

# Institute of Biology Sri Lanka

# Proceedings of the 41<sup>st</sup> Annual Sessions

# "Marching Towards a Bioeconomy"

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# Council of the Institute of Biology Sri Lanka 2020/2021



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### **INSTITUTE OF BIOLOGY**

## SRI LANKA

#### PROCEEDINGS OF THE 41st ANNUAL SESSIONS

Theme

Marching Towards a Bioeconomy

Institute of Biology, Sri Lanka 24<sup>th</sup> September, 2021

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#### Institute of Biology, Sri Lanka

Proceedings of the 41st Annual Sessions

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Editor, IOB

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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 575 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/seminars on current topics in biology on a regular basis. It also plays an important role in biology education to a wider spectrum of participants ranging from those in the industry, those seeking self-employment, school children and 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 in the country 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 41<sup>st</sup> time this year.

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#### **PRESIDENTIAL ADDRESS**

#### Marching Towards a Bioeconomy: Harnessing natural wealth in pest and mosquito vector control programmes in Sri Lanka

#### Professor Deepika Amarasinghe PhD (London), (BSc Kelaniya), C.I. BiolSL, F.I. BiolSL, Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya, Dalugama, Kelaniya

Good morning, Fellow members, Chartered members and life members of the Institute of Biology Sri Lanka, keynote speaker, Emeritus Professor Wimaladharma Abeyewickrema, Dr. Ivor Fernando, Invited guests, Ladies and gentlemen.

It is a great honour and privilege for me to be with you today to deliver the Presidential address of the Institute of Biology, the premier organization for biologists in Sri Lanka at its 41<sup>st</sup> Annual Sessions.

Our organization at present forms a single platform for over six hundred biologists in Sri Lanka and it has been a pillar of strength to its membership in many aspects which are of course embedded in the objectives of the Institute. I think it is not an exaggeration if I mention a few of such opportunities. The members get status, opportunities for networking with fellow professionals, update with information and key issues in the profession and even on some occasions improve their chances of promotions.

It is not only the IOBSL provides strength to its membership but has far more important obligations to look after the welfare of the nation. The IOBSL activities always committed to build social and economic capital of the country. The theme of the Annual sessions of the IOBSL this time is very timely selected, and I am thankful to the Council for approving the theme: "Marching towards a bioeconomy".

Eroded lands, polluted water, agricultural and food waste, landfill sites full to bursting, accumulation of plastics, toxins and chemical residues; these are some topics that we hear very frequently today in our country. They have become a cause of concern for everyone. Today the environment has been greatly damaged and many of the social and environmental problems has been caused predominantly by the reckless use of the natural resources as if they were limitless. As a solution to these challenges the world has now begun to use the sustainable technologies for production. These technologies are based on the use of the recyclable, biodegradable, renewable biological resources transforming the world to a bio-based economy.

The major driving force behind the sustainable use of the biological resources is centered on scientific findings in life sciences. The recent decision in our country to replace chemical agriculture with organic agriculture has given us, the biology professionals, a great challenge with respect to the availability of sustainable technologies. There are many questions that we should ask ourselves. Some immediate questions come to my mind are: Have we done enough in identifying floral and faunal resources that can be used as agro-inputs such as biofertilizers and biopesticides? Have we done enough to produce microbial enzymes or other products from microorganisms for industrial use? Have we identified biological controlling agents for

agricultural pests and disease vectors? Have we developed appropriate effluent management and pollution control methods? How far have we embarked on research on producing bioenergy? Have we done enough to exploit cell and tissue culture environments to produce high value compounds from plant and microbial resources? As IOBSL professional, it is true that we currently share our specialist knowledge with the decision makers, develop appropriate technologies through research, disseminate knowledge and conduct public awareness programmes. However, I believe, that there is a large space to be filled by all of us in these areas and our committed efforts will provide a better value for the public benefit which in turn will earn the public trust on biology professionals. The biologists therefore have a crucial role to play in a bioeconomy by their involvements in research and improvement of processes in health, agricultural and industrial sectors.

Obviously, agriculture is a major component of bioeconomy of a country. Not only it provides food and nutrition to the world, but it is also the main source of raw materials for bio-based industries. Harnessing the natural biodiversity to develop bio-based inputs and technologies for agriculture and also changing the agricultural biodiversity landscape to achieve sustainability have now become important areas of concern and biologists have a big role to play.

I wish to draw your attention on some of such areas.

#### **Crop diversification**

A recent report of Food and Agriculture Organization has shown that of over 6,000 plant species that have been cultivated worldwide for food, we rely on only 9 crops for 66 percent of our food production. This is affecting to reduce both the crop diversity on farm and food diversity on table. In Sri Lanka too, there is a big scope for research and development of many underutilized crops and, wild plants reported to be used for food which have to be explored from biodiversity by science. Crop diversification and increasing biodiversity in farmlands reduces the proliferation and prevalence of insect pests and pathogens compared to the large-scale mono-cropped areas.

#### **Biofertilizers and biopesticides**

Use of biofertilizers and biopesticides contributes to reduce the use of chemicals in agriculture. Depending on soil health and terrain of land, the nutrient use efficiency of chemical fertilizer added to the soil for different crops, is only around 10 to 30 percent. Lack of soil biodiversity too contributes to non-availability of nutrients caused by fixation. Organic manures such as compost and agro-waste; and biofertilizers which contain living microorganisms will be the best alternatives to reduce the dependency on chemical inputs for farming for better crop growth.

Plant materials which we use as mulches or cover crops, in addition to promote crop growth by reducing weeds and increasing the supply of nutrients, they too have the potential to reduce the soil plant parasites. One of our studies showed that in pot experiments, addition of dried plant parts of *Tithonia diversifolia*, and Tagetes erecta to cause a significant reduction in *Meloidogyne incognita*, root-knot nematode, infection in tomato plants. Nematicidal effect of aqueous extract of Tagetes erecta on *Meloidogyne incognita* was also detected. This shows the multitude of benefits that biological inputs can provides in agro-ecosystems.

Certain chemicals from biological origin whether they are from plants or microbe have shown their effectiveness against insects and nematode pests. Dried parts of plants have been used to protect stored grains from pest attacks for centuries. Pesticides based on botanicals have been used in the past as they are easily biodegradable. However, some formulations of plant-based pesticides were also hazardous to people. Researchers have the challenge to develop pesticides of biological origin which shows no health and environmental hazardous. The plants having a long history of safe human use are good candidates for such explorations. Bioprospecting insecticidal compounds from plants will be an important step when introducing such products to be used as safe pesticides.

A recent study reported for the first time, the volatile profiles of different parts of the brinjal plant, Solanum melongena that attracts the major insect pest, *Leucinodes orbonalis*. Identification of the active compounds using gas chromatography coupled with electroantennogram (GC-EAG) is continued to develop an eco-friendly attractant-based trap to capture the flying adult moths.

Now let me talk about a little on the exploitation of the biological methods in vector control strategies with a special reference to the controlling of the vector mosquitoes. Use of insecticides to control the mosquitoes has been the main method of control but has associated disadvantages. Development of resistance to insecticides, effect on non-target organisms and negative effects on environment are some main concerns on the insecticides. Moreover, the Sri Lankan government has now tightened the laws on importation of pesticides on the grounds of their bad effects on human health and environment. All these reasons have now called for a situation where the biologists have to introduce sustainable and efficient biological methods for controlling mosquito vectors.

#### **Biological methods of controlling vector mosquitoes**

#### Plant extracts

The plants for development of natural products to control mosquito vectors should accompany the ethnobotanical studies which involves the traditional use of plants by human beings. The rich biodiversity coupled with the traditional knowledge and new formulation technologies is an opportunity for us to develop new biopesticides for mosquito vector control.

We have observed in our research that Annona glabra, an alien plant having medicinal properties are effective against the dengue vector mosquito larvae. It was found that more than 70% of third instar larvae of *Ae. albopictus* and *Ae. aegypti* were killed by 5-5.9 mg/L crude leaf extracts of this plant after 24 hours exposure.

#### Bacillus strains

Use of the *Bacillus thuringiensis*: a soil bacterium to control the mosquito vectors has been well recognized. Several formulations of *Bacillus thuringiensis* are available that can be applied by hand or with conventional spray equipment in a variety of mosquito breeding habitats. Local strains of *Bacillus thuringiensis* have also been isolated, tested and used to produce formulations in Sri Lanka for the control of dengue vector mosquito larvae.

#### Antagonistic fungi

Not many studies have been conducted using fungal isolate to control vector mosquito larvae. Potential of extracellular metabolites of two fungal species, *Trichoderma harzianum* and *T. viride*, as novel strategies to control dengue vector larvae have been reported recently in Sri Lanka. In this study, they have shown that these metabolites are effective against the third instar larvae of *Aedes albopictus* and *Ae. Aegypti*.

#### Microbiome and microbiota

The field of microbiome research has evolved rapidly over the past few decades and has become a topic of great scientific and public interest. The microbiome is defined as a characteristic microbial community occupying a reasonable well-defined habitat which has a distinct environment defined by physio chemical properties. The microbiome not only refers to the microorganisms involved, but also encompass their theatre of activity, which results in the formation of specific ecological niches. The microbiome, which forms a dynamic and interactive micro-ecosystem prone to change in time and scale, is integrated in macroecosystems including eukaryotic hosts, and here crucial for their functioning and health. Therefore, use of microbial communities for vector mosquito control interventions has taken as the recent emerging field of research.

#### Microbiota and microbiome in vector mosquito breeding habitats in Sri Lanka

Only a few studies on associated microbiota in mosquito breeding habitats have been carried out locally and internationally. Comprehensive studies or checklists on associated microbiota, specifically targeting a variety of mosquito breeding habitats have been developed in Sri Lanka recently. Total number of 83 naturally occurring microbiota species has been identified from variety of mosquito breeding habitats that are associated with mosquito larvae in Sri Lanka. Microbiota species identified were belonged to 11 phyla. Majority of microbiota species that were associated with mosquito larvae belonged to Phylum Rotifera. Identification of naturally occurring microbiota and their interactions on mosquito larvae, would be beneficial for developing novel larval controlling approaches in an environmental-friendly manner. Three epibiont ciliates have been identified of which two were found to cause mortalities in mosquito larvae. *Vorticella macrostoma* gave the 100% mortality in third instar larvae of *Culex* species.

#### Microbiota and microbiome of the midgut of vector mosquitoes in Sri Lanka

Larval and adult mosquito stages harbor different extracellular microbes exhibiting various functions in their digestive tract including host-parasite interactions. Midgut symbiotic bacteria can be manipulated to altering vector competency and potential for disease transmission. Therefore, identification of mosquito gut inhabiting microbiota is important in novel vector control strategies such as Incompatible Insect Technique (IIT) in dengue vector control. Recently a study was undertaken in Sri Lanka to identify the midgut bacteria of larvae and adults of dengue vector mosquitoes, by sequencing ribosomal gene fragments. Results showed that adult and larvae of *Ae. egypti* and *Ae. albopictus* harboured 25 bacterial species. *Bacillus entophyticus* and *Pantoea dispersa* were found more frequently in field-caught *Ae. albopictus* adults respectively. *Lysinibacillus sphaericus* was a common species

in adult and larvae of laboratory reared *Ae. Aegypti*. More research is needed to exploit this aspect of the research.

The transition toward a bioeconomy will rely on the technological advancement through research, public awareness and enabling policy environment. For all these aspects the biologist's role is indispensable. So Ladies and Gentlemen, let me invite all of you, the members of IOBSL to take part in this national endeavour to develop a stronger bioeconomy.

Ladies and gentlemen, let me close this address by expressing my sincere gratitude and appreciation for your attention.

Thank you.

#### **FELICITATION OF**

#### **DR. I.V.S FERNANDO**

**Citation Presented by** 

#### Professor Mangala Ganehiarachchi Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya



I consider it as an honor conferred on me, the opportunity of delivering the felicitation speech on Dr. Ivor Fernando, popularly known as Ivor Sir, teacher of many of us who were/are in the academic staff of Department of Zoology and Environmental Management, University of Kelaniya and some office bearers of Institute of Biology, Sri Lanka. He got his retirement in 2006 after a dedicated career spanning over 36 years shaping the destiny of many students who graduated from then Department of Zoology, University of Kelaniya. Of course, this felicitation is an overdue activity, however, I am glad that we are doing it today.

Ivor Victor Senathi Fernando was born on 02<sup>nd</sup> November 1940 in Colombo and lived nearly thirty years in Moratuwa which became his hometown. He had his early education from Prince of Wales College Moratuwa and then from St Thomas college, Mount Lavinia. He entered the University of Ceylon in 1963 and studied at both campuses of the University in Colombo and Peradeniya. He graduated in 1967 obtaining a BSc Special degree in Zoology with Chemistry as the subsidiary subject. In 1968, he proceeded to England to pursue postgraduate studies in Applied Entomology at The Imperial College of Science and Technology (University of London) and obtained the MSc degree as well as the Diploma of Membership of Imperial College in 1969. In 1970, he joined the Department of Zoology of Vidyalankara University (now University of Kelaniya) as a Probationary Assistant Lecturer. In 1971, he proceeded to England on a Colombo-Plan Scholarship to pursue research in Insect Ecology at the Department of Zoology of the University of Manchester. He obtained a PhD in Zoology in 1975, returned to the Department of Zoology, University of Kelaniya and served as a Senior Lecturer and Associate Professor until his retirement. Dr. Fernando rendered an invaluable service to the Department of Zoology, University of Kelaniya by playing a key role in preparing the curricula of the subject Zoology for threeyear and four-year degrees, especially for the course modules of General and Applied Entomology. He was instrumental in introducing many course modules such as agricultural entomology. When he started this course module, very little was taught on Agricultural entomology to university students following courses in entomology in the existing universities with the exception of the Agriculture Faculty in Peradeniya. With a grant from Natural Resources Energy & Science Authority (NARESA) Sri Lanka (presently NSF), Dr. Fernando collected insect pests of local vegetables, growing many of them within the University premises. Pests of rice and many fruit crops were collected from the vicinity of university and during field studies. He had them identified by the Commonwealth institute of Entomology in London. A reference collection was established in the Department of Zoology (now Dept. of Zoology and Environmental Management) so that the identities of specimens collected in the field and studied during laboratory sessions could be easily determined by reference to it.

It is my pleasure to share with you all present in this gathering that I was fortunate to be one of his close associates as a research student when I was following my BSc (Special) degree in Zoology and again as a postgraduate student in the field of agricultural entomology at the University of Kelaniya. As a mentor, he possessed excellent qualities that are essential for guiding and motivating students. He inspired his students by not only providing mentoring but also by offering the comfort of fatherly advice. I can state with confidence that Dr. Fernando carried out whatever responsibility he was entrusted with, wholeheartedly and to the best of his ability. He repeatedly mentioned that his passion was for imparting knowledge and teaching his students how to think independently in analyzing the results of their research works. He derived immense satisfaction by looking at their subsequent achievements feeling happy about whatever small contribution he was able to make towards their success. His notion was that students must surpass teachers with respect to knowledge, skills, and attitudes; otherwise, no country will develop.

Dr. I.V.S Fernando made a significant contribution to national development through his involvement in a collaborative research project on the Biological Control of Salvinia between the Sri Lankan and Australian Governments and Co-ordinated by Natural Resources Energy & Science Authority (NARESA) from 1984 – 1987. This project was managed by a Technical Committee comprised of members from several Government Departments and Universities. Technical support and the Control Agent were provided by the Commonwealth Scientific and Industrial Research Organization (CSIRO) of Australia. Dr. Ivor Fernando with his scientific collaborator, Dr. Peter Room from the CSIRO introduced the Brazilian weevil *Cyrtobagous salviniae* in 1986 into Sri Lankan inland waters as a biological control agent of the invasive aquatic weed, *Salvinia molesta*. By 1990, spectacular control of *Salvinia* was achieved in over 100 large irrigation tanks throughout the country. These included several tanks in Mahaweli systems B and C such as Maduru oya, Ginnoruwa and Belagan wewa.

His contribution to the field of Entomology outside the University of Kelaniya too is commendable. He has served as a visiting lecturer to Colombo University, Ruhuna University and Moratuwa University and served as an external examiner of the Department of Zoology, University of Jaffna. He served as a chief examiner for the subject Zoology of the GCE Advanced level Examination for many years and a member of setting panel in Zoology for GCE Advanced level Examination for several years until retirement. He has also functioned as a member of the teams that carried out EIAs for the Southern Expressway, Katunayake Expressway and the Railway Extension Project from Matara to Kataragama. He served as a resource person for the Popularization of Science Project of the SLAAS along with Prof. Nandadasa Kodagoda, Prof Sena de Silva, Prof Gamini Widanapathirane and Dr. Nanda Dharmawardene and visited many parts of Sri Lanka to record Quiz programs for school children which were subsequently broadcast over Radio Ceylon (now Sri Lanka Broadcasting corporation). He also took part in weekly Panel discussions on popular biological topics at Radio Ceylon along with Prof. Chandrasiri Palliyaguru as the compere and Prof Sarath Kotagama and Dr. Jayampathy Samarakoon as panelists. Dr. Fernando also served as the President of Section D of Sri Lanka Association for the Advancement of Science (SLAAS) in 1996. He also served on Annual Research Review Committees of the Agriculture Department, Coconut Research Institute, and the Sugarcane Research Institute.

In preparing this speech, I discussed with some of Dr. Fernando's past students who got the opportunity to streamline their education using the guidance given by him from the time he returned to the Department with his PhD. They still admire his utmost dedication for dissemination of knowledge without any complaint of deficiencies of facilities then available in the faculty/department (46 years ago). According to them, Dr. Fernando had the habit of taking students to the University library to explain how to refer relevant textbooks, journals, and encyclopedias and "he himself is an encyclopedia of entomology". He earned the due respect and regard from all the academic and non-academic staff and all students of Faculty of Science, University of Kelaniya. According to their observations, Dr. Fernando's advice was sought by many Commercial Enterprises and Exporters of products as diverse as biscuits, herbal teas, rubber gloves, ceramic tiles and even electronic components having curious problems with insects which affected their businesses adversely. His advice and solutions were always given free of charge in the national interest and highly appreciated by the businesses concerned.

Within this personality that is being felicitated today, is an ever-loving husband to his wife and a loving and caring father to his adorable two sons. Dr. Ivor Fernando and his beloved wife have made selfless sacrifice on the education of their two sons whom they are very proud of today. Presently, Dr. Fernando is a grandfather and living a contended life taking care of his wife. His faith in God ornately designed the beauty of his life and made him courageous; this must be the answer to how he was able to endure the tides of life with a smiling face, all the time. Many who were fortunate to work with him were inspired with a great commitment for teaching and by his exemplary life too.

I am taking this opportunity to thank the officers and members of the Institute of Biology for felicitating a great academic and a scholar of the highest caliber. I conclude my brief note of appreciation of my dear teacher quoting this statement. "As students, many teachers and educators will pass through our lives without remembrance. Yet, a special few will leave a lasting impression, not only as teachers or mentors but also as kind-hearted persons, who care for their students. Thank you for being one of the special educators who will live on forever in the minds and hearts of the students whose lives you have touched ". Sir, we all wholeheartedly wish you long life with good health.

# ABSTRACTS

Parallel Session - 1 (Zoological Sciences)

#### Approaches to Gender Determination of Indian Crested Porcupine (Histrix indica)

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Determining of gender in naturally existing species is essential in understanding mating behavior and population structure. The Indian Crested Porcupine (Histrix indica) belongs to the family of Old-World porcupines (*Histricidae*) of the order Rodentia. Determining the gender of this species has proven to be a challenge, pertaining to minimal distinguishing variations between adult boar and sow individuals. This study was carried out over a period of one year from January 2020 to December 2020 at Udawattekele Reserve Forest, Kandy (URF) (7°17'58" N80°38'20E) to differentiate between boar and sow individuals of the Indian crested porcupine and to monitor mating behavior and identification of family units. Measurements were taken from five captive boars and sows as clearance to capture wild inhabitants was not permitted. A variation in body length resulted in ranges between 35cm and 56cm for boars and 30cm to 45cm in sows. Measurements of the circumference of the eyes in boars were in the range of 3cm to 5cm and 2cm to 3cm in sows. Further observations on the coat of quills were recorded using a Munsell's soil color chart and over 60% of boars were recorded to be a dark black brown at 2/value and /2 chroma and 3/value and /2 chroma (10YR) while 80% sows revealed a much lighter brown or tan coloration between 4/ value and /3 chroma and 4/ value and /5 chroma (2.5 Y). The location of mammary glands posed a significant find. Located on the sides of the dorsal region closer to the lower abdomen and hind feet and protruding outwards rather than underneath the abdomen, accommodating easy suckling of young and avoiding injury. The observations made from captive animals helped identify boar to sow ratio at URF to be 3 sows: 1 boar, with subtle differences in size, coloration, quill length and quill distribution being an important factor in identifying individual porcupines in the reserve specifically during nighttime counts. Mating behavior and rituals showed a display of quills and grunts and hissing by both boar and sow. It was determined that porcupines are monogamous and family units comprised of two adults and one or two pups.

Keywords: Quills, Monogamous, Mating, Family Units, Mammary Glands

#### Using Morphometrics to Identify Beetles: Distinguishing the Genera of Subtribe Tricondylina (*Coleoptera, Cicindelinae, Collyridini*) of Sri Lanka

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Accurate identification of insects has often been a challenging problem due to the high morphological similarity of closely related taxa and in many species occurring together in large numbers. Therefore, morphological identification has been occasionally replaced by morphometric comparison which uses the linear distances between points on each body part However, in certain taxa these defining trait measurements rely on the sex of the species and different value ranges are recorded for males and females of the same species. This evolutionary phenomenon is explained by the sexual size dimorphism theory (SSD). Taxa that display high morphological similarity and does not reveal SSD are more suitable for being successfully distinguished by morphometric analysis. The present study intends to investigate the possibility of using morphometrics to distinguish between two genera of arboreal tiger beetles, Derocrania and Tricondyla of sub-tribe Tricondylina of Sri Lanka. The two genera differ in the shape of the excavation of the vertex, shape of pronotum, body size and length, and pronotal indices, which range between 1.5 and 2.5 in Tricondyla, and between 2 and 3 in Derocrania. The present study records ten morphometric parameters for genera Derocrania and Tricondyla, compares the differences between males and females of each genus for SSD and compares the differences between the two genera in order to select the most appropriate morphometric parameters to distinguish *Tricondyla* from *Derocrania*. Thirty beetles of genus Derocrania (17 males, 13 females) and twelve beetles of genus Tricondyla (9 males, 3 females) were used for the study. Body weight, body length, and measurements of the head, mandible, pronotum and elytra of each specimen were recorded using standard methodology. Measurements of the male and females of the same genus, and measurements of the beetles of the two genera were compared using statistical software. The study revealed the absence of SSD in both genera. Significant differences in the morphometrics of the body, head, mandible, pronotum and elytra were evident between the genera Tricondyla and Derocrania in which all the measurements with the exception of the pronotal index were larger in *Tricondyla* when compared with Derocrania. The pronotal index of Tricondyla (1.62-2.19) was significantly smaller than that of *Derocrania* (2.20–3.14) and were in the value ranges suggested by previous literature.

#### Assessment of Heavy Metal Accumulation in Different Bird Species Occupying Diverse Trophic Levels Using Contour Feathers

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The accumulation of three heavy metals (Zn, Pb, and Cd) in six avifaunal species [(Corvus splendens - House Crow (scavenger), Columba livia - Feral Pigeon (granivore), Pelecanus philippensis - Spot-billed Pelican (Piscivore), Psittacula krameria - Rose-ringed Parakeet (frugivore/granivore), Acridotheres tristis - Common Mynah (insectivore), and Spilornis cheela - Serpent Eagle (carnivore)], each occupying different trophic levels was investigated. The assessment was done using contour feathers collected from different locations in the Colombo District. The feathers were well laundered for removal of adsorbed particles and acid-digested for heavy metal analysis using the Microwave Plasma Atomic-Emission Spectrometry (MP-AES). The results showed significant differences in Zn, Pb, and Cd levels across the different avifaunal species (P<0.05). The highest mean value recorded was 7.40mgkg-1 for Zn in Parakeets (frugivore/granivore), whereas the highest levels of Pb and Cd, 0.23mgkg-1 and 0.12mgkg-1 respectively, were recorded in the Serpent Eagle (carnivore). The metal levels in ascending order were Zn – Pelican (piscivore) < Pigeon (granivore) < Myna (insectivore) < Crow (scavenger/omnivore) < Serpent Eagle (carnivore) < Parakeet (frugivore/granivore); Pb - Parakeet (frugivore/granivore) < Myna (insectivore) < Pigeon (granivore) < Pelican (piscivore) < Crow (scavenger) < Serpent Eagle (carnivore); Cd – Myna (insectivore) < Pelican (piscivore) = Crow (scavenger) < Serpent Eagle (carnivore). No Cd was detected in the feathers of Pigeons (granivore) and Parakeets (frugivore/granivore). The Pearson's multiple correlation tests showed a significantly positive correlation between the Pb and Cd levels (P<0.05, r=0.81), suggesting that the species having high Pb levels also accumulated high levels of Cd. Overall, this study shows that the heavy metal accumulation was greater in bird species belonging to higher trophic levels than those at lower trophic levels, although some exceptions were also noted.

#### Comparative Effect of Parasitism of Ciliated Protists Associated with Selected Vector Mosquito Larvae in Selected Rice Field Habitats in Ganewatte, Kurunegala District, Sri Lanka

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Mosquito breeding sites provide habitats for diversifying naturally occurring microbiota and favour different types of interactions including parasitism. This study was carried out to determine the natural occurrence of ciliated protist parasites associated with selected vector mosquito species inhabiting rice fields in Ganewattha area in Kurunegala district in Sri Lanka and to compare their parasitic effects on the selected vector mosquito larvae. Mosquito samples were collected from five rice field sites during the period from June to December 2020. The samples were observed for epibionts/parasitic infections under the microscope. Moribund or dead mosquito larvae in each sample collection were observed for internal parasitic infections. A comparative study was carried out to determine the lethal effect of ciliated organisms on Culex tritaeniorhynchus (n=50) and Culex gelidus larvae (n=50) in replicated trials (R=3). The minimum number of V. microstoma that cause a lethal effect on Cx. *tritaeniorhynchus* third instar larvae (n=100) was determined by a bioassay with ten replicates and two controls per each replicate. A total of 1650 third instar mosquito larvae of *Culex* tritaeniorhynchus (62.8%) and Culex gelidus (37.2%) were collected in this study. Ciliated protists namely Vorticella microstoma, Zoothamnium spp. and Chilodonella spp. associated with both species of mosquito larvae were identified. Results revealed that *V. microstoma* has the potential of infection to cause 71.33 ( $\pm 5.23$ ) mean percentage mortality of Cx. tritaeniorhynchus larvae. A minimum of 1000 V. microstoma is required to kill a third instar larva of *Cx. tritaeniorhynchus* at 69.60 (±2.40) hours of exposure. *Culex gelidus* larvae showed only 41.33 (±3.43) mean percentage mortality. This study concludes that *V. microstoma* is the most successful ciliated parasite as a killing agent of *Culex tritaeniorhynchus* vector mosquito larvae. Their abundance and effectiveness may contribute to develop them as an effective biocontrolling agent in the future.

Keywords: Culex, Ciliated parasites, Vorticella, Zoothamnium, Chilodonella

## Potential of aquatic carnivorous plants; *Utricularia vulgaris* and *Utricularia reticulata* as biological control agents for the larval stages of dengue vector, *Aedes aegypti*

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Species in the genus Utricularia are aquatic carnivorous plants that are known as bladderworts. They have the potential to trap a wide range of prey, causing the prey die due to anoxia. This study was conducted to assess the effectiveness of bladderworts as controlling agents of Aedes aegypti larvae. Utricularia vulgaris and U. reticulata species were collected from natural water bodies in Dankotuwa and Kandy, respectively. The experiment was set with 10 larvae of Ae. aegypti (early [i & ii] and late [iii & iv] instars, separately) in 250 mL of water with bladderwort containing approximately 100 bladders. The experiment was repeated 50 times for both plant species to achieve a total sample size of 500 mosquito larvae each from early and late instars. A control test was performed without plant species at each setup. The survival of larvae was recorded daily until death or adult emergence and only the larvae found whole or partially inside the bladders were attributed to predation. The cox-regression and Mantel-Cox log-rank test was used to assess the larval survival probability. The highest predation by both U. vulgaris (97.8%; n= 489) and *U. reticulata* (82.8%; n= 414) was observed in early instars. The mortality due to predation by *U. vulgaris* was significantly higher (HR=60.71, CI; 5.69–999.25, P<0.05) than *U. reticulata*. The mortality rates of late instars were low in both *U. vulgaris* (82.6%) and *U. reticulata* (74.8%). The highest predacious efficacy was detected from *U. vulgaris* (HR=45.02; CI: 5.96–850.51, P<0.05) even in late instar stages. Overall, >70% of cumulative predation was evident within 72 hours of exposure for early and late instars in both species. Hence, the adults emerge from the breeding site could be minimal. Therefore, U. vulgaris and U. reticulata can be used as potential bio-control agents for *Ae. aegypti* larvae.

Keywords: Aedes aegypti, carnivorous, Utricularia, predator, larvae, control

#### The Association of Fish Abundance and Composition with Water Quality Parameters in the Lower Kelani River Basin, Sri Lanka

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The present study investigates the relationship between selected water quality parameters and fish abundance and composition in the lower Kelani River basin. Fish specimens from thirteen sites of the lower Kelani river basin were sampled once in three months. Sampling was conducted from May 2019 to May 2020 using a cast net for fish capture. The water quality parameters, temperature, pH, dissolved oxygen, turbidity, electrical conductivity, total suspended solids, biological oxygen demand, chemical oxygen demand, alkalinity, total hardness and nutrients were measured in all sampling sites, once in three months for a year, using standard methods and equipment. The number of fish caught for each species per the unit operation of the cast net was used for the calculation of frequencies and relative abundance of fish. Multiple regression analysis was performed to study the relationship between fish abundance and water quality factors. A total of 34 freshwater fish species belonging to 12 families were identified from the selected locations during the study. A majority of (19) species belonged to family Cyprinidae. Rasbora daniconius, Dawkinsia srilankensis, Pethia reval and Garra ceylonensis, were found to be the most abundant Cyprinid fish species in the lower catchment. Panagoda (L9) had the highest fish abundance of 87.01% of Cyprinidae and 12.99% of other fish families. Further, 17.04% from the total fish count was recorded in the L9 location. Mattakkuliya (L1) had the lowest percentage fish count of 1.99%. Considering the water quality data L9 had favorable conditions to fish communities as none of the parameters exceeded the tolerance limits and L1 had deviations with tolerance limits for most of the parameters selected. Most locations of the lower catchment of the Kelani river had polluted water. The loading plot for abundance data showed that *Dawkinsia srilankensis* and Garra ceylonensis are positively correlated with all the water quality variables except water temperature, turbidity, dissolved oxygen and pH, indicating their ability to withstand a wide range of water quality changes. Further, Pethiya reval and Rasbora daniconius were negatively correlated with many water quality variables indicating their sensitivity to polluted water. The findings of the present study highlight the need of unpolluted water to accommodate a rich fish species diversity and abundance while emphasizing the fact that many species face threats due to pollution in the lower reaches.

Acknowledgement: The National Aquatic Resources Research & Development Agency, Ministry of Fisheries and Aquatic Resources Development.

#### Foraging Microhabitat Preferences of Aquatic Birds in an Agricultural Wetland, Meegoda, Sri Lanka

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As natural wetlands continue to decline, the use of manmade agricultural wetlands such as rice paddies by aquatic birds for foraging and nesting has increased. This study investigates the microhabitat preferences among aquatic birds using a large paddy field in Meegoda, Western Province, Sri Lanka. Birds were enumerated along a line transect (1km) covering the entire length of the paddy field, weekly from 0700-1000 h from November 2020 to January 2021 (n=10 days). A total of 287 sightings were made and this included the Cattle Egret (Bubulcus *ibis*) (n=138), Intermediate Egret (*Ardea intermedia*) (n=19), Little Egret (*Egretta garzetta*) (n=3), Indian Pond Heron (Ardeola grayii) (n=59), White-breasted Waterhen (Amaurornis phoenicurus) (n=36), Black-headed Ibis (Threskiornis melanocephalus) (n=18) and Asian Openbill (Anastomus oscitans) (n=14). Six microhabitats were used by these birds which included areas where paddy grew, ditches (small streams, irrigation channels), levees, patches of grass, marsh (boggy areas with/ without herbaceous plants) and ponds (stagnant water). The differences in foraging habitat preferences were evaluated using the Correspondence Analysis (CA). Accordingly, B. ibis mainly foraged in areas with paddy, A. intermedia preferred areas with paddy and marshy areas, E. garzetta marshy areas and ditches, A. grayii areas with paddy and levees, A. phoenicurus marshy areas, T. melanocephalus the ponds and levees, and A. oscitans ditches and levees. Areas with grass (3.48%) and ponds (1.40%) were the least utilized microhabitats by the species. Considering that the food habitats of the seven species are broadly similar, partitioning of the microhabitats within rice paddies might serve as a means of co-existence among these seven common wetland birds.

PROCEEDINGS OF THE 41st ANNUAL SESSIONS OF INSTITUTE OF BIOLOGY, SRI LANKA HELD ON 24TH SEPTEMBER 2021

# Length-Weight Relationship and Maturity Analyses of Male Silky Sharks (*Carcharhinus falciformis*) Landed in Negombo Fishery Harbour

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Carcharhinus falciformis is a viviparous shark that belongs to family Carcharhinidae. It is necessary to study biological information of silky sharks, in order to manage their fisheries and to conserve them. The length-weight relationship is widely used in fisheries management to understand health status and habitat condition of fish. The present study was conducted to investigate length-weight relationship of silky sharks and maturity status of male silky sharks landed in the Negombo fishery harbour. Total length, weight, sex and clasper length of 98 silky sharks captured by single and multi-day boats were obtained from August to September 2020. Out of that, 43 were males and 55 were females. The total length was measured to the nearest 0.1 cm and body weight was measured to the nearest 0.1 kg. The total length (TL) of the captured silky sharks ranged between 39.0 – 285.0 cm and weight ranged between 3.5 -75.0 kg. The logarithmic transformation of length-weight relationship of male, female and the pooled sample of silky sharks were  $\log W = 1.0644 \log TL - 1.0192$ ,  $\log W = 1.2769 \log TL - 1.4612$ and logW = 1.1781 logTL -1.2538, respectively. According to the results of one sample t-test, the 'b' values of males, females and the pooled sample were significantly different from 3 (P <0.05) and indicated a negative allometric growth. Three maturity stages of male silky sharks were identified according to the degree of clasper calcification as immature, maturing and mature. Highest number of male silky sharks were immature (51%) followed by maturing (33%) and matured (16%). According to the results, a higher proportion of immature male silky sharks are captured as by-catch in Negombo Fishery harbour. Therefore, it is necessary to implement management measures to reduce their proportion in fish catch with an aim to develop a sustainable fishery of silky sharks.

Keywords: Silky shark, Length-weight relationship, Viviparous, Allometric growth, Maturity

# Effect of Delayed Mating on Reproductive Performance and Life History Parameters of Dengue Vector *Aedes aegypti*

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Dengue is the fastest-growing mosquito-borne viral disease in the world. The primary vector that transmits the disease is *Aedes aegypti* of the family Culicidae. Recent methods to prevent the spread of the disease involves the release of sterile and genetically modified males, and the use of various pheromones which act as mating disruptors that will prevent or delay the mating of mosquitoes. The current study was conducted to determine the effect of delayed mating on the fecundity, fertility, life history parameters and longevity of *Ae. aegypti*. During the study, mating was delayed by 2, 5 and 8 days by keeping the male and female mosquitoes in separate cages. This was achieved through the separation of male and female mosquitoes at the pupal stage. Then the separated pupae were kept in separate cages to emerge into adults. Accordingly, the fecundity of females whose mating was delayed by 8days, was the lowest (38.47±1.10) and was negatively correlated with the age at the mating of the female. A 38% reduction of fecundity was observed after 8 days of delay in mating. Moreover, the fertility of the females whose mating was delayed by 8 days, was the lowest (65±6.43) and it was negatively correlated with the age at the mating of the females. Therefore, the fertility of the female Ae. aegypti was also declined by 24% when the mating was delayed by 8 days. However, the percentage larval mortality, total larval duration, pupal duration and longevity did not show any significant variation. Therefore, these results indicate that it is important to have a clear idea about the effect of delayed mating on the reproductive performance, life history parameters and the longevity of the vector mosquitoes as they correspondingly affect the success of vector control programs.

Keywords: Aedes aegypti, Delayed mating, Fecundity, Fertility

#### Can Pupal Dimensions of *Spodoptera frugiperda (Lepidoptera: Noctuidae*) be Considered as Predictors of Sexual Dimorphism Prior to Eclosion?

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Sexual dimorphism is a key feature mainly attributed to sexual selection and mating success. It is considered with importance when initiating management strategies for insect pests as the knowledge of gender-specific traits at the pupal and adult stages of pests have provided reliable information to manage pests and forecast outbreaks. Being a deadliest polyphagous pest of maize and other important crops, the morphology and morphometry of Fall Armyworm, Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) have shed light on successful identification of sex for its effective controlling. Therefore, the present study assessed variations in size and development duration of the pupal stage of *S. frugiperda*, to determine sexual dimorphic characters that could reliably distinguish sex before eclosion. Over six successive generations of laboratory reared 231 live pupae (141 females and 90 males) were observed for pupal stage duration (days), maximum pupal width (mm) and maximum pupal length (mm) and were analyzed. According to available literature, the only morphological feature to distinguish pupal males and females is that male pupae show a short distance between the genitalia and anal slit which is comparatively longer in the female. Since this only involves one parameter, other pupal dimensions were considered and thus, this study reveals that the pupal stage duration of males is significantly different to that of the females, and have a longer mean pupal duration (7.5 days) compared to the females (6.7 days). Early eclosion in females may increase early fecundity due to prematuration feeding. Delayed eclosion, may temporarily avoid mortality risks for adult males. Further, the length and width of male pupae were significantly larger than the length and width of the female pupae. This can be attributed to higher migratory propensity and flight capability in males. The study revealed that pupal dimensions and pupal stage duration can be used as predictors of sexual dimorphism in Spodoptera frugiperda.

Keywords: Spodoptera frugiperda, pupal dimensions, sexual dimorphism, morphometrics

#### Molecular Characterization of Midgut Bacteria in Larval and Adult Stages of *Aedes albopictus* in Gampaha District, Sri Lanka

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Bacterial species that are acquired from the aquatic larval and adult stages are established in the midgut of mosquitoes, exhibiting different functional tasks retaining in the gut as symbiotic species. The present study aimed on screening of midgut bacteria of larval and adult Ae. albopictus, as a fundamental pre-requirement to support the Sterile Insect Technique (SIT) and Incompatible Insect Technique (IIT) approaches which are in progress, in Sri Lanka. In novel techniques such as SIT, IIT or the use of genetically modified mosquitoes need artificial rearing of the life cycle stages of disease vectors followed by open releases into the environment and thereby reduce vector densities through population replacement or suppression. Sampling sites included Brandiyamulla, Gampaha, and Miriswaththa in Gampaha Medical Office of Health (MOH) area of Sri Lanka. Unfed adults and 3rd instar larvae, 250 in number were sacrificed using a cold shock and 70% Ethanol respectively. 70% ethanol followed by phosphate buffer saline (PBS) were used for surface sterilization. A homogenized lysate was prepared in sterile PBS (250µL), by pooling dissected midguts of ten individuals of larvae/adult mosquitoes. A dilution series (100-10-7) was made from lysate and 100 µL from each dilution was plated on Plate Count Agar and pure cultures for each microbe were obtained. Isolated bacteria were subjected to 16S rRNA amplification. A total number of 6 bacterial strains (Microbacterium trichothecenolyticum, Kocuria kristinae, Elizabethkingia miricola, Staphylococcus sciuri, Pantoea dispersa, Neisseria flavescens) were identified from 5 bacterial families; Flavoacteriaceae (22.05%), Neisseriaceae (11.76%), Micrococcaceae (10.29%), Staphylococcaceae (14.70%), and Erwiniaceae 35.29%) from field-collected adults, while 6 strains (Agromyces sp., Microbacterium paraoxydans, Microbacterium sp., Bacillus megaterium, Bacillus nanhaiensis, Bacillus sp.) were identified from the field-captured larvae. Species composition of gut microbes isolated from larvae was dominated by family Bacilliaceae (76.76%). Pantoea dispersa and Bacillus megaterium were the most prominent bacterial species isolated from midgut of adults and larvae respectively. Microbacterium genera was found as common for both adults and larvae, although no common bacteria were found up to species level. Midgut bacteria belonged to Bacteroidetes (Elizabethkingia miricola) and Proteobacteria (Pantoea dispersa, Neisseria flavescens) were only recorded from the midgut of adults. Larvae and adults in Ae. albopictus denoted different midgut bacterial species.

Keywords: insect, lysate, microbe, mosquito, strains

#### Uncovering the Diversity and Distribution of Scolopendrid centipedes in Sri Lanka

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Centipedes are nocturnal predators belonging to the Class Chilopoda of the Phylum Arthropoda and are divided into five orders. Of these, centipedes in the Order Scolopendromorpha are the most studied group in the world so that information on their identification, phylogenetic relationships, diversity and distribution are readily available in the published literature. In the Sri Lankan context, however, centipedes are a group of heavily overlooked animals so that no such information are available. In this study, we attempted to gather baseline information on the diversity and distribution of centipedes in the Order Scolopendromorpha among the major biogeographic zones of Sri Lanka. Live Scolopendrid specimens were captured from each biogeographic region and classified based on their morphological characteristics using the currently available identification keys for the Indian region. Data on their distribution and the abundance with respect to some biogeographic and climatic factors (average rainfall and elevation) were also collected and analysed as appropriate. In this study, one previously undescribed family (Cryptopidae), two previously undescribed genera (Arthrorhabdus and Digitipes) and two new species (Arthrorhabdus jonesii, Digitipes barnabasi) hitherto undescribed from Sri Lanka were identified. The distribution analysis showed that the abundance of *Scolopendrid* centipedes is higher in the wet zone of the country, but seasonal in both the dry and arid zones. Comprehensive future studies are recommended in order to gain further information of the origin and distribution of these centipedes within the Greater Indian Plate.

#### Teratogenic Effects of Albendazole and Levamisole on Embryonic Development of *Gallus gallus domesticus*

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Anthelminthic drugs are used in human and veterinary medicine for treating parasitic infections. Levamisole (LEV) and Albendazole (ABZ) are the most commonly used anthelminthic drugs in the poultry industry. These two drugs are also used as anticancer agents. The impact of residual levels of these two drugs in chicken eggs, on the embryonic development of chick, has not been studied so far. Therefore, an in ovo study was conducted to determine whether these two drugs have any teratogenic effects on chicken embryonic development. Fertilized eggs obtained from a hatchery was divided into three groups of forty eggs each, viz, control, albendazole and levamisole. The control group did not receive any injection while in the other two groups, were injected with  $100\mu l$  of  $1.03\mu g/g$  of ABZ or 0.8  $\mu$ g/g of LEV respectively. Another group of ten eggs were injected with 0.9% saline. The abnormalities, embryonic development, body measurements and stage viability, determination were conducted on day 5, 10, 15 and 20 of incubation. Finally, on 20th day gross anatomy of some major organs were examined. This study demonstrates that both drugs affect the embryonic development of chicken compared to the controls. The eggs treated with ABZ exhibited more lethality, inhibition of early embryonic development (90% of embryo inhibition at day 20) and initial abortions. The embryo weight (p=0.006) and embryo length (p=0.008) of LEV group showed significant growth retardation in embryonic development at day 20, which has been reported previously in mouse. The ABZ has the potential to inhibit the polymerization of tubulin protein and therefore can act as an anti-proliferative drug and thereby inhibit the initial embryonic development. The potential to cause temporary agranulocytosis and neutropenia may be the reason for the growth retardation observed in LEV treated group. Both drugs have influenced defects in cell division, which may be the reason for the observed results.

#### Gastrointestinal Parasites Diversity of Wild Products and Garbage Consuming Elephants in Southern Sri Lanka

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Disposal of municipal waste in open garbage dumps may result in alteration of foraging habits and diet constituents of diet of wild animals. Many elephants daily forage in open garbage dumps in Sri Lanka at many locations. Though it is commonly perceived that garbage consumption poses a risk of infections and adverse health conditions for wild elephants, there is no evidence of deaths or morbidity of elephants associated with garbage consumption. Changes in foraging habits and diet can change the parasitic load and diversity of animals. Persistent heavy parasitic infections can reduce the fitness of animals. We compared gastrointestinal parasites of elephants that consume garbage at Uddakandara dump with elephants that do not consume garbage, in the adjacent Yala National Park. A total of 30 fresh dung samples were examined from each location. Coprological analysis was conducted using direct smears, formalin-ether sedimentation and a modified Baermann funnel technique. We found 25 species of parasites in the dung of garbage consuming elephants and 15 parasite species in dung of elephants that did not consume garbage. The species that exclusively occurred in garbage consuming elephants were Taenia spp, Opisthorchis spp., Enterobium spp., Hymenolepis spp., Clonorchis spp., Trichuris spp., Hetarakis gallinarum, Cystoisospora spp. and two types of eggs that could not be assigned to species. Fasciola spp., Fasciola gigantica, Paramphistomum spp., Strongyle spp., Ascaris spp., Strongyloides spp., Coccidian cyst, Moniezia spp., *Dicrocoelium* spp., *Tricostrongylus* spp., *Isospora* spp. and two types of cyst of unknown species were common to both groups. Gastrointestinal parasite prevalence (P=0.043), diversity (P < 0.05) and richness (P < 0.05) were significantly higher in garbage consuming elephants compared to that of non-garbage consuming elephants. Therefore, it was evident that garbage feeding behavior significantly increase the gastrointestinal parasitic infections in wild elephants.

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# Potential Contamination of Microplastics in Green Mussels (*Perna viridis*) Cultured in Negombo Estuary

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Microplastic pollution is a paramount environmental concern and contamination in organisms is a growing field of research. However, the information regarding the microplastic pollution in Sri Lankan marine environments is limited, especially on the culture systems. Present study investigated the prevalence of microplastics in water and potential contamination in edible green mussel, Perna viridis in the Negombo estuary in December 2020. Water and cultured mussel (134.49±7.01 mm) samples were collected from a mussel farm in the estuary. Microplastics were separated from water samples using a vacuum filtration system and microplastics were extracted from mussels following 10% KOH digestion method. Analysis for the presence of microplastics was carried out using a microscope (Nikon SMZ1270i). The polymer types of microplastics were identified using FTIR Spectroscopy and the possible sources were predicted. Microplastics were detected in all the water samples where mean microplastics concentration (±SD) was 4.0±2.9 items L-1. Microplastics recorded from the water samples were Nylon (Polyamide) fibres. Fibre colours were blue (60%), red (20%) and black (20%). Microplastics were detected in all the mussels (n= 30) with a mean concentration of 18.5±7.55 items mussel-1. Microplastics types in mussels were dominated by fibres  $(17.4\pm7.54 \text{ mussel}-1)$ , followed by, films  $(0.87\pm1.0 \text{ mussel}-1)$  and fragments  $(0.2\pm0.5)$ mussel-1). Microplastic fibres from mussels belonging to five colour categories were recorded (transparent-28%, blue-27%, black-27%, red-17% and green-1%). All the films were transparent and fragments of blue and red were recorded. Major polymer types identified in the mussels were High-density Polyethylene (HDPE), Low-density Polyethylene (LDPE), Polyamide (PA) and Polyethylene terephthalate (PET). The study concludes that the mussels, P. viridis cultured in Negombo estuary were contaminated with microplastics. The findings of this preliminary study suggest widening the monitoring programmes of microplastic contamination in marine environments of Sri Lanka.

Acknowledgement: Undergraduate research grant from the University of Colombo
## A Study on Aggressive Behavior and Locomotion of Zebrafish (*Danio rerio*) in Association with Polypropylene Microplastic Exposure

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Plastic pollution has become one of the most serious environmental issues. Most of the discarded end-of-life plastics are broken down gradually into microplastics of less than 5mm in length. Polypropylene microplastics are abundant in the environment however, its effect on freshwater organisms is poorly understood. The objective of this study was to investigate on aggressive behavior and locomotion of wild-type zebrafish (*Danio rerio*) in association with polypropylene microplastics exposure. During the study, fifteen adult zebrafish (n=15) of 160 days post fertilization were maintained in each of the control and treatment tanks. In the treatment tanks, zebrafish were exposed to environmentally relevant concentrations (1mg/L, 10mg/L) of polypropylene microplastics in the size range of 250 – 590 µm in triplicate for twenty days. Aggression was measured with a mirror stimulation test while locomotion activity was assessed using the line-crossings. Aggressive behavior and locomotion activity were significantly higher in control fish followed by those exposed to 1 and 10mg/L microplastics, difference being significant at 10mg/L concentration (P<0.05). Aggressive behavior as indicated by number of biting attempts were significantly higher in control fish when compared with 10mg/L microplastic treatment (Two-way ANOVA, F=17.45, DF=2, *P*<0.05). Further, fish locomotion was significantly higher in control tank when compared with 10mg/L microplastic treatment (Two-way ANOVA, F=20.33, DF=2, P<0.05). A significant negative correlation was observed between increased treatment duration and fish locomotion in 10mg/L microplastic treatment (r=-0.99705). It can be concluded that short-term exposure to polypropylene microplastics can have a significant impact on the locomotion and aggressive behavior of adult zebrafish. This model study calls for comprehensive physiological studies to explicate the impact of polypropylene microplastics on the behavior of adult zebrafish.

Keywords: microplastics, zebrafish, polypropylene, aggression, locomotion

## Marine Sponges of Coastal Waters of Mandaitivu Island, Northern Province, Sri Lanka

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Sponges belong to Phylum: Porifera play a vital role in the ecosystem as habitat forming organisms. Recent studies indicated that they are one of the important sources of bioactive compounds which show demand in pharmaceutical and biomedical industries. Even though more than 300 species recorded from Gulf of Mannar and Palk Bay region, the data on the diversity and distribution of marine sponges in Sri Lankan coastal waters especially in northern coastal waters has been limited. Therefore, the present study was carried out to identify the marine sponge diversity from the coastal waters of Mandaitivu Island, Sri Lanka. The sponges were collected from two sites (L1:9.60481°N, 80.00091°Eand L2:9.64379°N, 80.00254°E). The L1 is connected to the open waters with the sandy bottom and L2 is associated with the mangrove forest with the muddy bottom. Sampling area was limited to the 20 m from the coast and the sponges were collected by hand picking method during low tide period from October 2020 to February 2021. Species identification was performed by morphological characteristics, spicule preparations and histological sectioning and confirmed based on world Porifera database. In the present study, 30 species of Class Demospongiae representing 6 orders, 9 families, and 11 genera were recorded. Among the identified species, a *Callyspongia* sp was recorded in both locations, while others showed unique distribution such as 14 species namely Spongia officinalis, Hyattella intestinalis, one Hyattella sp and a species of Family: Spongidae, four species of Order: Haplosclerida, Clathria sp, Axinella agariciformis, two Axinella sp and two *Spheciospongia* sp were recorded at site L1; and 15 species namely, one species of Family: Spongidae, Dysidea fragilis, three species of Order : Haplosclerida, three Callyspongia sp, Mycale tenuispiculata, a species of Order: Poecilosclerida, Axinyssa sp, two Suberites sp, Amorphinopsis sp, *Spheciospongia inconstants* were recorded at site L2. This unique distribution could be due to the ecological features of the habitats. Molecular studies are underway to confirm the unidentified species.

Keywords: marine sponges, Mandaitivu island, Demospongiae, marine biodiversity

# A Preliminary Study of the Behavioural Ecology of Critically Endangered Horned Lizard *Ceratophora erdeleni* in Morningside Reserve of Sinharaja Rainforest During a Wet Spell

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Ceratophora erdeleni is a point endemic and critically endangered horned lizard restricted to Morningside forest reserve (60 22' to 60 26' N and 800 31' to 800 31' E) at an elevation of 1,060m. In-situ and ex-situ conservation of Erdelen's horned lizard need information on behavioural ecology as there is a dearth of data. Thus, the main objective of the study was to gather data on important aspects of behavioural ecology of the species. This species has occurred only in undisturbed natural forest habitats and preferred light gaps among forest edges. Study was conducted during a wet spell in December 2020. Focal animal sampling was conducted to study daily activity budget and behaviour of six adults. Direct observations were conducted for >200 hours while video techniques were employed to acquire more information on behaviour. Colour marking of focal individuals' forearm, using cotton thread was employed to determine the ranging behaviour. Daily activity budget depicted that only 8% time has been spent on active feeding, breeding and locomotory activities while the rest of the time is spent on roosting. Focal animals showed sluggish movements and preferred perches with an average height of 46-50 cm above ground and trees of greater than 6cm GBH. They were sub-arboreal as they foraged mainly on the ground layer. Their feeding guilds consisted mainly of Hymenopterans, especially ant species of the family Myrmicinae and Dolichoderinae. The thermal range was maintained at the range of 12°C to 20°C of body temperature. Mean home range of this species during the wet season was estimated as 12.6m<sup>2</sup> which is relatively small when compared to other genera of family Agamidae. Individual marking and focal animal sampling indicated that individuals show a site tenacity.

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PROCEEDINGS OF THE 41st ANNUAL SESSIONS OF INSTITUTE OF BIOLOGY, SRI LANKA HELD ON 24TH SEPTEMBER 2021

## Habitat Preference of Sympatric Agamid Lizards Inhabiting the Morningside Reserve of Sinharaja Rainforest During Wet Season

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Morningside reserve of the Sinharaja Forest (60 22' to 60 26' N and 800 31' to 800 31' E) is a rainforest that falls within the Rakwana mountain range. Despite being a small, relatively disturbed low-elevation cloud forest, this forest is recognized as a habitat par excellence for herpetofauna. Habitat loss and fragmentation due to anthropogenic activities is a major concern as the reserve harbours a rich assemblage of endemic and endangered amphibians and Agamid reptiles. Main objective of the study was to investigate the habitat preference of sympatric Agamid lizards inhabiting the reserve during a wet season (December 2020). Visual Encounter Survey on five belt transects (100m x 5m) and 32 quadrats (8m x 8m) were conducted in total to determine the population size of agamid lizards in four habitat types: undisturbed natural forests, undisturbed riverine forests, disturbed forests with cardamom and scrublands. Total agamid lizard density was higher in the scrubland (4.2 individuals/ha) than that of forest habitats (2.2 individuals/ha) due to the high abundance of native Green garden lizard *Calotes calotes*. The lowest density of agamid lizards (0.6 individuals/ha) was recorded for the riverine forests. Availability of microhabitats formed by light gaps among forest habitats seem to be a limiting factor. Endemic kangaroo lizard Otocryptis wiegmanni inhabited both undisturbed and disturbed forest habitats, however, did not occur among scrublands. Abundance of *O. wiegmanni* was higher in disturbed cardamom forests than natural or riverine forests. Endemic and critically endangered horned-lizard Ceratophora erdeleni occurred only in undisturbed natural forests. Erdelen's horned lizard confining to the undisturbed Natural forests indicate that human manipulations such as cardamom cultivation disturbing the forest floor could impose threat to their survival.

Acknowledgement: Undergraduate research grant from University of Colombo

## ABSTRACTS

## Parallel Session - 2 (Plant & Environmental Sciences)

## Assessment of the Effect of Mulching on Soil Quality and Plant Growth Parameters of Okra

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Mulching is application of living or non-living material over the soil surface as a soil management practice in cultivated landscapes. Present study assessed the effect of different mulches (rice straw, grass clippings, newspaper, black polythene) on physical and chemical properties of soil, weed occurrence, and the crop growth. The study was conducted as a pot experiment. Okra seed sown control (M0) pots (without mulch) and rice straw (M1), Grass clippings (M2), newspaper (M3), and black polythene (M4) mulches applied pots (10 replicate pots per control and each treatment) were arranged according to randomized complete block design. Plant growth parameters, chemical and physical parameters of soil and number of weeds in the control and treatment pots were measured over a culture cycle of Okra. Sampling was conducted at 30, 45, 60 days after sowing (DAS) and at the end of the culture cycle. Oneway ANOVA followed by Tukey's pairwise comparison in Minitab (Version 17.0) was used for statistical analysis. Okra plants in treatment M1 recorded significantly high plant height (55.0±0.01 cm, *p*=0.000) and flowers per plant (6.7±0.02, *p*=0.000). In addition, significantly high moisture content, organic matter content, electrical conductivity, extractable phosphorus content at every growth stage were recorded in the soil of M1treatment (p=0.00). M2 recorded significantly high (p=0.000) total N content at 45 DAS (4.2±0.1mg/g) and 60 DAS (3.3±0.3 mg/g). Significantly high (p=0.000) soil pH was recorded from M3 treatment at 30 (6.8±0.1) and 60 DAS (6.7±0.1). Significantly high total N content (5.2±0.2 mg/g) at the end of the culture cycle was recorded from the M3 treatment (p=0.000). Control pots recorded significantly higher number of weed plants per pot throughout the culture cycle (*p*=0.001). Results of the present study indicated that rice straw and grass clipping mulches are effective in improving the soil quality in terms of the nitrogen and phosphorous contents of soil.

Keywords: Mulch, Okra, soil quality, weeds

## Effects of Low-Cost Organic Fertilizer Derived from Invasive Alien Plants and Shade on Growth Performances and Yield of *Aloe vera (L.)* Burm.f.

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*Aloe vera* is a popular herb for cosmetic and medicinal industries at the global level. The study was conducted to evaluate the effects of organic liquid fertilizer (OLF) from invasive alien plant species (IAPS), Mimosa pigra and Panicum maximum on the growth and yield performance of A. vera under partial shade, full shade, and full sun conditions. Air-dried and crushed leaves of *M. pigra*, *P. maximum*, and *Musa* fruit peels were digested in water to prepare organic liquid fertilizer. The N: P: K ratio of OLF was 4.25:0.47:1.73. Three treatments; T1- (0.75g/mL of OLF), TC (control – distilled water), and TS (cow dung – fertilizer popularly use by farmers for A. vera) were investigated for growth performances under each shade condition. Aloe vera seedlings were planted in pots filled with solar sterilized sand, garden soil, compost (3:1:1 ratio), and fertilizer treatments were started one month after the seedling transplantation. Plant height, leaf length, leaf width, leaf thickness, the volume of leaves, number of leaves, number of offsets, fresh weight of leaves, fresh gel weight of leaves, and percentage of gel recovery were measured four months after the transplantation in 20 individuals/treatment. Statistical analysis of one-way ANOVA revealed that under both partial shade and full shade T1 showed significantly higher growth. Under full sun conditions, TS showed higher growth. Two-way ANOVA (*p*=0.05) results revealed that T1 under partial shade showed the highest significant growth performances. The percentage of leaf gel recovery, the most economically important yield parameter, showed significantly higher values in T1 (67.90%±5.57) than TS (49.23%±3.71) and TC (48.56%±2.11). The study recommended that the application of 0.75g/ml of *M. pigra* and *P. maximum* extract to *A. vera* under partial shade is the best, ecofriendly, and low-cost OLF. The study has further identified potential alternative management and control strategies of IAPS with their utilization.

**Keywords:** *Aloe vera*, Growth and yield, Invasive alien plant species, Organic liquid fertilizer, Shade condition

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## Assessment of the Presence of Heavy Metals in Recycled and Virgin Paper Waste Compared to Four Different Food Packaging Standards

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Out of numerous materials available, paper is a good alternative for food packaging. However, presence of heavy metals in paper may contribute to contaminate food. The main objective of this study was to assess heavy metals (Cu, Mn, Cr, Ni, Mn, Cd, Pb) in virgin wastes paper off cuts that can be used for recycling and in recycled papers. Four types of wastepaper offcuts; writing papers (70GSM), printing papers, (65 & 70GSM) printing paperboard ends (150GSM), and lottery papers (80 GSM) and three types of recycled papers; brown craft, yellow craft, and white were collected. 1.00 g of each wastepaper type was digested using a mixture of strong oxidizing agents; Conc. HNO<sub>3</sub>, 12.00ml, H<sub>2</sub>O<sub>2</sub>, 4.00ml and Conc. HCl, 2.00ml. Prepared samples were kept at 50 °C for 3 h then in room temperature for 24h to complete the digestion. Concentrations of heavy metals were determined in five replicates of each paper type using the Atomic Absorption Spectrophotometer. Zn showed significantly highest concentrations in all paper types; Writing Papers (6.5ppm), Printing Papers (6.74ppm), Printing paper board ends (7.01ppm), Lottery Papers (4.076ppm), Brown Craft papers (0.457ppm), Yellow Craft Papers (0.452ppm), White Papers (0.452ppm). Pb and Cd were detected only in three types of recycled papers while Ni was found only in virgin waste papers. However, Cu, Mn, Zn, Cr, Ni Cd and Pb in virgin wastepaper and recycled papers did not exceed the standard limits of EPA, 2012 legislation of US for paper-based food packaging and food packaging regulations (2010) Sri Lanka. Therefore, wastepaper offcuts and recycled papers considered can be recommended to produce food packaging without any prior treatments for heavy metals. However, according to the Council of Europe standard of food packaging, Cr, Ni, Cd and Pb concentration were exceeded the maximum permitted levels.

Keywords: Food packaging, Heavy metals, Recycled papers, and Wastepaper offcuts

## Morphological and Molecular Characterization of Medicinal Plants Commonly Known as Kalu Nika

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Two morphologically distinct species, [endemic *Litsea iteodaphne* (Family: Lauraceae) and an exotic species that morphologically resembles Vitex negundo (Family; Verbenaceae) but possess leaves with purplish underside] are commonly referred to as 'Kalu Nika'. This may lead to a controversy as the two distinct species could be used interchangeably for the therapeutic uses of 'Kalu Nika'. Interviews with indigenous medical practitioners revealed that no medical literature is available on 'Kalu Nika' but only traditional knowledge is disseminated. This study was focused on botanical identification, quantitative and qualitative comparison of the morphological features with botanical literature, and molecular characterization using DNA barcode by sequencing *rbcL* gene and analysing their phylogeny by the alignment of the *rbcL* gene sequence of other members in the same genus or family. Observations on qualitative morphological features (including leaf and stem colour, hairs) of *Litsea iteodaphne* matched with National herbarium records verifying that it is 'Kalu Nika'. It's uses also matched with information obtained from the medical practitioners. After comparing the quantitative morphological features of the exotic species (number of leaves per plant, length, widest breadth of leaves, length of the petiole, floral features) with Vitex negundo through independent sample t-test and with available literature proved that it is *Vitex negundo* var. *purpurascens*. The *rbcL* gene of this species was sequenced and the similarity test on GenBank BLASTn matched 99.82% with V. negundo var. purpurascens (Family Verbenaceae). Phylogenetic analyses revealed its relationship with other *Vitex* species. Sequence of *rbcL* gene of *Litsea* iteodaphne matched with available details of Litsea and Neolitsea species (Family Lauraceae), on BLASTn. It was also revealed that Litsea iteodaphne is closely related to Alseodaphne semecarpifolia (Family Lauraceae). The rbcL sequence of Litsea iteodaphne will be registered as DNA barcode. Further phytochemical studies will support the specific use of these plants in traditional medicine.

## Screening of Selected Pesticide Residues Present in Market Vegetables in Colombo Suburbs

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The use of pesticides has become a vital part of modern agricultural practices. This study was carried out to determine the presence and levels of five pesticides in Sri Lanka (profenofos, tebuconazole, metalaxyl, chlorothalonil and imidacloprid), in three selected leafy vegetables (Brassica oleracea var. capitata f. alba, Allium porrum, and Lactuca sativa), obtained from four popular supermarkets (LA, LB, LC and LD) and one public market (LE) in the areas of Nugegoda, Kohuwala and Kalubowila, and farmlands in Nuwara Eliya. Sample collection was carried out from July to September 2020. QuEChERS method was used for sample extraction, and they were analyzed using Gas chromatography Mass spectrometry (GC-MS). Phytochemicals detected by chromatogram which are naturally present in selected leafy vegetables were omitted in this study. No pesticide residues, metabolites or derivatives were detected in the samples analyzed. However, a total of thirty-five harmful chemical compounds categorized as corrosive, acutely toxic, health hazardous, environmentally hazardous, flammable and irritants corrosive by the Globally Harmonized System of Classification and Labeling of Chemicals (GHS), were detected. Samples obtained from farmlands in Nuwara Eliya contained a lower number of compounds compared to those from marketplaces indicating that contamination of leafy vegetables may have occurred post-harvest, during transportation and storage in warehouse. Compared to the public marketplace, leafy vegetables obtained from supermarkets contained a lower number of compounds with high average percentage composition values. Marketplaces such as LB and LD appeared to be better sources for purchasing *B. oleracea var. capitata f. alba*, LD and LC were the better sources when purchasing *L. sativa* and *A. porrum*, respectively. Therefore, this study provided a valuable insight on selecting the better source when purchasing leafy vegetables.

**Keywords:** Pesticide residues, Leafy vegetables, QuEChERS, GC-MS, Harmful chemical compounds

## Assessing the Adaptive Response of Sri Lankan True Mangrove Species to Their Environment Using Leaf Characteristics

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Leaf characteristics of true mangroves show phenotypic variation in response to their unique environment and help explain their adaptations and limited occurrence. However, there have been limited studies on this aspect in Sri Lanka. The objectives of this study were to determine the variations of leaf anatomical characteristics and stomatal characteristics of selected true mangrove species among different climatic zones in Sri Lanka in order to determine the adaptive response of those mangrove species based on above leaf characteristics. Mature leaf samples of Avicennia marina, Avicennia officinalis, Bruguiera cylindrica, Bruguiera gymnorhiza, Bruguiera sexangula, Excoecaria agallocha, Lumnitzera racemosa, Rhizophora apiculata and Rhizophora mucronata were collected from Negombo, Chilaw and Kalpitiya lagoons which are from different climatic zones (wet, intermediate and dry zone respectively) of Sri Lanka. The thickness of leaf, palisade and spongy mesophyll, water-storing tissue and epidermis were measured using three plants and three leaves per plant. The stomatal characteristics observed were; stomatal density, stomatal index, guard cell length and potential conductance index. Along with the leaf anatomy observations, leaf mass per area, specific leaf area and relative chlorophyll content were measured. Analysis of Variance, cluster analysis and Principal Component Analysis were performed. Leaf characteristics of most species had a significant difference between one or two pair of climatic zones only. The cluster analysis showed the genetic influence rather than the climatic variation on clustering. Among the selected species, *R. apiculata, L. racemosa, E. agallocha, B. cylindrica* and *R. mucronata* were more phenotypically adapted to climatic variation in the locations than others. This study would be useful in species selection for mangrove restoration programmes and to understand the long-term adaptation of mangroves in the context of future climate change scenarios.

Keywords: Mangroves, Sri Lanka, leaf characteristics, adaptive response

## Predicting the Potential Distribution of Sri Lankan Endemic *Calophyllum* Using Ecological Niche Modelling: Impacts of Climate Change

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Climate change is considered a significant challenge to biodiversity conservation in the future. Such changes strongly influence the distribution patterns of range-restricted endemic plant species within the country. Genus *Calophyllum* is one of the endemic rich plant genera in Sri Lanka, where eleven out of thirteen species are endemic to the island and nine species are threatened. The present study aims to evaluate the effect of climate change on the current and future potential distribution of suitable habitats of four endemic *Calophyllum* species in Sri Lanka; C. bracteatum, C. moonii, C. thwaitesii and C. trapezifolium using MaxEnt modelling to implement sustainable conservation or adaptation strategy. Layers of bioclimatic variables and species occurrence data were used as the inputs for MaxEnt v3.4.1 software. Bioclimatic data were extracted from the WorldClim v2.1 database at 2.5 arc minute resolution. Species occurrence data were gathered from field sampling, National Herbarium, Peradeniya and virtual world herbaria. The future potential distribution of each species was projected for 2070 under SSP2-4.5 and SSP5-8.5 climate projections using CNRM-CM6-1 global climate model. Model performance was evaluated with the area under the Receiver Operating Characteristic (ROC) curves. The present study detected contractions of suitable habitats of all four species in 2070 under both SSP2-4.5 and SSP5-8.5 climate projections, with ROC curve values larger than 0.85 each time. The highest and lowest reduction of suitable habitats was reported in *C. moonii* and *C. bracteatum* respectively. The reduction of suitable habitats was higher in species with smaller present niches. Temperature seasonality had the highest contribution for the distribution of suitable habitats of *C. bracteatum*, *C. moonii* and *C. thwaitesii*. Precipitation of the coldest quarter had the highest contribution to the distribution of suitable habitats of *C*. *trapezifolium*. The findings of the study define high priority areas to consider in conservation planning or reintroduction of threatened *Calophyllum* species.

# Evaluation of *Vigna unguiculata* ssp. *sesquipedalis* Germplasm Accessions for Reproductive Traits

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Yard long bean (Vigna unguiculata ssp. Sesquipedalis), is cultivated in Sri Lanka mainly for its immature pods which is used as a popular vegetable. The present study aimed to assess the performance of 14 new local accessions of yard long bean (TJ-101, TJ-102, TJ-103, TJ-105, TJ-107, TJ-108, TJ-109, TJ-110, TJ-112, TJ-113, TJ-114, TJ-115, TJ-117 and TJ-151) against three recommended check varieties (Gannoruwa Hawari, Gannoruwa A-9 and DOA-Polon) based on reproductive traits. The study was conducted at the Agricultural Research Station in Telijjawila, Matara, during Maha season of 2020/21. Plants were raised in beds arranged in Randomized Complete Block Design with three replicates per accession. Results revealed that plants of the accession TJ-151 took the minimum number of days for first flowering (40.33±0.57), for 50% flowering (40.33  $\pm$  0.57) and for harvesting (56.00  $\pm$  0.00), performing significantly better (P<0.05) than the two check varieties, Gannoruwa Hawari and DOA Polon. Therefore, TJ-151 can be selected as a promising genotype for early flowering and harvesting. The yield performance of TJ-151 was also high (18.49±0.14t/ha) and was significantly better (*P*<0.05) than Gannoruwa Hawari and DOA-Polon. Accession TJ-112 produced the highest yield (19.14±0.71t/ha) as well as the highest mean number of pods per plant (30.36±1.95) and outperformed all three check varieties. However, TJ-112 took the longest time to flower, and the number of days for first harvest exceeded 67 days. Therefore, TJ-151 and TJ-112 can be identified as suitable accessions to be developed as high yielding varieties, with TJ-151 having the added benefit of early flowering and harvesting ability, for Matara District in the Maha season. Furthermore, number of pods per plant was correlated significantly (P<0.05) with the overall yield (0.824). Therefore, in terms of overall yield it is better to select varieties that show profuse fruiting rather than those that show longer or heavier pods.

Keywords: Germplasm evaluation, Yard long bean, Reproductive traits

### A Geoinformatics Approach to Identify the Effect of Land-Use Changes on Heat Distribution of Urban Lentic Landscapes

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Urban wetlands play a major role in making cities livable especially by reducing the heat. Colombo the commercial capital of Sri Lanka is consist of many urban wetlands, including many lentic systems and has been accredited as a RAMSAR Wetland city. However, with the development, the land use and land cover changes had severely affected the ecological landscape of these lentic ecosystems. Therefore, this study was conducted to analyse the land surface temperature (LST) patterns from year 2000 to 2020 with five-year internal and spatialtemporal variations along with three selected lentic systems; Thalangama, Boralesgamuwa, and Kesbewa. Remotely sensed data were extracted by using Landsat imagery for atmosphere radiance spectral radiance, top of atmosphere brightness temperature, land surface emissivity I and II, and LST. The land use and land cover changes were also assessed using NDVI and the maps were produced using Erdas Imagine and Arc GIS software. Based on the developed surface temperature maps the locations were categorized into four categories. In the year 2000, the maximum surface temperature near three lakes was 26.71°C, 26.96°C, 27.24°C and it has been increased up to 32.96°C, 30.75°C, 30.56°C in the year 2020 respectively. By 2020 the surface temperature severely invaded the western and central parts of the areas and the Thalangama lake area was the most affected. The NDVI analysis revealed that the vegetation cover surrounded by the three lakes has decreased over the past 20 years by replacing builtup areas (increased  $\sim$  40%). Further, paddy fields, home gardens and open spaces were decreased by 20%, 15% and 15% respectively. Compared to the year 2000 very high category areas were increased by 45.26%, 44.24%, and 37.29% in 2020 respectively. The temperature increments may negatively affect the biodiversity and ecosystem functioning of the lentic ecosystems. Therefore, conservation measures should be undertaken urgently and further research on the effect of heat on biodiversity and functioning of lentic ecosystem is recommended.

#### Enhancing Vase-Life of Chrysanthemum morifolium cv. 'Champagne Golden' Cut Flowers

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Vase-life of cut-flowers is a crucial factor of commercial value, customer satisfaction and repeat purchase. The aim of the study was to investigate the effect of vase solutions with different concentrations of selected chemical combinations, on vase-life extension of yellow Chrysanthemum (Chrysanthemum morifolium cv. 'Champagne Golden') cut flowers while maintaining their post-harvest quality. Table sugar and citric acid were used at concentrations of 4% (w/v) and 200ppm respectively for all treatments. The effect of  $Al_2(SO_4)_3$  and 8-Hydroquinoline sulphate (8-HQS) were investigated in three different concentrations, viz. 50ppm, 100ppm and 200ppm. All tested treatments were able to extend vase-life significantly compared to the control, tap water, which showed a vase-life of 15.66±1.33 days. The treatment 50ppm 8-HQS + 4% (w/v) sugar + 200ppm citric acid resulted in the maximum vase life, 31.33±0.33 days, extending vase-life by 15.67 days. Colour retention and overall appearance of the cut-flowers were maintained throughout the vase-life to a great extent. The holding solution that contained 4% (w/v) sugar + 200ppm citric acid + 100ppm  $Al_2(SO_4)_3$  was experimented with the Chrysanthemums of two more different shades; purple and red, Chrysanthemum cut-flowers of purple shade showed a notable extension of vase-life of 3 days on average compared to the control, the flowers of same shade held in tap water, which showed a vase-life of 16.00 ± 00 days, whereas no notable effect on vase-life extension was observed for the Chrysanthemum flowers of red shade. The treatment 50ppm 8-HQS + 4% (w/v) sugar + 200ppm citric acid can be suggested as an effective floral preservative solution developed under this study, for cv. Champagne Golden, and its suitability as a potential preservative holding solution may be further explored by testing on different shades of Chrysanthemum cutflowers as well as other species of flowers.

## Natural Dye Preparations from Sawdust of Jackfruit (*Artocarpus heterophyllus*) and Mahogany (*Swietenia sp.*)

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The demand for textiles dyed with natural pigments has re-emerged and the use of natural dyes can add value to textiles. Use of waste-material to obtain the natural dye would reduce the cost while being a sustainable approach. This study aimed to develop natural dyes from saw-mill wastes of two selected timber types, Jackfruit tree (Artocarpus heterophyllus Lam.) and Mahogany (*Swietenia sp*). Tannins and flavanols are known to be responsible for typical colour of Jackfruit wood whereas the anthraquinone rubiadin is known to majorly contribute to the colour of Mahogani wood or bark. The dye extraction procedures were optimized based on the dye yield determined as dry weight of the extract and absorbance at  $\lambda_{max}$  of each extract. The effect of different mordants in applying the extracted dyes to cotton fabric were compared. Color fastness to washing of the dyed fabrics was analyzed using MATLAB online software. Additionally, antibacterial activities of the extracts against two bacterial strains were also investigated using Kirby-Bauer Disk Diffusion Assay. The highest dye yields were obtained by the alkaline extraction method at pH 13, at 75°C for 30 minutes with 1:6 mass to solvent ratio for Jackfruit saw dust while the alkaline extraction performed at pH 13, at 75°C for 60 minutes with 1:6 mass to solvent ratio has shown to be the best extraction method for Mahogany saw dust. Acceptable color fastness to washing was observed in fabrics dyed with Jackfruit and Mahogany saw dust extracts and mordanted with a commercially available mordant. The dye extracts of Jackfruit and Mahogany showed antibacterial activity against Staphylococcus aureus but not against *Escherichia coli* at 0.4mg/disc and 0.8mg/disc under the experimental conditions used in the study. The findings of this study reveal that saw dust of Jackfruit and Mahogany are potential sources of natural colorants that can be used successfully for valueaddition in the textile industry.

Keywords: natural dye, saw-dust, Jackfruit, Mahogani

## Assessing Morphological Diversity of Sri Lankan Sesame (Sesamum indicum L.)

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Sesame (Sesamum indicum L., Family Pedaliaceae) is an important oil seed crop grown in the dry and intermediate regions of Sri Lanka. Detailed analysis on morphological diversity of sesame cultivars grown in Sri Lanka is scanty. Therefore, this study aimed to find the morphological diversity of Sri Lankan sesame. Sesame varieties (Uma, Malee, MI<sub>3</sub>) collected from Grain Legumes and Oil Crops Research and Development Centre, Angunakolapelessa and three landraces collected from Anuradhapura and Kurunegala districts were grown in common grounds. A total of 52 morphological characters were recorded for randomly selected five plants per variety. A and landrace according to the sesame descriptors recommended by the International Plant Genetic Resources Institute and analyzed using RStudio (version 1.3.1093). The quantitative data were subjected to Analysis of Variance and Pearson correlation test. All the morphological data were subjected to Cluster Analysis, and the dissimilarity matrix was obtained. The ANOVA revealed a significance variation among the varieties and landraces based on 14 quantitative traits ( $P \le 0.05$ ). The varieties have shown both the lowest and the highest values while the landraces showed in between values for most of the quantitative traits. The clustering pattern of the dendrogram indicated the more relatedness of *Malee* variety to landraces than the rest of the varieties studied. Correlation analysis mainly aimed to find the correlation between yield related characters to rest of the quantitative morphological characters. A strong positive correlation could be observed between number of seeds per capsule and plant height (r=0.621,  $p \le 0.001$ ), middle leaf width (r=0.740,  $p \le 0.001$ ), middle petiole length (r=0.650,  $p \le 0.001$ ) and capsule length (r=0.866,  $p \le 0.001$ ). The morphological markers used in this diversity analysis discriminates the local sesame varieties and landraces revealing the morphological similarity and diversity among them.

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## Influence of Herbaceous Ground Cover on Soil Fertility in an Immature Rubber-Land in Kalutara District, Sri Lanka

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Ground cover protects soil from erosion, control weeds and act as a supplementary source of nutrients during the first five years (immature) growth of rubber (Hevea brasiliensis) lands. The study aims to understand how the herbaceous ground cover of immature rubber lands influence the fertility of *Boralu* series of soil in four experimental sites, each containing 25 trees (400 m<sup>2</sup>). Living stand biomass and litter of the ground cover in four 1m x 1m sub plots with a cover crop (Mucuna bracteata - FM, Pueraria phaseoloides - FP), and control plots without a cover crop fertilized (FW) and non-fertilized (NFW) which maintained for two years were compared. Weeds were found in plots without a cover crop. Four composite soil samples collected at 0 – 15 cm depth from each site were tested for soil texture, bulk density, porosity, void ratio, pH, organic carbon (OC), cation exchange capacity (CEC), total nitrogen, available phosphorous, exchangeable potassium and magnesium, and microbial activity (CO<sub>2</sub> evolution method). Measurements taken from four sub samples were subjected to ANOVA followed by Tukey HSD ( $P \le 0.05$ ) using SPSS. Rubber trees showed similar girth patterns among sites. Living biomass and litter of the ground cover were highest in FM with the least soil microbial activity. Soil pH and Mg content were same among sites. The FM and FP sites had high soil nitrogen and CEC, respectively, compared to NFW. The FP site showed low bulk density, high porosity, and high phosphorus content compared to FW and NFW. Soil void ratio of FP differed only from FW, which had many herbaceous weeds, highest OC and Carbon: Nitrogen. As key soil parameters have been influenced by the ground herbs, a diverse ground cover (including weeds) would perform better over a single ground cover species in improving the soil fertility of rubber lands.

Keywords: rubber land, ground cover, soil fertility

## Antioxidant Activities of *Sargassum* sp. and *Gracilaria* sp. in the Thalpe Coast of Sri Lanka

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Marine macroalgae are considered as a source of bioactive compounds with a broad spectrum of biological activities. Antioxidants are compounds that inhibit oxidation that can produce free radicals, preventing damage to the cells of organisms. Antioxidants of different species of marine macroalgae have been reported from several countries. Even though the Sri Lankan coast has a rich diversity of macroalgae, knowledge of their antioxidant potentials is limited and the biochemical compositions of marine macroalgae are known to be affected by factors such as geographical location and environmental conditions. Therefore, it is beneficial to study the antioxidant potential of marine macroalgae in the Sri Lankan coast. Thus, this study was conducted to determine the antioxidant activities of *Sargassum* sp. and *Gracilaria* sp. that are commonly found in the Thalpe beach, Galle, Sri Lanka. Sargassum sp. and Gracilaria sp. were collected from the Thalpe beach in July 2020, air-dried and sequentially extracted into four solvents; hexane, chloroform, ethyl acetate and methanol. The antioxidant activity of the extracts (100-500µg/ml) was determined in triplicates, using the 1,1-diphenyl-2picrylhydrazyl (DPPH) free radical scavenging assay. The mean values were statistically analyzed through two-way ANOVA and Tukey's multiple comparisons using Minitab 17 software. The antioxidant activities of Gracilaria sp. and Sargassum sp. ranged from 17.74±1.41% to 93.82±0.78% and 6.54±0.59% to 77.06±0.24%, respectively. The antioxidant activity was the highest in the chloroform extract of *Gracilaria* sp. (93.82±0.78%) followed by the ethyl acetate extract of *Sargassum* sp. (77.06±0.24%) at 500µg/ml and these values were significantly higher than the other extracts. Hence, it was evident that the two algal species Sargassum sp. and Gracilaria sp. had antioxidant potentials. Further studies need to be done to identify compounds responsible for the antioxidant activity of these algal species.

**Keywords:** Antioxidant activity, DPPH free radical scavenging assay, *Gracilaria*, Marine macroalgae, *Sargassum* 

## Antioxidants and Antioxidant Activity of H Grade Bark of Ceylon Cinnamon (*Cinnamomum zeylanicum* Blume)

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Oxidative stress plays a vital role in the pathogenesis of several chronic diseases such as cardiovascular diseases, diabetes, neurodegenerative diseases and cancer. Combat of oxidative stress through natural antioxidants is increasing considerably throughout the world during the last few decades. We have previously reported alba grade bark of Ceylon cinnamon (CC) as a rich source of natural antioxidants and possess antioxidant activity through multiple mechanisms. However, to date antioxidants and antioxidant activity of other grades of the bark of CC are not yet reported. The present study therefore, evaluates the antioxidants and antioxidant activity of H grade bark of CC. Freeze dried hot water extract of H grade bark of CC was used in the study. Antioxidants [total polyphenolic content (TPC) and total flavonoid content (TFC)] and antioxidant activity [1, 1-diphenyl-2-picryl-hydrazyl (DPPH) and 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging and ferric reducing antioxidant power (FRAP)] were evaluated using 96-well microplates-based antioxidant bioassay protocols in vitro (TPC, TFC, DPPH, ABTS and FRAP n=3 each). Results revealed that hot water extract of H grade bark of CC possesses antioxidant and antioxidant activity. The mean TPC and TFC contents were 85.87±4.29 mg gallic acid equivalents/g of extract and 2.73±0.19 mg quercetin equivalents/g of extract respectively. The DPPH, ABTS and FRAP antioxidant activities were 65.19±1.25, 89.91±8.68 and 69.29 ±4.74mg Trolox equivalents/g of extract respectively. Further, IC<sub>50</sub> values for DPPH and ABTS radical scavenging activities were 133.18±2.58 and 71.17±7.09µg/ml respectively. In conclusion, hot water extract of H grade bark of CC possesses marked antioxidants and antioxidant activity. Further, the demonstrated antioxidant activity of bark extract is mediated by radical scavenging and reducing power mechanisms. This is the first report on antioxidants and antioxidant activity of H grade bark of Ceylon cinnamon.

Keywords: Ceylon cinnamon, H grade bark, antioxidants, antioxidant activity

## ABSTRACTS

Parallel Session - 3 (Molecular Biology & Biotechnology)

## Seed Priming with Polyamines Improves Germination of Rice (*Oryza sativa* L.) Under Aluminium Stress

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Aluminum (Al<sup>3+</sup>) toxicity is one of the major factors that limit rice (*Oryza sativa* L.) cultivation in acidic soils. Al stress causes delayed seed germination and consequently hinder seedling establishment and growth. Polyamines (PAs) are amine growth regulators that play key roles in diverse plant developmental processes and defence responses against an array of environmental stresses. The study was conducted to investigate the effect of seed priming with PAs; putrescine (Put), spermidine (Spd) and spermine (Spm) on seed germination and seedling growth of rice under Al stress. Surface sterilized seeds of Bg 368 rice variety were primed with 1.0mM of Put, Spd and Spm or treated with sterile distilled water (controls) for 24 hours. Primed seeds were then grown on filter papers moistened with 0 (control), 250 and 750µM of AlCl<sub>3</sub> solutions (pH 4.2). Three independent experiments with five replicates per each treatment (10 seeds/treatment) were done and results were analyzed with one-way ANOVA with Tukey's post hoc test of significance. PA-untreated control seeds showed significantly reduced germination at 250µM Al3+(14%) and 750µM Al3+(4%) compared to germination at Al-free growth conditions (26%) after 3 days of sowing. Put, Spd and Spm-primed seeds showed enhanced germination both under Al-stressed and control conditions. Under 750µM of Al<sup>3+</sup>, Put (28%), Spd (38%) and Spm (32%)-primed seeds showed significantly higher percent germination in comparison to PA-untreated control (4%). Germinating PA-primed seeds indicated increased  $\alpha$ -amylase activity compared to the PA-untreated controls under Al stress. The results exhibited that Bg 368 rice variety is highly susceptible to Al stress during germination. PA priming (1.0mM) resulted in higher percent germination compared with PAunprimed control clearly indicating positive effects of PAs in rice seed germination under Al stress.

Keywords: Aluminium, germination, polyamines, seed priming, stress tolerance

## Oxidation Stress and Antioxidant Response in *Piper longum* L. Subjected to Cryopreservation

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Piper longum L. commonly known as long pepper, which belongs to the family Piperaceae, is a valuable herb in traditional medicine and highly demanding spice in Asian countries. Cryopreservation using ultra-low temperatures (-196°C) in liquid nitrogen (LN) is a viable and cost-effective way for the long-term conservation of plant genetic resources of P. longum as an alternative to field collections. However, during the cryopreservation process, plant materials can be damaged due to the osmotic imbalance of cells, uncontrolled production of reactive oxygen species, excessive dehydration, chemical toxicity and the irreversible ice crystal formation at low temperatures, etc. The successful cryopreservation is based on the optimum conditions and the protective effect of preculturing and cryoprotective treatments. In this study, the use of plant vitrification solution 2 (PVS2) vitrification technique for cryopreserving nodal segments of *P. longum* was investigated. The oxidative stress and the antioxidant responses at different stages of the protocol were also evaluated using lipid peroxidation and catalase assays. Two sucrose concentrations (0.3 and 0.5M) and two application durations (20 and 40min) of PVS2 were tested in preculturing and PVS2 treatment respectively. A significant decrease (54.5%) in survival was observed when nodal explants were treated with LN. The best survival percentage of nodal explants of *P. longum* (42%) was observed in the treatment comprises of 0.3M sucrose, precultured for 5 days, and PVS2 application for 20min at 0°C before the LN exposure. At each treatment stage, there was significantly higher malondialdehyde (MDA) production, a lipid peroxidation agent, than in fresh untreated nodes. Furthermore, catalase activity was highest at PVS2 treatment stage and LN treatment stage, suggesting that nodal explants have been exposed to excessive oxidative stress during cryopreservation thus resulting low survival percentages. The development of vitrification technique by optimizing freezing and thawing rates, osmotic conditions, selection and concentration of cryo-protective agents (CPAs), and equilibration time in CPAs might result in better survival and of cryopreserved plant materials.

Keywords: Liquid nitrogen, Plant vitrification solution 2, Survival, Vitrification

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## Discovery of Genic and Intergenic SSR Markers from Black Pepper (*Piper nigrum*) Genome

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Piper nigrum (black pepper), dubbed the 'King of Spices' and 'Black Gold' is one of the oldest and most widely consumed spice in the world. Despite its importance, there have been a limited number of studies on genetic diversity analysis on black pepper using the available molecular markers. Even though Simple Sequence Repeats (SSRs) or microsatellites have been the best choice, limited availability of SSRs hinders such attempts in *P. nigrum*. Numerous algorithms/software packages are available for mining SSRs. In this study, an in-silico approach was employed to develop SSR markers from the *P. nigrum* genome assembly using two SSR mining tools/algorithms: Microsatellite Identification Tool (MISA) and Genome-wide Microsatellite Analyzing Tool Package (GMATA). In total 5,881,309 and 5,876,156 SSRs were detected using the MISA and GMATA tools, respectively; on average, 7.71 SSRs per kbp were mined. Among the different types of SSR motifs, mononucleotides were the most abundant, followed by dinucleotides; AT and TA repeats together represented 53.5% of the entire pool of SSRs (excluding the mononucleotide loci). In general, plant genomes are rich in AT repeats, thus the results were consistent with previous findings. Moreover, classification of identified SSRs based on their genomic location (genic or inter-genic) revealed that the maximum number of SSRs are located in the inter-genic region; on average, 34,380 and 170,048 SSRs were detected in genic and inter-genic regions, respectively. The highest percentage of SSRs found within the coding regions were tri-nucleotide repeats. It is reported that these repeats predominantly appear in coding sequences to avoid frame-shift mutations. Piperine is the major alkaloid responsible for the pungent flavour of black pepper. In the present study, 54 SSR loci associated with piperine biosynthesis-related genes were identified and primers were designed using Primer3. An in-silico PCR performed using FastPCRtool predicted 169 amplicons. Only 35 SSR loci were single-locus markers. These newly identified piperine biosynthesis-related markers may be a valuable resource for marker-assisted breeding of black pepper in the future.

Keywords: Piper nigrum, SSRs, piperine biosynthesis-related genes

## *In Silico* Analysis of Genes Responsive to Drought Stress in *Arabidopsis thaliana*: Potential Targets for Genetic Crop Improvement

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Drought is a crucial factor that significantly affects plant physiology. Understanding the molecular components and their interactions associated with drought responses has become an important area in developing drought-stress tolerant crop plants. Hence, the present study aimed to identify and characterize drought-responsive genes in Arabidopsis thaliana, employing a range of computational tools/software. Raw transcriptomic data derived from drought-induced tissues (flower (E-GEOD-55431), leaf (E-GEOD-16474), root and shoot (E-GEOD-5624)) were retrieved from the ArrayExpress database and analyzed using Bioconductor (affy package) R version 4.0. Genes with  $-1 \ge \lfloor Log2 \text{ fold change} \rfloor \ge 1$  (P-value  $\le$ 0.05) were considered as differentially expressed genes (DEGs). A total of 2874 DEGs were identified; 1290, 211, 567 and 330genes were differentially expressed specifically in flower, leaf, root and shoot tissues, respectively. Of these, 188 genes were transcription factors (TFs), and most of them belonged to bHLH (26), AP2/EREBP (25), MYB (22) and WRKY (10) TF families. Further, it was noted that two genes, Dicarboxylate-carrier2 (AT4G24570) and SAURlike-auxin-responsive-protein-family gene (AT1G72430) were differentially expressed in all tissue types in response to drought. As expected, Gene Ontology (GO) enrichment analysis of tissue-specific DEGs showed that they are enriched for GO terms related to response to abiotic stimulus, response to stimulus and response to stress. Moreover, Kyoto Encyclopedia of Genes and Genomes based pathway analysis of DEGs showed over-representation of genes related to metabolic pathways, biosynthesis of secondary metabolites, and plant hormone signal transduction. In addition, in silico promoter analysis of DEGs using Multiple EM for Motif Elicitation (MEME) tool discovered 30 novel motifs; these motifs exhibited biological roles such as response to water deprivation, ABA-mediated signaling pathway and stomatal movement. The drought-responsive genes reported in the present study could be potential targets for genetic crop improvement in the future to develop cultivars with improved drought tolerance across different crops.

Keywords: Arabidopsis thaliana, drought-responsive genes, in silico analysis

### Genetic Diversity Analysis of *Piper* Species in Sri Lanka Using PCR-Based SSR Markers

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Piper species in Sri Lanka include both wild and crop species. Economically important crop species are susceptible to several diseases. In contrast, wild species are known to harbour genes for resistance to biotic and abiotic stresses. Thus, evaluation of genetic relatedness among *Piper* species is useful to recognize cross-compatibility and to introduce desirable variation across species. Microsatellite or simple sequence repeats (SSRs) are the most widely used PCR-based markers for molecular diversity analysis because of their co-dominant inheritance, abundance, polymorphism and reproducibility. This study evaluated the genetic diversity among seven Piper species (P. nigrum, P. longum, P. ornatum, P. zeylanicum, P. chuvya, *P. walkeri and P. trineuron*) in Sri Lanka using eleven SSR markers; all SSR primer sequences used in the present study were derived from *P. nigrum* genome. The cross-species transferability of these SSR primers in the genus Piper was also studied. DNA extracted from immature leaves of *Piper* species using DNeasy Plant Mini Kit (Qiagen) was of high quality and used in subsequent analysis. All SSR markers (eleven) produced amplicons from all Piper species, of which eight displayed polymorphisms, with amplicons being present in some species and absent in others. Cross-species transferability of 11 SSRs from P. nigrum was highest in P. zeylanicum and P. trineuron (90.91%) and lowest in P. walkeri and P. chuvya (63.64%). The dendrogram produced with SSR data classified the seven Piper species into three main clusters. Cluster I consist of *P. chuvya* and *P. zeylanicum* whereas cluster II consists of *P. nigrum* and *P. ornatum*. Cluster III consists of *P. walkeri*, *P. longum* and *P. trineuron*. The results demonstrated that *Piper* species available in Sri Lanka can be differentiated from each other at the molecular level employing SSR markers. The polymorphic SSR markers confirmed in this study may serve as a valuable resource for future Piper genetic diversity and population genetic studies in Sri Lanka.

Keywords: Piper, Genetic diversity, SSRs, Cross-species transferability

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### Incubation Period Associated with the Weligama Coconut Leaf Wilt Disease in Sri Lanka

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Weligama Coconut Leaf Wilt disease (WCLWD) was first identified from the Southern province of Sri Lanka in 2006. It is a vector transmitted disease caused by Candidatus Phytoplasma with incurable nature. Traditionally expression of visual symptoms is used to identify diseased palms at the field level. Polymerase Chain Reaction (PCR) based pathogen detection is a wellestablished method for accurate detection of this pathogen. Since 2006, symptomatic palms have emerged on occasion in the Weligama area causing a severe threat to the coconut industry. The presence of an incubation period prior to symptom expression has been recorded for other Phytoplasma diseases of palms around the world. However, this phenomenon is not scientifically proven or systematically studied for WCLWD. Therefore, the objective of this study was to identify the incubation period prior to symptom expression in WCLWD infected palms. Five asymptomatic palms in the more or less same age and PCR positive for the WCLWD, were selected from a commercial coconut field with the disease history in Weligama area of Matara district. The nested PCR tests were positive only for bud leaf tissues and negative for inflorescence and root tissues of these palms by the time of sampling. Palms were observed in three-month intervals for disease symptoms and after one year resampled and subjected to a nested PCR test. Even though all the palms were asymptomatic after one year, bud leaf, inflorescence and root tissues were PCR positive for the Phytoplasma. Therefore, invasion of the pathogen from bud leaf to young inflorescence and roots were also detected during this time period. Estimation of the exact incubation period is not feasible in the field conditions since the date of first infection is unknown. However, with the available data, it is clear that the pathogen incubation period can be one year or longer. This finding is very important in planning and implementing future disease management programmes.

Keywords: Incubation period, Nested PCR, Phytoplasma, WCLWD

## Effect of Different Concentrations of 2,4-D and BAP on Callogenesis of *Munronia pinnata* (Wall.) Theob. (Binkohomba)

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Munronia pinnata locally known as "Binkohomba", a member of the Meliaceae family, is an economically valuable medicinal plant species. The production of high-quality planting materials for cultivation purposes is hindered due to some inherited characters of the species such as irregular flowering, low flower production & fruit yield, low seed germination rate & reduced viability of the seeds. Overexploitation from the wild has made *M. pinnata* an endangered species in Sri Lanka. Establishing a rapid and efficient callus culture system for this species is desirable not only for micropropagation but also for the quantitative analysis of bioactive compounds which later on can be optimized to cell suspension cultures. In the present study, the callogenesis potential of *M. pinnata* leaf explants (collected from 7-11months old mature, 3-leaflet morphotype plants) was tested in Murashige and Skoog's (MS) media supplemented with four 2,4-Dichlorophenoxyacetic acid (2,4-D) concentrations (1.0, 1.2, 1.4, 1.6mg/L) either alone or in combination with 0.3 or 0.6mg/L of 6-Benzylaminopurine (BAP). Further, callus multiplication was examined by subculturing the calli into best performing callogenesis media after three weeks. Significantly, higher callus induction was obtained in the MS media supplemented with 1.0mg/L 2,4-D along with either 0.3mg/L or 0.6mg/L BAP (64% and 63% respectively). The calli obtained from 1.0mg/L 2,4-D+ 0.3mg/L BAP treatment, showed the higher callus multiplication when subcultured into 0.6mg/L BAP+ 1.0mg/L 2,4-D treatment and recorded 79.50mg of mean fresh callus weight. The major percentage of calli were compact in nature even though friable calli were also visible. The protocol established here could be a useful tool for genetic transformation and metabolomic comparisons along with their regulation, which can demonstrate it towards be an asset to the pharmaceutical industry.

**Keywords:** 2,4-Dichlorophenoxyacetic acid, 6-Benzylaminopurine, Callogenesis, Callus multiplication, Friable calli, *Munronia pinnata* 

## Preliminary Assessment of Genetic Diversity in *Piper nigrum* Accessions Grown in Kolonna, Sri Lanka Using a Morphometric Analysis

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*Piper nigrum* L. (black pepper) belongs to family Piperaceae and is referred to as the "King of Spices" and "Black Gold". The center of origin of *P. nigrum* is Western Ghats in Peninsular India. Sri Lanka is considered as one of the earliest locations of the spice. Local *P. nigrum* varieties currently recommended for cultivation have been derived from a few farmer-selected landraces and therefore have a narrow genetic base. The Department of Export Agriculture of Sri Lanka has established 15 local P. nigrum cultivars collected from Ratnapura District in a field in Kolonna, Ratnapura for evaluation. Morphological characterization of this germplasm was carried out in August and September in 2020 using standard descriptors developed for black pepper. Data for 21 morphological characters (seven quantitative and fourteen qualitative) were recorded from 5 replicates from each accession. In addition, morphological data were obtained for eight *P. nigrum* accessions of Tanzania, Indonesia and India from published literature for comparative diversity analysis. There was no variation in qualitative characters among P. nigrum accessions in Kolonna except for floral bract type. All accessions showed absence of stem pubescence, horizontal lateral branch habit, even leaf margin, campylodromous venation, glabrous coriaceous leaf texture, ovate elliptic leaves, acute leaf base, filiform spikes, prostate spike orientation, round fruits, spicy fruits, round seeds and smooth seed texture. Two types of floral bracts were observed. ANOVA on quantitative characters showed that differences in the means between accessions were not statistically significant (p=0.05) for any of the seven traits (vine column height, vine column diameter, lateral branch length, number of nodes per lateral branch, leaf petiole length, leaf length and leaf width). Multivariate analysis using hierarchical clustering indicated that Kolonna accessions were more similar to some landraces from Tanzania than to accessions from Indonesia and Panniyur 5 from India. Further studies based on yield and molecular data are recommended to fully understand the level of genetic diversity in Kolonna black pepper germplasm.

Keywords: P. nigrum, Morphological characterization, Genetic diversity analysis

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#### In Silico Identification of Plant Derived Antimicrobial Peptides (AMPs)

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Amongst the numerous mechanisms and chemicals available in plants to defend against pathogens, antimicrobial peptides (AMPs) produced by plants, has become a topic of interest amongst the plant scientists today due to their immense diversity, versatility and effectiveness against plant pathogens. AMPs are frequently expressed as inactive precursors and later subjected to post-translational and posttranscriptional modifications in order to produce a mature peptide that acts as the AMP. The use of AMPs is becoming a novel strategy in the fight against plant pests and diseases, which can provide an environmentally safe and sustainable solution. Thus, the main focus of this study was to conduct an in silico analysis to identify and characterize AMPs of *Oryza sativa* L. ssp. indica (Rice). Initially the proteome of O. sativa L. ssp. indica (UP000007015) was downloaded from the UniProt knowledgebase. Already available plant AMP sequences in databases such as Uniprot knowledgebase (UniProtKB) and plant peptide database (PlantPepDB) were used to screen for candidate precursor AMPs in the proteome of O. sativa L. ssp. indica via sequence similarity searches. The resulted candidate AMPs were screened via SignalP online tool (to screen for secretory AMPs) followed by the AMP prediction tool AxPep, which resulted in 47 secreted candidate precursor AMPs. Analysis of the 47 precursor AMPs using the online web tool, AMPA (to derive candidate mature AMPs) resulted in 74 candidates mature AMPs (CMAMPs) and was further refined to yield 17 CMAMPs after taking the misclassification probabilities into consideration. The resulting 17 candidate mature AMPs were thereafter structurally and functionally characterized using appropriate web tools. InterProScan tool was used to identify functionally important protein domains, families and conserved sites while ClassAMP webserver was used to identify the functional classes to which the AMPs belong to. MEME suit and IAMPE were used to identify conserved motifsand classify AMPs based on physico-chemical properties respectively. The size of the 17 candidate mature AMPs discovered in the study ranged from 12 to 40 amino acids and belonged to the category of 'cationic antimicrobial peptides (CAPs). Moreover, the analysis revealed that a number of candidate mature AMPs contain domains such as Legume lectin, GNK2 and CAP, which are directly involved in antimicrobial activities. A database search revealed that a number of candidate AMPs (A2X7Q8, A2XME1, A2YK94, A2YHU5, A2YQS8, A2Z4V8, B8AXH6, B8AV16, A2XQ19) identified in this study have not been characterized as AMPs before, thus providing evidence for potentially novel AMPs in O. sativa L. ssp. indica.

Keywords: Antimicrobial peptides, Oryza sativa L. ssp. indica, in-silico

## In Silico Analysis of the Effectome of Ustilaginoidea virens

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Plants consist of passive and induced defense systems to protect themselves against pathogens. Induced defenses in plants are triggered upon the recognition of general elicitors and pathogen-derived specific elicitors, known as effector proteins (encoded by pathogen Avr genes), by host pattern-recognition receptors (PRRs) and R protein receptors (encoded by host R genes) respectively. Accordingly, the key objective of this study was to conduct an in-silico analysis to identify and characterize effector proteins secreted by the pathogenic fungus, Ustilaginoidea virens, and thereafter elucidate their interactions with host R proteins to provide an insight into effector-R protein interactions in host plants. For this purpose, the proteome of U. virens (UniProt proteome ID: UP000027002) was downloaded from UniProt Knowledgebase and taken sequentially through an optimized pipeline comprised of bioinformatics tools (SignalP, Phobius, Spoctopus, TMHMM, PredGPI, ProtComp, TargetP, WolfPSORT, ERPred, Myristoylator and EffectorP) to predict the secretome (collection of all secreted proteins) first and thereafter the effectome (collection of all effector proteins). The pipeline identified 244 candidate secretory proteins and 80 candidate secretory effector proteins (CSEPs) in the proteome of U. virens (8421 protein sequences). The functional domains, motifs and homologous genes of the CSEPs were then predicted using Expasy-Prosite, InterPro and tBLASTn tools respectively. Functional characterization managed to identify 10 CSEPs related to pathogenesis, which have not been reported so far. The conserved motif search in CSEPs showed a low conservation among the effector proteins except for a unique single motif (MKLLLLLAAA) confined to the signal peptide sequences of CSEPs. Moreover, possible interactions between known plant disease resistance (R) proteins of Oryza sativa subsp. japonica and CSEPs of *U. virens* (identified in this study) were elucidated, based on the gene-for-gene model using I-TASSER, PatchDock and FireDock web servers. The analysis revealed that CSEPs such as SCRE2, A0A063BP22 and A0A063BZU2 have significant probabilities to interact with R protein, RGA4R\_ORYSJ, while A0A063BQL7 interacts with RGA5R ORYSJ. The results generated in the present study could be used in future projects focused on developing natural resistance in crop plants.

Keywords: Ustilaginoidea virens, Avr genes, R protein receptors, effectome

## Analysis of Population Structure of *Ex-Situ* Conserved Sorghum (*Sorghum bicolor* (L.) Moench) Germplasm Accessions in Sri Lanka Using SSR Markers

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Sorghum (Sorghum bicolor (L.) Moench) is an important cereal crop in many tropical and subtropical areas of the world. The present study was carried out to elucidate the population structure of sixty ex-situ conserved sorghum germplasm accessions using sixteen SSR markers. The total genomic DNA was isolated from the ground leaf material using CTAB miniprep DNA extraction protocol. PCR was performed in two steps using a modified M13 tagged forward primer, a pig-tailed reverse primer and M13 oligo labeled primer with one of four fluorescent dyes; 6-HEX, FAM, TAMN or PET. Multiplexed PCR products were separated using capillary electrophoresis. Data analysis was performed using GeneMapper4.0, Structure 2.2 and STRUCTURE HARVESTER software. The admixtures of three subpopulations were observed in two germplasm accessions. The optimum number of subpopulation K which best explained the population structure was 3. The subpopulation 1 was comprised of twenty-eight germplasm accessions while fifteen germplasm accessions were grouped in each of subpopulation 2 and 3. The genetic similarity irrespective of the geographical origin was observed in this study. Fifteen local germplasm accessions in subpopulation 2 indicated their genetic uniqueness. Some local germplasm accessions with different vernacular names which are morphologically different were found in the subpopulation 2. The subpopulation 3 was observed with morphologically different exotic germplasm accessions belonging to different countries. The less genetically distinct nature of some germplasm accessions which are morphologically different could be due to various reasons. Out of them, the narrow genetic basis of the observed morphological differences, the farmer's directional selection for different morphological traits for different purposes and the non-detectable nature of observed morphological differences with neutral genetic markers are the main reasons. The results provide a technical guidance for efficient management of sorghum in breeding programmes and revealed the importance of collection of sorghum from different regions.

Keywords: sorghum, ex-situ, Sri Lanka, population structure, subpopulation, genetic similarity

## Identification of Differentially Expressed Long Non-Coding RNA in Soybean When Infected with *Phytophthora sojae*

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Long non-coding RNA (LncRNA) are defined as transcripts that are at least 200 nucleotides long (>200 nt) without a specified upper limit. Emerging evidence indicates that LncRNAs play a key role as regulatory molecules in plant disease response. However, the function of LncRNAs in soybean (*Glycine max*) disease resistance especially when infected with *Phytophthora sojae* is unfound in the literature. Therefore, we have used previously published RNA expression data to identify differentially expressed LncRNA transcripts during soybean-P. sojae interaction. This study has used ten Near Isogenic Lines carrying a unique resistance to *P. sojae* (Rps) gene/allele and each of these lines were either inoculated with the pathogen or mock treatment (control). Short-reads were downloaded from the National Center for Biotechnology Information's Sequence Read Archive. These short-reads were aligned to the previously identified soybean LncRNA transcripts and the aligned reads were counted to estimate the expression levels of LncRNA. Differential expression analysis was performed to find if the expression of LncRNA is significantly different between the mock and the pathogen inoculated samples. Seven differentially expressed LncRNA transcripts (adjusted p<0.01 and absolute (Log2FoldChange) > 0.58) were found in the infected soybean hypocotyl compared to mock inoculation. Five of these transcripts were located on the soybean chromosome eighteen and the other two are found in chromosome eight and chromosome three. These differentially expressed LncRNA transcripts can assist in uncovering the defence regulatory response of soybean LncRNA in the future.

Keywords: expression, *Glycine max*, LncRNA, Near Isogenic Lines, transcripts

## ABSTRACTS

Parallel Session - 4 (Microbiology & Chemical Biology)

## Hypoglycemic, Antibacterial, Angiogenic Activities and Toxicities of Two Decoctions Used in Sri Lankan Traditional and Ayurvedic Medicine Systems

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Hema Drumashya Panchanga Kasaya (HDPK) and Alu Kesel Ala Kasaya (AKAK) are two oral decoctions used to treat diabetes in Ayurveda and Sri Lankan traditional medicine respectively. This study evaluated the hypoglycemic, antibacterial, angiogenic effects and toxicities of HDPK and AKAK. Healthy male Wistar rats, A. salina, and chick embryos were used as animal models. The hypoglycemic effect was evaluated in rats by random and fasting blood sugar (FBS), oral glucose (OGT), and sucrose tolerance. Agar well diffusion assay against Gram-positive (S. aureus, B. subtilis) and Gram-negative (P. aeruginosa, E. coli) strains was conducted for antibacterial activity. Effect on angiogenesis was evaluated by chick chorioallantois membrane (CAM) assay. LC50 values were obtained from brine shrimp assay. Toxicity was evaluated by a 14-day acute toxicity study conducted using rats. Positive controls used in the hypoglycemic, antibacterial, angiogenic, and LC50 studies were Tolbutamide, Gentamicin, Naltrexone, K2Cr2O7 respectively. Except for the CAM assay (Saline) and LC50 assay (seawater), the rest used water as the negative control. Decoctions showed significant hypoglycemic effects compared to water (P<0.05). Compared to Tolbutamide, this was significant in AKAK (FBS) and HDPK (OGT) (P<0.05). Anti-bacterial activity against Gram-positive and Gram-negative strains was maximum in HDPK and AKAK respectively. Decoctions significantly reduced the length, size, number of junctions, and tubular complexes compared to the controls in CAM assay (P<0.01). This was maximum in AKAK (P<0.001). LC<sub>50</sub> of HDPK (3501 ppm) and AKAK (562 ppm) were above 25 ppm. Results for acute toxicity were statistically insignificant. Outcomes of the study proposed the safe and effective use of tested decoctions to treat diabetes, associated bacterial infections, and vascular complications. The current study provides the baseline in developing novel therapeutics to treat diabetes and the safe and synergistic impact of herbal medicine on diabetes-related complications.

**Keywords:** Diabetes mellitus, Hypoglycemic effect, Antibacterial activity, Anti-angiogenic effect, Acute toxicity, Decoctions

## Isolation and Identification of Bacteria with Antibacterial Activity from Cow Dung Samples Collected from Selected Areas in Sri Lanka

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Antimicrobial resistance and emergence of superbugs is a growing concern worldwide. There is a growing demand for new antimicrobial leads to combat this problem. Cow gut harbours a wide range of microbes that are a rich source of new metabolites which could be used as promising new compounds of pharmaceutical importance. The present study focuses on isolating cow gut microbes that secrete compounds with antibacterial activity. Eighteen (18) cow dung samples were collected from dry zone, wet zone-mid country, and wet zone-low country in Sri Lanka where farm and domestic/ free-ranging cows were considered. Dilutions were prepared from the samples, and bacteria present in them were isolated on nutrient agar medium using the streak plate method. Incubations were carried out overnight at 37 °C. Altogether, thirty-five (35) bacterial isolates were isolated. Each isolate was checked for its antibacterial activity against two gram negative and two gram positive bacteria; *Escherichia* coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus cereus respectively where 08 bacterial isolates out of the thirty-five demonstrated antagonistic activity against one or more of them. Morphological features of the isolates were observed and gram staining was carried out as preliminary identification methods. Molecular identification was achieved with DNA extraction followed by PCR amplication using 16S rRNA universal primers 27F and 1492R. The amplified PCR products were subjected to Sanger dideoxy sequencing. Similarity search of the sequences was performed using NCBI BLAST and the results predict that six bacteria showing antibacterial activity belong to the genus *Bacillus*. The remaining two belong to *Escherichia* or Shigella. These microbes can further be used for the isolation of the compounds having potential antibacterial activity.

Keywords: Cow dung, Bacteria, Anti-bacterial activity
# Cadmium and Chromium Concentrations in the Roots and Leaves of Leaf Lettuce (*Lactuca sativa*)

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Contamination of agricultural lands with toxic heavy metals is a widespread environmental issue due to intensive fertilizer applications. Leafy vegetables grown on contaminated soil can uptake heavy metals via root system and can be transported to the edible portions. Leaf lettuce (*Lactuca sativa*) is a popular green leafy vegetable in Sri Lanka due to quick and easy usability. This study was conducted to assess the Cd and Cr concentrations in the root zone soil (Cd<sub>soil</sub> and Cr<sub>soil</sub>), roots (Cd<sub>roots</sub> and Cr<sub>roots</sub>) and leaves (Cd<sub>leaves</sub> and Cr<sub>leaves</sub>) of L. sativa. Plants were grown with and without chemical fertilizer (Urea, MOP and TSP=2:1:1) applications in individual pots. Samples were collected at three stages (4 weeks, 8 weeks and 12 weeks after fertilizer application) and analyzed by atomic absorption spectrophotometry. The results indicated higher Cd and Cr concentrations in chemical fertilizer applied samples. The mean Cr concentrations were higher than those of Cd concentrations in all the sampling events. Highest mean concentrations of fertilizer applied samples were recorded as;  $Cd_{soil}$  (2.0±0.4mg/kg) <  $(20.3\pm0.7 \text{mg/kg});$  Cd<sub>roots</sub>  $(2.3\pm0.3 \text{mg/kg}) < Cr_{roots}$   $(10.7\pm0.5 \text{mg/kg});$  Cd<sub>leaves</sub>  $Cr_{soil}$ (0.5±0.2mg/kg) < Cr<sub>leaves</sub> (14.8±0.5 mg/kg). The mean concentrations variation pattern of Cd was Leaves < Soil < Roots and for Cr was Roots < Leaves < Soil. The present study showed that, mean bio-concentration factors (BCF) of Cr were greater than those of Cd;  $BCF_{Cd}$  (0.5±0.3) < BCF<sub>Cr</sub> (0.8±0.1). The leaf Cr and Cd concentrations of *L. sativa* harvested from fertilizer applied pots exceeded the safe limits for consumption recommended by WHO/FAO (Cr: 2.3mg/kg, Cd: 0.2mg/kg). Therefore, it can be concluded that there is a possibility of contamination of edible parts of *L. sativa* due to long term use of chemical fertilizer in the culture plots.

**Keywords**: heavy metals, bio-concentration factor, fertilizer applications, safe limits for consumption

Acknowledgement: This research was funded by Grant I-3-E-6048-1 by the International Foundation for Science (IFS), Sweden.

#### Compositional Changes of King Coconut (*Cocos nucifera* var. aurantiaca) Water and Kernel During Maturation

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King coconut (*Cocos nucifera* var. aurantiaca) is endemic to Sri Lanka and possesses several nutritional and therapeutic values. This study aimed to evaluate the physico-chemical changes of king coconut water (KCW) and kernel (KCK) at three different harvest maturities (6, 7 and 8 months after nut-set). A total of 60 nuts (n=20) of the variety "Nawasi Thembili" were harvested from randomly selected 5 palms located at commercial cultivation in the Western Province, Sri Lanka. King coconut bunches at each maturity were selected based on the "Leaf Counting Method" recommended by the Coconut Research Institute, Sri Lanka. Whole nut weight (kg), Nut circumference (m), Nut-water volume (L), Kernel weight (kg) were measured as physical parameters, while pH, Total Soluble Solids (TSS) (Brix), Titratable Acidity (as % ascorbic acid), Total sugar content (Glucose, Fructose and Sucrose in g/100 mL) and Mineral content (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>+2</sup>, Mg<sup>+2</sup>, Fe<sup>+2</sup> in mg/L) were evaluated as chemical parameters. All physicochemical parameters were assessed according to AOAC methods, while total sugars and minerals were determined using HPLC (Agilent 1200, USA) and ICP-MS (Agilent 7900, Japan), respectively. Results revealed that all tested physical parameters of the whole nut were significantly increased (p<0.05) during maturation. TSS, pH, acidity and total sugars of KCW were significantly increased (p < 0.05) from 6-8 months of maturity, while mineral content was significantly decreased (*p*<0.05) during maturation. It was found, tender KCW at 7 months of maturity was rich in invert sugars, while minerals were prominent at 6 months of maturity. Furthermore, a significant increase (*p*<0.05) in kernel development was observed during 7-8 months of maturity, while no kernel development was observed at 6 months of maturity. TSS and mineral content in KCK were significantly decreased (p < 0.05) during 7-8 months of maturity. Significantly higher (p < 0.05) sucrose content is reported in mature KCK compared to KCW. Ca<sup>+2</sup> and Mg<sup>+2</sup> were the most prominent minerals in both KCW and KCK. In conclusion, results suggested that the best harvest maturities with optimum physico-chemical characteristics should be selected based on the intended applications of KCW and KCK.

Keywords: King coconut water, king coconut kernel, maturity, physico-chemical changes

Acknowledgment: Financial assistance provided by the funded project (FP128) of Ministry of Science and Technology, Sri Lanka is greatly acknowledged.

#### Morpho-Molecular Characterization of Seed-Borne Mycoflora Associated with Stored Seeds of Selected Grains in Sri Lanka

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borne fungal pathogens to manage crop diseases better. However, there is a lack of research for the proper identification of seed mycoflora affecting the quality of seeds in Sri Lanka. Hence, this study strives to address the issue by identifying seed-borne fungal pathogens using morpho-molecular characterization. Fungal pathogens were isolated from ten surfacesterilized and ten non-surface sterilized seeds of Oryza sativa, Vigna radiata, and Vigna sinensis and five seeds of *Arachis hypogea* by the Agar plate method. DNA was extracted from the pure cultures on PDA using modified CTAB protocol followed by PCR amplification and DNA sequencing of the Internal Transcribed Spacer region. Contig sequences were used in molecular characterization. Germination percentage was calculated from pot experiments by sowing ten non-surface sterilized seeds of A. hypogea and 20 seeds of other seed varieties. Vigor index was calculated by multiplying the germination percentage by the sum of mean root and shoot length. A total of eighteen isolates were recovered from all four seed varieties. Aspergillus niger, Macrophomina phaseolina, Penicillium sp., and Rhizopus oryzae were isolated from the seeds of A.hypogea while Arthrobotrys foliicola, Bipolaris sivanesaniana, Daldinia eschscholtzii, and *Rhizopus oryzae* were isolated from the seeds of *O. sativa*. *Rhizopus oryzae* was the common isolate from the seeds of V. radiata whereas Aspergillus flavus and Aspergillus niger were isolated from the seeds of *V. sinensis. Arachis hypogea* showed the highest incidence of fungal pathogens (4 of 7 genera) resulting in low germination percentage (20%) and seedling vigor index (585) while no germination at all was recorded for the seeds of *O. sativa*. The seedlings of *V. radiata* and *V. sinensis* showed shoot and root abnormalities. This study will support future studies in determining optimal seed treatment strategies for the mentioned seed varieties in Sri Lanka.

Acknowledgment: This study was funded by the research grant RP/03/02/01/02/2020 from the University of Kelaniya.

#### Chemical Composition of Azolla - as a Livestock Feed in Sri Lanka

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Higher feed cost is a big challenge in Sri Lankan livestock industry. *Azolla* is an aquatic fern which can be grown in tropical countries in diverse fresh water systems such as ditches, ponds and paddy fields. Azolla can be used as an ideal feed for livestock. However, utilization of Azolla as a substitute feed is an emerging concept in Sri Lanka and also the production of Azolla at large scale is in its infant stage. Present study was an attempt to evaluate the proximate composition of Azolla pinnata. A temporary pit of 3m × 2m × 0.3m dimensions was created and covered with black plastic sheet. Little amount of soil mixed with cow dung and fresh water were added over the plastic sheet, then fresh Azolla was inoculated for cultivation. Harvested Azolla was washed with clean water and sun dried for three days until a constant weight. The collected dried Azolla was packed in polythene bags for further use. The proximate composition was estimated according to the standard AOAC method. Three replicate samples were analyzed for its dry matter content by using an oven at 105°C until a constant weight was achieved. Ash and organic matter content were calculated by incinerating in a muffle furnace at 550°C until constant weight and the crude protein content was estimated by Kjeldhal method. The ether extract was determined by the Soxhlet system using petroleum ether while the gross energy value was estimated using an E2K Bomb calorimeter. The dry matter content, total ash, organic matter, crude protein content, ether extract and energy of the Azolla sample used were 89.36%, 23.10%, 76.9%, 22.71%, 2.62% and 3052.58kcal/kg respectively. The study indicated that Azolla contains high crude protein, energy and ash content. Azolla is one of the best sources of high-quality protein for animal feed; therefore, effort should be taken to increase the Azolla production in Sri Lanka in a sustainable way to increase the productivity in livestock industry with minimal feed cost.

Keywords: Feed cost, Livestock feed, Proximate composition, Sun dried Azolla

#### Isolation and Identification of Endophytic Fungi Associated with Selected Marine Macroalgal Species in Sri Lanka and *In Vitro* Evaluation of Their Effect on the Growth of Selected Rice Pathogens

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Endophytic fungal assemblages of marine macroalgae are reported to constitute a promising source of novel bioactive compounds. However, the bioactivity of endophytes associated with marine macroalgal species of Sri Lanka has not been investigated adequately. Therefore, the fungal endophytic assemblages associated with selected marine macroalgal species collected from different areas of the coastline of Sri Lanka were isolated and identified with a view to evaluate their antimicrobial properties. Four *Gracilaria* species (Phylum Rhodophyta), one species of Halimeda (Phylum Chlorophyta), one species of Ulva (Phylum Chlorophyta), and two species of Sargassum (Phylum Phaeophyta) collected from Negombo, Hikkaduwa, Moratuwa and Beruwala were used for the isolation of fungal endophytes. As surface sterilization is a critical step in isolating endophytes, surface sterilization protocols for each algal species were optimized. The effectiveness of each protocol was evaluated by tissue imprint and spread plate techniques on PDA supplemented with artificial sea salt. Treating algal parts with ethanol (70%, 75%) for exposure times ranging between 15s – 25s proved to be optimal for effective surface sterilization. Sargassum sp. 1, Halimeda sp. and Gracilaria sp. 3 showed 60%, 35% and 10% frequencies of isolation of endophytic fungi respectively. Three fungal endophytes were isolated from *Sargassum* sp. 1. *Halimeda* sp. and *Gracilaria* sp. 3 yielded one fungal endophyte from each, whereas no fungal endophytes were isolated from any other algal species tested. Out of the five isolates, four were identified as *Aspergillus* spp., using colony and microscopic features. The antimicrobial activity of the endophytic fungal isolates on two rice pathogens, i.e. Bipolaris oryzae and Rhizoctonia solani was tested using the dual culture technique and two Aspergillus isolates showed the ability to control the growth of both pathogens significantly (p≤0.05). They were identified as *Aspergillus foetidus* (GenBank accession number of the most similar match – NR\_163668.1, Percentage identity – 100.00%, Query coverage – 100%, E value - 0.0) and Aspergillus costaricensis (GenBank accession number of the most similar match -MT558927.1, Percentage identity - 99.14%, Query coverage - 99%, E value - 0.0) through DNA sequencing and NCBI BLAST. The study showed the presence of fungal endophytes in Sargassum sp. 1, Halimeda sp. and Gracilaria sp. 3, collected from Sri Lankan coastline with the ability to control the growth of selected rice pathogens under *in vitro* conditions.

PROCEEDINGS OF THE 41st ANNUAL SESSIONS OF INSTITUTE OF BIOLOGY, SRI LANKA HELD ON 24TH SEPTEMBER 2021

#### Identification and Characterization of Decaying Hardwood Inhabiting Fungi in Sri Lanka in terms of Lignin Degradation and Laccase Production

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Fungi are a fascinating group of organisms with the ability to degrade lignin mainly using laccase. As a biocatalyst laccase often stands at a preferable position due to its low substrate specificity, high redox potential, ability to catalyze one-electron abstraction from various compounds reducing molecular oxygen to water and mediator dependent catalytic activity. In the enzyme industry, laccases have a high demand; 1 g of  $\geq 0.5$  U/mg laccase valued at more than 100 USD. The objectives of the present study were to isolate fungi from decaying hardwoods of the Dimbulagala forest reserve, identify them up to the species level and explore their lignin degradation and laccase producing ability. Thirty decaying hardwood samples were collected particularly targeting hardwood species such as Diospyros ebenum, Manilkara *hexandra* and *Vitex pinnata*. From the surface sterilized 0.5 mm length decaying wood strips and fruiting bodies, fungi were isolated into modified semi selective potato dextrose agar (PDA) amended with streptomycin and the carbendazim. A total of 50 morphologically different isolates were selected for further studies. Fungal DNA was extracted, rDNA-ITS regions were amplified by Polymerase Chain Reaction (PCR) using universal ITS 1 and 4 primers and the PCR products were sequenced by Sanger dideoxy sequencing. The sequences were compared with the GenBank using Basic Local Alignment Search Tool (BLAST). Species were characterized according to their lignin degradation and laccase producing abilities by performing staining lignin agar and guaiacol agar plate assays, respectively. Twenty-nine isolates were identified up to the species level and six isolates were identified up to the genus level. Twenty-one isolates degraded lignin and 11 species produced laccases including *Perenniporia tephropora*, *Flavodon* flavus, Schizophyllum commune, and Phlebiopsis flavidoalba. Some isolates having no laccase production ability, showed lignin degradation ability indicating the complex nature of the lignin degrading enzymes in the fungal kingdom. Identified laccase producing fungal species can be utilized as an initial point to start commercial laccase industry and apply in bioremediation processes in Sri Lanka.

Keywords: Dimbulagala forest reserve, Laccase, Lignin degradation, Wood decay fungi

Acknowledgement: This project is funded by ICGEB (CRP/LKA18-03), NSF (RG/2019/BT/03) and TWAS (18-020 RG/BIO/AS\_I) research grants.

#### Characterizing Antibacterial and Antibiofilm Activity of Traditional `Thuna-Paha' Spices on *Staphylococcus aureus*

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Five major spices in the traditional spice base 'Thuna-Paha' include Cinnamon, Cumin, Curry leaves, Fennel, and Coriander, which have also been used in ayurvedic medicine. We hypothesized that five spices in Thuna-Paha may have antibacterial and antibiofilm properties against *Staphylococcus aureus*, which is a Gram-positive opportunistic pathogen that may cause life-threatening human infections. S. aureus produces biofilms, where bacterial cells are embedded in an extracellular polymeric substance matrix. To prepare water extracts, spices were dried and ground individually to obtain a fine powder. Water containing 10% (w/v) spice powder was boiled for 15 minutes, filtered and then centrifuged to obtain a clear extract. Antibacterial properties were tested using the agar disk diffusion method. To test antibiofilm properties, S. aureus biofilm was formed in a flat bottom polystyrene 96-well plate. Spice extract treatment was done for 24hours using biofilms grown for 48hours. Crystal violet assay was performed to quantify the biofilm. According to the results, aqueous extract of coriander showed an average of 0.8±0cm diameter bacterial growth inhibition zone, indicating the presence of antibacterial compounds in coriander extract. Oxytetracycline, an antibiotic used as positive control showed 1.6±0.1cm bacterial growth inhibition. Coriander treatment did not significantly reduce the biofilm, indicating antibacterial tolerance of *S. aureus* biofilm. Fennel treatment significantly reduced the biofilm by 29.2%. Fennel, however did not show a bacterial growth inhibition zone in the disk diffusion test, revealing the presence of an antibiofilm mechanism that does not come from antibacterial activity. Cumin, cinnamon, and curry leave extracts did not show a significant antibacterial or antibiofilm activity. Further work is in progress to characterize the antibiofilm activity of fennel against *S. aureus*.



# **Schedule of the Scientific Sessions**

41<sup>st</sup> Annual Sessions of the Institute of Biology, Sri Lanka 24<sup>th</sup> September, 2021 via Zoom platform

Parallel Session	Session Chair	Link to join	
Zoological Sciences	Drof Mayuri Wijacingha	https://zoom.us/j/95283421014?pwd=WkRKU2pMbDQ4RGx	
Zoological Sciences	FIOI. Mayuri Wijesinghe	<u>TSjl6Qm0rYVJsUT09</u>	
Dlant & Environmental Sciences	Dr. Dinukshi Wimalasakara	https://us02web.zoom.us/j/89487550635?pwd=L3FjeHZLYWJtbIN	
Flant & Environmental Sciences	DI. KIIIUKSIII WIIIIalasekela	ra21UWIpCeWJHQT09	
Molecular Dielegy & Dietechnology	Dr. Couoni Calhona	https://us02web.zoom.us/j/89296850304?pwd=TmFIOTh5UE5sQ	
Molecular blology & blotechhology	Dr. Gayani Gamena	nZibGImT3dxb0FvQT09	
Microbiology & Chamical Diology	Dr. Chandrika Dorora	https://us02web.zoom.us/j/84840045962?pwd=RXdGOTc1UVArZGJ1bU	
Microbiology & chemical biology	DI. Chanulika Perera	<u>llcjUxRzNVQT09</u>	

### Parallel Session 1- Zoological Sciences

TIME	ABSTRACT NUMBER	TITLE
	1-01	Approaches to Gender Determination of Indian Crested Porcupine ( <i>Histrix indica</i> ) By <u>S.S.K.S. Thadhani</u> , D.S. Weerasekera
1.00–2.30 p.m.	1-02	Using Morphometrics to Identify Beetles: Distinguishing the Genera of Subtribe Tricondylina ( <i>Coleoptera</i> , <i>Cicindelinae</i> , <i>Collyridini</i> ) of Sri Lanka By <u>D.L. Abeywardhana</u> , Y.W. Mallawarachchi, C.D. Dangalle
	1-03	Assessment of Heavy Metal Accumulation in Different Bird Species Occupying Diverse Trophic Levels Using Contour Feathers By <u>W.M.A.N. Wanasinghe</u> , M.R. Wijesinghe, R.D. Wijesekara, S.M. Vithanarachchi

	1-04	Comparative Effect of Parasitism of Ciliated Protists Associated with Selected Vector Mosquito Larvae in Selected Rice Field Habitats in Ganewatte, Kurunegala District, Sri Lanka By <u>S. Wijesinghe</u> , L.D. Amarasinghe
	1-05	Potential of Aquatic Carnivorous Plants; <i>Utricularia vulgaris</i> and <i>Utricularia reticulata</i> as Biological Control Agents for the Larval Stages of Dengue Vector, <i>Aedes aegypti</i> <i>By <u>K.R.N. Perera</u>, P.A.D.H.N. Gunathilaka, L.D. Amerasinghe, N.W.B.A.L. Udayanga</i>
	1-06	The Association of Fish Abundance and Composition with Water Quality Parameters in the Lower Kelani River Basin, Sri Lanka By <u>S.R.C.N.K</u> . Narangoda, A.A.D. Amarathunga, C.D. Dangalle
2.30–2.45 p.m.		SESSION BREAK
2.45–4.15 p.m.	1-07	Foraging Microhabitat Preferences of Aquatic Birds in an Agricultural Wetland, Meegoda, Sri Lanka By <u>H.A.S.S. Alwis</u> , T.N.K. Jayawardena, M.R. Wijesinghe
	1-08	Length-Weight Relationship and Maturity Analyses of Male Silky Sharks ( <i>Carcharhinus falciformis</i> ) Landed in Negombo Fishery Harbour <i>By <u>H.M.S.G. Karunanayaka</u>, H.A.C.C. Perera</i>
	1-09	Effect of Delayed Mating on Reproductive Performance and Life History Parameters of Dengue Vector Aedes aegypti By <u>R.A.K.M. Gunathilaka</u> , G.A.S.M. Ganehiarachchi
	1-10	Can Pupal Dimensions of <i>Spodoptera frugiperda</i> (Lepidoptera: Noctuidae) be Considered as Predictors of Sexual Dimorphism Prior to Eclosion? <i>By <u>R.H. Kasige</u>, N. Pallewatta, C.D. Dangalle</i>
	1-11	Molecular Characterization of Midgut Bacteria in Larval and Adult Stages of <i>Aedes albopictus</i> in Gampaha District, Sri Lanka <i>By <u>H.A.K. Ranasinghe</u>, P.A.D.H.N. Gunathilaka, L.D. Amarasinghe, W.W.P. Rodrigo</i>

	1-12	Uncovering the Diversity and Distribution of <i>Scolopendrid centipedes</i> in Sri Lanka. By <u>A.P.P.T. Alexander</u> , J. Joshi, S.S. Seneviratne
4.15-4.30 p.m.		SESSION BREAK
	1-13	Teratogenic Effects of Albendazole and Levamisole on Embryonic Development of <i>Gallus gallus</i> domesticus By <u>Chandrakumar Anusha</u> , Devaka Weerakoon
	1-14	Gastrointestinal Parasites Diversity of Wild Products and Garbage Consuming Elephants in Southern Sri Lanka By <u>L.H.S. Wickramasooriya</u> , P. Fernando, I.C. Perera, P.N. Dayawansa
	1-15	Potential Contamination of Microplastics in Green Mussels ( <i>Perna viridis</i> ) Cultured in Negombo Estuary By <u>H.A.S.S. Alwis</u> , R.R.M.K.P. Ranatunga, A.S.L.E. Corea, P.N. Dayawansa
4.30–6.15 p.m.	1-16	A Study on Aggressive Behavior and Locomotion of Zebrafish ( <i>Danio rerio</i> ) in Association with Polypropylene Microplastic Exposure By <u>S.P. Vijayarathna</u> , R.M.U.M. Rathnayake, G. Rajapaksa
	1-17	Marine Sponges of Coastal Waters of Mandaitivu Island, Northern Province, Sri Lanka By <u>R. Nirujan</u> , W.S. Thulasitha
	1-18	A Preliminary Study of the Behavioural Ecology of Critically Endangered Horned Lizard <i>Ceratophora</i> <i>erdeleni</i> in Morningside Reserve of Sinharaja Rainforest During a Wet Spell <i>By <u>H.B. Amarasinghe</u>, C.D. Dangalle, P.N. Dayawansa</i>
	1-19	Habitat Preference of Sympatric Agamid Lizards Inhabiting the Morningside Reserve of Sinharaja Rainforest During Wet Season By <u>H.B. Amarasinghe</u> , C.D. Dangalle, P.N. Dayawansa

## Parallel Session 2 – Plant & Environmental Sciences

TIME	ABSTRACT NUMBER	TITLE
1.00-2.30	2-01	Assessment of the Effect of Mulching on Soil Quality and Plant Growth Parameters of Okra By <u>H.S.D. Madhubhashini</u> , W.M.D.N Wijeyaratne
	2-02	Effects of Low-Cost Organic Fertilizer Derived from Invasive Alien Plants and Shade on Growth Performances and Yield of <i>Aloe vera</i> (L.) Burm.f. By <u>A.R.B.W.M.C.D. Bandaranayake</u> , R.M.C.S. Ratnayake
	2-03	Assessment of the Presence of Heavy Metals of Recycled and Virgin Paper Waste Compared to Four Different Food Packaging Standards By <u>G.M. Indunil</u> , W.A.R.T.W. Bandara
p.m.	2-04	Morphological and Molecular Characterization of Medicinal Plants Commonly Known as Kalu Nika By <u>R. Vinushayini</u> , S.M.W. Ranwala
	2-05	Screening of Selected Pesticide Residues Present in Market Vegetables in Colombo Suburbs By <u>S.M.V.S.K. Samarakoon</u> , W.T.P.S.K. Senarath
	2-06	Assessing the Adaptive Response of Sri Lankan True Mangrove Species to Their Environment Using Leaf Characteristics By <u>G.S.C. Madhupanchanee</u> , H.I.U. Caldera
2.30–2.45 p.m.	SESSION BREAK	
2.45–4.15 p.m.	2-07	Predicting the Potential Distribution of Sri Lankan Endemic <i>Calophyllum</i> Using Ecological Niche Modelling: Impacts of Climate Change <i>By <u>P.R.G.K.T. Rankoth</u>, H.S. Kathriarachchi</i>
	2-08	Evaluation of <i>Vigna unguiculata</i> ssp. <i>sesquipedalis</i> Germplasm Accessions for Reproductive Traits By <u>L.E. Rathuge</u> , H.A.P.A. Shyamalee, T.D. Silva

	2-09	A Geoinformatics Approach to Identify the Effect of Land-Use Changes on Heat Distribution of Urban Lentic Ecosystem Landscapes By <u>M.D.K.L. Gunathilka</u> , Devanmini Halwatura, Lasantha Manawadu
	2-10	Enhancing Vase-Life of <i>Chrysanthemum morifolium</i> cv. 'Champagne Golden' Cut flowers By <u>G.U. Anushika</u> , P.S. Saputhanthri
	2-11	Natural Dye Preparations from Sawdust of Jackfruit ( <i>Artocarpus heterophyllus</i> ) and Mahogany ( <i>Swietenia</i> sp.) By <u>P.D.N.V. Alwis</u> , P.S. Saputhanthri
	2-12	Assessing Morphological Diversity of Sri Lankan Sesame ( <i>Sesamum indicum</i> L.) By <u>N.P.P.S Jayathilaka</u> , I.A.J.K. Dissanayake
4.15-4.30 p.m.		SESSION BREAK
	2-13	Influence of Herbaceous Cover on Soil Fertility in an Immature Rubber-Land in Kalutara District, Sri Lanka By <u>W.A.S.N. Sarathchandra</u> , R.P. Hettiarachchi, S.M.W. Ranwala
4.30-5.15	2-14	Antioxidant Activities of <i>Sargassum</i> sp. and <i>Gracilaria</i> sp. in the Thalpe Coast of Sri Lanka
p.m.		By <u>W.A.D.A.D. weerapperuma</u> , W.G.S.R. Thilakarathna, H.M. Herath, R.P.Wahigatunge

# Parallel Session 3 - Molecular Biology & Biotechnology

TIME	ABSTRCT NUMBER	TITLE
1.00-2.30 p.m.	3-01	Seed Priming with Polyamines Improves Germination of Rice ( <i>Oryza sativa</i> L.) Under Aluminium Stress By <u>P.C. Vileka</u> , R. Wimalasekera
	3-02	Oxidation Stress and Antioxidant Response in <i>Piper longum</i> L. Nodal Explants Subjected to Cryopreservation By <u>N.Y.M. Arachchi</u> , H.D.D. Bandupriya
	3-03	Discovery of Novel Genic and Intergenic SSR Markers from Black Pepper ( <i>Piper nigrum</i> ) Genome By <u>H.K.S.G. Gunasekara</u> , A. M. Wickramasuriya
	3-04	In Silico Analysis of Genes Responsive to Drought Stress in Arabidopsis thaliana: Potential Targets for Genetic Crop Improvement By <u>W.H. Kalpathara</u> , A.M.Wickramasuriya
	3-05	Genetic Diversity Analysis of <i>Piper</i> Species in Sri Lanka Using PCR-Based SSR Markers By <u>J.M.V. Lakshika</u> , A.M. Wickramasuriya, H.D.D. Bandupriya, K.G.S.U. Ariyawansa, W.G.N.A. Wimalarathna, J.M. Senevirathna, M.S.S. Munasinghe, D.G.H.M.K. Dissanayake, T.D. Silva
	3-06	Incubation Period Associated with the Weligama Coconut Leaf Wilt Disease in Sri Lanka
		By <u>P.H.P.R De Silva</u> , S.A.C.N Perera, K.P.R.N Attanayake
2.30–2.45 p.m.	SESSION BREAK	
2.45-4.15 p.m.	3-07	Effect of Different Concentrations of 2,4-D and BAP on Callogenesis of <i>Munronia pinnata</i> (Wall.) Theob. (Binkohomba) <i>By <u>K.T.R. Piyumal</u>, H.D.D. Bandupriya, K. Hirimburegama</i>
	3-08	Preliminary Assessment of Genetic Diversity in <i>P. nigrum</i> Accessions Grown in Kolonna, Sri Lanka Using a Morphometric Analysis By <u>W.G.N.A. Wimalarathna</u> , J.M.V. Lakshika, H.A.N.M. Waidyathilaka, J.M. Senevirathna, D.G.H.M.K. Dissanayaka, M.S.S. Munasinghe, H.M.G.I.C.K. Senevirathne, A.M. Wickramasuriya, K.G.S.U. Ariyawansa,

	H.D.D. Bandupriya, T.D. Silva
3-09	In Silico Identification of Plant Derived Antimicrobial Peptides (AMPs) By <u>H.H.H.N. Alwis</u> , K.G.S.U. Ariyawansa
3-10	In Silico Analysis of the Effectome of Ustilaginoidea virens By <u>H.K. Lokuyaddehige</u> , K.G.S.U. Ariyawansa
3-11	Analysis of Population Structure of <i>Ex-Situ</i> Conserved Sorghum ( <i>Sorghum bicolor</i> (L.) Moench) Germplasm Accessions in Sri Lanka Using SSR Markers <i>By <u>D.V.S. Kaluthanthri</u>, P.N. Dasanayaka, S.A.N. Perera</i>
3-12	Identification of Differentially Expressed Long Non-Coding RNA in Soybean When Infected with <i>Phytophthora sojae</i> <i>By <u>N.I.S. Mendis</u>, A.J. Wijeratne, R.M.T.S. Ratnayake, H.D.D. Bandupriya</i>

# Parallel Session 4 - Microbiology & Chemical Biology

TIME	ABSTRACT NUMBER	TITLE
1.00-2.30 p.m.	4-01	Hypoglycemic, Antibacterial, Angiogenic Activities and Toxicities of Two Decoctions Used in Sri Lankan Traditional and Ayurvedic Medicine Systems By <u>P.I.T. Liyanage</u> , V. K. Fernando, J.R.A.C. Jayakody, S.K.M.K. Herapathdeniya, I.C. Perera
	4-02	Isolation and Identification of Bacteria with Antibacterial Activity from Cow Dung Samples Collected from Selected Areas in Sri Lanka By <u>K.A.G. de Alwis</u> , S. Mauran, I.C. Perera
	4-03	Cadmium and Chromium Concentrations in the Roots and Leaves of Leaf Lettuce ( <i>Lactuca sativa</i> ) By <u>M.D.M.C.K. Amarasena</u> , W.M.D.N Wijeyaratne
	4-04	Compositional Changes of King Coconut ( <i>Cocos nucifera</i> var. <i>aurantiaca</i> ) Water and Kernel During Maturation By <u>M.D. Jayasinghe</u> , M.M.N.P. Gunasekara, M.G.D.S. Perera, G.U. Chandrasiri, K.D.S.M. Karunarathna, I.G.N. Hewajulige
	4-05	Morpho-Molecular Characterization of Seed-Borne Mycoflora Associated with Stored Seeds of Selected Grains in Sri Lanka By <u>A. Ganeshalingam</u> , D.A. Daranagama
	4-06	Chemical Composition of <i>Azolla</i> - as a Livestock Feed in Sri Lanka <i>By <u>K. Saruga</u>, K. Sivashanthini</i>
2.30–2.45 p.m.	SESSION BREAK	
2.45–3.30 p.m.	4-07	Isolation and Identification of Endophytic Fungi Associated with Selected Marine Macroalgal Species in Sri Lanka and <i>In Vitro</i> Evaluation of Their Effect on the Growth of Selected Rice Pathogens <i>By <u>C.U. Rodrigopulle,</u> N. Deshappriya</i>

	Identification and Characterization of Decaying Hardwood Inhabiting Fungi in Sri Lanka in terms of Lignin
4-08	Degradation and Laccase Production
	By <u>P. Perera</u> , G. Senanayake, H.M. Herath, W.R.P. Wijesinghe, R.N. Attanayake
	Characterizing Antibacterial and Antibiofilm Activity of Traditional `Thuna-Paha' spices on Staphylococcus
4-09	aureus
	By <u>T.J. Sarathchandra</u> , S. H. L. Fernando, E.A.P.T. Amarasekara, C.C. Kadigamuwa, P.M. Colonne



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