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**SRI LANKA**

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Theme

Biodiversity conservation: moving towards ecosystem services

Institute of Biology, Sri Lanka

September, 2015



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## About Institute of Biology, Sri Lanka

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The Institute of Biology is a leading professional body of biologists in Sri Lanka. Its current membership is over 400. 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 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 to 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 500 members, working in industry, research, education and healthcare. The institute also awards Fellowships and Charter of Biology status for members. There are 7 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), Associate Members, A.I.Biol (Sri Lanka) and Licentiates L. 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. Details of events are posted on the IOB website. The information provided on the web also keeps teachers informed on current events in the field of biology. 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. 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. It is continuing for the 35<sup>th</sup> time this year.

## Contents

<b>Council of the Institute of Biology, Sri Lanka 2014-2015</b> .....	3
<b>About Institute of Biology, Sri Lanka</b> .....	4
<b>PRESIDENTIAL ADDRESS Biodiversity conservation: moving towards ecosystem services</b> .....	10
<b>FELICITATION OF EMERITUS PROFESSOR NALINI BEATRICE RATNASIRI</b> .....	14
<b>ABSTRACTS OF PAPERS Parallel session 1</b> .....	17
Crown/Tree cover of ViharamahadeviPark, Colombo B D Madurapperuma and K A J MKuruppuarachchi .....	18
The responses of nursery plants of <i>Camellia sinensis</i> (L.) O. Kuntze to shade M S A E Cooray and H I U Caldera.....	19
Utilization of morphological and growth related traits for identification of water stress tolerant <i>Camellia sinensis</i> (L.) O. Kuntze cultivars prior to field planting H M Y E Herath and H I U Caldera .....	20
Comparative study of <i>Pongamia pinnata</i> , <i>Annona glabra</i> and <i>Moringa oleifera</i> extracts on growth performances of <i>Basella alba</i> L. (Spinach) T H Kahagalla and R M C S Ratnayake .....	21
Quantitative vegetation study in Namunukula forest A G S Arambawathta, H S Kathriarachchi, A M A S Attanayake, Y S Athugala .....	22
Potential of the common liverwort <i>Ricciasorocarpa</i> Bisch. as a bioindicator of selected heavy metals in the growth medium K Vignarasa, L N S Liyanage, K M Mohotti and P S Saputhanthri .....	23
A protocol to preserve flower texture___N P S N Karunarathne and P S Saputhanthri .....	24
<i>In vitro</i> propagation of the thalloid liverwort <i>Riccia sorocarpa</i> Bisch. N N Munasinghe, L N S Liyanage and P S Saputhanthri .....	25
Influence of <i>Chromolaena odorata</i> leaf extracts on seed germination, seedling growth and growth performance of <i>Abelmoschus esculentus</i> L. (Okra) and <i>Vigna unguiculata</i> L.(Bushita)___V W Rathnayake and R M C S Ratnayake .....	26
Floristic diversity of thotupolakanda upper montane rain forest H D R V L Harasgama ,R M C S Ratnayake <sup>1</sup> , A M A Attanayake and M P T Wijewickama .....	27
<i>Acacia auriculiformis</i> (Fabaceae), a threat to mangrove forest in Rekawa lagoon, Sri Lanka: A case study S K Madarasinghe, K A S Kodikara, N P Dissanayake and L P Jayatissa.....	28
A preliminary study on nutritional quality of an indigenous rice variety (Kuruluthuda) and a hybrid rice variety (BG 358)___P G I J Gamage and M D Amarasinghe .....	29
Effect of fungal endophyte <i>Arthrographison</i> growth of rice varieties Herath Banda andBg352___P V A R Ponnawila and N Deshappriya .....	30
Efficacy of liquid organic fertilizers on growth of <i>Anthurium andraeanum</i> L J M N P Jayasundara, L R Jayasekara and R M C S Ratnayake .....	31

Assessment of invasion of <i>Najas marina</i> , Linnaeus 1753 in Madu Ganga Estuary, Sri Lanka using ASTER data of Terra satellite	
T M S D G Silva, D D G L Dahanayaka and M J S Wijeyaratne.....	32
<b>ABSTRACTS OF PAPERS Parallel session 2</b> .....	<b>33</b>
Climatic and soil preferences of tiger beetles (Coleoptera, Cicindelidae) of Sri Lanka	
A Thotagamuwa, C D Dangalle, N Pallewatta and E Lokupitiya.....	34
Survey of molluscan shells from the Jaffna Estuary, Sri Lanka	
D Ai Reval and A Sivaruban.....	35
Enhancement of immunity in cultured shrimp, <i>Penaeus monodon</i> induced by <i>Achyranthes aspera</i> (Sin. Karal heba, Family: Amaranthaceae) compared to a commercial immune enhancer	
K V D H R Karawita and M Hettiarachchi .....	36
Protection of cultured shrimp, <i>Penaeus monodon</i> from white spot disease (WSD) with enhanced immunity induced by <i>Achyranthes aspera</i> (Family Amaranthaceae) compared to a commercial immune enhancer	
K V D H R Karawita and M Hettiarachchi .....	37
Possibility of preventing Acute Hepatopancreatic Necrosis Disease (AHPND), a killer disease in cultured shrimp caused by a unique strain of <i>Vibrio parahaemolyticus</i> if the strain enters into Sri Lankan culture systems	
M Hettiarachchi, D C Hettiararchi and K R P S Kumara .....	38
Selection of White Spot Virus (WSV) and Monodon Baculo Virus (MBV) free brood stocks of cultured shrimp <i>Penaeus monodon</i> , from Sri Lankan coastal sea to produce healthy post larvae	
K R P S Kumara and M Hettiarachchi .....	39
A simplified version of <i>ex ovo</i> cultivation method of chicken embryos as a model for evaluating venom toxicity	
M M Silva, C L Goonasekara, S S Senevirathn, D K Weerakoon .....	40
Occupational Paraquat exposure among sugarcane and vegetable farmers in Sri Lanka: A case study	
K S Mohammed Abdul, D V Eakanayake, T D K S C Gunasekara <sup>1</sup> , H A S D Perera, B C J De Silva, C Jayasumana, E P S Chandana, S S Jayasinghe and P M C S De Silva <sup>1</sup> .....	41
Establishing dietary and faecal relationships for crude protein and crude fibre in selected native herbivorous mammals in Sri Lanka	
A.U. Jayawardhana, C. V. Nelundeniya, R. D. Wijesekera and M. R. Wijesinghe .....	42
<i>In vivo</i> antioxidant activity of mature leaf concentrate of Sri Lankan wild type <i>Carica papaya</i> .L variety against carbon tetra chloride induced oxidative stress in rats	
C D Jayasinghe, W D Ratnasooriya and P V Udagama.....	43
Modulation of <i>in vitro</i> phagocytic activity, cell proliferation and cytokine production in the Wistar rat model by a Sri Lankan <i>Haliclona</i> ( <i>Soestella</i> ) sp sponge crude extract	
V K Gunathilake, W D Ratnasooriya and P V Udagama .....	44
Nest occurrence, mean nest density and relative nest abundance of <i>Aneuretus simoni</i> Emery and associated ant fauna in Meethirigala Forest Reserve	
R K S Dias and W S Udayakantha.....	45

An investigation of sex differences in feeding and vigilance behavior in Hanuman Langurs using fractal analysis_S Malluwawadu, K Premachandra, R Vandercone.....	46
Assessment of environmental pollutants using fledgling feathers of Little egret ( <i>Egretta garzetta</i> ) as a bio monitoring tool in Sri Lanka R L Jayaratne, I C Perera, D K Weerakoon, and S W Kotagama.....	47
Comparison of larvicidal and repellent efficacy of <i>Ocimum basilicum</i> (L.); “Maduruthala”, leaves and pods, against dengue vector, <i>Aedes aegypti</i> (L.) W L B P Abhayawickrama, G A S M Ganehiarachchi and P A Paranagama.....	48
<b>ABSTRACTS OF PAPERS Parallel session 3</b> .....	49
Superoxide and nitric oxide radical scavenging activities of bark and leaf of Ceylon cinnamon ( <i>Cinnamomum zeylanicum</i> Blume) <i>in vitro</i> W P K M Abeysekera, G A S Premakumara and W D Ratnasooriya .....	50
Antioxidant properties of brans of twenty nine rice ( <i>Oryza sativa</i> L.) varieties of Sri Lanka _W K S M Abeysekera, U K D S S Gunasekara, G A S Premakumara, W P K M Abeysekera and P Ranasinghe.....	51
Comparative GC-MS study of chemical constituents in essential oils of Ceylon Cinnamon ( <i>Cinnamomum zeylanicum</i> Blume) bark oils collected from different geographical locations__H D Weeratunge, S K Ganegamage and G A S Premakumara .....	52
Inhibitory effect on human leukemia (HL-60) cancer cell proliferation <i>via</i> caspase-3 mediated apoptosis by <i>Costus speciosus</i> (Koen.) Sm. leaf extract K W Samarakoon, H H C. Lakmal , P. T. Jayasooriya and Y-J Jeon.....	53
Microsatellite markers reveal the spatial genetic structure of dengue vector <i>Aedes aegypti</i> in selected areas in Colombo district . M D Nirmani, K L N Perera and G H Galhena.....	54
Nutritional composition, fatty acid profile and antioxidant activity of selected traditional rice ( <i>Oryza sativa</i> L.) varieties of Sri LankaY Sutharsana, M D W Samaranayake, W K S M Abeysekeraand H M T Herath .....	55
Chemical composition of inflorescence of <i>Alpinia calcarata</i> Rosc. (Zingiberaceae) grown in the western province of Sri Lanka S K Ganegamage and L D A M Arawwawala .....	56
Ethyl Methyl Sulfonate (EMS) induced herbicide resistance in seed-derived rice ( <i>Oryza sativa</i> ) callus__E M S I Ekanayaka, S R Weerakoon,T D Silva and S Somaratne.....	57
Antimicrobial potential of the endophytic fungal extracts of <i>Mangifera zeylanica</i> ( <i>Anacardiaceae</i> ), an endemic plant of Sri Lanka, against selected pathogenic bacterial species H D A A Senevirathna, E D de Silva, C D Wijayarathna and R L C Wijesundara.....	58
Sequence changes responsible for C <sub>3</sub> to C <sub>4</sub> transition of Phosphoenolpyruvate carboxylase (PEPC) of cereals at the DNA and protein levels using bioinformatics tools N M P M Nawarathna and T L S Tirimanne.....	59
Feasibility of using Exon-Primed Intron-Crossing (EPIC) markers to detect the genetic variation of a dengue vector in Sri Lanka	

M D Nirmani, K L N Perera and G H Galhena.....	60
<i>In vitro</i> Anti-5-lipoxygenase, anti-hyaluronidase and anti-oxidant properties of ethanol leaf extract of <i>Diospyros ebenum</i>	
H D S M Perera, R Samarasekera, S Handunnetti and O V D S J Weerasena .....	61
Antioxidant properties of leaves of <i>Aporosa lindleyana</i> Baill. (Kebella)	
S. Kathirgamanathar, D. M. K. P. Weerasinghe, W. P. K. M Abeysekera, P. Ranasinghe and A. M. C. U. Binduhewa .....	62
Isolation and cloning of thermostable alpha amylase gene for the production of recombinant enzyme for industrial purposes_ M S Thiwanka, W W P Rodrig, H H K Achala, A M M H Athapaththu and P A D H N Gunathilaka .....	63
A phylogenetic analysis of <i>Dinopium</i> woodpeckers in Sri Lanka using Cyt b and COI nucleotide sequences (Aves: Piciformes) S P Fernando and S S Seneviratne .....	64
<b>ABSTRACTS OF PAPERS Parallel session 4</b> .....	65
Comparison of four DNA extraction methods for target bacteria found in bovine milk for large scale detection of mastitis pathogens_ R M P C D Rajapaksha, D Senevirathna, I C Perera, D K Weerakoon, C M Nanayakkara .....	66
Rice rhizosphere manipulation with <i>Trichoderma virens</i> for effective phosphorous management_ P N Gallage, C M Nanayakkara and D N Sirisena .....	67
Screening of native actinomycetes for potential antimicrobial activity	
L D W Kekulandala and C M Nanayakkara.....	68
Morphological and reproductive characterization of <i>Colletotrichum</i> spp. causing anthracnose of papaya in Sri Lanka	
S Rasakulendran, R L C Wijesundara and C M Nanayakara.....	69
Antibiotic Sensitivity of <i>Bacillus thuringiensis</i> strains isolated from Sri Lanka_ R Y Baragamaarachchi, De Silva Kande Y, O V D S J Weerasena and R Samarasekara .....	70
Multivariate discrimination of inflorescence characters in conserved <i>Cocos nucifera</i> L. var. <i>typica</i> germplasm in Sri Lanka	
K N S Perera, D P S T G Attanayaka and S A C N Perera.....	71
Screening of rice for resistance against <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> through anther culture_ K A S R Perera .....	72
Determination of the antifungal, biochemical and physiological characteristics of <i>Trichoderma</i> spp. isolated from onion fields of Sri Lanka	
L N R Gunaratna, N Deshappriya and R G A S Rajapakse .....	73
<i>Sclerotinia sclerotiorum</i> causing cabbage head rot in Sri Lank	
B M A Guruge, K P Somachandra and R N Attanayake,.....	74
Antifungal effect of <i>Croton aromaticus</i> against <i>Rhizopus</i> spp. isolated from banana and papaya_ S A D T L Wijesundara and B T S D P Kannangara.....	75
Soil fungi of semi natural montane forest and adjacent pine plantation in Peacock hill, Pussellawa, in Nuwara Eliya district_ R G K Dharmasiri and B T S D P Kannangara .....	76
Evaluation of antibacterial properties of endophytic fungi of <i>Cyperus brevifolius</i> and <i>Cyperus melanospermus</i> _ R C Walgama, P B Ratnaweera, and E D de Silva.....	77



Evaluation of antibacterial properties of endophytic fungi inhabiting the grasses <i>Cyperus rotundus</i> and <i>Cyperus pilosus</i> _S H Liyanage, P B Ratnaweera, E D de Silva.....	78
Study of diversity and abundance of microfauna and microflora associated with selected mosquito breeding habitats in Gampaha district in Sri Lanka K D K M Karunathilake and L D Amarasinghe .....	79
Nematicidal activity of aqueous extracts and dry matter of <i>Tithonia diversifolia</i> , <i>Gliricidia sepium</i> and <i>Tagetes erecta</i> against root-knot nematode, <i>Meloidogyne incognita</i> (Kofoid and White) on tomato ( <i>Lycopersicon esculentum</i> Mill.) N W Premachandra and L D Amarasinghe.....	80

## **PRESIDENTIAL ADDRESS**

### **Biodiversity conservation: moving towards ecosystem services**

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#### **Human Population Explosion and its impacts**

The world has changed dramatically and in fact will continue to change melodramatically. We no longer live in a world that we lived in 20 years ago. Today, in the modern world, humans are intensely altering our ecological life support system and the natural environment has been altered to the extent that all living beings including the man himself are facing countless problems. Global human population growth amounts to around 75 million annually, or 1.1% per year. The global population has grown from 1 billion in 1800 to 7 billion in 2012. It is expected to keep growing, where estimates have put the total population at 8.4 billion by mid-2030, and 9.6 billion by mid-2050. It is fair to state that no other species that inhabited this planet underwent such a population explosion within such a short time.

These growing populations all over the world need shelter, food and other amenities, in fact, on a much larger scale than any other species living on earth. Each person uses far more land than the few feet they actually occupy. We use cropland to grow food, grazing land for meat and dairy, and oceans for fishing. We alter the land for habitation, agriculture, transportation, power generation and commerce. An average citizen in a developed country (European or American), owing to this luxurious lifestyle, needs 10-20 acres to supplement his needs. Our planet's ability to provide an accommodating environment for human wants (rather than needs) is being diminished continuously. This unrelenting growth of human population and overutilization of vast amount of resources by a single species has caused detrimental impact on natural resources. We have replaced natural forest with monocultures, polluted air and water bodies and exploited natural resources irresponsibly. Consequently, scientists claim that our species has caused 322 animal extinctions over the past 500 years, with two-thirds of them occurring in the last two centuries. It has been estimated that animals extinct 100 to 1,000 times (some scientists even claim 1,000 to 10,000 times) faster than at the normal extinction rate, which is about 10 to 25 species per year. If this trend continues many researchers warn that we are in the middle of a mass extinction even faster than the Cretaceous-Tertiary extinction which wiped out the dinosaurs.

Unlike the previous mass extinctions, the next extinction could be due to the actions of a single species, us humans. Although some may argue this is implausible, we only have to look at the numbers of animals that are extinct or threatened by our actions. It has been estimated that about 17,000 species are threatened with extinction in the foreseeable future. These include not only some unknown invertebrates, but also familiar species such as the Polar Bear, Hippopotamus, sharks, freshwater fish and Mediterranean flowers. Marine species are proving to be just as much at risk as their land-based counterparts.

## **Sri Lankan situation**

Sri Lanka, with a total land area of 65,610 km<sup>2</sup> is a tropical island situated in the Indian Ocean. The tropical climate, with evergreen natural forests supports a vast biological diversity in the island and is designated as one of the world's biodiversity hotspots. Sri Lanka has greater biodiversity per unit area than any other country in Asia. When the biological diversity of 8 Asian countries (Sri Lanka, Malaysia, Vietnam, Philippines, Thailand, Myanmar, Indonesia, India) is compared and ranked according to the average number of species per 10,000 m<sup>2</sup>, Sri Lanka has the highest number of species in mammals, amphibians, reptiles and flowering plants. As elsewhere in the world, Sri Lankan biodiversity is also threatened by the anthropogenic activities.

Along with the increase of the human population, the forest cover which had been approximately 80% of the total land area has now decreased to about 30% and the percentage of closed-canopy natural forest areas is 24%. Between 1956 and 1983 the natural forest cover of Sri Lanka fell from 2.9m ha to 1.75m ha. If this reduction continues at this rate, all natural forests will be gone by the year 2030. The loss of natural forests in Sri Lanka is directly related to the increasing human population. The human population that had been 2.3 million in 1870 has increased to 20.3 million in 2012. Natural forests in Sri Lanka has been cleared by colonial rulers for agriculture, mainly for tea and rubber. In the recent past, ambitious development projects such as accelerated Mahaweli scheme and recent agricultural projects such as Pelwatte Sugar Industries resulted in massive scale deforestation in the island. Mature and well-established forest plantations cover an area of about 72,340 ha (only 1.1 % of the total land area of the country). Out of this approximately, 15,600 ha is under Conifers, 8,400 ha is under Eucalyptus, and about 33,000 ha is under Teak plantations. In addition to the above, fuelwood plantations also occupy over 13,000 ha.

These natural forests are the home and breeding, foraging and roosting habitats of the fauna of Sri Lanka. The rapid loss of the natural forests in Sri Lanka had drastic in fact on most of animal species. According to 2012 IUCN report, five species of plants and 18 species of Amphibians have not been recorded in last 100 years or already extinct. Another 18 species of fish, 42 species of amphibians, 42 species of reptiles, 8 species of birds and 11 species of mammals are categorised as critically endangered.

## **Sri Lankan Bats**

The threat to our wildlife can be best illustrated by taking the population changes that have taken place in Bats. Sri Lanka was endowed with a very rich resource of bats, with 30 species. In fact Chiropterans are the largest order of mammals and bats comprise about 1/3 of mammals in Sri Lanka. Their status had been drastically changed in the recent past. Our studies since 1986 to date have shown that bats are one of the highest endangered species in Sri Lanka. Several species that had been described as common 100 years back are in fact rare or not been recorded since then. Four species of bats (*Tadarida aegyptiaca*, *Chaerephon plicatus*, *Falsistrellus affinis* and *Scotophilus kuhli*) have not been recorded after the survey by Phillips in 1933. About five species of bats (*Scotophilus heathii*, *Kerivoula hardwickei*, *Hesperoptenus tickelli* and *Saccolaimus saccolaimus*) have been recorded either only once or twice. The alarming factor is that the population sizes of even some common species are decreasing drastically. For example, in a survey carried out by Ruebsamen and coworkers, six species of cave dwelling bats have been recorded in 18 locations. When these roosts were revisited 7 years later, bats have disappeared from 8 locations. This threat to bats is still

continuing, perhaps on a larger magnitude. During a survey of Sri Lankan bats carried out by us between 1995 - 2000, it was noted that bats have disappeared from 17 locations, when the observed sites were re-examined at the end of the study. Our observations have shown that bats are under constant threat of direct and indirect actions of humans. Deforestations, destructions and disturbance in the roosting sites, as well as illegal hunting pose direct threat to the survival of bats. Caves are often visited by hunters and large numbers of trapped bats can be easily killed.

### **Valuing Nature – Ecosystem Services**

Biodiversity plays an important role in ecosystem function and in the many services that ecosystems provide. Some of these include nutrient and water cycling, soil formation and retention, resistance against invasive species, plant pollination, climate regulation, and pest and pollution control. Escalating biodiversity loss in any country will definitely have serious implications for human wellbeing and sustenance. Unfortunately, we have failed to realise this immense monetary value of the biodiversity, which probably is one reason for our failure to conserve biodiversity in Sri Lanka.

It is estimated that the monetary value of goods and services provided by ecosystems amounts to about 33 trillion dollars per year. An estimated 50,000-70,000 plant species are used in traditional and modern medicine worldwide. About 100 million metric tons of aquatic organisms, including fish, molluscs and crustaceans are taken from the wild every year and represent a vital contribution to world food security.

Insects are often considered as pests. Although some insects are pests and some are harmful, a large number of insects such as bees, wasps, butterflies, moths, hoverflies and beetles are extremely important for our survival. The estimated annual value of the ecological services provided by these insects in the United States alone is approximately \$57 billion. Many plants depend on insects for pollination. The value of crop production from pollination by native insects is estimated to be about \$3 billion in the USA alone.

The role played by bats (and many other species of wildlife) in maintaining a healthy environment and their immense economic potential had never been properly appreciated. Insectivorous bats may eat up to 80-100% of their body mass in insects on a nightly basis. A study carried out in the cotton dominated agroecosystems of southern Texas, USA, has shown that the contribution of bats to be \$12-\$173 per acre each year. By extrapolating these figures to the whole country, it has been estimated that economic contribution to the USA's agroecosystems to be between \$3.7 and \$53 billion/year. Fruit bats are known to pollinate over 500 plant species including mango, banana, cocoa, durian, guava and agave (used to make tequila). Some species of bats play a critical role in spreading the seeds of trees and other plants. Tropical fruit bats often carry seeds to a feeding site and then excrete the seeds far away from the original tree.

Our studies in Sri Lanka, carried out in two natural caves (Wavulgalge and Wavulpena cave) have shown that, insectivorous bat species in Sri Lanka, consume prey up to 30% of their body weight as determined by the weight difference before and after foraging. Our calculation showed that bat populations in these two caves consume over 1168 kg of insect prey each night. Thus, the weight of the insects consumed by these two cave population in a

year is estimated to be 426,420kg. The diet of these bats includes variety of insect prey from 15 insect orders.

We generally value those ecosystems and species we find useful or of interest to us, but we also have to recognize that other living beings have value aside from their utility. Each species (plant as well as animal) has a place in nature and has the right to survive. As humans, we have larger control over their existence. If we wish to, we can keep our population at sustainable levels and live in harmony with other species. If we do not appreciate the role played by other living beings and continue to disregard the right of other animals to share this planet with us, many species will soon disappear from this planet. If so, our descendants will not see the stars at night, will not experience wilderness and the incredible beauty of natural world.

## **FELICITATION OF EMERITUS PROFESSOR NALINI BEATRICE RATNASIRI**

Citation Presented by  
Dr. Nirmalie Pallewatta  
Department of Zoology, University of Colombo

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Prof. Nalini B. Ratnasiri hails from Ratnapura and had her early education at the Convent of Child Jesus, Ratnapura, and later at CMS Ladies College, Colombo. In 1960, she entered the University of Ceylon and read for a BSc Special Degree in Zoology and graduated with Second Class Upper Division Honours in 1964. Thereafter, she joined the University of Ceylon, Peradeniya Campus as an Assistant Lecturer in Zoology. In 1966, she decided to be an applied scientist and took up a research position at the Central Agricultural Research Institute of the Department of Agriculture, Gannoruwa. She proceeded for post-graduate studies to the University of Illinois, USA on a Fulbright-Hays Scholarship followed by a Ford Foundation Scholarship.

She returned to Sri Lanka in 1973 with a PhD in Entomology and served the Department of Agriculture as a Research Officer at the Dry Zone Research Station at Maha-Illuppallama for a short period of time. From 1974 to 1983, she worked at the Department of Forest Conservation as a full-time research entomologist. In this position she taught as a visiting lecturer to several universities, Kelaniya, Peradeniya, Sri Jaywardenapura, and the Faculty of Science, University of Colombo. In 1983, she joined the Open University of Sri Lanka as the Professor of Zoology in which position she continued up to the time of retirement in 2005. She is the founder Professor of Zoology at the Open University and became the Senior Professor of Zoology in 1986. Prof. Ratnasiri pioneered the teaching of Zoology in distance education giving

the academic and administrative leadership. In 1986, Prof. Ratnasiri became the Founder Dean of the Faculty of Natural Sciences at the Open University and was appointed to the Chair in Zoology. She served as the dean for six years.

She has played a key role in the rapid development of the Open University that took place during her tenure as an academic and as the dean. The programme leading to the BSc degree in Natural Sciences was developed under her leadership. She was also instrumental in developing the BSc degree programme in Nursing at the Open University, the first such university programme in Sri Lanka. In recognition of her expertise and contributions to distance education in science, the Open University awarded her the D.Lit. degree (*Hon. Causa*). She is an Emeritus Professor in Zoology of the Open University.

She has contributed significantly to national development in her fields of expertise and as an administrator from a scientific field. She has served as a Member and Vice-Chairperson of the University Grants Commission in 1994. She also rendered services to several government departments, by her appointment to key committees such as the Department of Examinations, The National Education Commission, Ministry of Higher Education and Sri Lanka Country Office of United Nations Development Programme. The Quality Assurance Programme of the Ministry of Higher Education has gained from her contribution to establishment of benchmarks for the subject of Zoology. She is a past member of the Governing Board of the Natural Resources, Energy and Science Authority and the Post-Graduate Institute of Science of the University of Peradeniya. She has served the National Science Foundation as the Chief Editor of its Journal from 2005 and continues to do so at present.

She was appointed to the National Science and Technology Commission as a member in 2004 and as Chairperson in 2006 and served in that capacity up to 2012. Her key contribution at NASTEC was the development of the current National Policy on Science and Technology.

Professor Ratnasiri is a highly respected and much admired person in the university system and outside of it. She has been a teacher, a guide and a mentor to many of us who are in this room today. She is known for her ready smile, friendliness and humble attitude despite all her achievements. Above all she is a very warm and genuine person who has been a friend to so many of us who have had the privilege of being her students and colleagues. Many are the

occasions where we have enjoyed her generous hospitality when we have visited her home to discuss academic or other official matters.

The field of Biology, especially Entomology in Sri Lanka has gained much from Madam Ratnasiri's tireless efforts to promote the discipline. At a time when entomologists are becoming an endangered species themselves in Sri Lanka, we should record our sincere gratitude to Madam Ratnasiri for her selfless contribution to it. It is indeed very fitting and timely that the Institute of Biology, Sri Lanka is paying tribute to Professor Nalini Beatrice Ratnasiri, the biologist, in the presence of this distinguished gathering of academics and scholars.

Madam, on behalf of the Institute of Biology I wish you and your family the very best in all your endeavours, good health and contentment.



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# **ABSTRACTS OF PAPERS**

**Parallel session 1**

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**Crown/Tree cover of Viharamahadevi Park, Colombo****B D Madurapperuma<sup>1\*</sup> and K A JMKurupparachchi<sup>2</sup>**<sup>1</sup>Department of Environmental Science & Management, Humboldt State University, Arcata, California, USA,<sup>2</sup>Department of Botany, The Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka.\*bdm280@humboldt.edu

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Recognition of existing landscape designs, suitable tree species and proper maintenance of trees in an urban landscape is beneficial for planning future green spaces in cities. This study examines site-specific tree crown cover and floristic composition in Viharamahadevi Park, Colombo. The tree cover of the area was determined by digitizing over a Google Earth image and the tree cover was compared with estimated crown cover using *in-situ* data. The vegetation parameters such as diameter-at-breast height (DBH), height, and crown radius were measured in each tree. The extent of the park is 24.27 ha and the estimated green cover using a Google Earth image is 14.39 ha (59% from the total). The estimated crown cover from the ground survey for the park is 12.25 ha (50%). A total of 1194 individuals belonging to 89 species, 69 genera and 32 families were recorded in the park premises. The largest contribution of crown cover is given by *Tabebuia rosea* (2.4%), *Terminalia catappa* (2.2%), *Cassia fistula* (2.1%), *Mangifera indica* (2.0%) and *Terminalia arjuna* (2%). Considerable numbers of park trees have been replaced by the invasion of *Ficus* species. We recommend the following steps for best park management: (i) training the park staff on sustainable park management systems, (ii) appropriate management practices of park trees, such as introducing indigenous trees for gap areas and treatment for disease trees and (iii) facilitating habitat formation and protection of existing habitat for birds and bats.

## **The responses of nursery plants of *Camellia sinensis* (L.) O. Kuntze to shade**

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Shade is considered as a necessary management practice in tea nurseries. Two cultivars (TRI 2026 and TRI 4006) of young tea plants were exposed to five different shade conditions (full sunlight, 35%, 50%, 80% and natural shade) for 15 weeks. Thereafter number of morphological, physiological, anatomical and bio chemical parameters were measured and data were analyzed using SPSS 20.0. From measured morphological characters including plant height, number of leaves/leaf buds/branches/fruits, leaf area parameters (total leaf area (TLA), leaf area (LA), leaf area index (LAI), leaf area ratio(LAR), specific leaf weight (SLW)) showed maximum value under full sun light and lowest under natural shade. Furthermore, for above morphological parameters TRI 2026 showed a higher value than TRI 4006. While Stomatal density (SD) and stomatal length (SL) did significantly change for both cultivars all other measured anatomical parameters showed a significant response to shade. Leaf thickness, palisade layer thickness, allometric ratios and specific leaf weight (SLW) decreased significantly with increasing shade. Except for relative water content (RWC), all other physiological parameters such as dry matter accumulation (DMA) in different plant parts, relative growth rate (RGR), C ratio in shoot: roots, stomatal conductance and leaf temperature significantly changed among shade conditions and cultivars. Photosynthetic pigments and proline content showed a significant difference amongst shade treatments but did not show a clear pattern. There was also a tendency for chlorophyll pigments to be higher under high shade while carotenoids were low under the high shade. According to principal component analysis LA, LAI, LAR, height increment, DMA, pigment content and SLW were the factors accounted that mostly for the total variance which can be used to future screening programs. Results showed considerable flexibility of tea in adaptation and acclimation to different shade conditions. Therefore this study emphasizes the importance of regulation of shade in tea nursery management.

## **Utilization of morphological and growth related traits for identification of water stress tolerant *Camellia sinensis* (L.) O. Kuntze cultivars prior to field planting**

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It is predicted that rainfall distribution patterns will alter under climate change. This may lead to enhanced depletion of available water content in global agricultural systems including tea plantations in Sri Lanka. Tea is affected as it is a rain-fed, perennial crop and low-country, young tea plantations are particularly affected due to climatic conditions. According to previous studies carried out using TRI 2000 and TRI 3000 cultivars, tea plants alter morphological and growth parameters under water deficit in soil. Altered parameters could be used to screen water stress tolerant cultivars at nursery stage. A pot experiment was conducted using six-month-old, vegetatively propagated tea cultivars (TRI 4006, TRI 4053 and TRI 4049). Plants were exposed to two watering regimes (field capacity and 50% field capacity) for ten weeks. According to the results, all cultivars showed morphological and growth alterations under water stress. TRI 4006 and TRI 4053 responded in a similar way. The height increment of TRI 4053, TRI 4006 and TRI 4049 was reduced by 76.86%, 61.45% and 13.80% compared to control. Reduction of number of leaves in main axis, total leaf area (LA) and leaf area ratio (LAR) was observed for all cultivars. In reference to the control treatment, 112%, 98.75% and 90% reduction was recorded for net assimilation rate (NAR) of TRI 4053, TRI 4006 and TRI 4049 respectively. Dry matter partitioning pattern among shoots, roots and leaves was changed in experimental plants belonging to three cultivars. There were no significant changes in number of newly emerged branches, number of leaves in the branches and root length under the experimental conditions. According to Principal Component Analysis, plant height increment, SGR, RGR, LA, LAI, stem girth, number of leaves in main axis, dry matter accumulation to roots, leaves and shoot: root ratio mainly contribute to identify water stress tolerant cultivars at nursery stage. Therefore, these parameters could be incorporated in to future breeding programs.

## **Comparative study of *Pongamia pinnata*, *Annona glabra* and *Moringa oleifera* extracts on growth performances of *Basella alba* L. (Spinach)**

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Excessive use of agrochemicals creates adverse health and environmental impacts. However, organic farming maintains sustainable agricultural systems while sustaining the health of human and ecosystems. This research aimed to study utilization of cow urine based leaf and seed extracts of *Annona glabra*, leaves of *Pongamia pinnata* and *Moringa oleifera* for enhancement of growth performances of *Basella alba*. The extracts were prepared by aerobic digestion of 100 g of dried leaves or seeds with 600 ml of cow urine for 2-weeks and the same procedure was done with water. Nutrient analysis of all extracts was done to determine the total nitrogen (Kjeldhal method), total phosphorus (Molybdo-vanadate method), potassium, magnesium, calcium, zinc and iron contents (atomic absorption spectrophotometry). Eight foliar application treatments of 1:15 diluted extracts were prepared by different combinations of the above extracts and applied to *B. alba* at twice a week for two months. *Basella* plants were grown in randomized block design with six replicates. Commercial fertilizer (Maxicrop) and distilled water were used as the standard and the control respectively. Shoot height, number of leaves, leaf area, stem girth and fresh and dry weights of shoot and root biomasses of *B. alba* were determined. The data were analyzed using ANOVA in MINITAB R16 statistical package. Analysis of cow urine based leaf extracts of all three species showed higher nutrient contents than the aqueous extracts. *B. alba* applied with the combination of *P. pinnata* leaf and *A. glabra* seed extracts showed the significantly high ( $p < 0.05$ ) mean number of leaves ( $15 \pm 0.70$ ), dry shoot biomass ( $14.5 \pm 0.96$  g/plant), fresh shoot biomass ( $35.7 \pm 5.63$  g/plant) and mean shoot height ( $33.0 \pm 3.55$  cm). *B. alba* applied with the commercial fertilizer showed the significantly lower ( $p < 0.05$ ) dry shoot biomass ( $9.8 \pm 0.09$  g/plant), fresh shoot biomass ( $10.17 \pm 2.20$  g/plant) and mean number of leaves ( $9 \pm 0.80$ ) than the combination of *P. pinnata* leaf and *A. glabra* seed extracts applied *B. alba*. Growth performances of *B. alba* treated with *M. oleifera* extracts was significantly lower than that of *P. pinnata* leaf and *A. glabra* seed extracts. The prepared organic extracts can be used to increase yield of *B. alba* hence reducing the usage of agrochemicals.

## Quantitative vegetation study in Namunukula forest

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Namunukula is a montane range located in south eastern corner of the central highlands (06° 56'N and 081° 07' E) in Badulla district. Patches of Tropical Montane Cloud Forests (TCMF) are randomly scattered over Namunukula montane range. A study was conducted to perform a comprehensive quantitative vegetation survey of Namunukula forest. Sampling was carried out in 40 randomly placed plots, each 10×10 m<sup>2</sup>. Within each plot, the individual plants with gbh (girth at breast height) greater than 15 cm, was used to determine the tree and understory of the Namunukula forest. Four 1×1 m<sup>2</sup> sub plots were randomly demarcated in same 10×10 m<sup>2</sup> to enumerate the ground layer. Floristic richness of the tree, understory and ground layers were determined separately by calculating the relative density, relative frequency, relative basal area and Important Value Index (IVI). Total of 152 angiosperm species belonging to 92 genera and 51 plant families were enumerated. Among them, 66 plant species are being threatened including 1 critically endangered, 29 endangered and 36 vulnerable species. The tree layer of the Namunukula forest consists of 100 species belonging to 57 genera and 29 plant families. The understory layer comprised of 102 species belonging to 59 genera and 34 plant families. In the ground layer 98 plant species belonging to 64 genera and 36 families were recorded. Among the sampled plots 47% endemic angiosperm species were recorded. According to IVI values, *Acronychia pedunculata* has become the dominant species in tree layer followed by *Actinodaphne ambigua* and *Syzygium revolutum*. *Actinodaphne ambigua* and *Symplocos cochinchinensis* are dominant plants in understory layer. Ground layer of Namunukula forest was dominated by *Lasianthus foetulentus*, *Hedyotis neoleSSERTIANA* and *Psychotria zeylanica*. Cluster analysis of plant abundance data of sampled plots, revealed six different clusters that separated based on altitude.

## Potential of the common liverwort *Ricciasorocarpa* Bisch. as a bioindicator of selected heavy metals in the growth medium

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All species or biological processes cannot act as successful bioindicators. The bioindicator species are said to have a moderate tolerance to the environmental variability. Bryophytes have been known as bioindicators of different environmental conditions. Being 'primitive' terrestrial plants, bryophytes lack protective structures such as the cuticle and also a proper root system which make them to show visible injury symptoms even in the presence of minute quantities of pollutants. The objective of this study was to investigate common liverwort, *Ricciasorocarpa*, as a potential bioindicator for selected heavy metals in the environment. *Ricciasorocarpa* was grown under greenhouse conditions in the growth medium consisting of a range of concentrations, i.e. from 0 to 8  $\mu\text{mol/l}$ , of  $\text{Pb}^{2+}$  or  $\text{Zn}^{2+}$ . Growth parameters such as the diameter of the colony, branch length and width of thalli were measured and the percentage increase or decrease in these parameters was determined. Also, the biochemical parameters such as the total chlorophyll and carotenoid contents for the plants were determined by spectrophotometric analysis. The increasing concentrations of the two metal ions  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$  reduced the growth of *R. sorocarpa* significantly, especially in terms of branch length and width of thalli. Under the increasing concentrations of  $\text{Pb}^{2+}$ , *R. sorocarpa* showed a significant reduction in both the total chlorophyll content and carotenoid content ( $P < 0.05$ ). Under the increasing concentrations of  $\text{Zn}^{2+}$ , only the carotenoid content was significantly reduced ( $P < 0.05$ ). The plants were able to survive under the test concentrations of  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$  but with significant effect on growth- and biochemical parameters tested. Therefore this study concluded that *Ricciasorocarpa* could be a potential bioindicator for selected heavy metal polluted environments.

## A protocol to preserve flower texture

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Preserving flowers has been practiced since ancient times for various purposes, including mainly ornamental or decorative purposes and scientific purposes such as for botanical specimens. Although there are many techniques available to preserve flowers in the dried form, preserving flowers with their natural shape and texture is a difficult task. The main objective of this study was to develop a protocol to preserve the texture of flowers while retaining their natural colour. The protocol developed consists of several steps, including initial dehydration and colour fixing, then permeation and substitution of moisture by a polymer, and finally removal of the excess polymer and drying. The initial dehydration was carried out using a reagent consisting of thiourea, tertiary butyl alcohol and citric acid/or sodium citrate as previously reported to successfully preserve colours of many flower species. Next, a series of polymers were tested for their ability to substitute internal moisture of the tissue. Twenty four different treatments with triplicates were tested initially on the extent of texture retaining in *Rosa* sp. In the first step, although the flower tissue was dehydrated while retaining the colour, during the permeation and substitution, the colour was lost. Among the series of polymers tested, polyethylene glycol (PEG) could successfully substitute moisture in the tissues, thereby retaining the texture and shape, even though the colour could not be retained. The flowers treated thus retained the texture and shape even after two weeks of drying in silica gel, compared to the other treatments and the control. In addition to *Rosa* sp., this protocol successfully preserved the texture of *Dendrobium* sp., *Alstroemeria* sp., *Gerbera* sp., *Dianthus* sp., *Clitoria ternatea*, *Nymphaea pubescens* and *Nymphaea stellata*. However, for *Bougainvillea* sp. initial dehydration with absolute ethanol was successful and rest of the procedure was the same as above. Although the above protocol could not retain the natural colour after substitution by PEG, when an artificial colourant (e.g: red colour food dye) was incorporated to the PEG solution, the colour was absorbed by flowers and was retained after drying in Silica gel. Commonly available cosmetic hairspray was found to be a promising surface coating that can be used to further protect the texture preserved and dried flowers. Improvements of the protocol to retain the natural colours along with the texture and shape are currently underway.



***In vitro* propagation of the thalloid liverwort *Riccia sorocarpa* Bisch.**N N Munasinghe<sup>1</sup>, L N S Liyanage<sup>1</sup> and P S Saputhanthri<sup>1</sup><sup>1</sup>Department of Plant Sciences, Faculty of Science, University of Colombo, Colombo 03

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Propagation of bryophytes using *in vitro* culture techniques would be useful in many ways, particularly in *ex situ* conservation which is a novel approach to Sri Lanka. The aim of the reported study was to develop an *in vitro* propagation protocol for the thalloid liverwort *Riccia sorocarpa* Bisch. Establishment of initial axenic culture was successful from the newly grown gametophyte (immature thalli tips of 5 mm length). The best surface sterilization was achieved by dipping thalli tips in 5% Clorox™ (5.25% active NaOCl) for 2 minutes followed by washing with 700 ppm fungicide Bullet-50™ (Carbendazim 50%) and bactericide, Ampicillin Sodium™ (250 ppm) and three rinses with sterile distilled water. With the optimized surface sterilization procedure, percentage of survival of the tested propagules was observed to be 66%. For *in vitro* regeneration, both direct regeneration method and callus induction method were used. In the first approach, surface sterilized immature thalli tips were placed on the nutrient medium under aseptic conditions, and incubated under continuous light conditions provided by white fluorescence light of 1210 Lux at 24 °C. Several media were tested and quarter strength Murashige and Skoog medium containing mineral salts and vitamins, 100 mgdm<sup>-3</sup> inositol, with 0.7% (w/v) agar was observed to be the best medium for regeneration of gametophytic thalli to be morphologically similar to the plants grown under natural conditions. Under the direct regeneration method regeneration was found to be 90 %. In the second approach, callus induction was achieved from the surface sterilized thalli tips on MS medium supplemented with 1.0 mgdm<sup>-3</sup> NAA and 1.0 mgdm<sup>-3</sup> BAP. From the green, compact calli that developed after 14 days, shoots could be induced in MS supplemented with 0.5mgdm<sup>-3</sup> NAA+ 1.0 mgdm<sup>-3</sup> BAP. Rhizoid induction was achieved in MS medium without sucrose after 7 days. Percentage of regeneration of plants via callus induction method was 100%. Thalli obtained through callus showed optimum growth in 1/4×MS medium. *In vitro* culture of *R. sorocarpa* Bisch. was successful. The plants regenerated by this method can be reintroduced to their natural habitats or may be cryopreserved as means of conservation.

## **Influence of *Chromolaena odorata* leaf extracts on seed germination, seedling growth and growth performance of *Abelmoschus esculentus* L. (Okra) and *Vigna unguiculata* L.(Bushita)**

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*Chromolaena odorata* was introduced as an ornamental plant but presently it is an invasive species to Sri Lanka. The present study was aimed to study the potential of using its leaf extract as a liquid fertilizer for Okra (*Abelmoschus esculentus*) cultivar “Haritha” and Bushita (*Vigna unguiculata*) cultivar “BS1”. Aqueous leaf extracts (25, 50, 75, 100 gL<sup>-1</sup>) of *C. odorata* was tested for seed germination and seedling growth in Petri dishes (four replicates for each treatment containing 20 seeds each of above crops) and seed trays (eight replicates for each treatment and sand as the growth medium) by adding 10 mL each test extract and distilled water used as the control. Growth and yield performance of Bushita and Okra were studied with the same concentrations of *C. odorata* extracts, with five replicates in a field with completely randomized block design and by adding 300 mL of the extracts every other day. Growth parameters of Bushita and Okra were recorded 60 days after seed sowing. Results were statistically analyzed by MINITAB R.16. The laboratory experiment results showed that seed germination, root and shoot lengths of both Okra and Bushita were significantly ( $p < 0.05$ ) reduced by *C. odorata* leaf extracts compared to the control. With increasing concentrations of *C. odorata* extracts, rates of inhibition of seed germination were proportionally increased. Addition of 75 gL<sup>-1</sup> *C. odorata* leaf extracts on germinated seedlings showed significantly ( $p < 0.05$ ) higher growth improvement in the mean leaf area ( $369.81^a \pm 5.35$ ), number of flowers ( $11^a \pm 0.5$ ), leaves ( $21^a \pm 0.4$ ) and fresh weight of fruit ( $28.4^a \pm 0.17$ ) in Okra. However in Bushita, only the 50 gL<sup>-1</sup> treatment has showed higher total fresh fruit weights ( $158.5^a \pm 2.66$ ) and leaf area ( $264.44^{a \pm} 13.97$ ) than the control ( $119.6^c \pm 3.99$ ) and ( $189.26^{c \pm} 11.13$ ) respectively (One way ANOVA,  $p < 0.05$ ) and no significant effects was recorded in the other measured growth parameters in the other treatments, compared to the control. Although *C. odorata* leaf extract has less promising effects on Bushita, leaf extracts of *C. odorata* (75 gL<sup>-1</sup> treatment) can be successfully used to enhance the growth and yield performance of Okra.

## Floristic diversity of thotupolakanda upper montane rain forest

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Due to paucity of detailed floristic diversity information on Thotupolakanda upper montane rainforest, the study investigates its floristics. Floristic heterogeneity along an altitudinal gradient was studied using stratified random sampling with a total of 31, 10 m × 10 m randomly distributed plots at lower elevation (2200 m -2270m), middle elevation (2270m - 2340m) and higher elevation (above 2340 m). Number of plants and basal area of overstorey vegetation (plant species taller than 1 m and gbh>10 cm) were investigated. The remaining plants in the understorey vegetation was sampled with two randomly selected sub plots (1 m × 1 m) located in each main plot. The identity of the collected plant specimens were confirmed with the specimen at the National Herbarium, Peradeniya. During the study 1824 individuals were enumerated and 108 species of 64 genera belonging to 39 families were revealed. The overstorey consisted of 42 plant species and understorey consisted of 66 plant species. Out of the total recorded plant species, 48% of the species are endemic to Sri Lanka while overstorey comprises 67% and understorey comprises 46%. *Syzygium rotundifolium*, *Syzygium revolutum*, *Syzygiums clerophyllum*, *Rhodomyrtus tomentosa* and *Ochlandras tridula* are the dominant overstorey species while Rubiaceae and Myrtaceae are the dominant overstorey families. The understorey vegetation are dominated by *Rubiocordifolia*, *Rubusellipticus*, *Hedyotis coprosmoides*, *Garnotia exaristata*, *Yushania densifolia*, *Plectranthus* sp., *Rubusrugosus* and dominant families are Rosaceae and Rubiaceae. Shapes of the rank abundance curves of both overstorey and understorey plant species is similar. Alpha diversity was calculated in terms of species richness (Menhinick's Index and Margalef's Index) and proportional abundance (Shanon - Weiner Index and Shanon evenness calculation). Overstorey of lower elevation was the most diverse strata recorded with highest species richness and plant distribution while it was lowest in the higher elevation. Understorey of middle elevation was the most diverse strata recorded with highest species richness while it was lowest in the higher elevation. Beta diversity was calculated by similarity and Jaccard indices. For overstorey vegetation, the highest similarity was recorded between middle and higher elevations while for the understorey was between lower and middle elevations. For both overstorey and understorey vegetation, highest dissimilarity was recorded between lower and higher elevations. Due to the high rich floristic diversity conservation is an important aspect at Thotupolakanda.

## ***Acacia auriculiformis* (Fabaceae), a threat to mangrove forest in Rekawa lagoon, Sri Lanka: A case study**

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Sri Lanka is one of the global biodiversity hotspots but the biodiversity of the country is highly affected by various factors. Based on preliminary observations, *Acacia auriculiformis* has invaded the Rekawa lagoon region (06°03'N–80°50'E). Therefore, the main objective was to study the distribution and the abundance of *A. auriculiformis* at the periphery of the mangrove belt of Rekawa lagoon. The recent (2015) Google Earth imagery combined with field based observations were used for the initial survey and then randomly selected eight quadrates (40x40m<sup>2</sup>) were fully studied in 5 km stretch in Rekawa lagoon to get the species composition, their abundance and size classes. Simpson diversity index and Dominance Index were calculated for each sampling site. The total area that has been invaded by *A. auriculiformis* was 0.256 km<sup>2</sup> (i.e. 4.91% to total land cover of Rekawa lagoon region). The results revealed that, seaward side of the lagoon is at high risk of further invasion. Moreover, *A. auriculiformis* dominated at the periphery of the mangrove belt while co-occurring with the true mangrove species, and replacing conventional mangrove associates like *Acrostichum aureum*. About 65% of the *Acacia* plants were grown up to tree level (10-12m in height, 25cm GBH) and about 20% was in sapling stage and the rest was seedlings. Simpson's Index of Diversity for sampled area was 0.76 (SD+/- 0.06) though Dominance Index for *A. auriculiformis* in the seaward side plots was 0.421 (SD+/- 0.3). These results revealed that *A. auriculiformis* has already become a serious threat, compared to non-invaded plots, particularly to mangrove associates as it dominates at the landward margin of the mangrove belt. Therefore, this should be considered as an alarm to take necessary action to protect the mangrove ecosystem in Rekawa lagoon which is considered as a prominent life support system for coastal livelihood.

## **A preliminary study on nutritional quality of an indigenous rice variety (Kuruluthuda) and a hybrid rice variety (BG 358)**

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Rice (*Oryza sativa*) is one of the most important cereals in human nutrition, consumed by about 75% of the global population and provides 60 % of the food intake in Southeast Asia. Rice has been considered as the queen among cereals for its nutritional quality and higher digestibility. The study was carried out to compare the nutritional qualities of two rice cultivars, i.e. an indigenous cultivar, Kuruluthuda that has grown without using agrochemicals and the hybrid cultivar, BG 358 grown with agrochemicals. All rice samples were milled and using a motor and pestle and the rice samples were fragmented and used in triplicate samples. Protein Content, reducing sugar content, non-reducing sugar content, fat content, amylose content and crude fiber content in two rice cultivars were determined. protein content was determined as total N present, using Kjeldal method. Sugar and amylose content were determined using starch – iodine titration method while lipid content was determined using chloroform extraction method. Crude fiber content was determined using Soxhlet extraction method. The rice data were checked for normality (Anderson- Darling test; available in MINITAB release 16.1) and two sample T-test and ANOVA were performed to determine significance difference between rice in two cultivars. Indigenous (Kuruluthuda) rice variety was contained 8.46% protein, 0.73% sugar, 0.26% fat, 0.32% amylose and 0.30% crude fiber. Hybrid (BG 358) rice variety was contained 7.43% protein, 0.85% sugar, 0.27% fat, 0.25% amylose and 0.20% crude fiber. The indigenous (Kuruluthuda) rice therefore contains more proteins, crude fiber, amylose and less sugar and fat, thus can be considered nutritionally superior to the hybrid (BG 358) cultivar.

## **Effect of fungal endophyte *Arthrographis* on growth of rice varieties Herath Banda and Bg352**

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Numerous chemical fertilizers are used in rice cultivations in order to increase yield. As these chemicals have deleterious effects on the environment as well as human health, the use of beneficial microorganisms as biofertilizers is a viable alternative. Endophytic fungi which reside inside the plants intercellularly or intracellularly have the potential to be used as biofertilizers as they are known to promote plant growth. With this in view, the effect of *Arthrographis*- an endophyte of rice plants, on plants of rice varieties Herath Banda and Bg 352 was determined by developing a hydroponic system and the results compared with those of pot experiments. Surface sterilized healthy seeds of rice varieties Herath Banda and Bg 352 were inoculated with the rice endophyte *Arthrographis* spp isolated previously from field grown plants of the two varieties. The soaked seeds of each variety were placed on the fungal culture grown in potato dextrose agar (PDA). After 5 days of incubation, the inoculated and germinated seeds were transferred to hydroponic systems and soil in pots to observe the effect of the fungal endophyte on the growth of Herath Banda and Bg 352 rice varieties. The hydroponic system was designed by floating rigid foam boards with 30 perforations in each board in 1.5l Hoagland solution placed in plastic trays. One inoculated rice seedling was placed in one perforation and 30 inoculated rice seedlings of each variety were placed in 30 perforations in rigid foam boards. There were 5 replicate trays for each variety. For the pot assay, soil from a paddy field was added to pots with 7cm diameter and 10cm height. 5 rice seedlings of each variety inoculated with the fungus were planted in each pot. Hydroponic trays and pots were placed in a greenhouse according to complete randomized block design at average temperature 30°C day and 20°C at night for 5 weeks. For the controls, seeds placed on PDA plates without the fungus were used for both pot and for hydroponics experiments. Dry weight, shoot length and root length of 10 rice plants selected randomly from hydroponic systems and 10 rice plants selected randomly from pots were measured at 2 week intervals. Results were analyzed using ANOVA and the pairwise comparisons using T test. Shoot length, root length and dry weight of two week old plants of both Bg 352 and Herath Banda varieties inoculated with the rice endophyte *Arthrographis* showed a significant increase in shoot length, root length and dry weight ( $P \leq 0.05$ ) when compared with non-inoculated plants grown in pots and in hydroponic systems. All growth parameters of Herath Banda and Bg 352 rice plants grown using the hydroponic system were significantly higher ( $P \leq 0.05$ ) than those grown in pots indicating that the effect of endophyte inoculation was significantly better manifested when the plants were grown using the hydroponic system.

## Efficacy of liquid organic fertilizers on growth of *Anthurium andraeanum* L.

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Liquid organic fertilizers (LOFs) are considered as important alternatives to synthetic fertilizers which cause negative implications on human health and environment. This study aimed at developing LOFs using widely abundant weeds in combinations with poultry manure or fish waste and to evaluate the potential of formulated LOFs in meeting nutrient requirements of *A. andraeanum*. Six combinations (F<sub>1</sub>: Poultry manure + *Tithonia diversifolia* + coconut husk ash, F<sub>2</sub>: Poultry manure + *Gliricidia sepium* + coconut husk ash, F<sub>3</sub>: Poultry manure + *Leucaena leucocephala* + coconut husk ash, F<sub>4</sub>: Fish waste + *Tithonia diversifolia* + coconut husk ash, F<sub>5</sub>: Fish waste + *Gliricidia sepium* + coconut husk ash, F<sub>6</sub>: Fish waste + *Leucaena leucocephala* + coconut husk ash) were prepared as water extractions. In each combination 360 g of leaves, 240 g of poultry manure or fish waste and 100 g of coconut husk ash were mixed with 6.0 L of well-water in closed plastic containers. Combinations were aerated for two hours daily for a six week period to facilitate decomposition. Based on the highest nutrient contents (N, P, K, Ca, Mg and Zn), F<sub>1</sub>, F<sub>2</sub> and F<sub>4</sub> were selected for the foliar application. Control plants were treated with well-water and commercial LOF "Maxicrop" was used as the standard. The pot trial was conducted in a complete randomized block design maintaining four replicates. Original, half and quarter strengths were applied on three months old *A. andraeanum* plants to evaluate the growth performance in terms of number of flowers, leaf area, shoot height and number of tillers. Growth parameters were compared by one way analysis of variance. The MINITAB 16 software was used for all analyses. The results revealed significant ( $p < 0.05$ ) difference in growth performance with different fertilizer treatments. The highest shoot height ( $26 \pm 0.3$  cm), number of flowers ( $2 \pm 0.4$ ) and number of tillers ( $2 \pm 0.4$ ) were observed with F<sub>2</sub> treatment. Therefore, F<sub>2</sub> proved to be the best for *A. andraeanum*. Application of half and quarter strengths of F<sub>2</sub> recorded  $2 \pm 0.0$  and  $1 \pm 0.2$  number of flowers respectively. Similarly half and quarter strengths of F<sub>2</sub> recorded  $1 \pm 0.4$  and  $1 \pm 0.2$  number of tillers respectively. These values were comparatively lower than the values obtained for undiluted F<sub>2</sub>. Therefore, original or undiluted F<sub>2</sub> LOF was the best strength for foliar application.

## Assessment of invasion of *Najas marina*, Linnaeus 1753 in Madu Ganga Estuary, Sri Lanka using ASTER data of Terra satellite.

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*Najas marina* (Family Najadaceae) is one of the nine invasive alien floral species that has been identified in the Ramsar site of Madu Ganga Estuary. Advanced Spaceborne Thermal Emission Radiometer (ASTER) is an imaging instrument onboard Terra, the flagship satellite of *National Aeronautics and Space Administration's* (NASA) Earth observing systems. The objective of the present study was to assess the invasion of *N. marina* (Spiny Water Nymph) in Madu Ganga Estuary using Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) data obtained from the Terra satellite. Cloud free ASTER imageries with 15m resolution of the study site were atmospherically corrected using the Fast Line-of-sight Atmospheric Analysis of Spectral Hypercubes (FLAASH) in Environment for Visualizing Images (ENVI) software version 5. The Normalized Difference Vegetation Index (NDVI) was calculated for the study site for each image and the distribution maps of *N. marina* were developed for December 2007, December 2009, December 2013 and April 2014. The map developed for April 2014 was validated using ground data and the Kappa coefficient of accuracy was calculated (K=1.0). The percentage coverage of *N. marina* was calculated through a supervised classification using ArcGIS software version 10.3. According to the derived distribution maps, *N. marina* was distributed in about 31% of the estuary in April 2014. The highest densities were mostly found in bay areas and peripheral areas except for the northern region of the estuary where it was found in the middle areas also. Maps developed for December 2007 (36%), December 2009 (14%) and December 2013 (24%) indicated that there is a temporal variation in the distribution of *N. marina* over the years. The overall distribution in of *N. marina* has decreased from December 2007 to December 2009 (36% - 14%) and increased from December 2009 to April 2014 (14% - 31%) reaching a coverage more or less similar to that of December 2007. Low water levels and stagnation of water appears to be conducive for the variation of this species. Hence, the future management of the invasion of this species may take these factors into consideration.



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# **ABSTRACTS OF PAPERS**

**Parallel session 2**

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## Climatic and soil preferences of tiger beetles (Coleoptera, Cicindelidae) of Sri Lanka

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Tiger beetles are active predators of small invertebrates in a wide spectrum of habitats. Over 2300 species of tiger beetles are recorded globally in both tropical and temperate countries. Fifty-five species of tiger beetles have been recorded from Sri Lanka of which thirty-two are known to be endemic to the country. Understanding habitat requirements of tiger beetles is important in identifying critical habitats for conservation, modeling distribution and indicator studies. The present study investigated microhabitat soil and climatic parameters for one hundred twenty-five locations to evaluate the preference criteria for tiger beetles. Locations were selected based on information in previous publications and collections in museums and other research institutions and represented all districts and provinces of the wet, dry and intermediate zones of Sri Lanka. Climatic and soil parameters of each location was recorded using standard equipment and methodologies and tiger beetles encountered in locations were collected and identified using appropriate taxonomic keys. Out of the one hundred twenty-five locations, tiger beetles were encountered in seventy-four locations of all districts, provinces and zones. Twelve species of six genera were identified with most species in genera *Cylindera*. Species displayed preference to habitats of coastal areas, reservoir and river banks, roads and footpaths and agro-ecosystems. Seven species were located on the banks of reservoirs and rivers, four on roads, footpaths and agro-ecosystems and one species inhabited the coastal areas. Statistical analysis of results using One-way Analysis of Variance and Tukey's pair comparison method of the Minitab 16.0 statistical software package indicated soil moisture and air temperature to be significantly different ( $p < 0.01$ ) in habitats of tiger beetles when compared to habitats from which they were absent. Tiger beetles preferred habitats with high air temperature ( $34.56^{\circ}\text{C} \pm 0.37$ ) and low soil moisture ( $12.28\% \pm 1.75$ ). The findings of the study reveals that tiger beetles are widely distributed in Sri Lanka and the majority of species inhabits locations associated with water bodies that have high air temperatures and comparatively low soil moistures.

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## Survey of molluscan shells from the Jaffna Estuary, Sri Lanka

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Gastropods and bivalves are known as shells that belong to the Phylum Mollusca. As far as Jaffna estuary is concerned, several studies have been published on fish, prawns, crabs and cephalopods. But there are no studies on cones, conches and bivalves inhabiting this water body. As such a study was conducted to document the cones and bivalve species inhabiting the Jaffna estuary along the entire coastal stretch. The study site was the entire coastal stretch of the Jaffna estuary from Ponnalai upto Killali and the study was conducted from June 2014 - January 2015. The sampling was done in each of 15 fish landing sites. Three landing sites were covered in one day and sampling time was 0630 hrs – 0900 hrs. Sampling was done weekly, sampling time for each landing site was 40 minutes and each site was visited 5 times. Samples were taken from coastal mud as well as the bottom mud of the estuary and by catches of trollers. The samples were brought to the laboratory and identified with the help of the standard keys. Altogether 28 species of shells were identified belonging to 12 families in 19 genera of which 19 were gastropods and 9 were bivalves. The percentage of occurrence of a species per site was calculated by the number of recordings divided by the number of visits. Then the average occurrence of a species with respect to all landing sites was also calculated by summing up the values divided by 15. The value range of 0-40%, 41%-80%, and above 81% were categorized as uncommon, common, and very common respectively. Three out of 15 species were uncommon though present in all landing sites namely *Oliva oliva*, *Murex haustellum* and *Meretrix* sp and others were common. The common species were namely *Trochus raditus*, *Umbonium vestiarum*, *Conus ceylanensis*, *Murex ternispina*, *M. trunculus*, *Chicoreussps*, *Donax deltoides*, *D. cuneatus*, *D. faba*, *Gafrarium tumidum*, *Meretrix casta*, *Clypeomorus* sp and *Turbirella pyrum*. Rests of the 13 were found to be uncommon and those were *Neritaplicata*, *N. polita*, *N.albicilla*, *Trochusraditus*, *Siratuspliciferoides*, *Bedevasps*, *Gelonia* sps, *Pecten fumatu,s* *Pecten* sp, *Lambis truncate*, *Lambis* sp, *Laevistrombuscanarium* and *Tonnadolium*.

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**Enhancement of immunity in cultured shrimp, *Penaeus monodon* induced by *Achyranthes aspera* (Sin. Karal heba, Family: Amaranthaceae) compared to a commercial immune enhancer**

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Use of immune enhancers is becoming popular in global shrimp culture industry in order to protect the health of shrimp; shrimp immune enhancers are commercially available in Sri Lanka. Karal heba, *Achyranthes aspera* is an indigenous medicinal plant and present study investigated the immune enhancing ability of ethanol extract of soft aerial parts (leaves, soft branches and flowers) of the plant in shrimp, *Penaeus monodon* using immune response indicators compared to a commercial immune enhancer termed for this study as "Immune x". From three groups of cultured shrimp ( $10 \pm 2$  g body weight; 4 replicates and 18 shrimp in each), one was fed with shrimp feed containing extract of the plant (experimental group), one fed with feed containing "Immune x" (reference group) and the other group was fed with normal shrimp feed (control) over four weeks and clotting time with other innate immunological parameters (measured by spectrophotometric readings) of haemolymph of shrimp in each group were recorded. Clotting time and superoxide dismutase activity (SOD) in haemolymph of experimental shrimp (5.67 sec and 0.238 respectively) were significantly lower than those of reference shrimp (63.86 sec and 0.384) and control shrimp (260.8 sec and 0.478 ;  $P < 0.05$ ). Prophenol oxidase activity (PO) and intra cellular super oxide anion activity (ISA) of experimental shrimp were significantly higher (respective values were 0.264 and 0.228) than those values of reference shrimp (0.136 and 0.131) and control shrimp (0.056 and 0.031;  $P < 0.05$ ). Tested innate immunological parameters of cultured *Penaeus monodon* were significantly enhanced by the ethanol extract of *Achyranthes aspera* compared to the commercial immune enhancer.

**Protection of cultured shrimp, *Penaeus monodon* from white spot disease (WSD) with enhanced immunity induced by *Achyranthes aspera* (Family Amaranthaceae) compared to a commercial immune enhancer**

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Ethanol extract of soft aerial parts (leaves, soft branches and flowers) of Karal heba, *Achyranthes aspera* could enhance the innate immunological parameters of cultured shrimp, *Penaeus monodon*. This study investigated whether the enhanced immunity could protect cultured shrimp from white spot disease (WSD), a killer viral disease. From four groups of shrimp ( $10 \pm 2$  g body weight; 4 replicates and 12 shrimp in each), first group was fed with the feed incorporated with ethanol extract of soft aerial parts of Karal heba (experimental group), second group was fed with feed incorporated with the commercial immune enhancer "Immune x" and the remaining two groups (positive and negative controls) were fed with normal shrimp feed over 4 weeks. Three groups except the negative control group were then challenged with white spot virus (WSV) by feeding equal weight of infected shrimp tissues. Shrimp in positive control group began to show disease symptoms within 2 days from challenge with 100% cumulative mortality within the post challenge period; natural mortality of shrimp was 8.33% (recorded in negative control). Shrimp that received "Immune x" began to show symptoms of WSD on the 5<sup>th</sup> day from challenge with 64.58% cumulative mortality which was significantly higher than cumulative mortality recorded for the experimental group (25%;  $P < 0.05$ ); there was no significant difference between natural mortality of shrimp and the mortality of experimental group ( $P > 0.05$ ). Histopathology and PCR showed that moribund shrimp in all groups except shrimp in negative control were infected with WSV. Results confirmed that the immunity enhanced by the ethanol extract of Karal heba plant could protect the recipient shrimp from WSD when challenged with the virus.

**Possibility of preventing Acute Hepatopancreatic Necrosis Disease (AHPND), a killer disease in cultured shrimp caused by a unique strain of *Vibrio parahaemolyticus* if the strain enters into Sri Lankan culture systems**

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Acute Hepatopancreatic Necrosis Disease (AHPND), initially termed as early mortality syndrome (EMS) is recognized as a newly emerging disease in cultured penaeid shrimp including black tiger shrimp (*Penaeus monodon*) and Pacific White shrimp (*Litopenaeus vannamei*). The disease was first reported in China in 2009 and over the last 5 years it spread through many Asian countries and even to Mexico. It is now confirmed that the causative agent of AHPND is a unique strain of *Vibrio parahaemolyticus* that cause massive sloughing off of tubular epithelial cells of hepatopancreas resulting 100% mortality in shrimp in grow-out ponds. AHPND bacteria are believed to colonize shrimp gut and release toxins that enter hepatopancreas. Sri Lankan shrimp farmers have not experienced massive mortality due to AHPND. However, monitoring total *Vibrio* and *V. parahaemolyticus* in culture water and in shrimp gut is very important in planning strategies for the prevention/control of AHPND if it is observed; bio-augmenters and probiotics could be used to control harmful bacteria. Therefore, *Vibrio* sp. and *V. parahaemolyticus* were monitored in culture water and in shrimp gut (using standard procedures) over two production cycles in two randomly selected groups of shrimp grow out ponds in the North Western province. Locally produced bioaugmenter /probiotic was used (for culture water and shrimp respectively) for the experimental group of ponds while control pond water and shrimp were managed under the normal procedures of farmers. Culture water of experimental ponds had a significantly lower count of *V. parahaemolyticus* ( $35.5 \pm 6.9$  CFU mL<sup>-1</sup>) and total *Vibrio* count ( $419.5 \pm 36.3$  CFU mL<sup>-1</sup>;  $P < 0.05$ ) compared to those values of control ponds ( $918.8 \pm 78.2$  CFU mL<sup>-1</sup> and  $3745 \pm 221$  CFU mL<sup>-1</sup>). Guts of shrimp from experimental ponds were negative for *V. parahaemolyticus* (0 CFU mL<sup>-1</sup>) and total *Vibrio* count in the gut was  $1.28 \pm 0.12 \times 10^4$  CFU mL<sup>-1</sup>; those values of control ponds were  $7.23 \times 10^2$  CFU mL<sup>-1</sup> and  $1.31 \pm 0.11 \times 10^7$  CFU mL<sup>-1</sup> respectively. Results show that the water in shrimp culture ponds as well as gut of shrimp are colonized heavily by different species of *Vibrio* including *V. parahaemolyticus* and the use of locally produced bioaugmenter /probiotic has the ability to control the populations of *V. parahaemolyticus* and other *Vibrio* sp. Further studies are required to find out whether the unique strain of *V. parahaemolyticus* that causes AHPND is present or not in our shrimp culture systems and if it is present whether that unique strain also could be controlled by the same local bioaugmenter/probiotic.

**Selection of White Spot Virus (WSV) and Monodon Baculo Virus (MBV) free brood stocks of cultured shrimp *Penaeus monodon*, from Sri Lankan coastal sea to produce healthy post larvae**

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White Spot Virus (WSV) and Monodon Baculo Virus (MBV) transmit vertically and horizontally. They have been identified as the major pathogenic viruses in cultured shrimp *Penaeus monodon*, both in hatcheries and grow-out farms of Sri Lanka, reducing the production in both sectors. A preliminary survey revealed that brood stocks of the shrimp used for the production of post larvae are collected from eight major sites along the coastal sea depending on monsoon seasons. Present study investigated the prevalence of WSV and MBV in brood stocks of *Penaeus monodon* collected from those sites over two calendar years. Each brood shrimp in each sample of brood stock collected from each site was transported separately to prevent cross contaminations. Tissue samples were used to screen for WSV by PCR technique and fresh fecal matter was observed for MBV occlusion bodies. Almost throughout the year WSV and MBV prevailed in brood stocks collected from Hendala and Negombo seas (prevalence of WSV varied between 14.2% to 92.9% and that of MBV ranged from 29.6% to 94.5%). Prevalence of WSV and MBV in brood shrimp was low from June to July in Beruwala (7.2% to 8.7% for WSV and 6.0% to 8.5% for MBV) and from January to March in Pottuvil sea (11.5% to 23.8% and 13.7% to 22.9% for the two viruses, respectively). It is recommended to collect brood stocks of *Penaeus monodon* from Beruwalasea and Pottuvil sea during the periods of low prevalence of WSV and MBV. After screening, WSV and MBV free brood shrimp should be maintained under strict biosecurity measures and better management practices in order to produce WSV and MBV free post larvae to be stocked in grow-out farms.

## **A simplified version of *ex ovo* cultivation method of chicken embryos as a model for evaluating venom toxicity**

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Modal organisms are important in studying various biologically important medical scenarios such as development, organogenesis, physiological activities. Unlike in *in vitro* assays, use of animal models always provide a wide window and strong insight into the functioning mechanisms of living systems. In this study *ex ovo* cultivated chicken embryos have been developed from consumable fertile chicken eggs to use as a model organism. The development of the embryos was carried out with very low cost and without high tech instrumentation. The embryos were grown by transferring fertile chicken egg yolks with the albumin onto hammocks consisting of thin plastic wraps suspended on water. The embryos were then incubated at 37°C till the desired growth stage is obtained. Successful cultivation generates full grown organisms even up to 20 days of developmental stage. As the embryonic model is a transparent organism, this provides a window to observe the interior organs up to day 5. We used this model to investigate the effects and the pathology of venom toxins of two snakes; *Daboia russelii* (Russell's Viper) and *Naja naja* (Common Cobra). With viper venom, a complete damage to extra embryonic vascular sac by degeneration/ hemorrhage of capillary network was observed while for cobra venom a clear and a visible cardiac arrest could be observed with a localized tissue damage. This model can be used in the preliminary trial of assessing the preclinical efficacy of anti-venoms and possible antidotes easier than in mice or rat models as it requires very low levels of skill in animal handling.



## Occupational Paraquat exposure among sugarcane and vegetable farmers in Sri Lanka: A case study

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Paraquat (1, 1 – dimethyl -4, 4- bipyridylium dichloride) is a contact herbicide, predominantly used in Sri Lanka. Despite the imposed ban in agriculture sector, Paraquat usage was seen among sugarcane farmers in Pelawatta compared to vegetable farmers in Nuwara Eliya. Therefore the level of Paraquat exposure was studied among sugarcane farmers comparing that with vegetable farmers. Sugarcane farmers [Warunagama GS, (n=44); Rahathangama GS (n=20)] and vegetable farmers (n=16) were selected based on pesticide usage and farming practices. Paraquat concentration in urine samples (U-PQ) were analyzed using competitive enzyme-linked immunosorbent assay (ELISA) method using commercial kits (US Biocontract Inc., San Diego, CA) and (U-PQ) concentrations were determined by 4PL nonlinear regression model. Differences of U-PQ and creatinine adjusted U-PQ among the locations were compared using one-way ANOVA followed by Dunnett's test using IBM statistics 22. Measureable levels of urinary-Paraquat (U-PQ) were detected in all study groups. Highest concentrations of U-PQ were detected in Warunagama sugarcane farmers (mean,  $3.25 \pm 0.29 \mu\text{g/g Cr}$ ) and lowest concentrations of U-PQ were detected in urine samples of Nuwara Eliya vegetable farmers (mean,  $0.603 \pm 0.03 \mu\text{g/g Cr}$ ). Urine samples of Warunagama sugarcane farmers recorded significantly higher levels of U-PQ compared to the control group and Nuwara Eliya vegetable farmers ( $P < 0.001$ ), indicated continuous usage of Paraquat by sugarcane farmers (Warunagama). But U-PQ concentrations in sugarcane farmers in Rahathangama were not significantly different from the control group and Nuwara Eliya vegetable farmers ( $P > 0.05$ ). As measurable levels of U-PQ was detected in urine samples of all study groups non-occupational exposure by Paraquat was evident which warrants further studies and remedial measures.

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## Establishing dietary and faecal relationships for crude protein and crude fibre in selected native herbivorous mammals in Sri Lanka

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The present study was to generate relationships between dietary and faecal crude protein and crude fibre contents for seven native herbivorous mammals i.e. three primates (Purple faced leaf monkey - *Semnopithecus vetulus*, Grey langur - *S. priam*, Toque macaque - *Macaca sinica*) and four ungulates (Spotted deer - *Axis axis*, Sambur - *Cervus unicolour*, barking deer - *Muntiacus muntjak*, mouse deer - *Moschiola meeminna*) held in captivity at the National Zoological Gardens, Dehiwala, Sri Lanka. Such relationships generated for captive mammals have been effectively used for ascertaining diet quality of wild-dwelling counterparts. Samples of food and faecal matter were collected from enclosures and analysed for crude protein using the Kjeldahl nitrogen macro method and for crude fibre using a process of ether extraction, boiling in H<sub>2</sub>SO<sub>4</sub> and NaOH, washing and subsequently incinerating at 600 °C. A total of three samples of food (leaves and fruits) and faeces were collected per day for each species over 15 days, yielding a total of 45 samples of food and faeces per species. The regression equations generated using dietary content (DC) and faecal content (FC) for crude protein were : Purple faced leaf monkey -  $FC = 2.62 DC - 19.41$ ; Grey langur -  $FC = 1.33 DC + 2.26$ ; Toque macaque - ; Spotted deer -  $FC = 19.36 DC - 214.14$ ; Sambur -  $FC = 22.68 DC - 253.1$ ; Barking deer -  $FC = 1.00 DC + 0.51$ ; Mouse deer -  $FC = 0.64 DC + 4.04$  and for crude fibre were : Purple faced leaf monkey -  $FC = 2.62 DC - 19.41$ ; Grey langur -  $FC = 1.33 DC + 2.26$ ; Toque macaque -  $FC = 0.65 DC - 11.53$ ; Spotted deer -  $FC = 8.01 DC - 54.91$ ; Sambur -  $FC = 10.87 DC - 94.52$ ; Barking deer -  $FC = 5.12 DC - 9.61$ ; Mouse deer -  $FC = 1.11 DC + 0.92$ . The R<sup>2</sup> values ranged between 63 % - 93% indicating that variation of a particular constituent in the faeces is to a large extent explained by that in the diet consumed on the previous day. It was revealed that there was considerable inter-specific variation in the regression coefficients suggesting that the species markedly differ in the degree of digestibility of each of the two constituents. For selected species, the generated relationships were made use of for predicting suitability of fodder consumed by individuals in the Yala National Park, using fresh faecal matter collected from this habitat. These analyses revealed that their diet, at least during the dry season when sampling was conducted, was poorer in protein content. Ensuring that diet quality of large herbivores is at an optimum is important as they play a vital role in maintaining the productivity of these natural ecosystems. Information generated through studies such as this would allow timely corrective measures to be adopted when managing natural habitats for wild species.

***In vivo* antioxidant activity of mature leaf concentrate of Sri Lankan wild type *Carica papaya*.L variety against carbon tetra chloride induced oxidative stress in rats**

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It has been reported that the mature leaf concentrate of Sri Lankan wild type *Carica papaya* (MLCC) possesses profound *in vitro* antioxidant activity. The present study was undertaken to evaluate the *in vivo* antioxidant properties of the MLCC against CCl<sub>4</sub> induced oxidative stress in the Wistar rat model. The MLCC was prepared by pulverizing mature leaves of wild type *C.papaya* using a mechanical juice extractor (Philips, 1861) without the use of water (at 10 g leaf blade/ 2 ml of concentrate). Adult male Wistar rats were divided into five groups (N=5/group); Group I received distilled water (DW) as the normal control. Groups II to V were subcutaneously treated with 1 ml/kg of CCl<sub>4</sub> diluted with olive oil at 1:1 ratio on days 01 and 06 to induce oxidative stress. Groups II, III, IV and V were orally gavaged with DW (induced control), 0.18, 0.36 and 0.72 ml/100g body weight (BW) doses of the MLCC, respectively, for 7 consecutive days. On day 8, blood was collected from rats and plasma levels of superoxide dismutase (SOD), Glutathione peroxidase (GSH), Glutathione reductase (GR) and liver enzymes (AST, ALT) were measured using standard kits. Statistical comparisons were made using the Mann-Whitney U test. The fresh MLCC was subjected to qualitative and quantitative phytochemical analysis. Oral administration of the MLCC at 0.36 and 0.72 ml/100g BW doses significantly (P<0.05) increased both SOD and GSH levels while the latter dose also significantly (P<0.05) increased the GR levels in rats compared with the induced control. Oral administration of the MLCC at 0.72ml/100g significantly (P<0.05) reduced the ALT levels while all three doses significantly (P<0.05) reduced the AST levels in rats. The MLCC was established to be rich in phenolics, flavonoids and vitamin C. Thus, the marked *in vivo* antioxidant property of MLCC may be attributed to its antioxidant phyto constituents. The present study established that the MLCC is rich in antioxidant phytochemicals, is orally active and effectively attenuate the CCL<sub>4</sub> induced oxidative stress in Wistar rats by enhancing antioxidant enzymes, and decreasing liver enzymes up to physiological levels.

**Acknowledgment:** Financial assistance by the Collaborative Research Grant (AP/3/2012/CG/29) of the University of Colombo

**Modulation of *in vitro* phagocytic activity, cell proliferation and cytokine production in the Wistar rat model by a Sri Lankan *Haliclona (Soestella)* sp sponge crude extract**

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Marine sponges, renowned for secondary metabolites with various bioactive properties, are efficient modulators of the mammalian immune system. This study reports for the first time, the effects on *in vitro* phagocytic activity, cell proliferation and cytokine production in a Wistar rat model by a Sri Lankan *Haliclona (Soestella)* sp crude extract (SCE). *Haliclona (Soestella)* sp, presumably a new species, was harvested from Unawatuna, Galle. Sponge samples were diced and refluxed with methanol/dichloromethane for 72 hours. The resultant extract was filtered, rotary evaporated and the SCE was used in a dilution series (10, 100, 500, 1000, 2000 µg/mL) to test for *in vitro* phagocytic activity (peritoneal macrophages- PM), cell proliferation (bone marrow cells [BMC] and splenocytes) and cytokine production (by BMC and PM). *In vitro* phagocytic activity was investigated using the NBT assay and the stimulation index (SI) was calculated. *In vitro* proliferation of BMC and splenocytes sans mitogen, were examined using the MTT assay. SI was calculated for each cell type while the IC<sub>50</sub> was calculated for percentage inhibition of BMC. Cellular aspirations of cultured PM and BMC stimulated with yeast were used to quantify cellular cytokine (IL-10, IFN-γ and TNF-α) levels using standard sandwich ELISA kits (BD Bioscience USA). The SI significantly decreased for PM (1000 and 2000 µg/mL doses), and for splenocytes (2000 µg/mL dose) (P<0.05; Mann Whitney U Test). A significant dose dependent reduction in the SI of the BMC proliferation was evident (P<0.05) while with a IC<sub>50</sub> (percentage inhibition) value of 0.026 µg/mL. IFN-γ and TNF-α levels of BMC cultures were significantly suppressed in all tested SCE dose (P<0.05) and in a dose dependent manner for the former, with no significant effect in IL-10. Conversely, there was no dose dependency in IFN-γ, TNF-α and IL-10 cytokine production by PM, although all cytokines were reported significantly higher than in the control (P<0.05). In conclusion, *in vitro* phagocytosis, cellular proliferation and cytokine production are modulated by *Haliclona (Soestella)* sp crude sponge extract in the Wistar rat model.

**Acknowledgement:** Financial assistance by HETC project, Ministry of Higher Education, Sri Lanka (SJP/O-AS/N1)

## **Nest occurrence, mean nest density and relative nest abundance of *Aneuretus simoni* Emery and associated ant fauna in Meethirigala Forest Reserve**

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The sole extant species of Aneuretinae, *Aneuretus simoni*, forms ground nests in various substrates in several forests in Sri Lanka. Nests of the species were surveyed outside the previously recorded habitats, in Meethirigala Forest Reserve, in Gampaha District. A preliminary survey conducted in the forest by placing honey baits, breaking of decaying wood pieces and sifting leaf litter in February, 2014 revealed the presence of workers of *A. simony* in the area. The frequency of nest occurrence (FNO), percentage nest abundance (NA%) and mean nest density (MND) of each ant species observed at three localities in three elevations of the forest were investigated in March, 2014 by laying 40 quadrats of 0.5 m x 0.5 m at two 50 m<sup>2</sup> plots in each locality. Number of nests occupied by each ant species within each quadrat was recorded. Air temperature (28°C - 29°C), soil temperature (25.3°C - 26.3°C), soil moisture content (6.0 % - 13.3 %), depth of litter (4 cm - 6 cm) and soil organic matter content (5.18 % - 11.3%) were also recorded. Nests of twenty one species of fourteen genera in five subfamilies including that of *A. simoni* were observed. Nests of *A. simoni* were found at the locality at 57m elevation, the highest value of FNO, 5/40 and 12.5% of NA were observed for the species and also MND of *A. simoni*, 0.5 m<sup>-2</sup>, was the highest value recorded among that of other species. Higher values of FNO were observed for *Odontomachus simillimus* Smith and *Technomyrmex albipes* Smith, respectively while *A. simoni* had 5/120 of FNO for the whole study region. The Meethirigala Forest Reserve in Gampaha District should be added to the list of habitats of the species and *A. simoni* is a resident species at 57 m elevation, which is the lowest elevation record for the species.

## **An investigation of sex differences in feeding and vigilance behavior in Hanuman Langurs using fractal analysis**

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Male and female primates experience different ecological pressures, which may be reflected in their behavior. Owing to gestation and lactation, female primates typically experience greater energetic demands than males. Similarly, male primates compete for mating opportunities. A number of studies have shown that behavioral sequences to have fractal-like properties. In this study we use detrended fluctuation analysis (DFA) to investigate long-range autocorrelations in separate, binary sequences of feeding and vigilance behavior of female and male hanuman langurs (*Semnopithecus entellus*) using focal animal sampling. Both foraging and scanning behavioral sequences of hanuman langurs exhibit long-range power law (auto) correlations ( $\alpha > 1/2$ ) and scale invariant (self similarity) properties or fractal-like properties. Albeit not statistically significant, adult female langurs show greater complexity in relation to feeding behavior than male langurs. Moreover, lactating females show greater complexity in relation to feeding behavior than non-lactating individuals. When scanning behavior is considered, adult females show less complexity than adult males. Lactating females show less complexity in relation to scanning behavior compared to non-lactating females. The higher complexity associated with feeding behavior in female langurs is probably adaptive, as it enables females to cope with fluctuating environmental conditions. The higher complexity in vigilance behavior in males could enable them to detect extra group males and predators under variable environmental conditions. Our finding also indicates that DFA is capable of detecting changes in complexity of behaviors performed by individuals in altered physiological states and hence could be employed to monitor the impact of factors such as ecotourism or habitat degradation on wildlife.

## Assessment of environmental pollutants using fledgling feathers of Little egret (*Egretta garzetta*) as a bio monitoring tool in Sri Lanka

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Bird feathers have been used as a non-destructive method of assessing levels of environmental pollutants. The objective of this study is to establish a baseline set of data for heavy metal contaminants in diverse ecosystems in Sri Lanka using bird feathers as a bio monitoring tool. Little egrets (*Egretta garzetta*) were selected as a model organism, since they are widespread and are higher in food chain. Five heronries of *Egretta garzetta* were identified from three areas, namely Gampaha, Kandy and Anuradhapura. Samples were collected from fledglings during May to July 2014 and Hg, As, Pb and Cd were analysed using Graphite Atomic Absorption Spectrometry. When the sites were compared for Hg, As, Cd and Pb levels, a significant difference of Hg and As ( $p < 0.05$ ) were detected. However such a significant variation was not detected for Cd ( $p > 0.05$ ). The concentration of As was significantly high in feathers (515.39  $\mu\text{g}/\text{kg}$ ) from Kadugannawa site in Kandy. The As concentration were significantly different from feathers from CTB depot (118.08  $\mu\text{g}/\text{kg}$ ) and Jaffna Junction sites (96.38  $\mu\text{g}/\text{kg}$ ) in Anuradhapura and Belummahara site (199.03  $\mu\text{g}/\text{kg}$ ) in Gampaha as well. Nevertheless the concentration of As in feathers from Kadugannawa did not significantly different from that of Kandy lake samples. Further the feathers from Kandy Lake presented high concentration of As (309.83  $\mu\text{g}/\text{kg}$ ) as well. The results show that Hg concentration in feathers was significantly high (1517.65  $\mu\text{g}/\text{kg}$ ) in Belummahara. According to the multiple comparisons Cd concentration of feathers was high (28.21  $\mu\text{g}/\text{kg}$ ) in Kadugannawa, however that not much different from Cd concentration of feathers in Belummahara (24.87  $\mu\text{g}/\text{kg}$ ). Interestingly Pb was not detected in feathers at any site. The results of the present study revealed that feathers of Little egret fledglings can be used as a bio-monitoring tool to measure the bio accumulation of Hg, As and Cd.

**Comparison of larvicidal and repellent efficacy of *Ocimum basilicum* (L.); “Maduruthala”, leaves and pods, against dengue vector, *Aedes aegypti* (L.)**W L B P Abhayawickrama<sup>1</sup>, G A S M Ganehiarachchi<sup>1</sup> and P A Paranagama<sup>2</sup><sup>1</sup>Department of Zoology and Environmental Management, University of Kelaniya, Sri Lanka.<sup>2</sup>Department of Chemistry, University of Kelaniya, Sri Lanka.

Mosquitoes transmit serious human diseases, causing millions of deaths every year. Among the disease causing mosquitoes, *Aedes aegypti* is the major vector of dengue and dengue hemorrhagic fever. Repeated use of synthetic insecticides for mosquito control has caused adverse impacts on the natural biological systems and led to resurgence in mosquito populations. Therefore, concern is raised to search for alternative mosquito control measures. Plant derivatives are considered as a rich source of bioactive chemicals and they have gained importance as an alternative source of mosquito control agents. Present study was carried out as an effort to find effective and affordable way to control *A.aegypti* by assessing the larvicidal efficacy and repellent efficacy of essential oil of leaf and pod extracts of *O.basilicum*L. (Lamiaceae) which is an indigenous plant in Sri Lanka. The essential oils of leaf and pod extracts of *O.basilicum* were extracted by steam distillation and their major chemical composition was determined using gas chromatography and identified using relative retention times. Twenty late third instar larvae of *A. aegypti* were exposed to various concentrations (50-600 mg/L) of essential oils of leaf and pod extracts for 24 hours. LC<sub>50</sub> and LC<sub>90</sub> values of the essential oils of *O.basilicum* leaf and pod were determined after 24 hours by following the Probit analysis and repellent efficacy was determined using a Y-tube Olfactometer. The LC<sub>50</sub> and LC<sub>90</sub> values with 95 % confidence limit for essential oil of leaf extracts are 141.51 and 357.71 mg/L respectively while same values for pod extract are 127.33 and 377.18 mg/L respectively. Further it was observed that there is no significant difference between the larvicidal effects of essential oils of leaf and pod extracts against the third instar larvae of *A.aegypti*. Essential oil of leaf extract and pod extract have 92.6% and 93.33 % repellency at a concentration of 800 mg/L respectively. Results of the GC analysis revealed that eugenol and methyl eugenol were found to be the major chemical compounds in both of the essential oils. The results suggest a potential utilization of essential oils of leaf and pod extracts of *O.basilicum* as a larvicide and a repellent against *A.aegypti*, thus creating new affordable and effective approaches to the control of dengue fever.



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# **ABSTRACTS OF PAPERS**

**Parallel session 3**

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## Superoxide and nitric oxide radical scavenging activities of bark and leaf of Ceylon cinnamon (*Cinnamomum zeylanicum* Blume) *in vitro*

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Ceylon Cinnamon (CC) (*Cinnamomum zeylanicum* Blume) known as 'true cinnamon' in the world is used as a spice in Sri Lanka for centuries. Although numerous publications on health properties of cinnamon are available, the use of authenticated true cinnamon in those publications is questionable. Further, superoxide and nitric oxide radical scavenging activities as antioxidant properties are less investigated worldwide. Moreover, superoxide and nitric oxide radical scavenging activity of leaf of CC is not reported to date. Therefore, present study evaluates the superoxide and nitric oxide radical scavenging activities of bark and leaf of Ceylon cinnamon *in vitro*. Freeze dried 95 % ethanol and 1:1 (v/v) dichloromethane: methanol (DCM:M) bark and leaf extracts of authenticated CC were used in this study. Different concentrations of bark and leaf extracts were studied for superoxide (bark and leaf: 37.5, 75, 150, 300, 600 µg/ml; n=4) and nitric oxide (bark and leaf: 15.62, 31.25, 62.5, 125, 250 µg/ml; n=4) radical scavenging activities using 96-well micro plate based assay protocols *in vitro*. Quercetin (12.5, 25, 50, 100, 200 µg/ml; n=4) and rutin (7.81, 15.62, 31.25, 62.5, 125 µg/ml; n=4) were used as the reference drugs for superoxide and nitric oxide radical scavenging assays respectively. Results demonstrated that both bark and leaf of CC possess dose dependant superoxide and nitric oxide radical scavenging activities. The nitric oxide radical scavenging activity is significantly higher (p<0.05) compared to superoxide radical scavenging activity in both bark and leaf extracts. Further, bark extracts showed high activity than leaf extracts for both radical scavenging activities studied. DCM:M bark extract showed the highest activity while DCM:M leaf extract showed the lowest activity for both radical scavenging activities studied. The IC<sub>50</sub> values of DCM:M bark for nitric oxide and superoxide radical scavenging assays were 35.89 ± 0.45 and 480.57 ± 15.67 µg/ml respectively. Similarly IC<sub>50</sub> values of DCM:M leaf extracts for nitric oxide and superoxide radical scavenging activities were 69.63 ± 0.56 and 1381.42 ± 98.30 µg/ml respectively. Compared to the reference drugs used in the study (IC<sub>50</sub> values: rutin:17.62 ± 0.01 µg/ml; quercetin: 75.58 ± 1.92 µg/ml) both bark and leaf extracts showed 2-4 times and 6-9 times less activity for nitric oxide and superoxide radical scavenging assays. In conclusion, both bark and leaf of Ceylon cinnamon possess moderate superoxide and nitric oxide radical scavenging activities. The activity showed by bark extract is higher than that of leaf. Further, this is the first report of nitric oxide and superoxide radical scavenging activity of leaf of Ceylon cinnamon worldwide.

## Antioxidant properties of brans of twenty nine rice (*Oryza sativa* L.) varieties of Sri Lanka

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Present study evaluated antioxidant properties (AP) of brans of 29 rice varieties (RV) of Sri Lanka. Freeze-dried 70% ethanolic extracts of brans of 21 improved, 2 old improved (OI) and 6 traditional RV were used in this study. AP were evaluated using total polyphenolic content (TPC: n=3), ferric reducing antioxidant power (FRAP: n=3), 1,1-diphenyl-2-picryl-hydrazyl (DPPH: n=3; % inhibition 25 µg/ml) and 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic) acid (ABTS: n=3; % inhibition 25 µg/ml) radical scavenging assay *in vitro*. The RV which showed highest radical scavenging activities were further studied to find IC<sub>50</sub> values in ABTS and DPPH assays (1.56, 3.12, 6.25, 12.5, 25, 50 µg/ml; n=3 each). Results clearly revealed significant differences (P<0.05) among bran extracts of 29 RV for the investigated AP (P<0.05). Mean TPC, FRAP, DPPH and ABTS radical scavenging activity of 29 RV were in the range of 21.91±2.68–2808.14±26.77 mg gallic acid equivalents (GAE)/100g bran, 1.71±1.37–58.01±0.64 mg Trolox equivalents (TE)/g bran, 5.02±0.55–68.58±3.88 % and 15.01±1.70–98.59±0.40 % respectively. Irrespective of improved, OI or traditional, bran extracts of red rice exhibited significantly high (P<0.05) activity compared to bran extracts of white RV. The Mean TPC, FRAP, DPPH and ABTS radical scavenging activity of red rice varied from 840.36±32.33–2808.14±26.77 mg GAE/100g bran, 25.70±0.79–58.01±0.64 mg TE/g bran, 33.16±1.02–68.58±3.88 % and 70.47±4.26–98.59±0.40 % respectively. Whereas those of white rice was in the range of 21.91±2.68–328.83±4.77 mg GAE/100g bran, 1.71±1.37–11.07±0.18 mg TE/g bran, 5.02±0.55–21.84±2.18 % and 15.01±1.70–42.06±0.95 % respectively. Further, the findings demonstrated that selected traditional red RV had significantly high (P<0.05) AP compared to selected improved red RV. Highest TPC and DPPH radical scavenging activity were demonstrated from Sri Lankan traditional red RV namely Kalu Heeneti (TPC: 2808.14±26.77 mg GAE/100g bran) and Beheth Heeneti (DPPH: IC<sub>50</sub>: 12.09±0.31 µg/ml). Further, high ABTS radical scavenging activities were evident for Dosthara Heeneti (IC<sub>50</sub>: 11.71±0.16 µg/ml) and Pachchaperumal (12.49±1.16 µg/ml). The OI red rice variety, H4 (58.01±0.64 mg TE/g bran) had the highest FRAP in this study. It is concluded that AP varies among the improved, OI and traditional RV of Sri Lanka. Red RV had significantly high activity compared to white RV. Further, traditional red RV showed high AP compared to improved red RV.

## Comparative GC-MS study of chemical constituents in essential oils of Ceylon Cinnamon (*Cinnamomum zeylanicum* Blume) bark oils collected from different geographical locations

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*Cinnamomum zeylanicum* (Lauraceae) is an indigenous perennial crop used as a spice, which belongs to the genus *Cinnamomum*. It is grown extensively in Sri Lanka besides a limited extent in Madagascar and Seychelles. Sri Lanka is the major true cinnamon producer as well as the best quality Ceylon cinnamon bark oil producer for the world market. The chemical fingerprint of Ceylon cinnamon bark oils extracted from plants in different districts will be useful to emphasize the unique characteristics of Ceylon cinnamon bark oil with reference to the geographical Indication (GI). The aim of this study was to investigate the variations in the chemical composition of Ceylon cinnamon bark oil extracted from H-grade cinnamon bark collected from different geographical sites of Sri Lanka and to understand the uniqueness of chemical fingerprint to use as a geographical indicator. The dried barks of H-grade Ceylon cinnamon were collected from different geographical locations: Ratnapura, Galle, Matara and Kalutara districts of Sri Lanka and subjected to extraction of oils by hydro-distillation using Clevenger arm. Each sample of bark oil was analyzed using Gas Chromatography Mass Spectrometry (GC-MS) for  $\alpha$ -pinene,  $\beta$ -thujene,  $\alpha$ -phelandrene, o-cymene, linalool,  $\beta$ -caryophyllene, *trans*-cinnamaldehyde, cinnamyl acetate, eugenol and benzyl benzoate to study variation in the chemical composition with respect to geographical location. The major compound present in the oil was *trans*-cinnamaldehyde and Matara had the highest composition [61.54% (53.36 - 67.69%)] followed by Galle [50.81% (43.18 - 64.37%)]. Cinnamyl acetate level in Ratnapura (14.80%) was approximately three times higher than the levels from other districts (Galle 5.08%, Matara 5.67%, Kalutara 4.27%). In contrast, *trans*-cinnamaldehyde, linalool and eugenol content in Ratnapura district were lower compared to other districts. Further, percentage of  $\alpha$ -Pinene,  $\beta$ -Thujene,  $\alpha$ -Phelandrene, o-Cymene, Linalool and Eugenol were significantly different ( $p > 0.05$ ) at least between two districts. However,  $\beta$ -caryophyllene and benzyl benzoate percentages were not significantly different among the four districts. The research indicated that GC-MS fingerprint could be an effective strategy to determine the geographical origin of the cinnamon bark oil.

### **Inhibitory effect on human leukemia (HL-60) cancer cell proliferation *via* caspase-3 mediated apoptosis by *Costus speciosus* (Koen.) Sm. leaf extract**

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Medicinal plants are widely used in several indigenous systems for the treatment of various disorders. Thebu leaf (*Costus speciosus*) extracts are a promising source for investigating medicinal value and usable applications. In this study, *in vitro* anticancer effects of *C. speciosus* leaf extracts, including methanol (T-ME), hexane (T-HE), chloroform (T-CE), ethyl acetate (T-EA) and aqueous (T-WE) extracts were investigated. *in vitro* anticancer activity was determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using three different cancer cell lines including human promyelocytic leukemia (HL-60), mouse melanoma (B16F10) and human lung carcinoma (A549). Among the *C. speciosus* leaf extracts, T-EA extract showed potent cancer cell growth inhibitory activity IC<sub>50</sub> value of 26.06 µg mL<sup>-1</sup> (HL-60), 31.02 µg mL<sup>-1</sup> (A549) and 30.58 µg mL<sup>-1</sup> (B16F10) compared to the other extracts. T-EA suppressed the HL-60 cell proliferation dose-dependently with evidence of Annexin-V fluorescein isothiocyanate (V FITC<sup>+</sup>) / Propidium iodide (PI<sup>-</sup>) staining under the flow cytometric analysis. z-DEVD-fmk, a caspase-3 inhibitor, significantly inhibited cell cytotoxicity, and apoptotic bodies which were induced by T-EA. As evidenced from western blot analysis, the activation of Bax (pro-apoptotic protein) and suppression of Bcl-xL (anti-apoptotic protein) through apoptotic inducing control of up-regulation caspase-3 proteins were confirmed dose-dependently. Therefore, *C. speciosus* leaf extracts may offer promising chemotherapeutic potentials and remedies for preventing cancers.

## Microsatellite markers reveal the spatial genetic structure of dengue vector *Aedes aegypti* in selected areas in Colombo district

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Dengue is among the most important arboviral diseases in the world. Its control relies mainly on eliminating the major vector *Aedes aegypti*. To yield success, these control strategies need to be designed after careful consideration of microevolution of vector populations. Accordingly, this study was conducted to determine the spatial genetic structure of *Ae. aegypti* in Colombo district using six microsatellite markers: CT2, AC7, Gyp8, BbB07, BbH08 and BbA10. The mosquito larval samples were collected from Dematagoda (n=26), Kirulapone (n=40), Grandpass (n=25) and Thummulla (n=28). DNA was extracted, PCR amplified and separated using 6% PAGE. Genotyping results were analyzed with Fstat 2.9.3, GenAEx 6.501 and Genpop 4.2. All populations were found to be polymorphic for the six microsatellite markers. Allelic diversity was high (5.167) with a total of 31 alleles. The highest number of alleles (9) was observed for BbB07. All markers conformed to Hardy-Weinberg Equilibrium (HWE). The overall  $F_{ST}$  estimate was 0.020 (0.015-0.026; 99% CI) indicating a significant but a low level population differentiation within the study area. Relatively high gene flow observed between the locations ( $Nm=8.281$  to  $100.739$ ) may have maintained the population structure at a lower level. This is the first report of the spatial genetic structure of local *Ae. aegypti* populations. The highly polymorphic nature of the selected microsatellite markers indicates that they are appropriate candidates to study population genetic parameters of *Ae. aegypti* populations in Sri Lanka. The observed subtle spatial genetic structure along with the high migration rates of mosquito populations suggests that the vector control strategies should be implemented in the entire study area considering it as a single unit to yield success.

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## **Nutritional composition, fatty acid profile and antioxidant activity of selected traditional rice (*Oryza sativa* L.) varieties of Sri Lanka**

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Rice is the staple food in Sri Lanka and the country holds thousands of traditional rice varieties which had been in the diet for centuries. Some of these varieties are claimed to possess functional properties according to Sri Lankan traditional knowledge and folklore. Recently antioxidant activity (AA) of some of these varieties was reported. However, nutritional composition (NC) and fatty acid profile (FAP) of these varieties are not well documented. The present study evaluates NC, FAP and AA of selected traditional rice varieties of Sri Lanka. Suwandhal, Nilkanda, Heeneti, Mawee and Kurulu Thuda were used in this study. As NC, flour of whole grains of these varieties was studied for moisture, fat, crude protein, ash, available carbohydrates, dietary fiber and sugar contents using standard analytical techniques (n=3 each). FAP (palmitic acid, oleic acid and linoleic acid) was studied for oil extracted from brans (n=3 each). AA was studied using ferric reducing antioxidant power (FRAP) and 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) radical scavenging activity for 70 % ethanolic extracts of rice brans (n=3 each). Results demonstrated that NC, FAP and AA varied significantly ( $P < 0.05$ ) among the varieties. Moisture, fat, crude protein, ash, available carbohydrates, dietary fiber and sugar contents varied from 13.36-13.84, 2.78- 3.28, 7.80-11.16, 1.50-1.68, 80.80-82.74, 2.68- 6.28 and 2.28-5.86% respectively among the varieties. Suwadal variety had the highest dietary fiber content while Mawee had the highest protein content for the studied varieties. FAP of these varieties showed that the most predominant fatty acid is oleic. The oleic, palmitic and linoleic acid contents ranged from 42.76- 49.48, 19.56-23.50 and 22.16- 31.86 % respectively among the varieties studied. Further, the variety, Kurulu Thuda exhibited the highest oleic and palmitic acid contents. AA of these varieties showed that Kurulu Thuda had the highest activity while Suwadal showed lowest activity. The order of potency for FRAP and ABTS radical scavenging activity were Kuruluthuda>Nilkanda=Heeneti>Mawee>Suwadal and Kuruluthuda>Nilkanda>Heeneti>Mawee>Suwadal respectively. It is concluded that NC, FAP and AA varies among the varieties and most predominant fatty acid is oleic in all the varieties studied. Further, variety Kurulu Thuda had the highest AA.

**Chemical composition of inflorescence of *Alpinia calcarata* Rosc. (Zingiberaceae) grown in the western province of Sri Lanka**

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*Alpinia calcarata* Rosk. (Family: Zingiberaceae) is a perennial rhizomatous herb distributed in Southern Malay Peninsula and Sri Lanka and considered as native to India. This plant is a major constituent of many formulations of indigenous system of medicine for relieving throat inflammation, stimulating digestion, purifying blood, improving voice and marinating youthful vigor. In this study, the essential oil composition of fresh inflorescence of *A. calcarata* was extracted by hrdrodistillation and analyzed using Gas Chromatography Mass Spectrometric (GCMS) method. Fresh inflorescence of *A. calcarata* was also subjected to extract by cold maceration process into hexane, dichloromethane (DCM) and ethanol (99.9%). These solvents also were analyzed using GCMS. The compounds with percentage (%) higher than 0.50 (0.50 %) were only considered for identification. Around 27 compounds were identified in hydro distilled oil. The major compounds present in the hydrodistilled oil were eicosane (12.17%), carotol (8.92%) and caryophyllene (7.29%). In hexane fraction, around 11 compounds were identified and major compounds were  $\alpha$ -pinene (46.34%), 2-methyleicosane (15.88 %) and trans-methyl cinnamate (17.04 %). In DCM fraction, 16 compounds were identified and major compounds were  $\alpha$ -pinene (27.14 %), trans-methyl cinnamate (20.84 %), eucalyptol (15.26 %) and caryophyllene (6.59 %). Among 5 compounds identified in ethanol fraction,  $\alpha$ -Pinene (53.75 %), trans-methyl cinnamate (27.77 %) and  $\beta$ -cis-Caryophyllene (8.74 %) were major compounds. In volatile oil, eicosane (12.17%) was the most abundant compound while in macerated fractions of hexane, DCM and ethanol,  $\alpha$ -pinene was recorded in highest (53.75, 27.14 and 46.34 % respectively) compared to other compounds.



## Ethyl Methyl Sulfonate (EMS) induced herbicide resistance in seed-derived rice (*Oryza sativa*) callus

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Weeds are the major biotic constraint to increased rice production worldwide affecting growth and leading to considerable yield reduction. Glyphosate, the most effective broad-spectrum herbicide decreases the final yield due to off target movements. These adverse effects may possibly be superseded by developing herbicide resistant (HR) rice. Glyphosate resistant crops are derived by mutating the gene coding for enzyme 5-pyruvyl shikimate 3-phosphate synthase (EPSPS). Ethyl Methyl Sulfonate (EMS), the most commonly used chemical mutagen in plants, causes mutation in EPSPS gene. Mutagenesis techniques in tissue culture are commonly used in developing resistant rice varieties. The present investigation was carried out to identify the response of rice seed callus to different concentrations of EMS and to evaluate HR in mutated calli. Seed-derived calli were obtained from Glyphosate susceptible rice variety (Bg250) and exposed to EMS at concentrations of 0.1%, 0.2%, 0.3% and 0.4 % (10 calli per treatment and three replicates from each treatment). The mutated calli were then treated with Glyphosate (0.2%) and resistance was observed. Tetrazolium test (1% TTC) was applied to identify cell viability in the calli before moving to plant regeneration stage. Results indicated that the efficiency of EMS was higher at lower concentrations (0.1 – 0.2%) in mutating the calli compared to higher concentrations. The analysis of variance clearly showed that there were considerable differences ( $p \geq 0.05$ ) between different EMS concentrations. Calli mutated using EMS was tested for Glyphosate resistance. About 60% of Bg250 calli exhibited resistance to Glyphosate at 0.2% concentration. Unmutated calli of Glyphosate resistant rice variety *Pachcaperumal* also showed positive results to TTC test. The study revealed that *in vitro* application of EMS at callus level has the ability to develop calli, resistant to Glyphosate which is an advantage in breeding programs.

**Acknowledgement:** The research grant provided by National Research Council in Sri Lanka (NRC 12-037) is acknowledged.

**Antimicrobial potential of the endophytic fungal extracts of *Mangifera zeylanica* (*Anacardiaceae*), an endemic plant of Sri Lanka, against selected pathogenic bacterial species**

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Endophytic fungi are symbiotic microorganisms that colonize within healthy plant tissues without manifesting apparent damage to the tissues. They are recognized as a highly productive source of bioactive secondary metabolites with varied biological activities. Due to the emergence of antibiotic resistant human pathogenic bacteria it has become critical that exploring of novel alternatives to the existing antibiotics. Endophytic fungi in endemic plants of Sri Lanka are relatively unexplored and are potential sources of novel antibacterial compounds, which can be developed as clinically useful antibiotics. In the present study, antibacterial activity of twenty endophytic fungi (AA-MS-01 to AA-MS-20) isolated from surface sterilized leaves of *Mangifera zeylanica* (Atamba) were investigated. Pure cultures of the endophytic fungi isolated from the leaves of *M. zeylanica* were grown on Potato Dextrose Agar (PDA) medium for 21 days at 28 °C. The ethyl acetate extracts of these cultures were tested for antibacterial activity using the disc diffusion method at 400 µg/disc against *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 35218) and *Candida albicans* (ATCC 90028). Fourteen fungal extracts manifested antibacterial activity against *S. aureus* and ten endophytic fungal extracts displayed activity against *B. cereus*. One extract inhibited the growth of *P. aeruginosa* and four were active against *E. coli* and *C. albicans*. Crude extracts of AA-MS-03, AA-MS-06, AA-MS-10 and AA-MS-11 fungal species displayed activity against Gram positive *S. aureus*, *B. cereus* and *C. albicans*. These active extracts were next tested at 100 and 50 µg/disc against these three organisms. At these concentrations only three extracts (AA-MS-03, AA-MS-06, and AA-MS-10) displayed activity. These results demonstrate that *M. zeylanica* harbors several endophytic fungi that are capable of producing antimicrobial substances against common pathogenic bacteria.

**Acknowledgment:** Financial support from the National Science Foundation (NSF), Sri Lanka, and grant number RG/2012/NRB/01 is gratefully acknowledged.

## Sequence changes responsible for C<sub>3</sub> to C<sub>4</sub> transition of Phosphoenolpyruvate carboxylase (PEPC) of cereals at the DNA and protein levels using bioinformatics tools

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Rice (*Oryza sativa*), is a C<sub>3</sub>-phototroph in the family Poaceae and is the staple food of >50% of the world's population. In C<sub>3</sub>-phototrophs, up to 25% of fixed Carbon is lost by photorespiration. C<sub>4</sub>-photosynthetic pathway is a natural adaptation to hot, dry low CO<sub>2</sub> environments with no Carbon loss. A green revolution is sought to meet the ever increasing demand for rice and engineering C<sub>4</sub>-rice is predicted to increase rice yield by 40%. Phosphoenolpyruvate carboxylase (PEPC) is the key enzyme catalyzing the carboxylation of CO<sub>2</sub> into organic acids in C<sub>4</sub> photosynthesis. Comparison of PEPC sequences of C<sub>3</sub> and C<sub>4</sub> plants in the family Poaceae and genus *Flaveria* using Bioinformatics tools could provide important clues to enable efficient C<sub>3</sub> to C<sub>4</sub> conversion of rice. Use of bioinformatics tools to detect these changes is highly efficient, cost effective and provide guidance to wet lab experiments. Gene Promoter, CDS and protein sequences of PEPC genes of the family Poaceae and genus *Flaveria* were used for this study since they contain both C<sub>3</sub> and C<sub>4</sub> plants. Maximum-Likelihood, Neighbour-joining and Bayesian-phylogenetic analysis were performed to identify the most closely related rice C<sub>4</sub>-specific PEPC gene. Comparison of regulatory-elements in the promoter region of C<sub>3</sub> and C<sub>4</sub> plants showed that essential C<sub>4</sub>-cis-acting elements such as MNF1, MEM1, light-responsive elements and direct repeats are missing from all the rice PEPC gene promoters. Other elements not directly related to PEPC function, such as GCN4-motif, ARE1 etc., are also absent in rice PEPC promoter, but present in C<sub>4</sub>-specific PEPC gene promoters. Comparison of conserved protein motifs of C<sub>3</sub> and C<sub>4</sub> PEPC showed that all the necessary conserved motifs such as active site, substrate-binding site are present in all the PEPC sequences tested including rice. However, overall secondary structure composition of rice PEPC did not tally with other C<sub>4</sub>-specific sequences. The Os01g0208700 was predicted to be the C<sub>4</sub>-specific PEPC gene in rice. To conclude, it could be predicted that rice PEPC activity may be enhanced if Os01g0208700 gene is engineered to have the necessary cis-elements and the overall secondary structure composition of C<sub>4</sub> PEPC genes.

## Feasibility of using Exon-Primed Intron-Crossing (EPIC) markers to detect the genetic variation of a dengue vector in Sri Lanka

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*Dengue* ranks as the most important mosquito-borne viral disease in the world. Its control relies chiefly on the management of the principal vector *Aedes aegypti*. However, to design sustainable mosquito control strategies, it is imperative to understand the microevolution of local vector populations. This requires an informative set of genetic markers. Although microsatellite markers are heavily used in this respect, they carry several limitations. Exon-Primed Intron-Crossing (EPIC) markers which detect polymorphisms across non-coding regions were shown to successfully address some of these limitations. Accordingly, this study was conducted to determine the feasibility of using EPIC markers to study genetic variation of *Ae. aegypti* populations in Sri Lanka. DNA from 22 *Ae. aegypti* larvae collected from Colombo district was extracted using phenol chloroform method and subjected to PCR amplification with two EPIC primers (RpL30a and RpS20b). Amplicons were separated on 6% PAGE. Resulting homozygous samples were sequenced and analyzed with BioEdit 7.2.5 and DnaSP 5.10. Departures from HWE were calculated using Genepop4.2. All samples were successfully amplified with both markers. PAGE separation of amplicons revealed two alleles for each marker; RpL30a:316bp (n=20), 322bp (n=12); RpS20b: 155bp (n=12), 157bp (n=17). The observed proportion of heterozygotes was higher in RpL30a (45.45%) compared to that of RpS20b(31.82%). Observed and expected heterozygosities did not differ significantly for both markers. Sequence variation showed relatively high nucleotide diversity [ $P_i(\pi)$ ; RpL30a: 0.01030 (1 mutation per 23bp); RpS20b: 0.00878 (1 mutation per 33 bp)] and indel diversity [ $P_i(i)$ ; RpL30a :0.00881 (1 indel per 20 bp); RpS20b (0.00184 (1 indel per 37 bp) values for both markers. This is the first attempt to use EPIC markers in Sri Lankan context. Conformance of both markers to HWE indicates that they are selectively neutral. The high sequence diversity and successful amplification in all instances suggest that they are suitable candidates to study genetic variation of local *Ae. aegypti* population.

**Acknowledgement:** University Research Grant, University of Colombo (AP/3/2/2014/RG/05) is acknowledged for funding.

***In vitro* Anti-5-lipoxygenase, anti-hyaluronidase and anti-oxidant properties of ethanol leaf extract of *Diospyros ebenum*.**H D S M Perera <sup>1</sup>, R Samarasekera <sup>\*1</sup>, S Handunnetti<sup>2</sup> and O V D S J Weerasena <sup>2</sup>

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Functional inhibition of 5-lipoxygenase (5-LOX) and hyaluronidase enzymes to reduce the generation of inflammatory mediators is considered as an effective approach in the treatment of inflammatory diseases. Medicinal plants continue to provide rich sources of new enzyme inhibitors and antioxidants. *Diospyros ebenum* (Ebenaceae) is an economically important tree grown in Sri Lanka with a medicinal value. The objective of the present study is to investigate bioactivities of ethanol leaf extract of *D. ebenum* using *in vitro* anti 5-lipoxygenase, anti-hyaluronidase and anti-oxidant assay models. The air-dried, powdered leaves (50.0 g) of *D. ebenum* were extracted with ethanol (100 × 3 mL) using cold extraction technique. A5-LOX and hyaluronidase enzyme inhibitory assays were performed to evaluate *in vitro* anti-inflammatory activity. Antioxidant activity was determined by DPPH free radical scavenging, ferric reducing antioxidant power (FRAP), ferrous iron chelating (FIC) and oxygen radical absorbance capacity (ORAC) assays. The total phenolic content (TPC) and total flavonoid content (TFC) were determined. The ethanol leaf extract of *D. ebenum* showed a good anti-A5-LOX activity (IC<sub>50</sub>: 141.78 ± 3.79 µg/mL) in comparison to the reference standard baicalein (IC<sub>50</sub>: 1.76 ± 0.15 µg/mL) and good hyaluronidase enzyme inhibition (42.43 ± 1.45 % at 500 µg/mL) as opposed to the reference standard tannic acid: (90.28 ± 0.91% at 500 µg/mL). The extract exhibited moderate DPPH free radical scavenging (IC<sub>50</sub>: 27.28 ± 0.34 µg/mL) against the reference standard trolox (IC<sub>50</sub>: 5.29 ± 0.09 µg/mL), low FRAP (369.15 ± 3.14 mg Trolox Equivalents (TE)/g) and ORAC (95.24 ± 0.00 mg TE/g) against standard green tea extract: 1662.82 ± 0.22 mg TE/g). The extract showed no FIC properties at the assay concentration of 1000 µg/mL in comparison to the reference standard EDTA-2Na (IC<sub>50</sub>: 13.07 ± 0.64 µg/mL). The TPC and TFC were quantified as 18.62 ± 0.65 mg Gallic Acid Equivalents (GAE)/g and 30.22 ± 1.01 mg Quercetin Equivalents (QE)/g, respectively. The anti-A5-LOX, anti-hyaluronidase and antioxidant activities of ethanol leaf extract of *D. ebenum* were significantly different from the respective reference standards (P < 0.05). The study conclusively demonstrates that, the ethanol leaf extract of *D. ebenum* possesses anti-A5-LOX and anti-hyaluronidase properties along with anti-oxidant properties. We record for the first time the ethanol leaf extract of *D. ebenum* as a good source of A5-LOX and hyaluronidase enzyme inhibitors, which is supported by anti-oxidant properties.

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## Antioxidant properties of leaves of *Aporosa lindleyana* Baill. (Kebella)

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Antioxidant protection in biological systems is a growing topic in biomedical sciences. Numerous epidemiological studies have shown that foods rich in antioxidants provide protection against multiple diseases. *Aporosa lindleyana* Baill. (Family: Euphorbiaceae) commonly known as Kebella in Sinhalese is used as a leafy vegetable in the country. The root and bark of *A. lindleyana* is reported to have many biological activities including antioxidant activity. However, antioxidant properties (AP) of leaves of *A. lindleyana* are not reported to date. The present study therefore, evaluates the AP of leaves of *A. lindleyana*, *in vitro*. Fresh *A. lindleyana* leaves were collected from Maharagama, in Colombo district Sri Lanka. Fresh leaves were cleaned, oven dried at 50 °C for 5-6 h and powdered. 95 % ethanolic and water extracts were prepared from powdered leaves. Antioxidants of 95 % ethanolic and water extracts of leaves of *A. lindleyana* were studied using total polyphenolic content (TPC), total flavonoid content (TFC) (TPC: Ethanol extract (EE) n=3 and water extract (WE) n=6; TFC: EE and WE n=3 each) and antioxidant activities using Ferric reducing antioxidant power (FRAP), 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and oxygen radical absorbance capacity (ORAC) *in vitro* antioxidant assays (FRAP: EE and WE n=3 each; ORAC: EE n =8 and WE n=4; DPPH and ABTS: EE and WE n=4 each). Both 95 % ethanolic and water extracts of leaves of *A. lindleyana* possess antioxidants in TPC and TFC and antioxidant activities in FRAP, DPPH, ABTS and ORAC. However, ethanolic and water extracts showed significant differences (P<0.05) among the investigated antioxidants and antioxidant activities. Ethanolic extract showed high activity for all the studied antioxidant activities compared to water extract and lower antioxidants in TPC. The mean  $\pm$  S.E values of TPC and TFC of ethanolic extract of leaves of *A. lindleyana* was 264.35 $\pm$ 3.84 mg gallic acid equivalents (GAE)/g of extract and 6.86 $\pm$ 0.12 mg quercetin equivalents (QE)/g of extract while the mean  $\pm$  S.E values of antioxidant activities of FRAP, DPPH, ABTS and ORAC was 369.87 $\pm$ 6.88 mg trolox equivalents (TE)/g of extract, 337.61 $\pm$ 10.15 mg TE/g of extract, 506.48 $\pm$ 5.27 mg TE/g of extract and 321.49 $\pm$ 5.95 mg TE/g of extract respectively. Similarly, water extract had 297.91 $\pm$ 2.75 mg (GAE)/g of extract, 0.94 $\pm$ 0.20 mg (QE)/g of extract of TPC and TFC while FRAP, DPPH, ABTS and ORAC had 329.94 $\pm$ 3.55 mg (TE)/g of extract, 277.73 $\pm$ 2.09 mg TE/g of extract, 470.55 $\pm$ 4.93 mg TE/g of extract and 304.79 $\pm$ 5.27 mg TE/g of extract respectively. In conclusion, leaves of *A. lindleyana* (Kebella) possess marked antioxidant properties. The ethanol extract has higher antioxidant properties than that of the water extract. Interestingly, this is the first report on antioxidant properties of leaves of *A. lindleyana* (Kebella) worldwide. Further, findings of this study indicate the potential of use of *A. lindleyana* (Kebella) leaves as a leafy vegetable for prevention and management of oxidative stress associated chronic diseases in Sri Lanka.

## **Isolation and cloning of thermostable alpha amylase gene for the production of recombinant enzyme for industrial purposes**

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Industrial enzyme production is a successful industry worldwide. Alpha Amylases (E.C.3.2.1.1), a widely used enzyme, biologically catalyze the hydrolysis of internal alpha 1,4-glycosidic linkages in starch in low molecular weight products, such as glucose, maltose and maltotriose units. Although Sri Lanka uses 200 billion rupees worth thermo-stable alpha amylase annually, it is not locally produced, and total enzyme requirement is imported. Incorporating the genetic engineering approaches to enhance the production of thermo-stable alpha amylases can be used in scaling up the production process to provide for the industry. The present work was carried out with the objective of cloning the thermo-stable alpha amylase gene from *Geobacillus stearothermophilus* in cloning vector pGEM<sup>®</sup>-T Easy, in order to express the enzyme in the highly efficient protein expression system *Pichia pastoris*. Genomic DNA was extracted from *Geobacillus stearothermophilus* by a low cost method developed in the laboratory. Thermostable  $\alpha$ -amylase gene was amplified by gene specific primers and expected size band was observed (1,670 bp) on an agarose gel. Amplified product was purified and cloned into pGEM<sup>®</sup>-T Easy vector (pGEM<sup>®</sup>-TEasy-Amy). Recombinants were screened by rapid screening method, colony PCR, restriction enzyme digestion and sequencing for confirmation. Currently work is underway to clone thermo-stable  $\alpha$ -amylase gene into expression vector.

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## A phylogenetic analysis of *Dinopium* woodpeckers in Sri Lanka using Cyt b and COI nucleotide sequences (Aves:Piciformes)

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Phylogenetic makeup of *Dinopium* woodpeckers was studied in two pairs of phylogenetic sister taxa, each consisting of a Sri Lankan endemic and its closest phylogenetic relative found in both Sri Lanka and India. Studies on mitochondrial regions of *Dinopium* from Sri Lanka and their genetic variability either within or between species are scarce. The present study investigates the genetic affinities of *Dinopium* woodpeckers in Sri Lanka and their allies using mitochondrial genes COI and Cyt b sequences. Birds were captured in transects spanning across the north to south of the island using mist nets. DNA was extracted using blood. Cyt b and COI genes were amplified by PCR. A total of 1134 base pairs from two genes were sequenced. Other species of *Dinopium* and an outgroup from a neighboring biogeographic region were downloaded from NCBI GenBank and all the sequences were multiple aligned with ClustalW. Maximum Likelihood (ML) and Neighbor-Joining (NJ) trees were generated using MEGA 5.2 using rapid bootstrap, for 1000 replicates with the GTR+I+G model. Concatenated tree was constructed using COI and Cyt b sequences. All red and yellow (parental) forms share haplotype sequence. In contrast orange forms show a combination of haplotype sequences of red and yellow forms. Both NJ and ML trees were concordant. Pairwise  $F_{st}$  values show a significant genetic distance between *Dinopium* species found in Sri Lanka. The sub species of *D. benghalense* found in southern India forms a very robust clade with ours and appears as the closest sister species to the local populations of *Dinopium*. The red colour form grouped into a separate clade. Intermediate phenotypes (orange type) are nested between pure red and pure yellow forms. ML and NJ trees of mitochondrial gene sequence data further supports *D. psarodes*'s recent elevation as a full species from *D. benghalense* cluster. Sri Lankan *Dinopium* complex is phylogenetically separated from other *Dinopium* species of Asia. The endemic red form comes out as a separate clade from other *Dinopium* species.

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# **ABSTRACTS OF PAPERS**

**Parallel session 4**

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## Comparison of four DNA extraction methods for target bacteria found in bovine milk for large scale detection of mastitis pathogens

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Direct isolation of high-quality DNA from the target bacteria found in milk is an essential task for PCR detection of mastitis pathogens in the dairy herds all over the world. But it is often problematic due to various factors such as small concentrations of the pathogenic bacterial DNA present in milk, the degree of cellular lysis and the presence of PCR-inhibitory substances. In addition, many current methods typically require multiple steps or specialized equipment, rendering them impractical for use with large sample numbers, while commercial kits are highly expensive. Thus this study is aimed at selecting the most efficient DNA extraction method which remains unaffected by potential inhibitors, high purity with maximum yield and minimum sample processing time as well as the cost per single extraction. Four published methods for DNA extraction from milk were used for the comparison, viz. Silica method, Sodium iodide method, Urea-SDS method and Phenol Chloroform method. Fresh Bovine milk samples were artificially inoculated with *Streptococcus agalactiae* having bacterial concentrations from 10<sup>3</sup> to 10<sup>8</sup> CFU/ml and then undergone to DNA extraction from each method. The yield and purity of extracted DNA was calculated using UV/visible spectrophotometer. The chemical cost and the total time for a single extraction was calculated separately for each method. A DNA fragment of the 16S to 23S rRNA spacer region of *Streptococcus agalactiae* was amplified in the PCR as previously described by Phuektes *et al.*, 2001. The amplified DNA was visualized in 2% Agarose gel and the minimum detection limit for each extraction method was determined. It was observed that Sodium iodide method has least processing time (80 min), sustainable cost (SLR 23.16), highest yield of DNA (405.0 ± 27.6 ng) and highest purity (1.93 ± 0.30) with up to 10<sup>4</sup> CFU/ml detection limit. So this method was identified as the most convenient and efficient method for the extraction of bacterial DNA from bovine milk for commercial scale detection of mastitis.

## Rice rhizosphere manipulation with *Trichoderma virens* for effective phosphorous management

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The use of *Trichoderma virens*, a highly rhizosphere competent phosphate solubilizer as a phosphorous (P) biofertilizer for organically grown rice was investigated. A pot experiment was conducted at the Rice Research and Development Institute, Batalagoda. The fertilizer treatments included no P (T<sub>1</sub>), Triple Super Phosphate (TSP) at recommended level (T<sub>2</sub>), TSP at half recommended level together with Eppawala Rock Phospahte (ERP) added to replace half recommended level of TSP quantitatively (T<sub>3</sub>), ERP at a rate to quantitatively replace the recommended level of TSP (T<sub>4</sub>) and two fold ERP equivalents to TSP at recommended level (T<sub>5</sub>). All treatments had Urea and Muriate of potash (MOP) at recommended levels. The inoculum was prepared in a mass cultivation medium containing rice straw and maize seeds at 1:1 ratio and 20 g of inoculum, was applied per pot. All the P treatments were supplemented with either 20 g of mass cultivation medium (MC) alone (I<sub>0</sub>) or with *T. virens* in MC medium (I<sub>1</sub>). Pots having zero level N, P, K fertilizers (C<sub>1</sub>); Urea, MOP but no MC medium and P (C<sub>2</sub>); and Urea, MOP, MC and P (C<sub>3</sub>) were maintained as controls. Completely randomized design was employed with 08 replicate pots and in total 112 pots, each pot containing 4 rice plants. Plant growth parameters and plant and soil P were quantitatively determined by destructive sampling of 04 replicates from each treatment at tillering and 50% flowering stages. The results obtained were analyzed using one way ANOVA and t test. TSP application exhibited a significantly superior performance compared to all other treatments in all parameters tested (p= 0.000). In all treatments having ERP with fungal inoculum, a significantly higher performance was observed when compared with its counterparts with no inoculum. The plant P levels also showed the pattern the same as treatment effect indicating that the fungus has the potential to supply adequate P for the plant whenever needed irrespective of the soil P level. The recommended level of TSP replaced with ERP completely and ERP at double recommended level of TSP showed similar growth responses. Additionally, the ratio of the shoot dry weight to root dry weight values in the treatments showed the potential of *T. virens* in enhancing root development.

## Screening of native actinomycetes for potential antimicrobial activity

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This study was undertaken with the aim of isolating soil and leaf litter actinomycetes and screening them for antimicrobial activity. Soils collected from 5 different niche habitats of Sri Lanka were subjected to pre-treatments, *viz.* enrichment with CaCO<sub>3</sub>, treating soil at 70 °C for 15 min, 100 °C for 1h, 40 °C for 1h and 50 °C for 1h. Pretreated samples were plated on five different media: Starch casein nitrate agar (SCA), Actinomycete isolation agar (AIA), Half strength nutrient agar, Yeast extract malt extract agar, and Potato dextrose agar (PDA). Out of the 10 actinomycete isolates, only four were identified using the keys of Bergey's Manual of Determinative Bacteriology (1994). They were belonged to four genera; *Kibdelosporangium*, *Intrasporangium*, *Actinoplanes* and *Saccharomonospora*. Three unidentified actinomycetes were morphologically characterized. The best medium for actinomycete isolation was Actinomycete isolation medium which yielded five isolates, whereas heating of dry soil for 15 minutes at 70 °C proved to be the best pretreatment method. Primary screening of the antimicrobial activity against bacteria and yeasts employed Cross-streak and Agar overlay methods employing *Pseudomonas aeruginosa* (ATCC® 27853™), *Staphylococcus aureus* (ATCC® 25923™), *Escherichia coli* (ATCC® 25922™) and *Candida albicans*. The disk diffusion method was used to further evaluate antibacterial activity and to detect the antifungal activity against *Curvularialunata*, *Colletotrichum* and *Rigidoporous microporous*. Among the actinomycete isolates, four isolates showed either antifungal or antibacterial activity or both. None of the isolates inhibited the growth of the two gram negative bacteria: *P.aeruginosa* and *E coli*. Actinomycete isolates D003 and D005 (unknown genera) significantly inhibited the growth of *Candida albicans*, *Curvularia* and *Colletotrichum* and the bacterium *Staphylococcus aureus*. Hence, the isolation, characterization and investigating antimicrobial activity of actinomycetes from different habitats in Sri Lanka could be a pathway for discovery of new antibiotics.

## **Morphological and reproductive characterization of *Colletotrichum* spp. causing anthracnose of papaya in Sri Lanka**

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*Colletotrichum* is the major causal organism of anthracnose, which affects many economically important fruit crops including papaya. As *Colletotrichum* forms a species complex with noticeable genetic variation within groups, disease management strategies need to be oriented to address this diversity. As an initial step towards this goal, the present study attempts to group *Colletotrichum* species based on both morphological and reproductive characters. To isolate *Colletotrichum* species, a total of 67 papaya fruits with anthracnose symptoms were collected from 6 different districts: Jaffna, Anuradhapura, Kurunegala, Badulla, Monaragala and Ratnapura. From these samples, 20 *Colletotrichum* isolates were purified using single conidial isolation technique. Morphological characterization of the isolates was based on colony characteristics such as growth rate on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA), colour of the upper and lower surface of the colony, and microscopic characters such as appressorial length, width, colour and shape. Reproductive characterization was based on length, width, shape, and the yield of conidia. The colony characters and conidia yield were taken from seven day old cultures grown on PDA and MEA. The reproductive (conidial) and appressorial characters were taken from seven day old slide cultures. Initially, quantitative characters were analyzed separately with one-way ANOVA using SPSS 16.0 software which could not produce clearly defined clusters. Hence, both morphological and reproductive characters were pooled and analyzed with multivariate analysis using hierarchical cluster tool of SPSS 16.0 software. This resulted in a clear grouping of all 20 isolates into two clusters with two sub-clusters in each. Grouping based on both morphological and reproductive characters provide a well clustered dendrogram compared to one way ANOVA of the characters separately and hence can be used as a tool in grouping *Colletotrichum* sp. causing papaya anthracnose.

## Antibiotic Sensitivity of *Bacillus thuringiensis* strains isolated from Sri Lanka

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*Bacillus thuringiensis* (*Bt*) is a spore forming, entomopathogenic, gram positive bacterium. It produces, insecticidal  $\delta$  – endotoxins that are active against insects of different orders, mainly the orders of Diptera, Lepidoptera and Coleoptera. The objective of this study was to determine the sensitivity and/or resistance pattern shown by 10 *Bt* strains to different antibiotics. *B. thuringiensis* were isolated from soil from various regions in Sri Lanka using acetate heat treatment method and identified by phenotypic and molecular methods. Antimicrobial resistance/sensitivity of Amoxicillin, Ceftriaxone, Gentamicin and Netilin antibiotics was tested for 10 *Bt* strains; namely *Btkurstaki* (AB1, AB20), *Btgraciosaensis* (AB11, AB13), *Btpoloniensis* (AB17), *Btkonkukian* (AB21, AB23, AB59, AB63) and *Btkenyae* (AB155) by Bauer Kirby disc diffusion method on Mueller Hinton Agar (MHA). The zones of inhibition around the antibiotic discs were measured following 48 hours incubation period. The measurements were compared against the zone size interpretative chart for the antibiotics as per standards based by Clinical and Laboratory Standards Institute (CLSI). The results showed that 6 *Bt* strains were resistant to Amoxicillin whilst 4 *Bt* strains were sensitive, 6 *Bt* strains were resistant to Ceftriaxone whilst 4 *Bt* strains were sensitive, 5 *Bt* strains were resistant to Gentamicin while 5 *Bt* strains were sensitive and 1 *Bt* strain was resistant to Netilin whilst 9 *Bt* strains were sensitive. The 10 *Bt* strains used in the study displayed a significantly varied range of sensitive and resistance against different antibiotics tested. However, most of the strains were sensitive to the antibiotic Netilin, except one (AB155) whilst all the strains displayed significantly varied levels of resistance and sensitive to the other three antibiotics tested.

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## Multivariate discrimination of inflorescence characters in conserved *Cocos nucifera* L. var. *typica* germplasm in Sri Lanka

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Since the inception of the Coconut Research Institute in 1929, a coconut genebank was created by collecting local germplasm and importing exotic varieties. The germplasm has to be fully characterized and evaluated for them to be utilized effectively in breeding programs or to refine conservation strategies. Systematic characterization and evaluation of germplasm can be achieved by generating information on their morphological characteristics out of which inflorescence characters playing a significant role in implementing of breeding programs. The current study was conducted to characterize coconut inflorescence morphology to obtain information on the comparative inflorescence diversity of *Cocos nucifera* L. var. *typica* within different tall accessions collected from different geographic locations and conserved *ex-situ* in Pallama coconut field gene bank. A total of 8 descriptors for coconut outlined by Bioversity International for inflorescence morphology were recorded for 12 conserved accessions. The data were analyzed using Minitab Version 14 to derive Principal Components and Cluster analysis based on squared Euclidean distances. Principal component Analysis is used to describe the variation in the dataset. The first three principal components (PC1, PC2 and PC3) accounted for 68.1%, 10.5% and 6.9% of the variation respectively accumulating to a total of 85.5% of the total variability among the coconut accessions evaluated. The dendrogram showed two major clusters separated at 33.33% similarity level with one major cluster forming two separate groups at 66.67% similarity level. Further, two accessions formed a single group with standard Ambakelle special accession which has been extensively used in coconut breeding for higher yields and stability. These conserved *Cocos nucifera* L. var. *typica* accessions displayed considerable diversity for inflorescence morphology indicating the potential of them to be utilized in coconut breeding and the effectiveness of sampling in the conservation process.

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## Screening of rice for resistance against *Xanthomonas oryzae* pv. *oryzae* through anther culture

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*Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causes the most economically destructive bacterial disease, Bacterial Leaf Blight (BLB). It was reported that *Xoo* rapidly evolves into resistant strains evading successful control, necessitating continuous improvement of disease management and control programmes. Hence it was intended to develop a rapid screening method for BLB resistance rice varieties through anther culture. Three rice varieties with varying degrees of resistance to BLB were employed. They were: IRBB 60 (resistant), Bg 454 (moderately resistant) and Bg 450 (susceptible). Calli obtained were screened for survivability in the presence of *Xoo* toxins. The bacterium was grown in Lauria Bertani broth under oscillation for 24 h, 48 h and 72 h and filter sterilized. The filtrate was used as the source of bacterial toxin. Filtrates were incorporated into modified N<sub>6</sub> medium in 2:1 and 4:1 dilutions. Uninoculated broth incorporated modified N<sub>6</sub> medium served as the control. Five replicate plates containing ten pieces of calli per plate from each variety were employed to assess the mortality of calli. Generally at all filter concentrations obtained after 24 and 48 h post-inoculation, calli of IRBB 60 variety survived but most of the calli of Bg 450 (percentage mortality:  $p=0.00 < \alpha=0.05$ ) and Bg 454 ( $p=0.04$ ) did not. Calli of Bg 454 which is moderately resistant showed more survivability than Bg 450 variety. High survivability was exhibited by IRBB 60 variety with a zero percent mortality. Calli of all three varieties showed zero percent mortality on controlled media. 72 h old filtrate failed to show any effect on any of the rice varieties tested. This indicates that either the toxin is produced within the early stages of the growth by the bacterium or the toxin loses its activity within 72 h. Calli induced from anthers reflected considerable variability among the varieties with respect to *Xoo* resistance which is in accordance with their genetic characteristics. Therefore anther culture technique has the potential to be developed as a good tool in the screening procedure against *Xoo* resistance.



## Determination of the antifungal, biochemical and physiological characteristics of *Trichoderma* spp. isolated from onion fields of Sri Lanka

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*Trichoderma* species have been used as biological control agents of numerous fungal pathogens. In this study, *Trichoderma* species isolated from the soil of fifty five onion fields in different locations in the Matale and Anuradhapura districts were characterized. The antagonistic effect of three most frequently isolated *Trichoderma* spp. (Tr. 1, Tr. 3, Tr. 4) were evaluated using dual culture assays against fungal pathogens of onion *i.e.* *Fusarium* sp., *Colletotrichum gloeosporioides* and *Alternaria* sp. Four replicate plates were used for each treatment and the results analysed statistically using ANOVA. All tested *Trichoderma* spp. suppressed the mycelial growth of fungal pathogens tested. Tr. 3 caused a significantly high ( $P < 0.05$ ) reduction of growth compared with other species showing 55.7%, 76.6% and 57.7% growth inhibition of *Fusarium* sp., *C. gloeosporioides* and *Alternaria* sp. respectively. Tr. 1 showed less inhibition amounting to 31.9 %, 53.9% and 36.9% respectively. Microscopic observations indicated that the mode of action adopted by *Trichoderma* isolates to restrict the growth of pathogens was through coiling, formation of loops and attachment of hyphal tips to the hyphae of pathogens. Tests on extracellular enzyme production by Tr. 3 and Tr. 1 using plate assays indicated the production of Chitinase and CMCase. Proteolytic activity was determined using bromocresol green dye on casein agar plates and gelatin agar plate assay. Tr. 1 and Tr. 3 produced narrow zones of hydrolysis on both casein agar plates and gelatin agar plates indicating low proteolytic activity *i.e.* 1.25 cm and 0.5 cm respectively for gelatin agar plates. Crude extracts of Tr.1, Tr.3 dissolved separately in DMSO to a final concentration of 50 mg/ml were used in well diffusion assays. After incubation, zones of inhibition of growth of *Fusarium* sp. by two *Trichoderma* isolates were observed indicating antifungal activity. The effect of volatile metabolites produced by Tr.1 and Tr.3 on *Fusarium* sp. was evaluated by placing the pathogenic fungus and each test *Trichoderma* sp on the facing halves of a petri dish and sealing with parafilm. The effect of volatile metabolites was considered as growth inhibition of *Fusarium* sp. Tr. 1 caused a 15 % and Tr. 3 a 25 % inhibition of mycelial growth of *Fusarium* sp. indicating a low level of volatile metabolite production by both species. Therefore, out of the *Trichoderma* isolates characterized, Tr.1 and Tr.3 possessed an ability to control the growth of the three onion pathogens tested and the control was achieved by both physical and biochemical activities of the test *Trichoderma* spp.

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***Sclerotinia sclerotiorum* causing cabbage head rot in Sri Lanka**B M A Guruge<sup>1</sup>, K P Somachandra<sup>2</sup> and R N Attanayake<sup>1</sup>,<sup>1</sup>Department of Botany, University of Kelaniya, <sup>2</sup>Regional Agricultural Research & Development Centre, Bandarawela.

Cabbage head rot caused by *Sclerotinia* sp. has not been reported as an economically important disease in Sri Lanka until our unexpected finding of disease prevailing fields in Nuwara Eliya during 2013-2014. *Sclerotinia* is a well-studied genus around the world. However, no research has been done in Sri Lanka. During a preliminary survey it was found that farmers were unaware of the disease and employ improper cultural practices. The objectives of this study were to document the disease incidence of cabbage head rot in selected areas, to determine the pathogen species using morphological and molecular characteristics, to confirm pathogenicity and to test whether Maneb insensitive isolates are present in major cabbage growing areas in upcountry. Sampling was done from 15 randomly selected cabbage fields in Ambewela and Pattipola. A total of 35 sclerotia were collected separately from infected cabbage heads which were at least 3-6 m apart into paper envelopes and brought to laboratory. Sclerotia were surface sterilized, plated in antibiotic amended PDA plates and obtained pure cultures using hyphal tip method. Sclerotia size of each isolate varied from 4 to 10mm, which is the typical sclerotial size of *S. sclerotiorum*. All the isolates were identified as *S. sclerotiorum* based on morphological and cultural characters. Total genomic DNA was extracted from randomly selected two isolates and amplified the rDNA-ITS region using PCR. BLAST searches of ITS sequence confirmed that the sequence was 99% similar to *S. sclerotiorum*. Koch's postulates were carried out using a detached leaf assay on the cabbage cv. Bandon and confirmed the pathogenicity. During the survey no disease free field was found at the maturity of the crop and average disease incidence was 4-5.0 %. Twenty of the randomly selected isolates were tested for the percent inhibition of the mycelial growth by the fungicide, Maneb, amended PDA plates. At 250µg.a.i./ml discriminatory concentration, five isolates had less than 50% inhibition indicating Maneb insensitive isolates could be present in Sri Lanka. Therefore, early detection and management of the disease is important.

## Antifungal effect of *Croton aromaticus* against *Rhizopus* spp. isolated from banana and papaya

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Synthetic fungicides are widely used to control postharvest diseases of fruits all over the world. Numerous plant extracts have been identified to have antimicrobial properties *in vitro* and are potential alternatives for synthetic fungicides. *Rhizopus* species are common on fruits after harvest resulting in transit rot. Present study was an attempt to evaluate the antifungal effect of ethanolic extract of *Croton aromaticus* (Kappettiya) leaves *in vitro* against growth of *Rhizopus* sp. and *Rhizopus stolonifer* isolated from banana and papaya, respectively. Surface sterilized (NaOCl (3% W/V)) diseased banana and papaya fruit tissues were cultured on PDA plates in order to obtain pure cultures of possible fungi and they were identified by morphological and microscopic characteristics, following identification keys and by comparing with cultures available in the Department of Botany, University of Kelaniya. Inhibitory effect of the ethanolic extract of *C. aromaticus* was investigated by well diffusion method with 1 mg/ml, 5 mg/ml, 10 mg/ml, 30 mg/ml, 50 mg/ml, 100 mg/ml, 200 mg/ml and 300 mg/ml concentrations with the positive control (Captan) and negative control (DMSO). Significant ( $P < 0.05$ ) inhibitory effects were exhibited by the ethanolic extract of *Croton aromaticus* leaves against both test fungi. The highest mycelial growth inhibitions of *Rhizopus* sp. and *Rhizopus stolonifer* were observed at 100 mg/ml and 200 mg/ml concentrations respectively. *Rhizopus stolonifer* was inhibited more effectively than *Rhizopus* sp. by the above ethanolic extract. Minimum inhibitory concentration of mycelial growth of both fungi was 30 mg/ml. TLC analysis revealed the presence of four compounds with R<sub>f</sub> values of 0.6, 0.7, 0.8 and 0.9. Phytochemical analysis of ethanolic extract exhibited constituents including alkaloids, terpenoids, quinones, phytosterols and flavonoids. Hence, the results of the present investigation indicated the possibility of using ethanolic extract of *Croton aromaticus* leaves against transit rot mould *Rhizopus* isolated from banana and papaya.

## Soil fungi of semi natural montane forest and adjacent pine plantation in Peacock hill, Pussellawa, in Nuwara Eliya district

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Many ecosystem studies which were mainly for temperate situations have shown that the establishment of monocultures such as *Pinus*, *Eucalyptus* etc., affects soil microbial community and soil physicochemical properties. In Sri Lanka, there are only few recorded studies in this area and most of them are only on soil physicochemical properties of different ecosystems. Also, there is little published literature on identification of soil fungal species in different forest ecosystems in Sri Lanka. Hence, this study was carried out to investigate soil fungal diversity and their potential decomposing abilities in the soils of a *Pinus* plantation and adjacent semi natural montane forest in Peacock hill, in Pussellawa, in Nuwara Eliya district of Sri Lanka. Soil samples (15 replications) were collected following stratified random sampling technique from each of the two study sites and comparative studies of fungi in each sample were carried out according to the soil plate method. Potential decomposing abilities and metabolic capacities of fungi which were isolated in highest frequencies of occurrence (>30%) were tested using pure substrates, starch, pectin, cellulose and lignin. Results showed significantly higher fungal diversity in semi natural forest (32 fungal spp.) compared to *Pinus* plantation (9 fungal spp). *Trichoderma* spp. [*Trichoderma hamatum* (49.00±1.52), *Trichoderma piluliferum* (53.00±1.69), *Trichoderma polysporum* (39.33±2.24), *Trichoderma pseudokoningii* (62.00±1.21), *Trichoderma viride* (61.33±3.10), *Trichoderma* sp.1 (36.67±1.39), *Trichoderma* sp.2 (36.33±1.16), *Trichoderma* sp.3 (31.67±0.55), *Trichoderma* sp.4 (35.67±1.12)], *Mortierella* spp. [*Mortierella* sp.5 (35.00±2.80), *Mortierella* sp.6 (30.00±4.00), *Mortierella* sp.7 (59.33±1.73), *Mortierella* sp.8 (25.33±0.49)], *Penicillium* spp. [*Penicillium* sp.1 (43.00±1.36), *Penicillium* sp.2 (48.00±1.29), *Penicillium* sp.3 (27.67±0.66), *Penicillium* sp.4 (13.33±1.16)], *Acremonium* spp. [*Acremonium* sp.1 (8.30±0.71), *Acremonium* sp.2 (22.33±0.24), *Acremonium* sp.3 (19.67±0.81)], *Aspergillus* spp. [*Aspergillus* sp.1 (22.00±1.03), *Aspergillus* sp.2 (42.00±0.86), *Aspergillus* sp.3 (24.33±1.29), *Aspergillus* sp.4 (9.67±0.73), *Aspergillus* sp.5 (8.33±0.63)], *Rhizopus* spp. [*Rhizopus* sp.1 (22.00±1.63), *Rhizopus* sp.2 (26.67±0.73)] and white sterile spp. [White sterile sp.2 (28.67±0.87), White sterile sp.3 (14.00±1.61), White sterile sp.4 (17.66±1.37)] were isolated at higher frequencies from semi natural montane forest. Frequently isolated fungal species from the *Pinus* plantation were *Mortierella* spp. [*Mortierella* sp.1 (17.67±0.00), *Mortierella* sp.2 (10.67±0.00), *Mortierella* sp.3 (58.00±8.16), *Mortierella* sp.4 (23.00±4.90)] and Dark sterile sp.1 (83.33±17.15). *Penicillium* sp.1 and *Trichoderma viride* were common to both sites. Fungi isolated from both sites showed versatile abilities in utilization of many substrates such as starch, cellulose, lignin and pectin. Present study showed significantly negative impacts from exotic *Pinus* plantation on fungal community structure in the particular sites.

## Evaluation of antibacterial properties of endophytic fungi of *Cyperus brevifolius* and *Cyperus melanospermus*

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The defense oriented relationship between grasses and their endophytic fungi warrant an exploration into the secondary metabolites produced by the fungal symbionts. The present study aims to investigate antibacterial properties of the secondary metabolites produced by endophytic fungi of two species of the family *Cyperaceae* (*Cyperus brevifolius* and *Cyperus melanospermus*). Following surface sterilization, the plant parts were cut into pieces (1.5x1.5 cm<sup>2</sup>) and were placed on five growth media. The fungi that emerged from these tissue samples were sub cultured on Potato Dextrose Agar to obtain pure cultures. From both plant species, 28 morphologically distinct fungi (03 and 07 from aerial parts and roots, respectively, of *C. brevifolius* and 12 and 06 from aerial parts and roots, respectively, of *C. melanospermus*) were isolated. These fungal cultures were extracted into ethyl acetate assisted by sonication. The EtOAc extracts were utilized for standard disc diffusion assays at 400 µg /disc to assess antibacterial activity against Gram-negative and Gram-positive bacteria. All 28 fungi indicated inhibition against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) while six showed activity against *Escherichia coli*. One was active against *Salmonella enterica*, two were active against both *E. coli* and *S. enterica* and none against *Pseudomonas aeruginosa*. The assays were repeated at lower concentrations (200, 100 and 50 µg/disc) for nine samples (five from *C. melanospermus* and four from *C. brevifolius*) with the highest activity (inhibition zone >10 mm and activity against multiple bacteria). At 200 µg/disc, all nine fungi showed activity while at 100 and 50 µg/disc six fungi showed activity against two or more bacteria. For all bio assays, Gentamicin and methanol were used as positive and negative controls respectively. The extraction of genetic material for DNA sequencing for fungal identification is currently underway. This study revealed that both species of *Cyperus* host a large number of fungal symbionts with varying antimicrobial activity. Separation, isolation and characterization of these biologically active metabolites could lead to development of novel drug leads.

## Evaluation of antibacterial properties of endophytic fungi inhabiting the grasses *Cyperus rotundus* and *Cyperus pilosus*

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Grasses are reported as host rich with fungal endophytes and investigating their antimicrobial activity is an exciting frontier for the discovery of novel antimicrobial drug scaffolds. However, the endophytic fungal status of grasses in Sri Lanka and their antibacterial metabolites producing potential has not being investigated except for a recent report of a novel antibiotic compound from an endophyte of a *Cyperus* species. Thus, the objective of the current study is to isolate endophytic fungi of *C. rotundus* and *C. pilosus*, and evaluate their antibacterial activities. Healthy plants were collected from Homagama, and isolation of endophytic fungi from surface sterilized leaves and roots was carried out using potato dextrose agar (PDA), malt agar (ME), malt peptone dextrose agar (MEA), starch yeast peptone agar (SYP) and yeast peptone dextrose agar (YPD) media. After obtaining the pure fungal isolates, each were grown on 05 PDA Petri dishes, incubated 14-21 days and was extracted with ethyl acetate. The crude extracts were tested for their effect on *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella enterica* at 400, 200 and 100 µg/disc concentrations using agar disc diffusion assay. Gentamycin was used as the positive control while methanol was used as the negative control. Twenty four endophytic fungi, 11 from *C. rotundus* and 13 from *C. pilosus* were isolated. Among these, four endophytes were isolated using PDA medium while 07, 07, 03 and 03 were isolated using YPD, SYP, ME and MEA media respectively. Six fungal extracts, five from *C. rotundus* and one from *C. pilosus* inhibited the growth of at least one bacterium tested even at 100 µg/disc. Five and four extracts were active against the Gram positive *S. aureus* and *B. cereus* respectively, while two were active against each of Gram negative, *S. enterica* and *E. coli* at all three concentrations. Thus, it can be concluded that the extracts of endophytic fungi of the two grasses tested have the ability to control the growth of pathogenic bacteria. Further investigations are necessary to realize its true potential as drug leads.

## Study of diversity and abundance of microfauna and microflora associated with selected mosquito breeding habitats in Gampaha district in Sri Lanka

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The present study was conducted to study the diversity and abundance of microfauna and microflora associated in selected mosquito breeding habitats in selected areas of Gampaha district in Sri Lanka during the period from April to September, 2014. Some physico chemical parameters of water (Temperature, pH, Dissolved Oxygen concentration) were measured *in situ* conditions. The preserved water samples were observed individually under the high power microscope in laboratory and the count of each species of microflora and microfauna was taken. The gut contents of 10 larvae (4<sup>th</sup> instar stage) from each sample were pooled and the count of each food item in each sample was taken. *Aedes albopictus* (86.5%), *Culex quinquefasciatus* (11.5%), *Toxorhynchites* spp (3.8%), and *Mansonia* spp (1.9%) were found in outdoor habitats (23 habitats), where *A.aegyptii* (95.5%) and *Mansonia* spp (4.5%) were found in indoor habitats (6 habitats). A total of eight microfauna species/taxa discovered in different mosquito breeding habitats. They were; *Euglena tripteris* (45.4%), *Phacuscaudatus* (5%), *Onchocamptus mohammad* (5%), *Philodinaroseola* (3.6%), *Canthocamptus staphylinus* (3.6%), *Gloeobotryslimneticus* (1.8%), *Colpodasp* (7.2%) and unidentified insect sp1 (23.6%). Total of ten microflora species/taxa were encountered during the study. They were *Scenedesmusbijuga* (26.1%), *Volvox aureus* (13.8%), *Gloeocystisgigas* (52.3%), *Closteriumlunula* (3.0%), *Trinemalineare* (1.5%), *Ceratiumhirundinella* (1.5%), Blue green algae sp1 (4.6%), *Nitzschiasp* (1.5%), filamentous algae sp1 (1.5%), and *Surirellabiseriata* (1.5%). This study revealed that microfauna and microflora associated in outdoor mosquito breeding habitats covered with vegetation (12 habitats) were more diverse (Shannon-Weiner diversity index (H') = 2.31; Species richness=14) and significantly higher in abundance, than habitats not covered with vegetation (11 habitats, Shannon-Weiner diversity index (H') = 1.71; Species richness=11). Results have also shown that microfauna and microflora associated in outdoor habitats were more diverse than that of indoor habitats. Outdoor mosquito breeding habitats were dominated by two species of green algae *Scenedesmusbijuga* and *Gloeocystisgigas*. These habitats that covered with vegetation were dominated by *S.bijuga*, while habitats not covered with vegetation were dominated by *G.gigas*. However gut contents of *Aedes albopictus*; the dominating species of outdoor habitats and *Aedes aegyptii*; the dominating species of indoor habitats were both occupied by *Euglena tripteris* concluding that the main food item of these two mosquito species is *Euglena tripteris* in this study.

**Nematicidal activity of aqueous extracts and dry matter of *Tithonia diversifolia*, *Gliricidia sepium* and *Tagetes erecta* against root-knot nematode, *Meloidogyne incognita* (Kofoid and White) on tomato (*Lycopersicon esculentum* Mill.)**

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A study was conducted to determine the nematicidal effect of aqueous extracts of *Tithonia diversifolia*, *Gliricidia sepium* and *Tagetes erecta* on juveniles of *Meloidogyne incognita* (Kofoid and White) and to determine the effect of dry leaves of *T. diversifolia*, *G. sepium* and dry whole plant parts of *T. erecta* on the growth of potted tomato, *Lycopersicon esculentum* (Mill.) infected with *Meloidogyne incognita*. Nematicidal effects of aqueous extracts of; *T. diversifolia*, *G. sepium* and *T. erecta* (20 g/ 100 mL w/v) were evaluated at 0.05 g/mL, 0.1 g/mL and 0.2 g/mL concentrations in the laboratory bioassay. Results revealed that all concentrations of the extracts caused juvenile mortality in the laboratory within 48 hours. 0.1 g/ mL and 0.2 g/ mL concentration of *Tagetes erecta* and 0.2 g/mL concentration of *Tithonia diversifolia* were very effective in juvenile mortality by over 50% within 48 hours. *Tagetes erecta* plant parts were the most efficacious causing above 70% juvenile mortality in 48 hours. According to the one-way ANOVA test, the potted tomato plants infected with *M. incognita* in the *Tagetes erecta* dry plant parts (2% w/w) treatment showed significantly higher number of green healthy leaves and significantly lower number of yellow leaves. Significantly higher plant height, stem diameter, root length and root weight were also found in this treatment. Significantly lower number of root galls, gall index and significantly lower population of *M. incognita* in soil were also recorded in the same compared to other treatments. Results also revealed that the addition of botanicals; *T. diversifolia*, *G. sepium* and *T. erecta* were found to increase the plant growth of tomato. The infested plants in untreated control showed a significant reduction of plant growth and significantly higher number of galls, gall index and significantly higher population of *M. incognita* in soil. Overall, it can be concluded that the aqueous dry leaf extracts of *T. diversifolia*, *G. sepium* and aqueous extracts of dry plant parts of *T. erecta* showed nematicidal activity against root-knot nematodes. Addition of *Tithonia diversifolia*, *Gliricidia sepium* and *Tagetes erecta* botanicals enhanced the plant growth and significantly reduced the root-knot nematode infestation on tomato, *Lycopersicon esculentum*.



## Schedule of the Scientific Sessions

35<sup>th</sup> Annual Sessions of the Institute of Biology, Sri Lanka  
25<sup>th</sup> September, 2015 at Sri Lanka Institute of development Administration (SLIDA)

### Parallel Session 01

Time	Abstract Number	Title
1.00 pm	1-01	Crown/Tree cover of Viharamahadevi Park, Colombo B.D. Madurapperuma and K.A.J.M. Kuruppuarachchi
1.15 pm	1-02	The responses of nursery plants of <i>Camellia sinensis</i> (L.) O. Kuntze to shade M S A E Cooray and H I U Caldera
1.30 pm	1-03	Utilization of morphological and growth related traits for identification of water stress tolerant <i>Camellia sinensis</i> L. O. Kuntze cultivars prior to field planting H.M.Y.E.Herath, H.I.U.Caldera
1.45 pm	1-04	Comparative study of <i>Pongamia pinnata</i> , <i>Annona glabra</i> and <i>Moringa oleifera</i> extracts on growth performances of <i>Basella alba</i> L. (Spinach) T. H. Kahagalla and R. M. C. S. Ratnayake
2.00 pm	1-05	Quantitative vegetation study in Namunukula forest A G S Arambawaththa, H S Kathriarachchi, A M A S Attanayake, Y S Athugala
2.15 pm	1-06	Potential of the common liverwort <i>Riccia sorocarpa</i> Bisch. as a bioindicator of selected heavy metals in the growth medium K Vignarasa, L N S Liyanage, K M Mohotti and P S Saputhanthri
2.30 pm	1-07	A protocol to preserve flower texture N P S N Karunarathne and P S Saputhanthri
2.45 pm	1-08	<i>In vitro</i> propagation of the thalloid liverwort <i>Riccia sorocarpa</i> Bisch. N N Munasinghe, L N S Liyanage and P S Saputhanthri
3.00 pm	1-09	Influence of <i>Chromolaenaodorata</i> leaf extracts on seed germination, seedling growth and growth performance of <i>Abelmoschus esculentus</i> L. (Okra) and <i>Vigna unguiculata</i> L. (Bushita) V.W. Rathnayake and R.M.C.S. Ratnayake
3.15 pm	1-10	Floristic diversity of Thotupolakanda upper montane rain forest. Harasgama. H.D.R.V.L., Ratnayake. R.M.C.S. ,Attanayake. A. M. A and M.P.T. Wijewickama
T	E	A
4.00 pm	1-11	<i>Acacia auriculiformis</i> (Fabaceae), a threat to mangrove forest in Rekawa lagoon Sri Lanka: A case study Madarasinghe SK., Kodikara KAS., Dissanayake NP., Jayatissa LP.
4.15 pm	1-12	A preliminary study on nutritional quality of an indigenous rice variety (Kuruluthuda) and a hybrid rice variety (BG 358). P G I J Gamage and M D Amarasinghe
4.30 pm	1-13	Effect of fungal endophyte <i>Arthrographis</i> on growth of rice varieties Herath Banda and Bg352 P V A R Ponnawila and N. Deshapriya
4.45 pm	1-14	Efficacy of liquid organic fertilizers on growth of <i>Anthurium andraeanum</i> L. J.M.N.P. Jayasundara, L.R. Jayasekara and R.M.C.S. Ratnayake
5.00 pm	1-15	Assessment of invasion of <i>Najas marina</i> , Linnaeus 1753 in Madu Ganga Estuary, Sri Lanka using ASTER data of Terra satellite T. M. S. D. G. Silva, D. D. G. L. Dahanayaka and M. J. S. Wijeyaratne

Parallel Session 02

Time	Abstract Number	Title
1.00 pm	2-01	Climatic and soil preferences of tiger beetles (Coleoptera, Cicindelidae) of Sri Lanka Agasthya Thotagamuwa, Chandima D. Dangalle, Nirmalie Pallewatta and Erandathie Lokupitiya
1.15 pm	2-02	Survey of molluscan shells from the Jaffna Estuary, Sri Lanka. Dorin Arohani Reval and Abyerami Sivaruban
1.30 pm	2-03	Enhancement of immunity in cultured shrimp, <i>Penaeus monodon</i> induced by <i>Achyranthes aspera</i> (Sin. Karal heba, Family: Amaranthaceae) compared to a commercial immune enhancer K.V.D.H.R.Karawita and M.Hettiarachchi
1.45 pm	2-04	Protection of cultured shrimp, <i>Penaeus monodon</i> from white spot disease (WSD) with enhanced immunity induced by <i>Achyranthes aspera</i> (Family Amaranthaceae) compared to a commercial immune enhancer K.V.D.H.R.Karawita and M.Hettiarachchi
2.00 pm	2-05	Possibility of preventing Acute Hepatopancreatic Necrosis Disease (AHPND), a killer disease in cultured shrimp caused by a unique strain of <i>Vibrio parahaemolyticus</i> if the strain enters into Sri Lankan culture systems M. Hettiarachchi, D. C. Hettiararchi and K.R.P.S. Kumara
2.15 pm	2-06	Selection of White Spot Virus (WSV) and Monodon Baculo Virus (MBV) free brood stocks of cultured shrimp, <i>Penaeus monodon</i> from Sri Lankan coastal sea to produce healthy post larvae K.R.P.S. Kumara and M. Hettiarachchi
2.30 pm	2-07	A simplified version of <i>ex ovo</i> cultivation method of chicken embryos as a model for evaluating venom toxicity Madhushika M Silva, Charitha L Goonasekara, Sampath S Senevirathne, Devaka K Weerakoon
2.45 pm	2-08	Occupational Paraquat exposure among sugarcane and vegetable farmers in Sri Lanka: A case study K. S. Mohammed Abdul, D. V. Eakanayake, T. D. K. S. C. Gunasekara, H. A. S. D. Perera, B. C. J. De Silva, C. Jayasumana, E. P. S. Chandana, S. S. Jayasinghe and P. M. C. S. De Silva
3.00 pm	2-09	Establishing dietary and faecal relationships for crude protein and crude fibre in selected native herbivorous mammals in Sri Lanka A.U. Jayawardhana, C. V. Nelundeniya, R. D. Wijesekera and M. R. Wijesinghe
3.15 pm	2-10	<i>In vivo</i> antioxidant activity of mature leaf concentrate of Sri Lankan wild type <i>Carica papaya</i> L variety against carbon tetra chloride induced oxidative stress in rats Chanika D Jayasinghe, Wanigasekara D Ratnasooriya and Preethi V Udagama
T	E	A
4.00 pm	2-11	Modulation of <i>in vitro</i> phagocytic activity, cell proliferation and cytokine production in the Wistar rat model by a Sri Lankan <i>Haliclona (Soestella)</i> sp sponge crude extract Varuni K Gunathilake, Wanigasekara D Ratnasooriya and Preethi V Udagama
4.15 pm	2-12	Nest occurrence, mean nest density and relative nest abundance of <i>Aneuretus simony</i> Emery and associated ant fauna in Meethirigala Forest Reserve R. K. S. Dias and W. S. Udayakantha
4.30 pm	2-13	An investigation of sex differences in feeding and vigilance behaviour in human langurs using fractal analysis. Sanduni Malluwawadu, Kaushalya Premachandra, Rajnish Vandercone
4.45 pm	2-14	Assessment of environmental pollutants using fledgling feathers of Little egret ( <i>Egretta garzetta</i> ) as a bio monitoring tool in Sri Lanka R L Jayaratne, I C Perera, D K Weerakoon, and S W Kotagama
5.00 pm	2-15	Comparison of Larvicidal and Repellent efficacy of <i>Ocimum basilicum</i> (L.); "Maduruthala", leaves and pods, against dengue vector, <i>Aedes aegypti</i> (L.). W.L.B.P.Abhayawickrama, G.A.S.M.Ganehiarachchi and P.A.Paranagama

Parallel Session 03

Time	Abstract Number	Title
1.00 pm	3-01	Superoxide and nitric oxide radical scavenging activities of bark and leaf of Ceylon cinnamon ( <i>Cinnamomum zeylanicum</i> Blume) <i>in vitro</i> . W. P. K. M Abeysekera, G. A. S Premakumara and W. D Ratnasooriya
1.15 pm	3-02	Antioxidant properties of brans of twenty nine rice ( <i>Oryza sativa</i> L.) varieties of Sri Lanka. W.K.S.M. Abeysekera, U.K.D.S.S. Gunasekara, G.A.S. Premakumara, W.P.K.M. Abeysekera <sup>1</sup> and P. Ranasinghe
1.30 pm	3-03	Comparative GC-MS Study of chemical constituents in essential oils of Ceylon Cinnamon ( <i>Cinnamomum zeylanicum</i> Blume) bark oils collected from different geographical locations H.D.Weeratunge, S.K. Ganegamage and G.A.S.Premakumara
1.45 pm	3-04	Inhibitory effect on human leukemia (HL-60) cancer cell proliferation via caspase-3 mediated apoptosis by <i>Costus speciosus</i> (Koen.) Sm. leaf extract Kalpa W. Samarakoon, H.H. Chaminda Lakmal, Prasad Tharanga Jayasooriya and You-Jin Jeon
2.00 pm	3-05	Microsatellite markers reveal the spatial genetic structure of dengue vector <i>Aedes aegypti</i> in selected areas in Colombo district M. D. Nirmani, K. L. N. Perera and G. H. Galhena
2.15 pm	3-06	Nutritional composition, fatty acid profile and antioxidant activity of selected traditional rice ( <i>Oryza sativa</i> L.) varieties of Sri Lanka Y Sutharsana, M.D.W.Samaranayake, W K S M Abeysekera and H M T Herath
2.30 pm	3-07	Chemical composition of western province of inflorescence of <i>Alpinia calcarata</i> Rosc. (Zingiberaceae) Grown in Sri Lanka Ganegamage S.K. and Arawwawala L.D.A.M.
2.45 pm	3-08	Ethyl Methyl Sulfonate (EMS) induced herbicide resistance in seed-derived rice ( <i>Oryza sativa</i> ) callus E M S I Ekanayaka, S R Weerakoon, T D Silva, S Somaratne
3.00 pm	3-09	Antimicrobial potential of the endophytic fungal extracts of <i>Mangifera zeylanica</i> ( <i>Anacardiaceae</i> ), an endemic plant of Sri Lanka, against selected pathogenic bacterial species Senevirathna, H.D.A.A, de Silva, E.D., Wijayarathna, C.D., Wijesundara, R.L.C
3.15 pm	3-10	Sequence changes responsible for C <sub>3</sub> to C <sub>4</sub> transition of Phosphoenolpyruvate carboxylase (PEPC) of cereals at the DNA and protein levels using bioinformatics tools N. M. P. M. Nawarathna, T.L.S. Tirimanne
T	E	A
4.00 pm	3-11	Feasibility of using Exon-Primed Intron-Crossing (EPIC) markers to detect the genetic variation of a dengue vector in Sri Lanka M. D. Nirmani, K. L. N. Perera and G. H. Galhena.
4.15 pm	3-12	<i>In vitro</i> Anti-5-lipoxygenase, anti-hyaluronidase and anti-oxidant properties of ethanol leaf extract of <i>Diospyros ebenum</i> . Perera H.D.S.M, Samarasekera R., Handunnetti S., Weerasena O.V.D.S.J.
4.30 pm	3-13	Antioxidant properties of leaves of <i>Aporosa lindleyana</i> Baill. (Kebella) S. Kathirgamanathar, D. M. K. P. Weerasinghe, W. P. K. M Abeysekera, P. Ranasinghe and A. M. C. U. Binduhewa
4.45 pm	3-14	Isolation and cloning of thermostable alpha amylase gene for the production of recombinant enzyme for industrial purposes Thiwanka M.S., Rodrigo W.W.P., Achala H.H.K., Athapaththu A.M.M.H., Gunathilaka P.A.D.H.N.
5.00 pm	3-15	A phylogenetic analysis of <i>Dinopium</i> woodpeckers in Sri Lanka using Cyt b and COI nucleotide sequences (Aves:Piciformes) Saminda P. Fernando and Sampath S. Seneviratne

Parallel Session 04

Time	Abstract Number	Title
1.00 pm	4-01	Comparison of four DNA extraction methods for target bacteria found in bovine milk for large scale detection of mastitis pathogens. RMPCD Rajapaksha, D Senavirathna, IC Perea, DK Weerakoon, CM Nanayakkara
1.15 pm	4-02	Rice rhizosphere manipulation with <i>Trichoderma virens</i> for effective phosphorous management P. N. Gallage, C. M. Nanayakkara and D. N. Sirisena
1.30 pm	4-03	Screening of native actinomycetes for potential antimicrobial activity L. D. W. Kekulandala, C. M. Nanayakkara
1.45 pm	4-04	Morphological and reproductive characterization of <i>Colletotrichum</i> spp. causing anthracnose of papaya in Sri Lanka S Rasakulendran, R L C Wijesundara and C M Nanayakara
2.00 pm	4-05	Antibiotic Sensitivity of <i>Bacillus thuringiensis</i> strains isolated from Sri Lanka Baragamaarachchi R. Y. , De Silva Kande Y, Weerasena O. V. D. S. J. and Samarasekara R.
2.15 pm	4-06	Multivariate discrimination of inflorescence characters in conserved <i>Cocos nucifera</i> L. var. <i>typica</i> Germplasm in Sri Lanka. K.N.S. Perera, D.P.S.T.G. Attanayaka and S.A.C.N. Perera
2.30 pm	4-07	Screening of rice for resistance against <i>Xanthomonas oryzae</i> pv. <i>Oryzae</i> through anther culture K.A.S.R.Perera
a2.45 pm	4-08	Determination of the antifungal, biochemical and physiological characteristics of <i>Trichoderma</i> spp. isolated from onion fields of Sri Lanka L.N.R.Gunaratna, N. Deshappriya and R. G. A. S. Rajapakse
3.00 pm	4-09	<i>Sclerotinia sclerotiorum</i> causing cabbage head rot in Sri Lanka B M AGuruge, K P Somachandra, R N Attanayake
3.15 pm	4-10	Antifungal effect of <i>Croton aromaticus</i> against <i>Rhizopus</i> spp. isolated from banana and papaya S.A.D.T.L Wijesundara and B.T.S.D.P. Kannangara
T	E	A
4.00 pm	4-11	Soil fungi of semi natural montane forest and adjacent pine plantation in Peacock hill, Pussellawa, in Nuwara Eliya district R.G.K. Dharmasiri, B.T.S.D.P. Kannangara
4.15 pm	4-12	Evaluation of antibacterial properties of endophytic fungi of <i>Cyperus brevifolius</i> and <i>Cyperus melanospermus</i> R. Chandula Walgama, Pamoda B. Ratnaweera, and E. Dilip de Silva
4.30 pm	4-13	Evaluation of antibacterial properties of endophytic fungi inhabiting the grasses <i>Cyperus rotundus</i> and <i>Cyperus pilosus</i> Sajani H. Liyanage, Pamoda B. Ratnaweera, E. Dilip de Silva
4.45 pm	4-14	Study of diversity and abundance of microfauna and microflora associated with selected mosquito breeding habitats in Gampaha district in Sri Lanka K.D.K.M. Karunathilake and L.D. Amarasinghe
5.00 pm	4-15	Nematicidal activity of aqueous extracts and dry matter of <i>Tithonia diversifolia</i> , <i>Gliricidia sepium</i> and <i>Tagetes erecta</i> against root-knot nematode, <i>Meloidogyne incognita</i> (Kofoid and White) on tomato ( <i>Lycopersicon esculentum</i> Mill.) N.W. Premachandra and L. D. Amarasinghe

