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BOOK OF ABSTRACTS

1st International Conference on Recent Advances in Environmental Science and Biotechnology

(RAESB-2024)

September 18-19, 2024

Organized by

**Department of Environmental Sciences &
Department of Biotechnology, Shaheed Benazir
Bhutto University, Sheringal, Dir (U), KP,
Pakistan**

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September 18-19, 2024
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ORAL PRESENTATIONS...

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Lecturer, Biotechnology Deptt., SBBU, Sheringal

MESSAGE

It is my proud privilege and honor to welcome you all to the 1st International Conference on “Recent Advances in Environmental Science and Biotechnology (RAESB-2024). The objective of this conference is to provide a unique opportunity for Researchers, Scientists, and Professionals from around the world to come together and share their innovative ideas, experiences, and cutting-edge research in RAESB-2024 being held at Shaheed BB University Sheringal Dir Upper, Khyber Pakhtunkhwa, Pakistan. The RAESB-2024 will be an important network opportunity for faculty members, researchers, and students. RAESB-2024 is the result of the endless efforts of the organizing committee. The committee worked hard to organize the conference including local arrangements, inviting papers, and evaluation/screening of submitted papers. Therefore, I congratulate them for hosting such a wonderful event. I am also thankful to the reviewers for sparing valuable time for the review process and selection of top-quality papers for publication in the conference. I am hopeful that the contribution of the local and foreign scholars and researchers at this forum will strengthen the academia, industry, and R&D organizations’ future and ongoing endeavours. I am confident that this collection of quality papers and invited talks will provide you an insight into recent trends and research directions in emerging Environmental Science and Biotechnology areas.

May this conference be a complete success for everyone

Prof. Dr. Muhammad Shahab
Vice Chancellor SBBU/Patron in Chief
RAESB-2024

PREFACE

The 1st International Conference on “Recent Advances in Environmental Science and Biotechnology (RAESB-2024) will be held on 18-19 September 2024 at Shaheed Benazir Bhutto University Sheringal Dir Upper, Khyber Pakhtunkhwa, Pakistan. It has been co-organized by the Department of Environmental Science and Department of Biotechnology, Shaheed Benazir Bhutto University Sheringal Dir Upper, Khyber Pakhtunkhwa, Pakistan. Environmental Science and Sustainable Development Goals and Biotechnology and Sustainable Development Goals were selected as the themes of the conference. The conference has attracted 150 abstracts to be presented in the conference with more than 100 participants with different affiliations including researchers, academicians, and nascent students. From these abstracts, 100 abstracts are then shortlisted to be published in this proceeding. The papers presented here are organized into ten sub-themes namely: (i) Environmental Science and (ii) Biotechnology. The abstracts offered in this proceeding are expected to stimulate new perceptions in talking to promote science and technology culture in Pakistan. The main objective of the conference is to provide a dedicated platform for researchers, young inspired scientists, and academicians to share the knowledge that is still yet to be revealed in the field of environmental science and biotechnology at this 2-day event scheduled for September 18-19, 2024. The conference will comprise keynote addresses, oral presentations, and poster presentations. The event will bring together a unique mix of experts, researchers, graduate and undergraduate students, as well as decision-makers both from academia and industry across the country to exchange their knowledge, expertise, and research innovations to build an excellent “Environmental Science and Biotechnology” conference. We hope that you will find this 1st International Conference on “Recent Advances in Environmental Science and Biotechnology (RAESB-2024) perceptively inspiring and that the RAESB-2024 is a prestigious event organized with a motivation to provide an international platform for scientists and scholars around the world to share their research findings in the various research fields.

Dr. Allah Ditta
Conference Chair

Dr. Muhammad Asif Nawaz
Conference Co-Chair

ACKNOWLEDGMENT

It is our pleasure to welcome you to the 1st International Conference on "Recent Advances in Environmental Science and Biotechnology (RAESB-2024). We thank the authors for giving the content of the program in the form of posters and oral presentations. We are also grateful to the keynote speakers from academia, and research centers. These valuable speeches will guide us to a superior appreciation of the fields of Environmental Science and Biotechnology. We also thank our sponsors including the Higher Education Commission (HEC), Islamabad, Pakistan Science Foundation (PSF), Pakistan Scientific and Technological Information Center (PASTIC), Pakistan Academy of Sciences (PAS), and Shaheed Benazir Bhutto University Sheringal Dir Upper, Khyber Pakhtunkhwa, Pakistan, without their support, it would not be possible to hold this international conference. We are thankful to all organizers, who worked hard day and night to make this event successful.

Dr. Allah Ditta
Conference Chair

Dr. Muhammad Asif Nawaz
Conference Co-Chair

ABOUT PASTIC

Pakistan Scientific & Technological Information Centre (PASTIC) is a subsidiary organization of Pakistan Science Foundation (PSF), under the umbrella of Ministry of Science and Technology (MoST). PASTIC is a specialized premier organization in the field of S&T information handling and dissemination responsible for catering to information needs of R&D and industrial community across the country. The PASTIC National Centre is housed at Quaid-e-Azam University Campus, Islamabad having a network of 6 Sub-Centres at Karachi, Lahore, Peshawar, Quetta, Faisalabad and Muzaffarabad.

To begin with PASTIC supported research community across the country when S&T research infrastructure in Pakistan was at a nascent stage and provided facilities including supply of scientific and technical documents, abstracts and indexes, bibliographies, translations, patent information and patent indexes, science reference library service, technological information transfer service, dissemination of computer-based information services, reprographic and publication services.

For further details visit: www.pastic.gov.pk

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- Development of National Scientific & Technological Information (STI) resources (databases)
- Dissemination of Scientific & Technological Information through contemporary reference information tools
- Collaboration & Cooperation with institutional libraries/repositories for resource sharing
- Promotion of R&D based industrial development
- Printing of S&T/R&D Publications
- Capacity/skill development of researchers, information professionals, innovators & entrepreneurs
- Development of collaborations with national and international information networks

PASTIC Functions

S&T Publications

- *Technology Roundup:* Publish bi-monthly bulletin by repackaging of latest global Trade and Technology information.

- *Abstract Books of Conferences:* PASTIC supports publication/printing of Abstract Books of Conferences organized by various S&T universities (on request).

PASTIC Online databases

- *Pakistan Science Abstracts (PSA):* Abstracts of research published in Pakistani S&T Journals & Conference Proceedings etc.
- *National Digital Archive (NDA):* Full text digital repository of National Journals
- *PakCat:* Union Online Public Access Catalogue (OPAC) of books available in Scientific & Technological Libraries of Pakistan
- Digital Repository of Indigenous S&T literature
- *Directory of Scientific Periodicals of Pakistan*: An index of scientific periodicals (e.g., Journals, Magazines etc.) published in Pakistan.
- Database of R&D Projects executed in Pakistan
- Database of Books published by Pakistani authors
- National Scientists Directory (NSD)
- Industry related databases (e.g., Industries, Industrial challenges etc.)

Promotion of Commercializable Technologies & Industrial Products

Organize STEM and IT Expo for promotion of local R&D, SMEs, technologies/products/services, as well as empowering youth and general public on new and faster ways of delivering and accessing information.

National Science Reference Library Facility

A state-of-the-art Traditional Library facilitating the researcher through following services: Reference & Referral Services; Reader Service; Internet Service, Journal Listings; Photocopying & Scanning Services.

Skill Development/Capacity Building

Organize Seminars/Workshops /Trainings/ for capacity building of:

- Young Researchers on Data analysis, Reference Management etc.
- Women Entrepreneurs on E-marketing & E-business skills
- Library Professionals on Library automation & digitization
- Journal Publisher/Editors on E-Journal management & publishing
- Researchers and Innovators on Intellectual Property Rights, Media Information Literacy

CONFERENCE PROGRAM

Venue: Main Library, SBBU, Sheringal, Dir (U), KP Pakistan

Time	**Day-I** **September 18, 2024 (Main Library)**	
08:00 – 09:00	**Registration**	
Inauguration ceremony		
09:00 – 09:05	Recitation of the Holy Quran	
09:05 – 09:10	Conference Overview by Conference Chair, Dr. Allah Ditta	
09:10 – 09:15	Welcome Speech by Conf. Co-Chair, Dr. M. Asif Nawaz	
09:15 – 09:25	Opening Remarks, by VC, Prof. Dr. M.Shahab	
09:25 – 09:35	Opening Remarks by Pro-VC, Dr. Syed Abdul Khaliq Jan	
09:35 – 09:45	Address by Prof. Dr. M. Akram Shaikh (DG, PASTIC)	
09:45 – 10:15	Keynote Address 1	
10:15 – 10:30	**Tea Break**	
	Technical Session (Hall I)	
10:30 – 11:00	Keynote Address 2	Paper 1
11:00 – 11:30	Keynote Address 3	Paper 2
11:30 – 12:00	Keynote Address 4	Paper 3
12:00 – 12:30	Keynote Address 5	Paper 4
12:30 – 13:00	Keynote Address 6	Paper 5
13:00 – 14:00	**Lunch and Prayer Break**	
14:00 – 14:30	Keynote Address 7	Paper 6
14:30 – 15:00	Keynote Address 8	Paper 7
15:00 – 15:30	Keynote Address 9	Paper 8
15:30 – 16:00	Keynote Address 10	Paper 9
16:00 – 16:30	Keynote Address 11	Paper 10
Time	**Day-II** **September 19, 2024 (Main Library)**	
08:00 – 09:00	**Registration**	
	Technical Session (Hall I)	
09:00 – 09:15	Keynote Address 13	Paper 12
09:15 – 09:30	Keynote Address 14	Paper 13
09:30 – 09:45	Keynote Address 15	Paper 14
09:45 – 10:00	Keynote Address 16	Paper 15
10:00 – 10:30	**Morning tea**	
10:30 – 11:00	Keynote Address 17	Paper 16
11:00 – 11:30	Keynote Address 18	Paper 17
11:30 – 12:00	Keynote Address 19	Paper 18
12:00 – 12:30	Keynote Address 20	Paper 19
13:00 – 14:00	**Award and Closing Ceremony** **(Venue: Main Library Hall)**	
14:00 – 15:00	**Farewell Lunch and prayer break**	

NATIONAL INVITED SPEAKERS

Azeem Khalid
Department of Environmental Sciences, PMAS University, Rawalpindi

Sardar Khan
Department of Environmental Science, University of Peshawar, Pakistan; Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

Muhammad Ibrahim
Department of Environmental Sciences, GC University Faisalabad;

Afsheen Aman
Dr. A. Q. Khan Institute of Biotechnology & Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan

Ibrar Khan
Centre of Biotechnology and Microbiology, University of Peshawar, KP

Sohail Yousaf
Department of Environmental Sciences, QAU, Islamabad, Pakistan

Muhammad Arshad
Institute of Environmental Sciences and Engineering (IESE), School of Civil and Environmental Engineering (SCEE), NUST Islamabad, Pakistan

Iqbal Munir
IBGE, The University of Agriculture Peshawar-Pakistan

Ayaz Ahmad
Department of Biotechnology, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan

Sabir Hussain
Department of Environmental Sciences, GC University, Faisalabad

Iftikhar Ahmad
Department of Environmental Sciences, COMSATS, Vehari Campus

Muhammad Imtiaz
Soil and Environmental Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan

Muhammad Bilal

Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad Campus, Abbottabad, KP, Pakistan

Raham Sher Khan

Department of Biotechnology, Abdul Wali Khan University Mardan

Ghulam Hassan Abbasi

Institute of Agro-Industry and Environment, The Islamia University of Bahawalpur, 63100, Pakistan

M. Kamran Azim

Faculty of Life Sciences, Mohammad Ali Jinnah University, Karachi

Sarzamin Khan

Faculty of Animal Husbandry & Veterinary Science, The University of Agriculture Peshawar, Pakistan

INTERNATIONAL INVITED SPEAKERS

Sajid Mehmood

College of Ecology, Hainan University, Haikou City, 570100, P.R China

Qaisar Mahmood (TI)

Department of Biology, College of Science, University of Bahrain, Sakhir 32038, Bahrain

Yuan Yuan

School of Modern Industry for Selenium Science and Engineering, Wuhan Polytechnic University, China

Ghulam Murtaza

School of Agriculture, Yunnan University, Kunming, Yunnan, China

Urszula Guzik

University of Silesia in Katowice, Institute of Biology, Biotechnology and Environmental Protection, Poland

Engr. Leila Mercado Borbon-Laya

Senior Manager, Prime Integrated Waste Solutions Inc.; Former Vice President for Technology, Watermatic Philippines Corporation

Mohd Fadzelly Abu Bakar

Faculty of Applied Sciences and Technology, UTHM- Pagoh Campus, Hab Pendidikan Tinggi Pagoh, KM 1, Jalan Panchor, 84600 Panchor, Johor, Malaysia

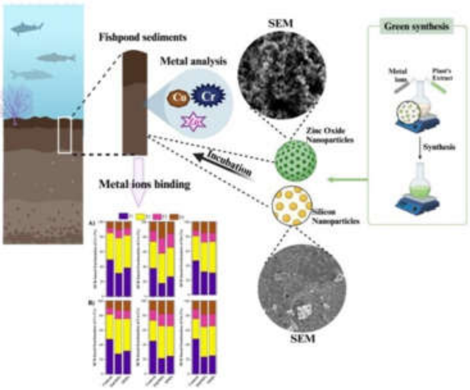
ABSTRACTS

Study on application of green synthesized ZnO and Si nanoparticles in enhancing aquaculture sediment quality

Yaoqiang Zhu[1,2#], Weidong Li[1,2#], Waqas Ahmed[1,2,] Mohsin Mahmood[1,2],
Muhammad Rizwan[3], Jochen Bundschuh[4], Muhammad Akmal[5], Sajid Mehmood[1,2*]
[1]College of Ecology, Hainan University, Haikou City, 570100, P.R China
[2]Center for Eco-Environment Restoration Engineering of Hainan Province, Hainan University, Haikou, 570228, China; [3]Agricultural Research Station, Office of VP for Research & Graduate Studies, Qatar University, 2713 - Doha, Qatar; [4]Faculty of Health, Engineering and Sciences, University of Southern Queensland, Toowoomba, 4350 QLD, Australia; [5]Institute of Soil and Environmental Sciences, PMAS- Arid Agriculture University Rawalpindi 46300, Pakistan
**Correspondence: drsajid@hainanu.edu.cn*

The exploration of simultaneous removal of coexisting pollutants from fishpond sediments (FPS) using nanomaterials synthesized by green method opens a novel and environmentally friendly pathway. Fishpond sediments, known for their richness in organic carbon and nutrients, hold great promise as fertilizers and soil conditioners. However, contamination of FPS with heavy metals (HMs) requires immobilization to ensure safe reutilization with reduced biohazards. This study assesses the efficacy of green-synthesized zinc oxide (ZnO) and silicon (Si) nanoparticles (NPs) derived from the aqueous plants Azzola pinnata and *Equisetum arvense* in immobilization (stabilizing) chromium (Cr), copper (Cu), and zinc (Zn), and enhancing nutrient content in FPS collected from San Jiang (SJ) and Tan Niu (TN), China. Green-synthesized nanoparticles (GSNPs), particularly GSZnONPs, notably decrease Cr, Cu, and Zn concentrations in both overlying and pore water, thereby reducing the leachability of metals from FPS compared to the control. GSZnONPs also effectively alter the acid-soluble metal fraction, reducing average concentrations of Cr by (31-28%), Cu by (18-21%), and Zn by (32-23%) in both SJ and TN sediments. Future research avenues could explore the optimization of nanoparticle concentrations, long-term effects on soil and plant health, and potential variations in different environmental conditions.

Keywords: Zinc oxide nanoparticles; Silicon nanoparticles; Fishpond sediments; Nanoparticle-sediment interactions; Heavy metal detoxification.



Graphical abstract

Green Technologies for Environmental Remediation

Qaisar Mahmood (TI)
Deptt.of Biology, College of Science, University of Bahrain, Sakhir 32038, Bahrain
Correspondence: qmahmood@uob.edu.bh

Environmental remediation has become a critical area of focus due to the increasing levels of pollution and degradation of natural ecosystems. Traditional methods of remediation often involve chemical treatments that can pose environmental risks. In response, green technologies have emerged as sustainable alternatives, offering effective solutions with minimal ecological impact. These technologies leverage natural processes and materials to detoxify and restore polluted environments, thereby aligning with the principles of sustainability and reducing the carbon footprint of remediation efforts. Among the most promising green technologies are phytoremediation, bioremediation, and the use of biochar. Phytoremediation utilizes plants to absorb, sequester, and degrade contaminants from soil and water. Bioremediation employs microorganisms to break down hazardous substances into less toxic forms, often enhancing natural degradation processes. Biochar, a carbon-rich material derived from organic waste, is used to immobilize heavy metals and organic pollutants, improving soil health while also sequestering carbon. The adoption of green technologies is driven by their ability to address a wide range of contaminants, including heavy metals, organic pollutants, and even emerging contaminants like microplastics and pharmaceuticals. Additionally, these technologies often require lower energy inputs and produce fewer secondary pollutants compared to conventional methods. However, challenges remain, including the scalability of these technologies, their effectiveness across diverse environmental conditions, and the time required for remediation. Future research is essential to overcome these challenges, particularly in optimizing the efficiency and cost-effectiveness of green remediation technologies. Advancements in biotechnology, materials science, and environmental engineering will play a crucial role in enhancing these technologies. As we move towards a more sustainable future, green technologies for environmental remediation represent a pivotal shift in how we approach the restoration of polluted environments.

Physiological and transcriptomic strategies related to photosynthesis of rice in response to vanadium stress

Yuan Yuan
School of Modern Industry for Selenium Science and Engineering, Wuhan Polytechnic University, China
Correspondence: yuany@whpu.edu.cn

Vanadium (V) is recognized as an environmental contaminant that affects plant growth and development. However, the photosynthetic response of plants to V stress at the molecular level remains unclear. In this study, we examined the photosynthetic responses of two rice varieties (Oryza sativa L.) with diverse V tolerance under various V levels (0, 10, 20, 30, and 40 mg L^{-1}) using physiological, cytological, and transcriptomic approaches. Our findings indicated that V stress significantly reduced the photosynthetic rate and chlorophyll content in both genotypes, with the V-sensitive rice displaying a more pronounced reduction. Furthermore, we observed numerous plastoglobuli, deformed stroma, and ruptured thylakoid membranes in the chloroplasts of the V-sensitive rice. In transcriptome analysis, we found 34 differentially expressed genes of 21 enzymes potentially affect chlorophyll metabolism. Among them, most of the genes involved in chlorophyll biosynthesis and phototoxic intermediate degradation were down-regulated under V stress, while chlorophyll degradation-related genes exhibited up-regulation. In addition, the different expression levels of genes encoding uroporphyrinogen III synthase (UROS), protoporphyrinogen oxidase (PPO), chlorophyllase (CLH), pheophorbide an oxygenase (PAO), and red chlorophyll catabolite reductase (RCCR) between the two rice varieties may indicate a potential tolerance mechanism in rice plants in response to V stress. Overall, this study contributes to our understanding of the molecular mechanisms underlying plant tolerance to V toxicity.

Accountancy of the Life Cycle Assessment Method to Account for Greenhouse Gas Emissions in Pakistan's Agricultural System

Ghulam Murtaza[1,2*]

[1]School of Agriculture, Yunnan University, Kunming, Yunnan 650504, China
[2]School of Ecology and Environmental Sciences, Yunnan University, Biocontrol Engineering Research Centre of Crop Diseases & Pests, Yunnan Province, Kunming 650500, PR China

[*]Correspondence: murtazabotanist@gmail.com

Agricultural systems account for around 33% of worldwide anthropogenic greenhouse gas (GHG) emissions and are a significant contributor to GHG output worldwide. Elucidating the greenhouse gas emission pattern from agriculture is crucial for developing an agricultural emission reduction mechanism and achieving Pakistan's dual-carbon goal. In this study, the greenhouse gas emissions from the agricultural system in China, which includes rice, corn, and wheat cultivation, as well as animal husbandry including sheep, pigs, camels, mules, donkeys, horses, and cows are completely quantified using the life cycle assessment method (LCA). This analysis encompasses the time frame from 2013 to 2023 and analyses the geographical dispersion, temporal patterns, and structural modifications in the GHG emissions within Pakistan's agricultural industry. The primary findings are as follows: (a) between 2013 and 2023, Pakistan experienced a steady increase in its overall agricultural greenhouse gas emissions, with an average yearly growth rate of 0.69%. Importantly, methane emissions were identified as the primary factor, exhibiting a generally variable trend. The emissions of carbon dioxide exhibited sporadic elevations with a significant yearly growth rate of 2%, indicating the fastest proliferation in this particular setting. Conversely, emissions of nitrous oxide decreased within the designated timeframe. (b) Greenhouse gas emissions

from agriculture are increasing, mostly due to the methane emissions from rice farming and carbon dioxide emissions from straw grinding. In contrast, greenhouse gas emissions resulting from animal farming decreased, driven mostly by methane gas emissions from animal enteric fermentation. The primary sources of agricultural nitrous oxide emissions are manure management and the application of nitrogen fertilizers. (c) The regional distribution of agricultural greenhouse gas emissions shows notable differences, shifting progressively towards the south zone of Pakistan. The main source of agricultural carbon dioxide is concentrated in dryland crop cultivating regions, namely the south zone of Pakistan. Conversely, the majority of agricultural nitrous oxide emissions are concentrated in South Punjab and West Punjab. The agricultural greenhouse gas emissions in Pakistan in 2023 exhibit a notable spatial clustering phenomenon, characterized by predominant hotspots in Bahawalpur, and other regions, while cold spots are mostly concentrated in the northern areas. The emission patterns of agricultural greenhouse gases are intricately linked to farming practices, levels of regional development, and national policy. Therefore, it is necessary to develop customized strategies for reducing emissions, taking into account particular types of crops, livestock categories, agricultural production operations, and regional development traits.

Keywords: Pakistan, GHG, Agricultural systems, management, strategies

Non-steroidal anti-inflammatory drugs degradation by immobilized bacteria

Urszula Guzik*

Univ. of Silesia in Katowice, Inst. Biol., Biotechnol. & Environ. Protect., Poland

Correspondence: urszula.guzik@us.edu.pl

The Biochemistry and Genetics of Microorganisms Group of the University of Silesia in Katowice has researched microbiological degradation of non-steroidal anti-inflammatory drugs for many years. These studies resulted in the collection of strains with increased degradation potential concerning selected NSAIDs. To use them in practice, protecting the strains from unfavorable and variable environmental conditions was necessary. One of the most commonly used methods of protecting microorganisms is their immobilization. The effect of the conducted research is the development of conditions for the immobilization of a strain capable of degrading naproxen on a natural carrier Luffa cylindrica characterized by a large specific surface area, low toxicity, and biodegradability. Promising results were also given by immobilization on a carrier based on xanthan gum developed by the team. The benefits of immobilizing microorganisms on these carriers include the possibility of using preparations in many degradation cycles, biodegradation of higher drug concentrations, and reducing the drug's toxicity towards immobilized microorganisms. Good survival of strains in the carrier environment was also demonstrated. The results indicate the possibility of using immobilized bacteria in the bioremediation of environments contaminated with NSAIDs, and they may also be valuable material supporting sewage treatment plants. This is all the more important

because many sewage treatment plants currently do not have efficient systems for removing these pollutants, resulting in drugs appearing in effluents.

Keywords: immobilization; Luffa; xanthan gum; microorganisms; NSAIDs
Acknowledgments: This research was funded by the National Science Centre, Poland (grant number 2018/29/B/NZ9/00424).

The Status of Water Supply, Wastewater Treatment, and Municipal Solid Waste Handling in the Philippines: How Public and Private Partnerships are paving the way for building critical infrastructures

Engr. Leila Mercado Borbon-Laya
Senior Manager, Prime Integrated Waste Solutions Inc.
Former Vice President for Technology, Watermatic Philippines Corporation
Correspondence: leilaborbon24@gmail.com

The Philippines, which is composed of 7,641 islands and has an annual average rainfall of 2,348mm still faces a water supply deficit. While the National Capital Region (Metro Manila) has nearly 95% access to safe water, to date the whole Philippines still has a 53% shortage because most of the islands rely on groundwater which is already brackish. The existing fresh water coming from lakes and rivers is not enough to provide for the 116 million population. To address the water supply deficit, especially in the highly urbanized cities located on highly developed islands, the Philippines has started shifting to seawater desalination through a Bulk Water Supply agreement. The wastewater treatment in Metro Manila has had significant progress since the year 1996 when the privatization of Utilities took place. To date, there are already more than 60 Wastewater Treatment Plants with capacities of up to 100 Million Liters per Day. Continuous building of infrastructure is still ongoing to reach 100% coverage by 2036. In terms of Municipal Solid Waste handling, the Philippines in 2023 started the industrialization of the Waste Sector, by building Modern Materials Recovery Facilities. These facilities aim to recover at least 80% of the waste into recyclables and refuse-derived fuel (RDFs), while the organics are sent to a composting facility. Waste Fuel and Waste-to-Energy are part of Phase 2 of industrializing the Waste Sector in the country. With the continuous cooperation between public and private sectors for the development of water, wastewater, and waste industries, it is possible to build critical infrastructures to meet the current and future needs in water and sanitation. Leila Mercado Borbon-Laya is a Registered Chemical Engineer by profession and has more than 18 years of experience in the industry – particularly in the engineering design, testing & commissioning, and project management of Water and Wastewater Treatment facilities. She has devised several water and wastewater treatment systems, including the treatment of Palladium wastewater which received an Excellence award at the 2010 Ibiden TPM convention in Ogaki, Japan. Leila has received President's Award from Manila Water in 2015, 2017, and 2018. She has worked on different projects across Asia and the Middle East.

Unveiling the Anti-Obesity Potential of Stingless Bee Honey: A Natural Approach to Weight Management

Mohd Fadzelly Abu Bakar

Faculty of Applied Sciences and Technology, UTHM- Pagoh Campus, Hab Pendidikan Tinggi Pagoh, KM 1, Jalan Panchor, 84600 Panchor, Johor, Malaysia

Correspondence: fadzelly@uthm.edu.my

Obesity continues to be a major global health crisis, contributing to numerous chronic conditions, including diabetes, hypertension, and cardiovascular diseases. While conventional treatments are often associated with adverse effects, there is growing interest in natural products with therapeutic potential. Stingless bee honey (SBH), produced by various species of stingless bees, has emerged as a promising functional food with unique bioactive compounds that may aid in weight management. This talk will explore the anti-obesity potential of SBH, focusing on its rich composition of phenolic acids, flavonoids, and organic acids, which contribute to its antioxidant, anti-inflammatory, and metabolism-boosting properties. We will discuss the current research findings that highlight SBH's ability to modulate key metabolic pathways, reduce lipid accumulation, and regulate appetite through hormonal balance. Additionally, the presentation will cover the challenges and considerations in the clinical application of SBH, including optimal dosing, safety, and standardization of its bioactive components. By integrating traditional knowledge and modern scientific insights, this presentation is to position stingless bee honey as a natural and effective adjunct in the prevention and management of obesity, encouraging further research into its potential health benefits.

NATIONAL INVITED SPEAKER

Climate change variables modify microbial community structure and soil enzymes involved in nitrogen and phosphorus metabolism

Azeem Khalid*

Deptt. of Environ. Sci., PMAS, Arid Agriculture University, Rawalpindi, Pakistan

Correspondence: azeem@uaar.edu.pk

Anthropogenic activities have threatened soil biodiversity which has a direct link with agricultural sustainability and ecosystem functionality. This study is aimed at investigating the changes in soil microbial biomass and enzyme activity in response to variations of primary climatic variables such as temperature and water regimes. Rhizosphere and non-rhizosphere soil samples were collected from an agricultural field of a rainfed area and transported to the laboratory for physicochemical analysis. These soil samples were preserved at 4 °C to be used for studying microbial biomass and enzymatic activities at varying soil temperatures (22, 33, and 44 °C) and water levels (30, 45, and 60% water holding capacity (WHC)). Urease and phosphatase activity showed a significant increase with increasing temperature. The urease and phosphatase values at a temperature of 44 °C after six weeks showed an increase of 19% and 67%, respectively, compared to the values before incubation. The microbial biomass carbon and the microbial biomass nitrogen decreased with increasing temperature and increasing time intervals. Under different temperatures, the diversity analyses of field samples showed maximum dominance of phylum proteobacteria with 70% relative abundance. With increasing water content, a relative decrease in the abundance of proteobacteria was observed. Water variability had non-significant effects on enzyme activity except at 30% WHC where a significant decrease (up to 14%) in urease activity was observed. The results showed a positive correlation between urease (r= 0.81) and phosphatase activity (r= 0.84) vs. an increase in temperature from 22 to 44 °C in soil. Based on these findings, it is concluded that changes in temperature and water levels modify microbial biomass, microbial community structure, and soil enzymatic activities involved in nitrogen and phosphorus metabolism.

The Role of Biochar on Arsenic Speciation in Paddy Soil, Rice and Human Health

Sardar Khan[1,2]

[1]Department of Environmental Sci., University of Peshawar, Peshawar, Pakistan
[2]Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

Correspondence: sardar.khan2008@yahoo.com; sardar@uop.edu.pk

The consumption of rice contaminated with potentially toxic elements (PTEs) is considered a major route/pathway for human exposure to PTEs. Biochar (carbon-rich material) is already recognized for different agronomic and environmental benefits. Two different experiments were conducted to investigate the role of biochar on As speciation and health risks in soil ecosystems and grown rice. In this study, the biochar materials were prepared from feedstocks such as sewage sludge biochar, rice straw, soybean, and mushroom waster using both pyrolysis and hydrolysis techniques. The pyrolyzed biochar derived from sewage sludge (SSBC) was used at 5% and 10% to minimize the PTE availability in the soil ecosystem and subsequently reduce their in rice grown in mining degraded soils. Results indicated that SSBC (10%) addition markedly ($P \leq 0.05$) reduced the daily intake of PTEs (As, Cd, Co, Cu, Mn, Pb, and Zn by 68, 42, 55, 29, 43, 38 and 22%, respectively). Biochar addition (SSBC 10%), reduced the health quotient (HQ) indices for PTEs (except for As, Cu, and Mn) up to <1. It means that SSBC minimizes the health risks associated with PTEs in rice. Furthermore, the addition of SSBC (10%) markedly ($P \leq 0.01$) reduced AsIII (72%), dimethyl arsinic acid (DMA) (74%), and AsV (62%) concentrations in rice. SSBC addition (10%), the incremental lifetime cancer (ILTR) value for iAs (AsIII+AsV) associated with the consumption of rice was significantly ($P \leq 0.01$) reduced by 66%. These findings suggest that SSBC could be a useful soil amendment to mitigating PTE exposure, through rice consumption.

Source identification and health risk assessment of atmospheric PM2.5 and PM10 – bound polycyclic aromatic hydrocarbons in Faisalabad: A future megacity in South Asia

Muhammad Ibrahim[1,2*], Afifa Aslam[1], Abid Mahmood[1,2], Umara Tabassum[1]
[1]Deptt. of Environmental Sciences; [2]Deptt. of Agricultural Sciences, GCU, Faisalabad, Pakistan
[*]Correspondence: ebrahem.m@gmail.com

The current study defines the characterization of PAH in PM2.5 and PM10 samples collected from five different sectors (residential, health, commercial, industrial, and vehicular zones) of Faisalabad, Pakistan. Regarding PAH (poly aromatic hydrocarbons) very little information is available which is a persistent environmental pollutant in Faisalabad, Pakistan. It was reviewed from previous studies that the concentration level of PAH was observed greater during winter because of climatic effects and changes in the sources of emission. The sampling sites were the busiest and heavy-traffic areas of Faisalabad. PAH's calculated through Gas Chromatography. PAH was found in the sequence of Naph (0.001) > Phe (0.001) > Fl (0.001) > Ant (0.01) > Ace (0.001) > Acy (0.001) against the chemical characterization of PM2.5 and PM10. The average concentration of PAH was found to be 110.97 and 115.24 µg/m3 for PM2.5 and 117.14 and 122.84 µg/m3 for PM10 in the summer and winter seasons, respectively. Among the determined polycyclic aromatic hydrocarbons Naph(0.001) was found to be in the highest concentration. We further evaluated the health impacts of fine and coarse particles bound PAHs in the ambient air.

Keywords: Fine particulate, coarse particulate, PAHs, health impacts, urban and suburban environments

Biopolymer-based Flocculants and Carbon Aerogels: Two Effective Green Biotechnological Approaches in Environmental Sciences

Afsheen Aman

Dr. A. Q. Khan Institute of Biotechnology & Genetic Engineering (KIBGE),
University of Karachi, Karachi-75270, Pakistan

Correspondence: afaman@uok.edu.pk; afsheen.aman@kibge.edu.pk

Biotechnology is a scientific field that holds the key to a sustainable future as it can assist in tackling issues from pollution clean-up to advanced nanotechnology. This innovative scientific domain is currently reshaping our understanding of living and non-living factors within our environment. Growing concern and awareness of climate change have made it evident that the environment and economy can no longer be considered separate entities as the recent climate crisis has drastically impacted not just the well-being of the people but also the economy of many countries. More sustainable biotechnological inventions are considered as compared to conventional methodologies. Among various biotechnological discoveries, two developments are making their way toward commercialization for the betterment of humanity. One is the utilization of flocculants for the removal of phenol-based pollutants and the second innovative approach is the use of carbon-based aerogels for the removal of atmospheric carbon dioxide. Recent flooding events have impacted human lives, and a long-term impact has been observed. When the ecological environment and the local fertile land are exposed to floodwater containing unwanted chemical pollutants, sewage material, and microorganisms, then the normal land dwellings are severely affected. Usually, the floodwater sediments bring along clay minerals which disturb the natural ecosystem. Conventional flocculation is an effective method to remove such types of suspended particles using either organic or inorganic flocculating agents while biopolymer-based flocculants could be used to promote solid-liquid separation activity in contaminated water bodies. Thus, the development of economical, safe, multifunctional biopolymer-based flocculants could offer a sustainable solution for removing hazardous contaminants as compared to synthetic flocculants. Another environmental issue is the excessive release of greenhouse gases that significantly impact the habitable zones and convert them into inhabitable regions. Increased carbon dioxide levels along with other related greenhouse gases significantly disturb the air-quality index and thus lead to various health-related issues. Biological and geological carbon sequestration naturally occurs at a very slow rate therefore, recently some advanced technological inventions have paved the way for the effective capturing of CO_2. Among different chemical processing techniques for environmental remediation, the utilization of aerogels has gained immense attention. Besides several synthetic aerogels, biological polymeric materials are now being explored for the aforementioned purpose. Carbon aerogels are a relatively new class of aerogels that are derived from organic matter which are specialized in capturing atmospheric CO_2. Hence, both these innovative approaches need to be explored at the local level.

Keywords: Aerogels, Bioflocculants, Carbon dioxide, Environment, Greenhouse gases

Extended-spectrum beta lactamases-producing *Escherichia coli* in retail chicken meat from Khyber Pakhtunkhwa, Pakistan

L. Zainab, K. Ibrar, A. Sadiq, A.K. Hamid, Midrar Ullah, R. Noor
Centre of Biotechnology and Microbiology, University of Peshawar, KP, Pakistan
Correspondence: ibrarkhan1984@uop.edu.pk

In the human diet, poultry meat is an important component due to the presence of vitamins, proteins, and minerals. But poultry meat can be contaminated by pathogenic bacteria which are responsible for food-borne infections. The current study was therefore aimed at the identification of Escherichia coli, a common pathogen causing food-borne infections, in chicken samples (n = 400) collected from three districts of Khyber Pukhtunkhwa; Peshawar, Kohat, and Nowshera. The isolates were identified by Gram staining, API strips, and through PCR (Universal Stress Protein). A total of 174 samples were positive for E. coli among the collected chicken samples. The isolates were resistant to TE, NOR and NA while were sensitive to MEM, TZP, and FOS. The results were statistically significant having a value P 0.05 in ANOVA. The isolates showed different antibiotic resistance genes; OXA-1, CTX-M15, blaTEM, QnrS, TetA, AAC, AAD, sul1, and sul2 which are the molecular explanations of their antibiotic resistance pattern. The PCR products were sequenced by Next Generation Sequencing (NGS) and the results revealed mutations in the AAC gene (M120T and R197T) and CTX-M15 (A85V, N122D, A148S and G247D). To prevent and treat pathogenic diseases, the use of antimicrobial agents in animal husbandry is of utmost concern. The overuse and misuse of antimicrobial agents have made pathogenic E. coli multi-drug resistant making it a causative agent for many diseases in human beings. The results of the current study may be helpful for physicians in the better management of the diseases caused by E. coli

Integration of Nano, Bio, and Phytoremediation for Degradation of Total Petroleum Hydrocarbons in Contaminated Soil

Sohail Yousaf, Amna Kiyani
Department of Environmental Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, 45320, Pakistan
Correspondence: syousaf@qau.edu.pk

Sustainable remediation aims to bring the concentration of pollutants to a level well below regulatory toxic limits so that it is no longer harmful to people and the environment. Advanced oxidative processes (AOPs) successfully transform toxic organic substances into more biodegradable products. One of the most used AOPs is photo-catalysis which involves radical generation, when solar light strikes the surface of a semiconductor called photo-catalyst, able to destroy

a wide range of contaminants. We hypothesize that photo-catalysis can be combined with biological treatments. The present study was designed to test the individual and combined application of TPHs degrading bacterial strain *Pseudomonas poae*, ornamental plant *Celosia argentea*, and copper sulfate nanoparticles as photocatalysts. The spiked soil was prepared by spiking agricultural soil with 3.4% (w/w) of crude oil. *Celosia argentea* seeds were sown and plants were harvested after 75 days. The results indicated that the combined treatment (N+B+P) with bacteria, plants, and nanoparticles showed the highest TPH removal of 74%. The highest bacterial counts (2.6 * 106 cells/g of soil) were noted in the N+B+P treatment. Higher fresh and dried plant biomass, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were noted in the N+B+P treatment as compared to other treatments. Significant reduction in MDA and H_2O_2 content, and improved soil enzymatic activities for catalase, dehydrogenase, and phosphatase were also observed in N+B+P, treatment. We concluded that combined nano-bio-phyto-remediation has great potential for field application for the remediation of contaminated soils as compared to chemical or biological methods alone.

Keywords: Non-volatile hydrocarbons, Nano-remediation, Ornamental plan, Bacteria, Plant growth.

Antibiotic-resistant crises: Evidence from the environmental samples and potential implications

Muhammad Arshad

Institute of Environmental Sciences and Engineering (IESE), School of Civil and Environmental Engineering (SCEE), NUST Islamabad, Pakistan

Corresponding author: marshad@iese.nust.edu.pk ; Tel: +92-51-9085-4309

Antibiotic resistance in the environment is a serious emerging concern for sustainability. Antibiotic susceptibility of the isolated strains was monitored against the five most commonly used antibiotics. The resistance was analyzed according to the Kirby – Bauer disc diffusion method. Results indicated that amongst all tested antibiotics, ampicillin, and levofloxacin resistance had the highest and lowest frequency respectively. Out of 109 strains of bacteria isolated, 91.74% were resistant to Ampicillin and 83.48% to Amoxicillin, 66.97% to Ofloxacin, 21.10% to Levofloxacin and 27.52% were resistant to Ciprofloxacin. 30.29% of the strains showed resistance to more than three drugs. Maximum resistance was observed in species from the genus *Escherichia* (57.14%), *Aeromonas* (56.25%), *Acinetobacter* (41.17%), *Proteus* (13.79%), *Pseudomonas* (8.33%), *Shewanella* (25%) while *Citrobacter sp., Comamonas sp.*, *Bacillus sp., Alishewanella sp.* showed 100% resistance to all the tested antibiotics. Incidence of Ciprofloxacin resistance in *Acinetobacter* sp., *Aeromonas* sp., and *E. coli* was higher than Levofloxacin resistance, while Levofloxacin showed intermediate resistance to a large number of isolates. Overall, these results highlight the severity of the problem and that can have serious effects on health and environmental suitability in the future which will be discussed in detail.

Keywords: Antibiotics, Antibiotic resistance, Multi-drug resistance, Pharmaceutical

Mitigating Climate Change with Advanced Biofuels: Engineering *Brassica juncea* for Superior Acetyl Triacylglycerol Production"

Iqbal Munir[1*], Ijaz Naeem[1], Timothy P. Durrett[2], Hamza Iqbal[1], Maaz Iqbal[1], Umair Munir[1], Aqib Iqbal[1], Fatima Tu Zuhra[1], Afaq Ahmad Mian[1]

[1]Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar-Pakistan; [2]Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS 66506, USA

Correspondence: iqmunir@aup.edu.pk

Climate change presents critical challenges for Pakistan, impacting agriculture, water resources, and economic stability, while the nation also grapples with severe energy shortages. To tackle these intertwined issues, the shift toward renewable energy sources is essential. Recent advancements focus on enhancing biofuels derived from renewable resources to offer viable alternatives to fossil fuels. A recent study addresses the potential of *Brassica juncea* (mustard seed) oil, which, despite its promise, faces drawbacks such as high viscosity and poor cold-weather performance. To improve its effectiveness, researchers turned to *Euonymus alatus* seed oil, which contains unique acetyl triacylglycerols (*acTAGs*) known for their lower viscosity and enhanced performance. The study employed advanced genetic engineering techniques to insert the *Euonymus alatus* diacylglycerol acetyltransferase (*EaDacT*) gene into *Brassica juncea*. This enzyme facilitates the production of acTAGs, aimed at improving the biofuel's properties. Despite initial challenges, including the absence of expected acetyl TAGs, a refined approach involving the silencing of specific TAGs synthesizing enzymes and overexpression of *EaDacT* successfully yielded a more effective biofuel. This innovative solution not only addresses Pakistan's urgent energy needs but also contributes significantly to climate change mitigation by reducing greenhouse gas emissions and promoting environmental resilience.

Engineering Brassica Plants for the production of Low Viscosity Biofuel

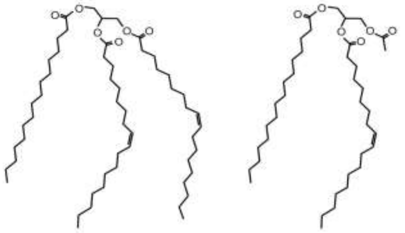
Iqbal Munir[1*], Ijaz Naeem[1], Timothy P. Durrett[2], Hamza Iqbal[1], Maaz Iqbal[1], Umair Munir[1], Fatima Tu Zuhra[1]

[1]Institute of Biotechnology and Genetic Engineering, the University of Agriculture Peshawar-Pakistan, [2]Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS 66506, USA

correspondence: iqmunir@aup.edu.pk

The development of viable alternative fuels from renewable resources is researchers' key target because of environmental concerns and energy crises. The use of *Brassica* 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 low-viscosity oil having 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 *Euonymous 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. To reduce the viscosity of *Brassica juncea* oil by synthesizing acTAG, we have developed transgenic *Brassica juncea* plants using different transformation methods. 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. The basta-resistant putative transgenic plants were developed and confirmed through Expression analysis. Biochemical analyses of the transgenic *B. juncea* seed revealed a modified fatty acids profile having no acetyl TAGs. An alternative strategy was adopted to silence genes encoding enzymes DGAT/PDAT along with overexpression of *EaDAcT*, which produced low viscous oil having acetyl TAGs. A technology was developed that could be exploited to use potential non-food crops for direct use as Biodiesel.



TAG

acTAG

Figure: acTAGs possess an sn-3 acetyl group that alters the physical and chemical properties of these molecules compared to regular TAGs.

Exploring the bio-catalytic role of *Pichia pastoris* for the cost-effective production of antidiabetic oligosaccharides from cress seed mucilage polysaccharides

Ayaz Ahmad, Imad Ullah Khan
Department of Biotechnology, Abdul Wali Khan University Mardan, KP, Pakistan
Correspondence: ahdayazb5@awkum.edu.pk

Diabetes mellitus is a heterogeneous metabolic disorder that poses significant health and economic challenges across the globe. Polysaccharides found abundantly in edible plants, hold the promise of managing diabetes by reducing blood glucose levels (BGL) and insulin resistance. However, most of these polysaccharides cannot be digested and absorbed directly by the human body. Here we report the production of antidiabetic oligosaccharides from cress seed mucilage polysaccharides using yeast fermentation. The water-soluble polysaccharides extracted from cress seed mucilage were precipitated using 75% ethanol and fermented with *Pichia pastoris* for different time intervals. The digested saccharides were fractionated through gel permeation chromatography

using a Bio-Gel P-10 column. Structural analysis of the oligosaccharide fractions revealed the presence of galacturonic acid, rhamnose, glucuronic acid, glucose, and arabinose. Oligosaccharide fractions exhibited the potential to inhibit α-amylase and α-glucosidase enzymes in a dose-dependent manner in vitro. The fraction DF73 exhibited strong inhibitory activity against α-amylase with IC_{50} values of 38.2 ± 1.12 μg/mL, compared to positive control, acarbose having an IC_{50} value of 29.18 ± 1.76 μg/mL. Similarly, DF72 and DF73 showed the highest inhibition of α-glucosidase with IC_{50} values of 9.26 ± 2.68 and 50.47 ± 5.18 μg/mL, respectively. In the in vivo assays, in streptozotocin (STZ) induced diabetic mice, these oligosaccharides significantly reduced BGL and improved lipid profiles compared to reference drug metformin. Histopathological observations of mice's liver indicated cytoprotective effects of these sugars. Taken together, our results suggest that oligosaccharides produced through microbial digestion of polysaccharides extracted from cress seeds mucilage have the potential to reduce blood glucose levels possibly through enhanced glucose uptake, inhibition of carbohydrates digesting enzymes, and regulation of the various signaling pathways.

Keywords: Diabetes mellitus; water-soluble polysaccharides; microbial digestion; Pichia pastoris; oligosaccharides; gel permeation chromatography; STZ-induced diabetic mice; blood glucose level.

Impact of soil physicochemical properties on dynamics of herbicides biodegradation and dissipation in agricultural fields: A case study of phenyl urea herbicide isoproturon

Sabir Hussain

Deptt. of Environ. Sciences, Government College University Faisalabad, Pakistan

Correspondence: sabirghani@gmail.com

Frequent use of herbicides in agricultural fields has resulted not only in the contamination of arable soils but also in the adaptation of the soil microflora to their rapid biodegradation. Microbial biodegradation plays a key purifying role by governing the natural attenuation of pesticides including herbicides in the environment. To further elucidate the factors affecting the purifying capabilities of soil microbial communities, we monitored the spatial variability of a herbicide isoproturon (IPU) mineralization/degradation activity of soil microflora in varying agricultural fields repeatedly exposed to this herbicide. The soil samples were collected over the field grid and analyzed for IPU degradation/mineralization. Several biological and physicochemical parameters including pH, relative humidity, CEC, organic carbon, total nitrogen, C/N ratio, microbial C biomass, cultural bacteria, and mineralization activity were estimated. The IPU mineralization/biodegradation was observed to be spatially variable. Pearson's coefficient of correlation indicated a weak relationship between the IPU mineralization and several physicochemical properties like pH and organic matter content. However, the geostatistical map of IPU mineralization rate (μm) showed important similarity with that of soil pH and organic matter content. Based on these findings, we conclude that pesticide

treatment is the key driver of pesticide degrading ability of the soil microflora and that soil physico-chemical parameters (pH and organic matter content) are influencing the expression of the IPU degrading potential of the soil microbiota.

Heavy Metal Stress Management in Plants through Bio-Organic Amendments

Iftikhar Ahmad[1*], Shehzad Mehmood[2], M. Atif Muneer[3], Aftab Jamal[4], Amjad Farooq[1], Umar Daraz[5], Ali Zakir[1], M. Farhan Saeed[1], Allah Ditta[6], Adil Mihoub[7]

[1]Department of Environmental Sciences; [2]Department of Biotechnology, COMSATS University Islamabad, Vehari Campus, Vehari-61100, Pakistan; [3]International Magnesium Institute, College of Resource and Environment, Fujian Agriculture and Forestry University, Fuzhou City, China; [4]Department of Soil and Environmental Sciences, Faculty of Crop Production Sciences, The University of Agriculture, Peshawar 25130, Pakistan; [5]Center for Grassland Microbiome, and College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, 730020, China; [6]Department of Environmental Sciences, SBBU, Sheringal, KP, 18000, Pakistan; [7]Center for Scientific and Technical Research on Arid Regions, Biophysical Environment Station, Touggourt 30240, Algeria

[*]Correspondence: iffises@yahoo.com; iftikharahmad@cuivehari.edu.pk

Bio-organic amendments including plant growth-promoting rhizobacteria (PGPR), compost, biogas slurry, and biochar application may have the potential to significantly improve plant growth and modulate metal toxicity and their translocations to plants. The purpose of this study was to see how inoculating PGPR with organic amendments reduces physiological stress in crop plants by regulating metal uptake and the antioxidant defense system. PGPR was equipped with aminocyclopropane-1-carboxylate deaminase (ACC deaminase), exopolysaccharide (EPS), and indole acetic acid (IAA). PGPR was applied alone and combined with BGS to crop plants in a pot experiment subjected to metal concentrations in soil. Integrated use of PGPR and organic amendments significantly ($P < 0.05$) increased plant biomass (up to 44%), relative water contents (up to 15%), photosynthetic rate (up to 20%), and stomatal conductance (up to 11%) of crop plants under normal and metal stress conditions, respectively. Possibly, metal-induced production of reactive oxygen species resulted in increased electrolyte leakage and malondialdehyde contents, which led to a reduction in growth and physiology, however, such negative effects were ameliorated by the regulation of antioxidants. Lipid peroxidation is repaired by the crop plants through enhanced activities of ascorbate peroxidase and catalase antioxidants; however, the combination of PGPR and organic amendments modulate these parameters to mitigate metal stress on crop plants.

Keywords: Antioxidants; *Bacillus* sp.; Biogas residues; Bioremediation; Heavy metals; Phytotoxicity

Climate change and agriculture productivity: can microbes be a solution?

Muhammad Imtiaz

Soil and Environmental Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan

Correspondence: m.imtiazpk92@hotmail.com

Global warming and irregular changes in temperature are serious threats to agriculture with a significant negative impact on yield. Global warming has resulted in an irregular rise in temperature posing a serious threat to agricultural production around the world. Temperature is a significant parameter in agriculture because it plays a vital role from seed germination to plant growth. A slight increase in temperature acts as stress and exerts an overall negative impact on different developmental stages including plant phenology, development, cellular activities, gene expression, anatomical features, and the functional and structural orientation of leaves, twigs, roots, and shoots. These impacts ultimately decrease the biomass; affect the reproductive process, decrease flowering, fruiting, and significant yield losses. Plants have inherent mechanisms to cope with different stressors including heat, which may vary depending on the type of plant species, duration, and degree of the heat stress. Plants initially adapt avoidance and then tolerance strategies to combat heat stress. The tolerance pathway involves ion transporters, osmoprotectants, antioxidants, and heat shock protein that help the plants to survive under heat stress. Developing heat-tolerant plants using the above-mentioned strategies requires a lot of time, expertise, and resources. On the contrary, plant growth-promoting rhizobacteria (PGPRs) are a cost-effective, time-saving, and user-friendly approach to support and enhance agricultural production under a range of environmental conditions including stresses. The microbes produce and regulate various phytohormones, enzymes, and metabolites that help plants maintain growth under heat stress. They form a biofilm, decrease abscisic acid, stimulate root development, enhance heat shock proteins, deamination of ACC enzyme, and nutrient availability especially nitrogen and phosphorous. The present talk will review the retaliation, adaptation, and tolerance to heat stress in plants using microbes and will shed light on how microbes can help to improve agriculture production under climate change scenarios.

Graphene Quantum Dots Decorated Metal and Nonmetal Doped Nanomaterials for Photocatalysis of Azo Dyes

Muhammad Bilal

Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad Campus, Abbottabad, Khyber Pakhtunkhwa, Pakistan

Correspondence: mbilal@cuiatd.edu.pk; malikmohammadbilal@gmail.com

The textile industry is recognized as a significant polluter of the freshwater environment. The high costs, toxic sludge generation, complex synthesis, and treatment systems pose limitations on the current techniques' proficiency in

decolorizing toxic dyes. Heterogeneous photocatalysis has been explored as a potential treatment solution for textile wastewater. The utilization of heterogenous photocatalysts, TiO2, is hindered by its large bandgap (3–3.2 eV) and activation under the UV region. In this research work, a series of photocatalysts (85 (number=*n*)) were synthesized in mono, binary, and ternary composite systems of TiO2. The best photocatalysts in mono (*n*=3), binary (n=3), and ternary (*n* =1) composite systems with high RB5 decolorization were optimized against reaction parameters. Different characterization techniques were used to study the physicochemical properties of the optimized photocatalysts. In a mono composite system, 0.1Fe-TiO2-300 and 20N-TiO2-300 were the best-performing photocatalysts. Isotherm studies exhibited the chemisorptive interaction between the dye and photocatalysts. In binary composite system 0.1Fe-20N-TiO2-300, GQD-0.1Fe-TiO2-300, and GQD-20N-TiO2-300 showed the best performance. GQD-0.1Fe-20N-TiO2-300 in ternary composite systems was proved to be the best choice with modified physicochemical properties like uniform size distribution, small crystallite size, reduced bandgap, and high surface area. In addition, 99.9 % decolorization was achieved with reduced reaction time (60 to 30 min) using GQD-0.1Fe-20NTiO2. The Langmuir-Hinshelwood isotherm fitness exhibited the simultaneous occurrence of adsorption and photocatalysis processes in the complete decolorization of RB5 dye. Photocatalysts reveals the RB5 decolorization order of TiO2 < GQD-TiO2 < 0.1Fe-TiO2 < 20N-TiO2 < GQD-0.1Fe-TiO2 < 0.1Fe-20N-TiO2 < GQD-20N-TiO2 < GQD-0.1Fe-20N-TiO2. Trapping study of the photocatalytic process depicted the two-way process i.e., both GQDs and doped TiO2 have an important role in harnessing the visible light and generation of electrons and holes to produce •OH. Recycling studies revealed that GQD-0.1Fe-20N-TiO2 could be used for four cycles. Moreover, GQD-0.1Fe-20N-TiO2 exhibited the best reaction energy efficiency and lower cost per 1000 L. Therefore, the GQD-0.1Fe-20N-TiO2-300 proves to be a potential photocatalyst for onward usage in binary and multi-solute systems.

Antagonistic and plant growth promoting potential of exopolysaccharides producing endophytic bacteria isolated from *Moringa oleifera*

Karim Gul and Raham Sher Khan

Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan

Correspondence: rahamsher@awkum.edu.pk

In recent times, bacterial endophytes have been used for their plant growth-promoting and inhibitory effects against phytopathogens, reflecting their remarkable contribution and significance in the industrial and agriculture sectors. Bacterial endophytes constitute an indispensable part of the plant microbiome and are involved in plant growth and developmental processes by employing different mechanisms. Exopolysaccharides (EPS), produced by these microbial consortia, have significant roles in plant-bacterial interactions and resistance to environmental stresses. In the current experiment, we isolated

forty-two strains of bacterial endophytes from the sterilized parts of the *Moringa oleifera* plant and then screened them out for the production of EPS. Out of forty-two, only three strains; KR-27, KL-4, and KS-7 produced 0.7, 0.5, and 0.9 g/l EPS, respectively. The antagonistic endophytic isolates, KR-27 and KL-4 were identified as *Serratia marcescens* and *Klebsiella aerogenes,* respectively based on 16s gene ribotyping. Among the identified isolates, KR-27 (*S. marcescens*) inhibited 59.33% and 49.62% mycelium development of *Alternaria alternata* and *Fusarium solani*, respectively. *K. aerogenes* (KL-4) showed percent growth inhibition, 41.3%, and 35.5% of *F. solani and A. alternata,* respectively. The KR-27 showed a significant inhibitory effect against *Ralstonia* and a moderate effect against *Xanthomonas*. In addition, *S. marcescens* produced phytohormones including indole acetic acid (IAA) (9.58 µg/mL), gibberellic acid (GA) (65.93 µg/mL), and salicylic acid (SA) (408.77 µg/mL). In addition, the strains also produced antioxidant compounds like siderophores, phenolics, and flavonoid contents. We also found the KR-27 isolate producing a significant amount of siderophores (~60%) and solubilized 30.46 µg/mL as compared to the KL-4 that solubilized 17.6 µg/mL of Pi. Thus it can be concluded that the endophytic isolates KR-27 and KL-4 have the potential to work two-fold by inhibiting the phytopathogens while enhancing the growth of host plants; thus can be applied in agricultural practices as potential biopesticides and biofertilizer.

Keywords: *Moringa oleifera,* endophytic bacteria, exopolysaccharides, bioactive compounds, antimicrobial activity

Water Purification through Constructed Wetlands Embedded with Graphene Oxide Zeolite Composite

Ghulam Hassan Abbasi*, Muhammad Irfan

Institute of Agro-Industry & Environ., The Islamia Univ. of Bahawalpur, Pakistan

Correspondence: ghabbasi@iub.edu.pk

Adequate water is necessary for various uses for a rapidly growing population and is one of the major challenges in recent years. However, it is becoming increasingly scarce and degraded through natural as well as anthropogenic sources. The major environmental issue facing the arid to semi-arid regions of the world is the treatment of nutrient and salt-enriched groundwater and effluent. Constructed wetland (CW) treatment systems could provide a simple and low-cost mechanism to remove these pollutants and their efficiency can be further boosted by the addition of active adsorbent material. Embedded constructed wetlands (ECW) are an efficient technology that uses the synergistic interaction between adsorbent material, plant roots, and microbes for in situ remediation of contaminated water. The mechanisms and effectiveness of ECW with salt-tolerant plants (*Typha latifolia* L. and *Spartina alterniflora* L.) were evaluated for the removal of saline and heavy metal elements at different adsorbent levels for 40 days. Two wetland setups (vegetated and unvegetated) were designed in which graphene oxide (GO), zeolite, and their composite were used as adsorbent materials. Pre and post-treatment analyses of water samples were done to determine different physicochemical parameters and heavy metal concentrations. The highest contaminant removal efficiency of >80% was obtained from the vegetated constructed wetlands (VCW) incorporated with GO-zeolite composite which was significantly higher than the unvegetated wetlands (UCW) (<60%) at $P \leq 0.05$ and the contaminant removal efficiency of both the wetland structures were decreased over time. After the allocated time for the experiment, the analysis of wetland elements of VCW showed that the maximum retention of major ions and heavy metals (26.4% and 52.6% respectively) was observed to be associated with adsorption on soil and adsorbent material. A considerable amount (7.1 and 4.7%) of contaminant ions was also uptaken by plants and 3.8% and 6.3% of salt and heavy metal ions were observed to be residual portions. In conclusion, the addition of adsorbent material can enhance the contaminant removal efficiency of CWs under saline conditions by boosting the function of both microorganisms and plants.

Keywords: Wetland, Water treatment, Graphene oxide, Zeolite, GO-zeolite composite

Metagenomics for biomedicine, public health, and environment

M. Kamran Azim

Faculty of Life Sciences, Mohammad Ali Jinnah University, Karachi, Pakistan

Correspondence: kamran.azim@jinnah.edu

Metagenomics is one of the advanced fields in biotechnology. The genomes of communities of bacteria, viruses, and fungi are studied in metagenomics. This technology is extending our comprehension of the diversity, ecology, evolution, and functioning of the microbial world. We have applied metagenomics sequencing using modern next-generation DNA technology in projects related to medicine, public health, and the environment. Metagenomic DNA was extracted and purified from the samples and subjected to NGS using Illumina technology. Detailed bioinformatics analysis of NGS data was carried out. Metagenomics research has contributed to the emergence of new applications in different areas including medicine, public health, and the environment. This lecture will cover the innovative and recent advances in methodological and applied areas of metagenomics.

ORAL PRESENTATIONS

Cloning and Functional Analysis of an ABA Receptor Gene *TaPYL2* from Bread Wheat (*Triticum aestivum* L.)

Faheeda Soomro, Jingyi Wang, Xingou Mao, Ruilian Jing
National Key Facility for Crop Gene Resources & Genetic Improvement, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China
Correspondence: jingruilian@caas.cn Tel/fax: +86(0) 1082105829

Drought stress is the major cause of the decline in wheat production. Hence finding candidate genes tolerant to drought stress in germplasm resources, and the development of functional markers will provide a new approach to enhance drought tolerance in wheat. PYR (pyrabactin resistance) /PYL (PYR-like) /RCAR (regulatory component of ABA receptor) consists of 14 family members in *Arabidopsis* that act as ABA receptors and are involved in activation of the ABA signaling pathway under abiotic stress. *TaPYL2* was isolated from wheat genome A, with a total fragment size of 3115 bp. Using diploid species and Chinese Spring nullisomic-tetrasomic lines *TaPYL2* gene showed a location on chromosome 3A. Using a DH (doubled haploid) population, the *TaPYL2* gene was mapped in the chromosome 3A region flanked by markers *Ax-111529842* (34.5 cM) and *Xwmc21* (65.4 cM). Relative expression results showed a downregulation pattern under ABA stress and expressed highly in roots in wheat seedlings. Association analysis results depict natural variation in which two SNPs were found in the promoter region at 1605 position (G/A) and the exonic region at 2547 position (C/A), it shows significant association with chlorophyll contents, spikelet per spike, yield per plant, and thousand kernel weight under different environments. SNP-wise geographic distribution in modern cultivars and landraces depicts that *TaPYL2-3A-A* was rare than *TaPYL2-3A-G* for the G/A polymorphic site while for C/A SNP *TaPYL2-3A-C* found to be rare than *TaPYL2-3A-A*. Therefore, the functional markers developed from the candidate gene will facilitate marker-assisted breeding and will help breeders develop drought-tolerant breeding lines and speed up the wheat breeding program.

Keywords: Wheat, ABA receptor, Drought stress, *TaPYL2*, dCAPS marker

Optimized peptide analogs for targeted fusion inhibition of SARS-CoV-2

Marriam Munawar, Sobia Jabeen, Waqar Siddique
Health Biotechnology Division, NIBGE, Faisalabad, Punjab, Pakistan
Correspondence: mughalmaryam502@gmail.com

The worldwide impact of viral diseases throughout history is substantial. Our research focus was SARS-CoV-2, which emerged as a substantial problem for the scientific community. Given the limitations of current therapies in terms of efficacy and availability, it is imperative to develop safe and effective inhibitors that interface between the virus and the ACE2 receptor. This study aims to synthesize peptides as ACE2 inhibitors and evaluate their efficacy in preventing SARS-CoV-2 infection via an α5β1 integrin-based mechanism. ATN-161 has shown high specificity and efficacy against SARS-CoV-2, demonstrating safety

in both in vivo and in vitro studies. Based on the reported ATN-161 structure, three peptide analogs were designed by strategically modifying the position of the proline in the amino acid sequence. The ability of proline to stabilize and enhance peptide activity was exploited to improve the analog properties. The synthesis of ATN-161 analogs was accomplished using solid-phase peptide synthesis (SPPS). The stepwise addition of protected amino acids to the growing peptide chain involved affixing, deprotection, amino acid coupling, and cleavage of the synthesized peptide from the resin. To assess their potential as anti-Covid agents, the peptides were evaluated on Vero (E6) cells. Experimental results demonstrated that one of the synthesized analogs reduced the virus-receptor interaction more effectively than ATN-161, demonstrating its role in mitigating SARS-CoV-2 infection. This is the first study reporting the potential therapeutic efficacy of synthetic analogs of ATN-161 as fusion inhibitors.

Keywords: SARS-CoV-2, ATN-161, ACE2 receptor, Antiviral, Peptide, Inhibitor

Staphylococcus aureus isolated from clinical samples and its antibiogram analysis

Mahwish Riaz, Asar Iqbal

Department of Microbiology, SBBW University Peshawar, Pakistan

Correspondence: mahwishriaz@sbbwu.edu.pk

Staphylococcus aureus is one of the most rapidly stated microbes among all pathogens that are present in hospitals. In the burn unit, *S. aureus* remains the main origin of diseases. *S. aureus* is also part of our normal flora but its infection begins when it gets entry into our body via cuts, burns, and wounds or through any other source. This study was conducted on isolating *S. aureus* from different clinically important samples of the patients and also to find an antimicrobial susceptibility pattern of the mentioned bacteria. Different Microbiological Standards and procedures including identification of colony morphology by growing bacteria on different media, gram staining, microscopy, and biochemical tests were used for the analysis of clinical specimens taken from blood, pus swabs, and urine. Out of 399 samples, 119 (29.82%) were positive isolates with a high percentage in pus swabs (44.01%), and the percentage of blood and urine was 16.32% and 11.95% respectively. According to the gender-wise distribution, the percentage in males was (53.78%) and in females was (46.21%). Antibiotic susceptibility procedures were applied in this study to find out the susceptibility as well as the resistivity of *S. aureus* to various antibiotics. *Staphylococcus aureus* showed resistance to Penicillins (100%), Ciprofloxacin (78.15%), Clindamycin (58.82%), Amikacin (56.30%), Cefoxitin (21.84%), Gentamicin (52.94%), Chloramphenicol (30.25%), Rifampicin (30.25%), Doxycycline (52.10%), and Minocycline (47.05%), while resistivity toward Vancomycin and Linezolid was 0%. Its sensitivity rate was as follows: Vancomycin (100%), Linezolid (100%), Cefoxitin (78.15%), Chloramphenicol (69.74%), Rifampicin (69.74%), Minocycline (52.94%), vi Ciprofloxacin (21.84%), Clindamycin (41.17%), Gentamicin (47.05%),

Amikacin (43.69%), Doxycycline (47.89%), while sensitivity toward Penicillins was 0%.

Keywords: Antibiotic resistance; Antimicrobial susceptibility; *Staphylococcus aureus*; Gram negative

Women's Indigenous knowledge and climate change adaptation in Southern Punjab Pakistan

Rizwan Hussain[1] and Mahwish Siraj[2]

[1]Department of Agricultural Sciences, Allama Iqbal Open University Islamabad, Pakistan; [2]Rural Development Allama Iqbal Open University Islamabad, Pakistan

Correspondence: mahwish.siraj@aiou.edu.pk

The study was conducted to explore the women's indigenous knowledge about climate change. The universe of the study was district, Multan. Administratively, the district is divided into four tehsils namely Multan Saddar, Multan City, Jalalpur Pirwala, and Shuja Abad. However, due to time and resources, tehsil Shuja Abad was selected. The data was collected from eight villages of Tehsil Shuja Abad through the proportion allocation method. A simple random sampling technique was used in this study's data collection from 200 female respondents. The findings of the study explained that the majority of the female farmers were between the age of 20-25 and were living in a joint family system. The majority (46%) of respondents were dependent on agriculture and 40% of respondents were illiterate. Furthermore, 78% of the respondents were aware of climate change. Additionally, 57% of the respondents highlighted that climate change was due to environmental degradation. They gained knowledge about climate change through traditional means. They were facing storms, floods, forest fires, and drought due to climate change. The majority of women agree that they were facing different types of challenges such as increased risk of violence, feeling insecure because of poor shelter, and increased risk of child marriage. Moreover, 40% of respondents reported that their health is also affected due to climate change patterns. The study concluded that although respondents had know-how about climate change, and they rely solely on agriculture there is a considerable literacy gap. The study further recommended that it is critical to address the educational discrepancies so, increasing educational opportunities and closing literacy gaps are critical steps in improving the livelihoods through the adoption of climate change mitigating strategies and futures of those who rely on agriculture.

Keywords: climate change, women, Multan, literacy

Identification of Polyethylene (PE) Degrading Bacteria and Assessment of Degradation Efficiency

Saba Farooq*, Saad Ghous, Uroosa Amjad

Department of Biology, Allama Iqbal Open University, Islamabad Pakistan

Correspondence: saba.farooq@aiou.edu.pk

In recent times, the introduction of plastic products has revolutionized invasive medical, industrial, and technological processes. Continuous input of plastic polymers has led to the alarming accumulation of plastic wastes in both aquatic and terrestrial ecosystems. The high stability and durability of plastic make it highly resistant to degradation in the natural environment. Inappropriate methods of disposal also make polyethylene (PE) and Polyethylene Terephthalate (PET) affect the environment, as these methods result in the release of several toxic compounds or pollutants that result in air pollution. The purpose of the current study was to identify potential plastic-degrading bacteria isolates and evaluate their degrading activity. For bacterial isolation, 10 soil samples were collected from Malote Village, District Bimbher, and Azad Jammu & Kashmir. After physicochemical analysis of soil, bacterial isolations were made. Bacterial isolates were identified by automated profile index and biochemical tests. Selected isolates were used for the degradation experiment. The experiment was set up using 2 types of polyethylene plastic, i.e. degradable and non-degradable. Plastic strips were weighed, treated, and transferred to minimal salt media (MSM) aseptically. Bacterial cultures standardized to 0.5 McFarland were inoculated in equal volumes to the test flasks and incubated on a shaking incubator for 7 days. Observations were recorded after 7 days for a total of 28 days. Out of 71 bacterial isolates, 04 were selected for the degradation experiment. Polyethylene strips weight evaluation and scanning electron microscopy (SEM) revealed that *Bacillus anthracis, Pseudomonas oryzihabitance, and Pseudomonas fluorescens* efficiently degraded polyethylene strips whereas *Enterobacter cloacae* were slow degraders. Active polyethylene-degrading bacteria could be further explored for on-site studies.

Keywords: Degradation, plastic polymers, polyethylene, degradable and non-degradable plastics

Production and characterization of α-1,4-glucosidase from *Bacillus licheniformis* KIBGE-IB4 by utilizing sweet potato peel

Muhammad Asif Nawaz[1], Sidra Pervez[2], Shah Ali Ul Qader[3]

[1]Deptt. of Biotechnology, SBBU, Sheringal, KP, Pakistan; [2]Deptt. of Biochemistry, SBBU, Women University, Peshawar Pakistan; [3]The Karachi Institute of Biotechnol. and Genetic Engineering (KIBGE), University of Karachi, Karachi Pakistan

Correspondence: asif_biotech@sbbu.edu.pk

Bioconversion of lignocellulosic biomass of agro-industrial waste utilizing microbial flora has become a potential source for the generation of various valuable metabolites. Various agricultural feedstocks have been exploited in different food, feed, and biofuel-producing industries due to their low cost, environmental biodegradability, and high product yield. The current study was designed to utilize various agricultural feedstocks for the production of α-1,4-glucosidase. Among various plant sources, a 1% concentration of dried and crushed sweet potato peel (*Ipomoea batatas*) was found to be the most inducible substrate for the production of α-1,4-glucosidase (6533.0 U ml^{-1}). Parametric optimization showed that Bacillus *licheniformis* KIBGE-IB4 required 0.4% peptone and meat extract while 0.1% yeast extract for maximum synthesis of the enzyme. Further optimization of physical parameters revealed that bacterial isolate released 6739.0 and 7190.0 U ml^{-1} of the enzyme at 40°C and pH 7.0, respectively. An improved and cost-effective growth medium design resulted in 8590.0 U ml−1 of α-1,4-glucosidase with a 1.3-fold increase as compared to the initial amount from *B. licheniformis* KIBGEIB4. This enzyme can be used to fulfill the accelerating demand of food and pharmaceutical industries. Further purification and immobilization of this enzyme can also enhance its utility for various commercial applications.

Keywords: Agro-industrial waste, Biodegradation, Fermentation, Industrial applications, Maltase

Thevetia peruviana extract mediated green synthesis of gold nanoparticles and their biological Screening

Saima Naz[1*], Abdur Rauf[2]

[1]Institute of Biotechnology and Microbiology, Bacha Khan University, Charsadda, KP, Pakistan; [2]Deptartment of Chemistry, University of Swabi, KP, Pakistan

Correspondence: saima_khan201164@yahoo.com

This research aimed to synthesize AuNPs from *T. peruviana's* aqueous extract to enhance the activity (both anti-bacterial and anti-fungal) of the title plant. For that purpose, the extract was subjected to synthesize AuNPs, and synthesized AuNPs were characterized by UV-visible, Fourier transform (FTIR) spectroscopy and SEM (scanning electron microscope) analysis. The active phytochemicals present in the extract are responsible for synthesizing AuNPs. Urease and α-glucosidase enzyme inhibitory activity of the extract was also checked which had 6.98 ± 0.98 µM and 700.7 ± 2.43 µM by using Thiourea (IC50 µM) as standard. It is concluded that *T. peruviana* extract is an outstanding enzyme inhibitor and is capable of making fine nanoparticles

Keywords: *Thevetia peruviana,* aqueous extract, biological activities

Effect of Medicinal Herbs against Multi-Drug Resistant (MDR) *Staphylococcus aureus* Isolated from Hospital Dry Waste in Saidu Teaching Hospital, Swat

Adnan Khan[1], Anwar Ali[1], Redaina[1], Muhsin Jamal[1]*, Shawkat Ali[2], Muhammad Nazir Uddin[3], Muhammad Asif Nawaz[4]

[1]Deptt. of Microbiology; [2]Deptt. of Pharmacy, Abdul Wali Khan University, Garden Campus, Mardan, Pakistan; [3]Centre for *Biotechnology and Microbiology, Univ. of Swat, Swat, Pakistan; [4]Deptt. of Biotechnology, SBBU, Sheringal, Pakistan*

Correspondence: muhsinjamal@awkum.edu.pk

The ratio of skin diseases is growing very rapidly worldwide including in Pakistan. The main reason for skin diseases is direct contact with affected persons, the environment as well as microbes. Hospital waste is a rich source disease disease-causing microbe. *Staphylococcus aureus* is one of them which mostly causes skin diseases. A microbe, that shows resistance to two or more drugs is known as multi-drugs resistant (MDR). In the present study, *S. aureus* was collected from Hospital dry waste from Saidu Teaching Hospital, Swat. The isolates were grown on Mannitol Salt Agar (MSA) and different antibiotics were applied to find MDR *S. aureus*. Then five medicinal herbs (*Ajuga bracteosa, Barberry lycium, Convolvulus arvensis, Rosa canina,* and *Rosa muschata*) extracts were applied against *S. aureus* and measured zone of inhibition (ZI). The most effective herb was *B. lycium* (31.33 mm) followed by *R. canina* (30.66mm), *R. muschata* (16.66mm), *A. bracteosa* (16.00mm), and *C. arvensis* (9.00 mm) respectively, with ethanol extract at 100 µL. while in water extract the most effective herb was *R. canina* (25.33 mm) with 100 µL extract followed by *R. muschata* (16.00mm), *A. bracteosa* (15.33mm) *B. lyceum* (14.66mm) and *C. arvensis* (8.33mm) respectively. The plant components were analyzed through FTIR showing sufficient concentration of chemicals having antibacterial activity. The results of this study concluded that the selected medicinal herbs were very effective and must be used in antibiotics against MDR *S. aureus*. This study could also result in awareness in the local community and patients in the hospital about microbial diseases and protective protocols to minimize health problems.

Key Words: Hospital waste, *Staphylococcus aureus*, Antibiotics, Medicinal herbs, FTIR

Characterization and immobilization of endo-1, 4-β-D glucanase

Muhammad Aslam[1], Muhammad Asif Nawaz[1], Sidra Pervez[2]

[1]*Department of Biotechnology, SBBU, Sheringal, Dir Upper, KP, Pakistan;*
[2]*Department of Biochemistry, SBBWU, Peshawar KP, Pakistan*

Correspondence: asif_biotech@sbbu.edu.pk

Enzymes have remarkable advantages over chemical catalysts but their use on an industrial scale is vulnerable due to a decrease in their stability, efficiency, operational stability, and inactivation in harsh environmental conditions. The current study is an attempt to increase the stability and efficiency of endo-1, 4-B-D-glucanase by its immobilization through the entrapment method. For this purpose, endo-1, 4-B-D-glucanase (cellulose degrading enzyme) was produced from a previously isolated strain of Bacillus subtilis and immobilized on natural and synthetic polymers by an entrapment method. Optimization of various parameters was carried out including polymer concentration beads surface area, pH, temperature, reaction time, substrate concentration, thermal stability, and reusability of the enzyme. All aforementioned parameters were then compared with those of soluble enzymes in the form of relative activity. The maximum immobilization yield was obtained using an 80% concentration of acrylamide. The lowest immobilization yield (75%) was obtained when entrapped with calcium alginate. The immobilized enzyme showed a wider pH and temperature profile as compared to the soluble enzyme. The optimum temperature of endo-1, 4-β-d-glucanase was shifted to 50°C, 60°C, and 70°C after immobilization within agar-agar, polyacrylamide and calcium alginate beads, respectively. Reaction time and pH optima were also changed after immobilization. After immobilization, the thermal stability was improved. Endo-1, 4-β-d-glucanase retained the highest reusability efficiency within agar-agar and acrylamide and showed activity after consecutive 12 and 8 cycles as compared to calcium alginate beads, respectively. Electron microscopy analysis showed that surface topology was changed after the entrapment of enzymes within polymers. Thus, it can be expected from current findings that the stability and recycling efficiency of immobilized enzymes was increased after entrapment and can be used as a potential bioreactor for commercial applications in different industrial applications.

Revealing the climate variability in the Hindu Kush: A tree rings-based temperature reconstruction

Fayaz Asad[1,2*], Haifeng Zhu[2,3], Ulf Büntgen[4]

[1]Department of Botany, Bacha Khan University Charsadda, KP, Pakistan; [2]State Key Laboratory of Tibetan Plateau Earth System, Resources and Environment (TPESRE), Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100101, China; [3]China Pakistan Joint Research Center on Earth Sciences, CAS-HEC, Islamabad 45320, Pakistan; [4]Department of Geography, University of Cambridge, Downing Place, Cambridge CB2 3EN, United Kingdom

**Correspondence: fayaz.asad79@gmail.com*

Annually resolved and dated proxy archives are necessary for contextualizing the rate and amplitude of anthropogenic climate change. High-resolution climate reconstructions are particularly important for remote regions where instrumental meteorological observations are limited in space and time. Here, we develop a robust, 260-year-long tree-ring width chronology from 32 Himalayan cedars (*Cedrus deodara* D. Don), which grew around 3000 m asl in the Kumrat Valley of the Hindu Kush region in northern Pakistan. This inverse relationship between radial tree growth and early spring temperatures, together with positive correlations against precipitation totals at the onset of the growing season, suggest that warming-induced drought is the most limiting factor of sub-alpine forest growth in the Kumrat Valley. We then use the inverse growth-temperature association to reconstruct March-April minimum temperatures from 1812 to 2018 CE. The reconstruction explains 40 % of the instrumental record back to 1967, and exhibits the coldest and warmest spring conditions in 1815–1864 (6.67 ± 0.21°C) and 1918–1927 (8.11 ± 0.43°C), respectively. This pioneering study is indicative of the dendroclimatological potential in the Hindu Kush, where samples from living and relict trees of different species and elevational bands should be collected for advanced temperature and hydroclimate reconstructions.

Keywords: Tree rings, *Cedrus deodara*, Dendroclimatology, Temperature reconstruction, Hindu Kush region

Insect Larvae as an Alternate Protein Source in Poultry Feed Improve the Performance and Meat Quality of Broilers

Muhammad Sajjad[1,2], Asif Sajjad[2*], Muhammad Binyameen[1*]

[1]Deptt. of Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan 60800, Pakistan; [2]Department of Entomology, Faculty of Agriculture and Environment, Islamia University, Bahawalpur 63100, Pakistan

* Correspondence: asif.sajjad@iub.edu.pk; mbinyameen@bzu.edu.pk

The primary challenge facing the global animal industry is the scarcity of protein feed resources. Various insects are gaining prominence as innovative feed sources due to their economic, environmentally friendly, and nutritious attributes. The purpose of the present study was to determine the effects of a partial replacement of soybean meal with fall armyworm Spodoptera frugiperda (Lepidoptera: Noctuidae) and black soldier fly Hermetia illucens (Diptera: Stratiomyidae) on the growth performances, blood parameters, gut histology, and meat quality of broilers. A total of 350 1-day-old (40 ± 0.15 g) male chicks (Ross 308) were randomly assigned to seven experimental meals. Each treatment was repeated five times with 50 birds per dietary treatment. The seven dietary treatments included 4, 8, and 12% replacements of SBM with larvae of *S. frugiperda* and H. illucens. SBM was the basal diet considered the control. The data showed that broilers fed 12% *S. frugiperda* or H. illucens exhibited a significantly higher ($p < 0.05$) live weight and average daily weight gain, and improved the feed conversion ratio. Meals with 12% *S. frugiperda* or H. illucens significantly enhanced ($p < 0.05$) hematological and gut histological parameters, including villus height, crypt depth, villus width, and villus height/crypt depth ratios. The meat of broilers fed the 12% *S. frugiperda* diet showed significantly higher ($p < 0.05$) lightness and yellowness. Replacing soybean meal up to 12% with either *S. frugiperda* or H. illucens larvae improves the growth performance, blood hematology, gut morphometry, and meat quality traits of broilers.

Keywords: broiler performances; *Hermetia illucens*; meat quality; soybean replacement; *Spodoptera frugiperda*

Genetic diversity and taxonomy of the genus Acanthodactylus (Squamata: Lacertidae) in Pakistan

Muazzam A. Khan, Daniel Jablonski, R. Masroor, M. Sajid Nadeem, Uwe Fritz
Deptt. of Botany & Zoology, Bacha Khan University Charsadda, KP, Pakistan
Correspondence: muazzamone@gmail.com

This study investigates the phylogenetic relationships within the Acanthodactylus genus in its easternmost range, particularly in Pakistan. Using a concatenated dataset of mitochondrial (16S, COI, cyt b) and nuclear (Rag1) genes, we reconstructed the phylogenetic relationships constructed by two tree methods that consistently revealed four, strongly supported major clades (A-D). These clades exhibit significant genetic differentiation, with the level of uncorrected p distances highlighting deep divergences among them. Clade A, consisting of a single sequence identified as A. micropholis, is the sister group to the remaining clades. Clades B and C, both identified as A. blanfordii from the Helmand Basin in northwestern Balochistan, are geographically separated by the Chagai Hills and show moderate genetic differentiation. Clade D, identified as A. cantoris, is the most divergent and widespread, yet it shows shallow intraspecific genetic variation through the Indus Valley. Additional analysis of 178 cyt b sequences, including data from GenBank, supported the division into the four main clades identified from the concatenated dataset. Notably, Clades B and C are newly identified in this study and represent novel phylogenetic entities within Acanthodactylus. Our research enhances our understanding of the evolutionary relationships and geographic distributions of Acanthodactylus lizards in the eastern edge of its wide distribution and provides a robust phylogenetic framework for further taxonomic and conservation studies.

Keywords: Afghanistan, endemism, Baluchistan, remote areas, speciation, taxonomy.

Comparative Analysis of the Plant Growth Regulators- and Carbon Nanotubes-Induced *In Vitro* Sugarcane Calli Culture Micropropagation

Aneela Iqbal, M. Usman, Muhammad Aizaz, Karim Gul, Raham Sher Khan*[1]

Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan

Correspondence: rahamsher@awkum.edu.pk

Sugarcane is an important perennial, polyploidy crop. Based on the growing demand, it has now attracted great attention as a cash crop. The tissue culture technique, employing nanoparticles as an alternative method for solving production problems was used in this study. Standardization of the protocol for induction and proliferation of callus was established through in vitro culture using thin cell layers of young leaf sheaths and leaf rolls of the sugarcane cultivar Israr Shaheed. The culture media supplemented with Carbon nanotubes (CNTs) and different auxins [Dichlorophenoxy Acetic acid (2,4-D), Naphthalene Acetic Acid (NAA)], cytokinin [Benzyl Amino Purine (BAP)] either alone or in combination with each other were evaluated. Among the different combinations of plant growth regulators (PGRs), 2,4-D in 2.0 mg/L was found best for callogenesis and resulted in the highest fresh (97.6±0.6 mg/g) and dry weight (69.5±0.5 mg/g) of the induced callus. In contrast, the CNTs-media resulted in 100% callus induction with the highest fresh (643.3±0.9 mg/g) and dry weight (591.9±0.8 mg/g). In the case of regeneration, amongst the various PGR combinations, BAP (2.0 mg/L) in addition to NAA (0.2 mg/L) resulted in higher regeneration capacities with maximum shoots per explant 26±1.1 and maximum shoot length 10.6±0.4 cm. While the CNTs in 15.0 mg/L produced the maximum number of shoots of 43±0.8 per explant with a shoot length of 21.1±0.4 cm. Similarly, NAA in 1-4 mg/L and CNTs in 5, 10, 15, and 20 mg/L were employed for root formation. The maximum number of roots (38±0.8) per explant with root length 6.9±0.1 cm was observed on 15.0 mg/L of CNTs-harboring media. These findings show that employing CNTs in a specific amount in media culture can enhance the plant's morphological and physiological characteristics and can be used as efficient plant growth regulators for enhanced production in crop plants.

Keywords: *Saccharum officinarum*, micropropagation, nanotechnology, Carbon nanotubes (CNTs)

Influence of environmental variables on regeneration potential of *Populus nigra* L. in Dir upper, Pakistan

Khalid Mahmood[1], Fayaz Ali[1*], Waqar Khan[2]

[1]*Department of Botany, SBBU, Sheringal, Dir Upper, 18050, Pakistan*

[2]*College of Horticulture, South China Agricultural University, Guangzhou, China*

Correspondence: fayazali@sbbu.edu.pk

Ecological studies focus on the vital role of forests in the global ecosystem, addressing factors influencing plant community composition, structure, and diversity. The spatial distribution of plant species and their natural regeneration is influenced by various environmental factors (climate change) and soil physiochemical properties such as nitrogen, carbon, and soil texture. Examining the case of *Populus nigra*, this work provides a detailed understanding of the relationship between growing stock variables and the natural regeneration of *Populus* within the natural habitats located in Dir upper Pakistan. Phyto-sociological data was collected in *Populus*-dominated forests (number = 31) by establishing quadrates of 10 × 10 m^2 and 5 × 5 m^2 for woody tree species and understory vegetation respectively. We also noted environmental characteristics like topographic variables (elevation, slope, aspect) and edaphic variables (texture, N (%), pH, organic matter, etc.) in each sampled site. Using the importance value index of 19 reported woody trees and 19 environmental variables, 31 sampled sites were classified into four distinct vegetation types through PC-ORD (version 6.1) by selecting the option of Ward's agglomerative clustering analysis technique. The resulting vegetation types were dominated by *P. nigra*, while the other close associates were *Ailanthus altissima*, *Quercus baloot*, *Pinus roxburghii,* and *Populus alba*. Furthermore, the impact of environmental variables on the resulting vegetation types was evaluated by performing Redundancy analysis (RDA). Results showed that topographic variables coupled with soil physiochemical properties highly influence the vegetation structure and composition in the studied area. Among the topographic variables, elevation was ranked on top and counted as the most influential variable followed by aspect. However, among edaphic variables, soil texture, nitrogen contents, and total dissolved solids were reported with a high impact on the vegetation types. We also assess the natural regeneration potential of the dominant species. The results indicated that the natural regeneration potential is at high risk due to heavy over-grazing, severe anthropogenic interference, and last but not least natural disturbances in the form of climate change. We concluded that *P. nigra* is an important (ecologically and economically) tree species in the area and is currently under severe pressure. Hence, urgent conservation programs both from government and non-government agencies need time for sustainable use.

Keywords: Growing stock; Regeneration; *Populus nigra* L; Barawal Valley.

Enhanced phytoextraction of cadmium through nitrogen and phosphorus fertilizers along with indole acetic acid supplementation in *Datura innoxia* Mill. and *Xanthium strumarium* L.

Fayaz Ali[1], Naveed Khan[1], Muradullah[1], Amin Ullah Jan[2], W. Khan[3], Rafi Ullah[4]

[1]Department of Botany; [2]Department of Biotechnology, Shaheed Benazir Bhutto University Sheringal, Dir Upper, 18050, Pakistan; [3]College of Horticulture, South China Agricultural University, Guangzhou 510642, China; [4]Dr Khan Shaheed Government Degree College Kabal Swat Khyber Pakhtunkhwa, Pakistan

Correspondence: fayazali@sbbu.edu.pk; waqar.khan399@gmail.com

Heavy metal contamination such as cadmium (Cd), in soil, poses significant risks to both global food security and human health. To address this issue, environmentally friendly and cost-effective technologies are required for managing such contamination. Plants that exhibit high resistance to Cd stress and substantial biomass production are promising candidates for phytoremediation. The current experiment was designed to assess the impact of fertilizers (nitrogen, phosphorus) and Indole acetic acid (IAA) on the growth, biomass production, and Cd phytoextraction potential of *Datura innoxia* Mill. and *Xanthium strumarium* L. Pot experiment was conducted using a completely randomized design with five replicates to evaluate the effects of various combinations of fertilizers and plant growth regulators. Following the harvest, plants were separated into different parts, including stems, roots, and leaves, and various growth, physiological, and biochemical parameters were measured and recorded. The results showed a significant decrease in growth, physiological, and biochemical parameters under Cd stress (T1 treatment). The Cd concentrations were increased with the addition of fertilizers having the highest concentration for T2 (Cd + Urea) of *X. strumarium* (15.25 ± 0.49 mg/Kg) followed by T5 (Cd + IAA) of *D. innoxia* (8.85±0.07). Chlorophyll contents and biomass were found to be enhanced by applying fertilizers. These findings demonstrate that Cd phytoremediation using *D. innoxia* and *X. strumarium* with soil amendments can greatly improve the quality of Cd-contaminated soils.

Keywords: *Datura innoxia*; *Xanthium strumarium*; phytoextraction; Cd-concentration; soil amendments; redundancy analysis

Isolation and characterization of siderophore-forming endophytic bacteria from *Moringa oleifera*

Nimra Gul, Shadab Murad[1], Sitara Andleeb, Karim Gul, Raham Sher Khan*

Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan

Correspondence: rahamsher@awkum.edu.pk

Siderophores are low molecular weight secondary metabolites, produced by bacteria and other microorganisms in iron-deficient areas. The present experiment was aimed to isolate and screen the siderophore-forming bacterial endophytes from *Moringa oleifera* and their Siderophore production ability. The siderophore occurrence, qualitatively, was noticed by the yellow-orange halo zone surrounding the spot in the CAS agar plate and quantitatively evaluated in

CAS liquid solution and shuttle solution. Its absorbance was measured at 650 nm. Siderophore production was found in all endophytic Bacterial isolates with varied levels. Among the isolates, NR-21 (50.96%) exhibited the highest production of Siderophore, followed by NL-3 and NR-33 isolates. Primary and secondary metabolites (IAA, Salicylic acid, phenols, flavonoids, carbohydrate, proline, phosphate) were found in all the siderophore-producing endophytic Bacterial isolates. EPS quantification was taken by mixing the supernatant in chilled ethanol, and all three isolates were found to be positive for EPS production. The catalase assay was done through the slide or drop catalase method, and the positive reaction was produced by all three Isolates. To examine resistance to chromium in our isolates, our isolates were cultured at various concentrations of heavy metal (Chromium). The growth kinetics of isolated endophytic bacteria were studied under chromium stress at increasing concentrations for 72 hours. The addition of Cr application significantly inhibited bacterial growth as compared to the control. By increasing chromium stress, the IAA production was significantly reduced in culture supernatant. By increasing chromium stress, the SA amount was increased except at 500 ppm. The plant growth-enhancing capability of the isolates was also investigated in the maize plant in the pot experiment. The results showed that siderophore-producing isolates encouraged the growth of maize under both normal and chromium stress in comparison with the non-inoculated plants. Moreover, isolates inoculated maize plants improved the morphological parameters, photosynthetic pigments, sugar, and IAA, with a decrease in proline content. Chromium stress reduced photosynthetic pigments, sugars, and IAA, amount while increasing proline value. The negative impact of chromium was eliminated by our selected endophytic bacteria. The results of this work show a variety of metabolic activities and the siderophore-producing capacity of *M. oleifera* endophytic bacteria, underscoring their significance in plant microbes' interactions and prospective applications in bioremediation and plant growth promotion.

Keywords: Siderophore, endophytic bacteria, *Moringa oleifera*, chromium

Analysis of susceptibility genes expression in potato against *Phytophthora infestans* in response to carbon nanotubes

Maria Gul, Amina Shumaila, Sumayya Kha, Raham Sher Khan*

Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan

Correspondence: rahamsher@awkum.edu.pk

The upswing in world population and food afflictions has commanded inordinate adversaries in agriculture and climatic situations. Potato (*Solanum tuberosum* L.) is the world's fourth most consumed but nutritious produce. It inclines to resource the amassed food and nutritional demand. However, it is faced with a hoard of biotic and abiotic stressors, amongst which *Phytophthora infestans* is found to be the prime challenger. The pathogen is known for its obnoxiously vicious nature as it abolishes the whole population in a few days' intervals. Potato cultivar Desireé is not acknowledged for having any resistance

genes (R-genes), nonetheless, certain susceptibility genes (S-genes) are found in abundance, whose silencing distresses the pathogen's route of infection and ultimately promotes resistance against the pathogen. In this current study, MWCNTs are utilized to interact with the genetic expression of the P. infestans susceptible gene (DND2) in the in vitro cultured potato cv. Desireé plants at different concentrations (5, 10, 15, 20 mg L-1). The morphological and biochemical analysis of these plant samples exhibited pronounced results in the increasing MWCNTs except at 20 mg L-1 concentrations as compared to the control sample. The antifungal potential of the plants against P. infestans also presented evident results with 15 ± 0.2 cm diameters of inhibition zones, due to higher production of antioxidant and phenolic compounds. Reverse transcriptase PCR analysis revealed that plant samples treated with MWCNTs did not amplify the DND2 gene but the gene was amplified in the control samples, which was a clear indication of the toxicity of MWCNTs towards expression of the S-gene in potato cv. Desireé against P. infestans. Therefore, carbon nanomaterials advocate promising advancements in the downregulation of S-genes for sustainable agricultural practices.

Keywords: Susceptibility genes, *DND2*, *Phytophthora infestans*, MWCNT

Aspergillus nomiae **and A.** ***fumigatus*** **ameliorating the hypoxic stress induced by waterlogging through ethylene metabolism in** ***Zea mays L.***

Khalil Ur Rahman[1], Salman Khan[1,] Noreen Nawaz[1], Afshan[1], Mamoona Rauf[2*], Muhammad Arif[1*]

[1]*Deptt. of Biotechnol.;* [2]*Deptt. of Botany, Abdul Wali Khan Univ. Mardan, Pakistan*

Correspondence: khalilurrahman@awkum.edu.pk

Maize (*Zea mays* L.) is an important crop but its production can be severely limited by waterlogging. The present research was rationalized to explore the potential of the newly isolated 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase-producing fungal endophytic consortium of *Aspergillus nomiae* (MA1) and *Aspergillus fumigatus* (MA4) isolated from the seeds of *Moringa oleifera* L., which able to produce a sufficient amount of IAA, proline, phenols, and flavonoids. The symbiotic association of MA1 and MA4 promoted the growth response of maize compared with the non-inoculated plants under waterlogging stress. The inoculated maize plants enhanced the production of total soluble protein, sugar, lipids, phenolics, and flavonoids, and also showed an increase in the DPPH activity, antioxidant enzyme activities of CAT and POD, along with an increased level of hormonal content (GA3 & IAA) and with a reduction in proline content H_2O2 production ABA and ACC contents under waterlogging. Stomatal activity and adventitious root formation increased in inoculated maize plants, with reduced lysigenous aerenchyma formation. A reduction in deformed mesophyll cells, and deteriorated epidermal and vascular bundle cells, as well as swollen metaxylem, phloem, pith, and cortical area, in maize plants under waterlogging compared to control. Moreover, the transcript abundance of ethylene-responsive gene *ZmEREB180*, responsible for the induction of the WS tolerance in maize, showed optimally reduced expression which was sufficient for induction in WS tolerance, in MA1 and MA4 inoculated plants under WS compared with the non-inoculated control. The current study supported the use of MA1 and MA4 isolates for establishing the bipartite mutualistic symbiosis in maize to assuage the adverse effects of WS by optimizing ethylene production.

Isolation and molecular characterization of growth-promoting fungal endophytes from *Phoenix dactylifera* and their role in enhancing wheat growth and drought stress tolerance

Salman Khan[1], Khalil Ur Rahman[1], N. Karishma[1], N. Nawaz[1], S. Laila[1], M. Rauf[2], M. Arif[1*]

[1]Deptt. of Biotechnol.; [2]Deptt. of Botany, Abdul Wali Khan Univ. Mardan, Pakistan

Correspondence: arif@awkum.edu.pk

This study investigates the isolation and characterization of growth-promoting fungus endophytes from *Phoenix dactylifera* (date palm) roots and evaluates their potential as biofertilizers. Six distinct fungi were isolated, and their metabolic profiles were analyzed. Among these, DT-2, identified as *Fusarium proliferatum*, exhibited superior production of secondary metabolites, including 45.86 µg/ml indole acetic acid (IAA), 260.3 µg/ml flavonoids, 179.6 µg/ml phenolic, and 15.4 µg/ml Proline. Inoculation of wheat seeds with DT-2 resulted in enhanced seed germination, root and shoot growth, and increased fresh weights (root: 1.73 g; shoot: 5.90 g). Additionally, DT-2 demonstrated the highest levels of secondary metabolites and photosynthetic pigments among the isolates. DT-2 was also found to significantly enhance wheat's drought stress tolerance. Under PEG-induced stress, DT-2-inoculated plants showed increased levels of beneficial hormones and antioxidants, demonstrating DT-2's capacity to boost wheat resilience against drought by modulating key metabolic pathways. These findings highlight the potential of *Fusarium proliferatum* as an effective biofertilizer, promoting plant growth and improving crop performance under stress conditions.

Keywords: Endophytic fungi, *Phoenix dactylifera*, biofertilizers, plant growth promotion, *Fusarium proliferatum*.

Exploring the role of endophytic fungi from *Olea european* in enhancing potato resistance against salt stress

Afshan[1], Shaista Afzal[1], Khalil Ur Rahman[1], Mamoona Rauf[2*], Muhammad Arif[1*]

[1]Deptt. of Biotechnol; [2]Deptt. of Bot., Abdul Wali Khan Univ. Mardan, Pakistan

Correspondence: Afshan.malik@awkum.edu.pk

Plants constitute dynamic systems with different mechanistic approaches to combat biotic and abiotic stress. Salinity (NaCl) stress is the abiotic stress that seriously the quality of soil, crop yield, photosynthetic rate, and plant growth and increases the accumulation of free radicals. Abiotic stress has to be mitigated by discovering synergistically positively and driving the ability to resist these stressful conditions. The main objective of current research is the identification of stress-tolerant and growth-promoting fungal endophytes for

Solanum tuberosum for facilitating stress tolerance including salinity. Aspergillus fumigatus (AF21) was isolated from the olive root which produced more amount of IAA, Proline, and phenol. The symbiotic association of AF19 and AF21 can promote the growth rate of potatoes compared with the non-inoculated plant under salt stress. Endophytic fungi AF21 colonized the root zone of potato (*Solanum tuberosum*). Furthermore, AF21 inoculation to potato plants enhanced the production of total soluble proteins, total contents of carbohydrates, sugar, Vitamin-C, phenol, and flavonoid, with the reduction in hydrogen peroxide. AF21 inoculated to potato plant evaluated an increase in the DPPH activity and antioxidants enzyme activities of SOD, APX, POD, and Catalase enzymes beside this IAA increased. AF21 inoculated potato plants reduced lysigenous aerenchyma and increased the tight and round shape of the cell without damage. Thus the present study might be helpful to enhance multi-stress resistance in *Solanum tuberosum* by exploiting fungal endophytes and identifying the molecular mechanism inducing stress tolerance response in *Solanum tuberosum*.

Keywords: Endophytic fungi, *Solanum tuberosum,* Abiotic stress, Aspergillus fumigatus and salinity

Cress polysaccharides mediated the synthesis of nickel oxide and silver nanoparticles for biological applications

Afghana Firdous, Imdad Ullah Khan, Yusra Jamil, and Ayaz Ahmad
Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan
Correspondence: ahdayazb5@awkum.edu.pk

Phytochemical-assisted biosynthesis of nanomaterials offers an eco-friendly alternative to traditional methods. This study presents the synthesis of silver (Ag-NPs) and nickel oxide nanoparticles (NiO-NPs) using cress seed mucilage polysaccharides (CSMP) as a reducing, stabilizing, and capping agent. $AgNO_3$ and $NiNO_3$ served as precursors in the hot plate combustion method. Characterization of the NPs was conducted using UV-visible and Fourier-transform infrared (FT-IR) spectroscopy. The biocompatibility of Ag-NPs and NiO-NPs was confirmed through hemolysis assays, showing no toxicity at low concentrations. Cytotoxicity assessments revealed CC50 values greater than 10,000 µg/mL for CSMP, 2346 µg/mL for Ag-NPs, and 1987 µg/mL for NiO-NPs, indicating their safety at lower concentrations. The antioxidant activity of the NPs, evaluated using the DPPH assay, showed Ag-NPs stabilizing DPPH radicals by 85.67 ± 1.01% at 400 µg/mL, comparable to ascorbic acid (96.91 ± 2.05%). Ag-NPs also exhibited significant inhibition of α-amylase (89.03 ± 3.71%) and α-glucosidase (90.83 ± 3.54%) at 400 µg/mL, surpassing NiO-NPs and CSMP, and approaching the effectiveness of the standard drug acarbose. Antibacterial assays demonstrated that both Ag-NPs and NiO-NPs effectively inhibited the growth of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, and Escherichia coli. These findings suggest that CSMP offers a sustainable and effective method for the green synthesis of bioactive Ag-NPs and NiO-NPs with potential applications in biomedicine and agriculture.

Keywords: Silver nanoparticles, nickel oxide nanoparticles, antioxidant, antidiabetic, antibacterial, green synthesis

Evaluation of in vitro and in vivo anti-inflammatory potential of bioactive polysaccharides from *Salvia hispanica L.* (chia) seed mucilage

Aiman khan[1], Imdad Ullah Khan[1], Y. Jamil[1], R. Wajid[1], A. Iqbal[2] , Ayaz Ahmad[1]

[1]Department of Biotechnology, [2]Department of Food Science & Technology, Abdul Wali Khan University Mardan, Pakistan

Correspondence: ahdayazb5@awkum.edu.pk

Inflammation is a crucial immune response to pathogens, cell damage, and harmful substances. Although various drugs are available to treat inflammation, they often have side effects. Plants, especially those with bioactive compounds, offer alternative therapeutic options. *Salvia hispanica* L, commonly known as chia, is a significant medicinal plant rich in bioactive ingredients and therapeutic polysaccharides. This study aimed to extract and fractionate the bioactive polysaccharides from chia seed mucilage and determine their anti-inflammatory effects in vivo and in vitro. Chia seeds were soaked in distilled water, and mucilage was precipitated using 75% ethanol. The resulting fraction underwent size-based fractionation using Bio-Gel-P-10 gel filtration chromatography, yielding 60 fractions screened for carbohydrate content. Biochemical analysis revealed the presence of uronic acids, pentoses, and hexoses. In vitro, anti-inflammatory activity was assessed using the HRBC membrane stabilization assay, while in vivo effects were evaluated with the carrageenan-induced paw edema model in mice. Results showed that the Ch19 fraction significantly stabilized HRBC membranes (EC50=8.75 μg/mL), and the Ch39 fraction at 1000 μg/mL resisted heat-induced membrane destabilization by 97.20%. Furthermore, the in vivo activity showed that the Ch31 fraction significantly reduced the volume of the mice's paw edema by 91.37%. These findings suggest that the ethanol-precipitated fraction of chia seed mucilage contains medium-sized polysaccharides with substantial anti-inflammatory potential, indicating their promise for further preclinical and clinical trials as treatments for inflammation and related diseases.

Keywords: Anti-inflammatory, Bioactive polysaccharides, *Salvia hispanica L.,* Mucilage

Exploring anti-diabetic and anti-inflammatory effects of endophytic fungi-derived exopolysaccharides from wheat and Parthenium

Yusra Jamil[1], Anam Pervaiz[1], M. Baqir Khan [1], H. Abbas[1], A. Iqbal[2] , Ayaz Ahmad[1]

[1]Department of Biotechnology; [2]Department of Food Science and Technology, Abdul Wali Khan University Mardan.

Correspondence: ahdayazb5@awkum.edu.pk

Endophytes are microbes that colonize the living, internal tissues of plants without causing any negative effects. In recent years, endophytic fungi have been demonstrated to be excellent exopolysaccharide (EPS) producers. EPS are high-molecular-weight carbohydrates with a wide range of biological activities. This study was, therefore designed to extract and purify EPS-producing endophytic fungi from wheat (*Triticum aestivum*) and Parthenium (*Parthenium hysterophorus*) and explore their biological applications using different bioassays. The purified EPS was labeled as EPSW1, EPSW2, EPSW3, EPSP1, EPSP2, and EPSP3. The purification of EPS involved inoculating isolated endophytic fungi into a broth medium at 25 °C for 32 hours on a shaking incubator and EPS precipitation using 95% ice-chilled ethanol. The purified EPS were processed for quantification of total carbohydrate content and bioactivity through α-amylase, α-glucosidase, and HBRCs membrane stabilization assay. The results indicated that EPSW1 inhibited α-amylase, showing an effective IC50 value of 265.75 µg/mL, while EPSP2 inhibited α-glucosidase with an IC50 value of 441.87 µg/mL. In terms of in vitro anti-inflammatory activity, EPSP1 significantly stabilized the HRBCs membrane, with an EC50 value of 8.75 µg/mL, followed by EPSW1 (155.17 µg/mL). Furthermore, the study assessed in vivo anti-inflammatory activity through a carrageenan-induced paw edema model in mice. Simultaneously, the in vivo antidiabetic activity was investigated in Balb/C mice by inducing diabetes via a 1% alloxan injection. Three concentrations (400 mg/kg, 200 mg/kg, 100 mg/kg) were tested for each activity. At 400 mg/kg, EPSP1 showed the highest anti-inflammatory impact (91.37%) while EPSW2 at the same concentration displayed a significant 74.85% reduction in blood glucose levels in alloxan-induced diabetic mice. In conclusion, the study emphasizes the potent bioactivity of EPS from wheat and Parthenium, showcasing therapeutic benefits, including anti-diabetic and anti-inflammatory properties.

Keywords: Endophyte, Exopolysaccharides, Anti-diabetic, Anti-Inflammatory, Wheat, Parthenium

Green synthesis of bioactive silver and nickel oxide nanoparticles using chia seed mucilage polysaccharides

Urooj Begum, Imdad Ullah Khan, Yusra Jamil, and Ayaz Ahmad

Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan

Correspondence: ahdayazb5@awkum.edu.pk

The eco-friendly synthesis of nanoparticles has gained attention due to their diverse biological applications. This study presents the sustainable production of silver (Ag) and nickel oxide (NiO) nanoparticles (NPs) using water-soluble polysaccharides extracted from chia *(Salvia hispanica)* seed mucilage. Polysaccharides were extracted via ethanol precipitation, and the biosynthesized NPs were characterized by UV-visible spectroscopy, revealing absorption peaks at 473 nm for Ag-NPs and 343 nm for NiO-NPs. The antioxidant potential of the NPs was assessed using the DPPH assay, with Ag-NPs and NiO-NPs showing EC50 values of 118.85 ± 2.52 µg/mL and 97.21 ± 1.39 µg/mL, respectively, compared to 80.78 ± 1.65 µg/mL for ascorbic acid. Additionally, both NPs demonstrated promising in vitro antidiabetic activity, with IC50 values of 87.70 ± 3.65 µg/mL (Ag-NPs) and 146.15 ± 1.94 µg/mL (NiO-NPs) against alpha-amylase, compared to acarbose (71.75 ± 2.63 µg/mL). The antibacterial effectiveness of the nanoparticles against three distinct variants of human pathogens was assessed using the agar well diffusion technique. The findings indicated that Ag-NPs effectively suppressed the growth of three pathogenic strains: *Staphylococcus aureus* (9.5mm ± 0.5mm), *Escherichia coli* (11.75mm ± 0.82mm), and *Hemophilus influenza* (8.5mm ± 0.82mm). These findings indicate that chia seed-derived polysaccharides are effective in synthesizing bioactive Ag-NPs and NiO-NPs with strong antimicrobial and antioxidant properties, making them valuable for biomedical applications. This study underscores the potential of mucilage polysaccharides as a sustainable resource for producing bioactive nanoparticles.

Keywords: Nanoparticles, polysaccharides, Chia, Antimicrobial, antioxidant

Antimicrobial efficacy of seed extracts from *Trigonella, Salvia, Ocimum*, and *Coriandrum* against resistant pathogens

Raeesa Wajid, Anam Pervaiz, Aiman Khan, and Ayaz Ahmad
Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan
Correspondence: ahdayazb5@awkum.edu.pk

The increasing risk of bacterial resistance to antibiotics and associated side effects has driven researchers to explore medicinal plants as natural alternatives to synthetic drugs. This study investigates the antimicrobial activities of four seeds (*Trigonella foenum-graecum, Salvia hispanica, Ocimum basilicum,* and *Coriandrum sativum*) commonly used in traditional medicine against five bacterial strains (*E. coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella spp.,* and *Staphylococcus aureus*) and one fungal strain (*Candida spp*) isolated from fruit samples. The bacteria were identified based on cultural morphology, Gram staining, and Analytical Profile Index (API 20E) testing. The antimicrobial activity of the seed extracts was tested using ethanol, methanol, acetone, and distilled water as solvents in a disc diffusion method. Ethanol extracts generally exhibited higher activity than methanol, acetone, and distilled water, except for *Trigonella foenum-graecum*, which showed better activity in methanol. *Trigonella foenum-graecum* demonstrated a significant zone of inhibition against *Proteus mirabilis*, while *Salvia hispanica* and *Ocimum basilicum* were particularly effective against *E. coli*. *Coriandrum sativum* exhibited broad-spectrum activity against all tested bacteria, with notable efficacy against *Salmonella spp*. None of the seed extracts in distilled water showed activity against the bacteria, and *Coriandrum sativum* did not exhibit antifungal activity against *Candida spp*. Antibiotic sensitivity tests with Azithromycin and Linezolid showed that the ethanol extracts were more effective than the reference drugs. The findings suggest that seed extracts could be a valuable source for developing new antimicrobial compounds

Keywords: Medicinal plants, Antimicrobial resistance, Antibacterial activity

Biosynthesis and antibacterial evaluation of iron oxide nanoparticles using neem leaf extract

Syeda Nabiha Bukhari, Maham, Kainat Ayaz, Nazif Ullah
Department of Biotechnology, Abdul Wali Khan University, Mardan, Pakistan
Correspondence: ullahnazif@awkum.edu.pk

Azadirachta indica, commonly known as neem, is a highly regarded medicinal plant native to India and widely distributed across tropical and subtropical regions. This study presents the green synthesis of iron oxide nanoparticles (Fe3O4-NPs) using neem leaf extract as both a reducing and stabilizing agent. The biosynthesized Fe3O4-NPs were characterized through UV-visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and X-ray diffraction analysis, confirming successful nanoparticle formation. The antibacterial potential of Fe3O4-NPs was assessed against five human pathogenic strains—Staphylococcus aureus, Salmonella typhi, Shigella spp., Klebsiella pneumoniae, and Salmonella spp.—using agar well diffusion, microbial biofilm inhibition, and minimal inhibitory concentration (MIC) assays. Results demonstrated that Fe3O4-NPs at 100 µg/mL produced inhibition zones of 8.78 mm against S. aureus and 8.7 mm against S. typhi, compared to 7.73 mm for neem leaf extract alone against S. aureus. The microbial biofilm inhibition assay revealed an IC50 of 15.67 µg/mL for Fe3O4-NPs, while neem extract exhibited an IC50 of 14.21 µg/mL against Klebsiella pneumoniae. MIC assays indicated that Fe3O4-NPs effectively inhibited the growth of S. aureus at a concentration of 28.12 µg/mL, with higher concentrations required for Shigella spp. (57.59 µg/mL), Klebsiella pneumoniae (87.49 µg/mL), and Salmonella spp. (54.22 µg/mL). These findings underscore the potent antibacterial activity of neem-based Fe3O4-NPs, highlighting their potential as a natural, eco-friendly alternative for biomedical applications in combating bacterial infections.

Green synthesis and characterization of *Triticum aestivum*-derived biocompatible silver nanoparticles and evaluation of their antidiabetic potential

Mahnoor1, Imdad Ullah Khan1, Yusra Jamil1, Amjad Iqbal[2], Ayaz Ahmad[1]
[1]Department of Biotechnology; [2]Department of Food Science and Technology, Abdul Wali Khan University Mardan, Pakistan
Correspondence: ahdayazb5@awkum.edu.pk

Current therapeutic options for various human ailments have faced challenges such as resistance, low efficacy, high costs, and increasing market demands, driving the search for novel, cost-effective natural alternatives. Nanoparticles, particularly those synthesized through green methods, have emerged as pivotal in biotechnology and therapeutics due to their high biocompatibility and potent bioactivities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer properties. This study focused on the biosynthesis of silver nanoparticles from endophytic exopolysaccharides (EPS) of *Triticum aestivum*. The EPS was extracted and precipitated using ethanol, followed by lyophilization. The presence of polysaccharides was confirmed through the phenol-sulfuric acid (PSA) method. Silver nanoparticles (AgNPs) were synthesized by treating the polysaccharide extracts with the respective metallic salts. The biosynthesis process was optimized using UV-visible spectrophotometry and characterized using FT-IR techniques to confirm the formation and stability of the nanoparticles. The in vitro potential of the synthesized nanoparticles was assessed through α-amylase and α-glucosidase inhibition assays. The results revealed that AgNPs significantly inhibited α-amylase activity, with an IC50 of 265.75 µg/mL, followed by EPS (357.18 µg/mL). NiO NPs, on the other hand, were found to significantly inhibit α-glucosidase, with an IC50 of 441.87 µg/mL, followed by EPS (590.68 µg/mL). These findings underscore the potential of biosynthesized MNPs as effective natural therapeutics, offering a promising alternative to conventional treatments, with significant implications for the development of cost-effective and biocompatible therapeutic agents.

Keywords: *Triticum aestivum*, Endophytic exopolysaccharides, Green synthesis, Silver nanoparticles

Evaluating the prebiotic effects of chia seed-derived oligosaccharides on human probiotics

Lubna Naz[1], Mansoor Ali[1], Imdad Ullah Khan[1], Amjad Iqbal[2], Ayaz Ahmad[1]
[1]Department of Biotechnology; [2]Department of Food Science and Technology, Abdul Wali Khan University Mardan, Pakistan
Correspondence: ahdayazb5@awkum.edu.pk.

Prebiotics, non-digestible compounds that enhance beneficial gut microbiota, have gained attention for their role in improving gut microbiome health. Mucilage from *Salvia hispanica* (chia), rich in polysaccharides, is a promising dietary component with multiple health benefits. The current research aimed to produce bioactive oligosaccharides from chia seed mucilage and evaluate in vitro prebiotic effects on human probiotics. Polysaccharides extracted from chia seed mucilage were precipitated with 75% ethanol, digested by *Pichia pastoris*, and purified via size exclusion chromatography. Biochemical analysis revealed that fraction ChF78 had the highest unsaturated uronic acid (7.79 ± 0.71 mg/mL), fraction EPP had the highest uronic acid (5.57 ± 0.32 mg/mL), and pentose (6.54 ± 0.30 mg/mL), while fraction ChF90 had highest hexose content (4.60 ± 0.12 mg/mL). Prebiotic effects were notable in fractions ChF91 and ChF90, which significantly enhanced the proliferation of *Bifidobacterium bifidum* and *Bifidobacterium clausi*, achieving cell counts of 9.11 ± 0.17 log10 CFU/mL and 9.96 ± 0.30 log10 CFU/mL, respectively, at 48 hours. The acidifying effect was strongest in EPP and ChF91 fractions, with pH levels dropping to 4.14 ± 0.13 and 4.46 ± 0.28. Prebiotic index and score analyses further highlighted ChF91 and ChF90 for their potent prebiotic potential. The prebiotic analysis highlighted ChF91 with the highest scores for both *B. bifidum* (5.67 ± 0.13) and *B. clausi* (5.47 ± 0.23), while ChF90 showed a strong prebiotic index (2.15 ± 0.07) for *B. bifidum* and (3.25 ± 0.12) for *B. clausi*. These findings support the potential for developing functional foods, paving the way for novel dietary interventions and functional food development to enhance gut health.

Keywords: Chia seed mucilage, Oligosaccharides, *Pichia pastoris* fermentation, Probiotics

Wheat exopolysaccharide-mediated green synthesis of nickel oxide nanoparticles with enhanced antioxidant activity

Faryal Ashfaq, Mahnoor, Yusra Jamil, and Ayaz Ahmad

Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan

Correspondence: ahdayazb5@awkum.edu.pk

Nanoparticles, fundamental to nanotechnology, are known for their distinct chemical, optical, and mechanical properties, which differ significantly from their bulk material counterparts. Due to increasing environmental toxicity concerns, this study focused on the eco-friendly biosynthesis of metallic nanoparticles (MNPs) using exopolysaccharides (EPS) extracted from endophytes of *Triticum aestivum* (wheat). The process involved dissolving 10 mg of wheat seed polysaccharides in distilled water, followed by mixing with nickel nitrate to achieve a final concentration of 10 mM. The formation of nickel oxide nanoparticles (NiO-NPs) was indicated by a color change and confirmed through UV-visible spectroscopy. The green-synthesized MNPs were characterized using double-beam UV and FT-IR spectroscopy to identify the specific biomolecules involved in their formation, with X-ray diffraction (XRD) analysis determining their crystalline structure. The biological potential of these nanoparticles was assessed through various bioassays, including antioxidant activity evaluation using the DPPH radical scavenging method and hydrogen peroxide inhibition. Among all screened samples, NiO NPs showed the most significant antioxidant potential, with an EC50 value of 37.93 µg/mL, followed by EPS at 66.04 µg/mL. At 200 µg/mL, NiO NPs effectively scavenged the H2O2 free radical by 81.9%±0.24, followed by EPS at 76.54%±0.65. These results highlighted the effectiveness of NiO NPs, emphasizing their potential for sustainable applications in nanomedicine and nanobiotechnology.

Keywords: Eco-friendly synthesis, Nickel oxide nanoparticles, Exopolysaccharides, *Triticum aestivum*, Antioxidant activity

Chemical composition and nutritional potential of some selected macro-fungi genotypes from the lower Hindukush Dir Upper, Khyber Pakhtunkhwa

Tariq ullah, Khan Sher, Muhmmad Ibrahim, Mohammad Abohashrh

Department of Botany, SBBU, Dir Upper, NIFA, Peshawar, Pakistan

Correspondence: khansher@sbbu.edu.pk

Naturally occurring wild mushrooms present an important source of Non-Timber Forest Product (NTFP) which comprises different substances and services. NTFP serves particularly as food and income through the hunting of locally dwelling communities all over the world. Twenty (20) naturally occurring mushrooms were collected from five (5) locations (Barawal, Sheringal, Usheray, Lowari top, and Upper Dir) of the Dir-Upper District of Malakand Division. These mushrooms are identified as medicinal (*Ganoderma applanatum, Astraeus hygrometricus, Pseudoinonotus dryadeus, Ganoderma adspersum, Ganoderma resinaceum), Edible/Nutritional (Perenniporia fraxinea, Agrocybe pediades, Pleurotus dryinus, Phallus hadriani*, *and Macrolepiota procera*), un-known status (*Bolbitus scoprophilus,* and *Russula grata*), and poisonous mushroom (*Russula cerolens*, *Amanita bisporigera*, *Agaricus californicus,* and *Scleroderma citrinum*). The mushrooms were analyzed for chemical and nutritional composition at the Food Testing Laboratory, Nuclear Institute for Food and Agriculture (NIFA), Peshawar. Percent (%) protein ranged from 5.5 to 26.7, where the highest value was recorded in *Astraeus hygrometricus* mushroom followed by 21.5 in *Amanita bisporigera* mushroom. The percentage (%) fat determined ranged from 1.7 to 9.9, where the highest value was recorded in *Phallus hadriani* (9.9%) which was followed by *Macrolepiota procera* (9.4%). Percent (%) fiber varied from 8.1 to 22.8, where the highest valve was recorded from *Ganoderma applanatum* followed by *Ganoderma resinaceum.* Sodium (PPM) ranged from 1.5 to 21.3, where the highest sodium value was recorded in *Macrolepiota procera* (21.3 PPM) followed by *Phallus hadriani* (14.9 ppm). Similarly, the potassium (ppm) value ranged from 17.3 to 175, where the highest (175) was recorded in *Ganoderma adspersum* followed by *Agaricus californicus* (123.9). Calcium (ppm) ranged from 4.8 to 54.2, which was high in *Ganoderma resinaceum followed* by *Ganoderma applanatum* (53.6). The mean (%) total phenols detected varied from 0.2 to 2.51% where it was highest in *Macrolepiota procera* (2.51) followed by *Phallus hadriani* (2.33). More studies are needed to explore macro-fungi (mushrooms) for their natural occurrence, types of mushrooms, and their utilization/export. Moreover, these studies will encourage local people to hunt important medicinal and nutritious mushrooms create self-employment, and increase their sources of income.

Keywords: wild growing, Macrofungi, Chemical composition, Nutritional potential, Hindukush, Dir upper, Pakhtunkhwa, Pakistan

Construction of Photobioreactor for Algal Cultivation: An Approach towards Eco-friendly Wastewater Treatment

Aqeela Bibi, Sidra Pervez

Department of Biochemistry, SBBU Women University, Peshawar, Pakistan

Correspondence: sidrapervez87@gmail.com

Photobioreactors (PBRs) are essential systems for cultivating algae in controlled environments, playing a vital role in various applications, including textile wastewater treatment. This research provides an overview of photobioreactor technology and its applications in algae cultivation, optimization, and use for textile wastewater treatment. PBR design, operation, and optimization are covered, with an emphasis on important variables that affect the development of algae and the effectiveness of wastewater treatment, including light intensity, temperature, pH, medium choice, and nutrient availability. Algal wastewater

treatment has drawn interest since it is both economical and environmentally beneficial. The primary goal of this study was to gather and analyze microalgae from local sources. Using microscopy, two types of algae (referred to as A and B) were identified. The best growth medium for these microalgae was subsequently identified by culturing them in three distinct nutritional media (BBM, Fog's, and M4N) with differing contents. The growing of microalgae samples in a photobioreactor involved the regulation of many parameters, including temperature, pH, and culture length. Anthrone, Lawry's, and DNS tests were among the techniques used to define the microalgae according to characteristics including total protein, total carbohydrate, and total sugar content. The results indicated that sample A was identified as Chlorella, while sample B was Spirogyra. The highest concentrations of carbohydrates (173.2mg/ml), protein (133mg/ml), and sugar (59.6mg/ml) were observed at 40°C in Fog’s medium for sample A, and at 25°C in BBM for sample B (carbohydrates: 208mg/ml, protein: 162.2mg/ml, sugar: 62.3mg/ml). Spirogyra exhibited its highest growth and concentration at pH 5 in Fog’s medium (carbohydrates: 185.7mg/ml, protein: 188.5mg/ml, sugar: 94.97mg/ml), and at pH 8 in BBM (carbohydrates: 208mg/ml, protein: 192.9mg/ml, sugar: 100mg/ml). The fifteenth day of culture showed the peak activity and distinct characteristics of the microalgae, with sample A reaching concentrations of 173.2mg/ml carbohydrates, 133mg/ml protein, and 59.6mg/ml sugar in Fog’s medium, and sample B reaching concentrations of 207mg/ml carbohydrates, 192.9mg/ml protein, and 100 mg/ml sugar in BBM. By using extracellular enzymes and photosynthetic assimilation of organic compounds, algae can effectively lower the COD levels (41%) from 2875 mg/L up to 1189 mg/L and BOD levels (20%) from 7521 mg/L up to 1510 mg/L in wastewater. Overall, monitoring both COD and BOD in algae-based wastewater treatment allows for a comprehensive assessment of the organic pollutant removal efficiency and the overall treatment performance.

Bio-Scouring of Cotton Fabric and Removal of Waxy Layer by Pectinase for an Eco-friendly Approach to Textile Processing

Salma Zeb, Sidra Pervez
Department of Biochemistry, SBBU Women University, Peshawar, Pakistan
Correspondence: sidrapervez87@gmail.com

Pectinases are a class of mixed enzymes that break down pectic materials, which are primarily found in higher plants and microbes. The group of enzymes known as pectinase catalyzes the synthesis of pectin-containing materials by microorganisms and plants. By eliminating hydrophobic and non-cellulosic elements from the fibers, such as waxes, pectin, and proteins, the process of scouring improves the absorbency and whiteness of the fabric. It is a more environmentally friendly process than traditional alkaline scouring. Which involves treating the fiber with powerful sodium hydroxide, which is efficient but harmful to the fabric. However, bio-scouring is a successful method of scouring the cloth that doesn't harm it or the environment. Yeast, bacteria, fungi,

and plants are the various natural sources of pectinase. Different microbial sources were employed and among them, *Aspergillus niger* was found to be the highest producer of this enzyme. Optimization revealed that the optimal enzyme concentration for the procedure adjusting the enzyme concentration to 5 ml at 50 degrees for 30 min at pH 10.5 this duration broke down the pectin’s effectiveness. The bio-scouring efficiency tests revealed a substantial 43% reduction in fabric weight, indicative of successful waxy layers removal furthermore by wicking test fabric was able to get wet up to 3 cm in 30 seconds. This improvement implies that the fabric structure's improved liquid absorption and transportation capacities were made possible by the bio-scouring treatment. In the degree of whiteness test performed on the bio-scoured cotton fabric. The treated fabric's appearance of being lighter and brighter specifically demonstrated how well the bio-scouring method improved the fabric's optical qualities. After applying congo red dye to the treated fabric, there was a distinct and evenly distributed area that showed active dye absorption and spreading. Hence, fungus-derived stable pectinase enzymes can reduce the environmental impact of chemical bio-scouring in textile processing, increase productivity, and promote safer, more environmentally responsible procedures in the textile sector.

Rice seeds biofortification using biogenic iron oxide nanoparticles synthesized by using *Glycyrrhiza glabra*: a study on growth and yield improvement

Sidra Ahmad, Nayab Ahmad, Iqbal Munir, Mian Afaq Ahmad
Inst. of Biotechnol. & Genetic Engg., University of Agriculture Peshawar Pakistan
Correspondence: sidraa267@gmail.com

Iron, a crucial micronutrient, is an integral element of biotic vitality. The scarcity of iron in the soil creates agronomic challenges and has a detrimental impact on crop vigor and chlorophyll formation. Utilizing iron oxide nanoparticles (IONPs) via nanopriming emerges as an innovative method to enhance agricultural efficiency and crop health. The objective of this study was to synthesize biogenic IONPs from Glycyrrhiza glabra (*G. glabra*) plant extract using green chemistry and to evaluate their nanopriming effects on rice seed iron levels and growth. The synthesized IONPs were analyzed using UV–Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), Scanning electron microscope (SEM), Transmission electron microscopy (TEM), and Energy-dispersive X-ray (EDX) techniques. The UV–Vis peak at 280 nm revealed the formation of IONPs. SEM and TEM showed that the nanoparticles were spherical and had an average diameter of 23.8 nm. Nanopriming resulted in a substantial enhancement in growth, as seen by a 9.25% and 22.8% increase in shoot lengths for the 50 ppm and 100 ppm treatments, respectively. The yield metrics showed a positive correlation with the concentrations of IONPs. The 1000-grain weight and spike length observed a maximum increase of 193.75% and 97.73%, respectively, at the highest concentration of IONPs. The study indicates that *G. glabra* synthesized IONPs as a nanopriming agent significantly

increased rice seeds' growth and iron content. This suggests that there is a relationship between the dosage of IONPs and their potential for improving agricultural biofortification.

In vitro antibacterial and antifungal activities of different solvent-extracted samples from the stem of *Euphorbia heliscopia*

Jehan Bakht, Faiza, Mohammad Shafi and Arshad Iqbal

Inst. of Biotechnol. and Genetic Engg., University of Agriculture Peshawar Pakistan

Correspondence: amarjan8922@yahoo.com

This paper presents the anti-microbial potentials of methanol, ethyl acetate, n-hexane, butanol, and water-extracted samples from the stem of Euphorbia *heliscopia* against S. aureus (Gram-positive), B. subtilis (Gram-positive), P. aeruginosa (Gram-negative), K. pneumonia (Gram-negative), E. coli (Gram-negative), C. albicans (fungal specie) by discs diffusion susceptibility assay using 0.5 and 1mg [disc. sup.-1] concentrations. Our results showed that all the extracted samples from the stem of *E. heliscopia* exhibited varying degrees of antimicrobial activity. Ethyl acetate extracted samples measured maximum activity against the studied microbial species followed by the n-butanol and crude methanolic extract; n-hexane extracted samples inhibited the growth of all microbial species except P. aeruginosa and E. coli at lower concentrations. Aqueous fractions showed inhibitory activity against B. subtilis, K. pneumonia, and C. albicans. The most susceptible gram-positive bacteria were S. aureus while B. subtilis was the most resistant one. Among Gram-negative bacteria, P. aeruginosa showed more susceptibility while K. pneumonia was resistant.

Persimmon (Diospyros Kaki) and date plum (Diospyros lotus) leave hidden phytochemicals and health claims

Sundus Malik[1], Dawood Ahmad1, Aqib Iqbal[2], Iqbal Munir1, Syed Tariq Shah[3]
Amir Muhammad Khan[1], Sara Khan[1], Muhammad Tayyab[1]

[1]IBGE, University of Agriculture, Peshawar; [2]Deptt. of Agronomy, Abdul Wali Khan University, Mardan, Pakistan; [3]NIFA, Peshawar, Pakistan

Correspondence: Sundusmalik651@gmail.com

Diospyros kaki and Diospyros lotus commonly known as Japanese persimmon and date plum belong to the Ebenaceae family and are known for their medicinal properties. The presence of bioactive compounds in different parts of Diospyros kaki and Diospyros lotus have an effective role in the treatment of various diseases especially the leaves of these plants possess alkaloids used for pain relieving, anticancer, antihyperglycemic, analgesic, and anti-inflammatory activity. The presence of flavonoids responsible for the radical scavenging activity, antimutagenic activity, and reducing inflammation in the body, tannin is known for its nutritional value and is helpful against hyperlipidemia, hyperglycemia, dropping the rate of strokes and also possesses antimicrobial activity. The presence of phytosterols has anti-inflammatory, antipyretic, anticancer, anticholesterol, anti-diabetic, and treating obesity. The present study aimed to evaluate the methanolic, n-hexane, ethyl acetate, N-butanol, and aqueous extracts of Diospyros kaki and Diospyros lotus leaves for its phytochemical study. The methanolic, ethyl acetate and N-butanol ethanolic extracts of Diospyros kaki and Diospyros lotus leaves showed the presence of alkaloids, flavonoids, tannins, saponins, phytosterols, and proteins. The isolation of these bioactive compounds can be of great significance in treatments.

Keywords: Diospyros kaki, Diospyros lotus, Ebenaceae, extracts, persimmon, date-plum, antimicrobial activity.

Pharmacological and Therapeutic Potential of *Azadirachta Indica* (Neem) Aerial Part.

Junaid Zada , Saima Naz , Iqbal Munir, Faiza Aamir

IBGE Agriculture University Peshawar

Correspondence: Junaidzada90@gmail.com

The use of medicinal plant extract-mediated treatment was found to be very effective in controlling bacterial infection. The plant A.indica aerial parts were evaluated against gram-negative pathogenic bacteria *S. aureus*, *E. coli*, *S. typhi*, and *P. aeruginosa*. The methanolic extract of A. indica aerial parts also possesses moderate antifungal activity against *F. oxysporumand*, *A. Niger*. The MeOH. Ext and Aq Ext of A. indica aerial parts were screened for insecticidal activity, against three different insect species i.e. Triboliumcastaneum, Rhyzoperthadominica, and Callosobruchusanalis, revealed that MeOH Ext and Aq Ext of the plant showed significant activity against the tested insects except MeOH and aqueous Extract. The methanolic extract showed significant results in growth inhibition at concentrations of 1000 and 100 µg/ml, respectively. No phytotoxic activity was observed at a concentration of 10 µg/ml. The methanolic Ext and Aq Ext of A. indica aerial parts show no hemagglutination activity against human red blood cells.

Keywords: Antibacterial activities, Fungicidal activities, Anti insecticidal, Hemagglutination activity.

Development of industrial dye decolorization agents using bacterial strains for bioremediation of polluted water

Misbah ul Haq*, Muhammad Amin

Department of Environmental Sciences, SBBU, Sheringal, Dir Upper, KP, Pakistan

Correspondence: misbahulhaqsgl@gmail.com

Dyes have been in use from the age of antiquity and has consumption increased over time as the population and industries have extended. The synthetic dyes are increased as a result, which pollutes the environment severely and has a worse impact on the surroundings. The azo dyes degrade the environment because of their huge consumption which needs proper attention to be handled. Biological solutions are developed to make the issue normalized, as the bacteria are considered the best organism for the said remedy. The current study tries to provide bioremediation through biological organisms which is helpful to provide costless and non-toxic procedures. The discharge of wastewater from textile and dye industries causes acute environmental issues. Therefore, the current study

tries to provide bioremediation through biological organisms. It is observed that the physical and chemical characteristics of the textile effluents decreased significantly by the consortium BP to a lesser level below the standard limit. Now it is possible for irrigation of rural areas and agriculture should be maximized as treated wastewater, is purified in a huge amount. Because of phototoxicity testing consortium BP is a good source to mineralize this dye into nontoxic metabolites, as it is observed by the tests. This fact was discovered when the research was conducted for the elimination of Chemical Oxygen Demand (COD) and Total Oxygen Demand (TOC). The consortium BP and individual strains are considered more effective in the decontamination of the chemical concerning biodegradation investigation by the creation of both metabolisms. the collected data from the biodegradation process build up the best knowledge about the variation of anthraquinone-based dyes in the environment which contribute to coop with the problems that are present at low and high industrial levels.

Investigation of groundwater quality in Sheringal Valley, Dir Upper, Pakistan

Said Hakim, Fazli Aziz*, Allah Ditta, Aroosa Sher

Department of Environmental Sciences, SBBU, Sheringal, Dir Upper, KP, Pakistan

Correspondence: aziz@sbbu.edu.pk

Water especially clean and safe drinking water plays a vital role in human life. Though its importance is non-ignorable its quality is deteriorating day by day due to various natural and anthropogenic operations. The objective of the study was to assess the physicochemical and bacteriological parameters of the drinking water quality of Sheringal Valley. Twenty-six samples were collected/obtained from bore wells and were checked for several various parameters following standard protocols. The results obtained were within the permissible limit of the World Health Organization (WHO) standard except few factors that were slightly crossing the established limit (turbidity and Electrical conductivity). It was found that the level of potassium in most samples was less than the permissible limit of WHO. It is concluded that the status of water quality of the area was safe for drinking. A Piper plot was used for water classification and water chemistry was measured via Gibbs diagram. The rock-water interaction was mostly involved in water chemistry. Further research on metal contamination and the possible source identification is recommended in the area.

Clinical and molecular Diagnosis of Marfan Syndrome (OMIM:154700) for mutation in FBN1 gene

Munir Alam[1], Raqeeb Ullah[1,2]

[1]Qurtuba University of Sci.& Information Technol.; [2]Bacha Khan Univ., Charsadda

Correspondence: nraqeeb@gmail.com

Marfan syndrome is an autosomal dominant disorder of connective tissues that occurs due to a change in the FBN1 gene; responsible for an extracellular matrix

protein formation that plays a crucial role in soft connective tissues. This syndrome is clinically characterized by Arachnodacty, Scoliosis, Ectopia lentis, and Aortic heart aneurysm. In minor criteria, it also includes *Pectus excavatum* and wrist and thumb signs. Due to the high frequency of this disease and our interest in genetic anomalies, we target Marfan syndrome to investigate its genetic causes in district MALKAND especially in tehsil DERGAI. For this purpose, the most affected family was targeted and recruited 200 individuals from the surrounding population. For clinical confirmation in the selected family of MFS, we took the help of different specialized and concerned physicians (Cardiologists, Dentists, Ophthalmologists, and Orthopedics) and for genetic analysis, we extracted their DNA from venous blood through organic phenol method and Sanger sequenced it, after receiving fast q file the Insilco analysis through online and offline software was carried out and find out that there is a deletion mutation in exon 49 (6012-6013del), (TYR2004ter) of the affected individuals of the targeted family while the unaffected members of the family and controlled group stayed un mutated for this disease.

Eco-Friendly Synthesis of Iron Oxide Nanoparticles from *T. ammi* Extract: Impact on Rice Growth and Iron Uptake

Nayab Ahmad, Sidra Ahmad, Iqbal Munir, Mian Afaq Ahmad
Correspondence: nayabahmadkhan8314@gmail.com

Iron is a crucial micronutrient essential for the growth and health of plants. Its deficiency poses a significant challenge in agriculture, impacting the nutritional quality and health of key crops. This study explores the green synthesis of iron oxide nanoparticles (IONPs) using *T. ammi* seed extract and evaluates their effect on rice plant growth and iron uptake. Characterization of the synthesized IONPs was conducted using UV-visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and Fourier transform infrared spectroscopy (FTIR). UV-visible spectra revealed a peak at 358 nm, while XRD analysis indicated a crystallite size of 5.50 nm. SEM showed nanoparticles with an average size of 4.8 nm, and EDX confirmed elevated concentrations of iron and oxygen. FTIR analysis identified various functional groups involved in the nanoparticle synthesis. Foliar application of IONPs at a concentration of 100 mg/L led to significant improvements in rice growth, including increased root and shoot length (117.6% and 75% enhancements, respectively) and higher root and shoot weight compared to control plants. Additionally, chlorophyll a and b levels increased by 115% and 146%, respectively. Iron content in treated plants showed remarkable improvements, with root and shoot iron levels rising by 318.4% and 466.4%, respectively. These findings suggest that iron oxide nanoparticles positively influence rice plant growth and iron translocation. The potential for applying these nanoparticles to other crops like wheat and maize is also promising.

The potential of rhizospheric fungi to reduce copper and lead toxicity in Triticum aestivum L.

Naveen Dilawar, Anwar Hussain
Department of Botany, Abdul Wali Khan University Mardan, Pakistan
Correspondence: naveendilawar26@gmail.com

Plants are exposed to various heavy metals and other environmental stresses during anthropogenic evolution. The most common heavy metal contaminants are lead (Pb) and copper (Cu), which can be found in both natural and industrial settings. Microorganisms play a crucial role in the biotransformation of heavy metal-contaminated environments. In this study, four rhizospheric fungus strains were extracted from *Parthenium hysterophorus* L. and tested for their tolerance against lead and copper. These strains were selected for their ability to promote plant growth under heavy metal stress. When inoculated to Triticum aestivum L. under heavy metal stress, the fungal isolates NMG showed promising results in the form of increased secondary metabolites, hormones, and enzymes. Different concentrations of specific heavy metals (Pb25, Pb75, Cu100, Cu200, Pb25+Cu100, and Pb75+Cu200g/mL) were applied to wheat plants. The plants exposed to metal stress without microbial inoculation displayed a reduction in growth. However, when rhizospheric fungal inoculation restored plant growth and development, showed the symbiotic relationship between the fungus and the host plant roots under heavy metal stress conditions. The isolates can transform HMs, making them unavailable for plant uptake and accumulation, which prevents harm to plants. Apart from their bioremediation properties, some isolates can create secondary metabolites like IAA, GA, MDA, ABA, H2O2, Electrolytic leakage, SA, lipid, DPPH, flavonoids, phenols, and proteins, which can stimulate plant growth and make them suitable for use as bio-fertilizers and bio-remediating agents in areas with high levels of heavy metal contamination.

Acinetobacter bamannii: A rising threat in nosocomial infections in Islamabad, Pakistan

Naila Imtiaz[1], Irum Khalil[1], Hazir Rahman[2], Raham Sher Khan[1*]
[1]Deptt. of Biotechnol.y; [2]Deptt. Microbiology, Abdul Wali Khan University Mardan
Correspondence: rahamsher@awkum.edu.pk

Acinetobacter Baumannii has been a primary cause of nosocomial infections worldwide including Pakistan, causing significant morbidity and mortality. The purpose of this study was to investigate the recent infection frequency and antimicrobial resistance over the last 6 months in multiple tertiary care centers in Islamabad Pakistan. This prospective study investigates the occurrence and antimicrobial resistance of *A. baumannii*, recovered from clinical specimens that were referred to the Microbiology Laboratory of Islamabad Diagnostic Center Islamabad Pakistan. All bacterial strains were confirmed by MALDI-TOF. Susceptibility testing was done through the Modified Kirby Bauer Disc diffusion method. The results were interpreted according to the Clinical and Laboratory Standards Institute (34th Edition). Only the hospitalized patient's specimens were included in the study to know the actual frequency of this pathogen among the nosocomial infections. The data from January 2024 to July 2024 was recorded and analyzed. Sociodemographic characteristics and laboratory record data were analyzed using SPSS, version 25. A chi-square test was applied to see the significance. Of 11000 clinical samples, *A. baumannii* strains were detected in 64 of them. The majority of the clinical samples, 19 were from pus swabs (29%) followed by sputum and tracheal as 10 (15%) of total samples, 3 each from blood, tissue wound ETT CSF, and Tracheal fluid (4%), 1 sample from each of synovial fluid and psoas abscess had the pathogen. Further, *A. baumannii* has been found in 35 men (54.68%) and 29 women (45.31%). In all patients, sensitivity for minocycline (MN30 µg), colistin (CT10µg), polymyxin B (PB 300µg), and tigecycline (15 µg) were observed at 100% while tobramycin (TOB10µg) was 25% sensitive which indicated the potential viability of these drugs to treat highly resistant MDR and XDR *Acinetobacter* infections. The increased frequency of XDR supports the need for continuous surveillance to determine the prevalence and evolution of XDR A. *baumannii* in Pakistan. Colistin, tigecycline, minocycline, and polymyxin B remain the possible line of drugs to treat XDR Acinetobacter.

Keywords: A. baumannii group, Acinetobacter spp., antimicrobial resistance, extended-spectrum β-lactamases, multidrug resistance

Green Synthesis of ZnO Nanoparticles Using *Selenicereus grandiflorus* for Managing *Meloidogyne incognita* in Sustainable Agriculture

Sidra Ahmad, Nayab Ahmad, Iqbal Munir, Mian Afaq Ahmad

Institute of Biotechnology and Genetic Engineering, UAF, Peshawar Pakistan

Correspondence: sidraphd267@gmail.com

Nanotechnology offers innovative approaches to enhance crop yields and address the rising global demand for food, feed, and energy while promoting sustainable agricultural practices. Meloidogyne incognita, a significant plant-parasitic nematode, inflicts substantial damage on a wide range of vegetable crops. This study aimed to investigate the phytochemical profile of *Selenicereus grandiflorus*, synthesize zinc oxide nanoparticles (ZnO NPs) using this plant, and assess the efficacy of these nanoparticles in managing M. incognita. ZnO NPs were synthesized from *S. grandiflorus* extract and characterized using UV-Vis spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDX). The nematicidal effects of both the plant extract and its ZnO NPs were evaluated against M. incognita. Phytochemical analysis of *S. grandiflorus* extract revealed various active compounds. ZnO NPs demonstrated superior nematicidal activity compared to the plant extract alone. At 24 hours, the highest nanoparticle concentration (1000 µg/ml) achieved 88.5% mortality, while the lowest concentration (100 µg/ml) resulted in 25.33% mortality. After 48 hours, the highest concentration led to 96.3% mortality, with the lowest concentration resulting in 33.5%. At 72 hours, the highest concentration reached 99%

mortality, and even the lowest concentration showed effective activity at 87.83%. Increasing the nanoparticle concentration and incubation period significantly improved mortality rates. These findings suggest that ZnO NPs synthesized from *S. grandiflorus* have promising potential as eco-friendly bionematicides. They offer a sustainable alternative to conventional chemical nematicides and may also have medicinal uses.

Preparation of modified g-C_3N_4 photocatalyst for the degradation of organic pollutants in water

Abdul Khaliq Jan

Department of Chemistry, SBBU, Sheringal Dir, Khyber Pakhtunkhwa, Pakistan

Correspondence: abdukhaliq@gmail.com; abdukhaliq@yahoo.com

With the advancement in human lifestyle, industrialization, and human population, pollution is an emerging global concern, as it negatively impacts various water bodies and poses severe threats to both human and aquatic life. Among other techniques, Oxidation-Reductive is one of the valid options to eliminate undesired compounds from water, due to their wide applicability, efficient removal of pollutants without producing other harmful compounds, and rapid reaction rates. For an effective photocatalyst, the band gap, thermal stability, sufficient absorption of visible light, high electronic conductivity, and recombination rate of photogenerated electron-hole pairs are the big issues. To overcome these problems and improve the photocatalytic performance catalyst, diverse strategies have been developed in recent years. Doping of nonmetals to extend the visible light absorption by creating surface states; Coupling of nanosized metal oxides onto the surface of g g-C_3N_4 for enhancing the charge separation. Modification of the surface of g-C_3N_4 with inorganic acids for promoting the O_2 adsorption. Our current research is based on the synthesis and characterization of Graphitic carbon nitride (g-C_3N_4), a semiconductor polymeric photocatalyst. It has attracted growing attention in the field of visible-light-induced photocatalysts, because of its facile synthesis procedure, low band gap energy, light absorption in the visible spectrum, easy functionalization, attractive electronic band structure, and high physicochemical stability. Various techniques such as UV/Vis., IR, XRD, etc. techniques are used to characterize the synthesized Graphitic carbon nitride products. Photocurrent activity and bandgap are identified through standard techniques.

Keywords: Energy, environment, synthesis, photocatalyst, g-C_3N_4

Synthesis, Spectroscopic Characterization, and Application of Ag-Cu Hybrid Nanoparticles for Electrochemical Sensing of Alizarin Red S

Urooj Shahzadi*, Latif Ur Rahman

Institute of Chemical Sciences, University of Peshawar, KPK 25120, Pakistan

Correspondence: latif.wardag@uop.edu.pk

An efficient, simple, and highly sensitive electrochemical sensor for the detection of Alizarin Red S (ARS) in aqueous solution employing a glassy carbon electrode modified by drop coating of bimetallic Ag-Cu hybrid nanoparticles along with a conducting polymer polyvinylpyrrolidone is reported in this work. In this regard, bimetallic Ag-Cu hybrid nanoparticles have been fabricated using the polyol chemical reduction method. The structural characterization of the synthesized NPs was investigated by X-ray diffraction (XRD) spectroscopy, Ultraviolet-visible spectroscopy, scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS). The crystallinity degree and phase transformation of the nanoparticles were seen in the XRD pattern. The proposed BNP-based electrochemical sensor was connected to simultaneously identify ARS. The true surface of developed composite electrodes as well as their surface charge, permeability, and charge transfer resistance have been determined using electrochemical characterization techniques i.e., Electrochemical impedance spectroscopy (EIS), and cyclic voltammetry (CV). A low limit of detection (LOD) of 0.86 µM and a sensitivity of 0.4218 $\mu A \cdot \mu M^{-1}$ resulted when the calibration curve was plotted under optimal conditions, in the concentration range of 1.5 µM to 13.5 µM, suggesting that the Ag-Cu (1:1) designed electrochemical sensor exhibited optimal sensing characteristics for the detection of Alizarin Red S at its lowest possible concentrations.

Keywords: Electrochemical Sensors, Nanoparticles, Dyes,

Synthesis and Characterization of Ag-Ni Bimetallic Nanoparticles, and their Application to Sense Alizarin Dye (Alizarin Red S)

Abdullah, Latif-ur-Rahman*

Institute of Chemical Sciences, University of Peshawar, KP, Pakistan

Correspondence: latif.wardag@uop.edu.pk

Ag-Ni Bimetallic Nanoparticles (BNPs) were synthesized using the co-precipitation method for the electrochemical sensing application to detect Alizarin Red S (ARS). The as-synthesized Ag-Ni BNPs were analyzed using Four Transfer inferred spectroscopy (FTIR), Ultraviolet-visible spectroscopy, Scanning electron microscopy (SEM), Energy dispersive spectroscopy (EDS), and X-ray diffraction (XRD) spectroscopy. Ag-Ni-BNPs exhibit a stretching band at 439.8 cm^{-1} in their FTIR spectra, which could be indicative of metal-metal or metal-oxygen bond vibrations. The surface morphology and average particle size were determined by the SEM to be 20 nm, whereas EDX analyzed the nanocomposite's compositional analysis. Ag-Ni BNPs' face central cubic (fcc) crystal structure and crystallinity were confirmed by XRD investigation, which further verified their average crystallite size to be 23.37 nm. The electrochemical methods were applied using cyclic voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) techniques. A polished glassy carbon electrode (GCE) was modified by the synthesized Ag-Ni BNPs employing the drop coating method. The ARS depicts the adsorption-controlled process. The obtained linear regression, characterized by R^2=0.98, shows a good limit of detection for Ag-Ni 3:1, with a limit of detection of 8.5 µM and a limit of quantification of 25.6 µM respectively. Ag-Ni 3:1 electrode exhibited good sensitivity and reliability in the simultaneous electroanalysis of ARS.

Keywords: Electrochemical Sensors, Bimetallic Nanoparticles, Dyes

Application of acetylsalicylic acid to study growth of wheat under saline conditions

Zafar Hayat Khan*, Rafi Ullah & Waleed Khan
Department of Agronomy, Abdul Wali Khan University Mardan
** Corresponding Author: zafarhayat@awkum.edu.pk*

The purpose of this study was to find out how Acetylsalicylic acid (ASA) affected wheat (*Triticum aestivum*) growth in saline environments. Utilizing a fully randomized design (CRD) with three levels of salinity (0 mM, 50 mM, and 100 mM NaCl) and three doses of Acetylsalicylic acid (0 mM, 2.5 mM, and 5 mM), the experiment was carried out in the Department of Agronomy at Abdul Wali Khan University Mardan. The main goals were to assess how ASA affected the length of the roots and shoots, the fresh and dry weights of the roots and shoots, and the amount of chlorophyll in wheat at varying salt stress levels. The results revealed significant differences among treatments. For root length, plants at 0 mM NaCl and 2.5 mM ASA exhibited the longest roots (25.88 cm), while the shortest roots (14.41 cm) were observed in plants at 100 mM NaCl and without application of ASA. Average shoot length was highest (27.27 cm) in plants treated with 2.5 mM ASA at 0 mM of NaCl; lowest shoot length (15.74 cm) was recorded in untreated plants (0 mM ASA) at 100 mM NaCl. Root fresh weight was maximum (1.25 g) at 0 mM NaCl with 2.5 mM ASA while it minimized (0.54 g) at 100 mM NaCl without ASA. Similarly, shoot fresh weight peaked (1.414 g) at 2.5 mM ASA dose under 0 mM NaCl and was lowest (0.74 g) at 100 mM NaCl without ASA application. Dry weights followed similar trends. Root dry weight was highest (0.13 g) in plants treated with 2.5 mM ASA under 0 mM NaCl while lowest (0.05 g) at 100 mM NaCl without ASA. Shoot dry weight was also highest (0.14 g) at 2.5 mM ASA and 0 mM NaCl while recorded lowest (0.074 g) at 100 mM NaCl without ASA. Chlorophyll *a* content was significantly enhanced by ASA treatment, with the highest content (27.07 mg/g) at 2.5 mM ASA and 0 mM NaCl and the lowest (16.69 mg/g) at 100 mM NaCl without ASA. These findings demonstrate that ASA, particularly at a concentration of 2.5 mM, significantly alleviates the adverse effects of salinity on wheat growth by improving root and shoot lengths, fresh and dry weights, and chlorophyll *a* content. This study highlights the potential of Acetylsalicylic acid as a growth regulator to enhance wheat performance under saline conditions. Further research should explore the underlying mechanisms and field-level applications to validate these results.

Keywords: Salinity, Acetyl Salicylic Acid, Wheat**,**

Genetic associations of factor II and factor V Leiden with thrombosis in affected individuals of district Peshawar

Deena Jamal and Sajid Ali*
Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan

*Correspondence: sajidali@awkum.edu.pk

Cardiovascular disease (CVD) is an umbrella term for conditions affecting the heart or blood vessels. It is generally associated with an increased risk of blood clots and atherosclerosis, or the build-up of fat deposits within the arteries. The factor V Leiden and the factor II prothrombin mutations are prevalent genetic variables linked to thrombosis. They increase the risk of thrombotic events by altering the activation of protein C resistance and prothrombin levels, respectively. Genetic screening for Factor II Prothrombin and Factor V Leiden variants is essential for risk assessment, diagnosis, and treatment to enable targeted interventions such as anticoagulant therapy and thrombotic patient management. According to some clinical research, high-risk cardiac families had higher frequencies of factor II and factor V type mutations. This investigation aims to examine the variants rs6025 for Factor II and rs1799963 for Factor V and determine their association with cardiovascular disease pathology in Peshawar, Khyber Pakhtunkhwa. The rs1799963 and rs6025 of factors V and II will be examined for genotype and allele frequencies in the cardiac patients using ARMS-PCR, and their association will be determined using logistic odd ratios. Blood samples from cardiac patients in Pakistan who are infected will be obtained, and routine phenol-chloroform extraction will be performed. Moreover, SPSS, or the Statistical Package for the Social Sciences, will be used for statistical analysis. Critical understanding gained from these findings will be crucial for future improvements in both epidemiology and therapy.

Keywords: Factor II, Factor V, rs6025, rs1799963, Khyber Pakhtunkhwa

Cost-effective production of oligosaccharides from cress seed mucilage via microbial bioprocessing for biological applications

Imdad U. Khan[1], Yusra Jamil[1], Aqsa[1], Mansoor Ali[1], Amjad Iqbal[2], Ayaz Ahmad[1*]
[1]Department of Biotechnology; [2]Department of Food Science and Technology,
Abdul Wali Khan University Mardan KP, Pakistan
**Correspondence: ahdayazb5@awkum.edu.pk*

Oligosaccharides are getting attention for their health benefits, but their production is often costly. The current study introduces a novel, cost-effective, and sustainable method for converting cress seed mucilage polysaccharides into valuable bioactive oligosaccharides using *Pichia pastoris*. This research investigated these oligosaccharides' antioxidant, anti-inflammatory, antidiabetic, and prebiotic effects. Polysaccharides extracted from cress seed mucilage were subjected to fermentation and Size Exclusion Chromatography based separation. The results revealed that *P. pastoris* effectively digested and converted them into oligosaccharides. The gas chromatography-mass spectrometry (GC-MS) analysis showed that the oligosaccharides fractions comprise key monosaccharides components, including galacturonic acid, glucuronic, rhamnose, arabinose, and maltose. The fraction DF73 exhibited significant anti-inflammatory effects in the carrageenan-induced paw edema model, reducing inflammation by 92.60% in experimental mice. Similarly, in the STZ-induced diabetic mice model, DF73 and DF72 substantially reduced blood glucose levels by 91.50% and 83.35%, respectively. Additionally, these oligosaccharides improved the lipid profile by reducing triglycerides, low-density lipids, and total cholesterols while enhancing the high-density lipids. Also, the digestion of polysaccharides with *P. pastoris* produced oligosaccharides with strong prebiotic potential, particularly DF73, DF72, and DF53, which significantly promoted the growth of *B. bifidium* and *B. clausi* by proliferating the probiotic growth from 2.86 ± 0.14 $\log_{10}$CFU/mL at 0 h to 5.70±1.04 $\log_{10}$CFU/mL after 24 h and 9.76 ± 0.29 $\log_{10}$CFU/mL after 48 h. These findings support the sustainable development of nutraceuticals through a one-step microbial digestion process, warranting further research into these bioactive oligosaccharides' detailed structure and mechanism.

Keywords: *Lepidium sativum*, mucilage, polysaccharides, nutraceuticals, microbial bioprocessing, prebiotics, antioxidants, inflammation, diabetes mellitus.

Advancing Vaccine Design Strategies against *Salmonella typhimurium*: An Integrated Approach Harnessing Computational and Immunoinformatics Techniques for Precision Customization

Yusra Jamil, Adnan Khan, Fazal Rabi, Ayaz Ahmad

Department of Biotechnology, Abdul Wali Khan University Mardan, KP, Pakistan

Correspondence: ahdayazb5@awkum.edu.pk

Salmonella typhimurium, a gram-negative bacterium, is a leading cause of gastroenteritis worldwide, with frequent outbreaks across various regions. Despite its significant global impact, no widely available vaccine exists for human use against this pathogen. Complicating the challenge, *S. typhimurium* has developed resistance to multiple antibiotics, making infection management increasingly difficult. Given its prevalence and growing antibiotic resistance, there is an urgent need for an effective vaccine. In this study, we leveraged vaccinomics-assisted immunoinformatics techniques to identify potential vaccine candidates using the *S. typhimurium* full proteome data. The focus was on pathogen-essential proteins that are dissimilar to human proteins and those found in the human gut microbiome. Employing a reverse vaccinology approach, four antigenic outer membrane proteins were prioritized for lead epitope prediction. Lead B and T cell epitopes were then fused with appropriate linkers and adjuvant peptide sequences to design a multiepitope-based chimeric vaccine, termed ST-MEVC. The ST-MEVC construct was evaluated based on several criteria, including immunological, physicochemical, and immune receptor docking scores. Immune simulation studies predicted a strong immunogenic response potential for the proposed vaccine formulation. Molecular dynamic simulations further confirmed stable interactions between the vaccine construct and host receptors. In silico restriction and cloning, analyses suggested the feasible cloning potential of the ST-MEVC construct within an E. coli expression system. The proposed vaccine design is expected to elicit robust immune responses against *S. typhimurium* infections, demonstrating safety, efficacy, and potential for further investigation in in-vitro and in-vivo assays.

Keywords: *Salmonella typhimurium*, gastroenteritis, vaccine, vaccinosis, immunoinformatics

Isoeugenol improved cognitive deficits against cadmium chloride by inhibiting NLRP3 inflammasome in albino mice

Shoukat Ali, Zia Ur Rahman Khalil, Shahid Ali Shah

Department of Zoology, SBBU, Sheringal Dir (U), Khyber Pakhtunkhwa, Pakistan

Correspondence: shoukatalizoology@gmail.com

Cognitive impairment is a multifactorial and multi-step pathological process that places a heavy burden on patients and society. Neuroinflammation is one of the main factors leading to cognitive impairment. The Inflammasomes are multi-protein complexes that respond to various microorganisms and endogenous danger signals, helping to initiate innate protective responses in inflammatory diseases. The present study aimed to investigate the NLRP3 Inflammasome inhibitory potential of isoeugenol (IE) against Cadmium Chloride (CdCl2) induced NLRP3 Inflammasome activation mediated cognitive dysfunction in mice model. Male adult mice of 7-8 weeks old were randomly divided into four experimental groups i.e. 1. Control group (0.9% normal saline-treated), 2. CdCl2 induced group (1mg/kg), 3. CdCl2 plus IE group (1mg/kg and 10mg/kg, respectively) and 4. IE group (10mg/kg). Morris water maze and Y-maze tests were performed to assess their memory and western blotting techniques were used to evaluate the NLRP3 Inflammasome inhibitory potential of IE in CdCl2-induced neurodegeneration in mice. According to the western blot results, IE significantly inhibited the CdCl2-induced NLRP3 Inflammasome complex protein markers such as NLRP3, IL-1β, Caspase-1, and ASC in the brain homogenates. Similarly, IE significantly reduced the protein expression of Iba-1 (microglia) and GFAP (Astrocytes). Furthermore, IE improved the Cognitive deficits induced by CdCl2 in Albino mice. In summary, this study reveals the NLRP3 Inflammasome inhibitory capability of IE in the CdCl2 mice model. Further in-depth study is required to highlight the therapeutic potential of IE in neurodegenerative diseases.

Keywords: Oxidative Stress, Isoeugenol, NLRP3, ASC, Y-maze.

Correlation of age, gender, and blood group with leukemia susceptibility

Inam Hussain, Javeria Gul

Department of Biotechnology, SBBU, Sheringal Dir (U), KPK, Pakistan

Correspondence: inamm871@gmail.com; javeria@sbbu.edu.pk

Leukemia is a type of blood cancer caused by mutation in bone marrow cells which leads to the production of functional less blood cells (WBCs) There are various risk factors contributing to leukemia development, including smoking, Exposure to certain chemicals, Chemotherapy in the past, Radiation exposure, Rare congenital diseases, Certain blood disorders, Family history, age and gender. Leukemia is divided into four main types (1) acute lymphoid leukemia (ALL), (2) acute myeloid leukemia (AML), (3) chronic lymphoid leukemia (CLL), and (4) chronic myeloid leukemia (CML). Leukemia can be treated through Chemotherapy, Targeted therapy, Radiation therapy, Bone marrow transplant, Immunotherapy, and Engineering immune cells to fight leukemia. This study aimed to investigate the correlation of age, gender, and blood group with leukemia susceptibility. A comprehensive analysis of 103 patients with leukemia was conducted, examining the distribution of age, gender, and blood group (ABO and Rh) concerning leukemia and it is typed. A significant correlation between age and leukemia susceptibility, with increased risk observed in children followed by adults and older adults. ALL is more common in children, AML is more common in adults and ALL, AML, CLL, and CML are in the same ratio in older adults. Gender and leukemia susceptibility, with a higher incidence in males than females. Blood group and leukemia susceptibility, with higher risk associated with A+ followed by B+, O+, AB+, B-, O-, A- and AB-. These findings have important implications for understanding leukemia risk factors, personalized screening, and prevention strategies.

Keywords: leukemia, age, gender, blood group, susceptibility, risk factors.

Isolation and Characterization of Plant Growth Promoting Bacteria for the Zinc and Iron Bio-fortification

Ayesha Ikram*, Fathia Mubeen, Kalsoom Akhter, Muhammad Imtiaz, Aniqa Nawaz, Usama Marghoob, Summiya Sana

National Institute for Biotechnology and Genetic Engineering (NIBGE), P.O. Box 577, Jhang Road, Faisalabad 3800-Pakistan.

Correspondence: aysha.ikram@yahoo.com

Zinc (Zn) and Iron (Fe) are essential micronutrients crucial for the growth and development of all living organisms, including humans and plants. According to a World Health Organization report, the deficiency of micronutrients such as Zn and Fe poses a significant challenge to global health and sustainability. Highly adaptable bacteria have developed various mechanisms to manage elevated concentrations of Zn and Fe. One promising approach to addressing micronutrient deficiencies is the biofortification of cereal crops like rice. In this study, rhizospheric soil samples were collected from different districts of Punjab to isolate Zn- and Fe-solubilizing bacteria. The bacteria were isolated using the serial dilution method and then screened for Zn and Fe solubilization on minimal salt media containing 0.1% zinc oxide and CAS agar medium with bound FeCl3, respectively. Out of 220 isolates, twelve were capable of solubilizing Zn, and 10 could solubilize Fe. Two isolates, RL-16 and KH-3, demonstrated high zinc solubilization indices (ZSI) of 3.4 and 3.33, respectively, while two iron-solubilizing isolates, R-5 and R-7, exhibited iron solubilization indices (ISI) of 3.6 and 3.62, respectively. These four isolates were further characterized for plant growth-promoting (PGP) attributes. Morphologically, all

four were rod-shaped, with Zn-solubilizing isolates KH-3 and RL-16 being gram-negative, and Fe-solubilizing isolates R-5 and R-7 being gram-positive. Additionally, RL-16 showed the ability to solubilize silicon, potassium, and calcium. R-5 and R-7 were identified as potential biocontrol agents against Streptomyces scabies. Furthermore, KH-3 demonstrated tolerance to 7.5% NaCl stress and was identified as an effective nitrogen fixer. Based on their PGP characteristics, these isolates have potential as bio-fertilizers, contributing to sustainable agriculture.

Influence of protease enzyme, organic acid, and essential oils individually or in combination on growth, blood biochemistry, humoral immunity, gut pH, ileal microflora, and nutrient digestibility of broiler fed on poultry by-product meal

Ziaul Islam[1], Asad Sultan[2], Sarzamin Khan[2]
[1]Department of Basic and Applied Zoology SBBU, Sheringal Dir Upper;
[2]Department of Poultry Science, The University of Agriculture Peshawar Pakistan
Correspondence: ziaulislam@sbbu.edu.pk

This study aimed to determine the effects of a feed protease enzyme derived from Bacillus lachiniformis, alone and in combination with an organic acid blend and phyto-essential oils, on the immunity, gut health, blood biochemical parameters, and nutrient retention of meat-type birds at day 35. A total of 720 Cobb-500 day-old broilers were divided into six duplicate (n=6) feeding treatments; control (CON), basal diet; negative control (NC), which consisted of 6% poultry by-product meal (PBM); other groups were NC+Prot, NC+Prot-OA, NC+Prot-EO and NC+ Prot-OA-EO which was the same as NC but supplemented with protease; organic acid; phyto-essential oil; and combination of protease, organic acid and phyto-essential respectively. Compared to the birds in the negative control (NC) group, the nutritional interventions significantly ($p<0.05$) improved the birds' body weight gain (BWG) and feed conversion ratio (FCR). The treated group NC+Prot-OA-EO had the highest BWG (1905.83), whereas the birds in the NC group had the lowest BWG (1764.7). The treatments enhanced FCR considerably ($p<0.05$). In comparison to the NC group, the group NC+Prot-OA-EO showed the greatest improvement (27%), (16%), and (18%) during the starter, finisher, and overall production periods, respectively. Carcass yield increased significantly ($p<0.05$), while the weight of the lymphoid organs did not change over the trial. The presence of harmful bacteria like Salmonella, Escherichia coli, and Clostridium perfringens decreased significantly in birds supplemented with protease enzyme, organic acid, and photo essential. The treatment group Prot-OA-EO showed the greatest change in the relative abundance of Salmonella, Escherichia coli, and Clostridium perfringens in ileal digesta, with 47%, 54%, and 42 %, respectively, when compared to the NC group. `In contrast the population of beneficial Lactobacillus increased by 48% in groups receiving protease and the combination compared to the NC group. When comparing the dietary treatments to the negative control, the duodenum and jejunum showed a substantial ($p<0.05$) decrease in digesta pH. Dietary interventions increased apparent metabolizable energy, crude protein (CP), and dry matter (DM) digestibility significantly ($p<0.05$). In summary, dietary supplements of protease enzyme combined with organic acid and phyto essential improved growth performance, immune responses, increased Lactobacillus population, decreased pathogenic bacteria, and increased nutrient retention in boiler fed with poultry by-product meal.

Keywords: Growth performance, gut microbiota, Nutrient digestibility, Animal protein source

Development of a Fusion Protein-Based Chimeric Multi-Epitope Subunit Vaccine (CMESV) to Combat Co-Infection by Respiratory Syncytial Virus (RSV) and Human Metapneumovirus (hMPV), Using In Silico Strategies

Syeda Javiria Zahir, Abdullah Shah, Sikandar Khan

Department of Biotechnology, SBBU, Sheringal, Dir (U), KPK, Pakistan

Correspondence: shahbiotech3@sbbu.edu.pk

Respiratory syncytial virus (RSV) and Human metapneumovirus (hMPV), both belonging to the Paramyxoviridae family, are critical respiratory pathogens that contribute to significant morbidity and mortality, particularly in pediatric, elderly, and immunocompromised individuals. Emerging research highlights that co-infection with RSV and hMPV significantly increases the severity of infections. This synergistic effect of co-infection remains unaddressed by existing vaccine efforts, which target each virus individually. Therefore, this study utilized immune informatics tools to target the antigenic and conserved fusion proteins of hMPV and RSV and to design a chimeric vaccine against co-infection by these viruses. Initially, cytotoxic T lymphocyte (CTL), helper T lymphocyte (HTL), and B cell lymphocyte (BCL) epitopes were predicted from the fusion proteins of both viruses. Following evaluation based on key parameters, 3 CTL, 3 HTL, and 3 LBL epitopes from each virus were selected. These epitopes were then combined with appropriate linkers and an adjuvant to construct a multi-epitope vaccine. The resulting vaccine construct exhibited high antigenicity, non-allergenicity, solubility, and favorable physicochemical properties. The 3D structure of the vaccine and its potential interaction with Toll-Like Receptor-2 (TLR2) were also predicted. Finally, codon adaptation and in silico cloning confirmed the vaccine construct's high expression rate in the K12 strain of Escherichia coli (E. coli). While these findings are promising, in vitro and animal studies are necessary to validate the potency and safety of this vaccine candidate.

Keywords: Co-infection, Epitopes, Vaccine, Toll-Like Receptor-2 (TLR2), Adjuvant.

Molecular analysis of *CLPG* gene polymorphism in endangered Kutta sheep breed of Pakistan

Syed Ansar Ahmad[1], A. Shah[1], M. Asif Nawaz[1], Amin Ullah Jan[1], Ziaul Islam[2]
[1]Deptt. of Biotechnolog; [2]Deptt. of Zoology SBBU, Sheringal, Dir (U) KP, Pakistan
Correspondence: aminjan@sbbu.edu.pk

Scientists and animal breeders around the world are actively involved in utilizing the genetic variations in animals to tackle the problem of meat scarcity. The Callipyge (CLPG) gene mutation, a well-documented natural mutation in the ovine genome, is responsible for postnatal muscular hypertrophy in the pelvic and loin regions of the ovine. Thus keeping in mind the economic importance of the endangered Kutta sheep breed of Pakistan and meat scarcity in the country the present study was designed to execute the CLPG gene polymorphism in the Kutta sheep breed. For this, 100 individuals of Kutta sheep were randomly selected and their genomic DNA was extracted from their blood samples. The compact, extracted genomic DNA samples were subjected to PCR amplification of the targeted CLPG gene fragment (214bp). The PCR amplified product was treated with AvaII restriction enzyme and the resulting product was assessed by agarose gel electrophoresis for CLPG gene mutation. Interestingly, the results revealed that the selected population of the Kutta sheep breed was polymorphic concerning CLPG gene mutation. All three types of genotypes, wild type homozygous (NN), heterozygous (NC), and CLPG homozygous (CC) were observed. Furthermore, the wild-type genotype (NN) has the highest ratio (0.88) followed by heterozygous NC (0.10) and CLPG mutant homozygous CC being the lowest (0.02). Moreover, the Chi-square (X2) test showed that the studied population of the Kutta breed is not in hardy Weinberg equilibrium. One of the reasons might be the selective breeding of the studied population of the Kutta sheep breed. Keeping in view the significance of the CLPG gene mutation in muscular hypertrophy, a breeding program of the Kutta sheep breed should be designed to breed a maximum number of NC and CC genotypes with wild-type genotype NN.

Keywords: Muscular hypertrophy, Mutation, PCR-RFLP, Genotype.

Investigation of A Lytic Bacteriophage Potential to Control Multi-Drug-Resistant *Escherichia coli*

Noor-ul-Ain Ali [1], Atif Jamal[1] , Redaina[1], Muhsin Jamal[1]*, M. Asif Nawaz[2]
[1]Deptt. of Microbiology, Abdul Wali Khan University, Garden Campus, Mardan, Pakistan; [2]Deptt. of Biotechnology, SBBU, Sheringal, Dir (Upper), Pakistan
Correspondence: muhsinjamal@awkum.edu.pk

Escherichia coli is a gram-negative rod-shaped bacterium that mainly causes urinary tract infection (UTI), meningitis, and blood septicemia. It also causes nosocomial infections. E. coli has acquired multi-drug resistance and also has a great tendency for biofilm formation on different biotic (living) and abiotic (nonliving) surfaces. This biofilm further complicates bacterial resistance towards different drugs/ antibiotics. Therefore, an alternative therapy such as phage therapy is required to control this biofilm-producing MDR bacterium. The main objective of the current study was to isolate and characterize a bacteriophage to infect the multi-drug-resistant E. coli and also to investigate its potential to control host bacterial planktonic cells and its biofilm. A new bacteriophage designated MNA-1 was isolated from wastewater against MDR *E. coli*. Different characteristics of phage MNA-1 such as host range, heat, and pH stability, one-step growth, and Ca^{2+} and Mg^{2+} ions effect on phage adsorption rate to its host bacterium were studied. Phage MNA-1 produced clear plagues with well-defined boundaries ranging in size from 2.0 to 3.5mm possessing a narrow host range infecting very few isolates of selected bacteria under study. It was also quite stable at pH values ranging from 5 to 11. Phage adsorption rate to its host bacterium was enhanced when the bacterium was treated with $MgCl_2$ or $CaCl_2$ solutions as compared to the control. The phage showed a latent time of 27 min with a burst size was 450 virions per cell. We finally evaluated the phageMNA-1 lytic activity against *E. coli* planktonic cells and biofilms. This phage showed a significant reduction in the log-phase growth of bacterial planktonic culture as compared to control at shaking conditions. Phage MNA-1 was also evaluated for its anti-biofilm activity against *E. coli* biofilm developed in 96-well plates for different time intervals. It showed a significant reduction in the biofilm biomass of the host bacterium. Biofilm formed by *E. coli* for 24, 48, and 72 hours was treated with phage MNA-1 for about 6 hours and showed 2.1, 3.0, and 3.5 fold folds biofilm biomass reductions, respectively. Therefore, we concluded from this study that phage MNA-1 was a lytic phage with high potential to eradicate the biofilm-producing multi-drug resistant *E. coli* under static conditions but for complete eradication of biofilm, a phage cocktail will be suitable to avoid biofilm resistance to phages.

Keywords: Wastewater, *Escherichia coli*, Antibiotics, Biofilms, Phage cocktail

Physiological and biochemical characteristics of salt stress tolerance in selected varieties of sunflower under various treatments of potassium, zinc, and gibberellic acid

Amin Ullah Jan

Department of Biotechnology, SBBU, Sheringal Dir Upper, KPK, Pakistan

Correspondence: aminjan@sbbu.edu.pk

The effects of potassium, zinc, and gibberellic acid foliar spray on salt stress tolerance, free proline, total phenolics, carbohydrates, and chlorophyll contents in sunflower varieties were evaluated. Eight varieties of sunflower were grown in the glass house and NaCl (150 mM) was added to roots alone and in combinations with KNO_3, $ZnSO_4$ and GA3 foliar spray. Different varieties were evaluated for salt tolerance through leaf disc assay (LDA). Various test solutions of NaCl were used to induce salt stress. T1 treatment (150 mM NaCl only) significantly decreased the greenness % in all eight varieties of sunflower as compared to control C. The T2 treatment (150 mM NaCl + Potassium) enhanced the greenness % and maximum greenness % was observed in the SMH-0917 (76.00 %) followed by Ausigold-7 (75.50 %). The treatment T3 (150 mM NaCl

+ Zinc) and T4 treatment (150 mM NaCl + 100 ppm of GA3) retained the greenness % in leaf discs compared to control. The free proline and total phenolic contents significantly increased in all eight varieties of sunflower when treated with K, Zn, GA3, and NaCl. A positively significant correlation was found between greenness % with proline ($R^2 = 0.86$) and total phenolics contents ($R^2 = 0.83$). Conclusively, salt tolerance could be increased by the application of K, Zn, and GA3.

Keywords: Sunflower; salinity; Potassium; Zinc; GA3; Proline; Phenolics

Floristic Composition and Biological Spectrum of Khwazakhela, District Swat, Pakistan

Khaista Rahman and Amin Ullah Jan

Department of Botany, Islamia College University Peshawar, KP, Pakistan; Department of Biotechnology, SBBU, Sheringal Dir Upper, KP, Pakistan

Correspondence: aminjan@sbbu.edu.pk

The present study was conducted during 2015-2016 to explore the floristic list life form and leaf size spectra of the flora of Khwazakhela Swat, Pakistan. The area is home to 63 plant species belonging to 58 genera and 38 families in which Asteraceae (12.69%, 8 spp) was the leading dominant family followed by Lamiaceae (11.11%, 7 spp), Papilionaceae, and Rosaceae (4.76% 3 spp) for each. Four families contributed 2 species for each to the floristic list of the area while the other 26 families were monspecific. Therophytes (23 sp., 36.51%) were dominant followed by nanophanerophytes (13 sp., 20.63%) and chamaephytes (8 sp., 12.70 %). In the assessment of leaf form spectra, the dominant leaf form was nanophylls (20 sp., 31.75%) followed by microphylls (17 sp., 26.98%), mesophylls (15 sp., 23.81%) and leptophylls (7 sp., 11.11%).

Keywords: Floristic list, Life form, Leaf Size Spectra, Khwazakhela, District Swat, Pakistan.

Enhanced activity of *Anabaena variabilis* phenylalanine ammonia-lyase by site-directed mutagenesis for L Phenylalanine Production

Amin Ullah Jan[1], Muhsin Jamal[2]

[1]Department of Biotechnology, SBBU, Sheringal Dir Upper, KP, Pakistan; [2]Deptt. of Microbiology, Abdul Wali Khan University, Garden Campus, Mardan, Pakistan

Correspondence: aminjan@sbbu.edu.pk

L-phenylalanine is an important amino acid for mammals and has an important role in human health. The phenylalanine ammonia-lyase (PAL) from Anabaena variabilis (A. variabilis) catalyzes the amination of substituent trans-cinnamic acid (t-CA) to L-phenylalanine in the presence of high ammonia concentrations. Meanwhile, neither cofactor reprocessing nor extra additives are required. In the present study, increasing the activity of this therapeutically important enzyme has been explored by the introduction of a hydrogen bond into the structure. Site-directed mutagenesis was performed with a quick-change PCR method. Recombinant wild-type and mutated enzymes were expressed in Escherichia coli (E. coli) and His-tagged proteins were affinity purified. The formation of a hydrogen bond was confirmed by the computational simulation (docking) method, and then the reaction rate, substrate inhibition, and kinetic behavior of the mutated enzyme were compared with the wild type (WT). Based on our results, the N103R mutation showed an 11-fold enhanced enzymatic activity compared with the AvPAL WT. Reaction rates were proven experimentally, while kinetic parameters and pH aspect of enzyme activity were persevered unaffected.

Keywords: L-Phenylalanine, Anabaena variabilis, Amination, Mutagenesis, Recombinant

Resolving bioactive polysaccharides from Lepidium sativum seed coat mucilage

Fareeha Shams1, Imdad Ullah Khan[1], Amjad Iqbal[2], Ayaz Ahmad[1,*]
[1]Department of Biotechnology; [2]Department of Food Science and Technology,
Abdul Wali Khan University Mardan Khyber Pakhtunkhwa, Pakistan
**Correspondence: ahdayazb5@awkum.edu.pk*

Since the last decade, plant mucilage has been a very rich source for the development of desired pharmaceutical dosage formulations. *Lepidium sativum* Linn. (Family: Brassicaceae), commonly known as garden cress, is one of the mucilage-containing, fast-growing, edible annual herbs, and its different parts have been used for various human ailments. This study focused on investigating the biological potential of cress seed mucilage and establishing its anti-inflammatory effect. Mucilage was extracted upon soaking the seeds in water. The crude mucilage was fractionated in 75% ethanol, resulting in soluble and insoluble fractions. Comparative biological potential of crude, soluble, and insoluble extracts was determined using antibacterial, antioxidant, and in vivo anti-inflammatory assay. The results show that soluble fraction was able to inhibit the growth of human pathogenic *Staphylococcus aureus* (3.2 mm ± 0.14 mm), *Klebsiella pneumonia* (5.1 mm ± 0.31 mm), *Escherichia coli* (4.6 mm ± 0.12 mm) and *Shigella* (4.9 mm ± 0.19 mm). Antioxidant capacity was determined through DPPH assay. The soluble fraction was the most potent, having an IC50 value of 376.60 µg/mL, followed by the insoluble fraction with an IC50 value of 525.86 µg/mL. Each fraction was further processed to determine the cytotoxic effect following the hemolysis assay, where no extract showed any significant hemolytic effect. Alpha amylase inhibition assay of the extract shows that soluble fraction has an IC50 value of 571.03 µg/mL, followed by insoluble and crude fractions having IC50 values of 840.45 µg/mL and 1684.78 µg/mL, respectively. The fractions' in vivo anti-inflammatory effect was determined by carrageenan-induced paw edema in the mice model. The result revealed that 400mg/kg soluble fraction could reduce the paw edema volume from 4.9 mm ± 0.18 mm to 2.09 mm ± 0.12 mm, comparable to the standard drug diclofenac sodium. These results suggest that cress seed mucilage is a rich source of bioactive polysaccharides. Further study is needed to characterize the biochemical profile of these fractions to identify and purify the target bioactive compounds.

Keywords: *Lepidium sativum*; Polysaccharides; Mucilage; antibacterial, antioxidant, anti-inflammatory

On-demand Antibiotic Delivery from Xenobots at Multidrug Resistant Infection-Microenvironment

Zul Kamal[1, 2]

[1]Department of Pharmacy, SBBU, Sheringal, Khyber Pakhtunkhwa, Pakistan;
[2]School of Pharmacy, Shanghai Jiao Tong University, 200240, China

Corresponding: xulkamal@sbbu.edu.pk

Multidrug-resistant (MDR) infections pose a significant threat to global health, necessitating innovative solutions for targeted antibiotic delivery. "Xenoboots," a novel pharmaceutical biotechnological platform for on-demand antibiotic release in response to specific microenvironmental cues. Xenoboots system leverages biomimetic nanotechnology and supramolecular chemistry to create adaptive, responsive nanocarriers that will diagnose and selectively target MDR infection sites. Upon exposure to infection-specific stimuli, Xenoboots release encapsulated antibiotics, enabling precise, high-dose treatment while minimizing off-target effects. In vitro and in vivo studies demonstrate the efficacy of Xenoboots in eradicating MDR bacterial infections, including *methicillin-resistant Staphylococcus aureus* (MRSA) and *carbapenem-resistant Enterobacteriaceae* (CRE). This groundbreaking approach of “Xenoboots”, which is considered a future pharmaceutical biotechnological product, will offer a promising strategy for combating MDR infections, reducing antibiotic resistance, evading the immune system, prolonging circulations, infection diagnosis, and on-demand antibiotic delivery, which will be improving patient outcomes in prospects.

Keywords: Antimicrobial resistance, biotechnological product, Multidrug-resistant, Xenobots, *methicillin-resistant Staphylococcus aureus*, *carbapenem-resistant Enterobacteriaceae*

Expression and Purification of Head Domain of Fowl adenovirus serotype-4 fiber-2 Protein for Protection Studies against Inclusion Body Hepatitis Hydropericardium Syndrome in Poultry

Gul Wali Khan[1], Essa Hassan[1], Ayan Khan [1], Rahim Ullah[1] and M. Asif Nawaz

Department of Biotechnology, SBBU, Sheringal Dir Upper, KPK, Pakistan

Correspondence: asif_biotech33@sbbu.edu.pk

In recent bygone, contagions caused by the very hyper-virulent Fowl Adenovirus serotype 4 (FAdV-4) have been elevated. The outbreaks of FAdV-4 lead to severe economic losses worldwide including Pakistan. The upsurge in the infection cases and inability of the control strategies is highly attributed to the inefficacy of the available commercial vaccines against the FAdV-4. Lower effectiveness and ultimately lower antigenicity of the full-length subunit peptide vaccine is also another factor in the failure in prevention of the disease. The current study explored the possibility of the surface exposed, head domain of short fiber 2 protein (fiber-2) of FAdV-4. The head domain of fiber-2, (fiber-2

HD) was identified in the protein structure using bioinformatics tools and homology model was generated for the known fiber-2 structure in protein data bank. The DNA fragment responsible for coding the HD was cloned in Escherichia coli (E. coli) based expression vector. The designed clone was revamped into E. coli and isolated by miniprep after propagation in the bacteria. The designed-clone then was revamped into the BL21 (DE3)-expression strain of E. coli. The protein was expressed successfully using Isopropyl βD-1-thiogalactopyranoside as inducer at 16 °C. The protein was then purified from soluble fraction using metal affinity chromatography. To investigate the immunogenicity of the fiber-2 HD, the purified protein was injected into chickens along with appropriate adjuvant. After booster dose with the protein + adjuvant, the chickens were confronted with infective FAdV-4 virus. In parallel the efficacy of the commercially available, formalin killed vaccine was also checked. Similarly, appropriate negative and positive controls were kept for the authenticity of the experiment/s. The results indicated higher protection of the chickens vaccinated using fiber-2 HD against the virus in comparison to commercial vaccine and unimmunized group. The corresponding results recommended that the cleansed, recombinant fiber-2-HD protein can be a perfect vaccine (subunit-vaccines) to eradicate IBH-HPS.

Key words: Fowl Adenoviruses Serotype-4**,** Inclusion Body Hepatitis Hydropericardium Syndrome, *Escherichia coli* and Fiber-2 protein

Prevalence of gastrointestinal symptoms associated with diabetes mellitus

Sadaqat Hussain, Jaweria Gul

Department of Biotechnology SBBU, Sheringal Dir Upper, KPK, Pakistan

Correspondence: sh3559sbbu@gmail.com; jaweria@sbbu.edu.pk

This research aims to discern the prevalence of gastrointestinal symptoms within the diabetic population, utilizing data collected from diverse areas and hospitals. Our sample comprises 100 diabetic patients, including 39 males and 61 females. Of the males, 12 exhibit gastrointestinal symptoms, while among females, 26 display such symptoms, resulting in an overall prevalence of 38 out of 100 patients. Demographic analysis reveals varying age distributions, with higher diabetic rates observed in older age groups, particularly those aged 45-65. Constipation emerges as the predominant symptom, occurring frequently on a weekly or daily basis, significantly impacting energy levels. Remarkably, a considerable number of patients do not regularly monitor their glucose levels, prompting inquiries into the correlation between glucose levels and gastrointestinal symptoms. Furthermore, identified triggers include specific foods (eggs, potatoes, wheat, and certain vegetables) and common medications, notably metformin, with a propensity to induce constipation in diabetic patients.

Genetic Diversity of Plasmodium falciparum and Plasmodium vivax field Isolates from Nowshera District of Pakistan

Chandni Hayat and Sahib Gul Afridi*

Department of Biochemistry Abdul Wali Khan University Mardan, Pakistan

**Correspondence: drafridi@awkum.edu.pk*

The genetic diversity of malaria parasites contributes to their ability to adapt to environmental changes, develop drug resistance, and escape from the host immune system, hence very important for control measures of malaria. This study aimed to analyze the genetic diversity of the pfmsp1 and pfmsp2 genes in P. falciparum and the Pvmsp-3α gene in *P.vivaxisolates* from District Nowshera in Pakistan. Blood samples from 124 consented patients with uncomplicated malaria presenting to different hospitals of district Nowshera, were collected during March-August 2019, representing 28 P. falciparum and 96 P. vivax isolates. DNA of all samples was subjected to nested-PCR-based allele-specific markers analysis. Pvmsp-3α amplified fragments were further treated with Restriction fragment length polymorphism (RFLP) based Hha1 restriction enzyme. In P. falciparum 21 alleles were detected, including 14 alleles for Pfmsp-1 and 7 alleles for Pfmsp-2. Sub-allelic families MAD20 (50%) in Pfmsp-1 and FC27 (75%) in Pfmsp-2 family were predominant. The multiplicity of infection (MOI) was calculated as 1.4 and 1.2 for Pfmsp-1 and Pfmsp-2 respectively, with an overall mean MOI of 1.34. In *P.vivax*, 4 allelic variants as Type A-D were detected for Pvmsp-3α through nested PCR while after RFLP digestion of amplicons, 9 sub-allelic variants (A1-A4, B1, B2, C1, C2, and D1) were observed at Pvmsp-3α locus. This is the first-ever report of molecular characterization of P. falciparum and P. vivax genotypes from District Nowshera Pakistan. Both P. falciparum and P. vivax field isolates exhibited moderate to high allelic diversity in the district of Nowshera, Pakistan.

Keywords: Malaria, *Pfmsp-1*, *Pfmsp-2, Pvmsp-3*, genetic diversity, Nowshera, Pakistan

Unravelling Malaria's Genetic Landscape in Pakistan: Implications for Targeted Interventions and Vaccine Innovation

Sahib Gul Afridi

Department of Biochemistry Abdul Wali Khan University Mardan 23300, Pakistan

Correspondence: drafridi@awkum.edu.pk

Malaria remains a significant public health concern in Pakistan, with a considerable burden on the population and healthcare system. Pakistan is one of the seven Eastern Mediterranean countries that account for 98% of the total malaria burden in the region, where 371,828 cases of malaria were reported in 2020, with 217 million people at risk of malaria infection caused by *P. vivax* (70%-85%) and *P. falciparum* (15-30%) parasites. Despite various interventions in place, several challenges persist, hindering the progress of malaria control and elimination among which the emergence and spread of drug-resistant parasites are serious. Molecular and genomic interventions in malaria are essential to address the genetic diversity of malaria parasites, manage drug resistance, and enhance vaccine development and diagnostic accuracy. These approaches enable targeted control strategies and improved surveillance, crucial for effective malaria management and prevention. Our research delves into the genetic landscape of malaria in Pakistan, employing advanced molecular and genomic techniques to address the complex issues of parasite diversity, drug resistance, and vaccine development. By examining key antigens such as Apical Membrane Antigen-1 (AMA-1) and Merozoite Surface Proteins (MSPs) in *P. vivax* and *P. falciparum*, along with conducting genomic analyses of CSP, MSP1, GLURP, DBP, and the VIR gene family, we aim to elucidate genetic variations that impact vaccine efficacy and drug resistance. Our research highlights significant genetic variations that influence treatment outcomes and vaccine responses, informing the development of more effective and targeted malaria interventions. Ongoing investigations are focused on identifying additional genes associated with drug resistance, vaccine development, G6PD deficiency, and diagnostic markers, crucial for tailoring control strategies and improving surveillance.

Keywords: Pakistan, Malaria, interventions, Molecular analysis, vaccine, drug resistance

Comparative Study of Salt Tolerance in Different Land Races of Moringa (*Moringa oliefera* L.)

Fatima Farooq, Muhammad Imtiaz

Soil and Environmental Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan

Correspondence: m.imtiazpk92@hotmail.com

The popularity of Moringa oleifera as a superfood is primarily attributed to the presence of various essential phytochemicals in its leaves, pods, and seeds. While moringa is known for its hardiness and ability to thrive in diverse environmental conditions, including saline soils, there is still much to be explored regarding its responses to salt stress and its potential for improved production under such conditions. A pot experiment was conducted to investigate the impact of salinity levels (1.5 dS m-1, 3.5 dS m-1, 7.5 dS m-1, and 11.5 dS m-1) on emergence, growth, and biochemical traits of moringa landraces under completely randomized design having three replications. Four landraces of Moringa oleifera (Faisalabad black seeded moringa (MFB), Patoki black seeded moringa (MPB), Faisalabad white seeded moringa (MFW) and Rahim Yar Khan black seeded moringa (MRB) were selected for experimentation. All the salinity levels significantly affected the emergence parameters (time to emergence start, time to 50% emergence, mean emergence time, emergence index, and final emergence percentage) of moringa landraces. However, 1.5 dS m-1 and 3.5 dS m-1 were found more favorable. Higher salinity levels (7.5 dS m-1 and 11.5 dS m-1) significantly minimized the root surface area, root projected area, root volume, and root density as compared to 1.5 dS m-1, and 3.5 dS m-1. Several branches, leaves, leaflets, and leaf lengths were also adversely affected by 7.5 dS m-1 and 11.5 dS m-1. Maximum seedling

fresh and dry weights and seedling length were recorded at 1.5 dS m-1 followed by 3.5 dS m-1. Chlorophyll a and b contents, carotenoids, and membrane stability index were also observed highest at a salinity level of 1.5 dS m-1. In the case of moringa landraces, MRB performed better regarding emergence attributes, growth parameters, and biochemical analysis followed by MFW as compared to MFB and MPB. Moringa landraces i.e. MRB and MFW were found more tolerant to salinity stress as compared to MFB and MPB.

The Role of Biotechnology in Crafting High-Yield and Thermotolerant Wheat for Sustainable Development Goals

Aleeza Shaffaq, Muhammad Imtiaz

Soil and Environmental Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan

Correspondence: m.imtiazpk92@hotmail.com

Wheat is a staple food in Pakistan and contributes to 70% of the total energy intake. However, Pakistan is among the countries that are facing worse food insecurity. According to the National Nutrition Survey 2018 (NNS-2018), the prevalence of food insecurity is about 37%. Many factors such as chronic poverty, repeated disasters, political and economic instability, high population growth, food inflation, and extreme weather are the major causes. Climate change, with its rising temperatures and unpredictable weather, is making it harder to grow wheat. This situation threatens global food supplies and could worsen hunger and malnutrition in other vulnerable areas including Pakistan. Wheat is one of the most susceptible crops to the heat stress. To address this, developing wheat varieties that produce high yields and can withstand extreme weather is now the need of the hour. High-yield wheat varieties can be designed to produce more grain from the same cultivation area. This also reduces the pressure to convert natural areas into farmland, protecting the environment and supporting SDG 15: Life on Land by conserving the environment. This goal can be achieved by using different strategies like the modification of the genes by using biotechnological applications, omics technology, and microbes which could prove as a cheap and faster way to get the thermotolerant wheat. Even a slight increase in temperature can lead to lower yields. Using thermotolerant wheat helps ensure food security despite the challenges posed by climate change and supports SDG 13: Climate Action. High-yield and thermotolerant wheat often requires fewer resources, such as water and fertilizers, making them more efficient and environmentally friendly. In summary, such measures need to be taken that help combat food insecurity in developing countries to achieve SDG 2: Zero hunger. These countries can be on track to achieve other SDGs. These wheat varieties not only help ensure a steady food supply but also contribute to broader goals of sustainability, making agriculture more resilient and capable of meeting future challenges.

Microalgae-associated bacteria and the cumulative effect of this biological system to mediate the burden of environmental pollutants in wastewater

Rabia Rehman, Farhan Hafeez, Mohammad Bilal, M. Irshad, Rashid Nazir*

Department of Environmental Sciences, COMSATS University Islamabad - Abbottabad Campus, Pakistan

Correspondence: rashidnazir@cuiatd.edu.pk

The rapid increase in population has increased the urbanization-cum-industrialization and subsequently environmental pollution. Water is utilized as millions of gallons in different industrial processes and is released as polluted into the environment with no or mere (primary) treatment. Such wastewater is enriched with contaminants like heavy metals, micronutrients, antibiotics, dyes, pesticides, etc. These constituents, released (directly or indirectly) into wastewater, degrade the quality of natural water reservoirs. Moreover, the enriched wastewater may also act as a growth medium for the cultivation of different microorganisms like microalgae and bacteria. Some wastewater pollutants e.g. antibiotics are not efficiently removed by conventional wastewater treatment systems. Bacteria present in wastewater can acquire resistance against antibiotics, evolving into antibiotic-resistant bacteria (ARB) with antibiotic-resistant genes (ARGs), and ultimately causing multidrug resistance development (MDR). Such scenarios may pose a serious risk to human health and the environment. In our work, 57 strains of aquatic microalgae were isolated, characterized, and screened for rapid growth and valuable algal products. Four different microalgae systems were subsequently deployed for wastewater treatment. Results revealed a reduction of COD, NH4-N, PO43--P, and TP (all beyond the permissible limits) as well as bioresources accumulation (up to 78.1 mgL-1d-1 biomass productivity, with 38.1 mgL-1day-1 lipid productivity). For molecular analyses, out of 14 tested bacterial genes, tetO, tetW tetX, and ermB were reduced beyond detection within the first 4 days of treatment in all the cultures. Other genes, including blaCTX, sul1, cmlA, aadA, int1, and uidA were also reduced beyond the 2-log reduction value (LRV). The mobile genetic element, int1, correlated positively with most of the ARGs, especially sul1 (up to $r = 0.99$, $P < 0.01$) and aadA ($r \leq 0.97$, $P < 0.01$). Similarly, the Escherichia coli indicator gene, uidA, correlated positively with the study genes, especially with aadA, blaCTX, blaTEM, and cmlA ($r \leq 0.99$, $P < 0.01$). The synergistic relationship between microalgae and microalgae-associated bacteria has been proven to be helpful for both as for microalgae growth, bacteria provide CO2 and other vitamins and in return, microalgae provide O2 and other organic products for bacteria. These microalgae-bacterial consortia not only reduce the chemical pollutants but also reduce the number of ARGs and mitigate the health threats, potentially caused by these pollutants.

Role of non-timber forest products in the livelihood of local communities in Dir Kohistan KPK Pakistan

Riffat Aziz[1], Allah Ditta[1,2*]

[1]*Department of Environmental Sciences, SBBU Sheringal, Upper Dir, KP, Pakistan;* [2]*School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia*

**Correspondence: allah.ditta@sbbu.edu.pk; riffataziz87@gmail.com*

Forest and forest resources contribute significantly to the welfare of the local community in many ways like fodder, wood, non-timber forest products (NTFPs), fuel wood, and their livelihood. The extensive and improper use of forest resources by the local population for their livelihood results in increased deforestation rates and forest degradation. The current study was carried out to determine the socio-economic status and contribution of local forest resources in the livelihood of rural communities residing within the selected research areas i.e. Kumrat, Thal and Badgowai, Khyber Pakhtunkhwa as well as to assess the reasons behind forest depletion. The study also addressed suggestions regarding effective approaches related to forest conservation and ultimately the livelihood of local people involved. Field visits, structured questionnaires, and interviews were key tools in the data collection. A total of 240 respondents were selected for data collection. The results showed that 20% of the respondents used forests as a primary source of their income. The most virulent factor for forest degradation was fuel wood collection as 50% of respondents used forest resources for fuel wood. The annual fuel wood consumption was estimated at 92.2097 kg capita^{-1} year^{-1} and 116.45 kg household^{-1} year^{-1}. Several NTFPs like walnuts, medicinal plants, mushrooms, and brooms were collected from the local forest but walnuts were the most abundant one with a contribution of 36% of the total NTFPs obtained. Half of the total respondents claimed to receive PKR 25000-35000 as their seasonal income from the sale of NTFPs. Moreover, 47% of the respondents were dependent upon agriculture for their income and 39% of respondents claimed that agriculture was the most effective approach for local livelihood enhancement. In conclusion, forest resources play an important role as an energy and income source and ultimately in the livelihood of the local people involved but these resources have been badly affected by human encroachment and also due to the 2010 flood. Moreover, there was a lack of government policies for forest conservation. In the future, the provision of an alternative source of energy for the local people while policy regarding restriction on activities of the local community within forests is recommended to avoid forest degradation.

Dimeric Napthoquiones from *Diospyros lotus* and its biological screening for discovery of new Drug

Abdur Rauf[1*], Saima Naz[2]

[1]*Deptt. of Chemistry, University of Swabi, Anbar 23561, KPK, Pakistan;* [2]*Institute of Biotechnology and Microbiology, Bacha Khan University, Charsadda, KP, Pakistan*

Correspondence: mashaljcs@yahoo.com

Diospyros lotus Linn, known as date plum, has multiple usages in the traditional system. Various parts of Diospyros lotus are used for curing bleeding, insomnia, hiccups, lumbago, and constipation. The extracts and their fractions have been reported for diverse biological activities such as anti-inflammatory, soothing, antioxidant, anti-microbial, febrifuge, vermifuge, and anti-hypertensive. Diospyros lotus is a rich source of producing valuable natural products most precise and effective way. The promising biological profile of Diospyros lotus is due to the presence of bioactive molecules. The main objective of this finding is the isolation of Di-naphthodiospyrol (A- H) from Diospyros lotus. The defatted chloroform extract was subjected to normal phase column chromatographic analysis which afforded eight new dimeric naphthoquinones namely; Di-naphthodiospyrol (A-H). The chemical structures isolated from Di-naphthodiospyrol (A-H) were elucidated by advanced spectroscopy including ^{1}H-NMR, ^{13}C-NMR, HMBC, HSQC, NOSEY, and mass spectrometry. The isolated Di-naphthodiospyrol in bulk quality was assessed for various in vitro and in vivo biological activities. The crude extracts, fractions, and their compounds exhibited anti-nociceptive, sedative, anti-inflammatory, antipyretic, and acute toxicity. The tested compounds showed significant antiproliferative and reversal of MRD in mouse lymphoma cells. The crude extract and isolated compounds were also screened for enzyme-inhibitory activity. Among the entire extracts, the chloroform and ethyl acetate extract exhibited very strong activity as compared to the standard drug. The compound isolated in bulk was also screened for analgesic, antipyretic, and sedative activity. The tested compounds showed promising analgesic, antipyretic, and sedative effects. Di-naphthodiospyrols isolated from Diospyros lotus are also assessed for muscle relaxant effect which showed excellent activity as compared to standard drugs.

Keywords: Diospyros lotus, Napthoquiones, biological activities.

Dietary protein level and stocking density influence the growth and nutritive value of mealworm (*Tenebrio molitor*) larvae

Ihsan Ullah[1], Kamran Khan[1,*], Sarzamin Khan[2]
[1]*Deptt. of Basic and Applied Zoology, SBBU, Sheringal Dir Upper, Pakistan;*
[2]*Department of Poultry Science, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar, Pakistan*
**Correspondence: dr.kamran@sbbu.edu.pk*

The animal feed industry faces a growing challenge in finding cost-effective, sustainable, and resource-efficient solutions due to increasing feed ingredient costs. Insects, specifically *Tenebrio molitor* L. (yellow mealworm), offer a promising solution. This study aimed to evaluate the impact of stocking density and dietary protein on insect biomass production and nutrient composition. A total of 540 two-week-old mealworm larvae were randomly selected and grouped based on larval density and dietary protein levels. The larvae were divided into six groups, each with three replicates (30 larvae per tray), and received two different diets (16% and 24% CP). The results showed significant effects of dietary protein levels ($P < 0.001$), rearing density ($P < 0.003$), and their interaction on feed intake, weight gain, and feed conversion ratio. Higher protein levels (24%) and higher stocking density (1.35 cm^2 per larva) improved feed intake, weight gain, and feed conversion efficiency. Diet composition and rearing density significantly influenced protein and fat content. In conclusion, this study demonstrates that both larval density and nutrient composition significantly impact mealworm larvae performance and body composition, providing valuable insights for optimizing rearing conditions.

Keywords: Stocking density, dietary protein, insect biomass, nutrient composition

Super worm (*Zophobas morio*) pupa-meal-based diet as an alternative protein source for rainbow trout (*Oncorhynchus mykiss*)

Irshad Ullah, Kamran Khan*, Nimra Saeed

Department of Basic and Applied Zoology, SBBU, Sheringal Dir Upper, Pakistan

**Correspondence: dr.kamran@sbbu.edu.pk*

The aqua-feed industry faces challenges in finding cost-effective, sustainable, and environmentally friendly feed ingredients. Conventional options like fishmeal and soybean are costly and pose sustainability concerns. Insects, specifically yellow mealworm larvae, offer a promising alternative due to their high protein levels and low environmental impact. This study investigated the effects of superworm pupa meal (SWPM) supplementation in fish diets on growth indices and nutrient digestibility. Two hundred forty fingerlings rainbow trout (*Oncorhynchus mykiss*) were divided into four groups, each with three replicates (20 fish per replicate). The control group received a commercial concentrate as a basal diet without SWPM while testing groups received the same basal diet supplemented with SWPM at 5% (SWPM5), 10% (SWPM10), and 15% (SWPM15), respectively. The SWPM5-based diet showed significantly higher weight gain (WG) and better feed conversion ratio (FCR) than other groups. Notably, higher SWPM levels (10% and 15%) did not further improve WG and FCR. Protein and dry matter digestibility were significantly higher in the SWPM5-based diet, while ether extract digestibility was higher in both the control and SWPM5-based diets. This study suggests that supplementing diets with 5% SWPM improves WG, FCR, protein, and dry matter digestion in rainbow trout.

Keywords: Zophobas Morio, growth performance, digestibility, rainbow trout

Isolation, screening, and characterization of arsenic-resistant bacteria from contaminated soils in Patrak, Khyber Pakhtunkhwa, Pakistan

Naeem Ullah, Allah Ditta

Department of Environmental Sciences, SBBU, Sheringal, Dir (U), KP, Pakistan

Correspondence: naeemullah473@gmail.com

Arsenic like other heavy metals is toxic and hazardous to plants, animals, and humans too. Microbes tend to resist and bio-remediate heavy metals along with arsenic. The study was conducted to isolate, screen, and characterize arsenic-resistant bacteria from the soil samples collected from various areas located at Patrak, Khyber Pakhtunkhwa. The isolated bacteria resistant to different arsenic (As) concentrations were tested for various parameters like colony color, gram staining, molecular identification, and plant growth-promoting trait i.e. phosphate solubilizing activity. The isolated strains were diverse in color. The highest number of bacteria isolated were of off-white (NU-2, NU-3, NU-5, NU-6, NU-7, NU-8, and NU-10) color, followed by white (NU-4, NU-11, NU-12, NU-13 and NU-14). Only two strains (NU-1 and NU-15) with yellow color and one (NU-9) with light yellow color were observed. The percentage of bacteria with off-white, white, yellow, and light-yellow colors was 47, 33, 13, and 7%, respectively. The isolated strains mostly belonged to the gram-negative group. Only two isolates were gram-positive bacteria. The isolates bacteria with gram-negative stain were (NU-1, NU-2, NU-3, NU-4, NU-6, NU-8, NU-9, NU-10, NU-11, NU-12, NU-13, NU-14 and NU-15). Similarly, gram-negative isolates were (NU-5 and NU-7). The isolated strains exhibited as resistance under different as concentrations. Most of the isolated strains were resistant to higher concentrations of As. The bacterial isolates exhibiting the highest concentration of as i.e. 50 mM were NU-1, NU-7, NU-9, NU-11, NU-14, and NU-15. In conclusion, the bacterial isolates i.e., NU-7, NU-9, NU-11, and NU-14 exhibited the highest concentration resistance and can be used in bioremediation studies in future studies.

Keywords: Arsenic, PGPR, heavy metal resistant bacteria, isolation, gram-positive

POSTERS

Bioremediation Potential of *Serratia marcescens* Extracted from *Moringa oleifera* in bioremediation of Heavy metals

Syed Muhammad Shafi Shah, Karim Gul, Atta Ullah, Raham Sher Khan*
Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan
Correspondence: rahamsher@awkum.edu.pk

The present research aimed to explore the potential of *Serratia marcescens* bacteria extracted from the *Moringa oleifera* plant for bioremediation. Twenty-five bacterial endophytes were isolated, in five of the bacterial isolates were further evaluated and assessed for their minimal inhibitory concentration (MIC) in the presence of four heavy metals. Among the five isolates tested, strain SR-5 (*S. marcescens*) was found the most resistant to cadmium (250 mg/L), followed by chromium (200 mg/L), lead (250 mg/L), and arsenic (200 mg/L). SR-5 bacteria have also the ability to produce secondary metabolites, indoleacetic acid, siderophores, 1-aminocyclopropane-1-carboxylate (ACC) deaminase and biosurfactants, and solubilize phosphates. Various growth kinetics and parameters of the *S. marcescens* in the presence of heavy metals stress were observed, and the growth kinetics of bacteria were found to change slightly. The pH and temperature affect heavy metal reduction rates. The research findings highlighted that the bacterial strain SR-5, identified as *S. marcescens*, exhibited an amazing ability to tolerate and thrive in environments contaminated with heavy metals. This resilience makes SR-5 a highly promising candidate for bioremediation applications due to its potential for active uptake and sequestration of heavy metal in its cellular structure and reducing its bioavailability and toxicity. Additionally, it also produces plant growth-promoting hormones and solubilized essential nutrients in the symbiotic relationship that can enhance the process of phytoremediation where plants with their microbes work together for the remediation of polluted environments.

Keywords: Bioremediation, Serratia marcescens, Moringa oleifera, heavy metals

Effect of foliar application of acetic acid on chlorophyll & carotenoid contents in transgenic brassica napus under cadmium stress

Muhammad Abdullah, Muhammad Tayyab
Inst. for Biotechnol.& Genetic Engg., The University of Agriculture Peshawar
Correspondence: muhammad.abdullah@aup.edu.pk

Researchers highly valued carotenoid overproduction due to its medicinal and health-promoting properties i.e. anti-inflammatory, antibacterial, antidiabetic, and neuroprotective, etc. Due to the excessive production of carotenoids by transgenic brassica napus, it also has some pleiotropic effects i.e. reduced photosynthetic pigments, slow growth rate, and delay in maturation time. In our study, we applied foliar application of acetic acid to enhance photosynthetic pigments. Our data suggested that 30mM of acetic acid foliar applications increase root length in wild-type and transgenic plants from 33cm to 44cm (33%) and 24.7cm to 37cm (49%) respectively. After cadmium stress applied to

the plants it was decreased from 33cm to 29cm (13%) & 24.7cm to 24.5 (1%) and in combination with Cd stress and acetic acid, it was increased from 33cm to 36 9% and 24.7cm to 31.2cm (26%) as compared to their respective control plants. Plant height in WT and transgenic plants also increased from 83.1cm to 112.7cm (35%) and 20.85cm to 35.89cm (72%) respectively. After Cd stress applied to plants it was decreased from 83.1cm to 74.1 (11%) and 20.85cm to 16.9cm (19%) and in combination with Cd stress and acetic acid it was increased to 93.9cm (12%) in WT and decreased to 19.73cm (17%) in transgenic plants as compared to their corresponding control. The chlorophyll and carotenoid contents in WT were increased from 37.1 to 45.8 µg/g F.W (23%) and 1.04 to 1.44 µg/g F.W (38%) while in transgenic plants it was increased from 28.2 to 31.9 µg/g F.W 13% and 1.88 to 2.23 µg/g F.W (18%) respectively. After Cd stress was applied it was decreased to 28.5 µg/g F.W (24%) and 0.58 µg/g F.W (45%) in WT and 20.7 µg/g F.W (27%) and 1.49 µg/g F.W (21%) in transgenic plants while in combination of Cd stress with acetic acid, the chlorophyll content was decreased to 36.02 µg/g F.W (3%) and 20.7 µg/g F.W (11%) and the carotenoid content was increased by 1.07 µg/g F.W (2%) and 1.89 µg/g F.W (1%) both in WT and transgenic plants respectively. After foliar application of acetic acid, the proline content was increased from 0.08µg/g to 0.66µg/g fresh weight and sugar content from 0.68µg/g to 1.35µg/g F.W in wild-type plants. Similarly, in transgenic plants, the proline content was increased from 0.18µg/g to 0.42µg/g F.W and sugar content from 0.40µg/g to 0.65µg/g F.W which indicates the stress response towards cadmium. Acetic acid can boost chlorophyll content along with the increase of production in both wild-type and transgenic Brassica napus. It is concluded from the above study that acetic acid is a possible stimulant for enhancing plant physiological, morphological, and tolerance towards cadmium stress.

Immobilization of Glucosidase using Agar and Alginate as Support System for Enhancing Stability and Reusability

Somayya, Sidra Pervez

Department of Biochemistry, SBBU Women University, Peshawar, Pakistan

Correspondence: sidrapervez87@gmail.com

The primary role of glucosidase, the most significant member of the glycosidase family, is to hydrolyze glucosidase bonds and liberate glucose. The entrapment method of enzyme immobilization has several benefits, including speed, economy, non-toxicity, and adaptability. Glucosidase is more active and reusable when immobilized by agar and alginate. This work addresses the entrapment method of glucosidase immobilization within agarose and alginate. Using guar as substrate, maximum entrapment yield (89%) was obtained at a 5% concentration of agar. The activity of glucosidase was checked by optimizing different parameters like bead size 0f 1.7, enzyme concentration(5ml), bead quantity (1g), and substrate concentration (1.25%) maximum activity of 90% was achieved. The kinetic behavior was slightly changed after immobilization as the activity of the enzyme was increased at 5.0 min (soluble) and 25.0 min (immobilized) in agar and higher reaction temperature activity was evaluated at 50 °C of both soluble and immobilized enzyme. Immobilized glucosidase was more stable at pH 6 and soluble enzymes were stable at pH 5. The highest activity of immobilized glucosidase was detected in a 50 mM citrate phosphate buffer after 25 minutes. While maximum activity of soluble glucosidase was noticed in 50 Mm citrate buffer after 5 minutes. Immobilized glucosidase within agar was reused for up to eight cycles. At the first cycle, the enzyme activity was 83% then a gradual decrease up to eight cycles. Storage stability of immobilized glucosidase within agar at -4˚C retains 32% and at Room, temperature retains 42% activity after 30 days. Optimum sodium alginate concentration was found to be 4%, 0.1M of $CaCl_2$, bead size 0.2mm, beads quantity 1g,2ml of enzyme concentration,0.25% substrate concentration and curing time on $CaCl_2$ 20 minutes were carried out and immobilization yield reach to 84%. The optimum temperature of the immobilized enzyme was 60 ˚C. The optimum pH, buffer, and ionic strength was 8, citrate buffer and 50mM respectively. By using these parameters maximum yield of up to 93% was obtained. Immobilized glucosidase within CaAlg support was reused for up to 6 cycles. Remarkable thermal and storage stability was observed. Thus, the study shows that agar and calcium alginate offer effective support for glucosidase immobilization and may have benefits for industrial applications.

Enhancing Stability and Reusability of Entrapped Mannanase Using Agar and Alginate as Support

Sumbal Kamal, Sidra Pervez
Department of Biochemistry, SBBU Women University, Peshawar, Pakistan
Correspondence: sidrapervez87@gmail.com

The enzyme mannanase has numerous uses in various biotechnology sectors. An ideal foundation for boosting the enzyme’s access to the substrate with greater activity over an extended length of time is provided by enzyme immobilization. In this study, the entrapment approach was utilized to immobilize Mannanase within an agar-agar and calcium alginate matrix and its kinetics and immobilized parameters were studied. Using guar as a substrate, the enzymatic activity of soluble and entrapped Mannanase was evaluated. Under optimal conditions, the maximum yield was found at 4% agar concentration with bead size 0.7mm, and enzyme concentration 5ml. The optimum temperature of immobilized mannanase within agar and soluble Mannanase is 50 ˚C and its yield was 85%. Immobilized mannanase was most stable at pH 5 while soluble enzyme was at pH 6. The highest activity of immobilized mannanase was detected in a 25 mM citrate phosphate buffer after 20 minutes. Immobilized mannanase within agar was reused for up to eight cycles. The optimum sodium alginate concentration was found to be 3%, 0.3M of $CaCl_2$, bead size 0.5mm, 0.5ml of enzyme concentration,1.25% substrate concentration, and curing time on $CaCl_2$ 10 minutes was carried out and immobilization yield reached 95%. The optimum temperature of the immobilized enzyme was 60°C. The optimum pH, buffer, and ionic strength was 8, citrate buffer and 50mM respectively. The thermal stabilities of free enzymes are low as compared to immobilized enzymes. Immobilized enzymes within agar were stable at 60 ˚C after 30 minutes while immobilized enzymes within CaAlg were stable at 50 ˚C after 60 minutes. Hence the study concludes that calcium alginate and agar provide efficient support for the immobilization of Mannanase and may have advantages for industrial use.

Enhancing Fabric Bleaching and Dyeing Efficiency through Enzymatic bioprocessing with glucose Oxidase: A Sustainable Approach

Nida Israr, Sidra Pervez

Department of Biochemistry, SBBU Women University, Peshawar, Pakistan

Correspondence: sidrapervez87@gmail.com

A well-characterized enzyme called glucose-1-oxidase (GOD) catalyzes the conversion of beta-D-glucose to D-gluconolactone and hydrogen peroxide, which is used to defend against electromagnetic radiation by employing metalized fabrics. The sustainable alternative for bleaching cotton textiles is to use glucose oxidase to produce hydrogen peroxide enzymatically. Bio-bleaching of cotton using H_2O_2 generated from GOD underscores the viability of enzymatically produced H_2O_2 for fabric bleaching A naturally occurring enzyme generated by certain fungus and insects is glucose oxidase. By reducing the use of harsh chemicals and high temperatures, enzymatic bleaching can result in decreased energy consumption, reduced water pollution, and enhanced worker safety. It is a more environmentally friendly process than chemical bleaching which involves treating the fiber with hydrogen peroxide which highly damages the fabric. Optimization revealed that the optimal enzyme concentration for the procedure adjusting the enzyme concentration to 0.5 ml GOD 50ml buffer sodium acetate at 37 degrees for 30 min at PH 5 in The bio-bleaching efficiency tests revealed a substantial 49% reduction in fabric weight, indicative of successful contaminants remove furthermore by glucose concentration The GOD-PAP reagent was used and mixed with enzyme solution at 37-degree 3ml reagent 30µl of leftover enzyme solution was mixed. The reaction produced a colored compound whose intensity is proportional to the glucose concentration. The absorbance of the solution was measured by using a spectrophotometer at the wavelength speclized at 540 nm. In the degree of whiteness test performed on the bio-bleached cotton fabric. The treated fabric's appearance of being lighter and brighter specifically demonstrated how well the bleaching method improved the fabric's optical qualities. This duration removes contaminants effectively and causes the least amount of damage to the fabric. Enzymatic bleaching with GOD not only provides environmental benefits but also carries socio-economic implications.

Cultivation of indigenously isolated microalgae for dye degradation

Hadia Qureshi, Sidra Pervez

Department of Biochemistry, SBBU Women University, Peshawar, Pakistan

Correspondence: sidrapervez87@gmail.com

Microalgae are tiny creatures found in both terrestrial and aquatic environments. As a result of that, the group of species known as microalgae is extremely diverse and can be found in a variety of environments. Three essential elements are needed for microalgae to grow: carbon sources, water, and sunlight. The aim of the present study was the collection and characterization of microalgae from indigenous sources. Through the use of microscopy, both of the algae samples, designated as (A and B), had been recognized. Different methods (total carbohydrate, total protein, and total sugar) are carried out for the characterization of Microalgae by different tests such as Anthrone, Lawry's, and DNS tests. Azo dye degradation was performed for the ability of microorganisms (microalgae) to decolorize the dyes. The fifteenth day was when the microalgae displayed the highest activity and most distinct characteristics i.e., with 173.2 mg/ml of carbohydrates, 133 mg/ml of protein, and 59.6 mg/ml of sugar in Fog's media while in BBM (208mg/ml carbohydrate, 192.9mg/ml protein, and 100mg/ml sugar content). It was found that *Spirogyra sp.* can degrade the dyes greatly at higher concentrations i.e., 2% and 2.5%. The conclusion is that *Spirogyra sp.*, a microalga, has a great deal of promise for dye degradation and was well characterized at the particular parameters.

Evaluation of *Salvia hispanica* seeds as a source of potent bioactive carbohydrates

Shaista Afzal, Afshan, Imdad Ullah

Department of Biotechnology, Abdul Wali Khan University Mardan, KP, Pakistan

Correspondence: shaistaafzal880@gmail.com

Natural mucilage and gums isolated from plants especially seeds have been widely used in different capacities including as excipients, therapeutic formulations, intensifying agents, gelling agents that increase food shell life, and other functional food products. Chia seeds release a high amount of mucilage when immersed in water which has been determined to have several bioactive roles. Chia seed is an annual herbaceous plant that came from Mexico and Northern Guatemala. This study aimed to optimize the process of mucilage extraction and to fractionate and evaluate the biological role of polysaccharides present in the chia seed mucilage. Mucilage was extracted from chia by immersing chia seeds in autoclaved distilled water. The obtained mucilage was dried with the help of a lyophilizer and the dried mucilage was fractionated in 75% v/v ethanol. The biological activities of crude and each fraction was determined using various biological assays. The antimicrobial activity was performed using the well agar well diffusion method. The results revealed that among the screening fractions ethanol precipitated fraction was the most potent

one which induced a 3.5mm zone of inhibition in S. typhi, and 5.2mm in S. aureus while no significant zone of inhibition was observed in the crude fraction. Radical scavenging activity of the test samples was determined using DPPH where ethanol non-precipitated fraction was found to have the best IC50 value which is 536 µg/mL followed by ethanol precipitated fraction of 570 µg/mL. Alpha amylase inhibition of the extract was determined through standard alpha amylase inhibition assay where ethanol non-precipitated fraction showed an IC50 value of 546 µg/mL. Ethanol non-precipitated fraction was found to significantly reduce the paw edema in the carrageenan-induced paw edema mice model. None of the fractions was found to be inducing any significant hemolytic effect. The results concluded that among all the chia seed mucilage fractions, ethanol non-precipitated fraction exhibited significant biological activity which could be further screened and investigated for potential antidiabetic drug development. The evidence resulting from this study will further strengthen the search for other plant-based milage and their development as alternative therapeutic measurements. Furthermore, biochemical and molecular characterization of these mucilage fractions will understand and increase our knowledge of the exact molecular mechanism involved in its bioactivity.

In vitro prebiotic activity of oligosaccharides from cress seed mucilage

Mansoor Ali[1], Lubna Naz[1], Anam Pervaiz[1], Amjad Iqbal[2], Ayaz Ahmad[1*]
[1]Department of Biotechnology; [2]Department of Food Science and Technology, Abdul Wali Khan University Mardan, Pakistan
Correspondence: ahdayazb5@awkum.edu.pk

Probiotics are beneficial microorganisms known for their positive impact on human health and require specific nutrients to thrive and exert their health-promoting effects. Oligosaccharides, a class of dietary carbohydrates have gained attention as potential prebiotics, selectively nourishing beneficial gut microbiota. The current study aimed to isolate oligosaccharides from *L. sativum* mucilage and evaluate their in vitro prebiotic effects on human probiotics. Mucilage was extracted from cress seeds in distilled water overnight, and polysaccharides were precipitated with 75% ethanol. The precipitated polysaccharides were fermented with Pichia pastoris to produce bioactive oligosaccharides followed by fractionation through size-exclusion chromatography. The fermentation process showed substantial growth of P. pastoris, with cell counts increasing from 4.98 ± 0.13 log CFU/mL to 8.76 ± 0.17 log CFU/mL over 6 hours. Biochemical analysis revealed that DF73 had the highest carbohydrate content (3.14246 mg/mL), while EPP contained the most unsaturated uronic acid (5.54436 ± 0.35138 µg/g) and hexose (6.54849 ± 0.30852 µg/g). DF58 exhibited the highest uronic acid concentration (7.98201 ± 0.73763 µg/g), and DF72 had the highest pentose content (4.54738 ± 0.11939 µg/g). The prebiotic effects on *Bacillus clausii* and *Bifidobacterium bifidum* showed that DF73 had the strongest acidifying effect (pH drop to 4.74333 ±

0.27538) and significantly supported bacterial growth. DF72 and DF73 had the highest prebiotic. These findings highlight the potential of cress seed-derived polysaccharides, especially DF72 and DF73, as effective prebiotics for enhancing gut microbiota, offering a promising strategy for developing functional foods aimed at improving gut health.

Keywords: Cress seed mucilage, Oligosaccharides, Pichia pastoris fermentation, Probiotics.

Exploring the therapeutic potential of exopolysaccharides from wheat-derived endophytic fungi

Anam Pervaiz[1*], Mahnoor[1], Faryal Ashfaq[1], Amjad Iqbal[2], Ayaz Ahmad[1]
[1]Department of Biotechnology; [2]Department of Food Science and Technology, Abdul Wali Khan University Mardan.
Correspondence: aanampervaiz@gmail.com

Endophytic fungi residing inside plants have emerged as an intriguing source of bioactive secondary metabolites with diverse biological applications. Exopolysaccharides (EPS) have gained significant attention for their potential therapeutic role in various human ailments. In the current study, fungal endophytes were isolated from various parts of the wheat plant and identified as Wh1, Wh2, Wh3, and Wh4. The fungi were cultured in a broth with yeast extract, peptone, and dextrose at 25 °C for 7 days, and EPS was precipitated with ethanol. Biochemical analysis revealed significant carbohydrate content in all EPS, with Wh4 having the highest total carbohydrate content (10.8 µg/mL). The EPS composition included various concentrations of uronic acid, unsaturated uronic acid, pentose, and hexoses. The biological potential of the EPS was determined using various biological assays, including the DPPH assay, α-amylase assay, and anti-inflammatory and antibacterial assay. Biological assays demonstrated strong antioxidant potential in Wh2 (73.00% ± 3.22) and Wh3 (77.82% ± 1.42) at 1000 µg/mL. Wh4 showed notable anti-inflammatory activity (88.34% ± 1.14) with an IC50 of 163.60 µg/mL. Wh3 exhibited the highest anti-diabetic activity with α-amylase inhibition of 87.17% ± 4.21 and an IC50 of 301.39 µg/mL. All EPS exhibited significant antibacterial activity against E. coli and S. aureus, with Wh2 showing inhibition zones of 6.75 mm against E. coli and 10.75 mm against S. aureus at the highest concentration. This study highlights the therapeutic potential of EPS from wheat endophytic fungi, suggesting their promising role in developing natural, sustainable solutions for human health and biomedical research.

Keywords: Endophytic fungi, Exopolysaccharides, Wheat plant, Bioactive compounds.

Biotechnological Aspects of Assessing Clinical Parameters in Rheumatoid Arthritis: Correlations between tender and swollen joints, morning stiffness and Laboratory Findings: A cross-sectional study in Peshawar, Pakistan

Abdullah, Danish Khan, Rahimullah

[1]*Department of Biotechnology, SBBU, Sheringal Dir upper KP, Pakistan*

Correspondence: abdullahsbbu2@gmail.com

Rheumatoid arthritis (RA) is a chronic autoimmune disorder that significantly impacts quality of life through joint inflammation and pain. The integration of biotechnological approaches in assessing clinical parameters has opened new possibilities for understanding the disease's progression and management. This study examines the correlations between key clinical features; tender and swollen joints, morning stiffness, and laboratory findings to provide insights into RA among diverse ethnic groups in Peshawar, Pakistan. Cross-sectional was conducted from January 15 to July 15, 2024, at the Rheumatology Department OPD of Lady Reading Hospital (LRH) involving 110 RA patients, representing various ethnicities such as Pashtun Punjabi and others. By converging on the biotechnological assessment of disease parameters, the study aimed at correlating clinical manifestations with laboratory results by counting erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), cyclic citrullinated peptide (CCP) and complete blood count CBC. The demographic and age-wise analysis revealed a higher prevalence of RA among females (70%) compared to males (30%) and about 60-70 % in individuals aged 40-45, moderate incidence (25-30%) in the 25-35 age groups and with no reports under age group 25. Socioeconomically RA distribution was highest (80%) in the middle class compared to the poor (50%) and rich (15-20%). Morning stiffness, a critical parameter, was observed to last between 20-35 minutes in most patients, correlating with the severity of joint inflammation and tenderness. Laboratory findings demonstrated that patients with more severe symptoms had elevated ESR and CRP levels, positive CCP, and abnormal CBC results highlighting the utility of these biomarkers in RA management. The findings suggest that tailored biotechnological approaches could enhance RA management by allowing for more precise correlations between clinical symptoms and laboratory biomarkers. This could lead to better-targeted treatments, improved patient outcomes, and a deeper understanding of RA's multifactorial etiology in different ethnic groups.

Keywords: Rheumatoid Arthritis, Complete blood count, Morning stiffness, Cyclic citrullinated peptide, Erythrocyte sedimentation rate.

Elucidating plant-cell melatonin interactions in the production of medicinal metabolites using in vitro culture of *Moringa oleifera*

Atta Ullah, Muhammad Aizaz, Karim Gul, and Raham Sher Khan*

Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan

Correspondence: rahamsher@awkum.edu.pk

Melatonin is a pleiotropic molecule that regulates key biological pathways in plants including growth, rooting, seed germination, photosynthesis, and defense against biotic and abiotic stresses. *Moringa oleifera*, commonly known as drumstick tree, is a nutritional plant found across the globe. The current study aims to evaluate the effect of melatonin on the in vitro callogenesis and regeneration in Moringa and to analyze the biochemical and metabolomics profile of the callus and regenerated plants. Moringa seeds were cultured on MS basal medium containing 3% sucrose, and 0.9% agar supplemented with different concentrations of melatonin (0, 5, 10, 15, 25, 35, and 50 mg/L) and incubated at 25 ± 1°C for germination, and germination data was recorded. In another experiment, Moringa seeds were cut down into 2 to 4 pieces and then cultured on the same medium with the addition of BAP (1.0 mg/l) and 2,4-D (2.0 mg/l). Data on callus induction and callus morphology, fresh and dry biomass, and biochemical parameters of the callus and regenerated plants were recorded. The results revealed that melatonin supplemented with BAP and 2,4-D, significantly improved the quality and biomass production. Biochemical characterization shows that enhanced concentrations of phenolics, alkaloids, and terpenoids were produced using in vitro Moringa plant and callus. From these preliminary results, we can conclude that melatonin can be used as an effective elicitor in improving the quality and quantity of bioactive compounds in *M. oleifera* cultures. However, further studies are needed to explore the molecular pathways, involved in melatonin-induced metabolites production, contributing to the production of plant-derived pharmaceuticals.

Keywords: Melatonin, *Moringa oleifera*, in vitro culture, bioactive metabolites

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