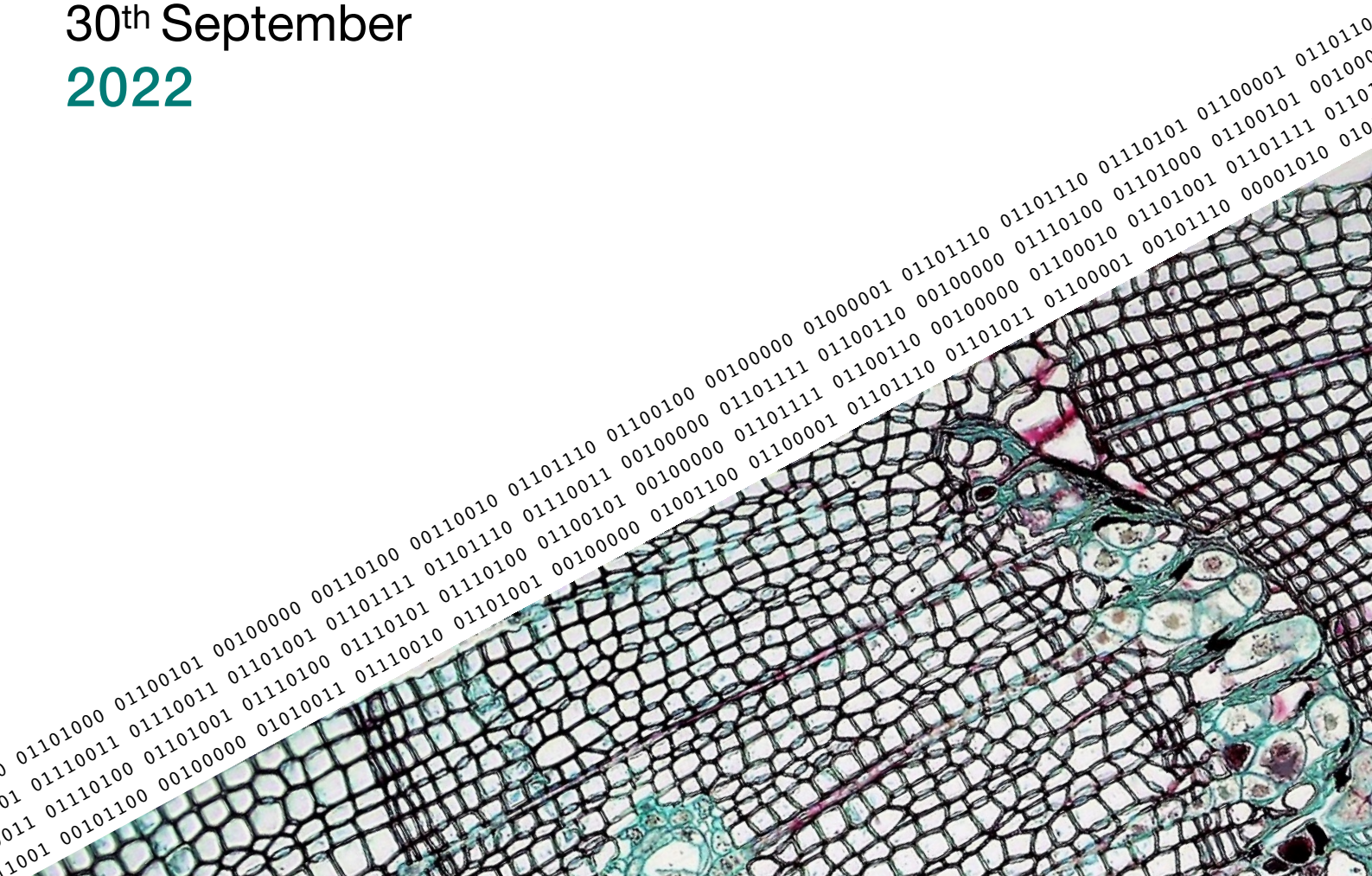
The Institute of Biology
Sri Lanka

# Towards a Paradigm Shift in Biology

## Proceedings of the 42nd Annual Sessions

30th September
2022



# Council of the Institute of Biology Sri Lanka 2021/2022

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Seated (from left): Dr. I. A. J. K. Dissanayake, Prof. P. B. Ratnaweera, Dr. H. D. D. Bandupriya, Dr. G. H. Galhena, Dr. R. Wimalasekera, Prof. S. A. C. N. Perera, Dr. K. G. S. U. Ariyawansa

Absent: Dr. W. K. S. M. Abeysekera, Prof. L. D. Amarasinghe, Prof. C. K. Beneragama, Prof. Nissanka De Silva

# INSTITUTE OF BIOLOGY

# SRI LANKA

PROCEEDINGS OF THE 42nd ANNUAL SESSIONS

Theme

## *Towards a Paradigm Shift in Biology*

Institute of Biology, Sri Lanka

30th September 2022

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Cover design: A microscopic view of a plant leaf. Image credit: Fayette Reynolds M.S. from Pexels. The binary code reads "The 42nd Annual sessions of the Institute of Biology, Sri Lanka". Designed by Rovindu Kudaliyanage.

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# Annual Sessions of IOBSL 2022

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# About the Institute of Biology, Sri Lanka

The Institute of Biology is a leading professional body of biologists in Sri Lanka. The institute was formulated in a small way by a group of Sri Lankan biologists led by late Prof. B. A. Abeywickrama (Emeritus Professor of Botany, University of Colombo) in 1981. It became an incorporated organization by the Act of Parliament No 22 in 1984.

**The objectives of the institute are:**

1. To promote and advance the science of biology and its applications in Sri Lanka.
2. To advise the government, and give counsel to public corporations, local bodies and other institutions on all matters connected with the application of biology in the progress and development of the country.
3. To promote acquisition, dissemination and interchange of biological knowledge by providing a forum for the presentation of original communications and discussions and maintaining libraries which publish matters of interest to the profession of biology.
4. To promote education in biology at all levels.
5. To promote, encourage and foster original research in biology.
6. To ensure the maintenance of high standards in the professional activities and the general conduct of its members.
7. To establish liaison with other scientific organizations.
8. To establish and enhance the status of the profession of biology in Sri Lanka.

**Membership**

The institute has around 600 members, working in industry, research, education and healthcare. The institute also awards Fellowships and Charter of Biology status for members. There are seven categories of membership and members are encouraged to transfer to other grades in due course. Eligibility for each category depends upon a combination of professional experience and academic qualifications. Fellows are entitled to use the abbreviated designation F.I. Biol (Sri Lanka) while the Chartered Members are eligible to use C. Biol (Sri Lanka), Members M.I. Biol (Sri Lanka). The designation 'Chartered Biologist' endorses the high standards expected of biologists and is for international recognition as a hallmark of professional competence and ethical conduct.

**Activities**

The Institute organizes workshops and seminars on current topics in biology on a regular basis. It also plays an important role in biology education on a wider spectrum of participants ranging from those in industry, those seeking self-employment, school children and the general public. The Biology Olympiad Competition organized solely by the Institute of Biology is a hallmark event in the country which provides opportunities to students to become champions in biology both locally and internationally. "Inter-University Biology Quiz Competition" and "Inter-University Biology Challenge", are two initiatives taken to promote and popularize education in biology among the undergraduates in the stream of biological sciences of the state universities in Sri Lanka. Details of events are posted on the IOB website and the newsletter "BIO NEWS" re-launched as the official e-newsletter of the IOBSL. The information keeps

readers informed on current events in the field of biology. Sri Lankan Journal of Biology (SLJB), a biannual open access journal published by the IOBSL, creates the platform for researchers to disseminate the findings of biology related research under a Creative Commons Attribution 4.0 International License. The annual session provides a forum for both senior and junior biologists to present their research findings for a complex audience of scientists, policy makers and implementers. The annual sessions continue for the 42nd time this year.

In 2022, IOBSL initiated a few more activities. 'Biology Oration' was introduced with the objective of recognizing outstanding research contributions in biology providing a platform to promote awareness of the IOBSL among relevant stakeholders in the field of biology in Sri Lanka. Further, the 'Young Scientist Award' was launched to recognize outstanding contributions and achievements in research of the early career corporate members of the Institute. The web page, and Facebook were open for the publication of research news stories of its membership with the aim of recognizing the valuable contribution made by the institute membership toward research and development and bridging the gap between researchers and the general public. A national competition on Biology photography was also initiated by the IOBSL to create an opportunity for professional, amateur, and youth photographers in Sri Lanka to communicate with the general public on the biology related issues.

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# PRESIDENTIAL ADDRESS

## Human Genetic Research in Sri Lanka – Biologists' Standpoint

**Gayani Galhena, PhD, C. Biol**

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I consider it a singular privilege to address this distinguished gathering of Biologists as the President of the Institute of Biology Sri Lanka at its 42nd Annual Sessions. In keeping with the theme of this year's Annual sessions, Towards a Paradigm shift in biology, I like to focus on a field that has witnessed a drastic change over the last few decades, human genetics. However, I do not intend to discuss the cutting-edge technologies that have opened up the flood gates of information or the controversy over transhumanism. In the Sri Lankan context, such phenomena are too sophisticated yet, to be our primary focus. Hence, in my speech, I want to draw your attention to where we stand as biologists in the landscape of human genetic research in Sri Lanka to understand what hinders us from moving along with the shifting paradigms in biology.

### Early developments

Let me first start by taking you to the early days of genetic research to show you the cradle of modern genetics. It dates to Gregor Mendel's work on pea plants, which was published in 1865. Though he was able to postulate about 'factors' of inheritance using his experimental observations, he wasn't aware of the existence of chromosomes. It was another biologist, Walther Flemming, who first discovered the chromosomes in 1882 from the salivary gland cells of midges. However, he was not aware that these chromosomes were housing the 'factors' of inheritance that Mendel was referring to. Thus, it took another biologist Thomas H. Morgan in 1910 to demonstrate using his experiments on fruit flies, that genes, Mendel's so-called 'factors' of inheritance, were in fact lined up within chromosomes. Later, two more biologists James Watson and Sydney Brenner together with other physicists and chemists laid the foundation to construct the central dogma of molecular biology, which explained the flow of information from DNA, the building block of heredity to the construction of the phenotype.

### Debate over genetics vs. molecular genetics

These findings paved way for a new era of genetic research, the era of molecular genetics. This brings us to an interesting question. What is the difference between molecular genetics and genetics? It can be explained using two fundamental concepts in biology, 'reductionism' and holism'. Reductionism explains how a complex system can be understood by studying its component parts and has been a powerful strategy in biology. 'Molecular genetics' uses this approach by studying how the elements of heredity work, starting at the DNA level.

Holism on the other hand points out that a higher level of order cannot be meaningfully explained by examining component parts in isolation. For example, a cell dismantled to its chemical ingredients can no longer show its unique property - life. 'Genetics' use this holistic or organismic approach and studies the effect of heredity over a broader range of phenotypes. However, in the 21st century, is this division between 'molecular genetics' and 'genetics' realistic? As Prof. C. Kenneth Waters, Research Chair in Logic and the Philosophy of Science, University of Calgary put it, "The term 'molecular genetics' is now redundant, because

contemporary genetics is thoroughly *molecular*. Genetics is not made up of two sciences, one molecular and one non-molecular." Obviously, contemporary genetics has a unifying approach. It studies the mechanism from the molecular level upward to the organismic level to understand the full effect of heredity over the organism. At the same time, it also tries to appreciate the interactions between different regulatory layers in bringing out the phenotype. In addition, the term genetics has now gone beyond its original meaning of single genes and captures the genomic landscape, which incorporates both coding and noncoding DNA.

With these expanded boundaries, contemporary genetics have branched out in such a sophisticated manner, with many overlapping fields. If you carefully look at these fields, we can identify three discrete criteria used for this categorization; 1) the approach used for analysis, such as candidate gene analysis, whole genomic analysis, epigenomic analysis etc. 2) the aim of the research, or what we try to achieve; i.e. whether we are conducting basic research to understand molecular pathways or a clinical application geared to fight diseases, etc. 3) the organism on focus; i.e. microorganisms, plants, animals, or humans. The combination of these elements has produced this seemingly complex landscape of genetic research in which human genetics is only a somewhat recent development. As per the observation by the famous American Geneticist, Alfred H Sturtevant in 1954, "Man is one of the most unsatisfactory of all organisms for genetic studies. Obviously, no geneticist would study such a refractory object."

## Rise of human genetics

Accordingly, the term 'human genetics' came into notice only around 1949 when the genetic basis of some well-known human Mendelian diseases such as sickle cell anemia were identified. This opened an era that shift the focus of genetics from basic biology to clinical genetics. As a result, the human genome project as well as many subsequent mega genomic projects like ENCODE (Encyclopedia of DNA Elements), International HapMap and the 1000 Genomes project, primarily targeted the identification of medically important genes.

However, human genetic research does not comprise only of medical genetics. Like bacteria, who have no defined niche within the classical botany-zoology division, the human is also an organism with a bit of an awkward position. Its whereabouts tends to swing between the purview of zoology and medicine. Thus, human genetics is in fact an umbrella term that cover many different branches of biology other than medical genetics such as cytogenetics, forensic genetics, population genetics, molecular anthropology, and behavioral genetics.

## Biologists' plights: accessibility to human specimens

Nevertheless, within the Sri Lanka context, human genetic research is still revolving largely around medical aspects and is dominated by the medical professionals. Even many biologists consider it to be under the purview of medicine. The Importance of physical and psychological wellbeing of humankind is not the only reason governing this irrational situation. Partly, it is due to the easy accessibility of human samples by medical professionals. The sample types that can be used for human genetic research contain a diverse array of specimens from hair roots to buccal swabs and blood to biopsies. Among them, blood samples are the most popular specimen type due to the high DNA yield it can provide even from minute volumes. However, the collection of blood needs training in phlebotomy, a skill that most of us, as biology researchers has never accomplished. This creates an unnecessary barrier between biologists and human genetic research requiring the assistance of medical professionals, even in non-medical research fields like forensic genetics or molecular anthropology. It is my view that this barrier needs to be addressed urgently and effectively to ensure human genetics is a research

field accessible to biologists in Sri Lanka. And obviously, the simplest way to overcome this obstacle is training biology researchers in phlebotomy.

Similar to the training given in animal handling, perhaps it is high time now for us to consider incorporating basic phlebotomy training in relevant undergraduate and postgraduate programmes which deal with human genetic research. In fact, the practice can be adapted to all programmes related to biomedical sciences. We can also consider training the relevant laboratory technicians in phlebotomy. And if we as biologists can qualify at least to fingerpick an individual to collect a couple of blood drops, I believe that will be adequate to cover sampling in all human genetic research areas except medical genetics and cytogenetics.

**Human subject definition for ethics review purposes**

However, getting access to human specimen would not remove the barrier entirely. There is an even bigger obstacle in the form of obtaining ethical clearance for human research. According to international research ethics guidelines, research does not qualify to be considered as human research simply because the specimens have originated from human beings. The concept behind the human subject research is much broader than that and is aimed specifically at minimising possible physical as well as psychological/social harm through loss of privacy and confidentiality.

The table below illustrates the general federal guidelines for human research.

<table>
<tr><th>Specimen type</th><th>Collection time</th><th>Sample</th><th>Recommended level of reviewing</th><th>Consent</th></tr>
<tr><td>Specimens without identifiers - minimal risk - no subject contact</td><td rowspan="3">Already collected</td><td rowspan="3">Blood, biopsy specimens, images, records</td><td>Do not qualify under human subject research</td><td>Not applicable</td></tr>
<tr><td>Access to identifiers, but identifiers are not recorded or linked to specimens - minimal risk - no subject contact</td><td>Exempt from reviewing</td><td rowspan="2">Adheres to the scope of research allowed by the original consent or apply for informed consent waiver</td></tr>
<tr><td>Access to and recording of identifiers - minimal risk- no subject contact</td></tr>
<tr><td>Minimal risk- subject contact</td><td>Collected after commencing the research</td><td>Blood or surgical/ diagnostic specimens that would be discarded</td><td>Expedited review</td><td>Consent required</td></tr>
<tr><td>Procedures pose greater than minimal risk</td><td>Drug trials/ behavioral study types</td><td></td><td>Full review</td><td>Consent required</td></tr>
</table>

According to this, already collected specimens without identifiers (characters that help identifying a person such as name, address, national identity card number, photographs etc.) such as blood or biopsy samples do not qualify to be considered under human subject research. Likewise, human research guidelines do not apply for the research conducted with samples taken from the dead bodies either.

On the other hand, if the identifiers are available or accessible to the researcher, but not linked to the already collected specimens, then the risk of psychological harm through the loss of privacy is minimum and such research can be exempted from ethical review. This means the majority of the preexisting samples that are obtained from medical professionals for genetic research are either not considered as human specimens or can be exempted from a full ethical review. Even those specimens that we collect prospectively during research, can be considered under expedited review, if it is causing only a minimum risk or if it is collected as part of a diagnostic or surgical intervention and are meant to be discarded afterwards.

However, those human subject research that are likely to cause more than the minimal risk and require contact with human subjects continuously over the period of the research, need a full ethical review i.e., drug trials or behavioral research. The human genetic research that we are discussing within the present context in Sri Lanka does not generally involve those. We usually contact human subjects only once, at the beginning of the research for specimen collection. Likewise, the data we require are generally either biochemical, behavioral or demographic parameters that would not make unique identifiers of individuals.

And another important point I want to highlight in this guideline is the flexibility of informed consent. Preexisting human specimens, which cannot be connected to their doners, are eligible for informed consent waiver. Further, this guideline also facilitates making use of already collected and stored human samples that will be discarded otherwise. Such specimens represent a valuable tressure in terms of human genetic research that can be utilized in medical, forensic and population genetics to address many scientific questions.

Now, I would like to ask a question from the researchers, who are working with human specimens. Is this your ethics review committees experience? I am sure many of you have encountered much more stringent reviews for research conducted with human specimens that drag the commencement of your research for many additional months. I believe this practice needs to be changed and that it is high time to revisit the human research guidelines pertaining to minimum risk research conducted in Sri Lanka like human genetic research. We need to ensure that the ethics review committees address the fundamental purpose of human research review guidelines; that is minimizing the physical, psychological and social harm to the subjects. This needs a protective approach, not a restrictive approach.

**Orientation of ethics review committees**

It is also interesting to note that the majority of ethics review committees which undertake reviewing of human research in Sri Lanka are established in medical settings, either in medical faculties or in hospitals. Out of those ERCs registered under the Forum of Ethics Review Committees in Sri Lanka (FERCSL), only one has a nonmedical background. This placement of ethics review committees within medical setups is likely to have contributed towards the mixing up of minimum risk genetic research with high-risk intervention studies.
I believe it is high time now to establish ethics review committees that are independent of medical institutions which have the mandate to review human research, within the boundary of minimal risk. And I am happy to say that we as the institute of Biology came forward to take up this responsibility. Our ethics review committee is now authorized to review human

research that come under the purview of biology, within the boundary of minimal risk. I would like to take this opportunity to thank the council and Prof. MJS Wijerathna, the Chairperson of our Ethics Review Committee for agreeing with this bold and important step, a step towards a higher level of independence with respect to human research in Sri Lanka.

**Bioinformatics: the void**

Another barrier to human genetic research in Sri Lanka, especially to be on par with international research, is the limited knowledge in bioinformatics. As many of you are aware, bioinformatics and genetics go hand in hand. There is a huge void in bioinformatic knowledge within the Sri Lankan context especially when it comes towards the analysis of data generated from cutting edge tools like next generating sequencing and SNP microarrays. At present, within the government sector, we have six next generation sequencing machines, which are thoroughly underutilized. The shortage of trained bioinformaticians, who are capable of processing and analysing genome, transcriptome and proteome data is the main reason behind this deadlock. Indeed, financial constrains also plays a role in this, but improving finances will not help in coming out of this situation until we become self-sufficient with respect to our technical expertise.

As such, providing training in bioinformatics should be one of our priorities as biologists to transcend Sri Lankan genetic research to the next level. We as the IOBSL have shouldered this task this year by introducing six different short courses in bioinformatics covering genetics, genomics proteomics as well as metagenomics. We had 227 Participants for these six courses which reflects the dire need for training in this field.

**Collaborative research framework**

Before I conclude my speech, I would also like to draw your attention to an aspect that is generally brushed under the carpet. That is the issues associated with collaborative research. Especially when it comes to medical genetics. Be it molecular genetics, immunogenetics or pharmacogenetics, genetic data needs to be analysed relevant to either biochemical or immunological parameters of patients under study to make it more meaningful and applicable in a clinical setup. Accessing such databases requires collaboration with medical professionals. But how structured are these collaborations and to what extent do they achieve the expected outcomes? Within the current research context, most collaborations are based on unwritten, unstructured, and implicit understandings. As biologists who have limited access to clinical setup by default, we expect medical professional to collect the human specimens and to share the relevant biochemical and clinical data with us during medical genetic research. Whether or not our collaborators are of the same view or whether they are ready to share data to the level of our expectation is unknown to us until we reach the latter part of the study. If they take a more reserved approach in data sharing, or if there are disputes in sharing credit, the productivity of the research could be drastically affected. Like bad marriages, bad collaborations too would bring in lot of stress, wasted energy and disappointments and sometimes aggression as well.

So how can we avoid it? Do we have a mechanism in the Sri Lankan context to streamline research collaborations? Not that I know of. I personally believe that there is an urgent and pressing need to device a mechanism to facilitate collaborations not only between different researchers or institutions but also between different disciplines, especially within the purview of the changing paradigms in science that has brought a multitude of disciplines together. We must develop a formalized and a structured method to distribute responsibilities, obligations, and benefits. Since such aspects are part of the ethical conduct of researchers and are core

elements that determine the successful completion of projects, I propose ethics review committees to come forward and assist us in this. The simple inclusion of a section on research responsibilities and credit shearing in the ERC application would facilitate ice breaking among the researchers on such topics and would initiate discussions. When we consider our cultural upbringing, where interpersonal relationships and professional hierarchy can have undue influence over the balance between obligations and credit, such a mechanism might assure overall fair play among researchers. Having a proper framework for research collaboration would also remove the barriers of mistrust that hinders researchers from coming together and would allow them to have a more realistic view of the tradeoffs between individual research and collaborative research.

**Way forward and our responsibility**

Charles Darwin once said, "In the long history of humankind, those who learned to collaborate and improvise most effectively have prevailed." I think this statement is extremely valid for the research in human genetics. Especially in the current context of the financial crisis. Just look around to see the stored human specimens and clinical, demographic, phenotypic and genetic data already available and are qualified for minimal risk guidelines. With effective collaborations and an open mind set, these can be easily utilized to generate a wealth of knowledge in the field of human genetics while eliminating those expenses associated with sample collection, completely and partially eliminating those related to sample processing. Collaborations would also help us in better analysis, data interpretation and publication through resource sharing.

And establishing such collaborations requires the leadership of a solid, reputed reliable professional body. And I believe for us biologists, there is no organization other than the Institute of Biology that fits these specifications. The potential of the Institute of Biology to support and foster the advancements in biological research is immense. Tapping its full potential however is the responsibility of us as its membership.

# FELICITATION OF

## Mr. Rohan Pethiyagoda

Citation Presented by

**Professor Devaka Weerakoon**
**Department of Zoology and Environment Sciences, Faculty of Science, University of Colombo, Colombo 03**

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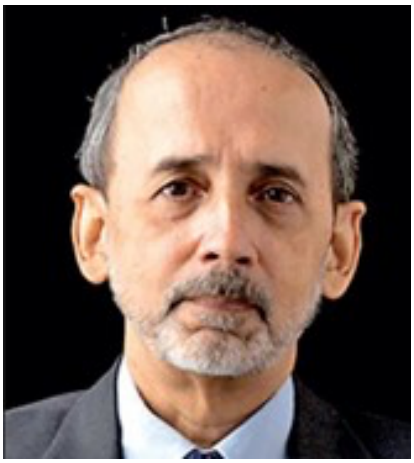

I consider it a great privilege and honor to introduce my colleague and long-standing friend, Mr. Rohan Pethiyagoda, the recipient of this year's Lifetime Achievement Award presented by the Institute of Biology. Mr. Pethiyagoda is a man of many talents, and this award is given to him in recognition of his contributions to the field of biology, especially taxonomy and phylogeny of vertebrates as his work has led to a spate of new discoveries of freshwater fish, frogs, reptiles, and mammals as well as clarification of taxonomic status and phylogeny of these taxa.

Born and raised in Colombo, Rohan received his secondary education at St. Thomas' College, Mount Lavinia. He obtained his bachelor's degree with honors in Electrical and Electronics Engineering from King's College, University of London in 1977 and MPhil in Biomedical Engineering from the University of Sussex in 1980.

In 1981, he returned to Sri Lanka and joined the Ministry of Health and served as an engineer in the Division of Biomedical Engineering. In 1982 he was promoted to the post of Director of the Division of Biomedical Engineering, a post he held till 1987. From 1984 he was concurrently appointed as the Chairman of Sri Lanka's Water Resources Board. He returned to government service in 2015 when he was appointed as the Chairman of Sri Lanka Tea Board, a post he held till 2018, which would have brought a lot of nostalgic memories to him, for he is a son of a tea planter.

Rohan also has a spectacular record of service to our profession as he has served in the board of directors or as an officer of numerous professional societies. He served as the deputy chair

of the IUCN Species Survival Commission, deputy chair of the Assurance group of the British American Tobacco Biodiversity partnership, in the board of trustees of the International Trust for Zoological Nomenclature and the board of directors of the declining Amphibian Population Task Force. In 2009 he was appointed as a Research Associate of the Australian Museum.

I believe the entry of Rohan to the discipline of natural science in Sri Lanka is denoted by his landmark publication in 1991, the Freshwater Fishes of Sri Lanka, treating the diverse ichthyofauna of Sri Lanka more comprehensively than ever before, including the description of two new species. Mr. Pethiyagoda used the profits from the book to setup the Wildlife Heritage Trust (WHT) that embarked on a journey of exploration, discovery and documentation of Sri Lanka's biodiversity and elucidation of phylogenic and biogeographic relations of Sri Lankan ichthyo and herpetofauna. The work done by Rohan and his colleagues of the WHT has led to the discovery and or description of more than 100 new species of vertebrates, including fish, frogs, lizards, and shrews as well as 43 freshwater crabs in Sri Lanka. In addition, his work inspired several other young naturalists in Sri Lanka, which has contributed further, to improve our knowledge on Sri Lankan taxa.

Over the last three decades, Rohan led, guided, and contributed to an extraordinary array of people, projects, events, and developments, many of which have proven critical to the field of vertebrate taxonomy and phylogeny as it is now constituted. In the process he has helped the development of many young scientists including myself. Many of the young scientists that worked with Rohan in WHT such as Mr. Mohomed Bahir, Prof Suyama Boyagoda, Mr. Madura de Silva, Mr. Dinesh Gabadage, Dr Kalana Maduwage, Mr. Kelum Manamendra-Arachchi, Prof Madhava Meegaskumbura, Mr. Sudath Nanayakkara, Prof Anjana Silva and Mr. Hiranya Sudhasinghe have gone on to become highly accomplished naturalists who have contributed a great deal for the enhancement of our knowledge about Sri Lanka's biodiversity.

His influence transcends the boundaries of Sri Lanka as his collaborative research efforts had a catalytic influence on neighboring countries, especially India. Prof. S. D. Biju, inspired by Rohan's work in Sri Lanka, undertook similar studies in India that has resulted in the discovery of a number of new frog species. Further, Rohan's work on ichthyofauna of Sri Lanka has helped to resolve the taxonomic status of the ichthyofauna and herpetofauna in the south Asian region. In recognition of this contribution, a team of Thai, Chinese and Indian Scientists led by Prof. Bidju has named a new genus of Asian tree frogs, Rohanixalus in honour of Rohan. This is also the first instance where a non-Sri-Lankan genus has been named after a Sri Lankan. In addition to this, many species [Dawkinsia rohani, Rasboroides rohani, Uperodon rohani, Calotes pethiyagodai, Onomastus pethiyagodai and Macromidia donaldi pethiyagodai] have been named after him.

Rohan has received so many honorary awards and has been elected by his peers to so many important posts that space does not permit a full listing of each. However, I must mention a few that underscore his extraordinary leadership skills, fundamental to this award. In 1989 Rohan and WHT embarked on a challenging project to convert 25 ha of degraded tea lands in the Agarapathana region to montane forest that harbours many of Sri Lankas endemic and threatened species. His initiative was recognized by Rolex Award for Enterprise in 2000 given to exceptional individuals who have the courage and conviction to take on major challenges; by initiating extraordinary projects that make the world a better place. In 2022 he was bestowed the coveted Linnean Medal given by the Linnean Society of London in recognition of his significant contribution to the science of natural history. Mr. Pethiyagoda is the first Sri

Lankan to receive this award, which has been presented annually since 1988, usually to a botanist and a zoologist.

His program of original and collaborative research (both in Sri Lanka and overseas) has resulted in a large number of publications on many zoological groups in addition to fishes. His impact on biodiversity research in Sri Lanka and beyond through his output is another remarkable achievement of this modest man. Further, WHT under his leadership printed a number of books, authored by him as well as others on various taxa of Sri Lanka that have helped further our knowledge about Sri Lankas biodiversity. In 1997, his global contributions have been acknowledged by his election as a Fellow of the National Academy of Sciences of Sri Lanka.

Mr. Pethiyagoda has made an immense contribution to the Natural History of Sri Lanka, and I know that all of whom were touched by him during a career spanning over three decades will share with Rohan, this timely acknowledgement of his life's work. I would also like to point out that though we are bestowing him with this lifetime achievement award, he will continue to contribute to the discipline of Natural History in years to come. I wish him all the very best in his future endeavors.

# ABSTRACTS

# Studying the Microbial Community Interactions of Sri Lankan Cattle Milk Microbiota Under Different Climatic Conditions

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The makeup of the milk microbiota has a critical impact on the shelf-life of raw milk as well as the quality of dairy products. Therefore, it is vital to discover community interactions in milk microbiota and the keystone genera that contribute to such consequences. The emergence of high-throughput sequencing has enabled the investigation of community ecology in a variety of contexts using a variety of omics methods. For the first time in Sri Lankan history, such a study was conducted on milk microbiota using 16s rRNA gene sequencing in 2021. The study recorded the abundance of different microbes in milk samples obtained from different locations representing Sri Lanka. In the present study, the above-mentioned abundance data were analyzed further in order to identify the co-occurrence patterns in different climate zones (wet, dry, and intermediate) and the keystone genera of the milk microbiome in Sri Lankan cattle milk using microbial co-occurrence networks. The SparCC method was used for the construction of the co-occurrence network. In addition, the FAPROTAX tool was used to investigate the functional relationships behind the co-occurrence patterns. The present study was able to identify 12 keystone taxa in the wet zone and significant co-occurrence patterns in the microbiota of the wet zone and the dry zone. In addition, the co-occurrence networks demonstrated a higher diversity in the wet zone compared to other zones. The analysis provided evidence for the functional relationships behind co-occurrence patterns. The pipeline developed in the present study lays the foundation for numerous future studies to be conducted on Sri Lankan milk microbiota using different variables (cattle breed, lactation phase, etc.). Furthermore, the results obtained in the present study provide the initiatives for wet-lab experiments to investigate methods to control the harmful microbiota of Sri Lankan milk.

**Keywords**: Sri Lankan milk microbiota, Co-occurrence network, Keystone genera

# Effect of Curcumin Extract on Inhibition of Coconut Oil Rancidity

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Coconut (*Cocos nucifera*) oil (CO) is the most commonly used edible oil in Asian countries, including Sri Lanka. Various processes are used to obtain CO from coconut. Several factors reduce the quality of coconut oil through the oxidation process known as rancidity and it is accelerated by an increased level of unsaturated fatty acids. Curcumin is a natural antioxidant that can be used as a substitute for synthetic antioxidants since their long-term usage can cause health effects. The objective of this study was to inhibit the rancidity of coconut oil using an optimum concentration of curcumin while improving its quality. Rancidity-induced virgin coconut oil (VCO) was incorporated with two different concentrations of curcumin (500 ppm and 1000 ppm). Tocopherol at 500 ppm and 1000 ppm were used as control samples. On the 1st, 7th, 14th and 21st days, physiochemical parameters such as free fatty acids (Acid value test), peroxides (Peroxide value test), pH and unsaturation levels (Bromine water test) were assessed. The sensory evaluation was conducted to determine the acceptability of the samples. Free fatty acids, peroxide value, pH and unsaturation levels of normal VCO and rancid oil samples increased with time. According to all tests, 1000 ppm had the highest rancidity reduction percentage (reduction of acid value-27%, peroxide value-11.38%, unsaturation-16% and increment of pH-21%) than 500 ppm (reduction of acid value-2%, peroxide value-11.4%, unsaturation-16% and increment of pH-22%) after 21 days of storage, despite the fact that both curcumin concentrations had a positive impact ($p>0.05$) on quality characteristics of coconut oil. ANOVA was performed using R studio version 4.0.2, followed by Dunn's test. The 1000 ppm curcumin sample surpassed the 500 ppm curcumin sample in terms of overall sensory acceptability. Furthermore, the 500 ppm tocopherol sample was more effective than the 1000 ppm tocopherol sample in reducing rancidity. It can be concluded that the 1000 ppm curcumin was effective in reducing rancidity compared to the control which therefore suggests that curcumin can be used as a natural inhibitor of coconut oil rancidity.

**Keywords:** Coconut oil, Rancidity, Oxidation, Curcumin

# Evaluating Plant-derived Antifungal Substances for the Effective Management of Seed-borne Fungi of Selected Crop Species

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Seeds are the most vital input for crop production. Seed-borne fungal pathogens cause infections and diseases, leading to enormous crop losses, and a reduction in yield and productivity. Cereals and legumes contribute as the most important food sources in Sri Lankan diets. Therefore, environmentally sound, and economically feasible pathogen management strategies such as plant extracts are much needed for controlling the seed-borne fungi and increasing seed quality. This study was aimed at identifying the antifungal efficacy of *Allium sativum*, *Aloe vera*, *Azadirachta indica*, and *Zingiber officinale* extracts and their effective concentrations to control the seed-borne fungal pathogens; *Aspergillus flavus*, *Aspergillus niger*, *Orbilia foliicola*, *Rhizopus oryzae*, and *Talaromyces oumae-annae* isolated from *Arachis hypogea*, *Oryza sativa, Vigna radiata,* and *Vigna sinensis*. Determination of antifungal efficacy was performed using the agar well diffusion method and poisoned food technique. Plant extracts' effectiveness for seed germination and seed quality was evaluated via pot experiments. Experiments were arranged into a completely randomized design and data were statically analyzed using Minitab (version 17). *Zingiber officinale* crude extract exhibited the highest antifungal activity against the tested pathogens which was as effective as Captan 50%, a commercial fungicide. Preliminary results, of this study, showed that the poisoned food technique is a promising alternative for the determination of the antifungal activity of fungi, overcoming the limitations of the agar well diffusion method. Further analysis of the results from the pot experiment revealed that *O. sativa* and *V. radiata* seeds treated with *A. indica* and *Z. officinale* aqueous extracts showed a 100% germination percentage and it was similar to the germination percentage obtained by the positive control (Captan 50%). Furthermore, *O. sativa* has greater vigor indices 2824, and 3079 with *A. indica,* and *Z. officinale* respectively. *Azadirachta indica* and *Z. officinale* aqueous extracts are the most effective extract in promoting seed germination and seedling vigor while *A. vera* extract is the least effective extract. Comparing the aqueous and methanolic extracts, aqueous extracts are notably effective in promoting seed germination and increasing seedling vigor.

**Keywords**: Antifungal, Fungal pathogens, Plant extracts, Seed treatments

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# Wound Healing Enhancing Terpenoids from *Vernonia zeylanica* (L.) Less.

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*Vernonia zeylanica,* an endemic plant of Sri Lanka and its aerial parts are used to treat wounds in Ayurveda. Even though, the wound healing potential of this plant has not been evaluated to date. Assessment of the wound healing potential of aerial parts of *V. zeylanica* and identification of active compounds by bioactivity directed chemical investigation. Hexanes, dichloromethane, ethyl acetate and methanol extracts of the aerial parts of *V. zeylanica* were obtained by sequential extraction at 30 (±2 °C) for 24 h. Each extract was assayed for cell migration enhancing ability through scratch wound assay (SWA) at a concentration of 20 mg/L on Madin-Darby Canine Kidney (MDCK) cells. The cell migration ability was expressed as mean percentage wound closure at 24 h. The SWA guided fractionation of hexanes extract which showed the highest activity led to the isolation of two fractions HF5A and HF5B each of which was found to be an inseparable mixture of two terpenoids. The terpenoids present in these two mixtures were identified by GC-MS data together with NMR spectroscopic data and confirmed by comparison of NMR spectroscopic data with those reported. The SWA was performed for these two inseparable mixtures at a concentration of 5 mg/ L. It was established that the mixture HF5A contained lupeol and $\beta$-amyrin while the mixture HF5B contained glut-5-en-3$\beta$-ol and friedelin-3$\beta$-ol. The mean percentage wound closures for these two mixtures HF5A and HF5B at 5 mg/ L and for the positive control (asiaticoside;12.5 $\mu$M) were found to be 83.9%, 78.3% and 89.5% respectively. While the negative control (1% DMSO in 20% DMEM) showed 19.5% mean wound closure. The two terpenoid mixtures (HF5A and HF5B) containing lupeol /$\beta$-amyrin (HF5A) and glut-5-en-3$\beta$-ol /friedelin-3$\beta$-ol (HF5B) were identified as the wound healing enhancing constitutes from the aerial parts of *V. zeylanica*.

**Keywords:** *Vernonia zeylanica,* Asteraceae, Madin-Darby Canine Kidney (MDCK) cells, Scratch Wound Assay (SWA), Wound healing activity

**Acknowledgment:** Financial assistance from NSF is gratefully acknowledged (Grant No: NSF/PSF/ICRP/2017/HS/02)

# Antibacterial Activity of Entomopathogenic Fungi Isolated from a Thorn Treehopper in Sri Lanka

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Antibiotic resistant crisis has become a significant public health issue in the current world. Thus, there is an urgent need to find novel alternatives to the existing antibiotics. Entomopathogenic fungi who are known to secrete bioactive metabolites are an untapped resource for discovering novel antibacterial chemical scaffold. The main aim of the current study was to investigate the antibacterial activity of secondary metabolites secreted by entomopathogenic fungi isolated from a thorn treehopper in Sri Lanka. Freshly dead thorn treehopper (Family Membracidae) cadavers were collected from a tree branch and surface sterilized using 70% ethanol for 45 sec, 1% Clorox for 30 sec, again with 70% ethanol for 45 sec and finally with sterilized distilled water for 30 sec. The surface sterilized cadavers were crushed and spread on antibiotic (Ciprofloxacin, 150mg/L) enriched potato dextrose agar media plates. After incubation the emerging fungi were isolated, pure cultures were obtained and extracted into ethyl acetate. The fungal crude extracts were tested for antibacterial activity against *Staphylococcus aureus* (ATCC 25928), *Bacillus cereus* (ATCC 11718) and *Escherichia coli* (ATCC 35218) using agar disc diffusion method at 400 and 100 µg/disc concentrations. Gentamycin and ethyl acetate were used as positive and negative controls respectively.Three morphologically different entomopathogenic fungal strains (SMF, LF, BWF) were isolated from the treehopper cadavers. BWF extract was active against the Gram positive *S. aureus* and *B. cereus* bacteria at 400 and 100 µ g/disc concentrations. Other two fungal extracts were only active against *S. aureus* at 400µg/disc concentration. None of the extracts were active against Gram negative *E. coli*. The fungi were preserved for the identification process. Entomopathogenic fungi are potential sources for discovering novel antibacterial drug leads.

**Keywords:** Antibiotic resistance, Entomopathogenic fungi, Antibacterial, Secondary metabolites, Treehopper

# *In vitro* Bio Efficacy of *Beauveria* sp. Against Tea Shot- hole Borer (*Euwallacea fornicatus*, Eichh.)

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Tea shot-hole borer (TSHB) *Euwallacea fornicatus* (*Xyleborus fornicatus* Eichh.) is a serious pest of tea and is managed by adopting Integrated Pest Management (IPM) strategies. Strengthening IPM with microbial biopesticides would be one of the economical and environmentally safe approaches. The present study focused on the Isolation, identification and evaluation of the entomopathogenic fungus *Beauveria* sp. against TSHB. *Beauveria sp*. (BBWg) was isolated from freshly collected dead white grubs from tea soil and identified using morphology. The growth of BBWg was evaluated in vitro in different media at different temperatures. In vitro compatibility of BBWg was tested with agrochemicals used in tea*; Dolomite, Hexaconazole EC, and Glyphosate*. Laboratory bioassays for TSHB were carried out using fresh, 5-7 cm long, pencil-thick stems of cultivar *TRI 2025.* Stem pieces were dipped in the fungal spore suspension ($8.625X10^8$) and distilled water for 2 minutes for treatment and control, respectively. Two stem pieces were placed in petri dishes and 10 beetles were introduced to each replicate. The petri dishes were sealed and kept under dark conditions at room temperature for 9 days. Mortality was recorded at 3-day intervals. Three days after treatment application, there was no significant difference in TSHB mortality levels between the treatment (5.56%) and control (3.33%) groups. Beetle mortality was significantly increased to 55.56% and 100 % after 6 and 9 days, respectively when compared to the control (10.08% and 21.11%). The dead, treated beetles were completely covered by the fungus within 2-3 days. The suitable medium for BBWg was identified as Potato Dextrose Broth (PDB), and the highest growth was observed at $25^oC$. The fungicide Hexaconazole EC completely suppressed the growth of BBWg while Dolomite and Glyphosate did not. *Beauveria* sp. (BBWg) controlled TSHB *in vitro*, indicating that it has the potential to be used as a biopesticide.

**Keywords:** Bioassay, Biopesticides, Entomopathogens, In vitro test, *Xyleborus fornicatus*

**Acknowledgment:** The authors would like to thank Synkromax Biotech Pvt Ltd. India and Tea Research Institute of Sri Lanka for the financial support.

# A Microbial Cocktail Combined with Organic Fertilizer with the Potential of Providing Rice Growth and Yields Similar to 100% Urea Recommendation

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Nitrogen is required for rice cultivation as it has a direct impact on the growth and yield of the rice plant. The absence of sufficient amounts of urea fertilizer has led to many issues with rice yields. A Research team of University of Colombo has developed a successful microbial cocktail. This study was carried out to find out the possibility of incorporating a beneficial fungus *Trichoderma* spp. into the developed microbial cocktail (AAA) and to see the possible reduction of urea fertilizer application while using organic fertilizer with the new microbial cocktail (Mc-1). Also it was attempted to findout the possible reduction of urea fertilizer while using Organic fertilizer [OR] combined with the Mc-1. First AAA's ability to co-exist with *Trichoderma* spp. was conducted using the compatibility testing. It was indicated that all microbes (AAA and *Trichoderma* spp.) are compatible with each other. Then a field trial was conducted with rice variety AT 362. The main treatments included, Tx = 100% Urea, Ty = OR + Mc-1 and Tz = only OR, each treatment had three replicates. Flavonoid, Naringenin ($1\times10^{-4}$M) was also added to the soil. Mc-1 was added in 2, 4 and 6 weeks after transplantation. Vegetative and yield data were taken in 30, 60, 90 and 105 days after transplantation. The data was statistically analyzed by One-way ANOVA with Duncan's post-hoc test using IBM SPSS Statistics 26. It was observed that $T_y$ has the potential to provide several parameters similar to the use of Tx. It has provided significantly similar vegetative parameters (number of tillers and leaves) in 60 and 90 days and significantly similar yield parameters (panicles and grain weights per plot) when compared with the control Tx. But grain weight per hill data indicated that OR + Mc-1 (Ty) shows a significantly higher yield compared to the Tz treatments. This research indicated that there is potential to solve the issues with urea fertilizer usage faced by the country with the use of OR + Mc-1.

**Keywords:** Microbial cocktails, Organic fertilizer, Urea fertilizer reduction, Rice

# Rhizospheric Fungal Species of Selected Capsicum (*Capsicum annuum* L.) Varieties of Sri Lanka and their Ability to Control *Fusarium* sp., Causative Agent of Damping-off Disease in Capsicum

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Capsicum (*Capsicum annuum* L.), is a cash crop grown in Sri Lanka and an essential ingredient in Asian cuisine. Capsicum plants at the nursery stage are often affected by damping-off pathogens such as *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. Use of synthetic fungicides is the common practice used to control these pathogens. Rhizospheric microorganisms, particularly fungi are reported to have antagonistic effects on a number of fungal pathogens including damping-off pathogens. The present study focused on the isolation and identification of rhizospheric fungi of commercially grown capsicum varieties of Sri Lanka and the evaluation of their ability to control a *Fusarium* sp., causing damping-off disease of capsicum. Soil was collected from the rhizosphere of commercially grown capsicum varieties, Muriya, C.A.8, and Hungarian Yellow Wax (HYW) in Anuradhapura, Kandy, Colombo, Kalutara, and Nuwara-Eliya districts. Fungal species in the collected soil samples were isolated using the dilution plate technique. The ability of isolated rhizospheric fungi to control the growth of pathogenic *Fusarium* sp. was evaluated using dual culture assays. The percentage inhibition (PI) of the radial growth of pathogen colonies by each rhizospheric fungal isolate was calculated. Fifty-two rhizospheric fungi were isolated and they were identified up to the genus level based on morphological features. *Aspergillus*, *Trichoderma*, and *Fusarium* were the most commonly occurring genera, with 29.8, 23.1, and 17.3% isolation rates respectively. Twenty-one isolates showed significant inhibition ($p \leq 0.005$) of the pathogen colony growth in dual culture assays. The highest PI showed by an *Aspergillus* sp. (79.3%), followed by a *Trichoderma* sp. (74.4%). The rhizosphere of capsicum varieties grown in Sri Lanka consists of a diverse assemblage of fungal genera. Some of the isolated fungal species had the ability to inhibit the growth of pathogen significantly and their mechanisms of control should be investigated further to be used as biocontrol agents of damping-off disease caused by *Fusarium* sp.

**Keywords:** Capsicum, Rhizosphere fungi, Biological control**,** Damping-off, *Fusarium* spp.

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# Antibacterial Activity of Selected Fungal Endophytes Isolated from Two Species of Pandanaceae

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Endophytic fungi inhabit plant tissues without apparent signs of infections for all or part of their life cycle. Much research had been focused on the analyses of bioactivities from endophytes in the recent past possibly due to the discovery of endophytes producing pharmacologically active substances with potential use in biotechnology. This study investigated the fungal endophytes present in the foliage of *Pandanus odorifer* and *Pandanus thwaitesii;* of the family Pandanaceae from the coastal areas of Northwestern Province, Sri Lanka. Species of this family have been used for medicinal purposes worldwide. A total of 194 leaf segments were sampled from the two species and 108 morphotypes were recorded of which 57 were from *P. odorifer* and 53 from *P. thwaitesii*. Of the 108 morphotypes, 16 morphotypes (8 from each species) were selected for antibacterial assay. Crude extracts of the fungal isolates were obtained from ethyl acetate and dissolved in methanol. The disk diffusion method was used for the antibacterial assays against the gram-positive bacterium *Staphylococcus aureus* (ATCC 25923) and the gram-negative bacterium *Pseudomonas aeruginosa* (ATCC 25853) on nutrient agar medium. All crude extracts exhibited inhibition zones against *Staphylococcus* sp. while 7 extracts exhibited inhibition zone areas equal to or surpassing the area of positive control Ciprofloxacin. In the assay against *Pseudomonas* sp., 11 extracts exhibited inhibition zones that had lower diameters than the inhibition zones of the positive control. The mean ranks of the diameters of the inhibition zones due to the different extracts were significantly different according to the Kruskal-Wallis test. Significantly higher inhibition zones were exhibited against *Staphylococcus* sp. according to the Mann-Whitney U test. It is evident from the results of this pilot study that the fungal endophytes of Pandanaceae possess compounds of interest with potential antibiotic activities. Further studies need to be conducted in order to identify their full extent and capacity.

**Keywords:** Antibiotics, Endophytes, *Pandanus*

# Effect of Cryoprotectants on Cell Viability and Biomass of *Chlorella* sp. and *Oscillatoria* sp. under Ultra Low Temperature

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Microalgae and Cyanobacteria are highly diverse groups of photosynthetic microorganisms that are commonly found in freshwater and marine ecosystems. A number of species in the groups of *Oscillatoria* sp. and *Chlorella* sp. are used in different applications such as biofertilizer and biofuel production and are identified as promising candidates in the future towards a bio-based economy. The effects of these cryoprotectants for the maintenance of *Oscillatoria* sp. and *Chlorella* sp. are not extensively studied. The present study aims for determining the effect of glycerol, methanol and polyvinylpyrolidone on the viability and biomass of *Oscillatoria* sp. and *Chlorella* sp. after storage under -20 °C and -80 °C temperatures. Previously identified pure cultures of *Oscillatoria* sp. and *Chlorella* sp. were obtained and the growth curves were developed by measuring the biomass at different time intervals. Cells harvested at the exponential phase of the two species were stored at -20 °C, and -80 °C with the three cryoprotectants (10%) along with controls in triplicate. After 5, 10, 15, 20, 25 days and at monthly intervals, cell viability was determined using dye exclusion method. The biomass was measured after inoculating the preserved cells in BG 11 media. Data were analyzed using ANOVA procedure in SPSS software. The highest specific growth rate was observed in *Chlorella* sp. (0.027 $hrs^{-1}$) while *Oscillatoria* sp. showed the longest exponential phase (144 hrs to 718 hrs). After one month of storage of *Chlorella* sp. at -20 °C, the highest mean biomass growth after thawing was observed with 10% methanol followed by 10% polyvinylpyrrolidone (PVP) and 10% glycerol compared with the control. At -80 °C storage of the species, biomass growth was not significantly different among the cryoprotectants. In *Oscillatoria* sp. highest mean biomass production at both -80 °C and -20 °C storage was observed in 10% glycerol. After five weeks of storage at -20 °C, *Chlorella* sp. indicated the highest cell viability with both glycerol (62.4%) and PVP (62.2%). This species showed contrasting results at -80 °C, with the highest viability in methanol treatment (60.5%). *Oscillatoria* sp. showed highest cell viability with glycerol (63.9%) at -80 °C and both glycerol and methanol resulted in significantly higher cell viability (59.4 % and 60.5 %) at -20 °C. This study has revealed that *Chlorella* sp. and *Oscillatoria* sp. can be effectively stored both at -20 °C and -80 °C temperatures for one month with high viability. Based on cell viability glycerol and methanol were the best cryoprotectants to store *Oscillatoria* sp. at -20 °C while glycerol has given highest cell viability at -80 °C. Glycerol and polyvinyloyrolidone were equally effective to store *Chlorella* sp. at -20 °C but methanol is the best cryoprotectant to store the species at -80 °C.

**Keywords:** Biomass Growth, Cell Viability, *Chlorella*, Cryopreservation, *Oscillatoria*

# Screening for Petrol Degradation Potential of Nine Bacterial Isolates Using a Redox Dye 2,6-Dichlorophenolindophenol

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Soil pollution caused by the deliberate or accidental release of petroleum products has become a global environmental problem. Petroleum contamination is a result of the spilling of petroleum products during transport and industrial processes. Some microorganisms are capable of utilizing petroleum pollutants such as petrol as their source of carbon and energy. This study aimed to validate the petrol degradation potential of bacteria isolated from petroleum-contaminated soil collected from the Kolonnawa Oil Installation plant in Sri Lanka. A total of 9 isolates, 7 from petroleum-contaminated soil and 2 isolates directly from petrol were screened for their ability to degrade petrol. The hydrocarbon degradation potential of the isolates was assessed using a redox indicator dye 2,6-Dichlorophenolindophenol (DCPIP) and oxidation levels were determined by spectrophotometry, which directly correlated to the efficacy of hydrocarbon degradation. Statistical analyses were performed using R studio version 4.0.2 and absorbance values from the 2,6-DCPIP assay were analyzed using Kruskal-Wallis test and Dunn's test. Results revealed that all the isolates were able to effectively utilize petrol as a source of carbon, indicating their ability to degrade hydrocarbons. Isolates B6.2 (60.22%) and B7 (48.97%) exhibited the highest oxidation rates for petrol. A highly significant difference in absorbance values for all 9 isolates was observed between day 0 and day 7 in the 2,6-DCPIP assay ($p$-value<0.05). Furthermore, 2,6-DCPIP assay results were validated using the redox dye 2,2-diphenyl-1-picrylhydrazyl (DPPH) in a previous study. This preliminary study revealed that some bacterial populations survive and thrive in petroleum-contaminated soil and that the petrol degradation potential of isolates from soil were higher than the bacterial isolates isolated directly from the petrol reference sample. This study can be expanded further to validate the petrol degradation efficiency, molecular identification and synergy of mixed bacterial consortiums in soil prior to implementation in bioremediation of petroleum-contaminated soil.

**Keywords:** Bioremediation, Petrol degradation, Hydrocarbon-degrading bacteria, Redox indicator

# Comparison of Phytochemicals of De-polysaccharide and Polysaccharide Rich Methanolic Extracts of Sri Lankan Marine Alga *Chnoospora minima*

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*Chnoospora minima* is a brown alga that belonged to the group of Phaeophyceae, which exhibited different bioactivities including anti-cancer, anti-diabetic, anti-inflammatory, antioxidant, etc. The present study aimed to investigate the phytochemical composition of the de-polysaccharide and polysaccharide-rich methanolic extracts and their fractions of *C. minima*. The powdered sample was extracted with methanol and obtained with polysaccharide-rich methanol extract. In contrast, a portion of the extracted sample was treated with 80% ethanol to remove polysaccharides and obtain de-polysaccharide methanol extract. The resulting two samples were partitioned using hexane, chloroform, and ethyl acetate with increased polarity while standard methods determined total polyphenolic content, flavonoid content, and alkaloids. A high total polyphenolic content (TPC) was observed in crude methanol extract of the de-polysaccharide sample (298.07 ± 0.003 mg GAE/g). The aqueous fraction (141.2 ± 0.002 mg GAE/g) of the polysaccharide portion showed high TPC. The highest level of total Flavonoid content (TFC) was observed in both aqueous fractions of de-polysaccharide (594.23 ± 0.001 mg QE/g) and polysaccharide-rich (113.46 ± 0.001 mg QE/g) samples of *C. minima*. Chloroform fractions exhibited the highest total alkaloid content (TAC) of both polysaccharides rich (2.91±0.26 mg PE/g), and de-polysaccharide (0.43±0.31 mg PE/g) samples *C. minima*. Based on the findings, it is noted that the highest total polyphenolic content (TPC), total flavonoid content (TFC), and total alkaloid content (TAC) are owed by de-polysaccharide *C. minima* fractions where polysaccharide *C. minima* fractions contain less. Therefore, the de-polysaccharide *C. minima* sample can further be utilized to determine bioactivities that lead to future drug development.

**Keywords:** Phytochemicals, Polysaccharide, De-polysaccharide, Alkaloids, Flavonoids

# Association of Selected Genetic Variants in *CBS* and *MTHFR* Genes in a Cohort of Children with Homocystinuria in Sri Lanka

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A defect in one of the enzymes of homocysteine metabolism at the genetic level such as *CBS* (Cystathionine *β*-synthase), *MTHFR* (methylenetetrahydrofolate reductase) genes or a nutritional deficiency in one or more of the co-factors (vitamins B2, B6, B12 and folate) leads to metabolic disruption and elevated levels of homocysteine and its metabolites, causing homocystinuria, a rare and a fatal inborn metabolism error and an autosomal recessive inherited disorder. This condition is detected by quantifying plasma total homocysteine levels in the blood. To study a cohort of children with homocystinuria in Sri Lanka for selected variants in the *CBS* and *MTHFR* genes of homocysteine metabolism. This study was designed to detect the selected variants, c.833 T>C/exon 8, and c.19del/exon 01 in *CBS* gene and c.665C>T/exon 5, c.1286A>C/exon 8 in *MTHFR* gene, which was selected based on literature. Blood samples from eight clinically confirmed children with homocystinuria were collected and DNA was extracted using the QIAamp DNA Blood Mini Kit and followed by PCR. Purified PCR products of c.833T>C and c.1286A>C were subjected to SNP mini-sequencing, and c.19del and c.665C>T were directly sequenced. Results of SNP mini-sequencing were analysed using Gene Marker software V2.6.4, while sequencing results were analysed using Mutation Surveyor®V4.0.9. Out of the eight patients, none had the c.833T>C, but four patients were in the homozygous state for the c.19del variant in the *CBS* gene. Furthermore, out of eight, seven were heterozygous for c.1286A>C, while one patient was heterozygous for c.665C>T in the *MTHFR* gene. According to the results, c.19del was common in the studied cohort of Sri Lankan children while c.833T>C was absent, whereas c.1286A>C was more frequent than c.665C>T. Further comprehensive studies are necessary with a larger sample size to establish the association of these variants with the disease in Sri Lanka.

**Keywords:** Homocystinuria, Methylenetetrahydrofolate reductase, Cystathionine β-synthase, Single nucleotide polymorphism, Mutation

**Acknowledgment:** This work was supported by the IBMBB and constituted as a part of the MSc studies of Dinithi Mahaliyanage and Nadeesha Samarasinghe

# Effect of Simulated *In vitro* Gastrointestinal Digestion on the Bioactive Properties of Phenolic Compounds from Edible Flowers

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Edible flowers used in the traditional medication system and for culinary purposes have recently regained popularity due to their phenolic composition and associated health benefits. However, there are no many investigations reported regarding the impact of simulated digestion on the edible flower phenolic compounds and their bioactive properties. The present study aims to assess the digestive recovery of phenolics and the variation in the bioactive properties of eight edible flowers after subjecting them to simulated gastrointestinal digestion followed by dialysis. *In-vitro* gastrointestinal digestion was performed by homogenizing the samples with simulated gastric and intestinal fluid. Bioassays were performed to evaluate the phenolic, flavonoid, and anthocyanin content along with antioxidant, anti-inflammatory, and anti-diabetic properties of ethanolic extracts and digested fractions. The presence of nine phenolic compounds in the ethanolic flower extracts was confirmed and quantified using LC-MS. Statistical calculations and the analysis were done using one-way ANOVA. Mean separation was carried out by Tukey's multiple variance test. A significant ($p<0.05$) reduction in the total phenolic content of all the flowers was noted after the gastric phase whereas a significant ($p<0.05$) increase in total flavonoid content was reported. The total anthocyanin content of four flowers has decreased significantly after the intestinal phase of digestion. The highest hydrogen peroxide scavenging activity (62.96± 0.87 % per g of dry weight) and nitric oxide scavenging activity (32.44±1.25 % per g of dry weight) in the dialyzed fractions were expressed by *Cassia auriculata*. *Hibiscus rosa-sinensis* expressed the highest activity in inhibiting protease (30.01±0.41 %) after dialysis. *Ocimum sanctum* flowers and *Calendula officinalis* flowers significantly inhibited the activity of the alpha-glucosidase enzyme indicating better anti-diabetic properties. The findings from the LC-MS data confirmed the presence of flavonoid kaempferol-3-O-glucoside in all the investigated flowers. It can be concluded that, though the phenolic content has decreased after gastrointestinal digestion and dialysis; a sufficient proportion was available after dialysis to demonstrate antioxidant, anti-inflammatory, and anti-diabetic activities.

**Keywords:** Anti-oxidant activity, Edible flowers, *In vitro* digestion, Phenolics

**Acknowledgement:** Financial assistance provided by National Research Council is acknowledged (Grant # 19-033)

# Genome-wide Identification of Gretchen Hagen3 (GH3) Gene Family in *Musa acuminate* L.

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Banana (*Musa acuminata*) is mostly cultivated in tropical and subtropical regions of the world for its edible fruit. Predictable climate changes and their variability, especially extreme temperature and rainfall changes affect bananas. The development of *M. acuminata* varieties that are better suited for abiotic stresses is critical for meeting the demand in the future. *Gretchen Hagen3* (*GH3*) genes participate in a wide range of developmental mechanisms and environmental reactions in plants by regulating the activity or availability of plant hormones and associated substances. In the present study, a comprehensive genome-wide analysis was conducted to identify GH3 protein-encoding genes in *M. acuminata* genome assembly. To identify *M. acuminata* GH3 protein sequences, GH3 protein sequences from *Arabidopsis thaliana* and *Oryza sativa* were used as query sequences in the similarity search. A total of 11 *MaGH3* genes were found in *M. acuminata*. Phylogenetic analyses revealed that *MaGH3* proteins could be classified into two main groups, group 1 and group 2, of the *GH3* family. However, Group 2 contained a higher number of MaGH3 family genes. It was hypothesized that diverse cis-acting regulatory elements (CREs) in the upstream promoter regions of*MaGH3*s would be crucial regulators of *MaGH3* expression patterns. *MaGH3* genes have few introns and are located unevenly across 10 chromosomes. An extensive amount of conservation in the exon/intron layouts and motif compositions of *MaGH3* genes were observed. It was discovered that there has only been one duplication event across all chromosomes. Analysis of the subcellular location of these proteins revealed that the majority are localized to the nucleus, cytoplasm or chloroplast. The current study offers a thorough analysis of anticipated genes from the *GH3* family of *M. acuminata* to direct future research into the roles of MaGH3 proteins during plant growth, development, and stress responses.

**Keywords:** Genome wide analysis, *Musa acuminata*, Abiotic stress, GH3 proteins, *In silico*

# *In vitro* Antioxidant Capacity and Lipoxygenase (5-LOX) Inhibitory Activity of Leaves of *Citrus aurantiifolia* (lime) and Chemical Profile of Leaf Essential Oils

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*Citrus aurantiifolia* belongs to the family Rutaceae and is used in traditional systems of medicine for the treatment of respiratory, stomach and skin disorders and believed to have health promoting properties. Further, bioactive constituents, phenols, flavonoids, carotenoids, and vitamins have been isolated from leaves, stems, roots, flowers, and seeds reported to have antioxidant, anti-inflammatory, and cytoprotective properties. The aim of this study was to analyse the ethanolic extracts of *Citrus aurantiifolia* leaves and isolate the essential oils (EO) from these leaves and evaluate the bioactivity. The EO was isolated from these leaves using Hydro-Distillation (HD) and Microwave Assisted Hydro-Distillation (MAHD). To evaluate the bioactivity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, ferric ion reducing antioxidant power (FRAP) assay, 5-lipoxygenase inhibition (5-LOX) assay, total phenolic content (TPC) and total flavonoid content (TFC) were performed. The EO was analysed for its chemical compositions using Gas Chromatography-Mass Spectrometry (GC-MS) to identify the chemical components for bioactivity. The ethanolic extract of *Citrus aurantiifolia* leaves showed the $IC_{50}$ value of 735.29 ± 38.01 µg/ml for DPPH free radical scavenging assay. FRAP, TPC and TFC values for ethanol extract were 13.35 ± 2.09 µg/ml Trolox equivalent (TE)/g, 16.41 ± 1.59 mg Gallic acid equivalent (GAE)/g and 21.28 ± 0.42 mg Quercetin equivalent (QE)/g respectively. The GC-MS results indicated that the major compounds of *Citrus aurantiifolia* leaf EO from HD were D-Limonene and Caryophyllene with the percentages of 35.65% and 27.06% respectively. MAHD essential oil showed Citral and D-Limonene as the major compounds with percentages of 62.33% and 17.29% respectively. Ethanol extract of *Citrus aurantiifolia* leaves and EOs of HD and MAHD dose dependently inhibit 5-lipoxyganase enzyme having the $IC_{50}$ values of 6.77± 0.34, 7.40 ± 1.46 and 6.52± 1.14 respectively, compared to the positive control baicalein $IC_{50}$ value 1.76± 0.15. The results of the anti-inflammatory and antioxidant activity of the *Citrus aurantiifolia* leaves and the leaf essential oils support the use of lime leaves to treat various inflammatory ailments in the traditional system of medicine, the Ayurveda, and various folk systems of medicine.

**Keywords:** Hydro-distillation, Microwave-assisted hydro-distillation, Gas Chromatography, Mass Spectrometry, 5-Lipoxygenase

**Acknowledgement:** Financial assistance by the Treasury Grant No. TG 21/203 received to Industrial Technology Institute.

# Computational Analysis of EMBRYO-DEFECTIVE (EMB) Genes in *Arabidopsis*

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Embryonic lethal or embryo-defective mutants represent the main source for the identification of genes needed for embryogenesis; the corresponding genes are collectively referred to as embryo-defective genes (EMBs). Significant advances have been reported in the identification of genes essential for zygotic embryogenesis (ZE). Recent studies have provided insights into the minimal gene set required for ZE in *Arabidopsis*. An updated dataset of 510 EMBs discovered by the *Arabidopsis* community is currently available for research. Unfortunately, many of these EMBs remain functionally uncharacterized. Hence, the present study aimed to perform a comprehensive analysis of EMBs employing a range of computational tools and databases. The analysis pipeline included the determination of protein characteristics and their subcellular locations, identification of conserved motifs in protein sequences using the MEME Suite, prediction of *cis*-acting regulatory elements (CREs) in the promoter regions employing the PlantCARE tool, and prediction of interacting proteins through protein-protein interactions (PPIs) through the STRINGdb. In general, the *Arabidopsis* EMB genes encode proteins with deduced amino acid sequences ranging from 81 to 2729, with molecular weights of 8.6 to 306.3 kDa. A total of 29 EMBs were predicted to contain signal peptides. The majority of the EMBs were localized to the nucleus. Interestingly, exon-intron analysis of EMB genes identified 40 intronless genes. The analysis of the promoter sequences of these EMB genes discovered the presence of CREs, especially related to light, stress, and phytohormone responses. Moreover, PPI network analysis revealed the presence of two distinct sub-networks within EMBs. Further analysis of extended networks showed that the two sub-networks were connected through two proteins which were PROLIFERATING CELL NUCLEAR ANTIGEN 1 (PCNA1) and PCNA2. Our study originally revealed the characteristics and PPIs of previously reported *Arabidopsis* EMB genes and provided novel insights into the functional characterization of EMB genes in other crops in the future.

**Keywords:** Embryo-defective genes, Arabidopsis, Protein-protein interaction network

# Molecular Docking for Discovering Lead Compounds of Fungal Origin for Quorum Quenching Agents Against *Pseudomonas aeruginosa*

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The phylogenetic and metabolic diversity of fungi provides a diverse array of secondary metabolites or mycochemicals of pharmacological significance such as antibiotics. However, the lenient usage of antibiotics has conferred antibiotic resistance to both pathogenic and opportunistic pathogens causing a surge of nosocomial infections by antibiotic-resistant bacteria. Both pathogenic and opportunistic bacteria rely on a cell-to-cell communication mechanism known as quorum sensing which triggers the expression of genes responsible for biofilm formation and pathogenic factors. Disruption of quorum sensing can prevent pathogenesis and increase the efficacy of conventional antimicrobials. A large number of mycochemicals are reported to have quorum quenching activity. In-silico drug screening or molecular docking is a feasible alternative for discovering possible quorum quenching lead compounds in a bottom-up approach. This study aims to identify common mycochemicals interfering *Pseudomonas aeruginosa* quorum sensing mechanisms, molecular docking was carried out with 68 compounds extracted from a wide variety of fungi recorded in the literature (as ligands), with 6 selected quorum sensing related proteins of *P. aeruginosa* - both generated from structure predictions by AlphaFold and determined by X-ray crystallography. The ligands were sourced from the PubChem database and the proteins were accessed via UniProt and downloaded from Protein Data Bank (PDB). Docking was conducted with PyRx, AutoDock Vina, and Autodock Tools 1.5.7. The study discovered Naringin, Rutin, Chlorogenic acid, Myricetin, Kaempferol, Ergocalciferol, and Brassicasterol among the highest scored binding affinities against all the six proteins (LasI, LasR, RhlI, RhlR, PvdQ, PqsH). Experimental evidence found in the literature for the aforementioned compounds confirmed the validity of molecular docking in discovering new quorum quenchers.

**Keywords:** Quorum quenching, Mycochemicals, Secondary metabolites, *Pseudomonas aeruginosa*, Molecular docking

**Acknowledgment:** Undergraduate research funding from the University of Colombo

# DNA Methyltransferases and Demethylases in *Theobroma cacao*: Genome-wide Identification, Genomic Structures and Phylogeny

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DNA methylation is an epigenetic mechanism that involves the transfer of a methyl group onto the 5′ position of the pyrimidine ring of cytosine in the DNA to form 5-methylcytosine. In eukaryotes, DNA methylation, and active DNA demethylation events are mediated by cytosine-5 DNA methyltransferases (C5-MTases) and demethylases (dMTases), respectively. Currently, no information on these gene families is available in *Theobroma cacao*, one of the most economically important crops worldwide. Thus, the main objective of this study was to perform a comprehensive *in silico* analysis of C5-MTases and dMTases in the cacao genome. Our study resulted in seven C5-MTases (TcC5-MTases) and three dMTases (TcdMTases) encoding genes in the cacao genome. These genes were randomly localized on chromosomes 1 to 5, 9, and 10. Further analysis of genomic structures and phylogenetic relationships revealed that TcC5-MTases are grouped into four subfamilies (METHYLTRANSFERASE (TcMET1), CHROMOMETHYLASE (TcCMT1-3), DOMAINS REARRANGED METHYLASE (TcDRM1/2, TcDRM3), and *de novo* DNA METHYLTRANSFERASE 2 (TcDNMT2)), while TcdMTases are grouped into three subfamilies (REPRESSOR of SILENCING (TcROS1), DEMETER (TcDME) and DEMETER-LIKE (TcDML)). Genes from the same subfamilies shared similar exon-intron structures and protein motif composition. Moreover, three-dimensional protein structures of all TcC5-MTases and TcdMTases exhibited an extensive level of conservation. Except for two C5-MTases, TcDRM1/2 and TcDRM3, all TcC5-MTases and TcdMTases were localized in the nucleus; TcDRM1/2 and TcDRM3 were localized in the chloroplasts. *In silico* promoter analysis of TcC5-MTase and TcdMTase genes revealed the presence of several important *cis*-acting regulatory elements (i.e., light-responsive, stress-responsive, hormone-responsive, and plant growth and development-related elements). Furthermore, we investigated the gene duplication, and synteny between the genomes of cacao and selected angiosperms to gain an insight into evolutionary characteristics; a segmental duplication event involving *TcDML1* and *TcDME2* was identified from all TcC5-MTases and TcdMTases. Collectively, the findings of this study enrich our understanding of C5-MTases and dMTases in cacao and provide insights into the functional characterization of these genes in the future.

**Keywords:** DNA methyltransferases, DNA demethylases, *Theobroma cacao*, Genome-wide analysis

# Prevalence of Angiotensin Converting Enzyme (ACE) Insertion/deletion Polymorphism and its Association with Oxidative Stress in a Subset of Sri-Lankan Pediatric Psoriatic Patients

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Pediatric psoriasis attributes to nearly one-third of the global psoriasis burden. Multiple lines of evidence have demonstrated the association between Angiotensin-converting enzyme (ACE) Insertion (I)/deletion(D) polymorphism with psoriasis susceptibility, and oxidative stress (OS) in psoriatic patients. However, such studies particularly, on pediatric psoriasis are scarce in the local setting. This study investigated the prevalence of ACE I/D polymorphism and its associations with oxidative stress in a subset of Sri-Lankan pediatric psoriasis patients. Twenty pediatric psoriasis patients (Mean age -10.73 ±4.54 years) were recruited for this study after obtaining ethical clearance (LRH/DA/29/2020). ACE I/D polymorphism was investigated using the polymerase chain reaction. Serum Nitric Oxide (NO) levels and the total antioxidant capacity (TAC) were measured using the Griess assay and the FRAP assay. Clinical details were obtained from the clinic reports. Statistical analyses were carried out using SPSS version 25. I/D was reported as the predominant genotype (60%) while I/I and D/D genotypes were recorded in 30% and 10% of patients, respectively. Significantly higher NO levels (µM) were observed in I/D (27.58±7.85) patients than I/I patients (20.40±4.14) ($p$=0.027: Mann-Whitney U test) but not with I/D and D/D (19.80±1.4) ($p$=0.09: Mann-Whitney U test) or I/I and D/D genotypes ($p$=1.00: Mann-Whitney U test). No differences in Total Antioxidant Capacity were reported among ACE genotypes ($p$=0.488: Kruskal-Wallis test). Moreover, an overall female predominance (75%), and the predominance of plaque psoriasis (65%) in patients infected with single type (60%) of psoriasis was observed. Plaque and scalp psoriasis co-existed in 62.5% of multiple psoriatic cases (40%). This pilot study for the first time revealed I/D as the most prevalent genotype with a significantly elevated NO levels than that of I/I pediatric psoriasis patients. However, it is prudent to increase the sample size to further validate the results.

**Keywords:** Pediatric Psoriasis, ACE I/D polymorphism, Oxidative stress, Sri Lanka

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# An ARMS PCR-based RT Coupled Method for Simultaneous Identification of SARS-CoV-2 Variants

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Severe Acute Respiratory Syndrome Corona Virus - 2 (SARS-CoV-2) created a global level pandemic and presumably the biggest socio-economic downfall of the century. Among its structural proteins, Spike glycoprotein is of utmost importance for survival and acts as a major antigen for therapeutic and diagnostic assays. The mutation process of SARS-CoV-2 ultimately resulted in a striking number of lineages arising from the native virus that emerged from Wuhan, China. Variants of Concern (VOC) were among the top evolved variants in delivering severe infections. Much attention was paid to developing early diagnostic tools. Whole genome high-throughput sequencing is costly, time-consuming as well as labour intensive. This study was carried out with the objective to develop a convenient, simple, and cost-effective diagnostic assay, by mapping out hypervariable regions of Spike glycoprotein, establishing an ARMS PCR/RT coupled method for SARS-CoV-2 variant identification, and combining assay results with the prediction of vaccine compatibility. In this study, hypervariable regions within the Spike encoding region were mapped, PCR assay on single variant-specific amplification and validation were established as well as multiplex PCR was attempted with designed primers, and the relationships between different variants and RNA-based vaccines; Pfizer/BioNTech and Moderna were analyzed. The designed ARMS PCR/RT coupled assay successfully amplified positive controls and available sample variants to obtain the expected PCR amplicons distinguishing variants based on amplicon size. *In silico* analysis revealed high variability in the receptor binding region. Delta (B.1.617.2) and Delta Plus (AY.1/ B.1.617.2.1) variants showed the highest similarity to Pfizer/BioNTech and Moderna vaccines and Omicron (BA.1/ B.1.1.529) showed the least similarity. Sequence similarity was considered synonymous with vaccine effectiveness. In conclusion, the designed assay can be successfully extended for the proposed methodology to be established as a diagnostic tool for SARS-CoV-2 provided with further confirmatory experiments.

**Keywords:** SARS-CoV-2, Spike, ARMS PCR/RT, Hypervariable region, Vaccine

**Acknowledgment:** Undergraduate research grant from the University of Colombo and the IDEA project is acknowledged for the funding and facilities provided.

# Investigating the Proteins Involved with Crosstalk Between Drought Response Sub-pathways in *Oryza sativa* Using a Network-based Approach

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Drought is a major climate concern that affects the yields of every crop in the world. With increasing erratic weather due to ongoing climate change, the effects of drought are predicted to get worse in the coming decades. Rice, being a Kharif crop, is particularly susceptible to drought conditions. Through the use of genetic modification techniques such as genetic engineering and selective breeding it is possible to enhance the natural drought tolerance of rice varieties. The complexity of environmental changes during drought leads to the participation of many different molecular pathways in the drought response in plants. The large number of pathways and their interactions provide an opportunity to control the different aspects of drought response indirectly and at a higher level via the manipulation of signaling elements that mediate crosstalk between these pathways. Understanding and characterizing these crosstalk related proteins would enable plant modification efforts to induce broad phenotypic changes with few actual edits to the genome. Protein-protein interactions (PPI) have previously been used to study multiple human diseases and predict novel gene candidates participating in them. The high quality (i.e. edge score >400) PPI network of rice from the STRING database was used along with verified drought-related proteins mined from the literature to find novel candidate proteins that participate in crosstalk between drought response sub-pathways. These would be represented as inter-modular hubs that connect different functional modules within the network. Using the Random walk with restart algorithm, 96 novel drought-related proteins were predicted. These were then used as an input for the Louvain community detection algorithm to predict the top 5 proteins that are most likely to engage in crosstalk. These results show the viability of network analysis for analyzing these complex phenotypes, and to guide future experimental studies that seek to explore them.

# Modelling Environmentally Suitable Areas for the Potential Introduction and Cultivation of the Ornamental *Cryptocoryne thwaitesii* in Sri Lanka

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*Cryptocoryne thwaitesii* Schot is a popular ornamental aquatic plant in the aquaculture industry, with high export potential. The demand for this plant is high as it is easy to cultivate, thrives for a long time, and has fascinating foliage patterns. As a result of deforestation, changes in river flow, climate change and sediment discharge, *C. thwaitesii* habitats are now at risk. These plants are indiscriminately harvested from the wild for the export market. Therefore, thorough knowledge of the distribution of *C. thwaitesii* is essential for implementing effective conservation strategies and selecting favourable areas to cultivate *C. thwaitesii* to meet the high demand in the market. Therefore, this study was conducted to evaluate the current and future potential distribution of *C. thwaitesii*, set conservation priorities, and propose areas for introduction/reintroduction of this species. For the present study, species occurrence data were collected from field sampling and the National Herbarium, Peradeniya. Climate data was downloaded from the WorldClim database. Soil and Elevation data were also used. The future potential distribution was modelled using the MIROC6 Global Climate Model and SSP 2-4.5 and SSP 5-8.5 climate projections for 2050 and 2070. The Area Under the receiver operating characteristic (AUC) curve was used to evaluate the model performance. This study successfully generated first-ever distribution models for *C. thwaitesii* with high prediction accuracy. Temperature seasonality (bio4) and precipitation of the Warmest Quarter (bio18) had the highest contribution to the distribution of suitable habitats for *C. thwaitesii*. Under the future climate conditions, *C. thwaitesii* has been predicted to lose its suitable habitats in 2050 and 2070. Apart from that, this study detected that lower altitudes of the South-western hills of Sri Lanka are suitable for the introduction/reintroduction of this species beyond its current habitats. This study serves as an effective reference for decision-makers to conservation and management of *C. thwaitesii*.

**Keywords:** Species Distribution Modelling, *Cryptocoryne thwaitesii*, Sri Lanka

# Morphological and Biochemical Characterization of Quinoa (*Chenopodium quinoa* Willd.) - A preliminary study

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Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal grown for its edible seeds and has been the staple crop in Andean region. Outstanding nutritional richness and resistance to abiotic and biotic stress conditions have led to global interest on cultivating quinoa. However, quinoa has not received due recognition as a high scope crop in Sri Lanka. The aim of the present study is to investigate morphological and biochemical characteristics of different varieties of quinoa grown in Sri Lanka. A preliminary pot experiment (27-31°C, 65-70 % RH) was conducted using three commonly grown varieties, namely, Amarillo Marangani, INIA 427 Amarillo Sacaca and Blanca de Junin. Morphological characteristics (height of the plant, diameter of the stem and number of branches) were recorded across 32-weeks (05 replicates each). Total chlorophyll and proline contents of the fully opened new leaves and total protein contents of the seeds were determined. Data were subject to descriptive data analysis using Minitab software (version17.1). After 32-weeks of growth, all the varieties reached an average height of 70-90 cm, and an average stem diameter of 7.0 – 9.5 mm. INIA 427 Amarillo Sacaca variety showed a rapid increment in height and stem diameter. Total chlorophyll contents were in the range of 2.8-3.3 µg /g and average proline contents were 4.7- 9.9 µmoles /g of fresh weight again recording significantly higher values for INIA 427 Amarillo Sacaca variety. Total protein content of 1300-1900 ppm were recorded from seeds and INIA 427 Amarillo Sacaca variety indicated the highest protein content. Preliminary data showed enhanced growth performance in INIA 427 Amarillo Sacaca compared to the other two varieties in green house conditions. Further, high proline accumulation in leaves of this variety suggests better stress tolerance ability and as a good source of protein.

**Keywords:** Growth, Proline, Protein, Quinoa

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# Prolonging the Shelf Life of 'Ambul' and 'Cavendish' Banana Using Potassium Permanganate and Activated Charcoal Based Sachet Fortified with Passive Modified Atmosphere Packaging

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The shelf life of banana (*Musa acuminata*) is short as it is a climacteric fruit. This study aimed at extending the shelf life of two banana cultivars, 'Ambul' and 'Cavendish', by controlling ethylene using a potassium permanganate ($KMnO_4$) and activated charcoal (AC) based sachet fortified with passive Modified Atmosphere Packaging (MAP). Altogether six treatments and control conditions were tested on each cultivar, starting at ~80% fruit maturity. Per 100g fruits 0 – 1% $KMnO_4$, and 0.5% or 1% of AC were filled into non-woven paper sachets and inserted into passive MA packages (20 µm polypropylene) containing the fruits. The controls were without $KMnO_4$ / AC and MA packaging. All the samples were kept at 23 ± 1 °C in Completely Randomized Design (CRD) with replicates. The physiological loss in weight, pulp: peel ratio, firmness, total soluble solids (TSS), pH and titratable acidity (TA) were measured weekly to determine the ripening level. Results were statistically analysed with RStudio version 1.3.1093 software using one-way ANOVA. The control samples of 'Cavendish' and 'Ambul' showed full ripening after 14 and 7 days of storage, respectively, with rapid changes in the tested parameters indicating higher pulp to peel ratio, lower firmness, higher TSS, lower pH and higher TA compared to the treatments. The $KMnO_4$ treatment prolonged the shelf life of 'Ambul' (0.1% $KMnO_4$, 0.5% AC) and 'Cavendish' (1% $KMnO_4$, 1% AC) up to 28 days and 21 days, respectively, without any significant changes to the physicochemical indices observed at the commencement of treatments. Sensory analysis conducted according to the standard methods showed higher quality scores for the flavour and taste in the treated samples compared to the controls, while there was no difference in the overall acceptance of the colour and appearance. Thus, the protocol developed could prolong the shelf life of the two banana cultivars without affecting their natural ripening ability.

**Keywords:** Banana, Shelf life, Ethylene, KMnO4, MAP

# Effect of the Organic Liquid Fertilizer Amended with *Trichoderma harzianum* on the Growth and Yield of *Capsicum annuum* cv. MI 2

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Application of inorganic fertilizers to crop fields lead to adverse environmental impacts. Organic fertilizers have emerged as eco-friendly alternatives for achieving sustainable agricultural practices while reducing environmental impacts. Amalgamation of organic fertilizers with bio-fertilizer is more advantageous than the individual application of either into the cropping field. Present study was conducted to evaluate the effect of formulated organic liquid fertilizer amended with *Trichoderma harzianum* on the growth and yield of *Capsicum annuum* cv. MI 2. The organic liquid fertilizer was prepared by aerobic digestion of *Crotalaria retusa, Colocasia esculenta, Eichhornia crassipes*, and *Pueraria Montana*, topsoil, cow dung, rock phosphate, ripe banana peels, fishbowl water, in well water. *T. harzianum* ($2.5 \times 10^5$ conidia/mL) was used as bio-fertilizer. Well water used as negative control and maxi crop organic seaweed fertilizer used as positive control. Treatments were applied foliar and soil on twelve replicates of *C. annuum* seedlings in pot trials. The results were analysed by using one way ANOVA. *T. harzianum* ($2.5 \times 10^5$ conidia/mL) inoculated organic liquid fertilizer (250 mL/L) treatment showed the highest shoot height (31.99 ± 0.49 cm), leaf length (4.94 ± 0.21 cm), leaf width (1.91 ± 0.05 cm), leaf area (8.31± 0.58 $cm^2$), number of pods (4.46 ± 0.31), length of pods (6.77 ± 0.08 cm), weight of pods (1.95 ± 0.06 g), fresh weight of shoot and root biomass (12.18 ± 0.60 g) and dry weight of shoot and root biomass (2.19 ± 0.17 g). Organic liquid fertilizer (250 mL/L) treated *C. annuum* seedlings showed a higher number of leaves (94.73 ± 7.45) and *C. annuum* seedlings did not show any significant difference in treatments with the number of branches and number of days for first flowering. Present study highlighted the use of combined application of formulated organic liquid fertilizer (250 mL/L) amended with *T. harzianum* ($2.5 \times 10^5$ conidia/mL) as an efficient method for improving the growth and yield of *C. annuum* cv. MI 2.

**Keywords:** *Capsicum annuum*, *Trichoderma harzianum*, Bio-fertilizer, Organic liquid fertilizer, foliar application

**Acknowledgment:** Research Grant (RP/03/02/01/03/2020) of University of Kelaniya, Sri Lanka gratefully acknowledged.

# Morphological and Phytochemical Characterization of *Cajanus cajan* L. (Pigeon pea) in Sri Lanka

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*Cajanus cajan* L. [vern. Pigeon pea (E); Ratatora (S)] is a phytochemical rich grain legume crop originated in India and have been cultivated in rain-fed agriculture in semi-arid tropics for nearly 3,000 years. However, in Sri Lanka, *Cajanus cajan* L. is recognized as an underutilized crop. Therefore, present research was focused on morphological and phytochemical characterization of *Cajanus cajan* L. to assess the diverse promising characters that can be used in crop improvement programmes. Morphological characterization was performed using selected eighteen qualitative and quantitative characters of leaves, flowers, pods with ten replicates. Seed and pod characteristics have provided a reliable source for field identification of *Cajanus cajan* L., resolving taxonomic ambiguity. Lanceolate shape of terminal leaflet, yellow color corolla, green color pods, light brown color mature seeds, linear-oblong and black color seed hilum are the most prominent diagnostic features. Total protein content, total phenol content, total flavonoid content and antioxidant activity in methanolic seed extracts were determined to evaluate the bioactive properties. According to the present study, seeds of *Cajanus cajan* L. has 0.04 ± 0.02 mg/g total protein content, 11.62 ± 0.05 mg GAE/g total phenol content, 55.5 ± 0.12 mg RE/g total flavonoid content and 82.94 ± 4.14 antioxidant activity, indicating the potential of developing the seeds as antioxidant rich food supplementary. Further, antioxidant property of seeds can be recognized as a promising trait for crop quality improvement of *Cajanus cajan* L. Less utilization of *Cajanus cajan* L. is mainly due to the lack of knowledge in its dietary importance hence, building awareness among community is recommended.

**Keywords:** *Cajanus cajan* L., Morphometric, Total protein content, Total phenol content, Total flavonoid content

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# Rooting of *in vitro* Developed Shoots and Stem Cuttings of *Passiflora edulis*

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*Passiflora edulis*, commonly known as passion fruit, is utilized world-over for nutritive, medicinal, and ornamental purposes. *P. edulis* is cultivated on a large scale in Sri Lanka for the production of fruit juice. The main disadvantage of the many protocols developed for *in vitro* clonal propagation of passion fruit is the difficulty of rooting the shoots, which limits its large-scale cultivation. This study focused on developing an efficient protocol for rooting of shoots produced *in vitro* and rooting of stem cuttings for conventional propagation in locally developed cultivars of *P. edulis*. For *in vitro* studies, shoot tips were cultured on Murashige & Skoog (MS) medium after surface sterilization with 70% ethanol for 1 min and 25% Clorox for 20 min. Effect of two auxins, Indole-3-butyric acid (IBA) and 1-Naphthaleneacetic acid (NAA), on the induction of roots from *in vitro* grown shoots was examined on a half-strength MS medium. For conventional propagation, three-nodal stem cuttings of different maturities were pulse-treated with commercially available rooting powder to induce adventitious roots. Roots were induced in 77.78% of the shoots cultured on the medium supplemented with IBA 0.5 $mgL^{-1}$, which was significantly higher than on media containing IBA 1.0 $mgL^{-1}$ (37.78 %), NAA 1.0 $mgL^{-1}$ (22.22 %) and NAA 2.0 $mgL^{-1}$ (13.33 %). The mean number of roots (6.71) and mean root length (12.24 mm) were also significantly higher in the medium supplemented with IBA 0.5 $mgL^{-1}$. During acclimatization, tissue-cultured plants showed significantly better survival on cocopeat medium (73.33%) than on sand (33.33%). In conventional propagation, cuttings from the mid-section of the vine showed a significantly higher rooting response (62%) compared to younger cuttings (22%) and more mature cuttings (36%). Although the mean number of roots (23.55) and mean root length (4.85 mm) were highest in the mature cuttings three weeks after planting in the sand, mid-section cuttings can be recommended due to their higher rooting response.

**Keywords:** *Passiflora edulis*, Rooting, Shoot tips, Cuttings

# Use of Plant-based Organic Fertilizer Paste Enriched with *Trichoderma* species for the Cultivation of *Basella alba* – An Alternative Solution to the Commercial Organic Fertilizer

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Human and animal health and the environment are significantly affected by the excessive use of synthetic fertilizers. This study aimed to develop an organic fertilizer (OF) paste from invasive alien plant species (IAPS) and *Trichoderma* spp. Air-dried, powdered leaves and immature shoots of selected IAPS such as *Annona glabra, Chromolaena odorata,* and *Clidemia hirta*, and a native plant *Pongamia pinnata* (2.0 kg each), were digested with distilled water (42.0 L) for a month to formulate an OF paste form. *Trichoderma harzianum* (KT852821.1) and *Trichoderma virens* (KP985643.1) were developed in a solid carrier material (compost, straw, clay, and cow urine; 2:1:1:1) separately. Six treatments of liquid OF (T10V, T20V, T25V - 10%, 20%, 25% *C. odorata*, *A. glabra*, *C. hirta,* and *P. pinnata* extract only incorporated with *T. virens*, respectively and T10H, T20H, T25H - 10%, 20%, 25% *C. odorata*, *A. glabra*, *C. hirta*, and *P. pinnata* extract only incorporated with *T. harzianum*, respectively), and positive and negative controls were applied to one-week-old *B. alba* (Spinach) seedlings planted in pots for 2 months on a weekly basis, in a complete randomized design with 15 replicates per treatment. The positive and negative controls used were commercial OF (Maxicrop®) and tap water, respectively. Shoot height, number of leaves, girth of the stem, leaf area, root length, diameter of the root, number of lateral roots, dry weight of the whole plant, dry weight of the shoot biomass, and dry weight of the root biomass measured as growth parameters in the T25V treatment vs. Maxicrop® were 48.33±8.20 cm vs. 59.07±7.06 cm, 31.53±2.41 vs. 37.80±2.26, 5.11±0.06 cm vs. 5.04±0.05 cm, 113.16±7.82 $cm^2$ vs. 129.17±8.06 $cm^2$, 27.51±1.38 cm vs. 25.61±1.20 cm, 4.67±0.07 cm vs. 3.92±0.13 cm, 33.00±1.35 vs. 29.00±1.19, 13.57±0.67 g/plant vs. 13.74±0.65 g/plant, 11.45±0.65 g/plant vs. 11.67±0.91 g/plant, and 2.45±0.11 g/plant vs. 2.69±0.12 g/plant, respectively. However, statistical analysis of one-way ANOVA (MINITAB 17) of these data did not show any significant differences ($P>0.05$) between the T25V treatment and the positive control. Therefore, T25V treatment can be recommended as effective as the commercial OF for the growth performances of *Basella alba.*

**Keywords:** *Basella alba*, Growth and yield, Invasive alien plant species, Organic fertilizer paste, *Trichoderma* spp.

# Investigating the Responses of *Sesamum indicum* L. (Variety ANKSE-3) to Drought Stress at Different Developmental Stages

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Sesame (*Sesamum indicum* L., Family Pedaliaceae) is the major oil seed crop grown in the dry areas of Sri Lanka. It is the only oil seed crop exported from the country. This study aimed to evaluate the drought responses of Sri Lankan sesame variety ANKSE-3, recently released by the Grain Legume and Oil Crops Research and Development Centre, Angunakolapelessa, Sri Lanka. The objective of this study was to determine the physiological, morphological and yield responses of ANKSE-3 to drought stress imposed during juvenile and late bloom stages. Two different pot experiments were conducted in a glasshouse, arranged in completely randomized design with ten replications per treatment. Drought conditions were imposed by withholding irrigation until the plants reached the initial wilting point. At the end of the stress period, effects on selected physiological parameters were recorded while morphological traits were recorded two days after recovery. Yield parameters were measured at the end of harvesting. Two-sample t-tests (unpaired) were conducted to analyse the data using the SPSS software (version 26.0). Results showed that the relative water content of leaves was depleted under drought stress at both the developmental stages (from 86.60% to 72.59% at juvenile stage; from 81.34% to 64.32% at late bloom stage, $p < 0.05$) while the proline content was significantly higher in the drought affected plants (1.105 mol/g at juvenile stage and 0.906 mol/g at late bloom stage) compared to the control plants (0.248 mol/g at juvenile stage and 0.375 mol/g at late bloom stage) at $p < 0.05$. Neither the relative chlorophyll content nor the chlorophyll fluorescence was sensitive to the water deficit caused by the drought treatment. Any difference was not observed in the leaf area, the number of capsules per plant and the number of seeds per capsule while the plant height was significantly decreased in drought affected plants (from 61.40 cm to 41.29 cm at juvenile stage; from 82.37 cm to 75.54 cm at late bloom stage, $p < 0.05$). However, sesame plants exposed to drought stress at the late bloom stage showed reduction in the yield by 8% and the thousand seed weight reduction by 13% (dry weight). It is concluded that drought stress provided at juvenile stage, did not show any significance effect on yield while sesame variety ANKSE-3 could survive under drought stress at the late bloom stage with a yield penalty.

**Keywords:** Sesame, ANKSE-3, Drought, Proline, Physiology

# Morphological Characterization of Selected *Phaseolus* Cultivars in Uva Province, Sri Lanka

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Genus *Phaseolus* L. contains 75 species, self-pollinated annuals that grow extensively throughout the tropical and temperate regions of the world. *P. vulgaris* L. and *P. lunatus* L. are available in Sri Lanka as two legume crop species. The main objective of the present study was to characterize five *Phaseolus* cultivars using ten replicates; three *P. vulgaris* cultivars and two *P. lunatus* cultivars using twenty-three morphological characters including 16 qualitative and 7 quantitative characters in Uva Province; Welimada. All the morphological characters were recorded through the observations made at the physiologically matured stage of the plants. Cluster analysis was performed and phenograms were constructed using PAST software. A multi-access key was prepared using gathered morphological characters and images using DELTA software for further references. According to the derived phenograms, two *Phaseolus* spp. formed separate clusters. Leaf and stem pubescent, shape of pod and seed, color of seed hilum, length and width of terminal leaflet, length of flower, width of pod and number of seeds per pod were observed as morphological differences between two *Phaseolus* spp. Pod shape, pod size, seed shape and seed color are used as main diagnostic features of the two species; *P. vulgaris* and *P. lunatus*. *P. vulgaris* cultivar 1 and cultivar 2 have shown highest close relatedness with respect to the morphological features. Separation of *P. vulgaris* cultivar 3 from, *P. vulgaris* cultivar 1 and cultivar 2 can be interpreted as due to the differences in color of pods and seeds. Quantitative characters of *P. vulgaris* cultivars were most likely similar and it has interpreted the phenogram. *P. lunatus* cultivar 1 produces blood red color seeds with white patches and seeds of *P. lunatus* cultivar 2 is light brown color with black spots. According to the clustering pattern, seed color, length of seed, color of corolla and color of pod can be recommended as reliable diagnostic characters for identification of selected *Phaseolus* cultivars. Seed color was identified as the best distinguishing feature to identification of five *Phaseolus* cultivars.

**Keywords:** *Phaseolus vulgaris* L.*, Phaseolus lunatus* L., Cultivars, Morphological, Phenograms

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# Green Synthesis of Zinc Oxide Nanoparticles Based on *Piper longum* L. Leaf Extracts

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The green synthesis of Zinc Oxide nanoparticles (ZnONPs) raises concerns since it is a safe, simple, cost-effective, and environmentally friendly approach. The current research describes the green synthesis of ZnONPs from *Piper longum* leaf extracts and its characteristics & bioactivity. ZnONPs were synthesized utilizing zinc acetate dihydrate solution using both fresh and dried leaf extracts of *P. longum*. Fourier-transform infrared spectroscopy (FTIR), UV-spectrophotometry, and scanning electron microscopy (SEM) were used to characterize the ZnONPs that were produced through biological means. Furthermore, the ZnONPs, methanol, and aqueous leaf extracts were assessed for their bio-efficacies. Antioxidant activity was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, total phenolic content (TPC) using Folin Ciocalteu assay, and total flavonoid content (TFC). Antidiabetic and anti-inflammatory properties were tested using alpha-amylase inhibition assay and inhibition of protein denaturation respectively. The formation of ZnO-NPs was demonstrated by the absorption peaks at 357 – 371 nm in the UV-vis spectrum. FTIR spectra revealed the existence of stretching vibrations of O-H, N-H, C-H, and C=C groups of the proteins, terpenoids, and phenolic chemicals found in *P. longum* leaf extract and suggested it may play a part in the stability and nucleation of ZnONPs. SEM imaging of ZnONPs reveals the spherical shape and low aggregation, and the sizes in the range of 50 - 150 nm. Yield percentages of four ZnONPs samples ranged between 66.29% - 99.92%. The current study showed that ZnONPs synthesized using leaf extracts of *P. longum* has DPPH radical scavenging activity (22.92% to 29.57%), antidiabetic (2.93% to 25.87%) and anti-inflammatory (16.36% - 19.57% at 500 µg/mL and 15.22% - 18.94% at 250 µg/mL) properties due to the presence of important functional groups. The results showed that biosynthesized ZnONPs have intriguing properties that make them suitable for a variety of potential industries such as cosmetic, food and biomedical in the future.

**Keywords:** Zinc Oxide nanoparticles, *Piper longum* L., Antioxidant, Antidiabetic, Anti-inflammatory

# Evaluation of Cytotoxicity of Fruit Extract of *Catunaregam spinosa* using Brine Shrimp Assay

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Ethnobotanical medicine is a resurging field in science sector. Despite the extensive uses, there is a still need to study the intrinsic toxicity of the-medicinal plants for a safety use. *Catunaregam spinosa* (Family Rubiaceae) is one of valuable medicinal plants that occupies in various phototherapy ailment with several pharmacological activities. Cytotoxicity is one of them which can be simply evaluated by brine shrimp lethality assay. The study aimed to explore the cytotoxicity of fruit of *C. spinosa* using preliminary test of brine shrimp lethality assay. Methanolic fruit extract was prepared using Soxhlet extraction. Phytochemicals were evaluated using conventional standard methods for each constituent. Lethality assay was conducted using 4th instar of *Artemia salina*. Stock solution (1000 µg/mL) was diluted to prepare 500, 100, 50, 10, 1, 0.5 µg $mL^{-1}$ concentrations. Each concentration was triplicated with twenty nauplii in a treatment. Potassium dichromate and 0.2 % DMSO were used as positive and negative controls respectively. Mortality was determined after 24 h and LC50 was calculated using probit analysis. Phytochemical screening revealed the presence of alkaloids, coumarins, flavones, glycosides, saponins, tannins and phenols in methanolic fruit extract. Concentration dependent mortality was observed. A 1.73 µg $mL^{-1}$ of median lethal concentration (LC50) was recorded in methanolic fruit extract which is significantly high compared to positive control (LC50, 26.55 µg $mL^{-1}$). No mortality was recorded in negative control. Literature mentioned the correlation between the cytotoxicity of above mentioned compounds. Thus, the results indicate the presence of cytotoxic compounds in fruits of *C. spinosa* may responsible for its cytotoxicity. It can be concluded that the methanolic fruit extract of *C. spinosa* possesses significantly high cytotoxicity against brine shrimps under the experimental conditions used in this study.

**Keywords:** *Catunaregam spinosa,* Phytochemical, Cytotoxicity, Brine shrimp lethality assay

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# Variations in Microclimatic Conditions Across Different Habitat Types in the Wasgamuwa National Park

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Vegetation characteristics are important in influencing the climate buffering capacity of forests. This study examined variations in microclimatic conditions across habitat types in a mosaic landscape in the dry zone of Sri Lanka. The study was conducted in the Wasgamuwa National Park (WNP), where four major habitats – Dry-Mixed Evergreen Forests (DMEF), Riverine Forests (RVF), Scrub Forests (SCF) and Grasslands (GR), were assessed. Forty-five sampling stations situated within the WNP were randomly picked – RVF (n=8), DMEF (n=15), SCF (n=11) and GR (n=11), and ambient temperature (T) and relative humidity (RH) were recorded at ground level and at 2 m, using sensors recording data every 30 minutes for 24 hours. The sensors were placed approximately at the centre of each of the habitat patches. Several microclimate metrics were extracted from the recorded data. There were considerable habitat-wise microclimatic differences in the WNP (one-way Anova $P<0.05$). The highest Tmax was recorded in GR and the lowest in RVF. Tmin was highest in GR and lowest in DMEF (at 2 m) and RVF (at ground level). Tmax – Tmin was widest in GR (15.7 $^0$C at 2 m) and narrowest in RVF (7.9 $^0$C at 2 m). The highest RHmin was in RVF and the lowest in GR. The lowest RHmax was in GR and the highest in DMEF (at 2m) and RVF (at ground level). RHmax – RHmin (range) was greatest in the GR (46 % at 2m), whereas it was less than half of this in RVF (22 %). The habitat-wise differences were significant ($P<0.05$) for Tmax and Trange (both at 2m), RHmin (at 2m) and RHrange (for both levels). Overall, the RVF and DMEF had moderate temperatures and relative humidity indicating a stable environment in comparison to SCF and GR where variability was high. This implies that the presence of a tree canopy helps in the modulation of both temperature and relative humidity. Given that forests would continue to decline globally and locally, these findings highlight the need to prioritize the conservation of tropical forests, particularly for mitigating climate change.

**Keywords:** Dry zone forest, Relative humidity, Temperature, Sri Lanka

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# Impact of COVID-19 Pandemic on Air Pollutant Emissions in Colombo District, Sri Lanka

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Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The impacts of the COVID-19 pandemic on different sectors can be analysed considering health and other aspects. COVID-19 has impacted both positively as well as negatively on the environment. The current study focuses on the status of air pollutant emissions during the COVID-19 pandemic period in Colombo district, Sri Lanka. The concentrations of Carbon dioxide ($CO_2$), Nitrogen dioxide ($NO_2$), Sulphur dioxide ($SO_2$), and Particulate Matter (PM) emissions from 2010-2020 were collected from National Building Research Organization (NBRO), Sri Lanka. The data were analysed for any trends, which were then used in forecasting the values for the year 2020 through time series analysis using R software. Several statistical models including Linear Trend- Seasonal models, Error Trend Seasonal (ETS) models, and Autoregressive Integrated Moving Average (ARIMA) models were employed to study the trends in forecasting the values for the year 2020. The forecasted values were then compared against the actual values. Compared to the forecasted concentrations in 2020, actual concentrations of $CO_2$, $NO_2$, $SO_2$, $PM_{2.5}$, and $PM_{10}$ emissions showed an average reduction of 7%, 27%, 36%, 36%, and 30% respectively. A significant drop in air pollutant emissions to the atmosphere was observed during the COVID-19 period mainly due to the closure of industries and restricted human activities during the lockdown time period. For a long-term drop in air pollutants released into the atmosphere, structural and transformational alternations should be introduced in the industrial, energy, and transportation fields while adopting more environment-friendly and sustainable lifestyle practices.

**Keywords:** Air pollutants, Emissions, COVID-19, Colombo District, Sri Lanka

# Effects of Sub-lethal Exposure of Ibuprofen on Laboratory-reared *Oreochromis niloticus* Juveniles: An Integrative Biomarker Study

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Toxicological impacts of pharmaceuticals on non-target species in aquatic and terrestrial ecosystems are still not fully understood. Ibuprofen, a non-steroidal anti-inflammatory drug (NSAID), has been reported in water samples collected from environmental samples. The present experiment followed the short-term effects of waterborne ibuprofen (20 µg/L) on laboratory-reared *Oreochromis niloticus* (Nile Tilapia) juveniles using selected biomarkers. The experiment was conducted for 15 days with static renewal of water having dissolved ibuprofen every 4th day in three replicates. Feeding and aeration were similarly maintained in the treatment and control groups, with regular monitoring of water quality. Data were collected on day 16 with respect to Fulton's condition factor, liver somatic index, erythrocyte nuclear abnormalities (ENA), total leukocyte counts, carbonic anhydrase enzyme activity, two behavioural endpoints (ventilation rate and response to tactile stimulus), and gill histology. Fifteen-day exposure of 20 µg/L ibuprofen resulted in significantly lower ($p<0.05$) Fulton's condition factor (1.62-1.74), and a significant induction ($p<0.05$) of total ENA and leukocyte counts in fish, compared to the controls. Ibuprofen exposure did not affect the liver somatic index, carbonic anhydrase activity, and behavioural parameters, but caused discernible gill histopathology, including lamellar fusion, and interlamellar hyperplasia. The results show that short-term exposure to sublethal concentrations of ibuprofen in aquatic environment may cause a multitude of effects on fish, including lowering the condition of fish, and inducing genotoxic, and hematotoxic effects.

**Keywords:** *Oreochromis niloticus*, Ibuprofen, Biomarkers of exposure, ENA induction, environmental pharmaceuticals

# Heavy Metal Accumulation in Selected Food Fish and the Level of Human Exposure Through Diet in Mahakanadarawa Wewa, Anuradhapura District

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Agrochemical contamination in food and water has been identified as a serious environmental issue causing adverse health consequences such as Chronic kidney disease of unknown aetiology. Fresh water fish is a popular food in the Dry zone of Sri Lanka, providing higher nutritional value. However, heavy metal contaminations of these food fish species have been reported in many instances. The present study investigated the level of heavy metal contamination of food fish varieties available in the dry zone of Sri Lanka, by considering Mahakanadarawa wewa as a typical dry zone reservoir that supplies food fish for the nearby inhabitants. The study tested the level of Pb, Cd and As contamination in different types of food fish species and the level of human exposure through daily fish consumption. Heavy metal accumulation was tested using -Inductively coupled plasma mass spectrometry (ICP-MS). The study attempted to correlate between the levels of accumulation with the amount of provisional tolerable weekly intake (PTWI) values. 30 food fish samples belonging to five food fish species; Catla (*Catla catla*), Theppili (*Trichogaster pectoralis*), Tilapia (*Oreochromis niloticus*), Kokassa (*Chitala* sp.) and Korali (*Etroplus* sp.) were identified. Different fish species are showing different levels of accumulations of heavy metals (0.003-0.079 ppm), with, lead (Pb) reporting the highest accumulation in all the species with Korali (*Etroplus* Sp.) reaching the highest (0.079 ppm) among them. Average weekly intake value (mg/week) for Cd and As showed the order of Theppili > Koraliya > Kokassa> Tilapia while that for Pb showed the order of Koraliya >tilapia >Theppili > Kokassa revealing differential availability and accumulation of metal species. None of the accumulation values exceeded the provisional tolerable weekly intake values, suggesting safe consumption of the food fish tested. The study highlights the importance of conducting extensive studies to test more fish species with different processing techniques.

**Keywords:** Heavy metal contamination, Food fish, Chronic kidney disease, Bioaccumulation

# Effect of Urbanization on Initiation of the Dawn Chorus of Home Garden Birds

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Many factors are known to affect the timing and occurrence of the dawn chorus of male birds. We investigated the impact of microclimatic conditions and urbanization on dawn chorus initiation in home garden birds. Audio recordings were done at 21 locations (covering urban, semi-urban, and rural areas) in the Western Province of Sri Lanka from 0430 to 0630 hours in January and February 2022. When a call was heard, the species and the time of its first call were recorded. Geo-references of the locations were used to obtain data on the astronomical twilight time (ATT) on different days, and values were generated for deviations of the time of the initial call of a species from the ATT. The data for temperature, relative humidity, cloud cover, and three proxies for urbanization (degree of human modification, population density, and extent of built-up areas) were obtained from websites. Calls of 21 garden birds were identified. Not all species were heard at every sampling location. Only eight species were recorded in over 50 % of study locations, whereas three species were recorded in single locations. The best-fitted model explaining the initiation of the dawn chorus included temperature, relative humidity, cloud cover, the index for human modification, and the species concerned. The Oriental Magpie-Robin (*Copsychus saularis*) emitted the earliest call. Interestingly, the majority of the initial calls (90.48%) were emitted after ATT, although some (e.g. Oriental Magpie-Robin and White-bellied Drongo (*Dicrurus caerulescens*) called prior to twilight at some locations, the latter suggesting the possibility of artificial light acting on call initiation at dawn. The findings that birds are receptive to factors related to urbanization for call initiation are especially relevant given that the dawn chorus has an important ecological and social function in avian communities.

**Keywords:** Avifauna, Calls, Urbanization, Twilight, Vocalization

# Acute Toxicity of the Herbicide Pretilachlor and its Effect on Mortality and Behaviour of Molly Fish (*Poecilia sphenops*)

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Pretilachlor (PTC) (2-chloro-2′, 6′-diethyl-N-(2-propoxyethyl) acetanilide) is a systemic herbicide that is used to control weeds in rice fields of Sri Lanka. Due to its chemical stability, it has the potential to accumulate in waterbodies posing a threat to aquatic animals. The aim of this study was to determine the acute toxic effects (on mortality and behaviour) in molly fish (*Poecilia sphenops*) exposed to four concentrations of PTC (15 ppm, 3 ppm, 1.5 ppm, and 0.75 ppm) during a four-day period. Trials were conducted in triplicate and controls without the herbicide were also maintained (N=15). Mortality and behaviour were monitored daily. In comparison to the control, the number of crossings per minute per individual decreased significantly ($p<0.05$) with increasing exposure time and decreasing concentrations. Mortality increased significantly ($p<0.05$) with increased duration of exposure and with increased concentration when compared with the control. The 24, 48, 72 and 96h $LC_{50}$ values of PTC for *P. sphenops* were 4.29 ppm, 2.18 ppm, 0.61 ppm and 0.37 ppm respectively. With time, behavioural alterations in fish were noticed, including impaired activity, swimming upside down, reverse swimming, slanting, and rapid gill movements at 15 ppm, 3 ppm, and 1.5 ppm concentrations. In conclusion, this study demonstrates that acute exposure to PTC can result in instantaneous fish death whereas long-term exposure to low PTC concentrations could result in behavioural abnormalities. This is an indication of threat to sensitive freshwater fish populations inhabiting rice cultivation areas, especially those in small streams directly receiving run off from paddy fields.

**Keywords:** Acute toxicity, Behaviour, Freshwater fish, Herbicide, Mortality

# Physicochemical Analysis and Toxicity Assessment of Sugarcane Distillery Spent Wash

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Sugarcane molasses-based ethanol industries in Sri Lanka generate large volumes (300 $m^3$/per day) of high-strength spent wash that may cause severe environmental issues. The potential toxicity of raw spent wash (RSW) on biological systems has been given less attention in Sri Lanka. The present study was conducted to assess the physicochemical characteristics of the RSW, and the possible cytogenotoxic effects of diluted RSW using *Allium cepa bioassay.* Selected physicochemical parameters and trace metal levels of RSW collected from the distillery industry of Sri Lanka were evaluated using APHA (2017) standard procedures. Toxicity assessment was carried out after exposure of *Allium cepa* bulbs to diluted RSW (1:8) following standard protocols. The physicochemical analysis revealed significantly high values of electrical conductivity (2 1.9 ± 0.09 mS/cm), COD (92,101 ± 0.33 mg/L), BOD (26,116 ± 2.33 mg/L) and total dissolved Solids (68,656 ± 0.13 mg/L) when compared with the tolerance limits for the discharge of industrial waste into inland surface waters ($p < 0.05$). High level of total suspended solids (4,076 ± 0.55 mg/L), nitrate (255 ± 0.04 mg/L), phosphate (38 ± 0.07 mg/L), and acidic pH (3.3 ± 0.08) and trace amounts of heavy metals (Cd, Cu, Ni, Zn, As, and Mn) were also reported. Significantly decreased root growth (92%) was found in *Allium* roots exposed to diluted RSW with the highest root growth delay (1.5 ± 0.62 cm) after two days of exposure (92%) compared to the negative control (20.73 ± 1.05 cm) ($p < 0.05$). The mitotic index did not show any difference in all exposure conditions. A significantly increased number of nuclear abnormalities were observed in root tip meristematic cells of diluted RSW compared to the negative control ($p < 0.05$) due to trace levels of heavy metals. Recorded physicochemical characteristics, trace metal levels and cytogenotoxicity bioassay tests indicated adverse environmental impacts on biota, and emphasized the need for advanced treatment before disposal.

**Keywords:** Spent wash, Bioassay, Cytogenotoxicity, Mitotic index, Nuclear abnormalities.

# Distribution of Endemic Plant Genera in Sri Lanka: Niche Breadth, Range Size and Altitudinal Range

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Endemic plant generics are not a uniform group in terms of distribution, range size, habitat preferences, and ecological plasticity. Based on comprehensive literature survey and field knowledge of experts, an inventory of 17 endemic plant genera was compiled in order to analyse their distribution patterns, and correlation among range size, niche breadth and altitudinal range. The species occurrence data were gathered mainly from the specimens deposited in the National Herbarium, Peradeniya, and national and international databases. Locality data of each endemic plant genus was mapped using ArcGIS v10.7.1 software to demarcate distribution patterns. Distribution data of endemic plant genera were overlaid with 16 floristic zones, in order to analyse their distribution pattern with respect to floristic zones. Heat map and grid network were generated to detect the centre of endemism for endemic plant genera in Sri Lanka. Niche specialists were identified using restricted niche width and range size. Most of the endemic plant genera confined to wet zone in Sri Lanka dominating lowland and central highlands. Majority of endemic plant generics distributed within the Lowland Wet Zone Rainforest, Peak Wilderness and Knuckles Region indicating centres of endemism for endemic plant generics in Sri Lanka. The Foothills of Adam's Peak and Ambagamuwa Floristic Zone was identified as floristic zone with highest number of endemic generics (35 species). *Chlorocarpa, Leucocodon, Davidsea, Pheniocanthus, Schumacheria* sp. and *Hortonia* sp. showed wide distribution range within several floristic zones. Endemic Species *Stemonoporus marginalis, Stemonoporus petiolaris, Stemonoporus gracilis, Stemonoporus mooni* and *Stemonoporus latisepalum* showed limited distribution and identified as range restricted species. *Stemonoporus marginalis, Stemonoporus bullatus* and *Stemonoporus mooni* were identified as species representing both low range size and narrow niche breadth. Altitudinal distributions of species of endemic plant genera exhibit a positive correlation with the range size.

**Keywords:** Distribution, Niche breath, Endemic plant genera, Sri Lanka, Range size

# Investigating Spatial Variation of Stomatal Traits of True Mangrove and Mangrove Associates in the Southern Province of Sri Lanka in Accordance with Climatic Adaptation

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Mangrove vegetation has unique traits to survive in their harsh habitat. Stomata, which are important in photosynthesis and transpiration, are highly sensitive to environmental changes. While there have been studies on Sri Lankan mangroves, few have focused on their stomatal characters. This study aimed to determine the spatial variation of stomatal characters of true mangrove and mangrove associate species in the Southern Province of Sri Lanka. Seven mangrove sites in the Southern Province covering the three principal climatic zones were selected. Mature leaves were collected at each site along with soil salinity measurements. Stomatal density, epidermal cell density, guard cell length (GCL), stomatal index (SI) and potential conductance index (PCI) were measured. ANOVA test and Cluster Analysis were carried out using RStudio software to determine whether there is a significant stomatal trait variation in relation to different climatic zones. The study observed varied stomatal trait values for different true mangrove and mangrove associate species that emphasized the varying degrees of ability to cope with different climatic conditions in their environment. For instance, *Acrostichum aureum* had higher GCL and PCI which enhances the photosynthetic efficiency. Furthermore, a variation in stomatal traits was observed in relation to different climatic zones. Some species including *Excoecaria agallocha* and *Clerodendrum inerme* showed a lower SI in the dry zone. A spatial variation could be observed in the clustering with members in the same family clustering together, showing a genetic influence. Species such as *A. aureum* inhabited different habitats without changing their stomatal traits. *C. inerme,* a mangrove associate species, had similar adaptive responses as true mangroves while *E. agallocha* showed a plastic nature and inhabited different locations. Overall, both true and mangrove associate species are likely to adapt to environmental changes under climate change scenarios. This study would support identifying more climate-resilient species for mangrove restoration programs.

**Keywords:** Mangrove, Stomatal traits, Climatic adaptation, Mangrove associates, Spatial variation

# Schedule of the Scientific Sessions

**42nd Annual Sessions of the Institute of Biology, Sri Lanka**
**30th September, 2022**
**At Marino Beach Hotel, Colombo 03**

## Parallel Session A

| TIME | ABSTRACT NUMBER | TITLE |
|---|---|---|
| 1.30–3.20 p.m. | A-01 | Studying the Microbial Community Interactions of Sri Lankan Cattle Milk Microbiota Under Different Climatic Conditions<br>*By U. Rajawardana and T. S. Artigala* |
| | A-02 | Effect of Curcumin Extract on Inhibition of Coconut Oil Rancidity<br>*By S. P. P. Amiyangoda, N. Balachandran and T. C. H. Gamage* |
| | A-03 | Evaluating Plant-derived Antifungal Substances for the Effective Management of Seed-borne Fungi of Selected Crop Species<br>*By W. N. Hansini and D. A. Daranagama* |
| | A-04 | Wound Healing Enhancing Terpenoids from *Vernonia zeylanica* (L.) Less.<br>*By W. M. P. Samarasinghe, G. M. K. B. Gunaherath, C. Ranasinghe, S. Somaratne and K. H. Jayawardana* |
| | A-05 | Antibacterial Activity of Entomopathogenic Fungi Isolated from a Thorn Treehopper in Sri Lanka<br>*By I. B. N. S. Sewwandi and P. B. Ratnaweera* |
| | A-06 | *In vitro* Bioefficacy of Beauveria sp. Against Tea Shot-hole Borer (*Euwallacea fornicatus* Eichh.)<br>*By R. D. S. M. Gamlath, P. D. Senanayake, G. D. Sinniah and R. G. S. C. Rajapakse* |

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|---|---|---|
| | A-07 | A Microbial Cocktail Combined with Organic Fertilizer with the Potential of Providing Rice Growth and Yields Similar to 100% Urea Recommendation<br>*By M. G. G. D. Kithmini and T. A. Perera* |
| | A-08 | Rhizospheric Fungal Species of Selected Capsicum (*Capsicum annuum* L.) Varieties of Sri Lanka and their Ability to Control *Fusarium* sp., Causative Agent of Damping-off Disease in Capsicum<br>*By P. U. N. E. Srimali, N. Deshappriya, M. S. W. Fernando, R. N. Attanayake and D. S. Manamgoda* |
| | A-09 | Antibacterial Activity of Selected Fungal Endophytes Isolated from Two Species of Pandanaceae<br>*By W. N. N. Dabarera, S. S. Ediriweera, C. M. Nanayakkara, K. G. S. U. Ariyawansa, N. N. Wijayawardene, R. P. P. K. Jayasinghe and S. C. Karunarathna* |
| | A-10 | Effect of Cryoprotectants on Cell Viability and Biomass Growth of *Chlorella* sp. and *Oscillatoria* sp. Cells under Ultra Low Temperature<br>*By B. L. W. K. Balasooriya, I. G. S. S. Ekanayaka and S. A. V. Viduranga* |
| | A-11 | Screening for Petrol Degradation Potential of Nine Bacterial Isolates Using a Redox dye 2,6-Dichlorophenolindophenol<br>*By A. M. Weerakoon, P. S. Wanigasooriya and S. R. Karunaratne* |

## Parallel Session B

| TIME | ABSTRACT NUMBER | TITLE |
|---|---|---|
| 1.30–3.20 p.m. | B-01 | Comparison of Phytochemicals of De-polysaccharide and Polysaccharide rich Methanolic Extracts of Sri Lankan Marine Alga *Chnoospora minima*<br>*By U. Bandaranayake, H. S. Kumarasinghe, T. L. Gunathilaka, P. T. Jayasooriya, P. Ranasinghe, L. D. C. Peiris and K. W. Samarakoon* |
| | B-02 | Association of Selected Genetic Variants in CBS and MTHFR Genes in a Cohort of Children with Homocystinuria in Sri Lanka<br>*By D. T. Mahaliyanage, M. H. N. J. Samarasinghe, S. De Silva, N. Punyasiri and E. Jasinghe* |

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| | B-03 | Effect of Simulated *In vitro* Gastro-intestinal Digestion on the Bioactive Properties of Phenolic Compounds From Edible Flowers<br>*By G. Janarny, K. D. P. P Gunathilake and K. K. D. S Ranaweera* |
| | B-04 | Genome-wide Identification of *Gretchen Hagen*3 (GH3) Gene Family in *Musa acuminata* L.<br>*By K.H.M. Jayasinghe and H. D. D. Bandupriya* |
| | B-05 | *In vitro* Antioxidant Capacity and Lipoxygenase (5-LOX) Inhibitory Activity of Leaves of *Citrus aurantiifolia* (lime) and Chemical Profile of Leaf Essential Oils<br>*By D. Jagoda, G. D Liyanaarachchi, H. D Weeratunge, S. M. Handunnetti, N. Fernando and J. K. R. R. Samarasekara* |
| | B-06 | Computational Analysis of EMBRYO-DEFECTIVE (EMB) Genes in *Arabidopsis*<br>*By K. H. N. Sandumina and A. M. Wickramasuriya* |
| | B-07 | Molecular Docking for Discovering Lead Compounds of Fungal Origin for Quorum Quenching Agents Against *Pseudomonas aeruginosa*<br>*By W. M. J. V. Wickramasinghe and I. C. Perera* |
| | B-08 | DNA Methyltransferases and Demethylases in *Theobroma cacao*: Genome-wide Identification, Genomic Structures and Phylogeny<br>*By W. M. A. Sanahari and A. M. Wickramasuriya* |
| | B-09 | Prevalence of Angiotensin Converting Enzyme (ACE) Insertion/Deletion Polymorphism and its Association with Oxidative Stress in a Subset of Sri- Lankan Pediatric Psoriatic Patients<br>*By S. A. K. Udayanga, J. Seneviratne, M. G. A. Saumyamala and A. D. D. S. Amarasekara* |
| | B-10 | An ARMS PCR-based RT Coupled Method for Simultaneous Identification of SARS-CoV-2 Variants<br>*By S. Liyanage, R. Anthonies, C. S. Sepalage, S. Siriwardana and I. C. Perera* |

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| | B-11 | Investigating the Proteins Involved with Crosstalk Between Drought Response Sub-pathways in *Oryza sativa* Using a Network-based Approach<br>*By J. W. J. K. Weeraman, T. L. S. Tirimanne and S. P. C. Fernando* |

## Parallel Session C

| TIME | ABSTRACT NUMBER | TITLE |
|---|---|---|
| 1.30–3.10 p.m. | C-01 | Modelling Environmentally Suitable Areas for the Potential Introduction and Cultivation of the Ornamental *Cryptocoryne thwaitesii* in Sri Lanka<br>*By K. A. M. R. P. Atapattu, P. R. G. K. T. Rankoth and H. S. Kathriarachchi* |
| | C-02 | Morphological and Biochemical Characterization of Quinoa (*Chenopodium quinoa* Willd.) - A preliminary Study<br>*By A. I. L. Silva and R. Wimalasekera* |
| | C-03 | Prolonging the Shelf Life of 'Ambul' and 'Cavendish' Banana Using Potassium Permanganate and Activated Charcoal Based Sachet Fortified with Passive Modified Atmosphere Packaging<br>*By M. A. Sandaru and P. S. Saputhanthri* |
| | C-04 | Effect of the Organic Liquid Fertilizer Amended with *Trichoderma harzianum* on the Growth and Yield of *Capsicum annuum* cv. MI 2<br>*By H. D. U. N. S. Senarathna, R. M. C. S. Ratnayake and B. T. S. D. P. Kannangara* |
| | C-05 | Morphological and Phytochemical Characterization of *Cajanus cajan* L. (Pigeon pea) in Sri Lanka.<br>*By H. D. N. H. Karunarathna, A. I. S. Priyadarshan and R. A. S. P. Senanayake* |
| | C-06 | Rooting of *In vitro* Developed Shoots and Stem Cuttings of *Passiflora edulis* |

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|---|---|---|
| | | *By D. M. N. L. Dassanayake and T. D. Silva* |
| | C-07 | Use of Plant-based Organic Fertilizer Paste Enriched with *Trichoderma* Species for the Cultivation of *Basella alba* - Alternative Solution to the Commercial Organic Fertilizer<br>*By N. N. Kalpani, B. T. S. D. P. Kannangara and R. M. C. S. Ratnayake* |
| | C-08 | Investigating the Responses of *Sesamum indicum* L. (Variety ANKSE-3) to Drought Stress at Different Developmental stages<br>*By W. K. P. Purnima and I. A. J. K. Dissanayake* |
| | C-09 | Morphological Characterization of Selected *Phaseolus Cultivars* in Uva Province, Sri Lanka<br>*By H. D. N. H. Karunarathna, A. I. S. Priyadarshan and R. A. S. P. Senanayake* |
| | C-10 | Green Synthesis of Zinc Oxide Nanoparticles Based on *Piper longum* L. Leaf Extracts<br>*By T. K. Rupasinghe, S. M. Vithanarachchi and H. D. D. Bandupriya* |

## Parallel Session D

| TIME | ABSTRACT NUMBER | TITLE |
|---|---|---|
| 1.30–3.10 p.m. | D-01 | Evaluation of Cytotoxicity of Fruit extract of *Catunaregam spinosa* Using Brine Shrimp Assay<br>*By P. K. Lawrence and W. T. P. S. K. Senarath* |
| | D-02 | Variations in Microclimatic Conditions Across Different Habitat Types in the Wasgamuwa National Park<br>*By A. D. Senevirathna, H. H.E. Jayaweera and M. R. Wijesinghe* |
| | D-03 | Impact of COVID-19 Pandemic on Air Pollutant Emissions in Colombo District, Sri Lanka<br>*By J. P. P. M. Jayalath, E. Lokupitiya and D. Halwathura* |
| | D-04 | Effects of Sub-lethal Exposure of Ibuprofen on Laboratory-reared *Oreochromis niloticus* Juveniles: An Integrative Biomarker Study<br>*By H.P.S.H. Wickramarathna, S.H.N.P. Gunawickrama, and K.B.S. Gunawickrama* |

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|---|---|---|
| | D-05 | Heavy Metal Accumulation in Selected Food Fish and the Level of Human Exposure through Diet in Mahakanadarawa wewa, Anuradhapura District<br>*By A.I. Wanasinghe, R.G.D.R. Jayawickrama, R.L. Jayaratne and U.A. Jayawardena* |
| | D-06 | Effect of Urbanization on Initiation of the Dawn Chorus of Home Garden Birds<br>*By M.G.D.D. Kariyawasam, G.D. Jayasinghe and M.R. Wijesinghe* |
| | D-07 | Acute Toxicity of the Herbicide; Pretilachlor, and its Effect on Mortality and Behaviour of Molly Fish (*Poecilia sphenops*)<br>*By* E.D.J. Chathurya, M.R. Wijesinghe and V.A.K. Fernando |
| | D-08 | Physicochemical Analysis and Toxicity Assessment of Sugarcane Distillery Spent Wash<br>*By H.D. Kuruppuarachchi, K.M.S. Ruvinda and J. Manathunga* |
| | D-09 | Distribution of Endemic Plant Genera in Sri Lanka: Niche Breadth, Range Size and Altitudinal Range<br>*By W.A.A.D.M. Viduranga and H.S. Kathriarachchi* |
| | D-10 | Investigating Spatial Variation of Stomatal Traits of True Mangrove and Mangrove Associates in the Southern Province of Sri Lanka in Accordance with Adaptation<br>*By W.R. Ranathunga and H.I.U. Caldera* |

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