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E-NEWSLETTER



Quarterly e-newsletter of the



**INSTITUTE OF BIOLOGY
SRI LANKA**

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COVER STORY

Flowering branch of *Strobilanthes sripadensis*

Strobilanthes Blume is a species-rich genus of the Acanthaceae family, consisting of more than 400 species of herbs and shrubs distributed mainly in tropical and subtropical regions of Asia. Many species within this genus possess medicinal and economic values.

Recently, *Strobilanthes sripadensis*, a new species of *Strobilanthes* Blume, was recorded from the Peak Wilderness Nature Reserve in Sri Lanka. According to the IUCN Red List Categories and Criteria, this species is classified as a Critically Endangered species.

Photograph by Mr. Bhatthiya Gopallawa
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Front view of the S. sripadensis flower

IOBSL NEWS AND EVENTS

INTERNATIONAL BIOLOGY OLYMPIAD SELECTION EXAM 2023

On April 21st, 2023, a selection exam for the IBO was held at the Blended Learning Center, Faculty of Applied Sciences, University of Sri Jayewardenepura. The eligible winners from the first round of the Sri Lankan Biology Olympiad were invited to participate in this exam. The IOBSL is organizing this prestigious competition, and four students will be chosen as the best performers in the selection exam. These outstanding students have the opportunity to showcase their knowledge and skills on an international stage and compete against the best biology students from around the world.





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Institute of Biology Sri Lanka

UPCOMING | 20 EVENTS | 23

**INTER-UNIVERSITY
BIOLOGY QUIZ
COMPETITION**

**E-PHOTOGRAPHY
COMPETITION**

**INTER-UNIVERSITY
BIOLOGY
CHALLENGE**

**YOUNG SCIENTIST
AWARD**

**ENTREPRENEURIAL
TRAINING
PROGRAMS/
WORKSHOPS**

**IOBSL
ORATION**

**THEMATIC
PUBLICATION**

**ANNUAL
SESSIONS**

**COURSES
WORKSHOPS
WEBINARS**

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INFORMATION**

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INTER-UNIVERSITY BIOLOGY QUIZ COMPETITION - 2023

IOBSL is organizing the Inter-University Biology Quiz Competition for the undergraduates of state universities for the 6th consecutive year in 2023. The competition aims at providing undergraduates with a novel educational experience and enhancing their interest in biology. The competition will also provide an ideal opportunity for collaboration and inspiration among students, academics and universities across the country.

Deadline for registration: 18th May 2023

IOBSL E-PHOTOGRAPHY COMPETITION 2023

The e-Photography Competition 2023 is now open for entries.

Theme: 'The Hidden Biological Wonders'

Awards:

- Best Biology Photographer of the Year - 2023 (18 years and over)
- Best Young Biology Photographer of the Year - 2023 (under 18 years)
- Most Popular Biology Photograph of the Year - 2023 (open)

Submission deadline: 30th June 2023

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INTER-UNIVERSITY BIOLOGY QUIZ COMPETITION - 2023

Eligibility
Current Undergraduates (Biology or allied streams) of State and Non state Educational Institutes
*Participants should be Sri Lankan Citizens

Structure of the competition

- Stage I - MCQ paper (online)
- Stage II - Viva and a Quiz

Registration

- Application closing date **Extended- 18th May 2023**
- Online registration - [Click here](#)
- Registration fee - 500/-

Important Dates

Awards & Certificates

- Champion: Gold medal & a certificate
- 1st runner-up: Silver medal & a certificate
- 2nd runner-up: Bronze medal & a certificate

For further details contact

[http://www.iobsl.org/
iobslquizcomp@gmail.com](http://www.iobsl.org/iobslquizcomp@gmail.com)

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**INSTITUTE OF BIOLOGY
SRI LANKA**

e-Photography Competition 2023

Open to entries on the theme of
'The Hidden Biological Wonders'

Submission deadline
30th June 2023

Scan to register &
submit entries

Competition
open to all
amateur photographers

For more information:

- <https://www.iobsl.org/>
- iob.ephotographycompetition@gmail.com
- +94 77 844 4161 - Mr. Sudesh Ruvinda (weekdays | 8 am - 4 pm)

IOBSL INTER-UNIVERSITY BIOLOGY CHALLENGE - 2023

Theme:

'Biological Wealth for Economic Prosperity'

Sub themes:

- Alternative energy sources
- Agriculture, forestry and fisheries products
- Bioprospecting
- Ecotourism

Target group:

Undergraduates of state universities in Sri Lanka

Deadline for registration: 1st June 2023



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INTER UNIVERSITY BIOLOGY CHALLENGE 2023

IOBSL is organizing an Annual Biology Challenge competition for interdisciplinary teams of undergraduates selected from state universities in Sri Lanka to develop an attractive and challenging proposal to nourish the IOBSL theme "Biological Wealth for Economic Prosperity"

Eligibility

Undergraduates of State Universities of Sri Lanka

Team composition

Four-member team consisting of students from different disciplines and at least one student from the discipline of biology

Structure of the challenge

Submission of a novel, feasible and cost-effective proposals and a presentation that nourishes the theme of IOBSL

Awards

1st, 2nd and 3rd places will be awarded with certificates at the Annual Sessions of IOBSL

- ✓ **Deadline for Registration**
1st June 2023
- ✓ **Deadline for submission**
1st July 2023
- ✓ **Notification of the winners**
1st of August 2023

Click here for [guidelines](#)

Register online- [click here](#)

For more information contact: email- iobchallenge2023@gmail.com

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43rd Annual Sessions IOBSL - 2023

BIOLOGICAL WEALTH FOR ECONOMIC PROSPERITY

Important Dates

Abstract Submission: Now Open
Submission deadline: 20th June
Notification acceptance: 10th September
43rd Annual Sessions: 22nd September

[Submission
guidelines](#)

[Declaration
form](#)

[Submit your
abstract](#)



For more information

Web: <https://www.iobsl.org/>

Email: iobabstract2023@gmail.com

CALLING ALL ABSTRACTS!

Present your discovery at the 43rd Annual
Sessions of the IOBSL

Deadline: 20th June

Submit your research to

Sri Lankan Journal of Biology (SLJB)

SLJB provides high quality, comprehensive and broad coverage in all areas of Biology. The journal publishes original scientific research articles that describe significant research findings. SLJB encourages the submission of original contributions in all fields of basic and applied research involved in Biological Sciences.

[Read the submission guidelines](#)



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Sri Lankan Journal of Biology (SLJB)
Published by The Institute of Biology, Sri Lanka

Invitation to submit manuscript

SLJB aims to set a platform to communicate original research to meet the needs of the demanding and growing disciplines of biology.

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FEATURE ARTICLES

Can Spices Be Used in the Treatment and Prevention of Cancer?



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The popularity of spices across the world dates back to ancient times, not only because of their importance in culinary use but also for their potential health benefits. Advances in scientific research have demonstrated that different spices can achieve their ability to maintain good health as well as fight against the damaging effects of harmful agents through their antioxidant and anti-inflammatory properties. *“Let food be thy medicine and medicine be thy food”* is frequently quoted by Hippocrates to demonstrate the strong link between nutrition and health, which is now strengthened by epidemiological, preclinical, and clinical evidence. Dietary factors, including herbs and spices, play a major role in maintaining the balance between delivering tumorigenic or anti-tumorigenic effects.

Cancer, being a multigenic disease causing nearly 9.6 million deaths worldwide, has become a global health burden. In addition to the dysregulation of multiple genes of an individual via germline mutations or epigenetic alterations, 90% of tumorigenesis is caused due to somatic mutations, environmental factors, and unhealthy lifestyles. The evidence indicates that 50% of cancer cases could be prevented by confronting its risk factors such as diet and exposure to pollutants which are also causatives of several chronic diseases. The cancer incidence rate is significantly low in countries such as India (94/100000) which consumes higher amounts of spices daily when compared with the United States (318/100000). Such epidemiological evidence further suggests the potential role of spices in cancer prevention.

To date, there are various approaches such as surgery, chemotherapy, immunotherapy, radiation therapy, stem cell therapy, combination therapy and vaccinations used to treat as well as prevent tumour growth and progression. However, due to the adverse effects of such approaches emerging interest has fallen upon promising natural compounds with fewer side effects, claiming for 70% of anti-cancer medications. Curcumin, capsaicin, vincristine, docetaxel and paclitaxel are some of the leading examples of such medicines derived from natural compounds used in clinics to treat a variety of cancer types. They act through their active phytoconstituents such as alkaloids, flavonoids, phenols, vitamins, essential oils, sugar, and amino acids, etc.

Globally, research has been conducted to identify possible health benefits of spices and to date, there are over 200 spice-derived compounds that reflect these effects. Black pepper, cumin, cardamom, cinnamon, clove, fennel, ginger, garlic, turmeric etc. are some of the commonly used spices which demonstrate such biological activities. Spices have been shown to demonstrate several anti-cancer properties such as reduction of free radical formation, regulation of inflammatory responses, pro-apoptotic and anti-proliferative effects on tumor cells. Further, various studies demonstrated the role of different spices on the regulation of expression patterns as well as the activity of numerous oncogenes, tumour suppressor genes, inflammatory markers such as pro-inflammatory cytokines, chemokines, enzymes, adhesion molecules, and certain growth factors which are involved in tumorigenesis.

To determine the effects of dietary spices on cancer cell proliferation, invasion, angiogenesis and metastasis, the majority of studies have focused on determining the role of spices in regulating signalling pathways such as Nuclear Factor-Kappa B (NF- κ B), Wingless-related integration site/ β -catenin (Wnt/ β -catenin), Tumour Necrosis Factor-alpha (TNF- α), Phospho Inositide 3-Kinase/AKT (PI3K/AKT), Janus Kinase/Signal Transducers and Activators of Transcription (JaK/STAT), Mitogen-Activated Protein Kinase (MAP) cascade by suppressing targets which are crucial for tumorigenesis, including cell cycle proteins.

As a community that consumes food rich in spices on a regular basis, it is important to know the possible health benefits of some commonly used spices on chemoprevention and tumor suppression. This article sums up research evidence on the anti-cancer properties of a few spices which are the main ingredients of the curry powder 'Thuna paha' commonly used in Sri Lanka.



Coriander (*Coriandrum sativum*) recognised as “කොත්තමල්ලි”

A number of experiments on animal models have demonstrated the ability of dietary coriander in the stimulation of hepatic antioxidant system and upregulation of the detoxifying enzyme, glutathione S-transferases (GST) activity by dietary coriander oil. Further, anti-cancer properties of coriander have been suggested via the promotion of oxidative stress in tumour tissues through linalool which is one of the principal constituents of coriander.

Caraway (*Carum carvi*) known as “meridian fennel” or “මහදුරු”

Chemical characterization of caraway essential oil has demonstrated its ability of free radical scavenging against diphenyl-1-picrylhydrazyl (DPPH) and its antioxidant potential has been demonstrated *in vitro*. Further, *in vivo* studies demonstrated a significant reduction of tumour promotion in mouse and rat models with chemically induced skin cancer and aberrant crypt foci by dietary caraway oil.

In mouse models, caraway has also been shown to increase the levels of GST in the liver, whereas it is negatively correlated with the levels of xenobiotic-metabolizing Cytochromes P450 (CYP) genes in rat models. Although various studies have been performed *in vitro* and in animal models to demonstrate the anti-cancer properties of caraway, only a handful have been performed with humans.

Cumin (*Cuminum cyminum*) also recognised as “සුදුර”

Thymoquinone (TQ), the main active ingredient of cumin has been shown to have antioxidant, anti-inflammatory and chemo-preventive properties in several *in vivo* and *in vitro* studies. Cumin-mediated suppression of tumour cell proliferation has been evident in colorectal cancer, breast adenocarcinoma, ovarian carcinoma, pancreatic carcinoma, osteosarcoma and myeloblastic leukaemia via downregulation of Bcl-xL, cyclin D1 and vascular endothelial growth factor (VEGF) gene expression.

Several *in vivo* studies in cancer xenograft models of prostate, bladder, lung, ovarian and gastric cancer have further strengthened the potential of anti-cancer properties of dietary cumin via depicting TQ-mediated suppression of NF-κB, STAT3, PI3/AKT and Extracellular signal-Regulated Kinase (ERK) pathways.

Cardamom (*Elettaria cardamomum*) commonly known in Sri Lanka as “එනසල්”

Cardamom is not only a prominent spice in Sri Lankan cuisine but is also well recognized in Asian, Arabic, and European countries for having antioxidant properties, primarily due to its main active ingredient, cardamonin. Its potential inhibitory effects on chemically induced carcinogenesis were suggested by *in vivo* studies performed on Swiss albino mice fed with cardamom oil. Similar to other spices such as caraway, a number of *in vivo* models have demonstrated an impressive rate of free

radical scavenging ability, an increase in the level of antioxidant enzymes (GST, catalase and superoxide dismutase) and a significant reduction of CYP levels in the liver due to dietary intake of cardamom.

Several *in vitro* studies have also demonstrated a cardamonin-mediated reduction of inducible nitric oxide synthase (iNOS), TNFα, and Interleukin 6 (IL-6) along with inhibition of NF-κB, STAT3, mammalian target of rapamycin (mTOR) and Wnt/β-catenin signalling, suggesting the potential of cardamom as an anti-inflammatory substance. Further, *in vivo* studies on dextran sulphate sodium (DSS)-induced colitis mouse model and xenograft model, have confirmed the role of cardamonin in the prevention of colitis incidence and inhibition of tumour growth and angiogenesis, respectively. Also, there are microRNA profiling studies performed on cardamonin which suggest its therapeutic potential against colorectal cancer.



Cinnamon (*Cinnamomum verum*) known in Sri Lanka as “කුරුදු”

Being one of the key ingredients in traditional Chinese medicine, the antioxidant properties of cinnamon have been extensively studied. Evidence includes a significant reduction of oxidative stress and enhanced levels of antioxidant-related enzymes in Wistar rats fed with cinnamon bark powder. Although clinical studies have not shown significant effects, several *in vitro* studies have demonstrated cinnamon-mediated growth inhibition of *Helicobacter pylori* which is recognised as a major risk factor in developing gastric cancer and possibly pancreatic cancer. Further, *in vitro* and *in vivo* analysis have demonstrated that water extracts of cinnamon could inhibit VEGF-mediated endothelial cell proliferation, migration and angiogenesis, which are key processes of tumour progression.

Fenugreek (*Trigonella foenum-graecum*) known as “උළුහාල්”

Diosgenin, the main bioactive compound of fenugreek, has been used to treat a variety of chronic diseases, including Alzheimer’s disease, osteoarthritis, breast cancer and chronic myeloid leukemia, due to its anti-inflammatory properties. Both *in vitro* and *in vivo* studies have shown a diosgenin-mediated upregulation of cancer cell apoptosis, reactive oxygen species (ROS)-dependent autophagy, and inhibition of cancer cell migration.

Clove (*Syzygium aromaticum*), commonly known in Sri Lanka as “කරුබු නැට්”

Cloves are well known for their potential anti-inflammatory and anti-tumorigenic properties. Numerous investigations have depicted the role of eugenol, the main active compound of cloves in suppressing cancer cell proliferation via inhibition of NF-κB and c-Myc and activation of apoptosis in gastric cancer, skin cancer and breast cancer cells. Interestingly, eugenol has been identified as a natural compound with the potential of enhancing the efficacy of anti-cancer drugs such as gemcitabine.



Since cancer is one of the diseases that can be prevented, there is growing proof that diet plays a crucial role in both cancer treatment and prevention. Furthermore, natural active compounds, such as those found in spices, have emerged as potential candidates in paving the way for the development of safer and more affordable therapeutics against chronic diseases, including cancer, due to the fatal side effects and high costs of modern therapeutics.

Although a variety of *in vitro* and *in vivo* experiments have demonstrated the spice-mediated influence on proliferation, apoptosis, angiogenesis, and immune competence, there

is a lack of information and confirmatory studies with regard to clinical trials. Studies reflect that even a small intake (1g/day) of some herbs or spices could significantly contribute to the total antioxidant intake of an individual. Further, some studies suggest the biased effects of spices depend on the cell type and the combinations. For example, an *in vitro* analysis of a combined treatment of cardamom aqueous extract with black pepper (*Piper nigrum*) has demonstrated a cell type-specific effect. Therefore, one of the main concerns with natural compounds/treatments is that although they possess tremendous health benefits, they must be further standardized for their doses, exposure, and authentication of raw material.

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UPDATES ON THE LATEST RESEARCH

Uncovering the Genetic Diversity of Cultivated Black Pepper (*Piper nigrum*) in Sri Lanka

In recent years, there has been an increasing interest from both academia and industry in spice crops, because of their biological, health, and economic importance. Black pepper (*Piper nigrum* L.), often referred to as the 'King' of spices, is indeed one of the most widely used spices in the world, with a long history of use in traditional medicine. The characteristic pungency of the dried mature fruits of black pepper is attributed to the non-volatile alkaloid, piperine and its isomers. The present literature describes numerous biological activities of piperine, including anti-inflammatory, antibacterial, antifungal, antidepressant, antimutagenic, and insecticidal activity.

Black pepper is a perennial vine that typically climbs on support trees with the aid of aerial clinging roots. The tropical evergreen forests of the Western Ghats in India are its native habitat, and presently it is widely cultivated there as well as in other tropical regions of Southeast Asia, Africa, and South America.



Dr. Anushka M. Wickramasuriya, M. I. Biol. (Sri Lanka)

Department of Plant Sciences, Faculty of Science
University of Colombo, Sri Lanka



Figure 1 Black pepper cultivation in Kandy

Photo credit: Mr. Bhathiya Gopallawa, Postgraduate Institute of Science, University of Peradeniya, Sri Lanka

Sri Lanka too has a rich genetic diversity of both wild and cultivated black pepper. According to the Food and Agriculture Organization of the United Nations statistics (FAOSTAT), Sri Lanka is the sixth largest producer of black pepper, with an average production of 42,485 tonnes in 2021. In addition to being an important export crop, black pepper is also used extensively in Sri Lankan cuisine and traditional medicine.

In 1974 and 1999, the Department of Export Agriculture, Sri Lanka launched programs for the collection, assessment, and selection of black pepper germplasm for high-yielding, high-quality and disease-resistant accessions. The present-day cultivars of black pepper consist of local selections, new hybrids, and

introductions, and their cultivation is mostly concentrated in Kandy, Matale, Kegalle, Kurunegala, Badulla, Monaragala, and Ratnapura districts (Figure 1). The area under black pepper cultivation was estimated to be around 46,235 hectares in 2021 (FAOSTAT) (Figure 2). The average yield of black pepper is estimated to be around 900 kg per hectare which is much less than the productivity of black pepper in other pepper-growing countries. Since 'Ceylon black pepper' is superior in quality due to its high piperine content and therefore has an increasing demand worldwide, improving the productivity and further enhancing the quality of this important cash crop could help to meet its growing demand while providing economic benefits to farmers and the country as a whole.

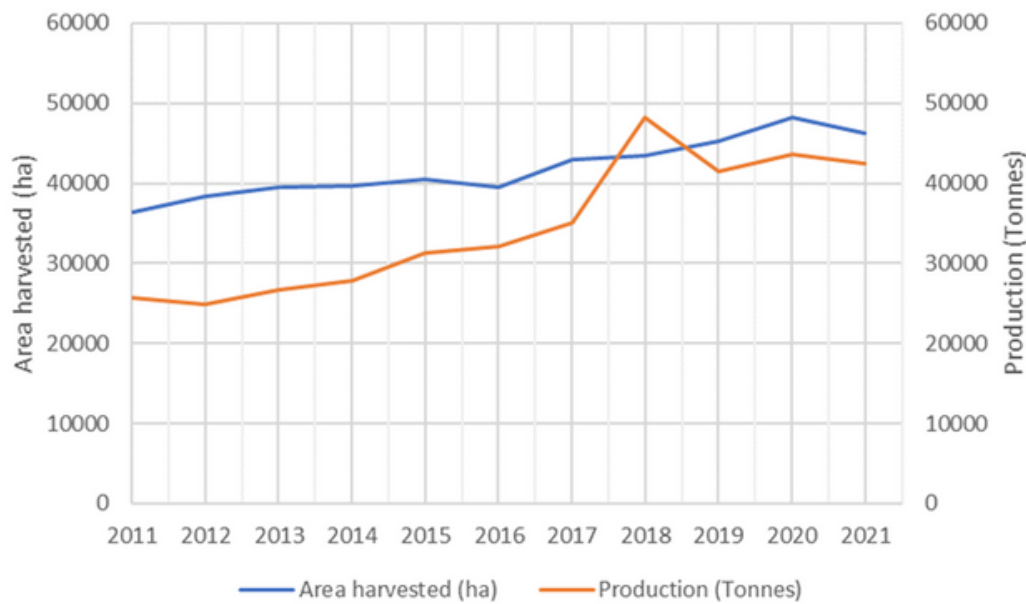


Figure 2 Black pepper production and area harvested from 2011 to 2021

Source: FAOSTAT, Production/yield quantities of pepper (*Piper* spp.), raw in world + (total) [online].

Available at: <https://www.fao.org/faostat/en/#data/QCL/visualize> (Accessed: 9 April 2023)

Assessment of genetic diversity using genetic markers

Studies on crop genetic diversity and population structure are recognized as a specific area of research, and the findings of such studies are of fundamental importance for the long-term sustainability of breeding programs and conservation efforts. The assessment of genetic diversity, relatedness, and population structure is usually performed using morphological, biochemical, and/or molecular (DNA) markers. Molecular markers can be further categorized as (i) first-generation molecular markers, including restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD), (ii) second-generation molecular markers, including amplified fragment length polymorphism (AFLP) and microsatellites or simple sequence repeats (SSRs), and (iii) third-generation markers, including single-nucleotide polymorphisms (SNPs).

Of these molecular markers, SSRs have been the most widely used marker system in studies of genetic diversity, population structure, genetic mapping, and evolutionary processes, due to their codominant inheritance, greater genome abundance, and reproducibility. During the last few years, markers based on SNPs have rapidly gained attention among plant molecular biologists. SNPs are defined as single nucleotide differences at a specific position in the genome sequence. They offer several advantages over SSR markers, including their biallelic nature, greater abundance in genomes, low mutation rate, and amenability for high-throughput detection formats and platforms.

However, the discovery of polymorphic SNPs can be more expensive and time-consuming than other marker systems. Nevertheless, recent advances in next-generation sequencing (NGS) technologies have made it increasingly possible for high throughput identification and

genotyping of large numbers of SNPs at low cost. High-density SNP genotyping arrays have a significant impact on many areas of biology, including population genetic studies at the genome scale and trait association studies in crops as well as in other organisms.

Despite the advantages of SNPs, there are only a limited number of studies that have used SNP markers in genetic diversity and structure analyses in plants. This is partly because of the cost of genotyping large numbers of samples and the lack of reference genomes. Until now, the studies performed on molecular characterization of black pepper genetic diversity have been limited to PCR-based molecular markers such as AFLPs, RAPDs, and SSRs.

RAD-sequencing - a valuable technique for next-generation population genetics

Restriction-site associated DNA sequencing (RADseq) is a powerful NGS-based method that has become increasingly popular for studying genetic diversity and population structure in non-model crops and organisms with limited genomic resources. It allows the identification of high-density genome-wide SNPs within and between individuals. Basically, the RADseq method involves the digestion of genomic DNA with chosen restriction enzyme(s) to produce sticky-ended fragments, the ligation of adapters containing a molecular identifying sequence to the sticky-end fragments, and the construction of libraries suitable for sequencing. Subsequently, the flanking restriction enzyme recognition sites are sequenced employing an NGS platform such as Illumina sequencing technology (Figure 3). RADseq method has been successfully applied in several crops, including rice, sesame, barley, maize, apple, and eggplant.

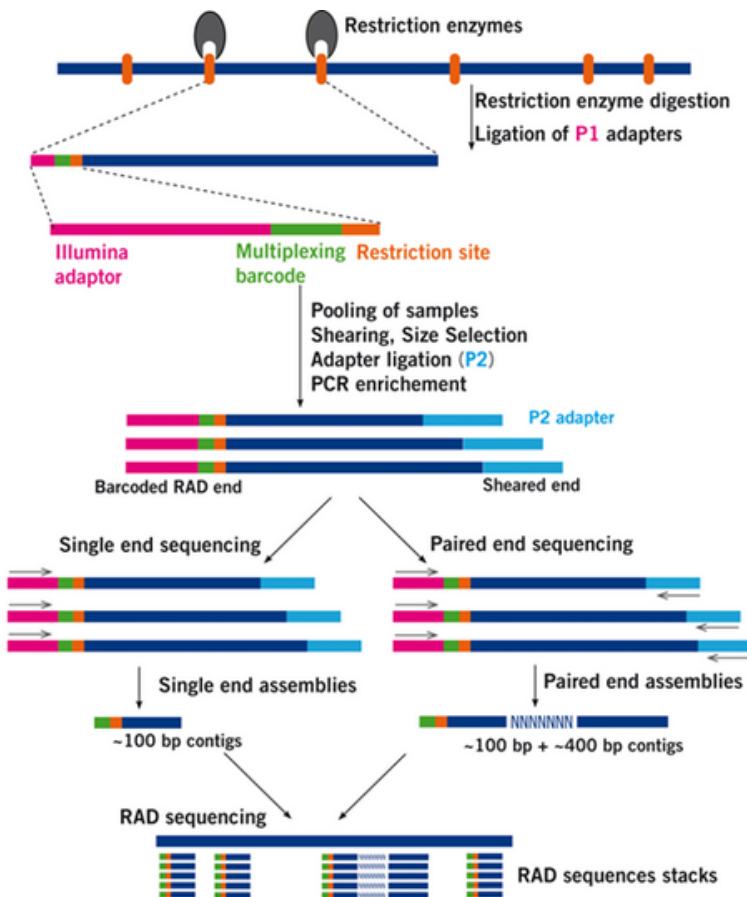


Figure 3 RADseq workflow

Source: Floragenex (2018) Technical brief: RAD sequencing [online]. Available at: <https://www.floragenex.com/rad-seq> (Accessed: 2 April 2023)

To our knowledge, the RADseq method has not been applied in previous studies to detect sequence polymorphism in spice crops. A research team led by Prof. Tara Silva at the Department of Plant Sciences, University of Colombo and Prof. Rosabella Samuel of the Department of Botany and Biodiversity Research, University of Vienna, Austria, is currently investigating the genetic diversity and population structure of cultivated black pepper populations grown in different agro-climatic regions of Sri Lanka using RADSeq. Ms. Nilni Wimalaratne, the Research Assistant who is currently pursuing her PhD studies at the University of Colombo, has succeeded for the first time in sequencing genomes of over 100 black pepper cultivars using RADseq. The polymorphic SNPs discovered in this study will

be used to estimate the genetic diversity, relatedness and population structure of cultivated black pepper in Sri Lanka. In addition, we have examined the percentage of bisexual flowers and their distribution along the spikes of a few genetically divergent selections of black pepper using three-dimensional computed tomography (Figure 4), since the percentage and the distribution of bisexual flowers in the spikes, have an impact on the yield of black pepper. The findings of this ongoing research project undoubtedly will provide novel insights into the genetics of population structure of black pepper and may serve as a valuable resource for more efficient utilization of black pepper germplasm for sustainable cultivation, genetic crop improvement and germplasm conservation.

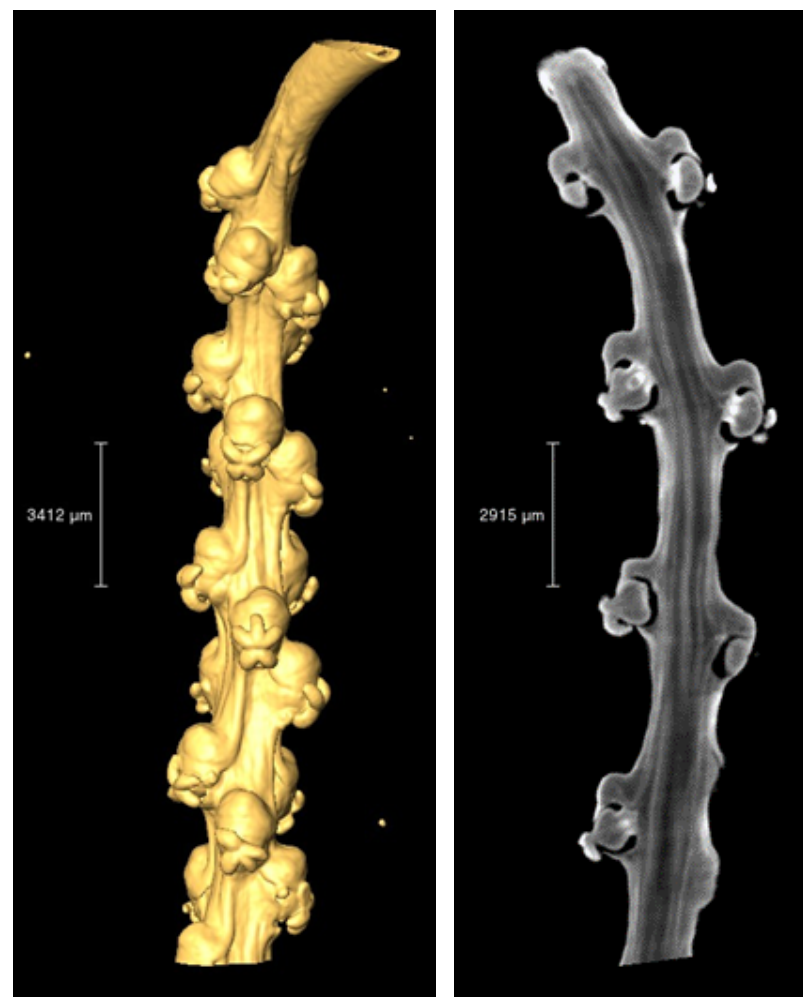


Figure 4 Microcomputed tomography images of a black pepper inflorescence showing numerous small flowers

Photo credit: Ms. Nilni Wimalaratne, Department of Plant Sciences, University of Colombo Sri Lanka

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Plant Growth in Space

Gravity sensing and plant growth

Gravitational force is a crucial factor that regulates plant growth and development. Plant roots are sophisticated organs that sense the gravity vector and respond to changes in the gravity vector by re-orientating the growth direction. Plant roots normally grow straight down when grown in an upright position (1g or 90°). Changing the gravitational direction by growing plants tilted at a surface angle of 45-60°, roots often show a wavy form, and extended periods of growth result in coil formation at the root tips. Lateral root development is also gravity-influenced. Shoots are able to sense gravity and alter hypocotyl elongation and cotyledon/leaf expansion in response to gravitational changes. In general, hypergravity causes growth suppression. Microgravity or near zero gravity is a feature of orbital flight in space. A limited number of studies conducted under space conditions have shown that root and shoot growth and morphogenesis can be greatly modified.

Space experiment - growing *Arabidopsis* under microgravity

BIOLAB is an experiment flight facility of the European Space Agency (ESA), accommodated in the International Space Station (ISS). The BIOLAB enables biological experiments that investigate the effects of space on microorganisms, cell/tissue cultures, small plants and animals.



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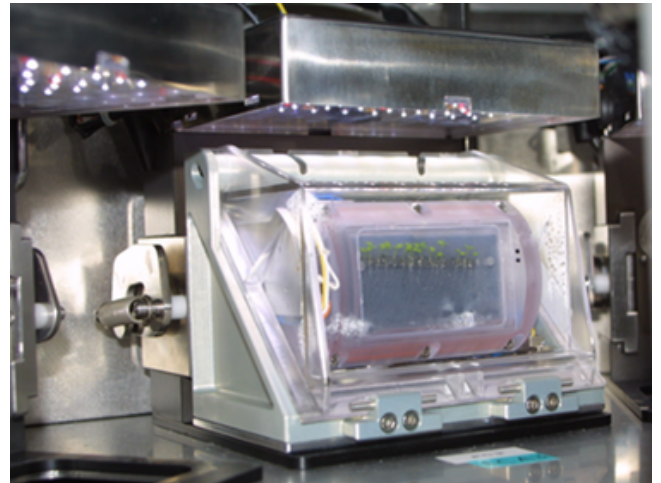
The present study was conducted at the BIOLAB facility aiming to strengthen the understanding of the microgravity on root and shoot growth using the model plant *Arabidopsis thaliana*. Surface sterilized and stratified seeds were glued on filter strips and mounted onto a stamp-like tool. On the ISS, the astronauts transferred the filter strips onto Murashige and Skoog medium (MS) (2% (w/v) agar) containing boxes. The boxes were then connected to experimental containers (ECs) and mounted ECs were placed in centrifuges. The mounted ECs were provided with LED light (24 h) and maintained at 23 °C. One of the ECs was maintained at 1g force as a control. Figure 1 shows an EC with grown plants. Continuous monitoring of the growth was recorded by inbuilt video cameras.

***Arabidopsis* plants grown in space**

The growth and development of *A. thaliana* is not completely impaired by the microgravity condition. However, microgravity-induced developmental changes were observed.

Compared to growth in 1g control, primary root growth was reduced in microgravity. The number of lateral roots increased significantly, and root tips produced an increased number of coils after 12 days of growth in microgravity (Figure 2). After a longer period of growth at microgravity, roots produced more coils. Seedlings exposed to microgravity led to increased waving of the roots.

(a)



(b)

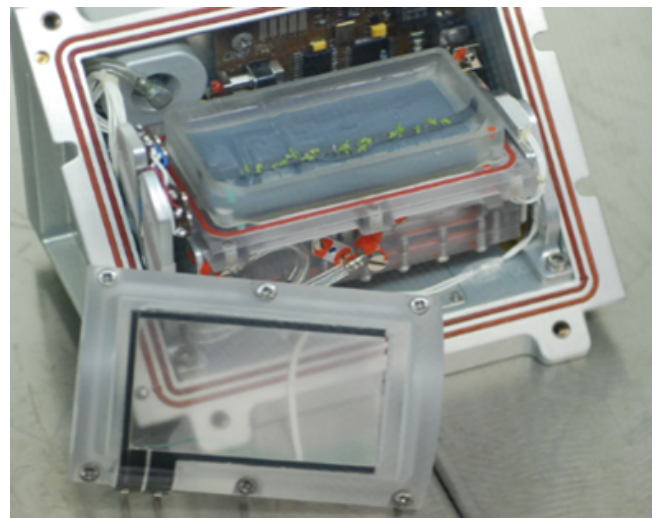


Figure 1 (a), Experimental container with *Arabidopsis* plants; (b), Opened agar container fixed to experiment container

In comparison to 1g grown seedlings, microgravity-grown seedlings exhibited an increase in hypocotyl length and an increase in petiole length. Cotyledon expansion is affected by microgravity conditions. However, leaf sizes were apparently not different between seedlings grown under 1g control and microgravity.

Plants grown in microgravity even for a short period of time show gravity-induced differences in root and shoot systems. The developmental changes are suggested to be caused at least partly by altered polar auxin transport and alterations in auxin signalling in microgravity.



Figure 2 Phenotypes of *Arabidopsis* seedlings grown in microgravity for 12 days

** Extracted from a research conducted under “WAICO” project led by Professor G.F.E Scherer from Leibniz University Hannover, Germany. The research group included K. Radatz, P. Pietrzyk, C. Labusch and R. Wimalasekera.

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INTERVIEW WITH A SCIENTIST

The youngest Professor in Sri Lanka, Professor Nayana Gunathilaka who has won the IOBSL Young Scientist Award 2022, talks to the BIO-NEWS, e-newsletter of IOBSL.



Prof. Nayana receiving the IOBSL Young Scientist Award 2022

Nayana Gunathilaka, Professor in Parasitology, at the Department of Parasitology, University of Kelaniya, is one of the youngest Sri Lankans to have secured a doctorate at the age of 27. He obtained his PhD in Molecular Medicine and Medical Entomology from the Faculty of Medicine, University of Kelaniya in 2014.

He received his foreign training at the World Health Organization Collaborating Centre for Reference and Research of Arbovirus and their Associated Vectors, Environmental Health Institute, Singapore.

His career begins as a Medical Entomologist at the Malaria Elimination Programme of the Tropical and Environmental Disease and Health Associates (TEDHA), which was funded by the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM). Thereafter, his career path took him as a Research Scientist in the Biotechnology Unit of the Industrial Technology Institute (ITI) in Colombo and finally to the position of a Senior Lecturer at the Department of Parasitology, Faculty of Medicine, University of Kelaniya.

Among the numerous awards Professor Nayana has bagged are the National Research Council Merit Award for Scientific Research in 2014 and 2016, the Postgraduate Research Merit Award for Medical, Dental and Veterinary Sciences by the Sri Lanka Association for the Advancement of Science (SLAAS) in 2015, and Presidential Research Awards for Scientific Publications in 2015 and 2017. His name was among the top 5 members in two award categories: Medical Innovation &/or Research and Academic Leadership &/or Accomplishment, in the Ten Most Outstanding Young Persons (TOYP) of Sri Lanka Awards - 2017 awarded by the Junior Chamber of International, Sri Lanka.



Prof. Nayana receiving the CVCD Excellence Award 2020 for the Most Outstanding Young Researcher

He has received the University of Kelaniya Vice Chancellor's Awards for Most Outstanding Young Researcher and Most Outstanding Senior Researcher for bringing honour to the university by winning prestigious national and international awards and being the researcher with the highest number of publications in the Science Citation Indexed (SCI) journals from 2016 to 2022. He has already secured three-lifetime awards, namely, the CVCD Excellence Award for 2020 for the Most Outstanding Young Researcher conferred by the Committee of Vice-Chancellors and Directors (CVCD), the Young Scientist Award – 2022 in recognition of excellence in conducting high-quality research and publishing in SCI Journals, academic accomplishment, leadership, research supervision, and national service by the Institute of Biology, Sri Lanka and Young Scientist Award – 2022 offered by the Young Scientist Forum of the National Science and Technology Commission of Sri Lanka.

Up to now, he has published over 70 full papers in SCI journals, 145 abstracts in national and international symposia and three book chapters. His research work has been cited over 889 times, with an h-index of 17 and i10-index of 32, according to Google Scholar. At the national level, Professor Nayana Gunathilaka has provided his technical expertise as the national expert for the health sector to the Third National Communication on Climatic Change and as the national consultant in the Food and Agriculture Organization (FAO) funded Biosafety Project in Sri Lanka under the component of genetically modified (GM) mosquitoes and insects.

He is also a working group member of the Malaria Research Colloquium at the Anti-Malaria Campaign, Sri Lanka and served as a technical committee member in the Integrated Vector Management technical advisory group at the Ministry of Health, Sri Lanka. He was a technical core group member of the GFATM-funded National Malaria Elimination Programme in Sri Lanka through Tropical and Environmental Diseases and Health Associates (TEDHA).

Professor Nayana, tell us about yourself and your family.

In my family, I have my father, mother, one elder brother, and a younger sister. I am the second in my family. My brother is a senior software engineer and my sister is a medical doctor who is also working as a Lecturer at a state University.

Did you always want to be a scientist when you were younger? How did you get started as a scientist? Tell us about your Science story so far.

My mother is a Science teacher. When I was at home, I used to look at her books and I saw how she conducted practical classes and experiments with her students. That motivated me to choose the path of Science.



Can you tell us a bit more about your current research interest(s)?

Infectious and vector-borne diseases, parasitological and molecular biological aspects of vector-borne disease pathogens, molecular diagnostics of parasitological infections

What do you think are the biggest challenges that Sri Lankan scientists are currently facing?

Funding for research work; difficulty in adapting to multidisciplinary research cultures and research collaborations

What would you regard as the most significant achievement in your career so far?

Building research collaborations with multidisciplinary researchers and expanding the research network

You were the recipient of last year's IOBSL Young Scientist Award, and you have also received many awards. In your opinion, how important is it to recognize the contributions made to Science?

Recognition of a scientist's contribution to Science in the form of awards is an encouragement for a scientist to work harder and to be motivated.

Tell us what motivated you to apply for last year's IOBSL Young Scientist Award.

IOBSL is a prestigious professional body in Sri Lanka which consists of eminent biologists. I am also a chartered member of the IOBSL and I felt it was an honour to be recognized by this IOBSL. So I decided to apply for this prestigious award.

What does a typical day at the university look like for you?

I often come to the university every five days, depending on the modules, I engage in teaching. Most of my time is spent in my laboratory guiding and supervising PhD and research students. Also, I do my bench work. I have allocated one day per week to engage in bench work. I also get involved in the administration work of the university. I have been appointed as a Chairperson for several committees and participate in several meetings during the day at the university.

What advice would you give to our young scientists?

My first piece of advice to young scientists is to extend the research collaboration. It is important to share resources, knowledge, and ideas among them. The facilities that we have developed should not be limited to a particular group of people; rather, we should share them with other scientists, which in turn facilitates national development.

The second piece of advice is that you should not restrict yourself to a specific or narrow field of study. If you choose a diverse field of study, you will get a chance to learn new knowledge, experience, and perceptions and to develop new skills. Then you will be well on your way to happiness.



How does a young professor spend his free time?

I like to play badminton. I used to play badminton in my school days. Also, I love to play cricket with some good neighbours. I also love to sing. Still, I take part in the church choir.

What are your professional goals? Where do you see yourself in 10 years?

I do not have many goals to achieve. I just want to keep this momentum going to continue my research, support fellow researchers, and contribute to the development of the country.



Interviewed by

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CORNER FOR YOUNG BIOLOGISTS

Is Caffeine Good or Bad for Human Health?



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Caffeine unarguably claims the crown as the most consumed nutritional supplement all over the world. It is naturally found in many types of foods or beverages that we take daily such as tea leaves, coffee beans, guarana berries, kola nuts, and cacao nuts. In a recent review article published in the journal 'Nutrients', many important aspects of caffeine use are explained. Chemically, caffeine is a purine alkaloid that is said to have some resemblance in action to the naturally occurring nitrogenous molecule adenosine (Figure 1). It is also artificially produced by the chemical synthesis of urea as a raw material, which is then combined with different chemicals such as methyl chloride and ethyl acetate.

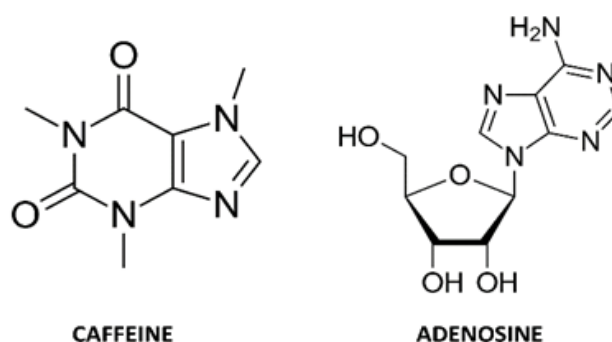


Figure 1 Chemical structures caffeine and adenosine
Source: Rodak et al. (2021)

Origin

People have enjoyed caffeine for over a thousand years. Its origin dates far back to China in 2437 BC when Emperor Shen Nung discovered tea. The pleasant aroma had reportedly fascinated the emperor who conjured the drink by boiling the twigs of the tea plant. Another historical landmark of caffeine according to Griffin M. in 'Coffee History' is when an Ethiopian shepherd noticed some wild berries that gave his goats a lot of energy and began consuming it as a drink, marking the origin of coffee as a commonplace drink. Since then, caffeine has been entwined with human history, helping billions of people burn the midnight oil or wake up every day.

Consumption

Consumption of caffeine is ubiquitous; almost every person fulfills their caffeine needs one way or another. Regardless of gender, everyone from children to adults happens to consume it through many sources including beverages but coffee, and tea being at the summit, followed by soft drinks. The use of caffeine in other food products, such as chewing gum was initially used in the military but soon found its way into sports. A distinct amount of the intake cannot be predicted as a whole due to the fact that the main source of caffeine changes from one country to another. As for the US and many European countries, the main source of caffeine is coffee while in the UK, the drink of preference is generally tea (Figure 2).



Figure 2 Sources of caffeine
Source: Rodak et al. (2021)

Mechanism of action

Once it is consumed, caffeine is rapidly ingested by the gastrointestinal tract of humans. Then it is taken to the liver, where it is metabolized. Caffeine is described as a psychoactive compound. This means that it penetrates the blood-brain barrier to commence its main mechanism of action. Caffeine has a similar structure to adenosine, which is a neurotransmitter in our brain. Due to its resemblance to adenosine, caffeine is found to be playing an antagonistic role, blocking the adenosine receptors. Four adenosine receptors can be found in our brain: A1, A2A, A2B, and A3. Among them, caffeine shows a preference toward the receptors A1 and A2A. Since adenosine is the neurotransmitter that promotes sleep, blocking it keeps the consumer awake while reducing fatigue. Studies have shown that it takes part in enhancing dopamine levels in the brain as well.

Positive effects

Caffeine enjoys its limelight due to several other important health benefits other than just helping us stay awake. There have been numerous studies done regarding caffeine and its role in human health. Because of the antagonistic role played by caffeine on adenosine receptors, increasing the amounts of neurotransmitters such as dopamine and norepinephrine leads to a short-term mood lift and improved alertness. Also, studies have shown that caffeine can boost our metabolism, which may assist in weight loss.

Caffeine has also been handy in aiding patients with Parkinson's disease, which is a long-term neurodegenerative disease that causes a progressive breakdown of the nervous system. Recent studies have revealed that caffeine is linked with decreasing the risk of contracting Parkinson's disease and may play a preventive role against its onset. Similarly, caffeine is reported to have some help in lowering the likelihood of Alzheimer's disease, which accounts for 50-70% of neurodegenerative diseases and dementia.

Studies have also shown that caffeine has some antioxidant properties and is therefore regarded as one of the promising anti-cancer agents. It is found to be especially important in cancer treatment because oxidative-antioxidative imbalance accompanies cancer progression and metastasis. Some studies have also shown that caffeine reduces the risk of liver and colon cancers as well as skin cancers induced by sunlight. Recent growth in non-communicable diseases (NCDs) is becoming a major health issue globally. Having elevated blood pressure, and high blood sugar levels combined with extra fat make us guilty of it. All such health issues combined are known as metabolic syndrome. Caffeine's ability to aid in weight loss may play a crucial role in reducing the overall risk of developing metabolic syndrome.

Negative effects

Just like anything else, caffeine also has its yin and yang. Although it has been scientifically proven to reduce depression, caffeine has a notable ability to increase anxiety levels. Also, overdosing can lead individuals to hallucinate and have panic attacks as well. Using caffeine while pregnant is not recommended.

Regardless of the form, caffeine consumption has always been engraved as a cultural heritage for its values. It will be interesting to uncover more intriguing facts about caffeine while enjoying your daily cup of tea or coffee.

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Marine Plastic Pollution: A Threat to Seafood Security

The ocean is a highly nutritious and protein-rich food supply source that greatly contributes to the healthy life of humans. Fish contributes to nearly 16% of global animal protein consumption. Consequently, the demand for seafood has been increasing over the past years with the rising human population. However, marine plastic pollution has been an immense threat to the oceanic ecosystem, inevitably affecting the world's seafood security. For example, 50-75 trillion pieces of plastic and microplastics are currently present in the ocean and some form of plastic pollution was found in approximately 33% of fish captured for human consumption. Marine plastic pollution leads to the destruction of the marine ecosystem by affecting seawater quality, destroying habitats of marine organisms, and changing their biological functioning, which directly leads to the destruction of marine food chains, the decline in the population of commercially important marine species and severe health impacts on humans due to the consumption of contaminated fish. This emphasizes the importance of addressing marine plastic pollution as a threat to seafood security and strategies to prevent pollution and ensure the sustainable distribution of quality seafood for human consumption.

Importance of seafood security

Seafood security is the physical and economic reachability to safe and nutritious seafood in sufficient quantity by every person, to fulfill their dietary requirements and food interests for a healthy and active life at all times. The global demand for seafood has been greatly increasing over the past few years. It was estimated at US\$113.2 billion in the year 2020 and was predicted to reach US\$1138.7 billion by 2027, growing at a compound annual growth rate of 2.9% throughout 2020-2027.



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The high demand for seafood is mainly due to its abundance of high-quality proteins, n-3 polyunsaturated fatty acids and other important minerals (Iodine, Selenium, Calcium, Zinc, Iron, etc.), vitamins (D, B12, A, E, etc.), and trace elements. Marine-sourced omega-3 fatty acids are important for retinal, brain and fetal development, improvement in psychological disorders such as depression, dementia, schizophrenia, and attention deficit hyperactivity disorder, protection against diseases related to the blood circulatory system, and maintaining immune functions.

Marine plastic pollution

Release of energy or substances into marine systems intentionally or accidentally by humans, causing damage to living resources, threats to human health, obstacles to marine activities and reducing seawater quality is defined as marine pollution. Annually, 300 million tonnes of plastic are manufactured for various purposes. A minimum of 14 million tonnes of plastics reach the ocean annually and contribute to producing 80% of marine debris from the water's surface to bottom sediments.

Nearly 80% of marine pollution is caused by land-based sources which are added to the ocean through runoff, rivers and direct release, while the remaining 20% accounts for marine shipping discharges; waste dumping at sea, and offshore industrial activities. Around 90% of global trade of goods between countries takes place via cargo shipping, as it provides an efficient and cost-effective means of transportation. Plastics used in various industries, including healthcare, textile, agriculture, etc., are widely traded across the globe. Hence, ship spills cause detrimental effects on biodiversity and seafood security. For instance, in May 2021, the explosion of the X-Press Pearl cargo ship at Colombo port, which carried about 300 MT of fuel in tanks, and 1500 containers comprised of harmful chemicals and plastic pellets, caused the greatest damage to the Sri Lankan biodiversity and seafood security (Figure 1 and 2). Consequently, in July, the bodies of 176 turtles, 4 whales and 20 dolphins had reported being washed ashore, damaging coral reefs and resulting in inevitable harm to the fisheries industry. Fishing was banned, and extremely rich feeding and breeding fish grounds collapsed. People were reluctant to consume fish due to a fear of contamination



Figure 1 Damage to fish by plastic pellets in the X-Press pearl disaster

Source: Karunaratne, C. (2021) Aftermath of Singapore-flagged container ship explosion [online]. Available at: <https://epaimages.com/> (Accessed: March 28, 2023)



Figure 2 Plastic pellets washed off to the beach from the X-Press Pearl cargo ship
Source: Liyanawatte, D. (2021) Plastic pellets washed off to a beach from the MV X-Press Pearl cargo ship are seen in Ja-Ela [online]. Available at: <https://www.reuters.com/news/picture/container-ship-sinks-off-sri-lanka-two-w-idUSRTXCVB7H> (Accessed: March 28, 2023).

Plastics are durable, stable, corrosion-free, difficult to break down and can remain for a prolonged time and move long distances in the sea. Initially, the plastic pollution may remain in coastal water, but it is soon picked up and transported to different locations by gyres, which leads to the formation of plastic accumulation areas (garbage patches). Plastic waste can degrade and form microplastics and nanoplastics due to mechanical abrasion; weathering and photodegradation. Plastics can damage living organisms via several processes, including, physical toxicity, particle effect and chemical toxicity.

Physical toxicity is mainly caused by macroscopic plastics and ghost nets.

Macroscopic plastic can be consumed by marine species, obstructing gastrointestinal tracts and leading to reproductive disabilities, malnutrition, and death. Industrial fishing is responsible for around 20% of marine plastic pollution. Ghost fishing with plastic nets, rings and fishing gear can kill or harm marine animals. Large plastic debris such as plastic bags and sheets can cover coral reefs and prevent the reaching of light, hence destroying the activities of phototrophic organisms that build reefs. The biodiversity in coral reefs is crucial for fisheries and seafood security, as they provide habitat for thousands of ecologically and economically important marine species.

The particle effect is mainly caused by microplastics and nanoplastics. Microplastics possess the capability to destroy cells, harm tissues and induce inflammation in living beings. Nanoplastics can even transport across the gut lining and collect in tissues, resulting in harmful effects. Chemical toxicity takes place due to toxic chemical additives and adsorbed or absorbed pollutants in microplastics and nanoplastics. Toxic chemicals added to microplastics include neurotoxins, carcinogens, and endocrine disruptors, which can reduce fertility, and cause birth defects and cancer. On average, a person ingests 74,000-121,000 particles of microplastics per year. Humans mainly get exposed to marine micro and nanoplastics and chemical toxins through contaminated seafood, including fish and filter-feeders (oysters and mussels), causing major medical conditions such as metabolic impairment, toxication and cellular damage, growth reduction, behavioural changes, negative impacts on reproduction, and high susceptibility to diseases. As marine pollution causes a major impact on marine ecology, strategies need to be implemented to protect the ocean and increase sustainable seafood security.

Establishing Marine Protected Areas (MPA)

MPAs are sections of environments that have been designated by the law to be protected by limiting anthropogenic activities in that area. These are established based on management objectives categorized by the International Union for Conservation of Nature (IUCN) and known to be an effective fisheries management and conservation tool.

MPAs protect marine ecosystems from pollution, restore marine biodiversity, increase species richness and population, and ensure sustainable utilization of marine resources. Globally, 7% of the ocean is defined as MPAs. However, a study has shown that only 3.6% were properly functional. Hence, it is essential to manage these areas strictly according to the defined objectives of the respective protected area category.

Reducing and preventing marine pollution

To prevent marine pollution, managing and controlling the entry of pollutants into the sea is important. Rivers associated with densely populated, rapidly developing coastal regions with inadequate waste collection systems are a major source of plastic waste in the oceans.

Laws and policy measures, including reducing plastic production, improving global waste management strategies and recycling, imposing strict bans and limitations on the usage of single-use plastics (e.g., straws, plastic packaging, etc.) and improving litter capture methods and designing innovative alternatives for plastic products, play a key role in preventing plastic pollution in marine environments.

Over the past years, international bodies have created various programs to protect oceans from pollution. Sustainable development goal 14 ('Life below water') aims to conserve and sustainably utilize the world's oceans, seas, and marine resources.

The first target of the goal was to prevent and notably reduce marine pollution of all kinds by 2025. To achieve this target, it is important to incorporate strategies such as zero waste policies, fishing and ocean certification systems, implement biomonitoring programs, review water quality standards, remediation and clean-up, and raise community awareness and empowerment.

The International Maritime Organization (IMO) is a specialized agency of the United Nations, that plays a main role in creating a fair, effective, universally adopted, and implemented regulatory framework for the shipping industry. It is responsible for the safety and security of shipping and the prevention of marine and atmospheric pollution by ships. In 2021, strategies were adopted by the IMO's Marine Environment Protection Committee (MEPC) to reduce marine plastic litter generated from and retrieved by fishing vessels, reduce the contribution of shipping to marine plastic litter, and enhance the effectiveness of port reception and facilities, and treatment in reducing marine plastic litter.

In conclusion, marine plastic pollution caused by anthropogenic activities leads to metabolic impairment, toxication and cellular damage, growth reduction, behavioural changes, negative impacts on reproduction and immunity, destruction of species' habitats, higher mortality, and decline in species abundance and distribution. It also affects food chains and the adaptations of species to the marine ecosystem. When pollution affects commercially important marine species, it significantly impacts seafood security.

To address this global challenge, establishing MPAs, encouraging activities to reduce and prevent marine pollution, strengthening the legal framework, and incorporating international bodies to implement conservation actions need to take place. As the fate of our lives is bounded by the ocean, it is the core responsibility of every citizen to protect this magnificent creation of nature.

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NATURE CAPTURES



Pseudophilautus stellatus (Kelaart's starry shrub frog)
Photograph by Mr. Buddhika Abeyrathne



Pseudophilautus stellatus (Kelaart's starry shrub frog)

IUCN Red List status: Critically Endangered (CR)

The Kelaart's starry shrub frog, also known as the spotted shrub frog (*Pseudophilautus stellatus*) is an endemic and Critically Endangered (CR) frog species in Sri Lanka. This species was initially described by Edward Frederick Kelaart in 1853. Afterwards, there were no descriptions until 2009, and this amphibian was lost for 157 years. This species was rediscovered at Peak Wilderness Sanctuary by Mr. Mendis Wickramasinghe, Dulan Ranga Vidanapathirana, and their team. This is a unique frog that lives in mountain forests over 1500 m elevation, is a canopy-dwelling species nearly 1–10 m above ground level, and is extremely rare to explore. They have a unique body colour that appears bright green with scattered pinkish-white yellow spots outlined by a brown band, making some kind of star shape.



Photographs by

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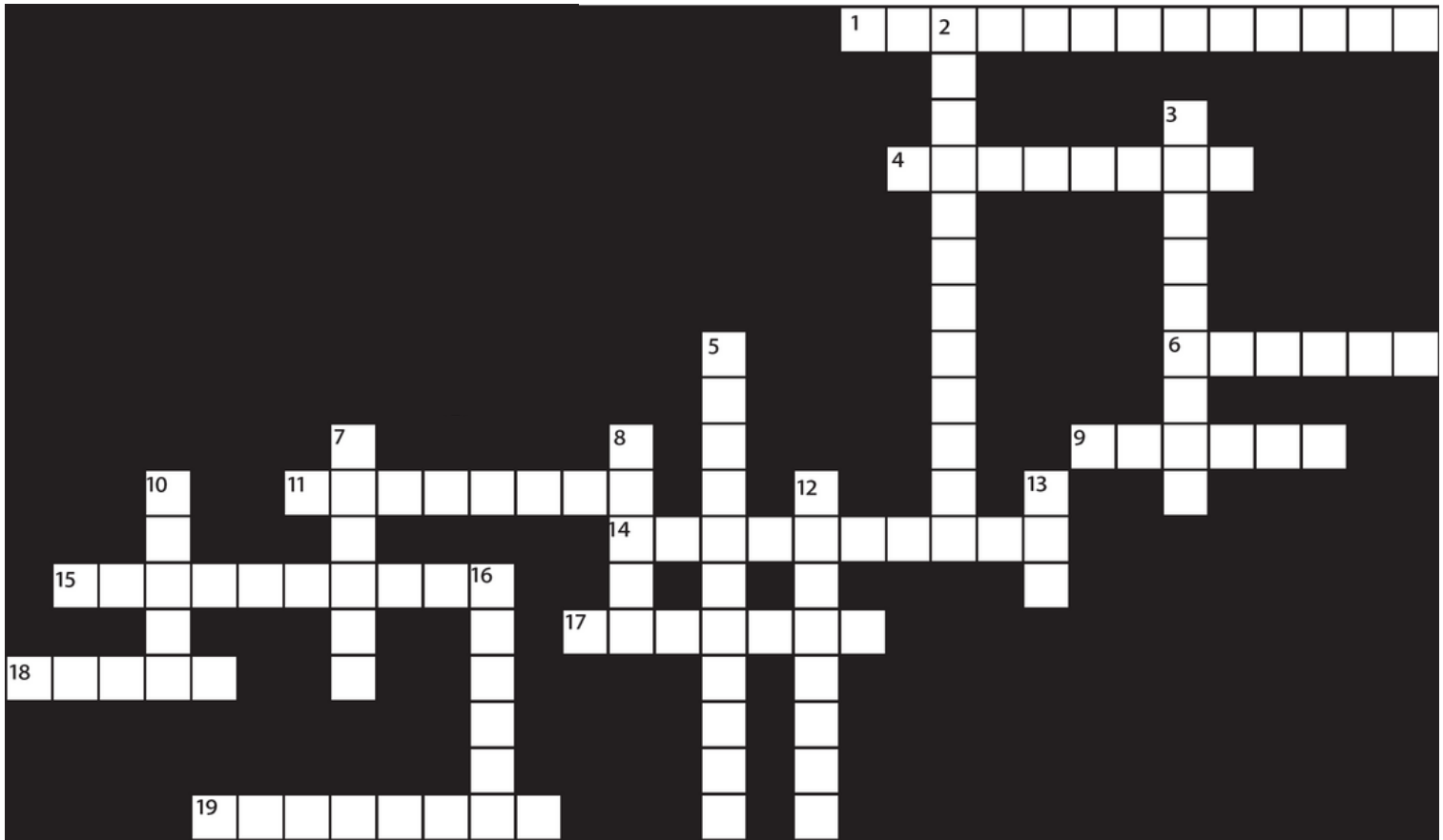


Pseudophilautus schmarda (Schmarda's shrub frog)

IUCN Red List status: Endangered (EN)

This Sri Lankan shrub frog species is endemic to the region and is commonly found in the central hills and montane forests at elevations ranging from 810 to 2000 m. It belongs to the family Rhacophoridae and was named in honour of Ludwig Karl Schmarda, an Austrian physician and naturalist. These canopy-dwelling creatures typically inhabit heights above 3 m above ground level and have rough, horn-like spinules and glandular folds on their upper body.

BIO-BrainBuster CROSSWORD



WORD BANK

ATAMBA
PATAU
LICHENS

RETROVIRUS
GOLDEN
PAEDOPHRYNE

HALMILLA
HORNBILL
TOQUE

RHODOPHYTA
STROMATOLITES
MIESCHER

NATIVE
RADIOBIOLOGY
SPLEEN

DARWIN
VESTIGIAL
MAY
PROTEINS

ACROSS

1. The living fossils and the oldest living life forms on the planet.
4. In 1868/9, Friedrich _____ performed experiments on the chemical composition of leukocytes that lead to the discovery of DNA.
6. _____ palm civet is endemic to Sri Lanka and is listed as Vulnerable on the IUCN Red List.
9. A wild species of mango tree endemic to Sri Lanka, which is in the Vulnerable category of the IUCN Red List.
11. *Berrya cordifolia* (Willd.) Burret is a deciduous native tree commonly known as _____ in Sri Lanka.
14. A group of marine algae.
15. A type/family of viruses that uses RNA as its genetic material.
17. Excellent bioindicators of atmospheric pollution.
18. The _____ macaque is a reddish-brown coloured Old world monkey endemic to Sri Lanka.
19. One of the most abundant biological macromolecules found in all living cells.

DOWN

2. A branch of science that deals with the action of ionizing radiation on biological tissues and living organisms.
3. Some consider the appendix to be a _____ organ in the human body.
5. A genus of microhylid frogs.
7. Indigenous plants are also called _____ plants.
8. Father of Modern Biology.
10. A syndrome caused by an extra copy of chromosome 13.
12. A family of tropical near-passerine birds found in the Old World.
13. Every year on _____ 22nd, the International Day for Biological Diversity seeks to increase awareness and understanding of biodiversity issues.
16. An abdominal organ that produces cells of the immune system.

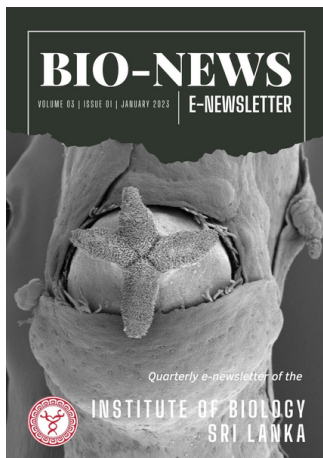
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BIO-NEWS

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Issue 1



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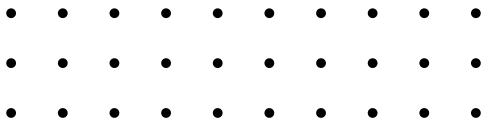
Issue 3



Issue 4

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