Plant Health 2019

Proceedings of the
First National Symposium of Sri Lanka Association for Mycology and Plant Pathology (SLAMPP)

Theme: "Ensuring safer plant produce for human consumption"

30th August 2019
Oak Ray Regency, Kandy

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Welcome to Plant Health 2019

"Ensuring safer plant produce for human consumption"

On behalf of the organizing committee of Plant Health 2019, we warmly welcome all participants, including the invitees, guests, presenters and sponsors to the Symposium at Oak Ray Regency, Kandy, Sri Lanka.

Plant Health 2019, the first ever Symposium of its kind to be held during the 150 year-history of Plant Pathology in Sri Lanka, is an occasion to reflect on the way in which Plant Pathology and Mycology developed in the Island.

Practice of plant health, over most of the past years, had been "traditional" often having to spend months-long spells for diagnosing of just a solitary disease or identifying a new fungal pathogen. The last few decades have seen many exciting developments in the discipline, firstly the arrival of Physiological Plant Pathology which followed Molecular Plant Pathology and Molecular Systematics, providing endless opportunities for Plant Pathological and Mycological activities, rapid disease diagnosis and pathogen identification, gene regulation and molecular systematics which has almost totally overridden the traditional and morphology-based approaches, practiced for centuries. Mycology as a discipline began in the country even before, in the last quarter of the 18th century which has been the most productive era in the history for fungal systematics.

"Ensuring safer plant produce for human consumption", has been a challenge today, particularly in the context of rapid human population growth, habitat destruction and urbanization. Increasing demand for safer produce has been one of the major concerns at consumer level as well as in national food supply chain and export agriculture in this era of global exchange of horticultural products and plant germplasm. Diseases and pests continue to threaten food crops in the field and as well as storage, leading to considerable annual crop loss worldwide. Similarly, the increased and excessive usage of synthetic pesticides and herbicides, not only affect the animal and human health adversely, but also disrupt food webs in natural and agro-ecosystems resulting in ecological instability of the environment.

It is the responsibility of Plant Pathologists, the world over, to focus more on safer and durable pest and disease management options as alternatives for synthetic pesticides, for field crops and harvested produce, and ensure high quality produce, free of chemical residues and hazardous microbial products, reaching the public for consumption and guarantee regional food security.

"Plant Health-2019" is the first national symposium organized by the 2018/20 Executive Committee of the SLAMPP. The aim the symposium is to connect national level researchers in academia and the R & D, in the government and corporate sector, engaged in various aspects of Plant Pathology and Mycology research and provide a forum for interaction, discuss new trends, opportunities and challenges arising in the field.

The year 2020, which is proclaimed as the "International Year of Plant Health (IYPH)" by United Nations primarily aims at increasing awareness among the general public and policymakers on plant health and food security. Plant Health-2019 symposium, is one of the key national initiative by the SLAMPP as a beginning of the series of events to be organized in future, in parallel to the global and regional ventures commemorating IYPH-2020.

Founded in 2007, the SLAMPP is a professional Association focused on promoting and disseminating knowledge in Mycology and Plant Pathology in the country and acting as the official mouthpiece of the Mycology and Plant Pathology community. SLAMPP is an Associate Member of the International Society of Plant Pathology (ISPP), ISPP council, the Asian Mycological Association (AMA) and the Association of Asian Societies of Plant Pathology (AASPP).

You are invited to join us in the journey of taking Plant Pathology and Mycology forward, and combat the threat of disease and pests that continue to challenge the Agriculture and Horticulture in the country.

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Plant Health 2019 *On the 30th of August 2019*

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About the keynote speaker…

Professor Saman Seneweera
Director
National Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka

Professor Saman Seneweera is the Director at the National Institute of Fundamental Studies (NIFS), Sri Lanka, Professor University of Southern Queensland, Honorary Prof University of Melbourne, Australia and Adjunct Professor of Ruhuna University, Sri Lanka. He has also worked at the University of Western Sydney, Australia, Tohoku University, Japan and the University of Illinois, Chicago, USA. Professor Seneweera's research focus is on understanding the impact of climate stress on the physiological processes of plants aiming to mitigate stress and improve quality and crop yield potential. His research also focuses on improving grain quality such as protein, iron and zinc of cereal crops and pathophysiology of crown rot. In his work, Professor Seneweera uses cutting-edge techniques, including genomics, proteomics, metabolomics and ionomics tools to identify new physiological traits to develop climate resilient crops. He has been instrumental in building the Australian Grain Free Air carbondioxide at the University of Melbourne and the Plant Biology Platform at the University of Southern Queensland. To date, Professor Seneweera has supervised over 25 PhD students internationally. He is in the editorial board of many journals and also provide reviews for large number of journals such as Nature, Plant Physiology, Plant, Cell and Environment and New Phytologist. Professor Seneweera has published more than 200 research articles in top ranking journals including Nature and Science. He has been an invited speaker at international conferences and has presented many keynote addresses. Professor Seneweera is the recipient of multiple awards including the 'MSLE Research Excellence Award – 2011' from the University of Melbourne, Japan Society for the Promotion of Science Fellowship, 2001, Science and Technology Fellow, Japan, 1999.

Summary of the keynote speech

Altered primary and secondary metabolism of plants under future CO_2 rich atmosphere could impact the development of pathosystems

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Over geological time scales, atmospheric CO_2 concentrations accompanying climate change has had a profound influence on the evolution, diversification and productivity of plants. The main factor contributing to climate change is rising CO_2 concentration, which is the primary substrate for photosynthesis. The CO_2 concentration will increase from 400 (current) to 550 L CO_2 L^{-1} by the middle of this century and will have a direct impact on the physiological processes in plants such as photosynthesis and transpiration. In addition, the plant's secondary metabolism, including carbon and nitrogen metabolism, cell cycle functions, hormonal regulation and plants defence could be altered. In the past, most studies have focused on the direct effects of CO_2 on plant processes, despite emerging evidence of the role elevated CO_2 plays in moderating secondary metabolism processes. Secondary metabolites have evolved to enhance plant fitness to interact with their environment. This group of plant metabolites also play an important role as a plant's defence system against range of diseases. Therefore, a better understanding of the defence response under high atmospheric CO_2 is essential to manage economically important plants effectively. Further, the changing climate can affect plant–pathogen interactions by altering the pathogen life cycle, expression of host resistance, disease epidemiology and severity of disease epidemics, development of new races and virulence. Therefore, disease management strategies should be reoriented in response to changing climatic conditions.

Keywords: disease epidemiology, host resistance, photosynthesis

About the plenary speaker…

Dr. Jayantha Bandara Senanayake
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Dr. Jayantha B. Senanayake is specialized in Virology, Molecular Biology & Biotechnology and Genetics. He has over 26 years of research experience in Plant Pathology and Biotechnology of field crops, including rice at the Department of Agriculture (DOA), Sri Lanka and management of plant diseases. He was instrumental in establishing a fully-equipped international level biotechnology laboratory at the Field Crops Research & Development Institute, Mahailuppallama and initiating Biotechnology research in collaboration with plant pathologists at the Institute. From 2015-2017, Dr. Senanayake has served as a principal scientist for NARP for fingerprinting of recommended chili varieties and from 2016, as the Deputy Principal Investigator of the NRC Mega Project, "Ensuring food security through developing climate smart crop varieties and cultivation techniques in Sri Lanka" with 18 sub-projects involving over 20 Research Scientists from three Institutes within the DOA, Field Crops Research & Development Institute, Rice Research & Development Institute and Natural Resources Centre of the DOA and prestigious Universities. He pioneered in identifying Chilli Leaf Curl Virus through sequencing the viral genome and developing virus specific primers, which are now utilized by local and international scientists for detection of similar viruses. He has developed micro-RNA constructs for resistance against potato X & Y viruses, which can be used to produce virus resistant potato transgenic varieties; collaborated in developing specific molecular markers for Okra Yellow Vein Virus disease in Sri Lanka. He has 40 publications on Biotechnology, Virology & Plant Breeding aspects in International and national journals including high impact factor journals. His present research involves development of quality protein maize, gene discovery for anthracnose resistance and development of pathogen derived resistance for chilli leaf curl virus and molecular identification of pests and diseases in rice and field crops.

Summary of the plenary speech

Sustainable plant disease management in field crop and rice sector in Sri Lanka – Biotechnology a possible tool

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Major diseases in the field crops, chilli and onion, are anthracnose (*Colletotrichum* spp.), onion bulb rot (*Ralstonia* spp., *Erwinia* spp.), wilt in chilli & tomato (*Ralstonia solanacearum* and *Fusarium* spp.), *Cercospora* leaf spot (*Cercospora* spp.), collar rot in cowpea (*Sclerotium rolfsii*), Mosaic in mungbean (*Mungbean yellow mosaic virus*) and yellow mosaic in okra (*Okra yellow mosaic virus*), Maydis leaf blight (*Bipolaris maydis)* and stalk rot (*Erwinia carotovora*) in maize, leaf curling in chilli (leaf curl viruses), purple stain in soybean, viral diseases caused by cucumber mosaic virus, chilli leaf curl virus, tomato spotted wilt virus etc. sesame phyllody (Phytoplasma), powdery mildew in legumes (*Erysiphe polygoni)*), and rust (*Puccinia arachidis*), leaf spot (*Cercospora* spp.) and bud necrosis (virus) in ground nut. Blast (*Magnaporthe oryzae*), bacterial leaf blight (BLB) (*Xanthomonas oryzae* pv *oryzae*) and sheath blight (*Rhizoctonia solani*) are major diseases in rice in Sri Lanka. No genetic resistance has been found with almost all of the above disease except for rice blast and bacterial leaf blight in rice and Mung bean yellow mosaic virus in Sri Lanka. A moderate resistance has been found in cowpea against collar rot and a considerable resistance to chilli leaf curl has been identified in some chili varieties. For the rest of the diseases genetic resistance has not been recorded. Generating resistance or increasing tolerant level using biotechnological tools is a possible solution when no native resistance is present against the pathogens in field crops and rice. The possible biotechnology based techniques under Sri Lankan conditions are the introduction of resistant genes from other sources (transgenic or cisgenic), siRNA or miRNA based mechanisms and gene editing to generate resistance/tolerance for major disease of field crops and rice avoiding the usage of large quantities of fungicides and insecticides. In the field crops sector generation of resistance for chilli leaf curl viruses is being attempted. In rice, several resistant genes have been pyramided using molecular tools to have a broader resistance for BLB. Similar attempts have been made and successful in the neighboring countries. However, commercial release of varieties, developed through biotechnological tools for disease resistance, has not taken place in these countries. However, commercial release of crops for insect resistance has already been done in those countries. The identification of pathogens associated with crop disease are done using molecular techniques in Sri Lanka. It also helps to reduce unnecessary application of chemical to control plant disease.

Keywords: resistance, diseases, field crops and rice

Theme 1: Detection and Molecular phylogeny of plant pathogens

Abstract No: SLAMPP/2019/104

Morphological and molecular characterization of *Colletotrichum* causing anthracnose in Sri Lankan *Begonia*.

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Anthracnose is a common and most destructive postharvest disease in many fruit species, vegetables, cut-flowers and foliage plants, especially under warm and humid climates. The causal agent of anthracnose disease in many hosts has been known for decades as *Colletotrichum gloeosporioides* or *C. acutatum*. The identification has been based mostly on morphological and cultural characteristics. Taxonomy of the genus *Colletotrichum* has been questioned since many *Colletotrichum* species have now been accepted as complexes of cryptic species using molecular approaches. Re-identification of *Colletotrichum* causing anthracnose disease to species level is carried out worldwide, using molecular data. Among the hosts that have been re-visited, the attention that has been paid to ornamentals is minimal. *Begonia* is an ornamental plant grown worldwide, while anthracnose is a common disease in *Begonia* in Sri Lanka which reduces the plant's horticultural value. Further, the disease in *Begonia* plants has not been studied or recorded in Sri Lanka. Anthracnose symptoms appear as brown color, irregular and often large, 2 –5 cm diameter necrotic lesions mainly on the leaves, towards the margins. *Colletotrichum* was isolated from anthracnose lesions on infected *Begonia* leaves, collected from the *Begonia* nursery of the Royal Botanical Gardens, Peradeniya, Sri Lanka. Among the thirty isolates made, seven isolates representing two morphological categories were subjected to DNA sequence analysis, using ITS, β-tubulin 2 (TUB2) and GAPDH as primers marker gene regions. For resulting sequences, the species affiliations and identities were determined through BLAST similarity searches of the NCBI GenBank Database. Considering >98% similarity, *C. siamense* and *C. truncatum* were identified as species associated with *Begonia* anthracnose. This is the first report of the association of *C. siamense* and *C. truncatum* with *Begonia* anthracnose in Sri Lanka and *C. siamense* for the first time in the world. *C. truncatum* was isolated at a lower percentage, (20%), compared to that of *C. siamense.*

Keywords: Anthracnose, *Colletotrichum*, *Begonia*

Abstract No: SLAMPP/2019/102

New records of *Hypoxylon* and *Hypomontagnella* species from Pilikuththuwa lowland wet zone forest in Sri Lanka

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Genus *Hypoxylon* (Bull.) is now belonging to the family Hypoxylaceae with more than 170 described species. Most of the species in this genus are saprophytic and some of them are considered as pathogens on wood too. They occur in a wide range of substrates, including wood, litter and soil. *Hypoxylon* species play a major functional role as ecosystem decomposers of angiosperms, pathogens and endophytes. The *Hypoxylon* of Sri Lanka are imperfectly known and only few species have been reported by various early mycologists. This study focused on providing comprehensive accounts of morpho-molecular identification of *Hypoxylon* species within Pilikuththuwa lowland wet zone forest. In this study, specimens of *Hypoxylon* species were collected during May-July 2018. Species recognition was carried out using both macroscopic and microscopic characters in all collected *Hypoxylon* species. DNA was extracted from pure cultures grown on MEA plates using modified CTAB protocol. Extracted DNA was subjected to the amplification of ITS and β-tubulin gene regions using ITS1, ITS4 primers and T1, T22 primers respectively. All the PCR products were purified and DNA sequencing was performed using the same primers. Raw sequences were assembled using Bioedit 7.1.3.0. Sequence homologies for the assembled consensus sequences were analyzed using BLASTn of the NCBI for the rough identification of fresh isolates used in the analysis. Phylogenetic species recognition was re-evaluated using a combined gene analysis of rDNA ITS and β-tubulin using *Daldinia concentrica* as the outgroup taxon. The phylogenetic tree inferred by combined analysis from Maximum likelihood (ML) analysis using RAxML-HPC2 on XSEDE tool available in the CIPRES Science Gateway provided the best resolution for species as compared to single gene analyses. *Hypoxylon nicaraguense, H. fuscopurpureum, H. curvirimum, H. flavoargillaceum, H. brevirimum, H. munkii, H. rubiginosum, H. piceum, Hypomontagnella submonticulosa* and *H. fuscum* were identified using phenotypic characters, while the identity of *Hypomontagnella monticulosa, H. hypomiltum* and *Daldinia eschscholtzii* were confirmed by the molecular characterization. Cultural characteristics were described for all isolate and identified taxa and their differences were recorded.

Keywords: Hypoxylaceae, phylogeny, taxonomy

Abstract No: SLAMPP/2019/107

Morphological and molecular identification of fungal pathogens associated with cultivated rubber trees in Sri Lanka

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Fungal pathogens are one of the major threats on cultivated rubber (*Hevea brasiliensis*) trees globally. Although rubber pathogens are traditionally known based on morphology, less or no molecular data are available for some pathogenic species in Sri Lanka. Emergence of pathogens can be due to climatic changes and introduction of new clones. Therefore, assessment of fungal pathogens associated with rubber trees in Sri Lanka is essential. The major objective of this study is to accurately identify foliar fungal pathogens associated with rubber plants in Sri Lanka based on morphology and phylogenetic analysis. Single spore isolation was carried out to obtain pure cultures of fungi from diseased leaf samples collected randomly from selected sites. Internal transcribed spacer (ITS) region of all isolates were sequenced to determine the generic placement of isolates. Phylogenetic analysis was performed based on five gene regions including partial actin (ACT), beta tubulin (TUB2), chitin synthase (CHS-1), glyceraldehyde-3 phosphate dehydrogenase (GAPDH) and ITS for the isolates within the *Colletotrichum gigasporum* and *C. truncatum* species complexes. Putative DNA lyase (Apn2), DNA lyase-mating type 1 intergenic region (Apn2-MAT1) and ITS regions were used for *C. gloeosporioides* species complex. The analysis was done based on ITS and GPDH for isolates belonged in *Curvularia*. According to the morphological and molecular data, *Curvularia verruculosa*, *Colletotrichum truncatum* and one unknown species belonged in *Colletotrichum gigasporum* complex identified in this study were the first global fungus-host association records on *Hevea*. Three isolates were first records of host-pathogen association in Sri Lanka, namely *Colletotrichum siamense*, *Curvularia senegalensis* and *Phyllosticta capitalensis*. Both *Curvularia* spp. recorded are first records of those fungi in Sri Lanka. Pathogenicity tests confirmed that *Colletotrichum* isolates leading to typical anthracnose symptoms are correlated with the species belong in gloeosporioides complex while *C. gigasporium* and *C. truncatum* species are also capable of successfully colonizing on rubber leaves. Hence, this study reveals a previously unknown diversity of foliar fungi associated with cultivated rubber trees in Sri Lanka.

Keywords: leaf diseases, plantation crops, species complexes

Acknowledgement — *This project was supported by University of Sri Jayewardenepura undergraduate research grant and Martin-Baker Research Award to D. Udayanga from Mycological Society of America.*

Abstract No: SLAMPP/2019/110

New records of *Exserohilum* (Pleosporales, Dothideomycetes) species from rice and associated weeds in Sri Lanka

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The order Pleosporales in the class Dothideomycetes comprised of several genera of graminicolous fungal pathogens including *Curvularia*, *Bipolaris*, *Exserohilum*, *Drechslera*, *Johnalcornia* and *Porocercospora*. Amongst them, *Exserohilum* is a well–known pathogenic genus of fungi causing important plant diseases such as leaf blights of corn and millet, leaf spots and foot rots of wheat, damping off of sugarcane seedlings. The genus composes around 35 species epithets in *Index Fungorum*. Although *Exserohilum* is an economically important genus, only two species, *E. turcicum* and *E. rostratum* have been recorded so far from Sri Lanka. The objective of this study is to identify the *Exserohilum* species from rice and associated grass species in Sri Lanka. During the current study, samples were collected from Kegalle and Gampaha districts. Fungi were isolated using the single spore isolation technique and colony characters were observed on three different culture media; Potato Dextrose Agar (PDA), Corn Meal Agar (CMA) and Malt Extract Agar (MEA). Fresh isolates were characterized based on morphological and molecular data. Phylogenetic analyses were implemented based on maximum parsimony and maximum likelihood criteria. The multi–gene phylogeny consisted of ribosomal Internal Transcribed Spacer (ITS) region and partial Glyceraldehyde 3–Phosphate Dehydrogenase (GPDH) sequence data from the GenBank and from the freshly collected isolates. Two isolates, identified as *E. rostratum* and *E. fusiforme*, were isolated from *Oryza sativa* and *Echinochloa oryzoides* respectively. Phylogenetic informativeness of ITS, GPDH, TEF and RPB gene regions were evaluated using Phydesign software. Highest phylogenetic informativeness recorded in the ITS locus for the genus *Exserohilum* although GPDH has reported as the highest informative locus for sister genera, *Bipolaris* and *Curvularia*. Both isolates appear to be novel host–fungal association records from Sri Lanka. Further studies are in progress to study the diversity of species of this poorly known genus.

Keywords: phylogeny, dematiaceous hyphomycetes, helminthosporoid fungi

Acknowledgement — *This project was supported by the University of Sri Jayewardenepura under the research grant no. ASP/01/RE/SCI/2018/36 and Emory Simmons research award from Mycological Society of America.*

Abstract No: SLAMPP/2019/106

Identification of fungal pathogens involved with rough bark disease of cinnamon

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The world-renowned Ceylon cinnamon is produced from the dried bark of *Cinnamomum zealanicum* Blume. Rough Bark Disease (RBD) has been reported as one of the major stem disease and contributes to heavy yield losses. The present study was conducted to isolate causal organism/s involved with RBD, confirm pathogenicity and identify them by molecular methods. Cinnamon stems with RBD symptoms representing initial, moderate and severe disease developmental stages were collected from 25 locations of Matara district, a major cinnamon growing area of Sri Lanka. Stem pieces were surface sterilized and pathogens were isolated using PDA and MEA media. Fungal cultures, consistently-isolated from the symptomatic specimens were purified and identified by colony and spore morphology. Spore morphology revealed that *Pestalotia* sp.-like fungal cultures were the mostly associated fungi with all three disease developmental stages of RBD (i.e. 74% at the initial stage, 70 % at the moderate stage and 75 % at the severe stage). Fungal cultures identified as *Colletotrichum* sp., *Bortryodiplodia* sp. and *Phomopsis* sp. were highly prevalent at the initial, moderate and severe stages of the disease (77, 45 and 52 %) respectively. Pathogenicity of each fungal isolate was confirmed through standard Koch's rules by inoculating on healthy cinnamon stems at semi hard wood stage under *in-vitro* conditions. Fungal isolates confirmed to be infectious were subjected to genomic DNA extraction and PCR amplification using ITS1 universal primers. Resulted in PCR products (550 bp) were sequenced and homology search was done. *Pestalotia* sp.–like cultures gave the highest homology (90% identity and 95 % query cover) with *Monochaetia kansensis, Pestalotiopsis* sp. (87-99% identity and 80-89 % query cover), *Neofusicoccum parvum* (99 % identity and 99 % query cover) and *Neofusicoccum ribis* (100% identity and 98% query cover). *Botryodiplodia*-like cultures gave the best match with *Lasiodiplodia theobromae* (98% identity and 98-95% query cover) and the *Phomopsis*-like cultures gave the best homology with *Phomopsis* sp. (94% identity and 89% query cover). To determine the involvement of unculturable fungi with RBD, genomic DNA was extracted from bark tissues of the three disease developmental stages. Genomic DNA was subjected to PCR amplification using ITS1 and ITS4 primers and the resulted in sharp PCR product (560 bp) was eluted from the gel and DNA sequenced. Homology search gave the best match with *Dothideomycetes* sp. (95% query cover and 90% identity). Results revealed that a complex of fungal pathogens is involved with RBD with a possible succession.

Keywords: *Monochaetia kansensis, Pestalotiopsis* sp., *Neofusicoccum* sp.

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Acknowledgement — *This project was supported by National Research Council (Grant No. 18-012).*

Theme 2: Plant diseases, disorders and their management

Abstract No: SLAMPP/2019/105

Efficacy of UV-C treatment on anthracnose disease control and postharvest quality enhancement of tomato

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Tomato (*Solanum lycopersicum*), a primary source of vitamins and antioxidants, is an extensively cultivated crop worldwide. Anthracnose caused by *Colletotrichum coccodes* is a significant postharvest disease in tomato. At present, commercial fungicides are used to eliminate the disease. However, there are negative effects of fungicide usage such as human health hazards and environmental impacts. Therefore an alternative method is needed to suppress postharvest diseases and at the same time to improve/ retain the postharvest quality. As a novel method of postharvest treatments, UV-C hormesis was tested in the study. 'Thilina' and 'Roma' varieties were used due to the availability in local and supermarkets. Five different UV-C dosages (0, 1, 2, 3, 4, 5 kJ/m^2) were applied to pure cultures of *C. coccodes* at an intensity of 254 nm (at a distance of 15 cm). The selected dosages (3.0 and 4.0 kJ/m^2) were tested (as before inoculation and after inoculation treatments) on the effect of anthracnose disease development of fresh tomato fruits and the change in postharvest quality parameters (weight loss, firmness, shelf life, antioxidant and total phenolic content). There was a significant difference ($P < 0.05$) of *in vitro* growth suppression at 4.0 kJ/m^2 dosage. Anthracnose disease development was suppressed at 4.0 kJ/m^2 in the 'Thilina' variety and at both 3.0 and 4.0 kJ/m^2 dosages in the 'Roma' variety. However, it was independent of the treatment time (before inoculation and after inoculation) for both varieties. With both dosages tested, the percentage weight loss of treated 'Roma' variety was significantly lower ($P < 0.05$), while that of 'Thilina' has no significant effect. The firmness has significantly enhanced compared to the control in treated 'Thilina' variety ($P < 0.05$) however, in 'Roma' there is no significant effect. Further the shelf life has significantly prolonged in both 'Roma' and 'Thilina' varieties ($P < 0.05$) by approx. 2 folds compared to the non-treated control. Antioxidant activity and total phenolic content of both varieties have increased after UV-C treatment. Improvement of postharvest quality varies between the two varieties and the two dosages used. Thus, UV-C treatment could be a potential method to suppress diseases while enhancing the postharvest quality in fresh produce.

Keywords: Tomato Anthracnose, *Colletotrichum coccodes*, UV-C hormesis

Abstract No: SLAMPP/2019/103

In vitro control of *Sclerotium rolfsii* causing stem rot disease in tomato using *Trichoderma* species and plant extracts

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Tomato (*Solanum lycopersicum*), a popular fruit among consumers due to its high nutritional properties, requires high humidity and temperature for its growth. Thus, it is highly susceptible to diseases. *Sclerotium rolfsii,* a fungal pathogen, causes stem rot disease in tomato. The pathogen can survive in soil for long periods by producing sclerotia, which function as dormant propagules. While fungicides are used to control the stem rot disease incidence and severity in tomato, it is essential to find an environmentally friendly, alternative method for the management of *Sclerotium rolfsii*. Five *Trichoderma* species isolated from soil from selected forests in Sri Lanka, namely *T. asperellum, T. harzianum, T. longibrachiatum, T. reesei and T. virens*, were used as bio control agents. Aqueous extracts of seven plant species namely *Lantana camara, Azadirachta indica, Piper betel, Sphagneticola trilobata, Ricinus communis, Allium sataivum* and *Cassia auriculata* were also used to study the effect on growth of *S. rolfsii.* Percentage growth inhibition of *S. rolfsii* by *Trichoderma* species in dual cultures and plant extracts at two concentrations (5% and 10%) in poison agar plates was studied. Captan fungicide was used as the positive control while sterilized distilled water was used as the negative control. *Trichoderma* species significantly inhibited the mycelial growth of *S. rolfsii* (44.56 ± 1.25 % to 60.00 ± 1.10 %) in comparison to the negative control after five days. Of the *Trichoderma* species, *T. virens* showed the highest antagonistic activity while *T. harzianum* showed lowest activity. Five plant extracts at 10% concentration resulted over 50% growth inhibition. 10% *Lantana camara* exhibited maximum growth inhibition of 64.22 ± 1.25% at 10% concentration while 5% *Sphagneticola trilobata* resulted 31.78 ± 2.37%. 10% *L. camara* and *T. virens,* resulted comparable growth inhibition percentage though that was significantly lower than Captan at 1.2 gl^{-1} (81.56 ± 1.14 %) at α= 0.05. The potential of *L. camara* and *T. virens* to control *S. rolfsii* in the field has to be further studied.

Keywords: *Sclerotium rolfsii*, plant extracts, biological control

Abstract No: SLAMPP/2019/ 118

Reducing postharvest disease development and increasing shelf-life of mango fruit cv. Willard with essential oil incorporated bio-safe fruit coating

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Use of biologically safe, edible fruit coatings or essential oil in-cooperated fruit coatings to reduce postharvest losses has become an attractive alternative to synthetic fungicides in recent years. Although essential oils have great potential in reducing postharvest disease losses, their high evaporation rate reduces application. Increase in shelf life due to reduced water loss and reduced evaporation of essential oils within the coating are some of the key advantages of fruit coatings. The effect of Bee's wax as a fruit coating with and without essential oil on the postharvest quality and Anthracnose disease development by *Colletotrichum gleosporioides* of mango (cv. Willard) was evaluated by conducting a laboratory experiment. Blemish free mango fruits at harvest maturity of cv 'Willard' were obtained from an orchard that does not practice the use of chemical fungicides. Pure Bees wax dissolved either in vegetable oil (1:3 w/v) or Petroleum ether (1:4 w/v) served as the coating material. Cardamom oil at 400 or 600 ppm was in-cooperated to the different coating material. The treatments were: Bees wax, pet-ether coating (BWPE), BWPE with 400 ppm cardamom, BWPE with 600 ppm cardamom, bees wax in vegetable oil coating (BWVO), BWVO with 400 ppm cardamom, BWVO with 600 ppm cardamom, fruits with no coating or essential oil- served as the control. Eight replicate fruits were used per treatment and two trials were performed. Treated fruits were arranged in a CRD and kept under ambient conditions. Daily observations were made on natural disease development and weight loss. The pulp pH, color, firmness, peel color, Total Soluble Solid content and titratable acidity were measured in treated fruits at eating ripe stage. Data were analyzed using ANOVA with SPSS package. Results indicate that essential oils in Bee's wax coatings delay fruit ripening thereby increasing shelf life, reduces weight loss. Further, it reduces and delays postharvest disease development.

Key words: cardamom oil, Anthracnose, Willard

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Abstract No: SLAMPP/2019/119

Identification and management of fruit rot causing agent in *Cucurbita moschata* in Trincomalee district

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Cucurbita moschata is a popular and nutritious vegetable consumed throughout American and Asian countries including Sri Lanka. *Cucurbita moschata* cultivation has been severely affected by fruit rot disease in addition to various other diseases. The present study was aimed to identify and control the causative agent of *C. moschata* fruit rot disease in Trincomalee district. Diseased fruit samples were collected randomly from four fields located in Nilaveli, Morawewa, Kinniya and Thambalagamam. Symptoms were characterized and pathogen was isolated on PDA medium. Culture and morphological characteristics were studied. Koch's postulates were carried out under aseptic conditions to confirm the pathogenicity. Four different concentrations (10mg l^{-1}, 100mg l^{-1}, 1000mg l^{-1} and 10000mg l^{-1}) of different fungicide suspensions (Captan, Mancozeb, Homai and Topsin) were tested for anti-fungal activity *in-vitro*. Antagonistic effect of *Trichoderma harzanium* and *T. viride* was studied against the fungal isolates. Under field conditions mulching and hanging of young plants were implemented to avoid soil contact of fruit along with management of soil moisture content. Fruit rots appeared as brown colour, sunken, water soaked spots which later turned into black, enlarged rot with moldy appearance. In PDA medium fungal cultures appeared white heavy, aerial mycelia which later becomes flat. Young hyphae were with swollen tips, while matured hyphae were hyaline, aseptate and dichotomously branched. Aplerotic oospores were surrounded by terminal oogonia. Motile zoospores arose from globose sporangium. Based on the morphological features the fungal isolates were identified as *Pythium* sp. There was no morphological variation among the isolates collected from different regions in Trincomalee district. Koch's postulates confirmed the pathogenicity of *Pythium* sp. *Trichoderma viride* and fungicides Homai (1000 mg l^{-1}) significantly ($p<0.05$) reduced mycelia growth *in-vitro*. Moreover the disease incidence was comparatively low in *C. moschata* plants which were not in contact with soil.

Keywords: *Pythium* sp., *Cucurbita moschata*, fruit rot

Abstract No: SLAMPP/2019/120

Internal Pulp Browning, a new disorder of ripe mango (*Mangifera indica* L.) fruit

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Internal Pulp Browning (IPB) is a disorder observed in ripe mangoes var. TomEJC. The main symptom is browning of pulp tissue just outside the seed on both sides. The affected fruits show no external signs of the disorder. Exhaustive literature reviews revealed that the disorder has not previously been recorded. The aim of the study was to understand the factors involved in the development of IPB and develop meaningful management practices. In TomEJC, the seed starts germinating while within the fruit. Length, width and thickness were measured in seeds from fruits affected by IPB and also in germinated and ungerminated seeds from healthy fruits. Additionally, daily rainfall was recorded during four consecutive seasons during 2017-2019. Measurements on seed dimensions indicated that the seeds swell during germination increasing their thickness, twice as much as that of ungerminated seeds. There was no significant difference in seed thickness between germinated seeds from fruit with or without IPB symptoms. However, in every fruit that showed IPB symptoms, the seed had germinated invariably while within the fruit and had increased its thickness. The mean length and width were not significantly different ($P > 0.05$) among the seed that had not germinated within the fruit, germinated but no IPB or germinated showing IPB symptoms. Incidence of IPB was only observed in the year-end season where monthly average rainfall was high. Percentage fruit with germinating seeds was determined at two harvesting maturity stages, 13 and 14 weeks after anthesis, and % incidence of IPB was also compared. The fruits harvested 14 weeks after anthesis showed 100% seed germination while it was 15% in fruits harvested after 13 weeks showing a positive relationship between seed germination and development of IPB. The seed expands in thickness during germination exerting pressure on the pulp tissue adjacent to the seed surface which could result in damage to the tissues and resin canals. Phenolic compounds released from damaged resin canals may undergo oxidation forming polyphenols and causing tissue browning. In conclusion, in-fruit seed germination and rainfall appear to be the main factors contributing IPB development. Harvesting fruits 13 weeks after anthesis reduced in-fruit seed germination and the incidence of IPB.

Keywords: fruit disorder, in-fruit germination, mango (*Mangifera indica*)

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Plant Health Posters

Abstract No: SLAMPP/2019/101

Identification and pathogenicity determination of fungal species associated with die-back disease of Anthurium in Sri Lanka.

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Anthurium andrenaum (family Araceae) is the most popular Anthurium species in Sri Lanka. Anthurium occupies an important place in cut flower trade due to its attractive, wide range of foliage and flower types. However, pathogen attacks on anthurium plant negatively affect its economic value. Pathogen attacks not only affect the appearance of the ornamental leaves, but it could cause a significant cut-flower loss due to reduced photosynthetic rate. This study was conducted to identify the pathogens associated with die-back disease on anthurium leaves, using Koch's postulates as a preliminary step to identify preventive measures for the disease. Diseased leaf samples with wilting, yellowing and browning were collected from Diwulapitiya, Gampaha District. Leaf tips were turning brown along the edges and eventually going through the whole leaf. For isolation of potential pathogens, small segments of diseased leaf samples were placed on PDA medium, after surface sterilizing in 5% Clorox for 2 minutes and the plates were incubated at room temperature for seven days. Potential pathogens were identified using morphological and microscopic characterizations. Molecular analyses were performed by extracting genomic DNA and targeting the internal transcribed spacers (ITS) region by Polymerase Chain Reaction (PCR) for further identification. Two different fungal species were isolated namely sp.1 and sp.2. Microscopic characterizations revealed sp.1 to be a *Colletotrichum sp.* and sp.2 as a sterile fungal species. PCR further confirmed the identification of *Colletotrichum* sp., demonstrating amplification fragment size about 500 bp and re-inoculation revealed the *Colletotrichum* sp. as the potential causative agent. However, further molecular analyses are needed to identify the relevant *Colletotrichum* species.

Keywords: ornamentals, Anthurium, *Colletotrichum*

Abstract No: SLAMPP/2019/113

Molecular and phenotypic variations of *Fusarium oxysporum* f. sp. *cubense* associated with Panama disease of banana in Sri Lanka

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Banana (*Musa* spp.), major fruit crop cultivated in Sri Lanka is threatened by the emergence of the devastating fungal disease, *Fusarium* wilt or Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). The soil inhabiting fungus invades the root system of the banana plant and colonizes the xylem vessels causing vascular wilt. Though the disease causes substantial losses in banana cultivations, developing an effective management strategy is complicated, partially due to the genetic variability of the pathogen. Therefore, this study was conducted to investigate molecular and phenotypic variations of ten Foc isolates. Based on the symptoms of the Panama disease, infected pseudostem samples of 'Silk' (AAB) banana were collected from different geographical locations in Sri Lanka representing Northern, Southern, Western, North Central, Uva and East provinces. Fungi were isolated following the surface sterilization and pure cultures were obtained from single spore isolation. Phenotypic variations in mean colony diameter and pigmentation were observed on different culture media; Potato Dextrose Agar (PDA), Corn Meal Agar (CMA), Czapek–Dox Agar (CDA), Sabouraud Dextrose Agar (SDA) and Malt Extract Agar (MEA). DNA was extracted from the ten isolates and PCR amplification was performed using primers for Internal Transcribed Spacer (ITS) region and first intron region of Translation Elongation Factor 1 - α (TEF 1- α). Resulted ITS and TEF 1- α sequences from the Sanger sequencing, along with the reference sequences downloaded from Genbank were subjected to phylogenetic analyses based on the maximum parsimony criterion using PAUP v.4.0b10. ITS region confirmed that the ten isolates belong in *Fusarium oxysporum sensu lato*. Resulted phylogram based on the TEF 1- α sequences revealed a considerable intraspecies variability and two distinct clusters were generated in both phylograms; ITS and TEF 1- α while the outgroup was *Fusarium redolens* YG1 (KF055839). The phylogenetic evidence, together with cultural variations, suggest a considerable genetic variability of the pathogen within Sri Lanka. Sampling size should be further increased to get a reliable estimate on genetic diversity of this species. In order to advance an effective management strategy for the phenotypically and genetically diverse isolates collected during this study, investigation of variations in the pathogenecity associated with them is timely important.

Keywords: pathogen, *Foc*, *Musa*

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Abstract No: SLAMPP/2019/116

Detection and estimation of Tea Blister using spectrorediometeric analysis

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Blister Blight caused by the biotrophic fungus *Exobasidium vexans Massee* is the most problematic foliar disease of tea in Sri Lanka. The disease attacks harvestable shoots, tender shoots emerging after pruning and young nursery plants. Crop loss due to blister blight is reported to be more than 30%. Estimation of blister blight severity is essential for screening cultivars, testing fungicides and other plant protection products, determining crop loss, etc. Currently, visual assessment method with disease assessment key is employed for the above purposes. Subjectivity, tediousness, rater dependency and intra and inter-rater variability are the main drawbacks associated with the existing approach. Recently, imaging techniques emerged as powerful non-destructive tools to overcome the drawbacks inherent to the visual estimation methods. This study evaluated the potential use of visible-near infrared spectroscopy for sensing and discriminating development stages of blister blight. Spectral signatures were collected from detached tea leaves and canopies under healthy and diseased conditions using PSR 1100f Spectroradiometer with a spectral range of 320 – 1100 nm i.e. visible and near-infrared (VNIR). Single leaf reflectance measurements were taken under laboratory conditions using a leaf clip. Tea cultivars TRI 2024, TRI 2043, CY 9 and TRI 3041 were used for both field and laboratory studies. Distinct spectral shift observed in NIR region (700 – 1000 nm) with disease development. As Blister blight progressed, reflectance decreased by 2.4%, 4.5% and 30.3% at translucent spot, mature blister and necrotic stage respectively, in comparison to those of healthy leaves. Spectral signatures acquired under field conditions also reflected the same scenario. The results showed the potential of spectral imaging in detection, distinguish developmental stages and severity assessment of blister blight in tea. The ground-based studies can be extended to investigate the prospective use of remotely sensed data in early detection and estimation of blister blight for precision management.

Keywords: disease assessment, image analysis, visible and near-infrared spectroscopy, precision agriculture

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Abstract No: SLAMPP/2019/121

Occurrence of Target Spot of Tomato caused by *Corynespora cassiicola* in Sri Lanka

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Tomato (*Solanum lycopersicum* L.) belongs to family Solanacea is one of the most important vegetable crops grown in Sri Lanka providing high levels of nutrients to the human diet. Many constraints affect productivity and quality of Tomato among which diseases cause by fungi, bacteria, viruses and phytoplasma play a major role. In February 2019, a new destructive leaf and stem spot disease reported from commercial tomato grown in Kimbissa area, Central Province in Sri Lanka which results significant loss in foliage. The disease severity was range from 50-70% in the infected tomato plants. Studies conducted at Horticultural Crop Research and Development Institute, Gannoruwa, Sri Lanka with the objectives of isolating and molecular identification of causal pathogen of the leaf spot disease of Tomato, their field symptoms and pathogenicity. Initially symptoms appeared as small, brown lesions with a yellow margin on lower leaves and later spread to the leaf petiole, stems and on fruits. The lesions coalesce leading to blighting of foliage. The potential causative fungus was isolated from the leaf and stem lesions on PDA. Pathogenicity test was carried out by inoculating single spore culture of isolated fungus, artificially to the healthy tomato plants. Leaf and stem spot symptoms similar to those observed in the field were observed after three days of inoculation and the fungus was re-isolated from the infected tissues, completing Koch's postulates. PCR amplification and partial sequencing of ITS regions of rDNA of isolated fungus using universal primers of ITS1 and ITS4 produced ~520bp amplification product. A BLAST search revealed 99.21% and 99.60% sequence similarity to *Corynespora cassiicola* in GenBank Accession No. JN662327.1 and MF542292.1.

Keywords: Molecular identification, Target spot, Tomato

Abstract No: SLAMPP/2019/122

Role of salicylic acid in growth and induction of plant defense enzymes in okra (*Abelmoschus esculentus*) plants

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Salicylic acid (SA) is a plant hormone plays a critical role in the plant defense against biotrophic pathogens. Salicylic acid is a chemical elicitor compound, which activate chemical defense systems in plants. Various biosynthetic pathways are activated by chemical elicitors in plants depending on the elicitors used. The objective of this study was to evaluate the growth parameters and plant defense enzymes activities such as chitinase, peroxidase (POD), phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and total chlorophyll content, hydrogen peroxide (H_2O_2), pH and total sugar content at seedlings of okra (*Abelmoschus esculentus*) plants tissues by two times exogenous application of different concentrations (1, 1.5, 2 and 2.5 mM) of salicylic acid (SA). Seeds of okra (variety -Ml5) were planted separately in plastic pots with 12.5 cm height and 10 cm diameter. A rate of 2 plants/plastic pot was allowed to grow. Pots were arranged according to a completely randomized design (CRD) with six replicates and covered with green net box in the plant house. First foliar application of five treatments were made using hand sprayer when seedlings had 2-3 true leaves and second application was made 2 weeks after first foliar application. Data were analyzed by variance (ANOVA) using a SAS statistical package and mean separation was done by Least Significance Difference (LSD). Plants treated with water were used as control. Exogenously applied SA resulted increased plant shoot and shoot lengths significantly. Plants responded to SA at 1.5 mM and showed higher induction of chitinas activity and total sugar content. Plants treated with SA at 2 mM showed increased total chlorophyll content. There were no significant difference observed among treatments and control in terms of POD, H_2O_2 and PPO activities. Plants treated with SA at 2.5 mM showed higher production of PAL activity. These results suggest that SA could be utilized for the induction of plant defense in okra plants.

:Keywords Salicylic acid, okra, plant defense enzymes

Abstract No: SLAMPP/2019/123

Promoting the DNA based identification of phytopathogenic fungi to ensure regional food security and sustainability in agriculture

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Plant pathogenic fungi are a diverse group of eukaryotes with a significant impact on crops, ornamentals and forest trees worldwide. Invasive fungal pathogens have caused severe epidemics historically, leading to major food security threats and even causing serious socio-economic impact. The accurate identification and precise naming of pathogenic fungi have a great impact on global plant health and food security and is considered to be crucial in the countries with lack of phytosanitary practices, including Sri Lanka. Although morphological characters of the fungi have conventionally been used in fungal identification, the past two decades have witnessed revolutionary changes with the implementation of DNA sequence data. The standard fungal DNA barcode, nuclear ribosomal internal transcribed spacer region (ITS) is extensively used for a quick and often approximate identification. However, due to limitations of single gene region in fungal species discrimination, Genealogical Concordance of Phylogenetic Species Recognition (GCPSR) which involved multiple gene regions has been the gold standard up to date. The identification of fungi in agricultural, biosecurity and quarantine practices are still widely based on morphology and tentative taxonomic assignments have increased the risk to regional and global food and fiber security. In order to overcome these challenges, we promote the need for (1) precise naming of pathogenic fungi in the era of one name for pleomorphic species; (2) the incorporation of molecular data in the identification of emerging phytopathogens; and (3) paradigm shifts in fungal identification practices in agriculture and food industry. We urge the relevant agencies of the countries lacking organized plant disease detection and surveillance practices, to recognize the need to confront the potential threats on their staple crops, fiber resources, export-crops and support appropriate research for DNA-based fungal identification and classification and application of accurate names to high priority phytopathogens and emerging species. This will enable effective management of plant diseases to ensure the food security and sustainability in agriculture both regionally and globally.

Keywords: DNA barcoding, molecular identification, invasive species, plant biosecurity, quartine

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Theme 3: Innovative and eco-friendly approaches of plant disease management

Abstract No: SLAMPP/2019/112

Root-application of rice husk ash as a natural silicon source enhances resistance against powdery mildew in bitter gourd (*Momordica charantia* L.)

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Beneficial effects of Silicon (Si) on growth, yield and disease resistance in many agricultural crops have been elucidated worldwide. We previously reported that soil amendment with soluble Si, in the form of potassium silicate, reduced pre-harvest and postharvest fungal disease incidence in bitter gourd (*Momordica charantia* L.) leaves. These changes were associated with increased Si levels and induced biochemical defenses in tissues. This study evaluated the effect of rice husk ash (RHA) as a natural Si source on bitter gourd-powdery mildew pathosystem. *M. charantia* cultivar 'Matale green' was grown in potting media amended with RHA at a rate of 200 mg Si/ kg soil, once a week, up to 28 days. Media without RHA were considered as controls. Each treatment consisted of 20 replicate pots. At 35 days after first Si application, *Erysiphe* sp., the powdery mildew pathogen, was inoculated to plants. Disease infection, total phenol content and peroxidase (POD), polyphenol oxidase (PPO), chitinase and glucanase activities in leaves were measured 3, 5 and 7 days after inoculation. Si accumulation in leaves was measured during the growth at 7-day intervals. Antifungal activity was tested by *Cladosporium* bioassay coupled with Thin Layer Chromatography. Data were analyzed by one-way ANOVA. RHA application significantly decreased the powdery mildew disease severity while elevating the levels of POD, PPO, chitinase, glucanase and total phenolic content in leaves compared to those in controls. RHA treated plants exhibited significantly higher ($p<0.05$) Si accumulation and a stronger antifungal activity. These results revealed the potential of RHA as a natural Si source for controlling powdery mildew in bitter gourd.

Keywords: silicon, bitter gourd, rice husk

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Abstract No: SLAMPP/2019/117

Comparison of endophytic fungal colonization rates in newly improved rice varieties Bw367 and Bg352 in selected geographical locations of Sri Lanka.

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Rice is the staple diet in Sri Lanka and the extensive levels of agrochemicals used in rice cultivation has caused serious environmental and health problems. Therefore, various microorganisms including endophytic fungi of plants are being investigated as an alternative for agrochemicals. As endophytic fungi have the ability to enhance plant growth and control diseases, they can be used to increase crop production as well as for disease management. Thus assessing the ability and rate of colonization of a particular plant by fungal endophytes is very important. Therefore, this study was aimed at assessing the colonization rates of endophytic fungi present in two newly improved rice varieties grown in selected geographical locations across Sri Lanka with a view of utilizing them for increased productivity and disease management of the two varieties. Healthy plant samples of Bg352 and Bw367 were collected during the Maha season (January, 2019) from fields in different climatic zones of Sri Lanka i.e. fields in Anuradhapura, Kurunegala, Gampaha and Kalutara districts. Endophytic fungi were isolated from a total of 480 plant segments including leaf, stem and root pieces of the two varieties onto Malt Extract Agar (MEA) after surface sterilization using previously optimized regimes. Colonization rates (CRs) of endophytic fungi were determined and the difference in the extent of colonization by endophytes between two varieties and between districts was analyzed separately by one-way ANOVA. A total of 92 isolates (35 isolates from Bw367 and 57 from Bg352) were obtained from all plant parts of both varieties collected from the four sites. The total CRs of endophytic fungi in all plant parts of Bw367 collected from Kurunegala, Anuradhapura, Gampaha and, Kalutara were 3.3%, 11.6%, 25%, and 16.6% respectively whereas for Bg352, the CRs were 26.6%, 16.6%, 28.3% and 23.3% respectively. The analysis of results showed that there was no significant difference ($P<0.05$) in the colonization rates of endophytes between the two rice varieties nor was there a significant difference ($P<0.05$) among the selected districts of the different climatic zones indicating that endophytic fungal colonization rates in Bg352 and Bw367 was not affected by varietal difference or by climatic and associated conditions of the different locations studied.

Key words: rice varieties, endophytes, diversity

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Abstract No: SLAMPP/2019/115

In-vitro efficacy of managing fungal diseases using Plant Growth Promoting Rhizobacteria (PGPR)

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Fungal diseases are one of major reason for heavy crop losses in fruits and vegetables. Fungicides, which has adverse effects to the human as well as environment, are commonly used to manage fungal diseases. Hence introduction of effective and environmentally- safer options are essential for the management of fungal diseases. Currently use of Plant Growth Promoting Rhizobacteria (PGPR) as alternative to fungicides are emerging trend. Therefore, this study was conducted to find the *in-vitro* efficacy of PGPR isolated from non-cultivating areas in Sri Lanka to manage fungal pathogens in Beetroot, Leeks and Strawberry. PGPR isolated and identified by a previous study were used in this experiment. Fungal pathogens were isolated from beet root, leeks and strawberry. Antagonistic activity against those fugal pathogens of the PGPR were tested by dual culture plate method. Inhibition zone were measured and data were analyzed by SAS. Fungal pathogens isolated from beet root and leeks were identified as *Fusarium* spp. by morphological features. Fungus isolated from strawberry could not identified based on morphological features. All the three fungal pathogens showed significant growth inhibition by an IAA producing PGPR isolate. *Fusarium* spp. and unidentified fungus isolated from strawberry showed significant growth inhibition by a phosphate solubilizing PGPR isolate and *Pseudomonas* spp. This unidentified fungus showed significant growth inhibition by a nitrogen fixing PGPR isolate. Further confirmation of PGPR isolates and fungal identification by molecular methods and finding the *in-vivo* efficacy of those PGPR isolates are required in future studies.

Key words: fungal pathogens, non-cultivated areas, PGPR

Abstract No: SLAMPP/2019/108

Mycolytic bacteria as potential biocontrol agents against phytopathogenic fungi of *Piper nigrum*

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Piper nigrum or black pepper is an important commercial spice crop often referred to as "king of spices", playing a significant economic role. Sri Lankan black pepper is of top grade, procuring a premium price in international spice trade. Black pepper market is ever growing and in constant demand. However, as all agricultural crops, black pepper too encounter diseases that adversely affect its production causing inevitable economic losses. Major phytopathogenic diseases that infect black pepper plants are Phytophthora rot, slow decline and anthracnose disease, caused by phytopathogenic fungi species *Phytophthora capsici*, *Fusarium solani* and *Colletotrichum gloeosporioides*, respectively. In this study, five mycolytic enzyme producing bacteria; *Acinetobacter calcoaceticus*, *Chryseobacterium indologenes*, *Serratia marcescens*, *Pseudomonas putida* and *Bacillus cereus* were tested against the three phytopathogenic fungi for effective biocontrol potential. Mycolytic enzymes such as chitinase, protease and glucanase are capable of degrading cell wall components of fungi. The efficacy of this antagonistic property was tested for each of the selected bacteria species against the phytopathogenic fungi using dual culture assay, double plate assay and mycelia growth test. Dual culture assay was a screening test to identify effective bacteria types, while double plate assay and mycelia growth test were confirmatory tests for antagonistic volatile and non-volatile metabolites produced by the effective bacteria. Efficacy was determined by calculating the percentage inhibition of radial growth (PIRG). It was found that *Phytophthora capsici* was effectively inhibited by all five mycolytic enzyme producing bacteria, however highest inhibition was achieved by *Chryseobacterium indologenes*. *Serratia marcescens* was found to produce non-volatile compounds antagonistic to all three phytopathogenic fungi.

Keywords: phytopathogenic fungi, biocontrol, mycolytic bacteria

Abstract No: SLAMPP/2019/109

Characterization of *Agrobacterium* strains from agricultural soils of Bandarawela, Sri Lanka

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Agrobacterium is a Gram negative, rod shaped, aerobic and motile soil inhabiting bacterium of the family Rhizobiaceae. It is well known as the causative agent of crown gall disease of many plant species around the world. However, not all the *Agrobacterium* strains are pathogenic and can cause galls. Only the virulent strains cause crown gall disease on number of plant species and are found only in contaminated soils. These virulent strains of *A. tumefaciens* harbor Ti plasmids with transfer DNA (T-DNA) region and virulence (*vir*) genes that are responsible for the pathogenicity. *virD2* gene codes for virD2 protein and the endonuclease domain of the virD2 protein cleaves T-DNA border sequences. The *ipt* gene is the T-DNA borne cytokinin synthesis gene. Therefore, the presence of *virD2* gene and *ipt* gene are useful in identifying pathogenic strains of *Agrobacterium*. The major objective of this research was to determine whether agricultural soils of Bandarawela were contaminated with virulence strains of *A. tumefaciens*. Soil samples were collected and bacteria were isolated using soil dilution method, and cultured on Yeast Mannitol Agar supplemented with Congo red and on Yeast Extract Peptone Agar. Five pure cultures of putatively *Agrobacterium* were further characterized using morphological and biochemical tests including Gram staining, catalase test, citrate utilization test, sugar fermentation test and 3-ketolactose test. These testes were often used for the species level identification of *A. tumefaciens*. Out of five isolates four were rod shaped with rounded ends and were either single or in pairs. However, the other isolate was in chains and long rod shaped. Interestingly, all the isolates were positive for all the biochemical tests. However, these tests do not help differentiating the virulence strains. Molecular characterization of all the soil isolates were carried out using universal 16s rRNA primers and *Agrobacterium* specific primers targeting *virD2* and *ipt* genes. PCR amplification with virD2 primers successfully amplified the targeted band of 224 bp in all five isolates while ipt produced the expected fragment of about 427 bp in three of the isolates. *virD2* gene sequences of selected soil isolates were 100-99% similar to the *A. tumefaciens* of the GenBank accession CP032925 and CP032929 reported from Taiwan. According to morphological, biochemical, and molecular characterization using *virD2* and *ipt* genes it was confirmed that the soil in the inspected field of Bandarawela is contaminated with pathogenic strains of *A. tumefaciens*. Therefore, farmers should maintain awareness when cultivating susceptible plant varieties in these fields.

Keywords: pathogenic, Ti plasmid, virD2 gene

Abstract No: SLAMPP/2019/111

Phosphate solubilising rhizospheric fungi as potential plant growth promoters of rice plants in Sri Lanka

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Phosphorus is one of the major limiting factors of crop production in many agro ecosystems as it is not readily available in the soluble form for uptake by plants. Rhizospheric microorganisms, including fungi are reported to have the ability to transform insoluble phosphate to the soluble form. In the present study, rhizospheric fungal spp. of the traditional rice variety Suwandel were isolated, identified based on their morphological and molecular characteristics, and the fungal spp. with phosphate solubilising ability were selected by a plate assay using Pikovskaya medium with a view to use them for growth enhancement of rice plants. The fungal isolates with the highest Phosphate Solubilisation Index proved to be *Penicillium* sp. 1, *Penicillium* sp. 2, *Aspergillus* sp. 1 and sterile species. These strains were evaluated for their effect on plant growth of rice varieties Suwandel and Bg 94-1 under greenhouse conditions by inoculating each fungal species to 3 days old rice seedlings of the two varieties. The inoculum was prepared by transferring actively growing mycelia from each fungal spp. into 1g of sterile rice husk medium. The seedlings of the two rice varieties were inoculated by placing the seedlings into rice husk medium in each fungal inoculum separately for 2 days. Then the seedlings were introduced to pots containing sterilized soil and the growth parameters of randomly selected plants were evaluated at two week intervals. The plants inoculated with all test fungal spp. showed a significant increase ($P \leq 0.05$) in shoot length, fresh weight and dry weight as compared to non-inoculated plants over a 4 week period. Therefore, these 4 different fungi may help to improve the rice plant growth. Also, Bg 94-1 variety showed a significant increase ($p \leq 0.05$) in fresh weight and dry weight when compared with Suwandel rice variety, after 4 weeks indicating that the fungal isolates were more effective in the promotion of growth of Bg 94-1 better than Suwandel variety and that the rhizospheric fugal spp. of Suwandel have the ability to promote the growth of Bg 94-1.

Key words: suwandel, phosphate solubilizing fungi, growth enhancement

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Effect of some selected essential oils against *Colletotrichum* sp. and *Lasiodiplodia* sp. causing postharvest diseases in papaya and mango: A preliminary study.

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Anthracnose caused by *Colletotrichum* sp. and stem-end rot (SER) caused by *Lasiodiplodia* sp. are common postharvest diseases affecting papaya and mango fruits in Sri Lanka. Diseases are managed by synthetic chemicals which are hazourdous. The use of essential oils (EO) has become a non-hazardous alternative. The present study aimed investigation of the antifungal potential of selected EOs against anthracnose and SER causing pathogens isolated from papaya and mango fruits *in-vitro*. Fungal pathogens causing anthracnose and stem-end rot were isolated from mango cultivar "Karuthacolomban" and papaya cultivar "Red Lady". Ten isolates from each fungal species of each commodity were identified based on morphological characteristics. Koch's postulates were performed to confirm the pathogenicity. A poisoned food bio-assay was carried out to evaluate the antifungal effect of EOs [basil (*Ocimum basilicum*), cardamom (*Elettaria cardamomum*), citronella (*Cymbopogon nardus L.*), orange (*Citrus sinensis*), mustard (*Brassica juncea L.*) and lemon (*Citrus limon*)] by mycelial growth inhibition of *Colletotrichum* sp. and *Lasiodiplodia* sp. The bioassay consisted of EOs at three standard concentrations (500, 750, 1000 $\mu l l^{-1}$) along with untreated control. Fungal colony diameter along four axes was obtained daily until the mycelium of the control completely filled the Petri plate. Three replicate plates were used per EO treatment and the experiment was performed three times. EOs that significantly inhibited growth of mycelia were selected and further bio-assays performed to optimize the effective concentrations. Results indicated that EOs Cardamom (1000 $\mu l\ l^{-1}$) and Citronella (750 $\mu l l^{-1}$) significantly ($P<0.05$) inhbited the mycelial growth of of *Colletotrichum* sp. and *Lasiodiplodia* sp. isolated from papaya fruit. Optimized concentrations of EOs Basil, Cardamom (700 $\mu l l^{-1}$) and Citronella (400 $\mu l l^{-1}$) significantly ($P<0.05$) inhibited the mycelia growth of *Lasiodiplodia* sp. from mango fruit. A higher optimized EO concentrations (basil 1250 $\mu l l^{-1}$and cardamom 1500 $\mu l l^{-1}$) were necessary to inhibit, mycelial growth of *Colletotrichum* sp. isolated from mango fruit; A potential for using Cardamom and Citronella oils for controlling anthracnose and SER in papaya fruits exists while EOs Basil and Cardamom were found to be effective against both the said pathogens of mango fruits and Citronella oil was effective only against the SER pathogen of mango fruit.

Keywords: postharvest diseases, anthracnose, stem-end rot, essential oils

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