to at coated and fresh pollen pellets. Similarly emergence of first worker from the first batch of colony also earlier ( $26.3 \pm 0.83$  days) at pollen patties without coated. Other two stage of the colony i.e. at colony foundation stage and colony maturation stage we observed that the pollen patties with one coat of beeswax was best for bumblebee rearing. According to consumption rate of pollen during the 24hr of observation we found that at colony initiation stage, colony foundation stage and colony maturation stage large amount of pollen consumption was found at fresh pollen pellets because to attack of wax moth on fresh pollen was also high. The best pollen consumption rate and growth rate was found at pollen patties with one coat of beeswax. So we found that at colony initiation stage pollen patties without coated was best and at colony foundation stage and colony maturation stage pollen patties with one coat of beeswax was best.

**Keywords:** *Bombus terrestris*, pollen patties, beeswax coated, colony development.

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## SCREENING OF THERMO TOLERANCE IN AROMATIC AND NON-AROMATIC RICE SEEDLINGS USING PHYSIOLOGICAL AND BIOCHEMICAL INDICATORS

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### ABSTRACT

Aromatic rice is distinguished by its superior grain quality and characteristic aroma but have low yield due to limited adaptive ability to cope new environmental conditions especially high temperature. While episodes of average heat stress causes an array of physiological, biochemical and morphological changes which ultimately affecting plant growth and aroma development. This study was designed to explore comparative responses of aromatic (Khushbo and Mehek) and non-aromatic (Sarshar, Shadab, Shandar and Shua) rice seedlings under different temperature regimes ( $40 \pm 1$  °C and  $42 \pm 1$  °C) for 16 h in terms of physiological and biochemical attributes. The results showed that heat stress detrimentally affected the growth and development of rice seedling of both aromatic and non-aromatic rice varieties but aromatic rice varieties were found more susceptible to heat as compared to non-aromatic rice varieties. Under heat stress, decrease in rice characteristic fragrance was observed, suggesting that high temperature hinders aroma development. Lower proline accumulation, higher lipid peroxidation, greater electrolyte leakage, and low content of total proteins in leaves during unfavorable temperature were observed in aromatic rice varieties especially in Mehak cultivar, making them heat susceptible. Among non-aromatic rice cultivars, "Shadab" showed lowest relative membrane permeability (EC), lipid peroxidation and H<sub>2</sub>O<sub>2</sub> level while high proline and protein accumulation under heat stress condition. Protein profiling on SDS-PAGE manifested the differential protein expression by up regulation of protein band of molecular weight approximately 90 KDa which is assumed to trigger the expression of heat shock protein (HSP70) conferring the protective role in thermotolerance.

**Keywords:** Thermotolerance, Aromatic Rice, Protein Profiling, lipid Peroxidation

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## CYTOGENETIC AND IMMUNOPHENOTYPIC CHARACTERISTICS OF CHILDHOOD ACUTE LEUKEMIA IN PAKISTAN AND THEIR OUTCOMES

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### ABSTRACT

**Introduction:** Acute leukemias account for 30% of childhood malignancies. The better outcomes in paediatric acute leukemia has been associated with certain cytogenetic and immunophenotypic characteristics. Following study investigates the outcomes of childhood leukemia and their association with cytogenetics and immunophenotype.

**Study design:** Prospective

**Duration:** Study spanned from 2006-March 2015.

**Patients and Methods:** 77 patients included in the study were diagnosed as acute leukemia on the basis of morphology, bone marrow examination. The diagnosis was confirmed using the cytochemical staining, immunohistochemistry, flow cytometry and cytogenetic analysis.

**Results:** Of the 67 patients, 55(82%) were diagnosed with ALL, 10(15%) with AML and 2(3%) with APML. Male preponderance was observed in both ALL and AML. In ALL, Pre-B Cell was diagnosed in 45(82%) and T-Cell in 10(18%) patients. With Pre-B Cell ALL, 27/45(60%) were in remission, 6(13%) relapsed, 4(9%) expired and 2(4%) were off-treatment. With T-Cell ALL, 4(40%) were in remission, 2(20%) relapsed and 3(30%) had expired. In ALL, 39/51(76%) with hyperdiploidy and 3/4(76%) with hypodiploidy were in remission. In AML, 2(20%) patients with t (8;21) were in remission while one each del 7q32 and t(6;11) had relapsed and expired despite treatment. In APML, both with t (15;17) were in remission. Cytogenetics was not performed in 10 patients out of which 6(60%) were in remission and off-treatment, 2(20%) had expired, 1(10%) had relapsed and 1(10%) was lost to follow-up.

**Conclusion:** In our cohort, the ALL patients had a much better outcome as compared to AML group. Although the importance of the bone marrow examination as an investigative tool is not less, still the cytogenetic analysis and their immunophenotyping helps in establishing prognosis as well as in designing of a more tailored regimen for both types of leukemia.

**Keywords:** ALL, AML, Cytogenetic analysis.

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## CHARACTERIZATION OF SINGLET OXYGEN DEPENDENT PHOTO-INACTIVATION MECHANISM OF PHOTOSYSTEM II IN CULTURED *SYMBIODINIUM* CELLS AND ITS INVOLVEMENT IN CORAL BLEACHING

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### ABSTRACT

Reef-building corals form an endosymbiosis with dinoflagellate algae from the genus *Symbiodinium* which reside within the endodermal tissue of the cnidarian. As a result of environmental stressors the symbiosis between the coral host and its symbiotic partner may terminate leading to the expulsion of the *Symbiodinium* and causing the phenomenon known as coral bleaching. This breakdown of the coral and algae symbiosis provides an opportunity to understand the molecular mechanism of this symbiotic association. The mechanism(s) that trigger the expulsion of the symbiotic partner are not yet fully understood. Singlet oxygen (<sup>1</sup>O<sub>2</sub>) has been implicated as an important mediator of light induced damage of the photosynthetic apparatus during photoinhibition in photosynthetic organism. Although various methods such as luminescence at 1,270 nm, fluorescent probes method and electron paramagnetic resonance (EPR) are available for <sup>1</sup>O<sub>2</sub> detection in isolated photosynthetic systems, these sensor molecules are unable to penetrate the cell wall of intact cyanobacteria and microalgae, which seriously limits the possibility to study the role of <sup>1</sup>O<sub>2</sub> in photoinhibition in vivo. In order to overcome this difficulty, we have developed a method for detection of <sup>1</sup>O<sub>2</sub> in intact cyanobacteria. The method is based on chemical trapping of <sup>1</sup>O<sub>2</sub> by histidine (His), which leads to O<sub>2</sub> uptake during illumination that can be detected and quantified by commercial oxygen electrodes.

Recently we have successfully employed this method for *Synechocystis* to understand the role of orange carotenoid protein (OCP) as <sup>1</sup>O<sub>2</sub> quencher and we showed that OCP is a very efficient singlet oxygen quencher. In this study, we have also employed chemical trapping method and our data demonstrates the production of <sup>1</sup>O<sub>2</sub> in intact *Symbiodinium* cells. The data show that <sup>1</sup>O<sub>2</sub> was enhanced during the heat and light stress conditions. Recently we proposed that the inhibition of the Calvin-Benson cycle by glycolaldehyde and potassium cyanide during thermal stress in *Symbiodinium* cells promotes <sup>1</sup>O<sub>2</sub> formation. Our study reveals that heat and light stress induce photo-inactivation of PSII and enhance <sup>1</sup>O<sub>2</sub> production, while histidine provides protection against PSII photo-inactivation and pigment bleaching. Based on our results, <sup>1</sup>O<sub>2</sub> induced inactivation of *Symbiodinium* cells, may be involved in triggering the expulsion of *Symbiodinium* cells from the coral host, which leads to coral bleaching. I will also report on the latest results obtained in our lab by using His-mediated chemical trapping techniques on the characterization of intracellular <sup>1</sup>O<sub>2</sub> detection in different cyanobacterial strains.

**Keywords:** Cyanobacteria, algae, *Synechocystis*, *Symbiodinium*, Photosynthesis, Photoinhibition, Photosystem II, Singlet oxygen, coral bleaching, chemical trapping.

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## EFFECTS OF ENVIRONMENTAL POLLUTION ON THE REPRODUCTIVE PERFORMANCE OF DAIRY ANIMALS

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### ABSTRACT

The rapid rise in industrialization, urbanization and use of insecticides with heavy metals has become a major health concern in dairy animals. These animals are exposed to a number of pollutants via drinking contaminated water and consuming fodder during their lifetime. These pollutants (pesticides and heavy metals) act as endocrine-disrupting chemicals, interrupt the synthesis, role and metabolism of reproductive hormones along with their receptors. The dairy animals suffer from the problems of reduced fertility through abortion, delayed ovulation, long calving interval and more number of services per conception. These pesticides and metals can also adversely affect the quality semen of male animals. In Pakistan, many pesticides and heavy metals have been identified, but their harmful effects on reproductive system of dairy animals are poorly understood. The rising level of arsenic in water in Pakistan poses the risks to fertility of dairy animals. Effective preventive measures must be adopted to save the environment from pesticides and heavy metals so that the risks of infertility can be avoided.

**Keywords;** Dairy animals, Pollution, Pesticides, Heavy metals

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## EVALUATION OF CHICKPEA FOR RESISTANCE TO *FUSARIUM* WILT

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### ABSTRACT

A total of ninety three genotypes of chickpea obtained from various sources were evaluated to identify resistance against *Fusarium* wilt caused by *Fusarium oxysporium* f. sp. *ciceri*, under artificial disease conditions. Among these, 69 genotypes were Desi and 24 were Kabuli. Disease observations were recorded at seedling and reproductive stages. A considerable variation among the genotypes was observed at both the stages. Disease incidence ranged from 0% to 100% at seedling stage and reproductive stages. At seedling stage, 37 genotypes (26 Desi and 11 Kabuli) were resistant, 19 genotypes (14 Desi and 5 Kabuli) were tolerant and 38 genotypes (29 Desi and 8 Kabuli) were susceptible. On other hand, 6 genotypes (one Kabuli SL-02-22 and 5 Desi NCS-611, 93127, ICC-6945, ICC-12968, EC.516957) were resistant at reproductive stage. However, 5 Desi genotypes (NCS-0605, 98004, ICC-14344, EC.516729, EC.516916) were tolerant and 82 susceptible. Chickpea genotypes identified as resistant during this study may be exploited in breeding programs to develop resistant varieties.

**Keywords:** Chickpea, wilt, screening, resistant sources, susceptibility

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## FLORAL HOST RANGE AND DISTRIBUTION OF INDIGENOUS BUMBLEBEE, *BOMBUS HAEMORRHODIALIS* SMITH IN RAWALAKOT, AZAD KASHMIR OF PAKISTAN

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### ABSTRACT

Bumblebees are important pollinator wild plants and commercial crops, especially those grown in greenhouses. Although several species of bumblebees are available for importation, they present a risk to local species through the inadvertent introduction of exotic parasites. This is especially likely when the local region includes wild bumblebee species, which may be susceptible to parasites of imported species. An alternative to importing bumblebees is learning to manage local species. Successful use of local species for agriculture requires understanding both the species-specific techniques of management and the diversity of plant species they prefer to visit. Here, we describe the distribution and host plant preference of *Bombus haemorrhoidalis* Smith the main bumblebee species of Rawalakot and its surroundings. Monthly surveys for *B. haemorrhoidalis* in three natural areas over two consecutive years identified forty three floral host plants from twenty plant families, with the greatest number of utilized hosts coming from the Asteraceae. *B. haemorrhoidalis* was active in the wild from April until November with the greatest number of bumblebee workers observed in August-September followed by production of males and daughter queens in October-November. The present study might help in conservation of bumblebees and facilitating ecological and biological interaction with other insect pollinators of Azad Kashmir, Pakistan.

**Key words:** Azad Jammu Kashmir, *Bombus haemorrhoidalis*, floral hosts, distribution

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## ANTIBACTERIAL ACTIVITY OF AJWAIN EXTRACT AND ENHANCEMENT OF ACTIVITY BY METAL SALTS

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### ABSTRACT

Medicinal plants are used as alternative of therapeutic agents all over the world due to facing the disaster of resistivity against antimicrobial drugs by micro-organisms. They are also used as flavor stimulating agents in Asian households. *Trachyspermum ammi* commonly known as ajwain in Pakistan, found in all Asian countries, grow in salty soil, and used against many digestive tract infections. The objective of the present study was to screen antimicrobial activity of ajwain extracts and their enhancement by the addition of trace metals. The antimicrobial activity of Methanolic and ethanolic extracts of ajwain against different bacterial species (*E. coli*, *P. aeruginosa*, *S. typhi*, *Proteus sp.*, *Klebsiella sp.*, *B. subtilis*, *S. aureus*) was performed in vitro by well diffusion and disc diffusion techniques. Moreover, the antagonistic and synergistic effect of metal salts with these extracts was also determined. Both extracts give great effect against all bacterial species but ZnSO<sub>4</sub> & CaSO<sub>4</sub> shows the antagonistic effect while CuSO<sub>4</sub>, MgSO<sub>4</sub>, & Na<sub>2</sub>SO<sub>4</sub> shows synergistic effect against all bacterial species. This study provide another justification in favor of the affectivity of antimicrobial activity of medicinal plant and enhancement of this activity by adding metal salts so it can use as bio-medicine for therapeutic purpose.

**Keywords:** Ajwain, Disc diffusion, Medicinal plant, Metal salts, Well diffusion.

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## SCREENING OF COMMERCIALY SIGNIFICANT ENZYMES FROM BACILLUS

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### ABSTRACT

Microbial enzymes are widely used because of their low cost, large productivity, chemical stability, environmental protection, plasticity and vast availability. The Gram-positive bacterium *Bacillus subtilis* is an important producer of high quality industrial enzymes. These include L-asparaginase, cellulases and hydrolases such as amylases, proteases and lipases. Utilization of bacterial strains specifically of genus *Bacillus* is gaining momentum because of their ability to resist and survive under harsh industrial conditions. Although this organism is regarded as (GRAS) generally regarded as safe. The objective of this study was to isolate bacteria from soil and screened for the production of different enzymes including; Lasparaginase, cellulase and amylase. Bacterial culture were identified as *Bacillus* species, based on their cellular morphology, gram staining and biochemical characteristics. Enzyme screening was performed by using plate assay. 80% of the isolates found to exhibit amylolytic and cellulose activity while 60% of the isolates exhibit L- asparaginase activity. From this study it was came to be known that the studied organism (*Bacillus subtilis*) isolated from soil can be used as an effective source for the production of industrially important enzymes.

**Keyword:** Asparaginase, Amylase, *Bacillus*, Cellulase, Plate assay.

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## SPORADIC GOITER PREVALENCE AND ITS ASSOCIATION WITH IODINE DEFICIENCY IN THE CAPITAL TERRITORY OF PAKISTAN

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### ABSTRACT

**Background and objectives.** Iodine deficiency is a major nutritional problem in Pakistan. The pregnant women and their neonates have been important target groups for the study of the prevalence of iodine deficiency in a community. The main goal of the present study was to assess the status of iodine deficiency in the pregnant women and their neonates, in Islamabad, Pakistan.

**Methods.** A hospital-based, cross-sectional screening study was conducted on 261 full term pregnant women, 125 neonates born to them and 50 controls (non-pregnant healthy women) in the Maternity Unit of the Islamabad Hospitals. The overall iodine status of the pregnant and non-pregnant women were estimated by measuring the urinary iodine concentration and neonatal thyroid function was estimated by measuring TSH levels in their cord blood, in Environmental Laboratory of Chemical and Materials Engineering Department at PIEAS, Nilore, Islamabad.

**Results.** A total of 73 percent pregnant women showed urinary iodine concentration > 20 µg/L and 50 percent showed UIC between 20-49 µg/L. The median UIC values in the pregnant women were found to be 30.37 µg/L. Statistically significant (p<0.05) difference was found between the UIC levels of pregnant and control women and non-significant difference was found in UIC levels between different age groups of pregnant women (p > 0.005). A total of 19 (14.84 percent) neonates had TSH values >10 mIU/L.

**Conclusions.** It can be concluded from the present study that pregnant women of the study area were found to be moderately iodine deficient and the neonates were mildly iodine deficient. These findings indicate that the use of iodized salt should be maintained in the study area and neonatal screening by measuring TSH levels is recommended in this area.

**Keywords:** Iodine, TSH, Urinary Iodide Concentration, Pregnant women

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## EGCG/LPS INDUCED HEPATOTOXICITY AND ITS INTERVENTIONS

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### ABSTRACT

Green tea (GT) is a widely consumed beverage and folk medicine worldwide, especially in East Asian countries. The main chemical constituents in green tea are caffeine and polyphenolic compounds known as catechins. The most abundant catechin present in green tea is Epigallocatechin-3-gallate (EGCG). Consumption of EGCG has resulted in clinical cases of hepatotoxicity. We have shown earlier a new murine model of hepatotoxicity by administering (1500 mg/kg) epigallocatechin-3-gallate (EGCG) intra-gastric (IG) for 5 days after a single intraperitoneal (IP) dose (6 mg/kg) of lipopolysaccharide (LPS). Here we report two different interventions that ameliorated the EGCG/LPS induced hepatotoxicity in mice. In one study administration of thymoquinone (up to 10 mg/kg for 8 days) before and concurrently with EGCG/LPS significantly reduced hepatotoxicity. Thymoquinone is a prominent constituent of the "black seed", *Nigella sativa* and is known for its anti-oxidant and anti-inflammatory activities. In another study we investigated the effect of intrahepatic (IH) injection of mouse embryonic stem cells (MESC) on the hepatotoxicity induced by EGCG/LPS in mice. Mice were administered EGCG/LPS and rested for 3 days. MESC were obtained from American Type Culture Collection and cultured in vitro for 4 days. Stem cells were injected IH. Seven days later, a single dose of LPS (6 mg/kg) followed by daily doses of IG administration of EGCG were re-administered for 5 days. At the end of the experiment, blood samples were collected for analysis of biochemical parameters associated with liver. Mice treated with EGCG/LPS showed elevated levels of liver toxicity markers alanine amino transferase (ALT), alkaline phosphatase (ALP), and bilirubin compared to the vehicle control group. In addition, higher albumin/globulin ratio and altered histopathological architecture was observed. Pre-treatment with thymoquinone significantly restored the levels of these parameters towards normalcy. Similarly, administration of MESC significantly protected the deleterious effect of EGCG/LPS. Besides, the group of mice treated with intrahepatic (IH) injection of mouse embryonic stem cells (MESC) showed less expression of oxidative stress biomarkers (oxidized low-density lipoprotein Ox.LDL and chemokine CXCL16), less expression of nuclear protein receptors (retinoic acid receptor and retinoid X receptor), and less expression of inflammatory biomarkers (tumor necrosis factor- $\alpha$  and transforming growth factor  $\beta$ 1) compared with other groups of mice that were not given MESC. In conclusion, we found that Thymoquinone or MESC can be protective interventions EGCG/LPS-induced hepatotoxicity in mice and can be used as potent strategies against liver toxicities.

**Keywords:** Epigallocatechin-3-gallate, Hepatotoxicity, Stem Cells, Thymoquinone.

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## PATHOGENESIS OF CHICKPEA WILT DUE TO *FUSARIUM OXYSPORIUM*

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### ABSTRACT

Pathogenesis study of *Fusarium oxysporum* f. sp. *ciceri* was made to correlate epidemiological factors with chickpea wilt. It was observed that seedling mortality increased with an increase in inoculum load, at the inoculum load of 3 g, seedling mortality was minimum and at 20 g it was maximum. Similarly, when *Fusarium* wilt was observed in different types of soils, it was found that the disease developed severe in sandy soil and least in clay soil. Soil moisture also played an important role in disease development. It was observed that seedling mortality was maximum (91%) when the soil moisture level was low (16.2%) and minimum (0.00%) when it was high (35.67%). Studies of disease development on sap extract of leaves and roots showed maximum growth of *F. oxysporum* f. sp. *ciceri* on root sap than leaves. Colony growth of *F. oxysporum* f. sp. *ciceri* was 3.2 to 5.9 cm at leaves sap and was 3 to 9 cm on root sap.

**Keywords:** chickpea, wilt, pathogenicity, inoculums, seedling mortality

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## EFFECT OF DIFFERENT PRUNING TIMES ON GROWTH AND YIELD OF BER (*ZIZYPHUS MAURITIANA* LAMK), CV. "ALU-BUKHARA"

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### ABSTRACT

To assess the effect of pruning times on the fruit characteristics and yield of ber (*Zyziphus mauritiana* Lamk.) cv. Alu-bukhara, an experiment was conducted during the year 2011-12 and 2012-13 at Horticultural Research Institute AARI, Faisalabad. Results showed maximum number of branches per tree (23.00), days to flowering (65days), early blooming (26<sup>th</sup> August to 30<sup>th</sup> October), highest fruit weight (32.45g), maximum fruit size (38.50 mm<sup>2</sup>), highest TSS (16.62%), highest sugar (6.25%) and maximum Vitamin-C contents (132.65mg/100mg) when pruning was done on 15<sup>th</sup> May, While minimum fruit weight (24.74g) and minimum fruit size (30.71mm<sup>2</sup>) was recorded when pruning was practiced on 15<sup>th</sup> June. Highest acidity percentage (0.57%) was recorded when pruning was done on 15<sup>th</sup> June. Fruit yield was also highest (208kg per tree) when pruning was done on 15<sup>th</sup> May while lowest yield was obtained when pruning was done on 15<sup>th</sup> June i.e. 176Kg per tree. It was concluded that 15<sup>th</sup> May is most suitable pruning time to maximize fruit yield and quality in ber cv. Alu-Bukhara.

**Keywords:** *Zyziphus mauritiana*; pruning time; fruit quality and yield

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## FREQUENCY OF CHROMOSOMAL BREAKS IN SUSPECTED CASES OF FANCONI'S ANAEMIA- A SINGLE CENTER EXPERIENCE

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### ABSTRACT

**Introduction:** Fanconi's (FA) anemia is the most frequently reported rare inherited bone marrow failure syndromes. FA accounts for about 25% cases of Aplastic Anaemia (AA). Screening of all patients undergoing bone marrow transplantation especially those suffering from AA should be done due to therapeutic implications and selection of donor.

**Objective:** To determine the frequency of chromosomal breaks in patients referred to Cytogenetics Department on the suspicion of FA/AA.

**Location & Study Design:** Retrospective study carried out from May 2010 - April 2015 in National Institute of Blood Disease & Bone Marrow Transplantation Karachi, Pakistan.

**Material & Method:** Chromosomal Breakages analyses were performed on patients diagnosed as AA or with suspicion of FA using stimulated 72 hrs cultures treated with Mitomycin C & without Mitomycin C employing solid Giemsa staining technique.

**Results:** Peripheral Blood samples of 223 patients were analyzed for chromosomal breaks including 143(64%) males and 80(36%) females with median age of 12 years (range 03 months-64 years). Out Of total, 58(26%) patients showed chromosomal breaks ranges in 05% to 100% metaphases. Most of the patients, 29(50%) exhibiting chromosomal breaks were suffering from AA. Chromosomal breaks also seen in 02(04%) donors of transplant patients, in siblings of FA patients 14(24%) and patients with phenotypic features suggestive of FA 13(22%).

**Conclusion:** Fanconi's Anaemia is diagnosed with increased frequency in centers managing haematological disorders. Most of the patients are diagnosed when they develop bone marrow aplasia. All patients of Aplastic Anaemia/Hypocellular Marrow and siblings of such patients are recommended to be screened for FA by Chromosomal Breakages Analysis.

**Keywords:** Aplastic Anaemia, Chromosomal Breakages, Fanconi's Anaemia, Mitomycin C

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## EVALUATING BIOSURFACTANTS PRODUCED BY *KLEBSIELLA PNEUMONIAE* MKOD36 FOR INHIBITION OF IN-VITRO BIOFILM INHIBITION ACTIVITY

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### ABSTRACT

Biosurfactants are amphiphilic compounds produced extracellularly by microorganisms on cell surfaces, or excreted extracellularly. Their role as antibacterial, antifungal and antiviral activities makes them relevant molecules for applications in combating many diseases and as therapeutic agents. The present study was done to evaluate production of biosurfactant and their ability to inhibit in-vitro biofilm activity. It was found that biosurfactant produced by *Klebsiella pneumoniae* cause significant inhibition of biofilm activity ( $30.20 \pm 0.15\%$ ). Additionally, it showed excellent hemolytic activity ( $83.70 \pm 1.85\%$ ) and thrombolytic activity ( $50.20 \pm 1.87\%$ ) which indicate their possible role in biomedical application and also decontamination purposes such as waste water treatment system where these microbes forms a biofilm hindering in remediation process.

**Keywords:** Biosurfactants, biofilm inhibition activity, hemolytic activity, thrombolytic activity

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## ASSESSMENT AND MOLECULAR CHARACTERIZATION OF CITRUS CANCER CAUSING PATHOTYPES IN SELECTED ORCHARDS OF KHANPUR

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### ABSTRACT

Citrus is one of the most popular and significant commercial fruit of Pakistan. Outbreak of Citrus Canker diseased in different varieties causes enormous economic loss. The present research work was conducted to identify and characterize citrus canker causing Pathotypes in citrus varieties from the orchards of Khanpur, district Haripur that were involved in degradation of quantity and quality of citrus fruit. The survey was conducted in orchards of Khanpur during March, 2015 to find out the incidence, prevalence, severity and disease index as well as for sampling from selected orchards of Khanpur KPK, Pakistan. Varieties encountered during the survey included Grapefruit, Malta (musambi), Red Blood, Ruby Blood and Lemon. Causal organisms were isolated and grown on nutrient agar (NA). Pathogens were initially identified on the basis of morphological characters. The incidence and severity on leaves of the orchards trees observed were observed about 49% and 18% respectively. Complete description of macro and microscopic characters was prepared. The purified cultures of bacterial pathogen was then identified and characterized. The identified microbial pathogens include *Xanthomonas* strains. *Xanthomonas* strains under specific condition showed discrimination of different Pathotypes and subgroups. DNA was successfully extracted and amplified by using primers *J-ptb 1* and *J-ptb 2* to confirm the identification by gene sequence. It is recommended on basis of present study that further experimentation on pathogenicity of these pathogen are required to find out the citrus varieties resistance against this disease. There is also need to control this pathogen using different biological/chemical agents and genetic engineering techniques.

**Keywords:** Orchards of Khanpur, Citrus fruit, Bacterial pathogens, Canker disease

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## COMPUTER GUIDED SYNTHESIS AND EVALUATION OF NOVEL IMMUNO-MODULATOR COMPOUNDS

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### ABSTRACT

Computer Aided drug discovery is an effective strategy for accelerating and economizing drug discovery and development process. Recently, a trend towards the use of *in silico* medicinal chemistry and molecular modeling for computer-aided drug design has gained significant momentum. Autoimmune diseases arise from an abnormal immune response of the body against own tissues [3]. The current regimen for the described condition is limited to immuno-suppressants having compromised pharmacodynamics and pharmacokinetics profiles [4]. One of the key player mediating immunity and tolerance, thus invoking autoimmunity is Interleukin-2; a cytokine influencing the growth of T cells [5]. The objective of present study is to devise small molecular inhibitors against interleukin-2 as immunomodulatory compounds. In the present study we have implemented *in silico* techniques to synthesize a series of novel inhibitors against interleukin-2 using fragment based drug design approach. Molecular Docking simulation was applied to identify the binding mode of the newly synthesized compounds. Analysis of docking results revealed potential hydrogen bonding network between ligand and the active site residues crucial for immuno-suppression. Inhibitory potential of the compounds showing promising results were analyzed using structure based 3D QSAR model. The predicted inhibitory potential of the compound suggests the synthesized compounds to be moderately active inhibitors of interleukin-2. To further investigate the mechanism of inhibition of compounds molecular dynamic simulation of (8) selected compounds was carried out using AMBER software suite. In addition to estimation of free energies associated with ligand binding, MD simulation yielded us a great deal of information about ligand- macromolecule interactions to evaluate the pattern of interactions and the molecular basis of inhibition.

### Keywords:

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## OCCURRENCE OF RABIES AND DOG BITE IN RAWALPINDI DISTRICT, PAKISTAN

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*\*Pir Mehr Ali Shah Arid Agriculture University Rawalpindi*

### ABSTRACT

A questionnaire survey of 1860 households was carried out from May, 2010 to December, 2012 in four areas of Rawalpindi city, in three towns and in 16 villages of Rawalpindi district randomly. The questionnaire was in local language and completed during a personal interview with the household head or adult person to gather information on occurrence of rabies in human and dogs and dog bites. A total of 1860 households, populated with 13641 people were surveyed and 1731 (93%) respondents were male while 129 (7%) were female members of the household. Only 356 respondents knew about the persons that died due to rabies. Out of these, 88.76% (316) were rabid due to dog bite, 8.43% (30) rabid due to jackal bite and 2.8% (10) rabid due to unknown reasons. The data given by the respondents were carefully analyzed and repeated rabies cases (reported by respondents of the same village/area) were fixed and concluded that 26 human deaths were reported during present survey from Rawalpindi district and 20 (76.92%) deaths were reported before 2000 while only 6 (23.08%) were reported after 2000 up to 2012. Only 955 (51.34%) respondents reported 1285 dog rabid in their area. In this regard, 14 households of Rawalpindi city reported 13 dog rabid, 132 town's households reported 185 rabid dogs, while 809 households reported 1087 rabid dogs in the area. Out of 1429 households, 1204 households (84.25%) killed rabid dogs, 196 households (13.72%) reported escape of rabid dogs while only 29 (2.03%) deliberately let the rabid dogs to go. Furthermore, 459/1860 household respondents reported dog bites in the house members and it was associated with males (332 cases). After dog bites, 24% people got spiritual treatments only and 40% go to hospitals only and 25% did both while 11% did nothing about treatment.

**Keywords:** Rabies, Rawalpindi district, questionnaire survey

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**IN-VITRO EVALUATION OF LACTIC ACID BACTERIA FOR PROBIOTIC SAFETY EFFICACY****Muhammad Shahid Riaz<sup>1</sup>, Samra Nawaz<sup>2</sup>, Tayyaba Shaheen<sup>1</sup>, Muhammad Tariq<sup>2</sup> and Arsalan H. Zaidi<sup>2</sup>***<sup>1</sup>Department of Bioinformatics and Biotechnology, GC University, Faisalabad, Pakistan**<sup>2</sup>National Probiotic Laboratory, Health Biotechnology Division,  
National Institute of Biotechnology and Genetic Engineering, Faisalabad, Pakistan***ABSTRACT**

Lactic Acid Bacteria (LAB) are the major constituents of the human intestinal micro flora. These have been considered as the major microbial group having probiotic potential. They are able to exert a wide range of beneficial health promoting effects that include inhibition of pathogen growth and production of antimicrobials and vitamins. In food industry, LAB have received considerable attention due to their probiotic activities. The probiotic strain's ability to resist unfavorable physiological conditions of the gastrointestinal tract (GIT) depends on various factors like tolerance to bile secretion and lysozyme resistance. The present study was conducted with the objectives of *in-vitro* screening of various indigenous LAB strains isolated from milk and yogurt in order to evaluate their probiotic potential. For probiotic potential, the pH sensitivity, bile resistance, H<sub>2</sub>O<sub>2</sub> and *lysozyme resistance* of LAB strains will be determined. Our *in-vitro* studies concluded that PL5, PL8, PL13 and PL14 proved to be most promising LAB isolates among all that exhibited a high resistance towards low pH, bile, lysozyme and H<sub>2</sub>O<sub>2</sub>. In future, these *in-vitro* studies will facilitate scientists to select suitable LAB strains and evaluate their probiotic properties *in-vivo* to understand how they affect human host and cope with adverse conditions in human GI tract. In future more potential properties of these strains may be checked, like folate & oxalate production, adhesion to mucin, production of  $\beta$ -galactosidase, cholesterol reduction mechanisms on LAB isolates. It will be helpful to design new protocols for in-depth studies related to their potential.

**Keywords:** Lactic Acid Bacteria, Probiotic Potential, GIT, microflora

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**THORACOPLASTY WITH PLICATION – A NEW APPROACH FOR HIGH RISK PULMONARY TB PATIENTS****Asif Asghar***Thoracic Surgeon PNS Shifa Hospital, Defence Phase 2, Karachi, Pakistan***ABSTRACT**

**Objective:** To evaluate the efficacy of thoracoplasty with lung plication for relief of fever and sputum production in pulmonary high risk patients with post tuberculosis cavities of lung.

**Methodology:** The study was conducted at Combined Military Hospital Lahore and PNS Shifa Karachi. 38 patients of all ages and sex with non-resolving thick walled post tuberculous lung cavities who were declared high risk for lung resectional surgery were selected. This group included cases with deranged lung function tests (FVC and FEV1 less than 40 % of predicted. Patients were evaluated for quantitative decrease in sputum and relief of fever after surgery. Thick walled cavities (wall thickness greater than 3 mm on CT scanning) usually do not resolve with conservative medical treatment. We think that by addition of apicolysis along with complete removal of three or four ribs which are overlying thickwalled cavities, we achieve better collapse as compared to thoracoplasty alone.

**Procedure:** All cases were operated using thoracic epidural block at T 4 or T 5 level. General anaesthesia was not administered. Four complete ribs were removed. Most of the patients were discharged on 4<sup>th</sup> post-operative day. Patients were assessed on 7<sup>th</sup>, 14<sup>th</sup> and 30<sup>th</sup> day as outpatients for quantitative decrease in sputum and relief of fever.

**Result:** We operated upon 38 patients. 11 patients were sputum positive for AFB. There were 3 cases with recurrence of symptoms over 03 months follow up. The remaining patients exhibited complete relief from sputum production and fever. There was one death due to postoperative pneumonia and respiratory failure.

**Conclusion:** Limited thoracoplasty with apicolysis and plication is a reliable procedure offering complete recovery without the ill effects of anaesthesia for nonresolving symptomatic apical tuberculous lung cavities

**Keywords:**

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## INNOVATIVE REPAIR OF LEAKING GASTRO- ESOPHAGEAL NECK ANASTOMOSIS FOR CARCINOMA OF ESOPHAGUS

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### ABSTRACT

**Introduction:** Leaking gastro esophageal anastomosis in neck is a complication usually managed conservatively by drainage. The leaks take two to three weeks to heal slowly and are usually associated with stricture. We devised a new procedure which is associated with minimum morbidity and can be performed under L.A. and is associated with early recovery and no stricture formation.

**Material and Methods:** We had four cases of cervical anastomotic leak over a period of 2 yrs. Reversed pectoralis major myocutaneous flap based on thoracoacromial artery was used to close the site of leak using local anaesthesia..

**Result:** There were no post procedure leaks. Barium swallow after 2 weeks revealed free flow of contrast distally with no narrowing at repair site.

**Conclusion:** Reversed pectoralis major myocutaneous flap coverage for a major gastro esophageal anastomotic leak in neck is a new and safe procedure and can be used in selected cases.

**Keywords:**

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## EVALUATION OF CORRELATION BETWEEN EXPRESSION OF P53 AND MALONDIALDEHYDE LEVELS IN PROSTATE CANCER PATIENTS

**Maria Arif, Amir Rashid**

### ABSTRACT

**Background:** Prostate cancer (CaP) is most commonly diagnosed non-dermatological solid malignancy with a high metastatic rate. CaP is the second most common cancer in males of USA and high incidence has been reported in United Kingdom, African, Brazilian and Pakistani men. Tumor suppressor gene, p53 plays a critical role in cancer prostate metastasis. Serum malondialdehyde (MDA) is a convenient and non-invasive biomarker in vivo index of lipid peroxidation in oxidative stress.

**Aim and Objective:** We have compared and assessed association of serum MDA with expression of p53 in prostatic carcinoma.

**Material & Methods:** Current study was designed to determine correlation between expression of p53 and MDA levels in CaP as compared to normal control. This is an analytical study which was conducted at Department of Urology, Department of Biochemistry & Molecular Biology Rawalpindi, National University of Science and Technology, Islamabad over a period of one year. Study included 32 samples. Expression of p53 and levels of MDA were determined by real time qPCR (quantitative polymerase chain reaction) and ELISA (enzyme linked immune sorbent assay) technique respectively.

**Results:** We have compared mean value of MDA in CaP and control group, the difference was statistically significant ( $p=0.002$ ). Gleason score 8 showed statistically significant increase in MDA as compare to control group among all other Gleason score (6, 7 and 9). Optimum annealing temperature required for annealing of our designed primers was 55.6°C. We have compared mean  $C_T$  value of CaP with control group, the difference was statistically significant ( $p<0.05$ ). Expression of p53 was 0.18 folds decreased in CaP as compared to control group. There was a weak inverse correlation between expression of p53 and MDA in CaP group.

**Conclusion:** MDA may be used as marker to determine prognosis of CaP. Expression of p53 may be help in diagnosis of CaP. Moreover, more studies should be carried out to find the pathway involved in inverse correlation of expression of p53 and MDA in CaP.

**Keywords:** Prostate cancer (CaP), Malondialdehyde (MDA), Gleason scoring

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## GENETIC ANALYSIS OF MULTI-DRUG RESISTANT *SALMONELLA TYPHI* ISOLATES IN PAKISTAN

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### ABSTRACT

Multi drug resistant *Salmonella typhi* (*MDR S. typhi*) a major public health problem in developing countries. More 75 % of the world population is facing 93% of global typhoid epidemics in developing world. The disease is a serious threat to the developed nations. More than 30 million new cases cause about 216,000 deaths each year. Typhoid represents the 4th most common cause of death in Pakistan. Emergence of ESBL has been identified in *MDR S. typhi* after fluoroquinolone resistance. Re-emerging sensitivity of 1st line anti typhoid and emergence of ESBLs has transpired the need for genetic analysis.

**Aims and objectives:** Drug resistant genes / mutations, antimicrobials susceptibility and incidence of ESBL in *S. typhi*.

**Patients, Materials And Methods:** Four thousands seven hundred and forty eight (n = 4748) *S. typhi* isolates were collected from blood culture samples, Identified by AP1-20E, susceptibility by Kirby-Baur and Production of ESBL using cephalosporin indicator discs in combination with co-amoxiclav. *S. typhi* DNA and drug resistant genes isolated by molecular method.

**Results:** Susceptibility of chloramphenicol, ampicillin and trimethoprim/ Sulfamethoxazole 87.3 %, 81.1 % and 78.2 %. Ciprofloxacin 45.1 %, ceftriaxone, cefotaxime and ceftazidime was found 95.7 %, 94.8 % and 94.6 % respectively. Only three isolates appeared ESBL producers 0.69% Imipenem and meropenem were 97.7% and 97.8% effective. PCR products sequencing showed Ser 83 to Phe (TCC to TTC) mutation in all the cases. No mutation a codon 87 was seen in any case.

**Recommendation for Physician:** If facilities exists molecular methods should be preferred.

**Conclusions:** *S. typhi* should be continuously monitored for the presence of plasmid carrying resistant markers against antimicrobials.

**Keywords:** *Salmonella typhi*, Genetic Analysis, Antimicrobial susceptibility

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## AWARENESS ABOUT THALASSEMIA IN A MEDICAL COLLEGE

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### ABSTRACT

**Objective:** The purpose of this research is to assess the awareness of thalassemia among students, faculty and admin staff of different departments at ANMCH.

**Method:** The research involves the filling of the questionnaire about thalassemia by the selected individuals. The results will be evaluated as to assess the overall awareness about thalassemia. The individuals who were selected are the students of MBBS and Nursing along with their faculty, the administration and staff of the Al-Nafees Medical College & Hospital and Isra College of Nursing.

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## COMPARATIVE PHARMACOKINETIC AND BIOAVAILABILITY OF CEFSPAN AND CEFORAL-3 IN ADULT HEALTHY FEMALE SUBJECTS

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### ABSTRACT

Comparative pharmacokinetic and bioavailability of two brands of cefixime, cefspan and ceforal-3, was carried out in healthy adult female volunteers after oral administration of 400 mg capsules with a wash out period of 7 days. Concentration of cefixime was determined in plasma by HPLC method. Plasma concentration versus time data was utilized for the calculation of pharmacokinetic and bioavailability parameters. One compartment open model was utilized for pharmacokinetic parameters. Mean $\pm$ SE values: half-life ( $3.99\pm0.54$  hr and  $3.12\pm0.39$  hr), volume of distribution ( $1.38\pm0.22$  l/kg and  $1.36\pm0.17$  l/kg), total body clearance ( $0.27\pm0.02$  l/hr/kg and  $0.31\pm0.02$  l/hr/kg), maximum plasma drug concentration ( $2.24\pm0.23$   $\mu$ g/ml and  $2.08\pm0.16$   $\mu$ g/ml), time to reach at maximum plasma drug concentration ( $4.05\pm0.35$  hr and  $3.87\pm0.32$  hr) and area under curve ( $27.12\pm2.25$   $\mu$ g.hr/ml and  $23.99\pm1.07$ ) remained non significantly ( $P > 0.05$ ) different from each other when compared by student's paired t-test. Relative bioavailability based on AUC and  $C_{\max}$  was found to be within the acceptable range for bioequivalence i.e. 80-125%. Hence ceforal-3, a test formulation and cefspan, a reference formulation were bioequivalent and replaceable in clinics.

**Keywords:** Pharmacokinetic; Bioavailability; Female; Cefspan; Ceforal-3; Bioequivalence.

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## SOURCE IDENTIFICATION OF PLANT PROTECTION PRODUCTS AND THEIR CORRELATION WITH COEXISTENT ANIONS IN SOIL OF COTTON/WHEAT FIELDS OF MULTAN

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### ABSTRACT

The indiscriminate use of plant protection products in cotton and wheat fields results in deterioration of soil which ultimately affects the food quality. This problem is much more aggregated in Pakistan especial in major cotton grown areas. Representative soil from Multan were collected and screened for common cotton and wheat pesticides by using modified EPA multiresidue pesticide analysis method and analyzed by GC-MS. Imidacloprid residues in these soil were determined by HPLC-UV. Most of soil samples from the study areas were highly contaminated with the residues of imidacloprid, chlorpyrifos and  $\alpha$ -cypermethrin. All of selected pesticides except imidacloprid were significantly correlated with each other at  $p < 0.05$ , while imidacloprid was negatively correlated with triazophos, bifenthrin,  $\lambda$ -cyhalothrin, deltamethrin, MCPA and 3-PBA at  $p < 0.01$ . The fenitrothion, MCPA, 3-PBA, triazophos, profenophos,  $\alpha$ -cypermethrin,  $\lambda$ -cyhalothrin and deltamethrin were significantly positively correlated with soil phosphate levels while negatively correlate with sulphate content of cotton/wheat soil at  $p < 0.05$ . Soil pH was positively associated with profenofos, triazophos and  $\alpha$ -cypermethrin residues while soil organic matter content was concomitant with bifenthrin,  $\lambda$ -cyhalothrin and deltamethrin residue. The varimax normalized factor analysis and cluster analysis further clarify that perhaps sulphate level of soil positively affects the imidacloprid persistence/mobility. The dendrogram of cluster analysis also support the above correlations.

**Keywords:** Plant protection products, cotton/wheat field's soil, HPLC, GCMS, multivariate statistical analysis

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## COMPARISON OF QUALITY OF LIFE OF CANCER PATIENTS UNDERGOING CHEMOTHERAPY IN A TERTIARY CARE HOSPITAL

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### ABSTRACT

**Introduction:** WHO defines QOL as individual perception of life, values, objectives, standards and interests in the frame work of culture. Cancer is one of the most important health concerns of today and evaluating QOL in cancer patients is an increasingly important issue

**Objective:** To compare the quality of life of cancer patients at different chemotherapy (CT) cycles.

**Methods:** A cross sectional study was conducted in a Tertiary Care Hospital in Rawalpindi. Non-probability purposive sampling technique was used to select a sample of 50 cancer patients undergoing chemotherapy. Participants were selected on the basis of number of chemotherapy cycles. After taking informed consent the data was collected using the European Organization for Research and Treatment of Cancer QOL Questionnaire (EORTC QLQ-C30) was used to measure QOL in the patients. Data was entered and analyzed using SPSS version 21.

**Results:** The study sample comprised of 31 males and 19 females .Mean age of participants was  $43.88 \pm 12.72$  years (Range 18 – 70 years). Out of all the participants 7(14%) were unemployed, 14(28%) were serving personnel, 18(36%) were doing household work and 11(22%) were business men. 16(32%) patients had lung cancer, 9(18%) had breast cancer, 13(26%) were suffering from genitourinary carcinoma and 12(24%) were suffering from oral cavity cancer. There was no association between the QOL and variables such as age, sex, and occupational status. There was a significant difference in symptom scale of group 2 which were was fatigue, pain, appetite loss and nausea and vomiting. While in case of functional scale only role functioning was significant in group 2. There was an overall improvement in Global Health Status (GHS) in group 2. Nevertheless, a significant difference was found between the level of QOL in patients with  $\leq 2$  CT cycles and with  $>2$  cycles ( $p < 0.001$ ).

**Conclusion:** This study suggests that the quality of life is directly related to chemotherapy cycles. Although QOL scoring system did not show significant improvement in all areas of our study, but the obtained results indicated a significant association between QOL and number of CT cycles in cancer patients.

**Keywords:** Cancer, QOL, Chemotherapy

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## KNOWLEDGE ATTITUDE AND PRACTICE OF CELL PHONE USE WHILE DRIVING AMONG UNIVERSITY STUDENTS

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### ABSTRACT

**Introduction:** Road traffic accidents (RTAs) are considered as one of the important public health problems around the world. Cell phone use is now estimated to be involved in on third of all motor vehicle crashes.

**Objectives:** The objective of the study was to assess the knowledge, attitude and practice of cell phone use while driving among university students and to recommend measures to prevent it

**Materials and Methods:** A descriptive cross sectional study was conducted in three premier Universities of Rawalpindi for the duration of 6 months (September 2014 to March 2015). Non-probability convenient sampling was used and a sample size of 264 was calculated using Raosoft sample size calculator with 95% confidence level and 6% permissible margin of error. University students between 18-25 years of age who were willing to participate and had personal cell phone, a car and drove at least three times a week were selected. However, those who did not have a driving license were excluded from the study. A self-made 19 item questionnaire was used for data collection and informed consent was taken prior to getting the questionnaire filled. Data was analyzed using SPSS version 22.0

**Results:** A total of 264 undergraduate students (60.2% male and 39.8% females) participated in the study. The mean age of participants was  $20.99 \pm 1.608$  years. According to our study, nearly 46% of the young drivers use cell phone while driving with males being 18.3% more likely than females to use their cell phones. 97% of the participants thought that cell phone use while driving is unsafe. Due to cell phone use, 26.9% individuals were involved in a close call or near crash. Moreover, 81.8% participants thought that strict action from police or risk of fine can reduce the cell phone use while driving.

**Conclusion:** Based on our findings, it is concluded that despite having knowledge regarding hazards of cell phone use while driving, majority of the students are still engaged in practice of cell phone use and there is a need for strict action and implementation of laws to avoid accidents.

**Keywords:** Knowledge, attitude, practice, cell phone use, driving, University Students.

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## PROTECTIVE EFFICACY OF COMMERCIAL WATER BASED VACCINE AGAINST HYDROPERICARDIUM SYNDROME

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### ABSTRACT

The current study was planned to check the efficacy of commercial water based vaccine against Hydropericardium syndrome virus (HSV). The characteristic features of the disease include accumulation of transparent or straw colored fluid in the pericardial sac with pulmonary edema, nephritis, and hepatitis with 30% to 70% mortality rate. The causative agent is a non-enveloped icosahedral fowl adenovirus (FAV) serotype 4, belonging to the Adenovirus genus of the family Adenoviridae. 10 days old group of broilers chicks were injected with commercial water based vaccine with a dose of 0.5 mL. Serum samples were collected weekly and immune response to the vaccine was confirmed qualitatively by agar gel precipitation test (AGPT). The vaccinated chicks were challenged with a Pakistani field isolate FAV-4 with a dose of 0.5 mL at day 38 post-vaccination to check the protective efficacy of the vaccine. No chick showed clinical manifestation of disease up to five days post challenge. No mortality was recorded. Broilers were effectively protected by commercial water based vaccine against FAV-4.

**Keywords:** Hydropericardium syndrome, Fowl Adenovirus Serotype 4, agar gel precipitation test, Hydropericardium syndrome virus, Mortality, Hepatitis, Nephritis.

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## GALECTIN-3: AN EMERGING BIOMARKER FOR HEART FAILURE

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### ABSTRACT

Heart Failure is an emerging epidemic. The increase in prevalence is due to aging population and prolongation of the lives of cardiac patients by modern therapeutic innovations. To facilitate clinical judgment for diagnosis and management of heart failure patients, cardiac biomarkers are used which makes it crucial that research in this field is actively pursued.

Galectin-3 has emerged recently as a prognostic marker in patients with heart failure. It is a chimera type galectin from a family of carbohydrate binding proteins that play a regulatory role in diverse disease states. In vivo experiments have shown that Galectin-3 is responsible for cardiac fibrogenesis and adverse left ventricular remodeling resulting in heart failure development and progression. Higher levels of Galectin-3 have been found to be associated with recurrent heart failure and increased risk of death. It is also helpful in predicting all-cause death and demonstrates a relationship with future heart failure and rehospitalizations in the general population. We have shown that Galectin-3 is increased at both transcriptional and translational level in the left ventricle in early post myocardial infarction period. It is expressed by cardiomyocytes, endothelial cells and neutrophils and was found to regulate proinflammatory and antiapoptotic mechanisms in the myocardium. Our work has shown that Galectin-3 acts as an “early warning” reflecting myocardial changes before the onset of hemodynamic strain.

**Keywords:**

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## CONVENTIONAL DIAGNOSIS OF FIELD ISOLATE OF FAV-4 CAUSING HYDRO PERICARDIUM SYNDROME (HPS)

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### ABSTRACT

Hydro pericardium syndrome caused by Fowl Adeno virus (FAV) serotype 4 belongs to Adenoviridae family associated with anemia, hemorrhagic disorders and hydropericardium in young chickens and drastically affects poultry industry of Pakistan having up to 80% mortality rate. Present study was designed for the conventional diagnosis of FAV-4. A field isolate of FAV-4 was recovered from Multan outbreak. Experimental infection was done by preparing 50% weight/volume suspension and 3 conventional methods for HPS diagnosis were employed including clinical signs, PM & AGPT. Clinical signs include abrupt mortality, lethargy, huddling with ruffled feathers and especially yellow mucoid droppings at onset of disease. Post mortem observation involved pathognomic lesions showing approximately 10ml of straw colored transudate in pericardial sac, generalized congestion, enlarged pale and febrile liver. Qualitative confirmation of FAV-4 was successfully done by agar gel precipitation test.

**Keywords:** Fowl adenovirus serotype-4, Agar Gel precipitation test, PM (post mortem)

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## ESTIMATION OF FLORFENICOL RESIDUES IN LAYER MEAT AND EGG SAMPLES

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### ABSTRACT

Antibiotics are widely used in the poultry industry to enhance the health and productivity of flocks which may pose an adverse health effect for the consumer. Screening of food products from animal origin for the presence of antimicrobial residues is necessary for the consumer's health. The present study was conducted to determine the Florfenicol (FF) residues in layer's meat and egg samples. A total 150 samples of meat (n=75) and eggs (n=75) were collected to determination of FF residues. The residual concentration of FF was determined by High Performance Liquid Chromatography (HPLC) with UV detector set at 223 nm using C<sub>18</sub> column. Ethyl acetate and phosphate buffer saline solution were used for extraction of FF from the samples. The mobile phase consisted of acetonitrile-water (27:73 v/v). Overall mean residual concentrations of FF in meat and egg samples were 61.56±13.19 and 281.08±57.46 µg/kg, respectively. This study revealed that 80% (60) meat and 72% (54) egg samples were positive for FF, out of these 86.7% (52) meat and 55.6% (30) egg samples were having residual concentration above the maximum residual limits. So it can be concluded that the usage of this contaminated meat may cause resistance in consumers and seems to be a public health threat as well as there is a need to educate the formers about the ill effects of residual drugs on human health and withdrawal time in poultry birds.

**Keywords:** Florfenicol, HPLC, residues, layer, meat, eggs

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## HEMATOPOIETIC POTENTIAL OF *ANGELICA SINENSIS* ROOT CAP POLYSACCHARIDES AGAINST LISINOPRIL INDUCED ANEMIA IN ALBINO RATS

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### ABSTRACT

The present study was designed to evaluate the protective hematopoietic potential of *Angelica sinensis* polysaccharides (ASP) against lisinopril induced anemia along with their toxic effects on kidney and liver cells. Thirty healthy adult male albino rats were randomly divided into five equal groups (n=6). Group I was control group. Group II was administered with Angiotensin converting enzyme inhibitor (ACEI, 20 mg/kg/day) to induce the anemia. In group III, ACEI was administered (20 mg/kg/day) in combination with erythropoietin (EPO, 100 IU/kg/each). Group IV was administered with ASP at the dose rate of 1 g/kg/day. In Group V, ACEI (20 mg/kg/day) was administered in combination with ASP (1 g/kg/day). After 28 days, blood and tissue samples were collected for hematological and histopathological analysis, respectively. The results showed that ACEIs significantly reduced the hemoglobin value (Hb), packed cell volume (PCV), red blood cell (RBC) count, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values. In group treated with ASP, significantly increased the Hb value and RBC count. The combination of ACE inhibitor and ASP led to the significant reduction in Hb, PCV, RBC count, MCV and MCH values. While histopathological examination of liver and kidney cells showed mild degree of toxicity in ASP treated group. APS may have effect against anemia but when it is administered simultaneously with ACEI to cure anemia then it showed unfavorable effect with more complicated anemia so it should not be used with ACEIs.

**Keywords:** ACE inhibitors, *Angelica sinensis*, RBC count, PCV, MCV, MCH

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## QURANIC PLANTS THE UNIVERSAL REMEDY OF MANY ILLS

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### ABSTRACT

The matchless style of the Quran and its superior wisdom are definite evidence that it is the word of God. In addition, the Quran has many miraculous attributes proving that it is a revelation from God. One of these attributes is the fact that a number of scientific truths that we have only been able to uncover by the technology of the 20<sup>th</sup> century were stated in the Quran 1,400 years ago. Hence, the purpose is to crystallize the Quranic scientific miracles by showing compatibility of quranic plants with modern scientific research. Cure of diseases through medicinal plants is always a salient feature of Islamic teaching and preaching. Keeping in view the importance of diverse medicinal flora and rich medicinal culture of Islam, research work was conducted to investigate ethnobotanical uses and create awareness about the plant species enlisted in Holy Quran for the welfare of human communities throughout the world. In the Holy Quran nineteen plants has been described in different verses for various aspects. In the current paper plants mentioned in Quran viz. *Phoenix dactylifera*, *Lawsoniainermis*, *Zizyphusmauritiana*, *Cucumismelo*, *Ficuscarica*, *Musaparadisiaca*, *Ocimumbasilicum*, *Allium sativum*, *Allium cepa*, *Zingiberofficinale*, *Vitisvinifera*, *Lens culinaris*, *Brassica nigra*, *Oleauropea*, *Punicagranatum*, *Tamarixaphylla*, *Salvadorapersica*, *Euphorbia species* and *Lagenariasiceraria* are described with reference to their therapeutic applications. Results were systematically arranged by alphabetic order of botanical names followed by English name, Arabic name, family, part used, medicinal uses and Characteristics Surah and Ayah in Holy Quran. Consequently, the Holy Quran is considered to be the reference for the citation of these plants.

**Keywords:** Quranic plants, Holy Quran, miracles, medicinal plants.

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## GASTROPROTECTIVE POTENTIAL OF *RUMEX HASTATUS* D. DON. ROOTS AGAINST DICLOFENAC-INDUCED GASTRIC DAMAGE IN RATS

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### ABSTRACT

According to our previous studies *Rumex hastatus* D. Don. possesses anti-inflammatory and antioxidant properties and in Pakistan the roots of this plant are traditionally used to treat emesis, ulcers, dysentery, stomach complaints, and liver disorders. So this study was conducted to evaluate the antiulcer effects of *R. hastatus* root methanol extract (RHME) against the diclofenac sodium-induced ulceration in rat. The RHME (200 mg/kg and 400 mg/kg body weight) was orally administered to rats once a day for 10 days in diclofenac-treated rats. The gastroprotective effects of RHME were determined by assessing gastric-secretory parameters such as volume of gastric juice, pH, free acidity, and total acidity. Biochemical studies of gastric mucosa were conducted to estimate the levels of nonprotein sulphhydryls (NP-SHs), lipid peroxidation [thiobarbituric acid reactive substances (TBARSs)], reduced glutathione (GSH), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), levels of scavenging antioxidants, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), and myeloperoxidase (MPO). In addition, stomach tissues were also tested for adherent mucus content and histological studies. Results showed that administration of RHME significantly decreased the ulcer index, TBARSs, H<sub>2</sub>O<sub>2</sub>, and MPO activity in gastric mucosa of the ulcerated rats. Activities of enzymic antioxidants, CAT, SOD, GSH-Px, GST and GSH, and NP-SH contents were significantly increased with RHME administration in the gastric mucosa of diclofenac-treated rats. Volume of gastric juice, total and free acidity were decreased, whereas pH of the gastric juice was increased with the administration of RHME + diclofenac. Our results show that RHME administration is involved in the prevention of ulcer through scavenging of free radicals. Results of histopathological studies supported the gastroprotective activities of RHME. Hence, it is concluded that antiulcer effect of *R. hastatus* roots might be due to its antioxidant ability.

**Keywords:** *Rumex hastatus*; antioxidant; antiulcer; gastroprotective; lipid peroxidation

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## BIOASSAY OF THE CRUDE EXTRACT FROM RHIZOSPHERIC *ASPERGILLUS* AND SOIL BORNE *PENICILLIUM* SPECIES

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### ABSTRACT

Crude extracts of two soil borne fungi i.e. *Aspergillus sp.* isolated from rhizosphere of *Mintha piperata* and *Penicillium sp.* obtained from soil of agricultural farm of Agriculture University Peshawar were screened for antibacterial, antifungal and cytotoxic activities. Antibacterial activities were performed against eight pathogenic bacterial strains while to test the antifungal ability the crude extracts of both fungi were tested against six pathogenic fungal strains. Cytotoxic activities were tested against brine shrimps eggs by hatching them in artificial sea medium. Results indicated that both ethyl acetate and n-hexane fractions of *Aspergillus sp.* showed better activities as compared to *Penicillium sp.* In case of antibacterial activities ethyl acetate and n-hexane fraction of *Aspergillus sp.* showed highest activity against *Bacillus subtilis* (47.5 mm) and *Salmonella typhi* (51 mm) respectively. Crude extracts of *Penicillium sp.* showed highest inhibitory activity against *Proteus vulgaris* (34 mm). Ethyl acetate fraction of *Aspergillus sp.* proved more effective against *Microsporium canis* (60.5%) while n-hexane fraction of this fungus was active against *Fusarium solani* (56.5%). Ethyl acetate fraction of *Penicillium sp.* was also active against *M. canis* (63.5%) while its n-hexane fraction showed considerable activity against *Candida glabrata* (28.5%). Extracts tested against brine shrimps revealed that *Aspergillus sp.* proved more toxic to brine shrimps with lethality rate of 98.33% as compared to 63.33% of *Penicillium sp.* at 1000 µg/mL.

**Keywords:**

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## LIGHT INTENSITY IMPROVES THE COPULATING BEHAVIOUR AND COLONY INITIATION OF BUMBLEBEE, *BOMBUS TERRESTRIS* (HYMENOPTERA: APIDAE)

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### ABSTRACT

Impact of mating tendency was investigated for *Bombus terrestris* under different light intensities (400, 800 and 1200 lux) with different age groups of daughter queens and males (3DF-5DM, 5DF-7DM, 7DF-9DM, 9DF-11DM) under controlled laboratory standard conditions. Percent mating, queens' survival after diapause and successful colony initiation were observed. Mating rate was more at light intensity of 800 lux with more than 80%. However, it was around 55% at 400 lux and 65% at 1200 lux suggesting 800 lux light intensity to be the best light intensity condition for improving success rate of mating. Mating tendency of more than 80% was achieved with 7-days old queens with 9-days old males with constant dark-treated queens. Among light intensity of 400, 800 and 1200 lux average copulation duration was highest ( $24.05 \pm 1.51$  min) at 400 lux. Among mating of 3, 5, 7 and 9 days old queen with 5, 7, 9 and 11 days old males average copulation duration was highest ( $24.4 \pm 1.62$  min) when mating of 7 days old queen with 9 days old male was done. These results represent the positive influence of light intensity to mating success rate, age-group for maximum copulating pairs and their possible impact on future generations.

**Keywords:** *Bombus terrestris*, light intensity, pre-mating exposure, mating, colony initiation.

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## COMPARATIVE PROTEIN PROFILING OF MILK OF NILI-RAVI BUFFALOES, SAHIWAL AND CROSS BRED COWS BY SDS-PAGE.

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Milk of different species may vary in protein quality and quantity. A proteomic approach for high-resolution analyses of the complex mixture of milk proteins by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) is of great importance due to its exceptional resolving capacity. Degreased milk samples (non-infected) of Nili-Ravi buffaloes, Sahiwal and cross-bred cows were used for comparative protein profiling using 12 % SDS polyacrylamide gels. Protein profiling clearly resolved the major milk peptides. Qualitative differences were observed in high, medium and low molecular weight (MW) zones. In high molecular weight (M. wt.) zone, proteins of ~208 kDa and ~190 kDa were detected in all tested samples. In medium M. wt. zone, three peptides i.e. lactoferrin (78.2 kDa), serum albumen (66.2 kDa) and heavy chain of immunoglobulin (IgG) (54 kDa) were detected in all samples while a prominent band of ovalbumin (45 kDa) was also detected mainly in cow milk samples. In low M. wt. zone, clear bands of milk caseins were detected. All four casein (CN) bands i.e.  $\alpha$ S<sub>2</sub>-CN (29 kDa),  $\alpha$ S<sub>1</sub>-CN (27 kDa),  $\beta$ -CN (24 kDa) and  $\kappa$ -CN (22 kDa) were detected in Sahiwal and cross-bred cows. However, in milk of Nili-Ravi buffaloes, three casein protein i.e.  $\alpha$ S<sub>2</sub>-CN (29 kDa),  $\beta$ -CN (25 kDa) and  $\kappa$ -CN (22 kDa) were detected. In milk of Nili-Ravi buffaloes,  $\alpha$ S<sub>1</sub>-CN (27 kDa) was not detected. Moreover, a band of  $\beta$ -lactoglobulin (~18 kDa) was detected in milk of cross-bred cows and not in other samples especially Nili-Ravi buffaloes. As the  $\alpha$ S<sub>1</sub>-casein and  $\beta$ -lactoglobulin are the major allergens, milk of Nili-Ravi buffaloes that lacks these peptides can be used for development of hypoallergenic or non-allergic dairy products. Differential peptides may also help to differentiate the milk from different tested breeds.

**Keywords:**  $\alpha$ S<sub>1</sub>-casein;  $\beta$ -lactoglobulin; kDa; lactoferrin

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## IMPACT OF REACTIVE OXYGEN SPECIES ON ANTIOXIDANT CAPACITY OF MALE REPRODUCTIVE SYSTEM

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### ABSTRACT

To study the mutual interaction of ROS and basal antioxidant capacity in male reproductive system and to establish the association between selected heavy metals and stress markers were the main focus of present research work. Total oxidant status (TOS) and total antioxidant status (TAS) of serum and seminal plasma were determined by automated photometric methods. The concentrations of Selenium (Se), Lead (Pb) and Cadmium (Cd) were determined by using atomic absorption spectrophotometer. The TOS was found highly significant ( $p < 0.05$ ) in seminal plasma as well as in serum of infertile group when compared with fertile group. On the other hand, TAS of infertile group was found noticeably decreased ( $p < 0.05$ ) as compared with the TAS of fertile group. Among the metals we measured, noticeably lower concentrations of Se were found in infertile group whereas markedly elevated levels of Cd and Pb were observed in infertile group as compared with the fertile group. Among the infertile group a significant inverse correlation ( $r = -0.521$ ,  $p < 0.05$ ) were observed between Se and TOS and between Cd and Pb ( $r = -0.407$ ,  $p < 0.05$ ). Contrarily among infertile group a considerable positive relationship was found between Se and TAS ( $r = 0.507$ ,  $p < 0.05$ ). It was concluded that the oxidant stress reduces the antioxidant activity in infertile men by elevating the production of ROS. Lower concentration of Se and elevated level of Pb and Cd explains the individual's exposure to these heavy metals. The study also revealed that the heavy metal toxicity contributes significantly in the male infertility.

**Keywords:** Infertility, Antioxidants, ROS, Selenium, Lead and Cadmium

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## USE OF ELEVATED TEMPERATURE FOR MANAGEMENT OF *TRIBOLIUM CASTANEUM* (HERBST) AND *SITOPHILUS ORYZAE* (L) IN STORED WHEAT

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### ABSTRACT

Insect development is directly affected with environmental factors. Temperature is given due importance to manage stored grain insect pests since history. Keeping in view the effectiveness of temperature as a limiting factor, best possible temperature with minimum time period was investigated. Adults of red flour beetle, *Tribolium castaneum* and rice weevil, *Sitophilus oryzae* were exposed to nine different temperatures starting from 36 to 60°C for fifteen time intervals from 01 minute to 72 hours were used in SANYO incubator. Three replicates of Petri dish with 20 adults of each species in CRD design were exposed for each treatment. Adults of *T. castaneum* showed 50% mortality at 45, 48 and 51°C in 24, 24 and 12 hours respectively and in case of *Sitophilus oryzae* showed 50 % mortality at 45, 48 and 51°C in 12, 2 and 1 hours respectively. *T. castaneum* showed 95 % mortality at 60, 57 and 53°C in 6, 8 and 15 minutes respectively. Although it was similar at 60°C for *S. oryzae* yet it took 6 minutes at 57°C and 14 minutes at 53°C. It can be suggested that high temperature of 60°C can be used for *T. castaneum* and *S. oryzae* for six minutes exposure to kill 95% adults of both stored grain pests of great economic and aesthetic importance.

**Keywords:** Temperature, *Tribolium castaneum*, *Sitophilus oryzae*, Time interval, Limiting factor

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## DETERIORATION OF PAINTED WALL SURFACES BY FUNGAL SAPROBES: ISOLATION AND IDENTIFICATION

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### ABSTRACT

Fungal saprobes were isolated from selected areas of deteriorated painted surfaces from downtown areas of Lahore, Punjab, Pakistan. The purpose of this study was to contribute the knowledge of mycoflora inhibiting the painted wall surfaces in buildings. The standard microbiological techniques were used for isolation and identification of fungal saprobes. The fungus isolated from deterioration of painted wall surface belongs to 12 different fungal species; the most prevalent represents genera *Penicillium*, *Aspergillus*, *Colletotrichum*, *Acremonium*, *Trichoderma*, *Fusarium*, *Curvularia*, *Mucor* and *Alternaria*. The use of antibiotic coated paint would possibly put off the bio-deterioration; improve the shelf life and retain the beauty of wall paints.

**Keywords:** Painted wall, deterioration, fungi, isolation, identification

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## EFFECT OF SMOKING ON SERUM AMYLOID A-LOW DENSITY LIPOPROTEIN (SAA-LDL) IN SPRAGUE DAWLEY RATS

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### ABSTRACT

**Background:** Cigarette smoking increases oxidative stress, which is a risk factor for several diseases like cancer, degenerative diseases and chronic inflammation. Serum amyloid A-Low density lipoprotein (SAA-LDL), formed by oxidative modification of low density lipoprotein (LDL), is considered a new and unique marker of oxidatively-modified LDL particles.

**Aims and Objectives:** The aim of this study was to investigate the effect of cigarette smoking on SAA-LDL in Sprague Dawley rats.

**Materials and Methods:** The study was carried out at National Institute of Health (NIH). 70 healthy Sprague Dawley rats were randomly divided into two groups. Group-I rats were not exposed while Group-II rats were exposed to cigarette smoke for 3months. SAA-LDL levels were determined using commercially available enzyme linked immunosorbent assay (ELISA) kit.

**Results:** Higher levels of SAA-LDL were found in exposed as compared to non-exposed rats. Mean values of SAA-LDL in control and smoker groups were  $1.51 \pm 0.29$  and  $2.99 \pm 0.59$  respectively. The difference was statistically significant ( $p < 0.05$ ).

**Conclusions:** Positive association was found between cigarette smoking and SAA-LDL. This shows that SAA-LDL, an oxidative stress marker, may be used as an early indicator of oxidative damage in smoke related disorders.

**Keywords:** Smoking, Cigarette, OxLDL, SAA-LDL, oxidative stress

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## EARLY SCREENING OF HPV16 IN BREAST CANCER AFFECTED PATIENTS OF PAKISTAN

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### ABSTRACT

Breast cancer is among the top three malignancies across the globe. Its prevalence is higher in less developed and developing countries. Involvement of different viruses in breast tumorigenesis has been observed. Association of Human Papillomavirus (HPV) as a potential risk factor for breast cancer is still controversial. Interestingly, several studies have reported the presence of HPV in breast cancer while some studies exonerate HPV influence in breast cancer. The studies related to screening, penetrance and involvement of HPV in breast women of Pakistani population are yet limited. The aim of the current study is to investigate the presence of HPV in breast cancer affected women. A cohort of patients suffering from mammary tumours (n=10) with a mean age of 49.5 yrs (ages ranged from 30-60 Yrs) was identified. Informed consent and prior ethical approval was taken before sampling. From each patient both tumor affected tissue along background tissue was collected and stored at per set standardized procedures. Isolation of DNA was carried out using organic protocol method from these biological samples. Primer 3 software was used to design HPV16 specific PCR primers. Amplification of DNA was carried out by conventional PCR technique to detect HPV16. GAPDH is used as an internal control. The results obtained showed the presence of HPV16 in 9 (45%) samples. Out of these, HPV16 has been detected in 5 (25%) breast tissue and 4 (20%) controls. Our results are consistent with earlier studies showing the presence of HPV in breast cancer. This supports the fact that HPV may have casual role in the pathogenesis of breast cancer. However, more evidence and tests need to be conducted to find the possible role of HPV in controls (surrounding or background tissue) and its prevention by HPV vaccines.

**Keywords:** HPV, PCR, Pakistan, Pathogenesis, Breast cancer, Amplification

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## INFLUENCE OF PRUNING ON FRUIT YIELD AND QUALITY OF GUAVA CV. GOLA

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### ABSTRACT

The study was conducted to find out the best pruning intensity level for better fruit yield in guava (*Psidium guajava*). Four pruning levels i.e. T<sub>0</sub> (no pruning), T<sub>1</sub> (25% pruning), T<sub>2</sub> (50% pruning) and T<sub>3</sub> (75% pruning) were used in this experiment. Pruning was done during the month of April (after harvesting winter crop). Data showed that maximum fruit weight (102 g) at 75% pruning level, while minimum (70 g) at T<sub>0</sub> (no pruning). At 25% pruning level fruit yield was maximum (172.6 Kg/plant) where as minimum fruit yield (70.11 Kg/plant) was at 75% pruning level, maximum fruit length (62.63 mm), fruit width (57.49 mm), TSS (8.11 %) and acidity (0.88 %) were recorded at 50 %, 75 %, control and 50 % pruning level respectively. Minimum fruit length (50.18 mm), fruit width (48.12mm) and acidity (0.5 %) was recorded in control, whereas minimum TSS (7.36 %) was observed at 75 % pruning level. The study provided useful information to determine the influence of different pruning levels on light penetration, flowering and fruiting.

**Keywords:**

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## EFFICIENCY OF *BURKHOLDERIA PHYTOFIRMANS* (PSJN) IN COMBINATION WITH *BACILLUS*, *ENTEROBACTER* AND *PSEUDOMONAS* SP. FOR INDUCING SALINITY TOLERANCE IN MAIZE (*ZEA MAYS* L.) UNDER SALT AFFECTED CONDITIONS

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### ABSTRACT

Food security is the most alarming issue of developing countries. These conditions compell us to increase yield per acre. Meanwhile, adverse conditions as a result of climate change and global warming (high temperature, high rainfall and more evapotranspiration) are converting the fertile land into salt affected, which covers almost 50% of the world area. These situations, demand efficient use of abandon salt affected/marginal lands by adopting different means. One option is inoculation with beneficial bacterial strains. Present study was planned to evaluate the effect of bacterial consortium under salt affected conditions. A field experiment was conducted at Postgraduate Agricultural Research Station, University of Agriculture, Faisalabad under randomized complete block design (RCBD) with three replications. For this, inoculum of pre-isolated *Burkholderia phytofirmans* (PsJN), *Bacillus*, *Enterobacter* and *Pseudomonas* sp. in different combinations were coated on sterilized maize seeds. Recommended dose of NPK was applied and standard agronomic and cultural practices were used. Data regarding growth, yield and physiological was taken and analyzed by standard statistical procedures. Results revealed that under salt affected condition, the yield of maize drastically effected but inoculation with PGPR consortium *Burkholderia phytofirmans*, *Enterobacter aerogenes* and *Pseudomonas fluorescens* significantly improved the yield of maize as compared to uninoculated control. The other growth contributing parameters were also significantly improves in response to inoculation with *Burkholderia phytofirmans*, *Enterobacter aerogenes* and *Pseudomonas fluorescens*.

**Keywords:** Consortium, Inoculation, PGPR, Rhizosphere and Endophytes

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## ANALYSIS OF MOLECULAR GENETIC DIVERSITY OF ENDANGERED PUNJAB URIAL (*OVIS VIGNEI PUNJABIENSIS*) BASED ON INTERLEUKIN 2 GENE SEQUENCES

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### ABSTRACT

Since sheep have a long history of domestication, subsequent formation of discrete breeds is inevitable. Punjab urial (*Ovis vignei punjabiensis*) is endemic to northern Punjab, Pakistan and also an endangered species according to International Union of Conservation of Nature and Natural Resources (IUCN) red list categories. Unfortunately, this species has been grossly neglected by the scientific community from conservation perspective and if timely attention is not paid for its conservation, it may become extinct. Better understanding of genetics of immune response can be helpful to design effective conservation strategies for Punjab urial. The objective of this study is to assess the molecular genetic diversity of endangered Punjab urial based on interleukin 2 gene sequences. Interleukin 2 (IL2) is a gene which encodes a cytokine significantly involved in some vital activities of immune response regulation. In this study, the IL2 gene (492 bp including regulatory TATA box, exon 1 and 2) was amplified and sequenced in DNA samples collected from wild as well as captive *O. v. punjabiensis*, followed by alignment and phylogenetic analysis. The neighbor joining tree constructed from MEGA6 showed, *O. v. punjabiensis* is closer to *Ovis ammon* (Argali) than the *Ovisaries* (sheep). According to the several analysis presented in the present study, *O. v. punjabiensis* is a unique isolated population found in Pakistan which is endemic as well as endangered. Stake holders such as, government institutions, wildlife authorities, naturalists and conservationists can consider the preservation of this endangered peculiar animals from extinction.

**Keywords:** Interleukin 2, Punjab urial, Endangered species, Phylogeny, Pakistan

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## MOLECULAR MEASURING OF BCR-ABL TRANSCRIPTS ON INTERNATIONAL REPORTING SCALE IN CHRONIC MYELOID LEUKEMIA PATIENTS

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**Introduction:** Since the start of targeted therapy in Chronic Myeloid Leukemia (CML) with Tyrosine Kinase Inhibitors (TKIs), the monitoring of response by cytogenetic analysis for Philadelphia Chromosome and BCR-ABL transcript levels by RT PCR have become a mandatory procedures in the follow up of these patients. The standardization of BCR-ABL transcript levels on international scale have removed the earlier discrepancies in reporting from different centers. Now molecular monitoring is done as early as three months of start of therapy to predict the major molecular response at 12 and 18 months.

**Study Design:** Prospective study design.

**Place and Duration:** The study was conducted in National Institute of Blood Disease & BMT (NIBD) and the data of newly diagnosed CML patients and as well as those on TKIs was analyzed from 2012 to April 2015.

**Patient and Method:** Real-time quantitative PCR method was used for measuring BCR-ABL mRNA transcripts levels in peripheral blood in CML patients. Transcripts quantification was done by using Nanogen advance diagnostic and later on *ipsogen*® qiagen kits and the results reported as a percentage of BCR-ABL/BCR on MMR-International scale (IS-Scale).

**Results:** Total 332 samples of 227 patients were analyzed, females were 111(48.8%) and males were 116(51.1%). Median age was 38(10-78) years. Mean baseline bcr/abl transcripts was  $63.3 \pm 62.3$ . Baseline analysis was done in 210(63%) and remaining 122(37%) were on follow ups. Among follow-up patients samples loss of molecular response was seen in 23(19%) patients and major molecular response (MMR) occurred in 99(81%) patient at median 6 months of therapy with TKIs.

**Conclusion:** We conclude that the quantitative measurement of BCR/ABL transcripts by real-time PCR should be done for monitoring minimal residual disease for the early detection of relapse and as an assessment of treatment response in all the patients of CML on TKIs.

**Keywords:** CML, RT PCR, BCR-ABL, MMR, IS-scale

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## ROLE OF PLANT GROWTH PROMOTING RHIZOBACTERIA IN ZINC FORTIFICATION OF CHICKPEA

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### ABSTRACT

Zinc is an important nutrient which is deficient in majority of human beings. This can be remediated through dietary diversification, mineral supplementation, food fortification and by increasing the concentration/bioavailability of mineral elements in food. Plant growth promoting rhizobacteria (PGPR) have already proved their capabilities for improving the growth and yield of various agronomic crops. However, their role for increasing the nutritional status of crop was less explored. In the present study a pot experiment was conducted to evaluate the potential of rhizobacteria for enhancing zinc concentration in chickpea. Seeds of chickpea were inoculated with selected PGPR isolates capable of zinc solubilization. The treatments were applied according to the completely randomized design (CRD) with 6 replications. In addition to zinc concentration effect of zinc solubilizing PGPR on growth and yield attributes of chickpea was also recorded. Standard statistical tools were used to analyze data. Results showed that zinc solubilizing PGPR significantly improved the zinc concentration in all tissues of chickpea as compared to uninoculated control. However, the contribution of different zinc solubilizing PGPR regarding physical, chemical and yield parameters of chickpea was inconsistent. Inoculation with isolates SB13 produced best result for improving the zinc concentration in chickpea plant tissue particularly in the grains. In addition to zinc concentration, SB13 also improved the nodulation (100%) and yield (96%) of chickpea. The study emphasized that the inoculation with zinc solubilizing PGPR could serve as an effective biofortification technique for mitigating the zinc malnutrition.

**Keywords:** Malnutrition, PGPR, microbial fortification and zinc solubilization

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## NOISE INDUCED HEARING LOSS (NIHL) IN TEXTILE WORKERS OF KARACHI

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Noise is considered to be an important industrial pollutant involving every industry and contributing to hearing loss in every country of the world. Worldwide, 16% of the disabling hearing loss in adults is attributed to occupational noise, ranging from 7 to 21% in the various sub regions. Majority of textile workers in Pakistan are also exposed to high noise produced by machines. Review of available literature shows that information regarding hearing loss due to noise exposure is scarce. Identification of the actual burden of noise induced hearing loss (NIHL) in textile workers will help in recognition of this important public health issue and will help in advocating related stakeholders to formulate guidelines for this important segment of population. This study aimed to assess the frequency of hearing loss in textile workers of Karachi and association between different factors with NIHL. A questionnaire based cross sectional survey along with audiometry was conducted in the six textile factories of different industrial areas of Karachi. A total of 264 workers were assessed for hearing loss with audiometric and otoscopic examination conducted by audiologist. Sample size was calculated using 95% confidence level, 5% bound on the error. Effect modifier were controlled through stratification of age, household income, marital status, and educational level to see the effect of these variables on outcome. Chi square was applied for the categorical variables. Univariate regression applied for calculating ORs with 95% CIs. All variables (age, duration of work, sound level, educational status, income, mill, ethnicity, symptoms on otoscopic examination) having significant p-value < .05 and those having biological plausibility were included in multivariate models. Mean noise level in these factories was  $97.6 \pm 2.05$  dB. About 79% workers had hearing impairment on audiometric assessment having hearing loss  $\geq 25$  dB. Out of these 19% of the workers had conductive deafness and 71% reports to have mixed hearing loss. Of them, 75% of these workers had symmetrical hearing loss in both ears. 63% of the workers in age group of 18-34 years reported to have hearing loss. Mean job duration of these workers was  $5.6 \pm 5$  years. After adjusting for age, monthly income and smoking status, noise was associated with hearing loss with OR 1.16 (95% CI 1.03-1.69) with  $p < .05$ . Hearing impairment affects a large proportion of the workers in Pakistan. There is a need for screening of workers exposed to occupational noise and guidelines to limit this exposure.

**Keywords:** Noise induced hearing loss, textile workers, determinants

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## EFFECT OF INTEGRATED USE OF DIFFERENT LEVELS OF CHEMICAL PHOSPHORUS, BIO-SLURRY PHOSPHORUS AND AUXIN ON GROWTH AND YIELD OF RICE

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### ABSTRACT

A study was conducted to check out the integrated effect of chemical fertilizer and biogas slurry as a source of phosphorus along with L-tryptophan (a precursor of auxin) on yield and other growth parameters of rice (*Oryza Sativa*). The experiment consisted eight treatments; T<sub>1</sub>: Control (Recommended NPK (100:67:62 Kg ha<sup>-1</sup>)), T<sub>2</sub>: Recommended Chemical P + BGS @ 600 Kg ha<sup>-1</sup> T<sub>3</sub>: Recommended Chemical P+BGS @ 600 Kg ha<sup>-1</sup> + L-tryptophane, T<sub>4</sub>: Recommended Chemical P + L-tryptophane, T<sub>5</sub>: 50% Chemical P + 50 % P from BGS, T<sub>6</sub>: 75 % Chemical P+25% P from BGS, T<sub>7</sub>: 50% Chemical P+50 % P from BGS + L-tryptophane, T<sub>8</sub>: 75% Chemical P + 25 % P from BGS + L-tryptophane. Results showed that at 30 DAT maximum plant height (73 cm), number of tillers (124 m<sup>-2</sup>), nitrogen (0.6 %) and potassium (2.1%) contents were observed in T<sub>8</sub> and maximum phosphorus by T<sub>3</sub> (0.288 %). At harvesting maximum plant height (131 cm), number of tillers (273 m<sup>-2</sup>), number of spikelets per panicle (106), 1000 grain weight (21.2g), straw yield (12.03 t ha<sup>-1</sup>), paddy yield (6.41 t ha<sup>-1</sup>) and total biomass yield (18.44 t ha<sup>-1</sup>) were shown by T<sub>8</sub>. Similarly T<sub>8</sub> also resulted in highest nitrogen (0.499%), phosphorus (0.336%) and potassium (0.929%) contents in straw and also highest nitrogen (0.709%), phosphorus (0.428%) and potassium (3.572%) contents in grain. Thus it is concluded that integrated use of biogas slurry, inorganic fertilizer and L-tryptophane resulted in increased rice growth and yield. Application of 75% P from biogas slurry and rest from chemical fertilizer along with application of L-tryptophane resulted in highest growth, yield and NPK contents in both straw and grains.

**Keywords:** Precursor, organic amendment, nutrient uptake, biogas slurry

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## A NOVEL APPROACH TO IDENTIFY GENOMIC REGIONS FOR GENOTYPING AND SUBTYPING OF SMALL GENOME PATHOGENS

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### ABSTRACT

Over the time, rapidly mutating viruses like hepatitis C (HCV) and Human immunodeficiency virus (HIV) produce resistant mutants. These rapid evolutionary dynamics not only allow them to evade the immune response but also make the identification/diagnostics of specific genotypes and subtypes difficult. Diagnostic of exact genotype is extremely important for clinicians to recommend the appropriate treatment regimens. Blind treatment practices without knowing the exact genotypes leads to drug resistance which is a great global concern. For this reason there is always a need of up gradation of viral diagnostic techniques/ strategies to determine exact genotype/subtype. Current study was a step ahead in this direction. Keeping HCV as a model, we analyzed its genome in a peculiar way combining manual sectioning and phylogenetic analysis. This novel approach also provided genome analysis options that are usually overlooked during sole automated analysis. The approach followed selection of an HCV reference data set comprising of whole genome sequences from all genotypes, based on its latest classification. All sequences were aligned along with numbering reference (variant H77 for HCV). The alignment was manually clipped into overlapping small sections of 500 bases 50 bases apart. Evolutionary inference for each section as well as the whole genome alignment was then estimated using neighbor-joining phylogenetic method. Finally each section phylogeny was manually inspected and compared with whole genome phylogeny to identify regions whose phylogeny compares whole genome phylogeny. These regions therefore represent the genetic heterogeneity as depicted by the whole genome and hence is sufficient for genotyping/subtyping. Furthermore phylogeny scanning at such small genomic scale reveals evolutionary relationships that are rarely explored. All computational analyses, alignment, phylogeny inference and visualizations were done using Molecular evolutionary genetic analysis software version 6.0.6 (MEGA6). On the whole this new genome analysis strategy can be adopted for other small genome pathogens as well or genome sections from large genome viruses for genotyping/subtyping and also very suited for in-depth evolutionary inference.

**Keywords:** HCV, genotyping, subtyping, phylogenetic analysis, evolutionary analysis, MEGA6

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## IN VIVO REGENERATION OF BETA CELLS' MASS BY COMBINED THERAPY OF METHANOLIC EXTRACTS OF GINGER AND TURMERIC CONTAINING BIOACTIVE COMPOUNDS: GINGEROLS AND CURCUMIN

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### ABSTRACT

To cure one of the world's leading morbid and mortal disorders: diabetes mellitus, has become one of the leading challenges of 21<sup>st</sup> century. In type 1 diabetes and end stage of type 2 diabetes, patients suffer from hypoinsulinemia due to significant reduction in islets  $\beta$  cells' mass. Currently used drugs, although may control blood sugar levels; but have lost their therapeutic potential due associated complications and cost related issues. Furthermore, they have no or poor effect on  $\beta$  cells' regeneration. The therapeutic use of methanolic extracts of ginger and turmeric containing gingerols and curcumin respectively, particularly after the WHO 2014-2023 recommended strategies of phytomedicines' uses, is a glimmer of hope in this regards.

**Materials and Methods:** Fifty two adult albino adult rats of either sex were be randomly divided into two equal groups of healthy rats and induced diabetic rats. Each group was further sub-divided into four groups of six rats (two rats from healthy and two from induced diabetic-dyslipidemic rats were sacrificed for pancreas' histopathological analyses at day 0). Group-I (normal healthy control) and group-V (diabetic control) were given no doses of methanolic extract of ginger and/or turmeric, while groups II, III and IV of healthy rats; and groups VI, VII and VIII of diabetic rats were given methanolic extracts of ginger or turmeric or ginger+turmeric (each extract was standardized for the presence of its respective main bioactive compound by HPLC analysis) for forty-two days at dose rate of 300 mg/100 mL dist. H<sub>2</sub>O/ kg body wt/day. Fasting plasma glucose and insulin levels were compared in each group by one-way ANOVA along with Post-hoc Tukey's multiple comparison test; while the rats' pancreatic histopathology in each category were compared to assess regeneration of beta cells' mass by aforesaid given treatments.

**Results:** The combined effect of both medicines was most significant ( $P < 0.05$ ) in comparison with their single effects. Furthermore, pancreas' histology of diabetic rats treated by combined therapy of both extracts were almost similar to healthy ones; and presented a considerable beta cells regeneration in comparison to diabetic control rats.

**Conclusion:** Methanolic extracts of ginger and turmeric, containing main bioactive compounds of 6-gingerol and curcumin respectively, may regenerate beta cells of islets and may effectively control hyperglycemia in diabetic patients.

**Keywords:** Diabetes, Beta Cells, Phytomedicines, Ginger, Turmeric, 6-Gingerol, Curcumin

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## PUBLIC AWARENESS AND TRAINING TO FOLLOW THE 3R-PRINCIPLES

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### ABSTRACT

A project against pollution was carried out from July 2014 to April 2015. The objective was to provide public awareness and training to follow the 3-R Principles among the residents of Rabwah, Pakistan (Population Size: 50,207 people). First of all a survey of the city was conducted and the situation was recorded, focusing on the pollution caused by plastic shopping bags, which is apparently the biggest cause of polluted environment. A series of seminars were held in ten different educational institutes to teach the Reduction Principle, to motivate purchase of biodegradable objects, to provide awareness of the health risks related to burning of plastic and, the overall effect on earth's climate leading to global warming. Through different questions, pre and post seminar responses were recorded. Brochures explaining 9 solid ways of reduction in use of plastic bags, were distributed as take home message. To train the community for the Reuse Principle, exhibitions were held in two institutes with themes such as, the effective reuse of plastic objects, introducing biodegradable items, producing minimum solid waste, constructing low cost kid's corners and garden arrangements. Exhibitions were visited by 2480 people 480 visitors were also made aware of the matter by informative presentations. For training the people helping in recycling process, triplet trash cans were introduced in different educational institutes (with 7121 students enrolled and 600 employed staff). Some of the pilot test were run for recycling plastic bags.

**Keywords:** Public awareness, plastic pollution, 3-R Principle.

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## THE EFFECT OF EXOGENOUS TRYPTOPHAN ON IMMOBILIZATION STRESS BY MODULATING SEROTONERGIC AND LEPTIN RESPONSES IN RATS

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### ABSTRACT

Recent studies show that together with serotonin (5-hydroxytryptamine; 5-HT), leptin also plays an important role in the aetiology of depression. Tryptophan, an essential amino acid, increases brain 5-HT but the effects of tryptophan on leptin and leptin responses to stress are not known. The present study was designed to evaluate the effect of tryptophan administration (300 mg/kg) on serotonin, leptin and behavioural responses to single and repeated immobilization stress. Acute exposure to 2 hour immobilization decreased food intake, body weight and elicited anxiety-like behavior. The deficits did not occur following repeated immobilization of 2hr/day for 5 days. Immobilization induced behavioral deficits were smaller in tryptophan than water treated animals. Single 2hr stress increased plasma leptin levels while these changes did not occur after repeated immobilization. Tryptophan administration also increased plasma leptin levels; the increase was smaller in rats exposed to first immobilization. Repeated administration of tryptophan did not increase leptin; tryptophan treated animals exposed to repeated immobilization exhibited smaller leptin levels. First exposure to immobilization decreased plasma tryptophan levels while these decreases did not occur after repeated immobilization. Both single and repeated immobilization decreased plasma tryptophan levels in tryptophan treated animals. Single 2hr stress increased brain tryptophan and 5-HIAA levels while repeated stress decreased brain tryptophan and 5-HT levels. Tryptophan administration increased brain tryptophan, 5-HT and 5-HIAA in unstressed animals. First exposure to immobilization increased brain tryptophan, 5-HT and 5-HIAA in tryptophan treated animals which did not occur following repeated immobilization. The results show that exogenous tryptophan can increase brain 5-HT metabolism in unstressed as well as stressed animals but attenuates stress effects on behaviour and circulating leptin. The findings may be useful in extending therapeutics in depression and other stress-related disorders.

**Keywords:** Stress, Tryptophan, Leptin, Serotonin, Depression

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## VALIDITY OF PRESERVED RATIO IMPAIRED SPIROMETRY (PRISM) IN SMOKERS AS A DIAGNOSTIC TOOL IN COMPARISON WITH GOLD DEFINED COPD

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### ABSTRACT

Worldwide spirometry is used to define Chronic Obstructive Pulmonary Disease (COPD<sup>1</sup>). It is however, well observed that people who smoke even if do not fulfill the GOLD definition, still continue to experience symptoms and behave in a similar manner. Spirometry pattern of FEV1/FVC ratio more than 70% but an FEV1 < 80% is given as a restrictive defect by Global Initiative of Obstructive Lung Disease (GOLD) registry, but numerous studies have demonstrate that smokers who exhibit this feature have evidence of increase in total lung capacity (TLC) as measured by total body plethysmography or CT scan<sup>2</sup>. Preserved ratio impaired spirometry (PRISM) is the term used to describe these patients<sup>3</sup>. The objective of this study was to compare the validity of PRISM as a diagnostic tool in comparison with GOLD defined COPD in smokers who present with symptoms. It was an analytical type of study carried out in the Department of Pulmonology, Fauji Foundation Hospital Rawalpindi. Sample size was calculated by WHO sample size calculator as 102. Patients were divided in two equal groups by non probability consecutive sampling. Current or former smokers with a smoking history of more than 10 pack years, between 40-80 years of age were included in the study. Patients with normal spirometry, radiological evidence of parenchymal lung disease, patients in exacerbation, non-smokers and huqqa smokers were excluded. Informed consent was taken. Demographic features including age and sex, and smoking history, patients symptoms, co-morbid diseases, body mass index were recorded. Dyspnea was measured by 6 minute walk test. Arterial oxygen saturation, chest x-ray were performed. Spirometry was done by spirometer manufactured by Schiller. Patients FEV1, FVC and FEV1/FVC ratio were noted. All patients underwent a high resolution CT chest (HRCT), to measure the total lung capacity that was taken as the gold standard<sup>4</sup>. Data was analyzed by calculating frequencies and percentages. Validity of PRISM and COPD groups was measured by checking the sensitivity, specificity, positive and negative predictive values

### Keywords:

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## PREVALENCE AND DISTRIBUTION OF HUMAN PLASMODIUM INFECTION IN FEDERALLY ADMINISTRATIVE TRIBAL AREAS OF PAKISTAN

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### ABSTRACT

Federally Administered Tribal Areas (FATA) perched on the border between Pakistan's Khyber Pakhtunkhwa Province and southern Afghanistan and is a residence of about 3.6 million Pashtun and over 1.5 million immigrants from Afghanistan. Malaria cases are very common in FATA, despite this no detail studies have yet been performed to reveal the actual situation of malaria in the local population and the epidemiological data is insufficient to elucidate the actual incidence of malaria in FATA. A malariometric survey of 691 patients of all ages and genders in seven agencies (districts) in FATA was carried out in 2013 using whole blood samples. Microscopically confirmed positive species were subjected to nested PCR for reconfirmation and detection of four species of Plasmodium (*P*) causing human malaria. From total 626 PCR positive samples, 81.1% were *P. vivax*, 13.8% *P. falciparum* and 4.9% mixed species containing both *P. vivax* and *P. falciparum*. *P. malariae* and *P. ovale* and were not found in any analysis. Sixty five microscopic positive samples were identified as negative by PCR. Incidence of *P. vivax* ranged from 13.4% in Orakzai Agency to 22.8% in North Waziristan Agency. The occurrence of *P. falciparum* ranged from 7% in Orakzai Agency to 19% in North Waziristan and Khyber Agency had the highest prevalence of 13% of mixed species. In Federally Administered Tribal Areas (FATA) *P. vivax* and *P. falciparum* are the main malaria causative agent and mixed species infections are also common. In addition, area variation in the incidence, frequency and species composition of malarial parasite is high.

### Keywords:

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## PROPAGATION DYNAMICS, SYSTEM STABILITY AND CONTROL OF HOSPITAL ACQUIRED – METHICILLIN RESISTANCE *STAPHYLOCOCCUS AUREUS*: A MATHEMATICAL MODELING APPROACH

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### ABSTRACT

**Background:** Methicillin resistant *Staphylococcus aureus* (MRSA) is endemic in many hospital settings which poses substantial threats and an added economic burden worldwide.

**Methods:** A couple of mathematical models are proposed to investigate the transmission dynamics of MRSA and to determine factors that influence the prevalence of MRSA infection when antibiotics are given to patients for treatment or prevention with either MRSA itself or other bacterial pathogens.

**Results:** Our results suggest that:

- MRSA persists in the hospital when colonized or infected patients are admitted;
- Longer the duration of treatment of infected patients and lower the probability of successful treatment, more will be the prevalence of MRSA infection;
- Longer the duration of contamination of health care workers (HCWs) and more the contact with patients, more will be the prevalence of MRSA infection;
- Possible ways to control the prevalence of MRSA infection include treating patients with antibiotic history as quickly and efficiently as possible, screening and isolating colonized and infected patients at admission, and compliance with strict hand-washing rules by HCWs.

**Conclusions:** The study offer an approach to investigate MRSA infection in hospital settings and the impact of antibiotic history on the incidence of infection. Our findings suggest important influences on the prevalence of MRSA infection which may be useful in designing control policies.

**Keywords:** *Staphylococcus aureus*, MRSA, Mathematical Modeling

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## EXPRESSION PROFILING OF HER 2 IN BREAST CANCER COHORT AND ITS ASSOCIATION WITH CLINICAL FEATURES

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### ABSTRACT

Breast cancer initiation, promotion and progression is influenced by numerous genetic factors. These factors include inactivation of tumor suppressor genes, activation of oncogenes and loss of control at cell cycle check points. Involvement of Human Epidermal Growth Factor Receptor Type2 (HER 2) as potential biomarker in breast cancer has already been established. Over expression of this molecule is significantly correlated with poor prognosis in breast cancer affected patients. At present, data regarding HER 2 expressional profiling in Pakistani mammary tumor affected females is limited. In the present study HER 2 expression in breast cancer cohort was explored at transcript levels and analyzed with various clinical features. About 94 freshly excised tumors along with background tissues were collected at the time of surgery from affected patients. Clinical data (like disease stage, grade, and lymph node) were also retrieved after a subsequent follow up. Isolation of RNA and cDNA synthesis was done using an already established protocol (Malik et al., 2009). Transcript levels of HER 2 were monitored using real time PCR technique.  $\beta$ -actin was used as an internal control in this regard. Tumor samples showed marked increase in HER 2 as compared to background tissues (p value=0.0004 at 95% confidence). In the given cohort, 33% patients were found positive for HER 2 expression. Interestingly, a significant association of HER 2 with tumor grade 3 had also been observed as compared to grade 1 and 2. Similarly, patients that entered the menopausal state <45 years of age showed strong HER 2 signals as compared to >45 yrs. Based on the present findings, (HER 2 positive in 31 cases out of 94 in the given cohort), HER 2 screening should preferably be incorporated for breast cancer patients. A concordance of these transcript findings along with protein expression for the given cohort is an avenue yet to be explored.

**Keywords:**

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## CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* STRAINS FROM SKIN AND WOUND INFECTION CASES IN HARIPUR CITY

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### ABSTRACT

*Staphylococcus aureus* is a bacterial species responsible for a high number skin and wound infections. Treatment of these infections has been complicated by emergence of antibiotic resistant strains. *S. aureus* strains tend to be resistant against a number of prescribed antibiotics by the physicians. Recent studies from different countries report that majority of the strains of this bacterial species have acquired resistance against penicillin class of antibiotics including penicillinase resistant penicillin called methicillin. In the present study over 100 *S. aureus* strains were isolated from skin infection cases. Antibiotic sensitivity testing was carried out by using disc diffusion assay, whereas presence of Panton Valentine Leukocidin (PVL) and *Staphylococcus* Cassette Chromosome (*SSCmec*) genes were determined by using PCR. The results of our study show high level of antibiotic resistance in the isolated strains. Over 70% are resistant to methicillin and bear *SSCmec* and PVL genes. Injudicious use of antibiotics may be responsible for dissemination of antibiotic resistance. Antibiotic resistance profiles of the isolated strains is also presented in the study.

**Keywords:** Antibiotic resistance, *Staphylococcus aureus*, Polymerase Chain Reaction, Panton Valentine Leukocidin

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## EVOLUTIONARY RELATIONSHIPS AMONG THE NS4B PROTEIN SEQUENCES OF DIFFERENT HCV GENOTYPES AND SUBTYPES.

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### ABSTRACT

**Background:** The NS4B of HCV is a non-structural hydrophobic protein involved in multiple activities such as the formation of membranous web, modulation of host and viral factors such as host ER activity, NS5B's RNA dependent RNA polymerase activity and possible involvement in liver carcinogenesis.

**Objectives:** Aim of the present study is to find out the evolutionary relationships among the NS4B protein sequences of different HCV genotypes.

**Materials & Methods:** Three hundred NS4B protein sequences of seven different HCV genotypes were selected from NCBI and European HCV databases. All the NS4B protein sequences were aligned using CLC main workbench software and finally a representative phylogenetic tree was developed.

**Results:** The analysis of phylogenetic tree revealed that an NS4B protein sequence of 3a Pakistani strain evolved first from the root of the tree. The NS4B protein sequence of 7a and all the rest of NS4B sequences evolved from two separate branches of tree. No evolutionary clustering was observed among the NS4B gene of Pakistani HCV strains.

**Conclusion:** A unique and distant evolutionary pattern was observed in NS4B sequences from Pakistani strains. Interestingly, the phylogenetic analysis of NS4B protein of HCV-3a genotype from Pakistan seemed to be a primitive sequence based on its evolutionary relationship with early ancestor sequence. This emphasizes the need to consider the local HCV strains for testing and designing novel HCV drug targets.

**Keywords:** NS4B protein, Membranous Web, Phylogenetic Tree.

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## GENETIC ASSOCIATION OF ADAM33'S SNP VARIANTS WITH ASTHMA IN THE POPULATION OF LAHORE REGION, PAKISTAN

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### ABSTRACT

Objective of this case control study was to investigate for any association of ADAM33 genomic region SNPs with the predisposition of asthma in local population of Lahore Region, Pakistan

A disintegrin and metalloproteinase ADAM33 gene is considered as an asthma susceptible gene due to its possible role in airways remodeling, abnormal cells proliferation and differentiation. Different Single Nucleotide Polymorphism (SNP) variants in ADAM33 have been studied in various ethnic populations which showed both type of results i.e., significant association in some and non-significant or no association in other populations. A large number of asthmatic patients are also present in Pakistan. So the current study was designed to evaluate any association of ADAM33 SNP variants with asthma in local population of Lahore Region, Pakistan. A total of 101 pairs of asthma patients and healthy controls were enrolled for the study. All samples of patients and controls were genotyped for eight SNPs [T+1(rs2280089), T2 (rs2280090), T1 (rs2280091), ST+5(rs597980), ST+4(rs44707), S2 (rs528557), Q-1(rs612709) and F+1(rs511898)] using single base extension method and capillary electrophoresis on automated genetic analyzers. Any association of these SNPs was investigated using allelic and genotypic models and it was found that the SNP i.e., S2 (rs528557) is independently and significantly associated with asthma in Lahore region population. Six SNPs [T+1(rs2280089), T2 (rs2280090), T1 (rs2280091), ST+4(rs44707), S2 (rs528557) and Q-1(rs612709)] were found to be in moderate to strong Linkage Disequilibrium (LD). In haplotype analysis of these six SNPs, one haplotype "GGAGGA" predicted the trend towards the association with asthmatic complication and another haplotype "AAGTCG" displayed as a protective factor against asthma susceptibility in local population of Lahore region. Although findings of this study indicate the association of ADAM33 gene with asthma susceptibility in Lahore Region's population, large population size study is proposed to confirm the current results in different ethnic groups of Pakistan.

**Keywords:** Haplotype, SNPs, Association, Asthma

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## CHARACTERIZATION OF MULTIDRUG-RESISTANT BACTERIA FROM PACKED FRUIT JUICES SOLD IN LAHORE CITY

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### ABSTRACT

Aim of the present study was to assess multiple drug resistance in bacteria isolated from packed fruit juices sold in Lahore, Pakistan. A total of two hundred and twenty six bacterial isolates including *Staphylococcus aureus* (n=49), *Escherichia coli* (n=27) and *Bacillus* spp. (150) were isolated from various fruit juices. Among *S. aureus* isolates, the highest resistance was observed against Cefamandole (94%) and the least resistance to Streptomycin (37%). *E. coli* isolates exhibited the highest resistance to Azlocillin (100%), and the least resistance to Chloramphenicol (11%). *Bacillus* spp. isolates showed 100% resistance to most of the beta lactams used and the least resistance to Tetracycline (23%). Among the 15 antibiotics tested, thirteen different resistance patterns were observed in *S. aureus*, twelve in *E. coli* and ten in *Bacillus* spp. isolates. 94% of *S. aureus*, 81% of *E. coli* and all *Bacillus* spp. isolates were found multiple-drug resistant. From cluster analysis, a significant dissimilarity in resistance patterns between antibiotics belonging to different groups and between different taxonomic groups of bacteria was observed. Significant correlation (p<0.05) was found between multidrug resistance,  $\beta$ -lactamase and ESBLs production in bacterial isolates. Measures should be taken to implement better hygienic conditions in the preparation of fruit juices.

**Keywords:** Packed fruit juices, Antimicrobial resistance, Disk diffusion method,  $\beta$ -lactamase, MDR bacteria

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## SURVIVIN AND CK2 CO-EXPRESSION IN HUMAN PROSTATE CANCER

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Dr. Shoaib N. Hashmi Dr. Fatima Qaiser Dr Aiza Sadia

### ABSTRACT

**Background:** Survivin, a substrate of CK2, is not detected/ has very low expression in normal tissues but is found to have strong expression in malignant lesions. In cancer cells, main role of survivin is inhibition of apoptosis. Protein Kinase CK2 is also a potent suppressor of apoptosis in cells and its expression is also deregulated in cancers. Since survivin is a CK2 substrate, the positive correlation between these two proteins in prostate cancer can determine their role in cancer phenotype.

**Objective:** The objective of this study is to check the co-expression of survivin and CK2 in the prostate cancer patients as compared to the BPH patients.

**Material & Methods:** The study was designed as a co-relational study and conducted at Armed Forces Institute of Pathology, Army Medical College Rawalpindi, from Dec 2012 to April 2014 after approval from institutional ethical committee approval. Expression of survivin was analysed by immunostaining in paraffin-embedded sections from 30 diagnosed cases of resected prostate cancer and 30 BPH cases that were immunostained for CK2.

**Results:** The CK2 and survivin were found to overexpress in prostate cancer as compared to BPH. Total scores of CK2 and survivin were strongly positive and significantly correlated in non-invasive cases as compared to BPH.

**Conclusion:** There is a strong positive correlation between survivin and CK2 over-expression in prostate cancer patients specifically non-invasive cases suggesting the coordination between the two proteins in early stages of prostate cancer progression.

**Keywords:** Casein Kinase 2, Survivin, Prostate Cancer, Co-expression

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## NATURAL INCIDENCE OF *ASPERGILLUS SPP.* AND THEIR TOXINS IN FRESH AND ENSILED CORN FODDER

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### ABSTRACT

Corn is most widely grown crop in Pakistan and preserved as silage in majority of countries worldwide. In Pakistan, silage making is in practice for last few decades to achieve high production parameters and provide a uniform diet to animals. However, mycotoxins contamination is generally ignored due to anaerobic and acidic environment of silage which is considered to be appropriate for inhibition of mycotoxigenic fungal growth. Furthermore, temperate and tropical climatic conditions of Pakistan along with inadequate feed storage provide ideal conditions for fungal (*Aspergillus*) growth, reduction of nutritional value and toxin production. In view of foregoing, present study was planned for screening of general mycoflora, quantification of total aflatoxins (TAF i.e. AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub>) and ochratoxin A (OTA). In addition, to ensure the quality of silage, nutritional profile was also assessed in fresh and ensiled corn fodder collected from various regions of Punjab. Mycoflora was determined by using oxytetracycline media (OGA), TAF and OTA was analyzed by HPLC coupled with Immunoaffinity columns. Nutritional profile was determined by following AOAC methods. Results revealed that *Aspergillus niger* (36.71 & 38.60%), *Aspergillus flavus* (32.19 & 27.87%) and *Aspergillus fumigatus* (35.80 & 37.22%) were prevalent in fresh and ensiled corn fodder respectively. Total fungal colonies were ranged from 1×10<sup>3</sup> to 6×10<sup>3</sup> cfu/ml for all species in both fresh and ensiled corn fodder. The findings revealed that total observed fungal density was less than safety limits i.e. 1×10<sup>4</sup> cfu/ml recommended by GMP (2005). Present study further depicted that aflatoxins B<sub>1</sub> (AFB<sub>1</sub>) detected in fresh (37.5%) and ensiled (41.66%) fodder with an average of 3.65 and 4.07 ng/g. Aflatoxin B<sub>2</sub> (AFB<sub>2</sub>) was detected in only two samples (1.60%) of fresh and ensiled corn fodder. Aflatoxin G<sub>1</sub> (AFG<sub>1</sub>) and G<sub>2</sub> (AFG<sub>2</sub>) were not detected in fresh and ensiled corn fodder. OTA was prevalent in fresh (54.16%) and ensiled (20.86%) samples with mean of 4.03 and 1.99 ng/g. Average detected value for TAFS and OTA was below the permissible levels of European commission (20 ng/g & 10 ng/g) respectively. Results of nutritional profile illustrate that pH was immediately drop up to 3.6 which is an indicator of good quality silage. Decline in pH and dry matter loss during ensiling of corn fodder indicate the preservation of nutritional value of silage.

**Keywords:** Ensiling; silage; corn fodder; Fungi; aflatoxins; nutritional profile

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## AFLATOXIN M<sub>1</sub> IN BULK TANK, UHT, PASTEURIZED, POWDER AND CONDENSED MILK SAMPLES COLLECTED FROM PUNJAB PROVINCE (PAKISTAN)

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### ABSTRACT

Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) is a hydroxylated metabolite of Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) as a result of hepatic cellular activity. It appears in milk, when lactating animals consume AFB<sub>1</sub> contaminated feed. Due to its carcinogenic nature, International Agency of Research on Cancer (IARC) has classified AFM<sub>1</sub> as class 2B (possible carcinogen). European Union and Food and Drug administration Agency (FDA) has defined permissible levels in milk and milk products i.e. 0.05µg/ml and 0.5 µg/ml respectively. Present study was planned to determine the incidence of AFM<sub>1</sub> levels in unprocessed (n=90) and processed (n=60) milk samples. The source of unprocessed milk was milk bulk tanks at dairy farms. However, processed milk i.e. UHT (n=36), pasteurized milk (n=12), powder milk (n=09) and condensed milk (n=03) were collected from the local market. Collected samples were purified by using AflaStar™ M<sub>1</sub> (IAC) column coupled with chromatographic determination. Limit of detection of method was 0.01ng/ml for liquid milk whereas 0.005ng/ml for powder milk. Highest incidence (100%) of AFM<sub>1</sub> was observed in UHT milk and pasteurized milk followed by bulk tank (86.66%) and powder milk (22.22%). However, none of the condense milk samples was found positive for AFM<sub>1</sub>. The positive data was further computed with respect to EU and FDA legislations. Results of present study depicted, AFM<sub>1</sub> levels in bulk tanks milk was 0.43ng/ml (mean) ranging from 0.17-1.63ng/ml followed by UHT (mean 0.22; range 0.01-0.95ng/ml), pasteurized milk (mean 0.11; range 0.07-0.15ng/ml) and powder milk (mean, 0.03; range 0.01-0.1ng/ml). All detected mean levels of AFM<sub>1</sub> were lower than the FDA regulatory limits but beyond the EC legislation except powder milk i.e. 0.03ng/ml. Briefly, the presence of AFM<sub>1</sub> in milk is an alarming situation as milk is an important constituent of human and particularly infant's diet. The need of the time is to manage the mycotoxin free feed through good agricultural practices to get quality milk.

**Keywords:** AFM<sub>1</sub>, Milk, IAC, Feed, Legislation

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## ANTI-INFLAMMATORY ACTIVITY OF PLANTAGO MAJOR L. LEAF EXTRACTS ON ORAL EPITHELIAL CELLS

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### ABSTRACT

The present study will be undertaken to reveal possible anti-inflammatory effects of *P. major* leaf extracts on oral epithelial cells *in-vitro*. Water- and ethanol-based extracts of *P. major* leaves will be prepared from freeze-dried plant material, and tested *in-vitro* using the oral epithelial cell line (H400). The anti-inflammatory activity of *P. major* will be tested against *E. coli* lipopolysaccharide (LPS) using the nuclear factor kappa beta (NF-κB) assay. It is assumed that water- and the ethanol-based extracts, as well as a combination of the two extracts, will show anti-inflammatory activity. The optimum concentration of each extract for anti-inflammatory activities will also be identified.

**Keywords:** Common plantain; Herbal medicine; NF-κB; Wound healing

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# CHARACTERIZATION OF *PSEUDOMONAS AERUGINOSA* STRAIN ZM24 FOR SIMULTANEOUS REMOVAL OF REACTIVE DYES AND HEXAVALENT CHROMIUM [Cr (VI)] AND EXPRESSION OF LACCASE ACTIVITY DURING DECOLORIZATION OF REACTIVE RED-120 (RR120)

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## ABSTRACT

Bioremediation of colored textile wastewater loaded with dyes and metals is a matter of great concern due to the hazardous risks associated with their untreated discharge into water bodies. The present study was conducted to isolate dye decolorizing metal tolerant bacterial strains from wastewater samples collected from outlets of different textile industries. Among the isolated bacterial strains, bacterial isolate ZM24 showed almost complete decolorization of different reactive dyes (reactive red-120, Reactive black-5, reactive orange-16) in the presence of four different heavy metals (Cr, Pb, Zn, Cd) and was identified as *Pseudomonas aeruginosa* strain ZM24 through 16S rDNA gene amplification and sequencing. The decolorizing ability of *P. aeruginosa* strain ZM24 was optimized using response surface methodology (RSM) following small composite design (SCD). The optimized values of independent input variables (salt content, pH, C source concentration and level of multi-metal mixture) given by the model were predicted to be 12.5 g L<sup>-1</sup>, 8.7, 4.75 g L<sup>-1</sup> and a multi-metal mixture (Cr: 5 mg L<sup>-1</sup>; Pb: 10 mg L<sup>-1</sup>; Cd: 5 mg L<sup>-1</sup>; Zn: 10 mg L<sup>-1</sup>), respectively, for highest response variable (% decolorization) by *P. aeruginosa* strain ZM24. Moreover, ability of *P. aeruginosa* strain ZM24 for simultaneous removal of reactive dyes and Cr(VI) was explored and considerable potential for removal of reactive dyes and Cr(VI) in the same medium was recorded. Furthermore, extracellular enzymatic activities of different oxidative and reductive enzymes were also examined. However, expression of laccase activity confirmed its role in decolorization of reactive red-120 dye by the bacterial isolate ZM24. Based on these findings, *P. aeruginosa* strain ZM24 with distinctive potential for simultaneous removal of dyes and heavy metals might serve as a potential bioresource for the biotechnologies involving textile wastewater treatment.

**Keywords:** Response Surface Methodology (RSM), Simultaneous removal, Reactive dyes, Cr(VI), *Pseudomonas aeruginosa* strain ZM24, Laccase activity

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# DIETARY EXPOSURE AND NATURAL OCCURRENCE OF *ASPERGILLUS* TOXINS IN BASMATI RICE COLLECTED FROM IRRIGATED AREAS OF PUNJAB (PAKISTAN)

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## ABSTRACT

Rice (*Oryza sativa*) as a cereal grain, it is the most widely consumed as staple food for a large part of the world's human population, especially in Asia. It is the agricultural commodity with the third-highest worldwide production, after sugarcane and maize (FAOSTAT, 2012). In Pakistan, during last decade, delayed rain fall during sowing season, heavy rain and flood before harvesting and high humid conditions may provide a chance for mycotoxins contamination. The production and occurrence of mycotoxins differs depending upon the geographic and climatic conditions. Climatic conditions of Pakistan favor the *Aspergillus* species responsible for producing some of the potent mycotoxins like aflatoxins and ochratoxin A. In view of above mentioned background, present study was planned to assess the mycotoxins (aflatoxins and OTA) contamination and estimation of daily dietary intake of these toxins in basmati brown and white rice (collected during period of 2009 to 2014-15) being consumed by Pakistani population. Method was optimized by matrix spike recoveries at four fortification levels of (0.1, 0.5, 2.5, and 12.50ppb) for total aflatoxins (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> & AFG<sub>2</sub>) and ochratoxin A (OTA). Samples were analyzed by high performance thin layer chromatography. The optimized method shows good linearity as R<sup>2</sup> is greater than 0.996. Detection limit of present method was 0.1ng/g for AFB<sub>1</sub>, AFG<sub>1</sub> & OTA while for AFB<sub>2</sub> and AFG<sub>2</sub> was 0.5ng/g. The results of present study shows that overall 2113 samples (75.73%) were found positive for aflatoxins B<sub>1</sub> while 452 samples (16.20%) were tainted with AFB<sub>1</sub> and AFB<sub>2</sub>. However, AFG<sub>1</sub> and AFG<sub>2</sub> were not detected in rice samples. Among these positive samples, 1091 brown rice samples (78.20%) were contaminated with AFB<sub>1</sub> and 247 samples (17.70%) were positive for TAFS. Incidence of AFB<sub>1</sub> in white rice was detected in 1022 samples (73.26%) whereas 205 samples (14.69%) were prevalent for TAFS. Similarly, samples were further analyzed for ochratoxin A (OTA) with percentage incidence of 73.33% & 93.33% in brown and white rice samples. The estimated amount of total aflatoxins intake for average rice consumer ranged from 0.16-4.75 µg/kg body weight per day that is much higher than the stated value of 1ng/kg body weight/day as defined by JECFA (1998).

**Keywords:** Total aflatoxins, Ochratoxin, Brown Rice, White rice, Dietary intake

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## INTERACTION OF HETEROTROPHIC AND AUTOTROPHIC BACTERIA DURNIG LEACHING OF SULPHIDIC ORE

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### ABSTRACT

Metal solubilization, applying bacteria *Acidithiobacillus* spp is being done for the recovery of copper and uranium by heap, dump and in situ leach techniques on a commercial scale, It is estimated that around 35 % of total copper is obtained through bacterial leaching processes. It is also observed that in the Biological community in an ore leaching process in addition to autotrophic bacteria (*Acidithiobacillus* spp., *Leptospirillum ferrooxidans* and *Sulfolobus* spp.) some heterotrophic microorganisms, including bacteria and fungi of various species are also found. These bacteria are also used as catalysts in the biomining process. To check the role of heterotrophic bacteria in presence of autotrophic bacteria lab scale experiments were performed. Carbonate bearing sulphidic ore from Rammelsberg mine in Goslar, Germany containing Cu, Fe, and Zn was tested for solubilization with strains of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* alone and in combination. The role of one of the heterotrophs in the microbial leaching process will be discussed. Chemical reaction of heavy metals sulphide by this acid as well as ferric iron resulted in production of sulphur in the form of passive film/layer. Effectiveness of *Acidithiobacillus thiooxidans* in removal of this passive film was also investigated. It was found that presence of heterotrophs in leaching process in lab scale studies negatively influenced the leaching efficiency of *Acidithiobacillus* spp.

**Keywords:**

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## THE ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF BACTERIAL ISOLATES CAUSING URINARY TRACT INFECTIONS

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### ABSTRACT

Increasing antibiotic resistance and ESBL occurrence are serious threats for public health. Present study was intended to explicate the antibiotic-susceptibility patterns of ESBL and non-ESBL producing *E. coli* and *K. pneumoniae* causing urinary tract infections in order to remove the ineffective antibiotics from the line of treatment. The bacterial pathogens were isolated from 6254 urine samples and were cultured for strain identification using API20E. Antimicrobial vulnerability and ESBL detection were done by Kirby-bauer diffusion technique. Almost 53.4% *E. coli* isolates and 24.5% *K. pneumoniae* isolates were found to be ESBL producers and revealed higher antibiotic resistance. The most efficient drugs against *E. coli* ESBL isolates were imipenem (99.54%), ampicillin-sulbactam (97.48%), piperacillin-tazobactam (96.86%), fosfomycin (94.51%), amikacin (92.26%) and nitrofurantoin (90.68%). The most useful drugs against *K. pneumoniae* ESBL isolates were imipenem (97.62%), piperacillin-tazobactam (95.35%), ampicillin-sulbactam (90.48%) and amikacin (88.37%). The antibiotics having the maximum resistance, particularly by the ESBL producers were amoxycillin clavulanic acid, sulphamethoxazole/trimethoprim, cefuroxime, cefpirome, ceftriaxone and ciprofloxacin. Most of the isolates exhibited multi drug resistance. To summarize, high prevalence of ESBL producing *E. coli* and *K. pneumoniae* was observed. Penicillins, cephalosporins and some members of fluoroquinolones were least effective against the common UTIs and are recommended to be removed from the line of treatment.

**Keywords:** *Escherichia coli*, *Klebsiella pneumoniae*, ESBL, antibiotic susceptibility

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## SYNTHESIS AND CHARACTERIZATION OF GOLD NANOPARTICLES WITH SELF-ASSEMBLED FERROCENETHIOPHENOL AS BIOLOGICAL PROBE

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### ABSTRACT

Synthesis, characterization, fabrication and potential applications of gold nanoparticles are new tier of nanotechnology. Fabricated gold nanoparticles have attracted scientists and clinicians worldwide due to their biocompatibility and optical properties. Their absorption and scattering capabilities in the visible and near infrared range make them suitable for *in vivo* and *in vitro* molecular imaging and molecular diagnosis. The optical properties of gold nanoparticles change upon slight modification of the vicinal environment which is the basis for the development of biosensors. Current study was performed to synthesize gold nanoparticles with the attachment of 4-ferrocene thiophenol and to investigate its potential applications. Gold nanoparticles were synthesized by seed-mediated method and fabricated with thiolated phenyl ferrocene to get a nanodevice which can be used for the detection of DNA due to its electrochemical properties. Hemolytic and anti bacterial assays were performed and the results confirmed no such activity of ferrocenethiophenol. This makes it a potent linker for attachment with gold nanoparticles. The coated nanoparticles were also labeled with radioactive element  $^{99m}\text{Tc}$ . This tagging can be helpful to probe the location of gold nanoparticles in the living systems. Overall results suggest that controlled synthesis and surface modifications of gold nanoparticles enhance their capacity and stability. Such modified GNPs can be used not only for detection of biological molecules *in vitro* but also for biological imaging and specific targeting *in vivo*.

**Keywords:** Gold nanoparticles, ferrocenethiophenol, DNA probe

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## ESTIMATION OF ANTI-LEISHMANIAL PROPERTIES OF ANTI-COAGULANT DRUG HIRUDIN

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### ABSTRACT

Hirudin is a clinically significant drug used for the management of cardiac diseases, but has never been explored for anti-Leishmanial potential. The present study was planned to establish the therapeutic efficacy of this drug against Leishmaniasis. Leishmanial parasites, brine shrimps and human macrophages were cultured according to the well-known protocols and treated with hirudin in dose-dependent and time-dependent approach. Glucantime and DMSO were used as positive and negative controls, respectively. Percentage survival and mortality were determined along with estimation of  $\text{IC}_{50}$  and  $\text{LD}_{50}$  values. Apoptosis-inducing potential was acquired by acridine orange assay. All experiments were done in triplicates. Hirudin showed promising activity against promastigote and amastigote forms of Leishmanial parasites with  $\text{IC}_{50}$  values of  $0.60 \pm 0.36 \text{ ng/ml}$  and  $0.43 \pm 0.23 \text{ ng/ml}$ , respectively in dose dependent assay. Both of these values were much less than the in-use drug glucantime. Time dependent assays also depicted enhanced activity of hirudin than glucantime. The cytotoxicity assays revealed no undesirable effects on brine shrimps and human macrophages with  $\text{LD}_{50}$  values of  $1082 \pm 45.45 \text{ ng/ml}$  and  $860.11 \pm 53.44 \text{ ng/ml}$ , respectively, which were much higher than glucantime. Hirudin caused Leishmanial cell death primarily by apoptosis as after 72 hours treatment, 74.6% parasites were found to be apoptotic.

**Conclusion:** The peptide drug hirudin showed very good anti-Leishmanial activity and much less cellular toxicity in comparison to commonly used drug glucantime. It should further be studied for its potential as the first-line therapy against Leishmaniasis.

**Key words:** Leishmania, hirudin, apoptosis, glucantime, promastigote, amastigote

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## THE IL-6 -174G>C GENE POLYMORPHISM AND RISK OF CORONARY ARTERY DISEASE CAD IN A PAKISTANI POPULATION

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### ABSTRACT

Coronary artery disease (CAD) is one of the most prevalent disorders worldwide. South Asians exhibit a special susceptibility for CAD that is not explained by traditional risk factors. Characterization of CAD associated genes would enhance the prediction of CAD and improve prevention in high risk populations in Pakistan. The aim of our study was to investigate the association between the interleukin-6 (IL-6) -174G>C gene polymorphism and CAD. In this study, 350 patients with documented CAD and 350 age and gender matched healthy control subjects were enrolled. Biochemical analyses were performed for the determination of serum lipid profile, uric acid and C-reactive protein (CRP) by using specific diagnostic kits. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism. The genotype and allele frequencies of IL-6 -174G>C in the study population according to different genetic models were analyzed. A significantly higher prevalence of variant genotypes (GC+CC) of IL-6 -174 (29%,  $P = 0.0007$ ) in the recessive genotype model was documented in CAD patients vs. control subjects. The C allele at -174 (OR = 1.75, 95% CI = 1.28-2.40,  $P = 0.0005$ ) locus showed significant association with CAD compared to controls in the additive model. These results strongly suggest the presence of C allele at IL-6 -174 as a risk factor for CAD. Additionally, the CAD cases bearing the IL-6 variant genotype showed elevated serum uric acid ( $P < 0.0001$ ) and CRP ( $P = 0.003$ ) levels compared to the wild type genotype. However, IL-6 -174G>C did not show any association with the CAD severity.

In conclusion, our findings support an association between the IL-6 -174G>C polymorphism and CAD risk in a Pakistani population.

**Keywords:**

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## TRACTION OF BIOFUEL FROM SPIROGYRA

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### ABSTRACT

Advanced biofuels are attracting intense interest from government, industry and researchers as potential substitutes for petroleum gasoline and diesel transportation fuels. Algae are the major photosynthetic component of the ecosystem. So we can use this photosynthetic component to produce environment friendly fuel in less time. Hence help in the reduction of rapid degradation of fossil fuel and to reduce environmental pollution. Algae's advantages as a biofuel feedstock are due particularly to their rapid growth rates and high lipid content. Maximum harvesting cycle for algae is less than any other crop as well as it does not require much aerated land as other crops, it can be grown in all types of water resources, it can use greenhouse gas CO<sub>2</sub> from the environment hence help in the carbon dioxide depletion from the environment and can convert 60% of its biomass into lipids. Once the oil was removed from algae, solvent extraction was used for further extraction of oil. Spirogyra is filamentous fresh water algae containing spiral chloroplast. It can grow under water in the form of filamentous mass. The best growing period of spirogyra is spring season. Spirogyra had been collected few days after rain from Chenab Nagar, then its morphology was studied under microscope. After studying morphology it was washed and put inside a tank that already contained the soil and sufficient nutrients required for the growth of spirogyra. The pH of water had been checked at regular interval as it had been harvested in tanks under controlled condition. The pH of tanks were kept between 6 to 7. The spirogyra had been harvested and regardless of using centrifugation machine, water was drained by using net cloth tying both ends. After draining water and mud from algae it had been dried under sunlight for 3 days. Drying proceeded by the addition of benzene which helped in coagulation of lipid components. The mixture was dried under fan and treated with methanol and sodium hydroxide. The whole mixture was heated and at last but not least the mixture of glycerol and oil has been obtained. After that solvent extraction was done in order to obtain oil from the mixture. At last oil has been obtained. If we use this at commercial level I hope world would be a greener planet to live one day and fossil fuel consumption will be prevented that is harmful for our environment.

**Keywords:**

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## SEQUENCE ANALYSIS AND COMPARISON OF METALLOTHIONINS FROM TWO DAPHNIDS

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### ABSTRACT

Metallothionins (MTs) bind heavy metals through the thiol group of cysteine residues and play an essential role in the detoxification process. MTs vary environmentally and between different zoological groups and can be used as biomarkers, if used wisely in well-designed environmental monitoring programs. *Daphnia magna* and *Daphnia pulex*, small freshwater crustaceans, are test species for ecotoxicity tests. They hold an important position in the aquatic food chain, respond to many pollutants, are easy to culture and have their genomes sequenced but sequence and structure of metallothionins from both these species has not yet been analyzed. We have attempted to compare MTs of *D. magna* and *D. pulex* to shed light on their sequence conservation, 3D structure and phosphorylation potential using bioinformatics based approach. Multiple sequence alignment results showed very low conservation among the isoforms, only a single glutamine at 9<sup>th</sup> position was conserved in all MTs. Physicochemical parameters and 3D structures also showed variation as observed from contact maps. Kinase mediated phosphorylation was predicted for all the MT isoforms but no consensus pattern/residues could be inferred. Lab studies are however, further needed to validate these findings. This baseline information regarding MTs of daphnids, might be of aid in making sozotechnical decisions for environmental monitoring of water.

**Keywords:** Metallothionins, *Daphnia magna*, *Daphnia pulex*, 3D structure, *in silico*

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## COMMUNITY PHARMACISTS, A MEMBER IN HEALTH CARE SYSTEM

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### ABSTRACT

As the experts in medicines, pharmacists have always been known as an accessible and trusted source of advice and treatment. Today, contribution of community pharmacist in health care is developing in new ways to support patients in their use of medicines and as a part of clinical decision-making across the range of specialisms. Pharmacies are open all day, are convenient for most people to get to and there is no need for an appointment to see the community pharmacist. All this makes pharmacies the natural first port of call for help with common ailments. Community pharmacists supply medicines in accordance with a prescription or, when legally permitted, dispense them without a prescription. Community pharmacists are in a distinctive position to identify, prevent, and resolve drug & related problems in ambulatory patients, and data suggests that community & based pharmacy services can improve health outcomes. Pakistan is a global face of rapidly changing developing nations, which requires strengthening its professional aspects of community pharmacy. With changing demands from educated and urbanized mass, there needs a reform in the policies and structure of the present community pharmacy setup. Community Pharmacy and Pharmacy Practice are yet to be established strongly and pharmacists working in community pharmacies do not provide patient counseling in the usual situation. We need to work closely with the pharmacist associations and share our common experiences and frame appropriate guidelines, so that community pharmacist who plays a major role in providing better health care's.

**Keywords:** Pharmacist, Health care system, Patients, Prescriptions, and Pharmacy Practice.

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## A NOVEL GYNECOLOGY CLASSIFICATION MODEL

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### ABSTRACT

According to UN estimates, after every two minutes, a woman dies during pregnancy or childbirth, thus worldwide nearly 290,000 pregnant women and 3 million newborns die each year. Considering this, eHealth systems targeting Obstetrics and Gynecology (OG) field were recently proposed in previous research that can automatically analyze data of patients in remote areas and then classify them as high risk or low risk. Unfortunately, these systems are hard to be adopted permanently in practice as these do not share insights about their decision making process with a medical expert and secondly, these studies have not clearly elaborated the challenges of OG data. In this study, we first perform a thorough analysis on challenges of OG data and then comprehensively evaluate a diverse set of rule learning classification paradigms on OG data. Our study finally leads to the design of an intelligent tool that can accurately classify OG patients (with above 98% accuracy) and also provide clear rule based insights to the medical experts about its decision making process. Further, experimental results show that our rule based approach can also be utilized by medical experts for exploring important hidden patterns in the medical databases of different diseases.

**Keywords:** Obstetrics and Gynecology, decision making, rule learning classification paradigms

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## APPLICATIONS OF MACHINE LEARNING TECHNIQUES IN MEDICINE

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### ABSTRACT

An efficient health system is required in order to improve the health status of people and to eradicate the financial consequences faced by people suffering from illness. However, due to shortages in medical staff, people living in remote rural areas face challenges of primary and secondary healthcare access. These problems demand the introduction of Data mining learning schemes in medicine to automate the disease detection process. But, there are several challenges encountered when applying learning schemes on biomedical data sets, as the data sets gathered from clinical databases include several systematic and human errors, mainly noise and missing values, which affect the classification accuracy of the machine learning schemes. In order to explore the effectiveness of Machine learning (ML) schemes in disease detection, we have conducted a detailed comparative study of a global probabilistic classifier, Naïve Bayes and a local Instance based learner (Ibk) in terms of how these algorithms perform in response to a variety of well-known medical data subsets extracted through different feature selection schemes. We have concluded that the accuracy of the learning schemes is affected by certain factors and that both the learning schemes perform differently, depending on the nature of the data sets. The results demonstrate that Ibik outperformed Naïve Bayes on the data sets having high imbalance ratio due to its ability to cope with the imbalance ratio while on the other hand, Naïve Bayes showed a much better ability of handling the class noise and missing values. Moreover, our study regarding attribute evaluators concludes that there is no evaluator which works best on all types of data sets for example Correlation based Feature Subset Evaluator could not perform well on the data sets having highly inter-correlated attributes whereas Consistency subset evaluator reduced the number of attributes to a very small subset and therefore, could not prove to be effective on the data sets already having less number of attributes.

**Keywords:** Machine learning, disease detection, Naïve Bayes, Instance based learner

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## COMPARISON OF DESTRUCTIVE & NONDESTRUCTIVE SAMPLING FOR BIOMASS ESTIMATION IN *OLEA FERRUGINEA* ROYLE.

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### ABSTRACT

Destructive sampling techniques, though more reliable method for estimation, may not be feasible in a country like Pakistan where the forest percentage of the country is already very low. In such developing countries nondestructive sampling techniques are more applicable. The present study was carried out in the subtropical forests of Pakistan at 33° 38' north and 73° 00' east latitude and longitude respectively and at an elevation of 917 m. In this study five *Olea ferruginea* trees of exploitable diameter were sampled destructively for the estimation of biomass. On the other hand biomass of five trees was calculated using the allometric equation. The results were then compared to see the accuracy of the two methods. The results showed the mean biomass value estimated destructively to be  $675.98 \pm 0.99 \text{ kg/m}^3$  whereas the average biomass value revealed by nondestructive sampling was  $692.52 \pm 0.56 \text{ kg/m}^3$ . Hence it can be concluded that although destructive sampling shows accurate results but can be replaced with nondestructive sampling depending on the situation.

**Keywords:**

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## DETECTION OF INTERFERON ALPHA RECEPTOR 1 (IFNAR1) IN CO-INFECTED PATIENTS OF HEPATITIS C AND HEPATITIS B RESISTANT TO INTERFERON THERAPY

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### ABSTRACT

**Background:** Hepatitis C (HCV) and hepatitis B (HBV) are the most common viral types of hepatitis with increased risk of liver cirrhosis and hepatocellular carcinoma worldwide. The prevalence of HCV is 5% and that of HBV is 2.5% in Pakistan. The disease is incurable or resistant to therapy due to various factors including viral, host and cellular. Patients commonly develop resistance to therapy and are at high risk of having more severe hepatic disease. Binding of interferon to its receptor is the initial step to initiate intracellular signaling cascade responsible for antiviral state established by interferon therapy.

**Objective:** This study has been designed for detection of mRNA for subunit 1 of the interferon-alpha receptor (IFNAR1) in blood and to detect the role of this cellular factor that might be responsible for interferon resistance in HCV/HBV non-responders.

**Study Design:** Case-control study

**Place & duration of study:** Army Medical College, NUST for a duration of 01 year

**Patients&Methods:** HCV/HBV 60 co-infected patients were studied who had undergone IFN-based therapy of 24 weeks duration. Patients were defined as responders or non-responders depending upon achievement of end-of-treatment response (ETR) after six months of interferon plus ribavirin therapy. IFNAR1 mRNA on peripheral blood leukocyte was detected by PCR.

**Results:** IFNAR1 mRNA was detected in 100% (n=30) of HCV/HBV responder to interferon therapy and 16 out of 30 patients (53.33%) resistant to interferon therapy

**Conclusion:** This investigation might be beneficial as a predictive marker and beneficial in developing therapeutics for patients lacking the interferon alpha receptor-1.

**Keywords:** Hepatitis C virus; Hepatitis B virus; Co-infection; Interferon alpha receptor 1; Interferon resistance; End-of-treatment response

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## MONOCYTE LYMPHOCYTE RATIO AS A POSSIBLE PROGNOSTIC MARKER IN ANTITUBERCULOUS THERAPY

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### ABSTRACT

**Background:** Tuberculosis is a major health threat with high mortality rate. This study was aimed to assess an inexpensive, less time consuming and routine biomarker to see the outcome of therapy and progress of disease by measuring monocyte lymphocyte ratio (M/L ratio).

**Methods:** We enrolled 45 newly diagnosed cases of tuberculosis from Military Hospital, Rawalpindi and 45 age and sex matched healthy controls from community. The study was designed to determine the baseline, initiation phase and 2-months maintenance phase treatment values of M/L ratio in newly diagnosed active TB cases. Blood complete picture was obtained from ethylene diamine tetraacetic

**Results:** The pre-treatment lymphocyte and monocyte count in the study group were significantly lower than the control group, while M/L ratio was similar in both the groups. The mean M/L ratio in cases and controls were  $0.24 \pm 0.14$  and  $0.24 \pm 0.07$  respectively. The M/L ratio significantly decreased after initiation phase and two months of maintenance phase of treatment from baseline  $0.24 \pm 0.14$  to  $0.20 \pm 0.10$  to  $0.19 \pm 0.10$  with p value of 0.006.

**Conclusions:** Tuberculosis is associated with increased M/L ratio, which declines and returns to normal with antituberculous therapy. It is therefore considered to be a surrogate prognostic biomarker of TB.

**Keywords:**

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## BIOINFORMATICS WORKBENCH FOR GENOME ANALYSIS

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### ABSTRACT

Genome is the most important part of every cell, thus in order to understand the functioning of the cell extensive work has been conducted in genome research. This has resulted in the production of vast genome data, in silico analysis of these data involves the use of computational tools and different analytic tools, which have been developed to speed up the analysis and annotation in the field of genomics. Due to the complexity in the functional properties of these tools, they demand technically complex input and generate output in unique formats. As a result it becomes difficult to utilize the output from one tool as an input to another, so the user is required to learn these input/output formats extensively. In order to address this problem and to help the researchers some time and effort, we have developed a Bioinformatics Workbench for genomic analysis. In this workbench the input and output of all the tools are handled in such a way that these tools can be integrated in the form of a pipeline to create a workflow which can automatically pass the output of any tool to the proceeding tool (as an input). The designed pipeline is time efficient by providing an understandable and user friendly interface to motivate biologists to use informatics with ease, which will ultimately boost their speed of research.

**Keywords:** Genome Analysis, Annotation, Bioinformatics Tools, Pipelines, Workflow.

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## EVALUATION OF *IN VITRO* CYTOTOXIC AND GENOTOXIC EFFECTS OF *DATURA INNOXIA*

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### ABSTRACT

*Daturainnoxia*, belonging to family *Solanaceae*, is used in conventional medicine due to its established antibiotic nature. Presence of highly toxic alkaloids are cytotoxic and genotoxic at high doses. Therefore present study was carried out to investigate *in vitro* cytotoxic and genotoxic activity of *Daturainnoxia*. Cytotoxic effects were screened through hemolytic, brine shrimp lethality assay and mutagenic assay. Crude methanolic extracts were further refined using parliament of the solvents; n-hexane, chloroform, acetone and n-butanol. Cytotoxicity results were contradicted against human red blood cells (RBCs) and Brine shrimp lethality assay. All extracts were evaluated at various concentrations. Maximum cell lysis was observed at concentration of 50 mg in acetone based extract and was up to 55.7%. But the LD<sub>50</sub> against newly hatched larvae of *Artemia salina* was very less. Moreover their genotoxicity against two common strains of *Salmonella typhimurium* TA98 and TA100 was highest. These results clearly reveal that the *Daturainnoxia* can be toxic and may cause some irreversible toxicity to different organs.

**Keywords:** *Daturainnoxia*, Hemolytic Assay, Brine Shrimp Lethality Assay, Mutagenicity

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## BRASSICA JUNCEA: A POTENTIAL CANOLA QUALITY OILSEED CROP FOR ARID AREAS OF PAKISTAN

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### ABSTRACT

Food security is the crucial global issue, especially in developing countries like Pakistan. Pakistan spends approximately US\$ 2.50 billion annually on edible oil import. The major share in imported edible oil is of palm oil which is low in quality and causes serious health problems. In Pakistan's Punjab province, 26,300 square kilometers area of Cholistan is lying mostly barren due to water scarcity. Development of a suitable oilseed crop for this area is a dire need to overcome the upcoming food and health threats. Canola quality *Brassica juncea* has been found as an appropriate solution due to its distinguished drought tolerance trait. The recently developed canola quality *B. juncea* line ZBJ-06012 (AARI Canola) at Oilseeds Research Institute, Faisalabad, Pakistan has shown good adaptability, early maturity, non-shattering, disease and drought tolerance traits with high yield potential in comparison with presently grown non canola mustard cultivars "Raya Anmol" and "Khanpur Raya". Due to the prominent features, this line has a great scope of motivating farmers to grow canola quality *B. juncea* compared to non-canola mustard cultivars. Future challenges demand further development of high yielding, short duration and aphid tolerant along with high oil content varieties/hybrids of canola quality *B. juncea*. There is a great potential of exploiting genetic variability in the existing *B. juncea* material to achieve the aforesaid goals by using conventional plant breeding techniques.

**Keywords:** *B. juncea*, food security, drought tolerance, short duration, genetic variability.

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## INHIBITORY EFFECT OF *AEGICERAS CORNICULATUM* EXTRACTS ON HUMAN PLATELET AGGREGATION IN VITRO

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### ABSTRACT

Platelets play an important role in hemostasis. Uncontrolled platelet activation under different pathophysiological conditions is associated with various pathophysiological conditions such as inflammation, thrombosis, hypertension and atherosclerosis. *Aegiceras corniculatum* is a mangrove plant traditionally used against inflammation, rheumatism, immune and cardiovascular disorders. It comprises of various novel chemical constituents including triterpenes (aegicerin and genin-A) saponins (corniculatonin) and polyphenols like tanins and lignin 1. In our previous study *A. corniculatum* extracts significantly inhibited inflammatory pathways *via* suppressing COX-1, 2 and 5-LOX metabolites as well as impairing oxidative stress 2. This study aimed to evaluate the antiplatelet aggregation and antithrombotic effect of *A. corniculatum* against thrombin, collagen, adenosine diphosphate (ADP), platelet aggregation factor (PAF) and arachidonic acid (AA). In our experiment, human platelet rich plasma (PRP) obtained from healthy donor and determine the platelet count by automatic counter, platelets (100  $\mu$ l)  $3 \times 10^8$  were pre-incubated for 10 min at 37 °C in the absence or presence of methanol and ethyl acetate extracts of *A. corniculatum* before stimulation. Aggregation of platelet were induced by different aggregating factors in all treated and untreated groups (negative control) and reaction was then allowed to proceed for 3 min followed by measurement of platelets aggregation by aggregometer in percent aggregation. Results showed that methanol and ethyl acetate extracts derived from *A. corniculatum* significantly inhibit thrombin and PAF induced platelet aggregation upto 90 % whereas ~ 50% inhibition was observed in ADP, collagen induced aggregation. Our study depicts that methanol and ethyl acetate extracts significantly reduced the platelet aggregation and thrombotic activities. It suggest that these extracts down-regulates the P-selectin expression, the mobilization of Ca<sup>2+</sup> and the elevation of cAMP. PAF and thrombin induced platelet aggregation activate the PLA2 pathway whereas ADP, collagen and AA involve the participation of receptors and thromboxane formation. Indeed, *A. corniculatum* extracts interfere platelet aggregation by multiple pathways and can be effective against inflammation e.g atherothrombosis.

**Keywords:** *Aegiceras corniculatum*, platelet aggregation, thrombin, thromboxane.

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## IMPACTS OF CLIMATE CHANGE ON CARBON STOCK PRODUCTIVITY OF *DALBERGIA SISSOO*

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### ABSTRACT

Forests occur across diverse biomes, each of which shows a specific composition of plant communities associated with the particular climate regimes. Predicted future climate change will have impacts on the vulnerability and productivity of forests; in some regions higher temperatures will extend the growing season and thus improve forest productivity, while changed annual precipitation patterns may show disadvantageous effects in areas, where water availability is restricted. Irrigated plantations in the province of Punjab-Pakistan are man-made forests and are in the shape of blocks of tree plantations in the canal-irrigated tract. These were raised originally to cater for the fuel wood requirements of the railway steam engines. The first artificial forest of this type was established at Changa Manga in 1866. The Irrigated plantations are spread over an area of 148968 ha in different districts of Punjab having irrigation water facilities. Although they have great commercial value but the highest concern of these plantations is environmental as these are the only green grooves of trees present in the central and southern Punjab. *Dalbergia sissoo* has been the main species in the irrigated plantations. It produces high quality timber as well as fuel wood. The present yield of 4-8 m<sup>3</sup> per hectare per year is low which can be increased three to four times the current yield. Data were collected from Changa Manga, Chichawatni, Bhagat, Shorkot, Daphar and Lalsohanra irrigated plantations to assess present carbon stock of trees. Volume Table constructed for *Dalbergia sissoo* during 1974 was taken as normal crop bench mark. The objective of the study was to estimate the gap between the existing carbon stock of *Dalbergia sissoo* to its normal to assess the extent of forest degradation. To estimate the present carbon stock and establish relationship between Diameter & Carbon stock, samples were taken randomly from each age group in each irrigated plantation for diameter and volume measurement. The equations developed for present and normal crop for carbon stock against diameter are 0.4449D<sup>2.2055</sup> and 0.204D<sup>2.4773</sup> respectively. The study reveals that present carbon stock of a *Dalbergia sissoo* tree with 65 cm diameter at breast height at the age of about 60 years i.e. rotation age is 4432 kg CO<sub>2</sub> in contrast to its normal productivity of 6321 kg CO<sub>2</sub>. The study strongly recommends improving the management skills to meet the gap between the existing and normal carbon stock productivity of *Dalbergia sissoo*.

**Keywords:** Carbon stock, Diameter, *Dalbergia sissoo*, Equations, Irrigated plantations

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## YIELD ADAPTABILITY AND STABILITY EVALUATION IN BRASSICA GENOTYPES THROUGH AMMI ANALYSIS TO COMPREHEND G × E INTERACTIONS

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### ABSTRACT

Plant breeders look for high yielding genotypes on the basis of both genotype and genotype x environment interaction for stability and adaptability in diversified environmental conditions. The present study comprised of 8 Brassica genotypes using a randomized complete block design (RCBD) with three replications at eight locations in Punjab, Pakistan to determine general and specific adaptability in 2013-14. Additive main effects and multiplicative interactions (AMMI) analysis showed that the environments (86.65%) have more influence to treatment sum of squares as compare to the G x E interaction (9.4%) and genotypes (2.65%) respectively, indicating the presence of sufficient genetic variability for effective selection to identify stable genotypes. G x E interaction was further partitioned by principal component analysis (PCA). The first four multiplicative axis terms (IPCA1, IPCA2, IPCA3 and IPCA4) explained 54 %, 28%, 9.9% and 5.6% of GEI sum of squares, respectively. The AMMI technique was used to identify appropriate genotype / genotypes to specific locations / environments. Results showed that genotypes RBN-08004, RBN-04021 and 11-CBN 006 were more stable with the lowest interaction and have general adaptability with yield close to mean yield. Genotype RBN-08004 has more than average yield, IPCA value closer to zero (1.8), genotype selection index (GSI) is 4 and least AMMI stability value (ASV) of 3.6 therefore considered the most stable. According to AMMI analysis, KN-253, KN-256 and RBN-08004 (adaptive group 1) exhibited specific adaptability for Bhakar and Khanpur with yield greater than mean and positive interaction. Genotypes RBN-04047 (adaptive group 2) revealed specific adaptation for Chakwal and Karore with yield less than mean yield and positive interaction.

**Keywords:** AMMI1, stability analysis2, GE interaction3, yield4, Brassica5

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## A REVIEW: BIODIESEL PRODUCTION FROM MICROALGAE

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### ABSTRACT

Biofuels has received much responsiveness for last few decades. To decrease the radiation of gasses like carbon dioxide and sulfur oxide, biofuels become more and more important. Almost 70% of fuels are being used to fulfill the demand of global energy especially in manufacturing and transportation. Recently biodiesel has become ever more valuable and beneficial due to two major perspectives. Firstly, it is made from inexhaustible resources and secondly due to its environmental benefits. The present study is to investigate the different skills for the production of biodiesel from microalgae. Four circumstances are available to produce microalgae those are Photoautotrophic, Heterotrophic, Mixotrophic and photoheterotrophic cultivations. Various methods have been discussed to improve the oil production in micro algal biomass. There is no comparable factor between the raceway and photo bioreactor productivity except the cost. Production of biodiesel from algae contracted more preference although different other new methodologies are developing to produce the oil from vegetable such as rapeseed, soybean, sunflower and palm. Microalgae obtained more attention due to significant increase in lipid content of microalgae. And all of this is possible from different heterotrophic cultivations and biotechnology/genetic engineering approaches.

**Keywords:** Microalgae, Lipids, Biofuels, Biodiesel, raceway ponds, photo bioreactor

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## DEVELOPMENT AND CHARACTERIZATION OF STARCH BASED NANO FILM

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### ABSTRACT

The study focus on starch nano film to enhance the mechanical and barrier properties of starch used for food packaging. The bio nano film was prepared by incorporating titanium oxide (TiO<sub>2</sub>) (0%, 5%, 10% and 15%) into the starch film by casting method. While comparing native starch film with TiO<sub>2</sub> loaded film (5%, 10% and 15 %), it was seen to decrease the crystallinity of the film. Water permilibility showed an increasing trend. Similar results are in case of water solubility. The structure of the film was characterized by FTIR, DSC and SEM. The increase in the concentration of TiO<sub>2</sub> in the starch based films showed increased values of tensile strength, % EAB and Tg and decrease in water solubility. This is because of the good interfacial instructions starch by hydrogen bonding and hence a strong effect of metal oxides. The hydrogen bond was formed in nano TiO<sub>2</sub>, structure and glycerol intermolecular hydrogen bonds of starch with glycerol and intermolecular hydrogen of starch was used by the addition of TiO<sub>2</sub> (metal oxides). Further the microbial analysis of the starch was also seen with treatment.

**Keywords:** Starch nano film, Indian Horse chest nut starch, DSC, FTIR

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## INHIBITORY EFFECT OF *AEGICERAS CORNICULATUM* AGAINST OXIDATIVE STRESS AND FREE RADICALS TO CONTROL INFLAMMATION

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### ABSTRACT

Oxidative stress is a result of imbalance between the production of reactive metabolites, free radicals like reactive oxygen species (ROS) and antioxidants. Reactive oxygen species (ROS) are highly reactive oxygen moieties including: O<sub>2</sub>•<sup>-</sup>, •OH, LOO• found to be involved in the activation of pro-inflammatory molecules i.e. transcription factors (NF-κB, p53, PPAR-α), chemotactic factor (leukotriene B<sub>4</sub>), lipid peroxidation (plasma membrane), cytokines (IL-1β, TNF-α) and DNA oxidation. Excessive oxidative stress can lead to inflammatory diseases including diabetes, atherosclerosis and cancer. Likewise, myeloperoxidase enzyme and nitric oxide are also involved in respiratory burst phenomenon. *Aegiceris corniculatum* a mangrove plant is used as a traditional medicine against inflammation and rheumatism. Ethyl acetate extract of *A. corniculatum* predominantly possess flavonoids (kaempferol, quercetin, and isorhamnetin) and novel triterpines (aegicerin, aegiceradienol, and genin-A) 1. In our previous studies, it showed anti-inflammatory activity *via* suppressing cyclooxygenase and 5-lipoxygenase enzymatic activity and protein expression along with interfering the cell migration<sup>2</sup>. This prompted us to explore its anti-oxidant potential at biochemical and cellular levels and *in vivo* pleurisy mouse model. *A. corniculatum* shown significant antioxidant effect against scavenging superoxide anion (O<sub>2</sub>•<sup>-</sup>) and hydroxyl radicals (•OH) in nitro blue tetrazolium reduction and deoxyribose degradation assays. Additionally, the MPO and NO were significantly decreased by 57-83% and 61-92% respectively in mouse plural fluid. Ethyl acetate extract has also suppressed the production of O<sub>2</sub>•<sup>-</sup> in human neutrophils activated by phorbol-12-myristate-13-acetate (PMA) and opsonized zymosan in dose dependent manner (IC<sub>50</sub> ~3–20 g/mL) and revealed that it is due to inhibition of NADPH oxidase activation and interfering the assembly of multi-component NADPH oxidase enzymatic at plasma membrane. In conclusion, ethyl extract of *A. corniculatum* inhibiting the production of NADPH oxidase and MPO and scavenging the superoxide anion O<sub>2</sub>•<sup>-</sup>, OH, NOO—showed potent & diverse anti-oxidant effect. Hence, it is effective against the production of pro-inflammatory mediators under oxidative stress and can be incorporated in drug discovery program against inflammation and immune response.

**Keywords:**

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## MANIPULATION OF *AtNCED3*, A MASTER SWITCH OF ABSCISIC ACID BIOSYNTHESIS IMPROVED DROUGHT TOLERANCE IN TOBACCO: FUTURE TRENDS FOR CROP DROUGHT TOLERANCE

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### ABSTRACT

Of various abiotic stresses, drought stress is the most devastating abiotic stress reducing crop growth and yield. Drought stress causes biosynthesis of abscisic acid (ABA) in roots, which is translocated to leaves via xylem thereby resulting in stomatal closure and reduces CO<sub>2</sub> fixation with subsequent generation of reactive oxygen species (ROS). ROS generation in peroxisomes and chloroplast also acts as signaling molecule which modulates cellular metabolism and growth of plants. Nine-cis-epoxycarotenoid dioxygenase (*NCED*) is key regulatory enzyme in the biosynthesis of ABA. It was hypothesized that transformation of *AtNCED3* in *Nicotiana tabacum* modulates chloroplastic antioxidative activity via ABA biosynthesis and thus induces drought tolerance. In present study, tobacco transgenic lines over expressing *AtNCED3* gene had higher biomass than wild type under drought stress. Among different transgenic lines, line L2A was maximal in growth. Growth reduction in tobacco plants was associated with reduction in plant water status and photosynthetic activity via stomatal closure. However, this adverse impact of drought on plant growth was less in transgenic lines probably due to improve plant water use efficiency, better antioxidant activity and low damages to membrane. These results were partially confirmed by proteomics approach. In future, quantification of ABA and components of ABA signaling pathway will be assessed to further reveal ABA-dependent molecular mechanism of drought tolerance. These strategies can be exploited in commercial crops.

**Keywords:** Reactive oxygen species, *Nicotiana tabacum*, abscisic acid, stomatal density

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## INHIBITORY EFFECT OF OPUNTIOLOPUNTIOSIDE-I AGAINST THE EXPRESSION OF CYTOKINE AND CHEMOKINE IN CARRAGEENAN AND ZYMOSAN INDUCED MOUSE INFLAMMATORY MODEL

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### ABSTRACT

Cytokines are signaling proteins including chemokines, interleukins, interferons, lymphokines and tumor necrosis factor that induce immune-based inflammatory responses. Elevated levels of cytokines provoke a broad range of cellular and physiological response and inflammation. *Opuntia dillenii* (KER-GAW) HAW belongs to genus *Opuntia* (Fam. Cactaceae) have shown remarkable effects as an anti inflammatory agent. In the present investigation we have evaluated novel compounds opuntiol and opuntioside-I derived from *Opuntia dillenii* have shown significant decrease in various cytokines (interleukins) and chemokines in a dose dependant manner. Cytokines i.e. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), Interleukin 1 $\beta$  (IL-1 $\beta$ ), interleukin 6 (IL-6), interleukin 10 (IL-10) and chemokine i.e. keratinocyte chemottractant (KC), JE, and monocyte chemottractant factor-1 (MCP-1) were evaluated in the absence and presence of opuntiol and opuntioside-I administered orally 60 minutes prior to induction of peritonitis via carrageenan and zymosan, respectively using ELISA. At highest dose 20mg/kg opuntiol/opuntioside-I inhibited IL-1 $\beta$ , IL-6, TNF- $\alpha$ , JE, KC and MCP-1 by 50-90%, whereas IL-10 levels were unchanged in all doses. Additionally, marked decline in mRNA expressions of pro-inflammatory cytokines (TNF, IL-1 $\beta$ , IL-6, IL-10) and chemokines (KC and MCP-1) were observed after i.p. treatment with 20mg/kg of opuntiol/opuntioside-I during zymosan induced peritonitis using RT-PCR. IL-1 $\beta$ , IL-6 and TNF- $\alpha$  activating NF-KB signaling pathway induced by JAK/STAT and I $\kappa$ B pathways which proceed with upregulation of various inflammatory gene such as COX2 and iNOS, growth factors, cytokines etc. Hence, opuntiol/opuntioside-I inhibiting the levels and reducing the expression of aforementioned cytokines and chemokine can be a potential immunomodulatory agent against Asthma, Rheumatoid arthritis, Atherosclerosis, Chronic bronchitis, Psoriasis etc.

**Keywords:** *Opuntia dillenii*, Cytokines, Chemokine, immunomodulatory agent.

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## ASSESSING THE ABILITY OF METAL RESISTANT BACTERIA FOR ACCUMULATION OF NI, PB AND CR

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### ABSTRACT

Present study intends to exploit the potentiality of indigenous bacterial isolates to remediate heavy metals from polluted environment. Removal through physicochemical techniques is not only expensive but also has environmental constraints. Therefore bioremediation is an option that offers the possibility to annihilate contaminants and render them innocuous using natural activity of ubiquitous living organisms. Bacteria isolated from industrial effluents of Hattar Industrial Estate, Haripur were checked for their bioaccumulation potential against Pb, Cr and Ni. Selected bacterial isolates were morphologically, physiologically and biochemically characterized. UV-Visible and atomic absorption spectrophotometric (AAS) analysis was applied to scrutinize the effects of different physical parameters including temperature, pH, inoculum size, incubation time and heavy metal concentration on growth as well as bioaccumulation potential of the isolates. Results revealed an enhancement in bioaccumulation potential with increase in incubation time and inoculum size. The isolates showed reduction in bioaccumulation at higher temperature (45°C) and metal concentration (500 µg/ml). Quantitative assessments exhibited that 83.51% of Cr by isolate D4-11, 85.30% of Pb and 48.78% of Ni by isolate A1-12 was accumulated at neutral pH, 37°C temperature and 500 µl inoculum size. Whereas isolate E5-Cr1 accumulated 88.33% of Pb at slightly acidic pH. However all isolates were capable to tolerate and accumulate heavy metal at pH 6, 7 and 8. The study therefore supports the effective utilization of indigenous bacterial isolates for comprehensive treatment of metal rich industrial effluents.

**Keywords:** Heavy metals, Metal accumulation, Effluent treatment

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## CHARACTERIZATION OF TWO UNIQUE ORGANOPHOSPHATE DEGRADING BACTERIAL STRAINS FROM MIANWALI DISTRICT, PUNJAB, PAKISTAN

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### ABSTRACT

Present study deals with the isolation, characterization and identification of indigenous organophosphate degrading soil bacteria from various agricultural soils of district Mianwali, Pakistan. Two isolates MB497 and MB504 were screened for their tolerance against three different OP pesticides (Chlorpyrifos, Triazophos, Dimethoate). The isolate MB497 showed tolerance up to 80g/l for CPF (Chlorpyrifos) which is the much higher concentrations of CPF reported so far. It could also tolerate Triazophos and Dimethoate up to 40g/l and 4g/l respectively. MB504 was able to grow in the presence of 8g/l CPF, 20g/l Triazophos and 1.2g/l Dimethoate. Both strains showed 41.56% and 63.56% degradation of CPF after 3 days of incubation, respectively. Both the strains were facultative anaerobes and mesophilic in nature. MB497 was alkaliphile (growing best at pH 9) and MB504 showed best growth at pH 6 (acidophilic). The bacterial isolates MB497 and MB504 were identified as *Bacillus* sp. and *Pseudomonas* sp. respectively using 16S rRNA sequencing. These isolated bacterial strains showed wide diversity in their morphological, biochemical and physiological characteristics and tolerance for heavy metals. Both strains also exhibited extraordinary high resistance to CPF, and other OP pesticides with considerable biodegradation. Therefore, these bacterial isolates have great capacity for degrading OP pesticides and can be used for bioremediation of OP pesticide contaminated soils under local conditions.

**Keywords:** Organophosphate, Pesticide degradation, Bioremediation

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## **IN-VIVO WOUND HEALING ACTIVITIES OF ETHANOLIC EXTRACT OF TURMERIC & HONEY IN DIABETIC MOUSE (*MUS MUSCULUS*)**

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### **ABSTRACT**

Diabetic wound management has always been a challenge for health care providers and biomedical researchers. The present study was planned for investigating the wound healing potential of South Asian spice turmeric, which is rich in polyphenols, and honey in Alloxan-induced diabetic mouse model. Wound healing was improved significantly in topically applied turmeric, honey and insulin (as positive control) when compared with diabetic non-treated control, however highest wound healing of 30.45% was observed in the case of turmeric. Similarly, diabetic mice on oral turmeric showed improved healing as compared to the controls. Moreover, diabetic mice on oral turmeric showed enhanced glucose tolerance test. On the basis of above results we surmise that turmeric improves the healing activity in diabetic wounds by enhancing the glucose sensitivity. Our data emphasize the idea of using turmeric as ethnomedicine for diabetic wound healing.

**Keywords:** Turmeric, honey, diabetes, wound healing, mice, GTT

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## **ROLE OF BLOOD GROUPS IN PATERNITY IDENTIFICATION: A REVIEW**

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### **ABSTRACT**

With the advancement in medical technology, certain productive tests are developed which give us the chance to resolve the paternity cases or disputes that are more accurate, reliable and cost effective as compared to old traditional methods used in paternity identification or testing (kinship investigation). There is also an important requirement of the tests to be fully accurate and giving fully fledged background history of the information we require. If it does not suit, or meet our requirement, may be the court discard it and adopt the old traditional methods. There are certain different tests developed for paternity testing which are accepted and rejected with times. For example different formulas such as probability formula called Bayes' Theorem which was latter neglected by scientists. In this review, a unique method in which blood groups role in paternity identification resolve many family disputes and world widely accepted. Different conventional DNA markers are used which are discovered with times found on alleles of blood groups and helped in distinguishing the suspect, moreover this blood group system clarifies us in such kind of disputes more precisely with low cost.

**Keywords:** Blood groups, DNA markers, Paternity Identification

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## EMERGING ISSUE OF E-WASTE IN PAKISTAN: STATUS, RESEARCH NEEDS AND DATA GAPS

Mehreen Iqbal

### ABSTRACT

Driven by the rapidly advancing technologies and increasing quantities of end-of-life electronic and electrical equipment, electronic waste is emerging as a global problem. Developed nations are shipping millions of obsolete electronics annually to developing countries and making them particularly vulnerable. Due to the lack of adequate infrastructure to manage waste safely, these wastes are often buried, burnt in the open air and/or dumped into the surface water bodies. This review article focuses on the current situation of e-waste in Pakistan with the emphasis on defining the major e-waste recycling sites, current and future domestic generation of e-waste, hidden flows or import of e-waste and discusses various challenges for e-waste management. Needed policy interventions and possible measures to be taken at governmental level are discussed to avoid the increasing problem of e-waste in the country. Our findings highlight that there is still a general lack of reliable data, inventories and research studies addressing e-waste related issues in the context of environmental and human health in Pakistan. There is therefore a critical need to improve the current knowledge base, which should build upon the research experience from other countries which have experienced similar situations in the past. Further research into these issues in Pakistan is considered vital to help inform future policies / control strategies as already successfully implemented in other countries.

**Keywords:**

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## EFFECT OF TURMERIC AND DONEPEZIL ON THE CHOLINERGIC SYSTEM IN THE BRAIN OF SCOPOLAMINE INDUCED CHOLINERGIC DYSFUNCTION IN MICE.

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### ABSTRACT

**Background:** Cholinergic system play an important role in higher cognitive functions and cholinergic dysfunction is a major cause in several neurodegenerative diseases. Turmeric is a yellow spice, also use as herbal medicine. Due to its neuro protective action, it plays an active role in the treatment of various central nervous system disorders. Donepezil hydrochloride is an acetylcholinesterase inhibitor (AChEI), promote neurogenesis, presently used for Alzheimer's disease (AD). Therefore, the turmeric and donepezil are important candidate to achieve its effects on cholinergic hypo function.

**Aim:** The aim of this study is to observe synergistic effect of Turmeric (20mg/Kg/day) and donepezil (0.5mg/Kg/day) treatment on cognitive function and gene expression in scopolamine (1mg/Kg) induced model.

**Methods:** Scopolamine was used to induce cholinergic hypo function in BALB/c mice. The effect of turmeric and donepezil on memory was investigated by Morris water maze (MWM) and social interaction test. Through RT-PCR the expression of muscarinic and nicotinic receptor genes was investigated in hippocampus and thalamus.

**Results:** Turmeric treatment significantly ( $p < 0.001$ ) improved spatial memory as compared to control and scopolamine groups. Donepezil and turmeric group  $68.03 \pm 0.9$  showed significant ( $p < 0.001$ ) synergistic effect relative to scopolamine group ( $27.62 \pm 0.29$ ). In social preference test, turmeric treatment in scopolamine group displayed significant ( $p < 0.05$ ) increase in sociability and social novelty. It decreased the expression of APP770 isoform and improved the level of APP695 in hippocampus although the result was not significant.

**Conclusion:** The synergistic effect of turmeric and donepezil can be a potential therapeutic and preventive strategy for cognitive deficits.

**Keywords:** Cholinergic hypo function, turmeric, donepezil, scopolamine.

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## OPUNTIAL AND OPUNTIOSIDE-I AN ANTI-ATHEROGENIC AGENT INTERFERING WITH CHEMOTAXIS VIA iPLA2 $\beta$ -DEPENDENT F-ACTIN POLYMERIZATION IN HUMAN MONOCYTE

Talat Roome<sup>1</sup>, Saeed Khan<sup>1</sup>, Anam Razzak<sup>1</sup>, Ayaz Noorani<sup>1</sup>, Rafiq Khanani<sup>1</sup>, Ahsana Dar<sup>2</sup>, Shaheen Faizi<sup>2</sup>, Lubna Abidi<sup>2</sup>, Lubna<sup>2</sup>, and Martha Cathcart<sup>3</sup>

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### ABSTRACT

Monocytes Chemoattractant Protein-1 (MCP-1) being a  $\beta$ -chemokine exhibits its most potent chemotactic activity towards monocytes and govern the pathogenesis of various inflammatory and immune diseases. We discovered earlier two intracellular signal transducing phospholipases (PLA2) calcium independent iPLA2 $\beta$  and calcium dependent cPLA2 $\alpha$  are critical regulator of monocytes chemotaxis in response to MCP-1. cPLA2 concentrated in endoplasmic reticulum and with its signaling molecule AA and DHETs regulate the speed of monocytes. These two phospholipases are translocated to different intracellular location in polarized, migrating monocytes with iPLA2 being distributed at the membrane enriched pseudopods (cell periphery) found to be associated with Cdc-42 (a Rho family member involved in cytoskeletal remodeling) and F-actin, whereas cPLA2 localizing to the endoplasmic reticulum behind the nucleus and closer to the uropod. We explored that MCP-1 induces association of iPLA2 $\beta$  with F-actin and actin polymerization significantly reduced due to inhibition of iPLA2 $\beta$  or its reduced expression. *Opuntia dillenii* (KER-GAW) HAW belongs to the genus *Opuntia* (Fam. Cactaceae) have shown remarkable effects as anti-diabetic, anti-oxidant, anti-hypotensive, anti-inflammatory and anti-viral agent. Most noticeable chemical constituents isolated so far include opuntiol and its glucoside called opuntioside-I. These active components did not cause any mortality at 1000 mg/kg *in vivo* using different routes of administration and found to be non-toxic at cellular level. In our present investigation we identified opuntiol and opuntioside-I reduced human monocyte migration (50-90%) dose dependently (2.5-10  $\mu$ M) against MCP-1 in microchamber chemotaxis assay and compared with iPLA2 $\beta$  inhibitor racemic-BEL. Laser Scanning Confocal Microscopy demonstrated Opuntiol and Opuntioside-I interfered with iPLA2 $\beta$  translocation to cell membrane in MCP-1 stimulated migrating cells using specific antibodies with fluorescent dye and its gradient dependent migration under-agarose assay was also suppressed. Furthermore, MCP-1 induced- and iPLA2 $\beta$  dependent- actin polymerization was also inhibited in cells treated with 10  $\mu$ M of Opuntioside-I and this inhibitory effect was restored in the presence of Lysophosphatidic acid (LPA). F-actin polymerization was studied using Phalloidin staining of filaments. In adoptive transfer mouse model, Opuntiol and Opuntioside-I at 10  $\mu$ g/ml inhibited cell migration by 80% induced by 4% thioglycolate. These compounds (20mg/kg) were also found to be effective upon oral treatment in mouse peritonitis model. These results suggest that Opuntiol and Opuntioside-I interfere with phospholipase A2 signaling in MCP-1 stimulated monocyte via inhibiting iPLA2 $\beta$  activity. We can conclude that these compounds are potential therapeutic agents to reduce MCP-1 dependent monocyte migration and its related immune and inflammatory diseases *e.g.* Atherosclerosis.

**Keywords:** *Opuntia dillenii*, Chemokine, Atherosclerosis, iPLA2 $\beta$  activity.

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## PREPARATION AND COMPARATIVE EVALUATION OF DIFFERENT ADJUVANTED TOXOID VACCINES AGAINST ENTEROTOXAEMIA

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### ABSTRACT

The objective of this study was to prepare and evaluate toxoid vaccines against enterotoxaemia in goats. Pure culture of *Clostridium perfringens* type D was used, already isolated and characterized by Institute of Microbiology, University of Agriculture Faisalabad, Pakistan. Epsilon toxin was extracted; the extracted toxin was subjected to LD50 determination and then with the help of formalin inactivation was done. The resulting toxoids were adjuvanted with two adjuvants including Montanide ISA 206 and Aluminium hydroxide gel (Alum). The pathogenicity and immunogenicity of prepared toxoid vaccines and commercial vaccine procured from VRI, Lahore, Pakistan were studied and compared initially in an experimental trial on rabbits and then in field trials on goat. Field trial was conducted on 72 goats divided into four equal groups (n=18). The Indirect Haemagglutination Test (IHA) was used to evaluate the antibody titer and challenge protection test was performed in rabbits. The results showed that antibody titers were significantly higher ( $P < 0.05$ ) for in group vaccinated with Montanide ISA-206 adjuvanted toxoid vaccine. The geometric mean titer of Montanide group was 203.19, 456 and 670 at 14, 21 and 28 days post vaccination as compared to 181.02, 298.63 and 456.14 of Alum group on same days. Montanide vaccinated group also exhibited highest protection percentage (100%) post challenge as compared to 85.71%, 71.42% and 14.29% of group B, C and D, respectively.

**Keywords:** *Clostridium perfringens*, Enterotoxaemia, Montanide, Aluminium hydroxide, vaccine

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## RISK ASSESSMENT OF TOTAL COLIFORMS, FAECAL COLIFORMS AND E.COLI ASSOCIATED WITH SALAD VEGETABLES

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### ABSTRACT

Advancements in food sector technologies have augmented health hazards particularly dissemination of foodborne pathogenic bacteria. It is imperative to make certain food safety by preventing foodborne diseases particularly pathogenic *E.coli* associated with salad vegetables; tomato, lettuce, cabbage and cucumber. The samples collected from three shops of three vegetable markets of Islamabad were analysed as unwashed and washed for aerobic plate count, total coliforms, faecal coliforms and *Escherichia coli*. Samples varied for their average pH values from 4.31(FMS 95abc, tomato, Aabpara) - 6.41 (FMS 101abc, Cucumber, Aabpara). Melody market contained highest number of coliforms in unwashed lettuce samples, followed by tomato, cucumber and cabbage. Washing prominently reduced total coliforms count. Highest number of total coliforms was noted on cabbage in Aabpara market while tomato showed minimum number of coliforms in Aabpara. Faecal coliforms revealed the highest number of faecal coliforms on lettuce vegetable sampled from melody market while cabbage was observed with least number of faecal coliforms. Aabpara market was found with highly contaminated cabbage samples while lettuce harboured minimum number of *E.coli*. Washing significantly reduced *E.coli* population, lettuce *E.coli* number was reduced to zero after washing. Sector G-9/4 also harboured higher *E.coli* counts where cabbage was found with least count. Washing effect was significantly observed on *E.coli* contamination. Paired T test showed non significant reduced *E.coli* count ( $p \leq 0.05$ ). Melody market provided 21 *E.coli* isolates of which 37 were of Biotype I and 20 were of Biotype II. One isolate showed atypical results. It was noted that leafy vegetable harboured more microorganisms and the count associated with such salads was high. Interactions of different factors did not produce significant results. Results of total coliforms, faecal coliforms and *E.coli* exhibited their contamination in all samples collected from all vegetable markets of Islamabad Pakistan. These findings urge for comprehensive sampling plans and a regular survey of vegetable markets after three months.

**Keywords:**

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## COMPUTER-AIDED FUNCTIONAL ANNOTATION OF THE ABC-TRANSPORTERS FOR SECRETION OF THE HETEROLOGOUSLY SYNTHESIZED BIOFUEL MOLECULES

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### ABSTRACT

Although algal oil is believed to be the most promising feedstock for biodiesel production yet pathogen load during cultivation, harvesting, dewatering and lipid extraction are the key challenges in the commercialization of algae based biodiesel. In the modern era of genetic engineering and synthetic biology, ABC transporters may be used to address the problems in the commercialization of algae based biofuels. Where, ABC transporters are ubiquitous proteins family and occur in all five kingdoms of life. Among ABC transporters, MsbA are involved in the export of the hydrophobic molecules into the extra cellular matrix. We hypothesized that the algal derived biofuel pathways may be installed into non-fermentative microbes and heterologously synthesized biofuel molecules may be secreted into the extracellular matrix via heterologous expression of the ABC-exporters. The present study was focused on *in-silico* characterization of the selected ABC-exporters (MsbA and its mutant) for extracellular secretion of biofuel molecules. The ABC-exporter proteins were predicted using Modeller9.1. The possible potential of the selected ABC-exporters to export the biofuel molecules was evaluated using AutoDockvina. Six hydrophobic molecules ( $\beta$ -carotene, botryococcene, canthaxanthin, zeaxanthin, squalene and heptadecane) were used in this study. Molecular docking analyses have shown the potential of selected MsbA transporters for the secretion of biofuel molecule. If successfully implied, these ABC-exporters may be exploited for the extracellular secretion of biofuel molecules. It will lead us to address the current problems regarding the cultivation, harvesting, drying and lipid extraction of biofuels.

**Keywords:** ABC transporters, secretion, biofuels, bioinformatics,

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## OPTIMIZATION OF TLC FOR CURCUMIN

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### ABSTRACT

Thin layer chromatography (TLC) is a technique used for the detection and separation of bioactive components from a complex plant extract. It is used for quantitative as well as qualitative analysis. The aim of present study is to optimize solvent systems (mobile phase) for curcumin (polyphenol), the active constituent of turmeric that gives characteristic yellow color and therapeutic properties to it. Best results were obtained when the Methanol and n-hexane were used as mobile phase in the ratio of 4:1; the  $R_f$  value was calculated to be 0.83. The separation of curcumin occurred (two distinct spots were obtained) when Methanol, n-hexane and water were used in ratio of 5:1:1 as mobile phase and the  $R_f$  values were calculated to be 0.76 and 0.94 respectively for both the spots. Chemical detection was used and different spraying agents were optimized for this purpose. The best results were obtained from  $FeCl_3$ .

**Keywords:**

*\*Correspondence author email:*

## THARPARKAR BLUE PEA FOWL (*PAVO CRISTATUS*) GENETIC ARCHITECTURE; EXPLORED BY MITOCHONDRIAL DNA D-LOOP MARKER

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### ABSTRACT

The blue peafowl from the genus *Pavo* is the largest species among both of its wild and domestic types, which is widely distributed in the habitats of Pakistan, India, Nepal, Sri Lanka and Bangladesh. The aim of this study is to have an insight of molecular diversity and phylogenetic analysis of *Pavocristatus* on the basis of mitochondrial D-loop region. A total of six samples were collected from the Lahore Zoo and Safari park Lahore. The whole genome was extracted by using standard protocol with minor modification. PCR amplification was done by using a set of mitochondrial D loop primer. CodonCode Aligner 5.1.5 was used for the sequence alignment and data analysis. Three C/T heterozygous loci were found at position 168, 170 and 223. One A/G heterozygous locus was observed at position 234. One insertion of A at 137, one transversion C>G at 190 and three transition mutation were observed at position 206, 222 and 236 respectively. Phylogenetic analysis was performed with the help of MEGA 6 software using neighbor joining method which revealed that our individuals are closely related with the Japanese *Pavo cristatus*. Moreover, this Japanese and Pakistan species sharing their common ancestor. Our samples are clearly placed themselves in one clade while all other related species are in another clade which predict the substantial divergence between Pakistani and other studies D loop regions of peacocks.

**Keyword:** *Pavocristatu*, mitochondrial D-loop, phylogeny, Tharparkar, Pakistan

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## PATHOGENESIS OF CHICKPEA WILT DUE TO *FUSARIUM OXYSPORIUM*

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### ABSTRACT

Pathogenesis study of *Fusarium oxysporum* f. sp. *ciceri* was made to correlate epidemiological factors with chickpea wilt. It was observed that seedling mortality increased with an increase in inoculum load, at the inoculum load of 3 g, seedling mortality was minimum and at 20 g it was maximum. Similarly, when *Fusarium* wilt was observed in different types of soils, it was found that the disease developed severe in sandy soil and least in clay soil. Soil moisture also played an important role in disease development. It was observed that seedling mortality was maximum (91%) when the soil moisture level was low (16.2%) and minimum (0.00%) when it was high (35.67%). Studies of disease development on sap extract of leaves and roots showed maximum growth of *F. oxysporum* f. sp. *ciceri* on root sap than leaves. Colony growth of *F. oxysporum* f. sp. *ciceri* was 3.2 to 5.9 cm at leaves sap and was 3 to 9 cm on root sap.

**Keywords:** Wilt, *Fusarium oxysporum* f. sp. *ciceri*, chickpea, germplasm

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## EXPRESSION STUDIES OF ACTIVE AND LATENT PHASE *MYCOBACTERIUM TUBERCULOSIS* ANTIGENS

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### ABSTRACT

TB (TB) is the foremost reason of death in the world from infection caused by Mycobacterium TB (M.tb) bacilli. Tuberculosis (TB) is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent. In 2013, 9 million people fell ill with TB and 1.5 million died from the disease. TB can be a difficult disease to diagnose, mainly due to the difficulty in culturing and not having a single perfect diagnostic tool. M.tb bacilli can produce latent TB in which patient is infected with M.tb but does not have active TB disease. Therefore a complete medical evaluation for TB is must before giving any treatment. Progression from infection to disease occurs when the TB bacilli overcome the immune system and begin to propagate. TB becomes active if immune system cannot stop them from growing. The diagnosis of M.tb infections remained practically unchanged for many decades and probably would have not progressed. Therefore to make TB diagnosis faster and easy, newer species-specific antigens from prevalent strain of M.tb associated with active and latent disease are necessary to be expressed and purified as an initial step for development of new diagnostic technology. Specific antigenic clones of active and latent M.tb genes i.e. Rv2626c, Rv3803c, Rv0934 and Rv1860 were obtained from TB Resource Center, Colorado State University, Boulder, CO, USA. Vectors containing antigenic genes were transformed in expression strains of E.coli BL21 (DE3) and BL21 (DE3) pLysS. After transformations recombinant plasmids were isolated. Isolated plasmids were restricted with NdeI/HindIII and NdeI/XhoI to confirm the presence of required gene. The restriction data revealed the presence of fragments of 431, 818, 1050 and 860 bp respectively when electrophorized on 1.2 % agarose gel. Expressions of positive clones were monitored by induction with different concentrations of isopropyl β-D-1-thiogalactopyranoside (IPTG) and time of induction for each antigen. Expression of antigenic proteins was confirmed by Dot blot and Western blot analysis using monoclonal antibodies raised against 6x His tag. Rv2626c and Rv3803c showed maximum expression at 0.2 mM while Rv0934 at 0.3 mM IPTG concentration after 5 hrs incubation. But in case of Rv1860 gene, the protein expression was very weak. After confirming cellular localization, proteins were subjected to immobilized metal affinity chromatography i.e. His.Bind® resin for purification. Batch purified proteins were subjected to SDS-PAGE and Western blot analysis for homogeneity determination and further confirmation of expressed proteins. These purified proteins are now used in development of multiplex immunoassay as new diagnostic tool for the detection of TB at any phase of infection i.e. active and latent.

**Keywords:**

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## ROLE OF ANGIOTENSIN CONVERTING ENZYME (ACE) INS/DEL POLYMORPHISM (RS4646994) IN THE ONSET OF MYOCARDIAL INFARCTION IN PAKISTANI POPULATION

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### ABSTRACT

**Introduction:** Cardiovascular diseases (CVDs) are a leading cause of morbidity and mortality in the developing countries such as Pakistan. Owing to its multi-factorial nature, both environmental and genetic determinants have been shown to have an important role in the onset of myocardial infarction (MI). High blood pressure is an important factor in the onset of MI because of its involvement in the renin angiotension system (RAS). A genetic variation of Alu repeat polymorphism (rs4646994) present in angiotensin converting enzyme (ACE) gene has previously been shown to be associated with increased risk of developing MI.

**Methodology:** In this case control study, MI patients were recruited from Rawalpindi Institute of Cardiology based on WHO standard criterion for MI patients with typical changes in ECG and positive cardiac markers. DNA was extracted from lymphocytes using a standard phenol-chloroform method and used for genetic screening. ACE gene polymorphism rs4646994 consisting of an Ins/Del was amplified through PCR. The amplified product was electrophoresed on 2% agarose gel and visualized under UV transillumination.

**Results and Discussion:** A case control study of 310 MI patients and 307 healthy control individuals was carried out to determine the role of ACE gene Ins/ del polymorphism in the onset of myocardial infarction in Pakistani population. The association of the genotype frequency was found to be non-significant in MI patients as compared to healthy controls ( $p > 0.05$ ,  $\chi^2 = 2.21$ ). The risk allele D was not associated in the onset of MI in patients ( $p > 0.05$ ,  $\chi^2 = 0.35$ , Odd Ratio (OR) = 1.07 (95% CI 0.85-1.33). Linear regression analysis also revealed no significant difference in genotype frequency of risk allele even when analyzed on the basis of gender ( $p > 0.05$ ). When the data were analyzed on the lines of positive and negative family history for CVD within the MI patients, a significant association of risk allele D was observed with the onset of disease in patients with a positive CVD history (50.8%) compared to CVD negative family history (41.8%) ( $p < 0.05$ ,  $\chi^2 = 4.3$ , OR = 1.43 (95% CI 1.01-2.02). Similarly, earlier onset of MI was observed in D allele carriers with a positive CVD family history (Mean age (Years)  $\pm$  StDev:  $51.6 \pm 10.3$ ) as compared to D allele carriers with a negative CVD family history ( $55.3 \pm 10.7$ ) with significant differences among the group ( $p < 0.05$ ).

In conclusion, ACE gene Ins/Del polymorphism was found to be associated with the onset of MI in Pakistani population with a higher penetrance in patients with a positive CVD family history.

**Keywords:** Myocardial Infarction, Angiotensin Converting Enzyme, Renin angiotension system, Alu repeat polymorphism, Odd Ratio (OR)

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## ASSOCIATION OF ENOS GENE WITH DIABETIC RETINOPATHY IN PAKISTAN

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### ABSTRACT

**Purpose:** Retinopathy is the non-inflammatory degeneration of the retina that leads to irreversible loss of vision. After cataract and glaucoma, diabetes induced retinopathy is the major cause of retinal degeneration worldwide. The aims of the present study was to investigate the role of genetic polymorphisms in the antioxidant pathway in the onset of diabetic retinopathy in the Pakistani population.

**Methods:** Variable number of tandem repeat (VNTR) polymorphism in endothelial nitric oxide synthase (eNOS) and two allelic polymorphisms of glutathione-S-transferases, the GSTM1 and GSTT1 were genotyped in 396 type 2 diabetic individuals (196 diabetic retinopathy (DR)+200 non-retinopathic (DNR)) and in 200 healthy persons. The genotyping was done through standard polymerase chain reaction (PCR).

**Results:** eNOS VNTR polymorphism was associated only with DR, while GSTT and GSTM were found to be associated with proliferative DR ( $p < 0.05$ ) but not with non-proliferative NPDR ( $p > 0.05$ ).

**Conclusion:** Our study results showed that eNOS gene has genetic role in DR susceptibility in Pakistan

**Keywords:**

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## THE MOLECULAR BASIS OF RETINAL DYSTROPHIES IN PAKISTAN

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### ABSTRACT

The customary consanguineous nuptials in Pakistan underlies the frequent occurrence of autosomal recessive inherited disorders, including retinal dystrophy (RD). In a number of studies, homozygosity mapping has been shown to be successful in mapping susceptibility loci for autosomal recessive inherited disease. RDs are the most frequent cause of inherited blindness worldwide. Till date there is no comprehensive genetic overview of different RDs in Pakistan. In this review, genetic data of syndromic and non-syndromic RD families from Pakistan has been collated. Out of the 132 genes known to be involved in non-syndromic RD, 35 different genes have been reported to be mutated in families of Pakistani origin. In the Pakistani RD families 90% of the mutations causing non-syndromic RD and all mutations causing syndromic forms of the disease have not been reported in other populations. Based on the current inventory of all Pakistani RD-associated gene defects, a cost-efficient allele-specific analysis of 11 RD-associated variants is proposed, which may capture up to 35% of the genetic causes of retinal dystrophy in Pakistan.

**Keywords:**

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## FREQUENCY DISTRIBUTION AND CHANGING PATTERN IN HEPATITIS C VIRUS GENOTYPES: A 10 YEARS DATA ON 20552 CHRONIC HCV CARRIERS

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### ABSTRACT

Information regarding the changing pattern in hepatitis C virus (HCV) genotypes/subtypes and resulting disease outcome is not well known. The specific objective of this study was to find out the frequency distribution of HCV genotypes and changing pattern of various HCV genotypes overtime in well-characterized Pakistani HCV isolates. The genotype distribution of HCV from all the four provinces of Pakistan was tracked for a period of 10 years (2000-2009) on total 20,552 consecutive anti-HCV and HCV RNA positive patients sample using type-specific genotyping assay. Of these, 16,891 (82.2%) samples were successfully genotyped. Of these 11,189 (54.4%) were males and 9363 (45.55%) were females. Of the successfully genotyped samples, 12,537 (74.2%) were with 3a, 1834 (10.9%) with 3b, 50 (0.24%) with 3c, 678 (3.3%) with 1a, 170 (0.83%) with 1b, 49 (0.24%) with 1c, 431 (2.1%) with 2a, 48 (0.23%) with 2b, 3 (0.01%) with 2c, 13 (0.06%) with 5a, 12 (0.06%) with 6a, 101 (0.49%) with 4, and 965 (4.7%) were with mixed-genotype infection. A changing pattern of HCV genotypes prevalence was observed in Pakistan overtime, with an increase in the relative proportion of genotype 3a and mixed genotypes and a decrease of genotypes 3b, 2b, 4, 5a and 2a. This changed HCV genotype pattern might have direct impact on HCV disease outcome and new therapeutic strategies may be needed.

**Keywords:**

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## HEPATITIS C VIRUS GENOTYPE 3A INFECTION AND HEPATOCELLULAR CARCINOMA: PAKISTAN EXPERIENCE

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### ABSTRACT

**Aim:** To assess the association between chronic hepatitis C virus (HCV) infection and hepatocellular carcinoma (HCC) in Pakistan, and the genotype distribution among these HCC patients.

**Methods:** One hundred and sixty-one subjects with HCC were included in this study. Liver biopsy was performed on 145 of the patients; sixteen were excluded because they failed to fulfill the inclusion criteria. Qualitative polymerase chain reaction (PCR) was performed for hepatitis B virus and HCV. Samples positive for HCV RNA were genotyped using genotype-specific PCR and confirmed by HCV 5' noncoding region sequencing analysis.

**Results:** Chronic HCV infection was identified a major risk factor (63.44% of tested HCC patients) for the development of HCC. The time from HCV infection to appearance of cancer was 10-50 years. In the HCC patient population, broader distributions of genotypes were present with genotype 3a as the predominant genotype. Using the type-specific genotyping method, we found HCV genotype 3a in 40.96%, 3b in 15.66%, 1a in 9.63%, and 1b in 2.40% of HCC tissue samples. About 28% of cases were found with mixed genotypes. Two cases were unable to be genotyped because of low viral load. Sixty-six percent of treated patients with cirrhosis had an end of treatment response, but unfortunately they relapsed quickly when the treatment was discontinued, and HCC developed during a median 3.8 years.

**Conclusion:** There was a strong association between chronic HCV infection and HCC in Pakistan, and between HCV genotype 3a and HCC.

**Keywords:** Hepatocellular carcinoma, Hepatitis C, Genotyping, Etiology, Prevalence

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## EPIDEMIOLOGICAL ASPECTS OF RHEUMATOID ARTHRITIS IN DISTRICT HARIPUR

**Sana Rukhsar, Darima Ashfaq, Afshan Saleem, Sadiq Noor Khan, Aamer Ali Khattah, Farrakh Javed.**

### ABSTRACT

Rheumatoid arthritis (RA) is a systemic and persistent disorder. It includes infection of many joints. This disease damages the lining of joints. Both environmental and genetic factors are responsible for pathogenesis of RA. Incidence of RA is evaluated to be 0.8 % universally. In women, this disease is double as compared with men. Its occurrence also varies by age within each sex. The purpose of this study is to focus on epidemiological aspects of this disease in district Haripur. To clarify the prevalence rate of RA, data was collected from the Yahya hospital during April to June. The blood samples collected from patients were tested by RA factor method based on the latex agglutination technique. The kit named was RA latex test kit. CBC and ESR further confirmed these samples. Total 300 samples were collected from both males and female. From these samples 15 were positive cases in males and 50 RA factor positive cases in females. Symptoms occur more frequently in the following sites like knee, lumber, shoulder and neck. In women this RA factor cases were more common at the age of >34. In men, more RA factor positive cases were seen at the age of >44. In Haripur, the prevalence rate is high in females as compared to males. Rheumatic symptoms in women are more frequent as compared to males. Out of 15 RA factor positive males, 9% were smokers and from 50 RA factor positive females 19% females were obese.

**Keywords:**

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## AWARENESS ABOUT CERVICAL CANCER, HUMAN PAPILLOMAVIRUS AND ACCEPTABILITY OF ITS VACCINE AMONG FEMALE UNIVERSITY STUDENTS OF PESHAWAR, PAKISTAN

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### ABSTRACT

Cervical cancer is the most common cancer among women all over the world. The major causative agent for cervical cancer is Human papillomavirus (HPV). Awareness and education about cervical cancer, HPV and its vaccination can help to prevent cervical cancer. Present study was a questionnaire based survey including 764 female students of Shaheed Benazir Bhutto women university Peshawar, Khyber PukhtunKhwa (KPK). KPK is considered as a relatively conservative society where talking about sexually transmitted diseases (STDs) is a taboo leading to a poor management of these STDs. Current study was conducted to assess the awareness of female university students about cervical cancer, human papillomavirus (HPV) and its vaccine. Majority of participants were unaware of cervical cancer and its causes. Only 23.20% knew that cervical cancer is a gynecological cancer. 93.50% of the participants had never heard about Pap (Papanicolaou) smear testing. Level of awareness about Human papillomavirus (HPV) as causative agent and availability of vaccine against HPV was just 11.53% and 10.87% respectively. Notably, acceptance of HPV vaccine among the female students was 95.49% indicating a positive attitude towards treatment if the facilities are provided. Majority of students want themselves to be educated by experts about HPV and its vaccination. Overall study indicated that although the educated young females of Peshawar are not very much aware of the cervical cancer, HPV and its vaccination but they are willing to have more awareness and proper medical facilities to address this issue. Being a citizen of developing and conservative country, a nationwide strong knowledge and awareness about cervical cancer, HPV and its vaccine is needed. This can lead to an early identification of the signs and symptoms of the disease following timely preventive measures and reduced cancer mortality rate.

**Keywords:** Cervical cancer – Pakistan- human papilloma virus - Peshawar – Pap test

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## CULTURE MEDIA STRESS ON THE ACTIVITY OF GLUTAMATE DEHYDROGENASE OF ANTIDIABETIC MEDICINAL HERB.

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### ABSTRACT

Activity of Glutamate Dehydrogenase (GDH) enzyme were studied in complete life cycle of antidiabetic medicinal herb *Argyrobium roseum* from *in vitro* developed conditions until the regenerated phase produced through indirect organogenesis. Culture medium was supplemented with different hormonal combinations along with additives to enhance the cell culturing process. Glutamate dehydrogenase showed inverse pattern during callogenesis as compared to *in vitro* grown seedlings. High activity of GDH was observed in callus as compared to *in vitro* studied explants. NADPH-GDH showed maximum activity at end of callus development and continued this pattern in proliferation phase. Leaf derived calli exhibited 25% increase in enzyme level as compared to stem 53% and roots 42%. NADPH-GDH showed descending pattern during regeneration period. Regenerated leaf exhibited 13.3% decline in enzyme level as compared to stem (17.05%) and roots (19.9%). Leaf, stem and roots showed 9.76%, 17.9%, 32.9 % activity respectively, at the end of acclimatized stage studied. High activity of NADPH-GDH was recorded in regenerated plants as compared to *in vitro* developed plants.

**Keywords:** Glutamate Dehydrogenase, *Argyrobium roseum*, *in vitro*, cell culturing.

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# VARIATIONS IN THE TESTICULAR HISTOMORPHOLOGY AND SERUM TESTOSTERONE CONCENTRATION OF HELMETED GUINEA FOWL (*NUMIDAMELEAGRIS*) DURING DIFFERENT REPRODUCTIVE PHASES IN PAKISTAN

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## ABSTRACT

Thirty healthy guinea fowls were slaughtered to peruse variations in annular testicular histomorphology and serum testosterone during reproductive phases viz, resting, progression and peak breeding in Pakistan. Samples of testis were stained with HE and histometric analysis was made with Image J®. Serum testosterone was measured by radioimmunoassay. Results revealed significantly ( $P<0.01$ ) greater values of weight, volume, length and circumference of testes during peak breeding as compared to progression and resting phases. However, no gross anatomical parameter showed significant variation between right and left testis during cycle. Histometric parameters like diameter of seminiferous tubules and its lumen, thickness of seminiferous epithelium showed a cyclic annual variations and their significantly ( $P<0.01$ ) higher values were recorded during peak breeding than other phases. Conversely, diameter and percentage of interstitial cells were significantly ( $P<0.01$ ) higher during resting in contrast to progression and peak phases. Serum testosterone showed significantly ( $P<0.01$ ) higher value during peak breeding which declined significantly ( $P<0.01$ ) during progression and resting phases. Moreover, histomorphometric changes positively correlated with hormonal profile during each phase. In conclusion, different reproductive phases influence annular testicular histomorphology and hormonal profile. Peak breeding activity of this bird is coincided with the increased steroid hormone synthesis under suitable circumstances.

**Keywords:** Guinea fowl, histomorphology, testicular cycle, seminiferous tubules diameter serum testosterone

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# PRE-HATCH GROWTH AND DEVELOPMENT OF DIGESTIVE SYSTEM AND SELECTED INTERNAL ORGANS OF DOMESTIC DUCK (*ANASPLATYRHYNCHOS*)

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## ABSTRACT

Normal growth and development pattern of selected internal organs of domestic duck (*Anas platyrhynchos*) was studied in this project. Tongue, esophagus, proventriculus, gizzard, small and large intestines, liver, kidneys, trachea, lungs, brain, eye balls and heart were studied for morphometric measurement. A total of 120 healthy fertile domestic duck eggs were equally divided into 24 groups ( $n=5$ ). Eggs were weighed, labeled and incubated. All groups were examined one by one on daily basis during incubation. Collection of embryos was started from day five of incubation but recordable observations were obtained from day ten. Means and standard errors of mass, length and width of selected internal organs were calculated for each age group till hatching. Growth rate of duck was measured by applying Janoscheck growth curve to age group means. Results revealed a sigmoid to exponential growth curves for organs' majority. Growth pattern grouped organs into: eyes, brain and trachea with early rapid growth; liver and some digestive organs having intermediate growth; while heart, lungs, kidneys and esophagus attained maximum maturity towards the end of incubation.

**Keywords:** Digestive system, Domestic duck, Embryo weight, Janoscheck growth curve, Incubation, Lungs, Kidneys, Brain

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# EFFECT OF TEMPERATURE ON LIFE TABLE AND PREDATION RATE OF *MENOCHILUSSEXMACULATUS* F. (COCCINELLIDAE: COLEOPTERA) ON *PHENOCOCCUSSOLENOPSIS* T. (PSEUDOCOCCIDAE: HOMOPTERA)

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## ABSTRACT

Age-stage, two sex life table studies and predation rate of *Menochilussexmaculatus* on *Phenococcussolenopsis* were examined at  $24\pm 1^{\circ}\text{C}$ ,  $27\pm 1^{\circ}\text{C}$  (60-70% R.H.) and  $32\pm 4^{\circ}\text{C}$ : 16-50% R.H. to see the effect of temperature on its different life stages, reproduction, population dynamics parameters and consumption rate. Total pre-reproductive duration was significantly greater at  $24\pm 1^{\circ}\text{C}$  than  $27\pm 1^{\circ}\text{C}$  and  $32\pm 4^{\circ}\text{C}$ . Adult pre-reproductive period showed non-significant variation and reproductive duration was significantly greater at  $24\pm 1^{\circ}\text{C}$  than other temperatures. Total adult longevity and total fecundity of beetle was significantly greater at  $24\pm 1^{\circ}\text{C}$  followed by  $27\pm 1^{\circ}\text{C}$  and  $32\pm 4^{\circ}\text{C}$ . Population dynamic parameters like intrinsic rate of increase ( $r_m$ ) and finite rate of increase ( $\lambda$ ) were recorded higher at  $27\pm 1^{\circ}\text{C}$ , followed by  $24\pm 1^{\circ}\text{C}$  and  $32\pm 4^{\circ}\text{C}$ . Net reproductive rate ( $R_0$ ) and gross reproductive (GRR) were significantly greater at  $24\pm 1^{\circ}\text{C}$  as compared to other thermal conditions. Survival rate, life expectancy and age-stage specific fecundity were greater at  $24\pm 1^{\circ}\text{C}$  than other temperatures. The predation rate was greatest in L4 stage among immature stages and in female among adult stages. Females consumed more nymphs at  $24\pm 1^{\circ}\text{C}$  than the other two temperatures. The sequence of net predation rate ( $C_0$ ) for beetle was  $24\pm 1^{\circ}\text{C} > 27\pm 1^{\circ}\text{C} > 32\pm 4^{\circ}\text{C}$ . The lowest transformational rate ( $Q_p$ ) from prey population to predator beetle egg production needs 25.62 nymphs at  $24\pm 1^{\circ}\text{C}$ . Although adult longevity, reproduction, immature duration and predation rate were higher at  $24\pm 1^{\circ}\text{C}$ ,  $27\pm 1^{\circ}\text{C}$  may be considered to the most appropriate for mass multiplication of *M. sexmaculatus* in laboratory based on higher demographic parameters like intrinsic rate of increase ( $r_m$ ) and finite rate of increase ( $\lambda$ ).

**Keywords:** *Menochilussexmaculatus*, *Phenococcussolenopsis*, life table, age-stage two sex, predation rate

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# DROUGHT STRESS EVALUATION OF SENSITIVE AND TOLERANT BARLEY GENOTYPES THROUGH PROTEOMICS ANALYSIS

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## ABSTRACT

Drought is a severe environmental constraint to plant productivity and an important factor limiting barley yield. To investigate the initial response of barley to drought stress, changes in protein expression were analyzed using a proteomics technique. Three-day-old barley seedlings of sensitive genotype 004186 and tolerant genotype 004223, were treated with 20% polyethylene glycol (PEG) and drought by withholding water. After 3 days of treatments, proteins were extracted from, separated by two-dimensional polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue. Among the common proteins between sensitive and tolerant genotype in response to drought, Vacuolar proton ATPase subunit E was increased and photosystem I reaction centre II was decreased. Many of the metabolism related proteins were decreased in the sensitive genotype under drought, however in the tolerant genotype metabolism related proteins were increased under drought stress. These results suggest that chloroplastic metabolism and energy related proteins might play a significant role in the adaptation process of barley seedlings under drought stress.

**Keywords:** Barley, Proteomics, Drought, Sensitive Genotype, Tolerant Genotype, Mass Spectrometry.

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## ANTIFUNGAL POTENTIAL OF DIFFERENT PARTS OF *OLEA EUROPAEA* AND *OLEA CUSPIDATA* GROWING IN AZAD JAMMU AND KASHMIR

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### ABSTRACT

Development of more effective and less toxic antifungal agents is required for the treatment of fungal diseases of plants and human being. The medicinal plants and their various extracts have been used as medicines against infectious diseases and found as potent against crops as well as human pathogenic fungal stains. In this study, the antifungal activity of *O. europaea* (cultivated olive) leaves, seeds and *O. cuspidata* (wild olive) leaves, roots bark, stem bark and seeds were evaluated against a range of human and crop pathogenic fungal species by using well diffusion assay. The extracts of investigated parts of *O. europaea* and *O. cuspidata* obtained with organic solvents were found to be effective against some of tested fungal strains particularly extracts of *O. cuspidata* parts proved to be more potent. The ethylacetate extracts of *O. cuspidata* leaves and seeds were found to be effective against fungal pathogens as compared to *O. europaea* extracts. Nystatin was also used as positive control and respective solvent as negative control. This study demonstrated that the use of wild olive leaves extracts as medicines may reduce the risk of fungal infections, particularly in situations where lengthy usage of synthetic fungicidal inspire growth of opportunistic contagions.

**Keywords:**

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## PRODUCTIVITY OF FOOD MUST BE RAISED ALMOST 40% TO ACCOMPLISH 33% NEEDS OF INCREASING POPULATION.

Aysha Mansoor, M Arshad

### ABSTRACT

To congregate these goals of huge productivity for prosperous population, food grains should be enhanced and development in agriculture sector should be certain. To achieve the improved food production, one of the major issues is the limited land resources. Soil, basically makes the earth layer with excellent rock particles and availability of nutrients to the plants is very small. Salinity and sodicity caused reduction of 59% in crop production. Lack of phosphorus and nitrogen are the major issues of Pakistani soils. Also the micro nutrients like iron, boron, and zinc are under consideration. Improper application of fertilizer and nutrients, improper management and misuse of organic matter are the main issues which influence fertilizer use efficiency. A study was conducted to delineate the status of soil fertility in two districts i.e. Lahore and Kasur and two tehsils i.e. Ferozwala and Pattoki. Soil fertility parameters were such as EC, pH, Organic Matter, available Phosphorous, available Potassium and soil saturation were observed to know the status of soil fertility. The data were collected, processed using ArcView GIS software and kriging method for interpolation of data. The extraction by mask method was used to extract the study area boundary from Pakistan map. It was concluded that District Kasur had 30% low soil fertility than District Lahore, while Lahore had 60% igh fertile soil due to presence of more organic matter in the soil. It was observed that Tehsil Ferozwala had 75% high fertile soil than Tehsil Pattoki and organic matter in Tahsil Pattoki was very low than Tehsil Ferozwala. It was found that there was a minute variation in available phosphorus and available potassium in both Districts and both Tehsils. Tehsil Pattoki had high available potassium than other areas.

**Keywords:**

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## STRUCTURAL CHARACTERIZATION OF LIPID A OF LIPOPOLYSACCHARIDES ISOLATED FROM INDIGENOUS STRAINS OF TYPHOIDAL PATHOGENS

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### ABSTRACT

Typhoid, a disease caused by *Salmonella enterica* serovar Typhi (*S. Typhi*), is a major public health problem worldwide. It is associated with about 33 million cases and resulting in 600,000 deaths annually. It is considered as the 4<sup>th</sup> largest cause of death in Pakistan. There is no single vaccine available which can cover all types of typhoidal ailments. To effectively combat this disease, development and use of efficacious vaccines are required. Like all other Gram-negative bacteria, the main virulence factor of *Salmonella* is the lipopolysaccharide (LPS), which is the key tool used in the development of effective vaccines. It has been reported that modification of *Salmonella* LPS results in attenuation of signaling through the TLR4 pathway and, therefore, may promote the evasion of the innate immune system during infection. Therefore, structural and functional study of LPS from various *Salmonella* strains is essential to understand the disease nature and to develop the effective conjugate vaccines against all typhoidal pathogens. On the basis of these facts, unambiguous structural analysis of LPS, purified from Vi-positive and Vi-negative strains of *S. Typhi*, have been pursued. LPS were isolated through conventional hot phenol-water method from the cell pellets of these strains. Mild acid hydrolysis yielded the lipid A and O-specific polysaccharides components from LPS. Lipid A analysis, conducted by GC-MS and ESI-MS/MS revealed that the tested lipid A samples are significantly different. Vi-positive sample indicated the higher occurrence of mono-phosphorylated tetra and pentaacylated lipid A structures. Whereas, Vi-negative strain represented the higher intensities of mono and bisphosphorylated hexa- and hepta-acylated lipid A variants. Similarly, both of the lipid A samples differ significantly with respect to covalent modification, i.e., addition of L-Ara4N and addition of 2-OH on fatty acyl chains. It seems that Vi-negative isolate, being without capsule around it, has invested more on its lipid A to make its membrane more compact and resistant by having higher amount of hexa- and hepta-acylated lipid A structures, as compared to Vi-positive strain. These findings might be helpful in understanding the pathogenicity of indigenous strains of typhoidal pathogens.

**Keywords:** Typhoid, *S. Typhi*, lipopolysaccharide, lipid A, GC-MS, ESI-MS/MS, covalent modification and pathogenicity

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## THERAPEUTIC POTENTIAL OF HYDROETHANOLIC EXTRACT OF *NERIUM OLEANDER* FLOWERS TO CHECK THE ANTIDIABETIC ACTIVITY

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### ABSTRACT

Diabetes Mellitus (DM) is a complicated metabolic illness that has greatly troubled human health and quality of life worldwide. Conventional methods are being used for treatment of diabetes; however they are not completely effective and are unable to maintain blood glucose level partially or temporarily. Medicinal plants with anti-hyperglycemic activities are being used at global level. In the present study, the therapeutic potential of ethanolic extract of *Nerium oleander* flowers was checked on diabetes *in vivo*. First of all hydroethanolic extract of plant and flowers was prepared and its toxicity was checked. Alloxan was used to induce the diabetes in rabbits. Treated rabbits were divided into two groups and given 200mg/kg body weight and 400mg/kg body weight per day respectively for 14 days. The decrease in blood glucose level was more significant in group II as compared to group I. Glucose level and histopathology was carried out to check the effect of extract of *N. oleander*. Results showed decrease in blood glucose level in treatment group II ( $118.7 \pm 7.4$ ) and group I ( $150.64 \pm 8.67$ ) as compared to diabetic control ( $308 \pm 7.75$ ). There was more pronounced decrease in blood glucose level in treatment group II as compared to group I. Serum insulin level was also improved in both treated groups i.e. in group I ( $7.67 \pm 0.18$ ) and group II ( $7.39 \pm 0.9$ ) versus diabetic control group ( $3.73 \pm 0.26$ ). Improvements in morphology of pancreas of treatment groups were observed. It was concluded that hydroethanolic extract of *N. oleander* flowers had tremendous effect against diabetes as it reduces the blood glucose level, improves glucose tolerance and also improves serum insulin level. Thus the current study showed the hypoglycemic effect of ethanolic extract of *N. oleander*. Oral glucose tolerance test (OGTT) was also performed and the results were quite satisfactory for treatment groups.

**Keywords:** Diabetes mellitus, *Nerium oleander*, alloxan, blood glucose level, serum insulin and histopathology

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## TREATMENT OF ALLOXAN INDUCED DIABETIC RABBITS WITH HYDROETHANOLIC EXTRACT OF *JUSTICIAADHATODA* LEAVES

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### ABSTRACT

Diabetes mellitus (DM) has become a common disease of all countries in the World. More than 371 million people are diabetic in the World. Diabetes has many complications like cardiovascular diseases, peripheral vascular diseases, stroke, diabetic neuropathy, amputation, renal failure and blindness are common. So prevention and control program to stop the rising epidemic of DM and its complications. Treatment with insulin and oral hypoglycemic agents showed a remarkable breakthrough, however, difficulty in repeated administration and risk of hypoglycemia with other side effects seriously influence the quality of life. Plant materials which are cheap and within range of village folks are being used World over as a remedy for DM. In this study, hypoglycemic effect of leaves of *Justiciaadhatoda* on alloxan induced rabbits is investigated. Pancreas of rabbits were infected by alloxan monohydrate and diabetes symptoms in them were confirmed on 5<sup>th</sup> day of alloxan injection. The diabetic rabbits were treated in three treatment groups i.e. with insulin, with 100 mg/kg and 200 mg/kg *Justiciaadhatoda* plant extract. The treatment duration was two weeks and random blood glucose levels of treating rabbits were daily monitored and the values were compared with normal and diabetic control rabbits and treated rabbits. Rabbits treated with 100 mg/kg of plant extract showed  $P < 0.05$  value and that of treated with 200 mg/kg of extract showed  $P < 0.001$  value which is more significant. Hence, it is concluded that dose of 200 mg/kg of *Justiciaadhatoda* plant extract once a day has more pronounced hypoglycemic effect as compared to 100 mg/kg of *Justiciaadhatoda* plant extract. Similarly, oral glucosetolerance test (OGGT), serum insulin and histopathological studies revealed that *Justiciaadhatoda* plant possess remarkable anti-diabetic effects in treated animals. So, further research on this medicinal plant can help to prepare commercial medicines for curing diabetes and its complications in humans.

**Keywords:** Diabetes mellitus, *Justiciaadhatoda*, blood glucose level, serum insulin, alloxan

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## ON-LINE PRECONCENTRATION AND DETERMINATION OF Cr (VI) BY *PSEDOMONAS AERUGINOSA* STATIC BIOMASS IMMOBILIZED ON EGG SHELLS

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### ABSTRACT

*Pseudomonas aeruginosa* biomass was immobilized on powdered egg shells, an effective and low cost solid support, for the determination of Cr (VI) in a flow through system. For the purpose a minicolumn filled with the immobilized microbial biomass was incorporated in a flow system for the preconcentration of Cr (VI) and its determination at ultratrace levels in standard solutions. The analytical methodology involved a double channel flow system holding the minicolumn and two injection valves placed at parallel positions. The procedure was based on preconcentration, washing and elution steps. Eluted Cr (VI) was mixed with a chromogenic reagent (1, 5 diphenyl carbazide) at mixing point followed by the measurement of absorbance of red colour complex at 545 nm. The effects of several variables on analytical signal were studied. Under optimal conditions calibration curve was found linear in the range of 0.1-100  $\mu\text{g L}^{-1}$  with detection limit of 0.05  $\mu\text{g L}^{-1}$  and relative standard deviation ( $n=5$  10  $\mu\text{g L}^{-1}$ ) of 1.8. The sampling frequency was 10 samples  $\text{h}^{-1}$  with preconcentration time of 5mins. Furthermore, after washing with buffer (0.1 M, pH 3.0) activity of the biosorbant was regenerated and remained comparable for more than 200 cycles.

**Keywords:** *Pseudomonas aeruginosa*, immobilization, flow injection system, Cr (IV)

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## COMPARATIVE IN VITRO EFFICACY OF DORIPENEM AND IMIPENEM AGAINST MULTI-DRUG RESISTANT PSEUDOMONAS AERUGINOSA

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### ABSTRACT

**Objective:** To compare in vitro efficacy of doripenem and imipenem against multi-drug resistant (MDR) *Pseudomonas aeruginosa* from various clinical specimens.

**Study Design:** Descriptive study

**Methodology:** A total 84 MDR *Pseudomonas aeruginosa* isolates from various clinical samples were included in the study. The susceptibility of *Pseudomonas aeruginosa* was performed by E-test strip and agar dilution methods. The results were interpreted as recommended by Clinical Laboratory Standard Institute guidelines (CLSI).

**Results:** The maximum number of *Pseudomonas aeruginosa* were isolated from pure pus and pus swabs (92%) followed by urine (90%). In vitro efficacy of doripenem was found to be more effective as compared to imipenem against MDR *Pseudomonas aeruginosa* with both E-test strip and agar dilution methods. Overall, a p value of 0.014 and 0.037 was observed when susceptibility patterns of doripenem and imipenem were evaluated with E-test strip and agar dilution methods.

**Conclusion:** Doripenem seems to be an invaluable option for treatment against MDR *Pseudomonas aeruginosa* in our set up especially in cases where carbapenem use is indicated.

**Keywords:** Agar dilution. Doripenem. Multi-drug resistant (MDR). *Pseudomonas aeruginosa*.

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## SCREENING PHYTOTOXIC POTENTIAL OF ORGANIC EXTRACTS FROM *PLEUROTUS OSTREATUS* AGAINST *LEMNA MINOR* (DUCK WEED)

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### ABSTRACT

This study was carried out to examine the herbicidal potential of the organic extract from *Pleurotus ostreatus* commonly known as Oyster mushroom. The Fruiting body, Mycelia and fermentation filtrate of *P. ostreatus* was extracted with ethyl acetate and this extract was screened for toxicity against *Lemna minor* (duck weed). Maximum mortality of duck weed was observed when 300  $\mu\text{g mL}^{-1}$  of mycelial extract was tested. Fruiting bodies of *Pleurotus ostreatus* culture were grown on agar plate culture on potato dextrose agar (PDA) and then inoculated on potato dextrose broth (PDB) medium for high production of mycelial extract. The crude extracts of these three cultures of *P. ostreatus* were tested against the *Lemna minor* as an indication of the potential of the extract possessing phytotoxic activity and prospect of developing future candidate(s) as herbicides. Different concentrations i.e. 100, 200 and 300  $\mu\text{g mL}^{-1}$  of each extract were prepared in ethyl acetate as test solutions and the toxicity of each concentration was compared with negative (blank) and positive control (Atrazine) for reference. Total 20 *Lemna minor* plants were raised on E-medium and test solutions were applied in triplicate to determine percent mortality. The results of the study revealed that the mycelia extract exhibited maximum toxicity towards the weed (70 % mortality) at 300  $\mu\text{g mL}^{-1}$  after 72 hours of exposure whereas the maximum  $\text{FI}_{50}$  was calculated (14.05  $\mu\text{g mL}^{-1}$ ) for extracts from fruiting bodies of *P. ostreatus*. On average the extract from fermentation filtrate showed least mortality with high  $\text{FI}_{50}$  Value (120.97  $\mu\text{g mL}^{-1}$ ). The mortality trend showed that the effect of organic extracts from different portions of *P. ostreatus* was both time and concentration dependent. It is opined that the extracts showing significant phytotoxic activity may contain useful natural products and could be utilized in weed management.

**Keywords:** *Pleurotus ostreatus*, Phytotoxicity, *Lemna minor*, duck weeds

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## PIXE ANALYSIS OF GROUNDNUT GENOTYPES FOR TOXIC ELEMENTS

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### ABSTRACT

Concentrations of Elements in nine selected groundnut genotypes have been analyzed by means of a PIXE (Particle Induced X-ray Emission) practice with an interior standard method to search traces of remaining agricultural chemicals or toxic elements in selected groundnut genotypes. We arranged the samples by separating seed of the groundnut into two cotyledons (seed leaves). The cotyledon recorded many elements but recorded none of the toxic element such that Pb, Hg, as and Cd. The peanut seeds used in the present amount are concerned.

**Keywords:** PIXE, Elements

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## IMMUNOMODULATORY, ANTIBACTERIAL AND TOXICITY POTENTIAL OF *ACONITUM NAPELLUS*

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### ABSTRACT

The present study was designed to evaluate the Immunomodulatory, antimicrobial, toxicity and beneficial effects of *A. napellus*. Various concentrations of leaf extract were tested for the sake of immunostimulatory activity and at 15.60mg/ml, maximum phagocytic concentration was achieved i.e 79 that generated potent 88.14% immunostimulation. The antibacterial activity of extract against fourteen gram positive and twenty two gram negative bacteria were explored in this study. Among them the best activity was achieved against *Acinetobacter baumannii*.e 21mm. Moreover, MIC against best tested strains were also done via Microtitre plate and quite promising results were obtained. Lethal toxic effects started at the dose of 300mg/kg and the LD<sub>50</sub> value of drug is calculated as 300 mg/kg. Whereas therapeutic dose ranges from 1-5mg/kg. The result of the oral toxicity study revealed death occurs at the dose of 500 and 1000 mg/kg body weight. However, the mice showed signs of fits up to the dose of 300 mg/kg. At the dose of 10 and below 10 mg/kg, there is no sign of toxic effect observed in behaviour. The gross organ toxicity at non lethal dose not show the sign of toxicity. The dose of 10mg/kg showed significant potential of analgesic activity and anti-inflammatory activity as it significantly reduces the number of writhes and edema at  $p < 0.05$ . Different neuropharmacological test were also performed. In these tests mice showed decrease in motor activity and exploratory behaviour at the dose of 10 mg/kg. In traction test mice showed similar behaviour like Diazepam and at 10 mg/kg the traction time of mice were increased and produced significant muscle relaxant effect. FTIR and HPLC analysis and antioxidant studies were also carried out.

**Keywords:** *Aconitum napellus*, Immunostimulatory, Antimicrobial, Toxicity

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## PATHOGENESIS OF CHICKPEA WILT DUE TO *FUSARIUM OXYSPORIUM*

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### Abstract

Pathogenesis study of *Fusarium oxysporum* f. sp. *ciceri* was made to correlate epidemiological factors with chickpea wilt. It was observed that seedling mortality increased with an increase in inoculum load, at the inoculum load of 3 g, seedling mortality was minimum and at 20 g it was maximum. Similarly, when *Fusarium* wilt was observed in different types of soils, it was found that the disease developed severe in sandy soil and least in clay soil. Soil moisture also played an important role in disease development. It was observed that seedling mortality was maximum (91%) when the soil moisture level was low (16.2%) and minimum (0.00%) when it was high (35.67%). Studies of disease development on sap extract of leaves and roots showed maximum growth of *F. oxysporum* f. sp. *ciceri* on root sap than leaves. Colony growth of *F. oxysporum* f. sp. *ciceri* was 3.2 to 5.9 cm at leaves sap and was 3 to 9 cm on root sap.

### Keywords:

\*Correspondence author email:

## TRANSGEIC APPROACH TO PRODUCE LOW VISCOSITY BRASSICA PLANT OIL TO BE USED AS BIODIESEL

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Energy crises along with environmental concerns are driving researchers to develop viable alternative fuels from renewable resources. The use of *Brassica juncea* oil as an alternative fuel suffers from problems such as high viscosity, low volatility and poor cold temperature properties. The seed of *Euonymus alatus* produces unusual triacylglycerol (TAGs) called acetyl triacylglycerol (acTAGs) where the sn-3 position is esterified with acetate instead of a long chain fatty acid. The enzyme *Euonymus alatus* diacylglycerol acetyltransferase (EaDacT) present in these plants is an acetyltransferase that catalyzes the transfer of an acetyl group from acetyl-CoA to diacylglycerol (DAG) to produce acTAG. In order to reduce the viscosity of *Brassica juncea* oil by synthesizing acTAG, we have developed an efficient and simple agrobacterium mediated floral depth transformation method to generate transgenic *Brassica juncea* plants. A binary vector containing the EaDacT gene under the transcriptional control of a glycinin promoter and with a basta selection marker was transformed into *Agrobacterium tumefaciens* strain GV-3101 through electroporation and ultimately to *B. juncea* through vacuum infiltration method. Basta is a herbicide which is used as a selection marker to allow us to conveniently screen very young transgenic plants from a large number of untransformed plants. The basta resistant putative transgenic plants were further confirmed by PCR.

**Keywords:** Biofuel; Acetyl TAGs; Biodiesel; *Brassica juncea*; Low viscous oil; Transgenic lines

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## ROLE OF CITRULLUS COLOCYNTHIS PLANT EXTRACT IN THE ALLEVIATION OF METABOLIC SYNDROME

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### ABSTRACT

Metabolic syndrome (MetS) is defined as collection of risk factors that augment the probability of acquiring diabetes mellitus and cardiovascular diseases. These risk factors primarily include central obesity along with any two of the following four factors; raised triglyceride (TG) level, reduced high density lipoprotein (HDL) cholesterol, raised fasting plasma glucose and raised blood pressure. Worldwide prevalence of metabolic syndrome in most countries is between 20% and 30% of the adult population. In Pakistani population the prevalence of metabolic syndrome is 18% to 46% according to different definitions. Primary intervention for MetS is management of underlying risk factors, mainly obesity, as it is most strongly associated with MetS. Disruption of adipogenic factors such as leptin and adiponectin occurs in obese individuals, with over expression of transcription factor of adipogenesis; peroxisome proliferator-activated receptor *gamma* (*PPAR-γ*). Drug therapy is, therefore, required for the management of obesity for restoration of adipogenic factors. In the present investigation, Citrullus colocynthis extract has been studied for reduction of MetS symptoms in cafeteria diet induced obese mice and their effects are being evaluated for restoration of adipogenic factors. The role of natural products on adipocyte function and growth will also be evaluated at gene and protein level. Our research may result in the discovery of a novel anti- obesity natural extract and ultimately provide a base for reducing the risk of diabetes mellitus and cardiovascular diseases associated with MetS in the general population.

**Keywords:** Metabolic syndrome; obesity; Citrullus Colocynthis; adipogenesis; leptin; adiponectin

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## HAPLOTYPE DIVERSITY ANALYSIS IN COLD TOLERANT RICE (ORYZA SATIVA L.)

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### ABSTRACT

Low temperature stress is one of the major abiotic stresses affecting plant growth and development and the rice growing areas of northern districts of Bangladesh are mostly affected by this stress, particularly at seedling stage. This problem can be overcome by developing high yielding cold tolerant rice varieties. Molecular analysis of genes or QTLs underlying cold tolerance is the best approach along with phenotypic cold tolerance screening in this regards. In this study twenty eight rice genotypes including 22 cold tolerant germplasms from home and abroad were evaluated under artificial chilling temperature for cold tolerance at seedling stage to investigate the level of cold tolerance and genetic similarity among them. Evaluation of germplasms under artificial cold temperature was done using three characters namely, leaf discoloration (LD) score, % survivability and % recovery. Higher level of leaf discoloration was observed in cold susceptible variety BR1 (8.57) followed by BRRI dhan28 and BRRI dhan29 (7.46 and 7.15). Lower level of discoloration was observed in IRGC55424 (ASOMINORT) (3.64). Two tolerant varieties, Hbj.BVI and BR18 also showed lower LD values of 4.74 and 5.67, respectively. LD Score, % survivability and % recovery among genotypes varied from 3.64% to 8.57%, 17.11% to 100% and 0.00% to 74%, respectively. UPGMA dendrogram based on LD score showed 70% similarity among the cold susceptible check varieties, tolerant donor parents (reported in literature) and the near isogenic lines of BRRI dhan29 clustering them into different clusters. Using 36 SSR primers linked to different QTLs for cold tolerance across 28 rice genotypes. UPGMA dendrogram constructed based Nei similarity indices of LD score grouped the cold tolerant donor genotypes (BR18, IR72, Dasan, Hbj.BVI, IRGC77142 (M-202, etc.) into a single cluster but the dendrogram based on SSR alleles that are linked to different cold tolerant QTLs cited in literatures grouped them in different clusters indicating differential genetic makeup of the tolerant genotypes. Furthermore, the donor parents shared 89.45% common alleles among themselves but they had up to 11.85% different alleles from the local cold tolerant check variety, Hbj.BVI. These findings indicated that Hbj.BVI has different genetic makeup up to at least 11.85% from the other donor for cold tolerance.

**Keywords:**

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## HYPOGLYCEMIC ACTIVITY OF *MONOTHECA BUXIFOLIA* AND ISOLATION OF THE ACTIVE CONSTITUENTS THROUGH BIOASSAY-DIRECTED FRACTIONATION TECHNIQUES

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### ABSTRACT

Hypoglycemic effect of *Monotheca buxifolia* was studied in vivo models in normal, glucose-hyperglycemic and streptozotocin-induced diabetic rats. The methanolic whole plant extract at high dose (500 mg/kg) exhibited significant antihyperglycemic activity. The methanolic extract also showed improvement in parameters like body weight and lipid profile. Histopathological studies reinforce the healing of pancreas, by methanolic *Monotheca buxifolia* extracts, as a possible mechanism of their antidiabetic activity. Through various bio-assay guided fractionation processes, some triterpenes were isolated by silica gel column chromatography as the main active constituents from the plant. The isolated bioactive components were elucidated on the basis of extensive spectroscopic (UV, IR, MS, <sup>1</sup>H and <sup>13</sup>C NMR) data analysis.

**Keywords:** *Monotheca buxifolia*, Hypoglycemic, Triterpenes

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## TO ELUCIDATE MALT LYMPHOMA PATHOGENESIS WITH REFERENCE TO DR4 EXPRESSION

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### ABSTRACT

Mucosa Associated Lymphoid Tissue lymphoma comprises 8% of all newly diagnosed Non-Hodgkin's lymphomas. TRAIL (tumor necrosis factor related apoptosis-inducing ligand) is involved in tumor immune surveillance and thus, a potential cancer therapy. TRAIL pathway has not been explored yet in MALT lymphoma so this study was designed to determine TRAIL receptor 1 (DR4) expression in cytogenetically normal and abnormal MALT lymphoma cases. Tissue biopsies were paraffinized followed by microtomy. Immunohistochemistry was used to determine the expression of DR4. MALT lymphoma samples were incubated with monoclonal DR4 antibody and standard protocol of immunohistochemistry was followed. Infiltrating plasma cells expressing DR4 were used as positive while tissues without primary antibody incubation were used as negative controls. The results of this study indicate that the expression of death receptor 4 was down-regulated in the evaluated MALT lymphoma samples leading to compromised TRAIL pathway signaling. DR4 was expressed in only 11/30 (36.7%) evaluable tissues of MALT lymphomas. We also correlated DR4 expression with cytogenetic status of patients and results indicated positive expression of DR4 in 47% MALT lymphoma samples having normal cytogenetic status while in patients with genetic aberrations, only (23.1%) of patients showed positive DR4 expression. We also correlated expression of DR4 with plasmacytic differentiation status and disease spread separately and results indicated no significant difference in the groups. The percentage of Ki-67 positive cells was more in DR4 negative tissues; while Ki-67 presence was less in DR4 positive patients. DR4 expression was also correlated with survival rate of patients and it was seen that the patients with positive DR4 expression represented a higher survival rate. In MALT lymphoma, DR4 expression is down-regulated which may be attributed to the lymphoid tissue of origin. Correlation of death receptor expression and cytogenetic aberrations indicate that down-regulation of death receptors expression is a required event in the pathogenesis of MALT lymphoma.

**Keywords:** MALT Lymphoma, DR4, TRAIL

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## PROFILE AND ANALYSIS OF FATTY ACID AND PROTEIN IN HUMAN MILK (KOHAT)

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### ABSTRACT

Milk is the fundamental food for humans. The best source for infants in early years of life is breast feeding and this is greatly encouraged for infants in early stage of life. Human milk is a complete diet for healthy infants. Human milk has the optimum amount of fats, carbohydrates, and proteins for infants that help in growth and development in early years of life. Fatty acid compositions of mature human milk of Kohat Khyber Pakhtun Khwa were studied by using the GC-MS. It is found that the majority of milk samples has higher percentages of saturated fatty acids which shows that mothers have high content of these saturated fatty acids in their diet. Results show that low percentage of mono unsaturated fatty acids and poly unsaturated fatty acids were present in these samples. These show that mothers' diet has not sufficient fats intake and has high intake of carbohydrates. Human milk has also low percentage of protein range between 0.8-0.94 in the given samples.

**Keywords:** Human milk, saturated fatty acid, unsaturated fatty acid protein.

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## IN-VITRO EVALUATION OF PROBIOTIC POTENTIAL AND SAFETY PROPERTIES OF INDIGENOUS *LACTOBACILLUS* ISOLATES FROM DAIRY ORIGIN

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### ABSTRACT

Probiotics are the health promoting viable microorganisms that exhibit beneficial effect on human gastrointestinal tract (GIT). Lactic acid bacteria, especially *Lactobacillus*, are the most commonly used microorganisms as probiotics because of the perception that they are desirable members of the intestinal micro flora and because these bacteria have "Generally Recognized as Safe" (GRAS) status. The *Lactobacillus* species have been considered as main group of microorganism that is believed to have probiotic characteristics. Survival and colonization in the GI tract are critical properties that confer health benefits to the host. The probiotic strain's ability to resist unfavorable physiological conditions of the gastrointestinal tract (GIT) depends on various factors like resistance to low pH, tolerance to bile secretion, resistance against lysozyme and H<sub>2</sub>O<sub>2</sub>. *In-vitro* screening of thirty three indigenous *Lactobacillus* isolates from the dairy origin to evaluate their functional and probiotic characteristics. All *Lactobacillus* isolates were able to grow at low pH 3.0 specifically PL5, PL7, PL13, PL14, PL16 and PL17. The growth inhibition of *Lactobacillus* isolates was observed with an increased concentration of bile such as 1.5% bile. However, all the *Lactobacillus* isolates were resistant at 800 µg/mL of lysozyme. Nominal growth was observed even at 80 µg/L concentration of H<sub>2</sub>O<sub>2</sub> while other *Lactobacillus* isolates were found to be sensitive. The selected *Lactobacillus* isolates showed the best probiotic potential and safety characteristics, and therefore could be used as potential probiotics in the production of microbial ecological agents and functional foods.

**Keywords:** *Lactobacillus*, *Lactobacillus* isolates, GIT, Probiotics, Probiotic Potential, Bile, Lysozyme, H<sub>2</sub>O<sub>2</sub>, pH, GRAS.

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## mRNA SECONDARY STRUCTURE ENGINEERING OF THERMOBIFIDA FUSCA ENDOGLUCANASE (CEL6A) FOR ENHANCED EXPRESSION IN ESCHERICHIA COLI

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### ABSTRACT

The sequence and structure of mRNA plays an important role in solubility and expression of the translated protein. To divulge the role of mRNA secondary structure and its thermodynamics in the expression level of the recombinant endoglucanase in *Escherichia coli*, 5'-end of the mRNA was thermodynamically optimized. Molecular engineering was done by introducing two silent synonymous mutations at positions +5 (UCU with UCC) and +7 (UUC with UUU) of the 5'-end of mRNA to relieve hybridization with ribosomal binding site. Two variants of glycoside hydrolase family six endoglucanase, wild type (cel6A.wt) and mutant (cel6A.mut) from *Thermobifida fusca* were expressed and characterized in *E. coli* using T7 promoter-based expression vector; pET22b(+). Enhanced expression level of engineered construct (Cel6A.mut) with  $\Delta G = -2.7$  kcal mol<sup>-1</sup> was observed. It showed up to ~45 % higher expression as compared to the wild type construct (Cel6A.wt) having  $\Delta G = -7.8$  kcal mol<sup>-1</sup> and ~25 % expression to the total cell proteins. Heterologous protein was purified by heating the recombinant *E. coli* BL21 (DE3) Codon Plus at 60 °C. The optimum pH for enzyme activity was six and optimum temperature was 60 °C. Maximum activity was observed 4.5 Umg<sup>-1</sup> on CMC. Hydrolytic activity was also observed on insoluble substrates, i.e. RAC (2.8 Umg<sup>-1</sup>), alkali treated bagass (1.7 Umg<sup>-1</sup>), filter paper (1.2 Umg<sup>-1</sup>) and BMCC (0.3 Umg<sup>-1</sup>). Metal ions affect endoglucanase activity in different ways. Only Fe<sup>2+</sup> exhibited 20.8 % stimulatory effects on enzyme activity. Enzyme activity was profoundly inhibited by Hg<sup>2+</sup> (91.8 %).

**Keywords:** Enhanced expression, endoglucanase, *Thermobifida fusca*, Synonymous mutations, Residual activity, *Escherichia coli*

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## PHARMACOLOGICAL ROLE OF *FOENICULUMVULGARE* IN CORTEX AND HIPPOCAMPUS OF LEAD INDUCED MOUSE MODEL OF OXIDATIVE STRESS

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### ABSTRACT

**Background:** *Foeniculumvulgare* possesses significant pharmacological properties that can be utilized to overcome the detrimental effects of oxidative stress. The aim of this study was the investigation of antioxidant activity of *F. vulgare* to counteract the effects of oxidative stress in cortex and hippocampus of lead induced oxidative stress in mouse model of oxidative stress.

**Methodology:** 100% and 75% extracts were given to mice mixed in feed for 21 days and brain dissections were performed on the following day. Proteomic profiling for these brain tissues was performed to investigate the alteration in protein expression in different groups of mice in comparison to control.

**Results:** The treatment group (75% ethanolic extract of *F. vulgare* + 0.1% Lead) showed down regulation in cortex and up regulation in level of protein expression. It was also seen that 75% ethanolic extract of *F. vulgare* was better at overcoming oxidative stress as compared to 100% ethanolic extract.

**Conclusion:** The study establishes a preliminary basis of potent antioxidant activity of *F. vulgare* in treatment of oxidative stress in brain tissues as a consequence of lead exposure.

**Keywords:**

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## GENETIC VARIATION FOR FLOWERING TIME AND HEIGHT REDUCING GENES AND AGRONOMIC TRAITS IN WESTERN CANADIAN SPRING WHEAT

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### ABSTRACT

Genetic variation is a prerequisite in global wheat improvement programs. High grain yield and protein content and early maturity are some of the major goals in global as well as Canadian wheat breeding programs. In current study, we investigated genetic diversity for earliness related and plant height reducing genes in 82 Canadian western spring wheat cultivars through eight diagnostic DNA markers. Allelic variation was observed at the *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Ppd-D1* loci but not for *Ppd-A1* and *Ppd-B1* loci in the studied wheat genotypes. Spring type allele of *Vrn-A1* was present in 94% cultivars, whereas only two cultivars carried spring allele of *Vrn-D1*. Among the four earliness related genes, the most frequent combination was *Vrn-A1a*, *Vrn-B1*, *vrn-D1* and *Ppd-D1b*, which was found in 32 Canadian spring wheat cultivars. As for the *Rht* genes, eight cultivars had *Rht-B1b* and 13 cultivars had *Rht-D1b*. Besides the known *Rht-1* genes, quantitative trait loci may also have a role in the variation in grain yield and plant height in the studied genotypes. Days to heading and maturity showed positive genetic and phenotypic correlation ( $r_g=0.65$ ;  $r_p=0.44$ ), and were also positively correlated with yield and kernel weight but negatively correlated with test weight and protein content. Plant height was positively correlated with protein content ( $r_g=0.53$ ;  $r_p=0.42$ ), but negatively correlated with grain yield ( $r_g=0.47$ ;  $r_p=0.14$ ). Grain yield and protein content showed negative genetic correlation ( $r_g=0.57$ ). Among the sixty cultivars from Canada Western Red Spring Class released over 100 years, the newest cultivars yielded 23% more grain and had 15% more grain protein than the oldest cultivars. Breeding efforts in western Canada have resulted in the incorporation of vernalization and photoperiod insensitive and height reducing genes in modern varieties.

**Keywords:** Wheat, Genetic Variation, Vernalization, Photoperiod, *Rht* genes

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## PATHOGENIC POTENTIAL OF FAV-4 FIELD STRAIN ASSOCIATED WITH HPS FROM PAKISTAN

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### ABSTRACT

Hydropericardium syndrome is an acute viral disease of broilers, breeders and layer birds caused by fowl adenovirus serotype-4 resulting in heavy economical losses in poultry industry. Present research was conducted to study comparative pathogenicity of HPS strain recovered from Multan field outbreak. 50% W/V viral suspension was aseptically prepared from HPS infected liver and different concentrations (0.6, 0.5, 0.4, 0.3, 0.2, 0.1,  $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$  ml) were injected to different groups of birds named as A, B, C, D, E, F, G, H, and I. The observed mortality rate in group A was 50%, B with 25%, C with 25%, and other with 0%. Postmortem observation showed clear signs and symptoms of HPS having straw colored fluid filled in pericardial sac and enlarged liver. Present study concluded that by increasing the concentration of virus, pathogenicity increases and 0.6 ml dose was considered as lethal dose 50 (LD<sub>50</sub>) of FAV-4 field isolate.

**Keywords:** Hydropericardium syndrome, Fowl Adenovirus Serotype 4, Lethal Dose-50, Weight/Volume

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