INDIAN PHYTOPATHOLOGICAL SOCIETY

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Abstracts

National Symposium On

UNDERSTANDING HOST-PATHOGEN INTERACTION THROUGH SCIENCE OF OMICS

March 16-17, 2015

ICAR-Indian Institute of Spices Research

Marikunnu P.O., Kozhikode -673012, Kerala, India

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on
Understanding host-pathogen interaction through science of omics

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ICAR - Indian Institute of Spices Research
Kozhikode (Calicut), Kerala

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March 16-17, 2015, Kozhikode, Kerala, India

Hosted by

ICAR-Indian Institute of Spices Research, Kozhikode, Kerala, India

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NATIONAL SYMPOSIUM

Understanding host-pathogen interaction through science of omics

ICAR- Indian Institute of Spices Research, Kozhikode, Kerala
March 16-17, 2015

Hosted by

ICAR-Indian Institute of Spices Research, Kozhikode, Kerala
Indian Council of Agricultural Research, New Delhi

Organized by

ICAR-Indian Institute of Spices Research, Kozhikode, Kerala
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New Delhi

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PREFACE

This national symposium is being jointly organized by the Indian Phytopathological Society, New Delhi and the ICAR-Indian Institute of Spices Research, Kozhikode, Kerala. The theme of the symposium is 'Understanding Host-Pathogen Interaction through Science of Omics'. The main objective is to provide a common forum for interaction among scientists, students, industry, extension and developmental agencies engaged in plant pathological research and development. Besides, it offers an opportunity for young scientists to acquire scientific knowledge through inspiring lectures by experts and participating in thought provoking discussions. The symposium will also provide a platform to address and discuss recent developments in the field of Plant Pathology.

Unraveling the molecular mechanisms that govern host-pathogen interactions is a subject of intense research in the field of Plant Pathology. The symposium will encompass discussions on the current gaps in our understanding of how plant pathogens manage to evade host immune surveillance and successfully establish acute and infection states. Sincere attempts will be made to highlight cutting edge approaches to describe recent advances in the fields of host-pathogen interaction in plants. Ergo, the symposium, among other things, will encompass pathogen characterization, diagnosis and epidemiology (Session I), pathogenomics, proteomics and host-pathogen interaction (Session II), host plant resistance and biotechnological approaches (Session III) and integrated disease management (Session IV).

This publication is a compilation of the abstracts of the Presidential address, various memorial award lectures, Prof. M.J. Narasimhan Merit Academic Award contest, lead talks and poster presentations encompassing the four sessions. The editors are indebted to all the contributors and hope that the publication would provide valuable information on research and development in host-plant pathogen interactions. We are also thankful to various agencies for providing their advertisements. Special thanks to Ms. P. Deepthi of the Indian Society for Spices, Kozhikode for her efforts in typesetting the manuscripts. The editors also gratefully acknowledge the cooperation of the organizing committee in bringing out this publication.

Editors
March, 2015

PRESIDENTIAL ADDRESS

The science of "omics" for plant pathologists

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The growing population of India is poised to overtake China in about a decade and half and India will be the most populous country in the world. This is putting the onus on the Indian Agricultural scientists to grow more food, feed, fibre and fodder under shrinking natural resources like land, water and non-availability of labour. The global warming and climate change could affect the plant diseases and there is increased pressure on the plant pathologists to minimize crop losses. There are ever increasing populations of aggressive pests and pathogens evolving to add to woes of global warming. There are reports of new strains of viruses on crop plants, new diseases such as late blight of tomato with newer strains of *Phytophthora infestans* that has created epidemics, new races of *Fusarium* on banana etc. Indian Phytopathological society being one of the largest societies, this august body of plant pathologists have always taken up the challenges and won several laurels. I am confident that the younger generations of plant pathologists are competent enough to meet the challenges and make use of the technologies available today.

The annual review of plant pathology is completing 52 years since it was first published and has been a source of information for researchers. The focus has been changing over the years and the reviews bring about latest information. A look at the latest issues reveals the focus is on climate change and the unraveling of host pathogen interaction and molecular level. The advances in bioinformatics along with genome sequencing technology have added to the growth of the new frontiers of "omics" sciences. It is providing newer tools to plant pathologists to understand the host-pathogen interaction and deploy newer and cheaper methods of pathogen control.

The advent of next generation sequencing platforms are the technological basis for the genome projects with their high throughput application for whole genome sequencing of all organisms. The "generation" refers to the chemistry and technology used by the sequencing process. First generation generally refers to Sanger sequencing. "Next-generation", is generally used to refer to any of the high-throughput methods which were developed after Sanger. The next generation sequencing tools emerged in 2005 as replacement to the low throughput and high cost of first-generation methods. With the advancement in Next gen sequencing technologies and less cost investment re-sequencing of any organism became easy. Whole genome re-sequencing aims to sequence the individual whose reference genome is already known. As reference genome sequences become increasingly available for many species, cataloguing sequence

variations and understanding their biological consequences have become major research goals. The DNA re-sequencing is of sequencing a DNA region for an individual given that a reference sequence for this region is already available for the specific organism.Targeted re-sequencing is a variation of re-sequencing where only a small subset of the genome is sequenced, such as the exome, a particular chromosome, a set of genes or a region of interest.

For plant pathologists taxonomy of the organism is very relevant. From phenotypic characterization to gene based sequences and the present multigene approaches gives a clear demarcation of the differences among pathogen populations. The fact that the number of *Phytophthora* species described after 2000 is far more in number than that has been descried till then is because of the molecular tools available presently. At Indian Institute of Spices Research, Kozhikode, whole genome sequencing of two isolates of *Phytophthora* infecting black pepper revealed the existence of enormous diversity. The genome size of 98-93 is 46.1Mb while that of isolate 05-06 is 66.8Mb. There were only 2039 genes common among both isolates whereas 6095 unique genes noticed in 98-93 and 4039 were unique to 05-06. The genome of 05-06 also revealed the presence of several effector genes some are common as reported in JGI and about 52 different effector type unique to this isolate. This clearly shows the diversity and non-conformity with the characters reported for *P. capsici* on other crops worldwide warranting re-description of the species complex found in black pepper.

The mechanism of gene action in plant pathogen interaction is not a straight forward transcription, translation and protein synthesis. There are ever complicated processes of alternative splicing and various regulations and post translational modifications. The knowledge on pathogenesis related proteins (PR) proteins not only would explore the mechanism of plant –pathogen interaction, but also would guide to develop the plant either through selection or to develop newer molecules to avoid the pathogen. The science of effectronomics, phosphoproteomics etc are paving way for better understanding of the complex processes involved. The science of proteomics can be better utilized for management of plant diseases. The scientists of Indian Institute of Spices Research have found a way to manage viral diseases in black pepper in the field by utilizing the knowledge of the interaction between the viruses and the host plant by using the proteomics information and by minimizing the damage and nursing back the vines to normal health by supplementing the nutrients and circumventing the pathogenic processes. This gives an alternate strategy to manage viral diseases in the field than the usual recommendation of 'cut and burn'. Similarly the transcriptome data generated during the pathogenic process between *Phytophthora capsici* and *Piper nigrum* and *P. colubrinum* is paving the way for better understanding of the infection process.

The responsibilities of plant pathologists increase with the new challenges facing crop production in the changing weather and emerging new diseases. The new tools available will certainly be useful to face all challenges swiftly and uphold the traditions of proving the critic wrong. The oldest science of "omics" relevant to plant pathologists is not the science as it is understood in genomic era but the art of managing diseases and the 'economics' of crop production.

DASTUR MEMORIAL AWARD LECTURE

A1

Recent changes in the *Phytophthora* populations led to severe outbreaks of foliar blights and fruit rots in vegetable crops in South India

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Diseases caused by stramenopile *Phytophthora* spp are emerging as a major production constraint for sustainable vegetable production in India. Since 2008, severe outbreaks of *Phytophthora* diseases such as late blight on tomato, fruit rot on tomato, brinjal and cucurbits, foliar blights and wilts in chilli were recorded in South India. One hundred and fifty-seven isolates of *Phytophthora* infestans, 63 from potato and 94 from tomato, were collected from major potato and tomato production areas of South India between 2010 and 2012. Their phenotypic and genotypic characteristics were determined and compared with reference isolates. Isolates were characterized based on mating type, *in vitro* metalaxyl sensitivity, mitochondrial DNA haplotype, RG57 DNA fingerprinting patterns, SSR markers and aggressiveness on potato and tomato, in order to monitor population changes in *P. infestans*. All isolates were A2 mating type, metalaxyl resistant, mtDNA haplotype Ia and had RG57 and SSR fingerprints almost identical to the 13_A2 clonal lineage reported in Europe. Variation at the D13 and SSR4 loci allowed discrimination of minor variants, designated as 13_A2_3, 13_A2_3b, 13_A2_3c and 13_A2_1. A comparison of the lesion diameters caused by 157 isolates on detached leaflets of three potato and tomato cultivars showed all isolates to be equally aggressive, confirming that the same clonal population is infecting both hosts. This study demonstrates that the 13_A2 lineage was responsible for severe late blight outbreaks on potato and tomato in South India and has replaced the prior population represented by the US-1 and other genotypes.Phytophthora isolates (86), recovered from blight affected leaf tissues of hot pepper from different localities in Karnataka and Tamil Nadu states between 2011 and 2012, were identified majority of the isolates as *P.boehmeriae* and few isolates as *P.capsici* based on morphology, a similarity search of ITS sequences at GenBank, PCR-RFLP patterns and species-specific PCR using PC1/PC2 and PB1/PB2 primer pairs. All isolates of *P. boehmeriae* were metalaxyl sensitive while *P.capsici* isolates were intermediate in sensitivity. *P. boehmeriae* isolates were highly aggressive and produced significantly larger lesion than those of *P. capsici* isolates. *Phytophthora nicotianae* isolates (76), recovered from brinjal (18), ridge gourd (45) and tomato (19) from different localities in these states during the June-to-December cropping season of 2012 and 2013 were characterized based on phenotypic, genotypic markers and aggressiveness. All brinjal and ridge gourd

isolates were A2 while tomato had both A1 (13) and A2 (6). All isolates were metalaxyl sensitive. They were monomorphic both at malate dehydrogenase (EC.1.1.1.37) and malic enzyme (EC 1.1.1.40) and also had identical protein profiles. In addition, isolates were genotyped for three mitochondrial (rpl5-rns, rns-cox2 and cox2+spacer) and three nuclear loci (hyp, scp and β-tub). All genes were polymorphic but nuclear genes were more variable than mitochondrial genes. Bayesian and net work analyses based on the combined data set of sequences of the three nuclear regions revealed host specific association. *P. nicotianae* isolates were highly aggressive and produced significantly ($P < 0.01$) larger lesions on their respective host than on their alternative host. Thus, the migration of 13_A2 genotype *P. infestans* was the cause of outbreaks of destructive late blight epidemics in India and stresses the importance of bio-security in agricultural trade. The emergence of *P. boehmeriae* was responsible for severe leaf blight epidemics on hot pepper in South India although it is not serious pathogen on any crop in any part of the world. The significant genetic variations in the population structure of *P. nicotianae* are responsible for severe outbreaks on brinjal, ridge gourd and tomato. These invasive and emerging *Phytophthora* species have epidemiological and management implications for the production of vegetable crops in India.

MUNDKUR MEMORIAL AWARD LECTURE

A2

Phylogeography and molecular evolution of begomo viruses infecting okra

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Bhendi yellow vein mosaic disease (BYVMD) is a serious disease of okra which has characteristic symptoms of yellow vein and chlorosis. Begomoviruses are plant-infecting viruses, which are transmitted by the whitefly vector *Bemisia tabaci* and have been known to cause extreme yield reduction in okra around the world. Several begomoviruses have been detected infecting okra in Asia, Africa and America. Small single stranded circular molecules, alphasatellites and betasatellites, which are about half the size of their helper begomovirus genome, have also been detected in plants infected by begomoviruses. Over the past 10 years, India has experienced epidemics of the disease, the most recent of which involved a virus and satellites that are resistance breaking. Loss of this conventional host–plant resistance, which saved the okra growers from ruin in the late 1990s, leaves farmers with only relatively poor host plant tolerance to counter the extensive losses the disease causes coupled with whitefly biotype emergence. Sequencing of viral genomes from okra resulted in the identification of many species of okra-infecting begomoviruses have been reported from both the Old World and New World. These include Cotton leaf curl Gezira virus (CLCuGeV) from Sudan, Okra leaf curl Cameroon virus (OLCuCMV) from Cameroon, Okra yellow crinkle virus (OYCrV) from Mali and Cameroon, Okra yellow mosaic Mexico virus (OYMMV) and Okra yellow mottle Igula virus (OKYMoIV) from Mexico, Okra yellow vein mosaic virus (OYVMV) from Pakistan, Okra leaf curl Oman virus from Oman and Sida micrantha mosaic virus and Okra mottle virus from Brazil. In India, distinctive monopartite begomoviruses such as Bhendi yellow vein mosaic virus (BYVMV), Cotton leaf curl Alabad virus (CLCuAlV), Bhendi yellow vein Bhubaneswar virus (BYVBhV), Cotton leaf curl Bangalore virus (CLCuBaV), Bhendi yellow vein India virus (BYVIV) and Okra enation leaf curl virus (OELCuV) have been found in okra. In addition to monopartite, two bipartite begomoviruses i.e. Bhendi yellow vein Delhi virus (BYVDV) and Tomato leaf curl New Delhi virus (ToLCNDV) were also identified infecting okra. Putative recombinations were detected in begomovirus genomes identified in okra, indicating that recombination is an important mechanism for their evolution. At least nine betasatellites and 12 alphasatellite molecules were associated with okra infecting begomoviruses. The recent advances made in understanding the molecular biology of the components of the disease complex and their interactions with host plants will aid in development of suitable management strategies.

M.S. PAVGI AWARD LECTURE

A3

Advances in phytonematology in India

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Plant Nematology or Agricultural Nematology also referred as Phytonematology, deals exclusively with those forms of nematodes which parasitize plants of economic importance to agriculture, horticulture and forestry. The first plant parasitic nematode was recorded in 1743, later on named as Anguina tritici. Since then, about 2500 nematode species are known to parasitize the lower as well higher plants at global level. Among these, about 600 species belonging to about 85 genera have so far been either described or reported to be associated with more than 700 plant species growing in diverse agro-climatic zones of our country. In India, many of them have been proved to be highly pathogenic and cause considerable economic yield losses in cereals, pulses, vegetables, fruits, ornamentals and forest crops.The historical perspective of the growth and development of Nematology in India have been traced out during the period from 1901 to 1958 that was marked by some important records of nematode parasites *viz.*, root-knot nematodes belonging to the genus *Meloidogyne* on tea and black pepper, ufra disease (*Ditylenchus angustus*) and white tip (*Aphelenchoides besseyi*) of rice, ear-cockle of wheat from various parts of the country. Among the several important developments that led in the expansion in the science of Nematology in India includes the pioneer work of scientists/workers at Aligarh Muslim University (AMU), Aligarh, Indian Agricultural Research Institute (IARI), New Delhi and Hyderabad, who have made the extensive surveys and worked on the taxonomy. In 1953's, the observation on the occurrence of root-knot disease on tomato was recorded by Prof. Abrar Mustafa Khan who had put foundation of Plant Nematology in AMU, Aligarh, which gained impetus so fast he had never realized. Later on after appearance of Molya disease due to Heterodera avenae, a serious problem of golden nematode (*Globodera rostochiensis*) in potato in Nilgiris during 1961 has also realized the importance of research in the field of Nematology. In 1961, a Division of Nematology was established at IARI which started post graduate teaching (M.Sc. and Ph.D. degree programmes) to produce trained manpower in Nematology. This was emerged as a discipline paying more attention to identification and study of disease problems than to taxonomy. Subsequently, the post graduate program was also started to award the M. Sc. and Ph. D. degree in Nematology in many SAUs, CAUs, CUs, DUs and other ICAR institutes. The organization of a series of training programmes by IARI, New Delhi and AMU, Aligarh also helped in the progress of this discipline in India. During 1967-68, First South-East Asia Post-graduate Nematology Course held at AMU, Aligarh and IARI, New Delhi in collaboration with the International Agricultural Centre, Wageningen (Netherlands). Moreover, between 1967 and 1975, five other South-East Asia Post-Graduate Training Courses were also organized at the IARI, New Delhi and AMU, Aligarh under the stewardship of Dr. AR Seshadri

and Dr. AM Khan, respectively. In 1969, Nematological Society of India was founded and First All-India Nematology Symposium was held at the IARI, New Delhi. The pace of publication on Nematological research gained appreciable momentum from 1971 when "Indian Journal of Nematology" started its publication from Nematological Society, IARI, New Delhi. In 1977, All India Coordinated Research Project (AICRP) on 'Nematode Pests of Crops and their Control' was launched and funded by Department of Science and Technology, and later (1979) by the ICAR. It started functioning at 14 centres all over India. With greater awareness about nematode problems and liberal financial assistance from ICAR under the aegis of AICRP, Nematology teaching and research centres have cropped up in several SAUs and ICAR institutes.

J.P. VERMA MEMORIAL AWARD LECTURE

A4

Diversity, diagnosis and management of bacterial diseases in India

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In India, about 200 plant diseases caused by different species and out of these, 75 have diseases have economic value. Among these diseases, bacterial wilt of solanaceous crops and ginger (*Ralstonia solanacearum*), bacterial blight of rice (*Xanthomonas oryzaepv. oryzae*), bacterial blight of pomegranate (*X. axonopodis pv. punicae*) black rot of crucifers (*X. campestrispv. campestris*), bacterial blight of cotton (*X. axonopodis pv. malvacearum*), black spot of mango (*Xanthomonas campestris* pv. *mangiferae-indicae*), citrus canker (*X. citri*), canker disease of grapes (*Xanthomonas campestris* pv. *viticola*), crown gall of pome fruits (*Agrobacterium tumefaciens*) and bacterial spot (*X. vesicatoria*) reduce the crop yield. Race characterization of *X. axonopodis* pv. *malvacearum*, *R. solanacearum* and *X. campestris* pv. *campestris* by using differential hosts has been studied in India. In *X. axonopodis* pv. *malvacearum*, out of 26 races described, race 32 is the most widely distributed and knocked out five bacterial blight resistant genes i.e. B7, B2, B4, BIn, and BN. *R. solanacearum* is a species complex, which comprises 6 biovars based on carbon utilization (bvs 1, 2, 2T, 3, 4 and 5), 5 races based disease reaction on the hosts (races, 1, 2, 3, 4 & 5) and 4 phylotypes (phylotypes I, II, III & IV) based egl gene. In India, 4 races (races, 1, 2, 3, 4), 5 biovars (bvs 1, 2, 2T, 3, 4) and 3 phylotypes (phylotypes I, II, & IV) have been reported in potato. In this pathogen most diversity is found, which was isolated from potato crops. Another bacterial pathogen *X. campestris* pv. *Campestris* has a lot diversity in India. Out of 10 races of this pathogen reported across the world. Race 1and 4 were prevalent in most of the cole crops growing states of India and race 6 was found only in Delhi, isolated from cabbage crops. In any plant diseases management, diagnosis is play vital role. Accurate routine disease detection requires high levels of specificity, sensitivity and speed. In this context, specificity is defined as the capability to detect the organism of interest in the absence of false positives and negatives. Sensitivity relates to the lowest number of pathogens reliably detected per assay or sample. The sensitivity levels of different techniques for detection in plant material are as follows (in colony forming units (cfu)/ml)]: isolation, about 10–102; immunofluorescence (IF) 103; conventional PCR, 103–104 and ELISA, 105–106. Besides classical methods and serological techniques, PCR based techniques has been used to detect plant pathogenic bacteria from seeds, asymptomatic plants, irrigation water and soils. In India, PCR based protocol has been developed to detect *X. campestris* pv.*campestris* (seeds, planting materials), *Ralstonia solanacearum* (asymptomatic

plants, tubers, irrigation water and soil.), *X. oryzae* pv. *oryzae* (Seeds, planting materials), *X. axonopodis* pv *punicae* (leaves and propagating material) and *X. axonopodis* pv. *malvacearum* (seeds) from different sources. The primers were designing by using nucleotide sequences different genes such as 16S rRNA, hrp B, hrp F, gyrB, which are conserved region for specific group of bacteria. The sensitivity of the primer to detect bacteria was further improved through Bio-PCR and Nested PCR and able to detect the bacteria below >100 cells/ mL. For management, preventive measures mostly sanitation is the best way to control the bacterial diseases. There is only two chemicals i.e. Streptocycline (10% oxytetracycline and 90% Streptomycin. Antibiotics in plants are locosystemic therefore their curative action is very poor and control is mainly due to their contact action. Early pruning in hot and humid area where monsoon prolong after first week of October must be avoided. Horticultural practices like of certification of disease free planting materials of pomegranate and grapevine is need to be standardized and insured that all the planters should get only disease free planting materials.

SHARDA LELE MEMORIAL AWARD LECTURE

A5

Trichoderma-Lab to Field

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Trichoderma is known and explored worldwide for its versatility as biological control and growth promoting agent and is rightly said as anchorage of basic and applied research. The success of biological control of plant diseases depend on the availability of effective formulations of biocontrol agents, their survival during storage and rapid multiplication and colonization after inoculation. However, in spite of the extensive research effort there are very few biological strains available at commercial levels. Selection and characterization based on antagonistic behavior pesticidal tolerance and evaluation of inter and inter species differences based on genetic diversity of effective biological control strains of *Trichodermaharzianum* and *Trichoderma viride* was being made. Different species of *Trichoderma* are known to produce different kinds of enzymes and active antimicrobial metabolites which have a significant role in biocontrol activity like cell wall degradation, biotic and abiotic stress tolerance, hyphal growth, antagonistic activity and biodegradation of pesticidal residues. By the advance techniques laid in the molecular biology, we can easily isolate, characterize, clone, sequence and express the functions of endochitinase, cellulase, Glucanase, Xylanase and can study their functions and role in the biocontrol mechanism. The success of bioagents *T.harzianum* in managing various soil and foliar diseases majorly Purple Blotch of Onion, Stalk rot and Black Rot and Damping-off of Cauliflower and tomato, Groundnut rot, Bacterial blight of rice and growth promotion in wheat. *Pseudomonas flourescens* in disease management under field conditions has been greatly attributed to the development of rhizospheric competent strains which establish in the root zones of different crops in different agroclimatic zones of the country. The consortial application of these bioagents were also found effective against blastof rice leads to development of bioformulation based on Integrated Pest Management (IPM) and established as a 'Biologically safe Technology' widely accepted by farmers of different states (J&K, U.P. H.P,Punjab, Haryana, Rajasthan) and commercialized successfully twice to Sai Bio Organics, Punjab in (September 2010) and Government of Rajasthan

PROF. M.J. NARASIMHAN MERIT ACADEMIC AWARD CONTEST

EASTERN ZONE

M1

On-farm production of indigenous AM fungus inoculum for improved phosphorus nutrition in upland rice using vermiculite based media

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Rice production in the upland ecology is severely impeded due to low phosphorus (P) acquisition from soil and chemical P fertilizers fixing in soil on application and soon becoming unavailable. The situation is further aggravated under nutritionally poor acidic soils and drought prone conditions of this ecology. Moreover, rice in this ecology is grown by resource poor farmers practicing subsistence farming. Arbuscular mycorrhizal fungi (AMF) can alleviate the problem of poor P nutrition by extending its extra-radical mycelium beyond the phosphate depletion zone into a new pool of solution P increasing its uptake. An *in-situ* developed mass-inoculum (MI) from the indigenous mycorrhizal consortium can aid in increasing rice yields in a way that is sustainable and cost-effective. The success of MI depends upon the substrate utilized. An attempt was made to improve efficacy of MI by modifying traditional substrate combinations (soil:sand:FYM) of nucleus inoculum (NI) which is multiplied in solarised soil to produce MI. In the present study a suitable substrate combination (Vermiculite: Soil: FYM or V: So: FYM) was selected from several combinations tested on the basis of highest root biomass of trap crop (*Sorghum bicolour*). It was fortified with different Hoagland solution nutrient regimens for encouraging root development to further support AMF multiplication in NI. V: So: FYM (70:25:5;v/v/v) enriched with 10 mL Hoagland solution/50g substrate/week was found most suitable. This improved NI was mass-multiplied in micro-plots *in-situ* to produce MI following standard protocol. Under field evaluation, application of improved MI showed to result in higher root colonization (%RLC), P uptake and grain yield of upland rice variety Vandana over control. The improved MI was further evaluated under fixed plots at varying doses (0.5, 0.75, 1.0, 1.25, 1.5, 1.75 t/ha for two consecutive years) to ascertain an optimum dose suitable for this ecology. It was observed that improved MI added in any proportion between 0.5 -1.75 t/ha gave significantly higher yield (21.4% - 25%) compared to control plots where no inoculum was added along with higher %RLC and P uptake. It was thus recommended that the improved MI is effective at doses as low as 0.5-0.75 t/ha as compared to that of 1.25 t/ha of traditionally prepared MI, without any significant reduction in yield. Further, as a decision making tool under precision farming system where inoculum needs to be applied based on site and system specific precise dose requirements to avoid excess or less (than requirement) application, mathematical models were developed for differentially AM responsive rice varieties to determine AM inoculum requirement under known soil P and AMF population (native) level for targeted P uptake by rice.

EASTERN ZONE

M2

Variability study of indigenous *S. rolfsii* isolates and host – pathogen interaction in cowpea – *S. rolfsii* system

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Sclerotium rolfsii is a soil-borne plant pathogenic fungus with a wide host range and worldwide distribution. It is characterized by the morphology of sclerotia in most cases due to rare availability of its basidial stage. Study on genetic variability of *S. rolfsii* and host-pathogenic interaction is the utmost need to control this pathogen in holistic approach. Nineteen mycelial compatibility groups (MCG) were found among the 28 isolates of *S. rolfsii* from different hosts. Most of the *S. rolfsii* isolates belonged to moderate to high virulence group on cowpea with no host specificity for the isolates from a particular host. Stepwise regression analysis showed among morphological parameters growth rate, biomass production were the two most significant predictors for predicting the virulence of *S. rolfsii* isolates upto 94% and among biochemical parameters cellulase production capability, pectinase production capability were most significant predictors for assessing the variation in virulence of *S. rolfsii* isolates upto 70%. The α and β esterase profiling revealed 13 and 7 Electrophoretic Phenotypes each indicating their suitability for fingerprinting of *S. rolfsii* isolates. Two low virulent isolates showed >80% similarity whereas, DB & Ch. PEA (Highly virulent isolate) showed 96% similarity during RAPD analysis. The BOX-PCR fingerprinting clearly demarcated three MCGs. In ITS-RFLP analysis, among 4 restriction enzymes studied Sau3AI revealed maximum genetic variability among *S. rolfsii* isolates. Sau3AI restriction pattern agreed with mycelial interaction reaction among the 6 MCGs. Biochemical analyses of various disease-responsive components have thrown focus on the pivotal role of different enzymes in cowpea–*S. rolfsii* host–pathogenic interaction. The suppressed activity of peroxidase, chitinase suggested their role in the disease development.

NORTH-EASTERN ZONE

M3

Effect of bioformulation of *Metarhizium anisopliae* in management of cow pea mosaic disease

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A liquid bioformulations of *Metarhizium anisopliae* (amended with oils and adjuvants) were prepared. Six oils *viz.*, sunflower, safflower, soybean, mustard, arachnid and coconut oils at three different concentrations and three adjuvants at different concentration were tested to

see their effect on growth and development of *M. anisopliae* in both the bioformulations. Liquid formulation of *M. anisopliae* amended with Glycerol (10.0%) + Sunflower oil (0.5%) was found significantly effective which showed 84.33% and 94.93% higher surface area covered and biomass production respectively than the control. Efficacy of the bioformulations was tested against cow pea aphid, *Aphis craccivora* in pot condition. Liquid formulation of *M. anisopliae* supplemented with Glycerol (10.0%) + Sunflower oil (0.5%) was found to be significantly effective causing aphid mortality of 80.00% 30 days after spraying with protection of secondary spread of cow pea mosaic disease up to 100.00%. Spraying liquid formulation of *M. anisopliae* amended with Glycerol (10.0%) + Sunflower oil (0.5%) at 15 days interval for twice proved to be the best treatment as compared to control.

NORTH-EASTERN ZONE

M4

Genetic diversity of banana bunchy top virus from Northeast India showed existence of distinct PIO isolates in naturally growing banana mats

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Banana bunchy top virus (BBTV) is considered as a major threat to banana (*Musa* spp.). Based on nucleotide sequence identity of DNA R, BBTV isolates are categorized into two groups: the "Pacific-Indian Oceans" (PIO) and the "Southeast Asian" (SEA) group. So far, the BBTV isolates from India have been characterized as the PIO group members. Recently, we have reported the most distinct PIO isolate of BBTV (BBTV-Umiam) from local banana mats growing in mid-hills of Meghalaya, India. Further survey has been conducted in different states of Northeast India. BBTV infection has been found in commercial orchards, road side banana mats and even in tissue culture raised plant materials (var. Grand Naine). Altogether, ten BBTV isolates distributed throughout the surveyed area were characterized based on DNA R segment. The full DNA R sequences of each isolate except the isolate from Mizoram shared >97.0% similarity

with BBTV isolates reported from plains of India. However, these isolates showed relatively less similarity (~95.0%) with BBTV-Umiam. Interestingly, the Mizoram isolate (naturally grown) shared only 91.0-92.0% similarity with both PIO and SEA group members. While, during phylogenetic analysis the Mizoram isolate including other isolates from Northeast India clustered within PIO group, but the clustering pattern indicated the distinctiveness of Mizoram isolate as of previously reported BBTV-Umiam from Meghalaya. The planting materials introduced from the plains of India might be resulting in predominance of the common PIO isolates of BBTV in this region. However, the existence of distinct PIO isolates in naturally growing banana mats of Meghalaya and Mizoram further strengthened the possibility of differential evolution of BBTV in this isolated region.

SOUTHERN ZONE

M5

Bioactive compound from *Ganoderma applanatum* with inhibitory effect against the oomycete pathogen *Sclerospora graminicola*

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Basidiomycete spp. produces a large number of antimicrobial metabolites and provides a good source of plant growth regulators. In present study, we report the efficacy of a pure active compound (G_app7) isolated by column chromatography from chloroform fraction of *G. applanatum* on inhibition of *Sclerospora graminicola*, as well as seed treatment effect on disease protection. G_app7 consistently demonstrated significant inhibitory effect against *Sclerospora graminicola* by recording 41.4% inhibition of sporangium formation, 77.5% inhibition of zoospore release and 91% inhibition of zoospore motility. The 2D NMR and LC-MS analyzes of the structure of the anti-oomycete G_app7 compound revealed close resemblance to metominostrobin, a derivative of strobilurin groups of fungicides. Further study revealed that G_app7 stably exhibited strong inhibitory effects at different temperatures and anti-oomycete activity was fairly stable for a period of 12 months at 4°C. Seed treatment with G_app7 resulted in a significant increase in disease protection (63%) under greenhouse conditions in comparison with distilled water control. The isolation of this functional anti-oomycete compound from *G. applanatum* provides a considerable agrochemical potential importance for plant protection against pearl millet downy mildew disease in an environmentally safe and economical manner.

SOUTHERN ZONE

M6

Disease incidence, severity and molecular characterization of *Phomopsis vexans* causing leaf blight and fruit rot of brinjal in Karnataka (India)

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Phomopsis leaf blight and fruit rot is a major constraint for the production of brinjal in Karnataka (India). The present study reports the prevalence, incidence and severity of *P. vexans* in six major brinjal growing agro-ecological zones. *P. vexans* isolated from the diseased leaf and fruit samples representing all the six zones were studied for their morpho-cultural and molecular characteristics. All the isolates were tested for their pathogenecity on 30 days old brinjal seedlings. Analyses of ITS regions of all the isolates of *P. vexans* were studied for their phylogenetic relationships. The study indicated that incidence of leaf blight and fruit rot disease was high in Northern Transition Zone (10.6-25.3 & 21-33.3%) followed by Southern Dry Zone (8.3-18 & 22.3-62%) and Central Dry Zone (10-17 & 29-39%). Highest disease severity was recorded in Southern Dry Zone (7.7-30.5 & 21.3-61.6%) followed by Central Dry Zone (5.4-14.2 & 33-64.6%) and Southern Transition Zone (11.5-18.3 & 23.3-48%). All the isolates studied showed variations in their colony morphology and 18 were belonged to G-type and the rest six isolates neither belonged to G type nor with W type colony. Out of 24 isolates 18 isolates produced leaf blight and fruit rot symptoms after 25-28 and 45-55 days after post inoculation respectively. Phylogenetic analysis of ITS region showed that all the isolates were grouped in to a single clade rooted to *Valsaambiens* and the complete ITS2 sequence analysis of isolates showed the presence of two distinct groups based on indels at three positions.

DELHI ZONE

M7

Genome characterization, infectivity and development of immunodiagnostics for a *Badnavirus* associated with banana

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A cryptic Badnavirus species complex, known as banana streak viruses (BSV) poses a serious threat to banana production and genetic improvement worldwide. Complete genome sequences of three episomal Banana streak MY virus (BSMYV) isolates sampled from triploid

banana hybrids (Chini Champa: AAB; Malbhog: AAB and Monthan: ABB), grown in North-East and South India was achieved by sequence independent improved rolling circle amplification (RCA). RCA coupled with restriction fragment length polymorphism (RFLP) revealed diverse restriction profiles in five BSMYV isolates including above three isolates. Episomal BSMYV isolates characterized in present study shared an identity of 45-50% with other BSV species and 43-44% with sugarcane bacilliform viruses (SCBV) and clustered in major Custer 1 along with other badnaviruses infecting banana, together with sugarcane infecting badnaviruses from Guadeloupe (SCBGAV and SCBGDV). Nucleotide substitution rates of BSMYV subpopulation and BSOLV subpopulation was in the range of 10-2 to 10-3, which indicated low subpopulation variation. In addition to the existence of extensive recombination within the banana streak viruses and sugarcane bacilliform viruses (intra-BSV and intra-SCBV recombination events), a total of 32 unique recombination events within banana and sugarcane badnaviruses (inter BSV-SCBV) were detected. The patterns of distribution of recombination events, hot-spots (intergenic region and C-terminal of ORF3) as well as cold-spots (distributed in ORF3) displayed the mirroring of recombination traces in both the group of badnaviruses. The putative coat protein (CP) coding region (p37) of BSMYV was identified in silico by comparison with caulimoviruses, retroviruses and Rice tungro bacilliform virus. The p37 was in vitro expressed as recombinant protein in pMAL system and used as antigen for raising polyclonal antiserum. The antiserum specifically reacted with BSMYV virions in immunosorbent electron microscopy (ISEM) and antigen coated plate-enzyme linked immunosorbent assay (ACP-ELISA). The anti MBP-p37 antiserum (1:2000) was successfully used in ACP-ELISA for specific detection of BSMYV infection. To further simplify the methodology of antigen preparation, synthetic peptides representing antigenic epitopes were successfully used for production of polyclonal antibodies to BSMYV. Two immunodominant linear epitopes were identified at N and C-terminal of putative CP of BSMYV (pep-I and pep-II), synthesized and used for polyclonal antiserum production. Only anti pep-I antiserum strongly reacted with BSMYV virions in ISEM and ACP-ELISA (1:2000 and 1:4000) in crude sap exhibiting >3 folds differences in optical density (OD) values of infected and healthy samples. Globulin (IgG) fraction of the anti pep-I antiserum was conjugated with alkaline phosphatase (ALP) and used successfully as secondary antibodies in double antibody sandwich-ELISA (DAS-ELISA) with good serological differentiation among healthy and infected samples. Employing the immunoreagents developed in present study a sensitive duplex-immunocapture-PCR (D-IC-PCR) was standardized for the sensitive, reliable and accurate routine indexing of episomal BSV infection in tissue cultured and field banana samples. In a survey 46% of the samples collected from North, North-East, East, West and South India were indexed positive for BSV infection indicating its widespread occurrence. A partial tandem dimer containing 1.8-mer of BSMYV-IN1 was constructed in binary vector. The agroinoculated banana plants were not positive for BSMYV DNA in RCA three months post-inoculation which might be due to the homology dependent silencing because of the presence of integrant eBSV sequences in inoculated banana plants.

DELHI ZONE

M8

Histone 2B interacts with replication initiator protein of *Chilli leaf curl virus* and confers resistance in chilli

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Chilli leaf curl virus (ChiLCV), a *Begomovirus* within the family Geminiviridae has emerged as the predominant species causing leaf curl disease in India. To explore the potential resistance factors in resistant *Capsicum annuum* var. Punjab Lal, suppression subtractive hybridization was performed. Out of 480 clones screened, 231 unique ESTs were known to be involved in different cellular and physiological processes including transcription, replication, photosynthesis and defense. Interestingly, histone H2B transcript accumulation was found to be persistently higher in the resistant variety Punjab Lal as compared to the susceptible one. For the study of global level post transcriptional modification (PTM) of histones, western blotting was performed using H3K4me3 and ubH2B specific antibodies. ChiLCV induces deposition of higher monoubiquitination of H2B and trimethylation of H3K4 in *Nicotiana benthamiana*. Further, chromatin immuno-precipitation coupled with polymerase chain reaction (ChIP-PCR) assay using H3K4me3 specific antibody revealed high virus titer in *N. benthamiana* and low in Punjab Lal. These results indicated reduced level of H3K4me3 deposition in the viral promoter region in the resistant chilli variety. Low level of H3K4me3 in virus promoter region was correlated with reduce deposition of monoubiquitinated H2B in Punjab Lal. To study the role of monoubiquitination machinery HUB1 (E3 ligase) and UBC2 (E2) in viral pathogenesis, *Tobacco rattle virus* based virus induced gene silencing (VIGS) was performed to knock down NbHUB1 and NbUBC2 genes in *N. benthamiana*. VIGS assays revealed mild symptom of ChiLCV and low viral accumulation in NbHUB1 silenced plants whereas UBC2 and UBC2+HUB1 dual silenced plants remained asymptomatic coupled with drastically reduced viral titer. Furthermore, NbHUB1 and ChiLCV-Rep gets localized in the nucleus. Bi-fluorescence complementation assay and Yeast two hybrid assay confirmed interaction of NbUBC2 and NbHUB1 with Rep of ChiLCV. Interestingly, ChiLCV Rep could interact with CaH2B but not with NbH2B. Interaction of Rep with CaH2B prevent the recruitment of monoubiquitination machinery on H2B. ChiLCV Rep protein impaired PTM of CaH2B, present in higher quantities in the resistant chilli var. Punjab Lal, which in turn yielded low level of H3K4me3 modification and reduced viral gene expression. Physical interaction between Rep with CaH2B prevents PTM of the later in virus promoter region, as a result of which H3K4me3 mediated activation of virus genes does not occur. The present study, for the first time, highlights role of H2B in begomovirus pathogenesis in a permissive host, *N. benthamiana*. This study demonstrates the indispensible role of histones in regulation of viral gene expression. Our study also indicated

that H2B mediated natural resistance in chilli possibly operates by two mechanisms. Firstly, naturally abundant H2B sequesters viral Rep protein and hamper the function of Rep in viral replication. Secondly, ChiLCV Rep can mask H2B present as one of the constituents of viral minichromosome and inhibit the activity of monoubiquitination machinery which in turn reduces the efficiency of H3K4me3 deposition.

WESTERN ZONE

M9

Analysis of proteomic profile of French bean (*Phaseolus vulgaris* L.) upon infection with begomovirus

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Begomoviruses have emerged as major constraint for vegetable production. French bean is one of the most important vegetable crops of India which is consumed widely. We have cloned and sequenced begomoviral genomic components associated with bean dwarf mosaic disease (BDMD) manifested leaf samples from Varanasi, India. Sequence analyses confirmed bipartite nature of begomovirus and *Mungbean yellow mosaic India virus* (MYMIV) is causal agent of BDMD. Agro-inoculation studies on *N. benthamiana* and French bean plants confirm bipartite nature of virus. Compatible pathogen incidence on plants changes spatial and temporal expression of genome of host plants. To explore the molecular mechanisms involved in the plant-virus interaction, we conducted proteomic analyses of leaf samples agroinnoculated with MYMIV. Twenty protein spots were identified and in response to MYMIV infection on French bean by Two-dimensional gel electrophoresis (2-DE). The relative expression levels of most of identified proteins were upregulated. These proteins were mainly involved in defense (25%) signal transduction (15%), energy (15%) and metabolic regulation (20%). In our studies, we found that, most of the proteins involved directly or indirectly to defense, shoots up at higher level when both DNA A + DNA B components inoculated than DNA A components alone. This suggested an activation of SAR in DNA A + DNA B inoculated plants, which restricts virus copy number accumulation, ultimately points out important role of movement protein and nuclear shuttle protein in disease establishment. More in depth insights into host protein modulation will clear defense pathways taken up by host plants. In summary, all of the above proteins showed changed abundances induced by MYMIV infection on French bean leaves. SAR and induced resistance might be a combinatorial effect of altered interactome of these proteins.

WESTERN ZONE

M10

Molecular characterization races of *Fusarium oxysporum* f.sp. *ciceri* through RAPD and ISSR markers

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In present investigation, a total of 30 RAPD and 16 ISSR primers were screened against four races of *Fusarium oxysporum* f.sp. *ciceri*, off which 23 RAPD primers produced scorable bands with an averages of 7 bands per primer. A total of 155 amplicons were amplified with 149 polymorphic bands. The level of polymorphism was 96.12%. The primer OPA-2 amplified maximum of 13 bands while primer OPA-12 and primer OPB-14 amplified minimum of three bands. Off 16 ISSR primers screened 15 primers produced 127 amplicons with an average of 9 bands per primer. Off 127 bands, 119 bands were polymorphic and level of polymorphism was 93.70%. The Primer (GA)8C and(GA)8YC amplified maximum twelve bands while primer (CT)8RG amplified minimum four bands. In the similarity co-efficient of RAPD and ISSR banding pattern analysis showed that Race-1 had the higher value of similarity coefficient 0.6948 and 0.7769 whereas Race-3 was had lower value of similarity coefficient 0.3701 and 0.3692, respectively. The analysis depicts that Race-3 is distinct from other three races of *Fusarium oxysporum* f.sp. *ciceri*. In combined analysis of genetic diversity of the 4 races of *Fusarium oxysporum* f.sp. *ciceri* by using 23 RAPD and 15 ISSR primers shown higher level of polymorphism. The Race-1 had higher value of similarity coefficient 0.7323, whereas Race-3 was had lower value of similarity coefficient 0.3697. The UPGMA analysis of RAPD, ISSR and RAPD-ISSR combine analysis grouped Race-1, Race-2, under cluster-A and Race-4 in cluster-B, whereas Race-3 showed distinct out-group type reaction with low similarity index and distinct from other races of *Fusarium oxysporum* f.sp. *ciceri*. The RAPD primers OPA-13, OPA-16, OPA-18, OPB-14 and OPB-15 showed monomorphic banding pattern and ISSR primers (AC)8YT, (AG)8G, (GA)8YT, (TG)8RT, (GA)8T, (ATG)6, (GA)9RY showed monomorphic banding pattern and ISSR primer (TG)8RT showed two monomorphic banding pattern with all the races, with the distinct band for Race-3, could be developed into SCAR (Sequence Characterized Amplified Regions) for identification of *Fusarium oxysporum* f.sp. *ciceri*.

Session I
PATHOGEN CHARACTERIZATION, DIAGNOSIS AND EPIDEMIOLOGY

LEAD LECTURES

L1

Morpho-physiological, pathogenic and molecular variability among, isolates of *Fusarium moniliforme* and *Fusarium oxysporum* from oil seeds

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The genus *Fusarium* (Link.) with its different species are known to attack variety of seeds both in field as well as storage and the results found to be responsible for major cause of seed deterioration and poisoning of the oil seeds under given set of environmental condition. The health of the oil seeds is the basic objectives of this investigation. It is certainly reveals that maximum number of *Fusarium* species have been recorded from oil seeds, However importance of seed- borne species of *Fusarium* and their different aspects like enzymatic nature and discoloration role in seed deterioration is very significant.In recent years many molecular techniques have been used to identify and differentiate fungi. Inter-simple sequence repeat ISSR markers were used to investigate genetic diversity of *Fusarium* species. RAPD technique to identify a species-specific amplification product also studied Intra and inter-specific polymorphisms among isolates of the *Fusarium* species which cause pathogenic effect on the oil seeds investigated by using 06 ISSR primers. Regarding the molecular properties of *Fusarium* species and their isolates were screened and determination of polymorphic and monomorphic bands and also determines maximum similarities and differentiation, among the isolates of *Fusarium* species by using phylogeny analysis.The impact of *Fusarium* species on the production of different toxins were remarkable when they were associated with the oil seeds (food grains) and their toxicity response, which may result into mycotoxicosis when consumed by animals and human beings. The toxins or fusotoxins like Moniliformin, Zeralenone, T-toxin, Fusaric acid, Fumonisins, Trichothecene, Fusarin-C, Fumagillin, Deoxynivalenol (DON), Nivalenol (NIV) and Diacetoxyscirpenol (DAS) were produced by the species of *Fusarium* among these the Deoxynivalenol (DON), Nivalenol (NIV) and Diacetoxyscirpenol (DAS) were detected. The attention is given for the alternative management of such virulent *Fusarium* species by adopting the fungitoxic extracts of different part of Botanicals with their different concentration and different solvent systems were beneficial to growth inhibition of *Fusarium* species.

L2

Barcoding of seed-borne fungal pathogens

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Early identification of seed associated pathogens is of utmost importance to prevent the invasive pathogens and in taking decisions on management strategy. Many morphologically similar species are genetically distinct. Further, most taxa in culture do not produce all spore states of asexual or the sexual morph. At global level International Barcode of Life (iBOL) project is the largest biodiversity genomics initiative that created digital identification system for life. Internal transcribed spacer (ITS) covering ITS1-5.8S-ITS2 region of DNA has been recognized as the official fungal barcode. Though, for oomycetes, COI sequence is more discriminative than ITS. Likewise, TEF-1α and β-tubulin are considered as barcodes for identification of Fusarium and Penicillium species, respectively. Best non-ITS barcoding genes to differentiate Colletotrichum gloeosporioides sensu lacto species complex are Glyceraldehyde-3-phosphate dehydrogenase (GPD), Glutamine synthetase (GS) and β-tubulin gene sequences. Curvularia species could be discriminated by ITS, GPD or ACT (Actin-like) barcodes complemented by calmodulin (CAL) or TEF-1α. Phoma is a highly polyphyletic genus with unclear species boundaries. Based on molecular phylogeny of the type species of the seven sections of Phoma, a new teleomorph family Didymellaceae has been established. In the case of Diaporthe species, the resolving power of Histone H3 (HIS), beta-tubulin (TUB) was better than ITS. Barcoding could be used even by a non-taxonomist to identify fungal pathogens; it helps in tracing the origin of pathogens and in identifying cryptic species. Barcoding has also helped in discrimination of endophytic, pathogenic and saprophytic fungi. It has become imperative to validate the fungal nomenclature with DNA barcode, though such a provision is not mandatory in the existing International Code of Nomenclature (ICN). A concerted network effort is required to develop barcode sequences and validate the identity of all the phytopathogens in India. This effort has several implications linked to quality assurance, trade and quarantine.

L3

Molecular characterization of mating type idiomorphs of *Erysiphe necator* from India

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Erysiphe necator, earlier known as *Uncinula necator*, causes powdery mildew disease in

grapevines. In many countries, both the asexual and the sexual stages of *E. necator* are reported. *E. necator* is a heterothallic fungus requiring the two mating typeidiomorphs, known as MAT1-1 and MAT1-2, for sexual reproduction to occur.The chasmothecia (cleistothecia) are initiated when the colonies of two opposite mating types meet on the infected plant parts and develop further if surface temperatures remains in favorable range for sufficient period of time. The teleomorph stage of *E. necator* is reported from Kashmir in north India, but not from peninsular India, even though powdery mildew is present since almost 100 years.Molecular analysis of one hundred and twenty *E. necator* field isolates collected from peninsular India,showed presence of a single band at 232 bp corresponding to MAT1-2. None of the samples gave band at 408 bp corresponding to MAT1-1. However, two bands of 232 bp and 408 bp were detected by this multiplex PCR method, in few samples collected from Srinagar, Kashmir. Thus, molecular analysis established that *E. necator* is not sexually reproducing in peninsular India due to presence of only one mating type idiomorph and explains the non-sighting of chasmothecia in vineyards in these regions.The study also brings out that MAT1-1 is not as common in nature as MAT1-2 and explains why in some other countries, too, chasmothecia were first observed as late as half to one century after start of grape cultivation.

L4

Morphological and molecular characterization of major fungal pathogens associated with diseases of cowpea in Karnataka State

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Cowpea is an important legume crop grown in arid and semi-arid regions of tropics. India is the largest producer of Cowpea among Asian countries. Field survey conducted during 2011-13 in Cowpea growing areas of Karnataka State revealed the occurrence of severe fungal diseases responsible for crop yield losses. In the present study, prevalence and incidence of fungal diseases in six major agro-ecological regions of Karnataka are presented. Diseased samples were collected and identity of the associated fungal pathogens was confirmed by microscopic, morphological and cultural characteristics. Morpho-cultural identification was supported by molecular identification through ITS region sequence analysis. Further, six major fungal pathogens were tested for their pathogenecity on healthy cowpea plants (cv.152) under green house conditions. A total of 14 fungal diseases were recorded and four among them were found severe in all the six zones. The major diseases include rust (*Uromyces vignae/U. appendiculatus*), Anthracnose (*Colletotrichum lindemuthianum*), leaf spot (*Alternaria tenuissima*)and leaf spot

(*P. vexans*). The results showed that theincidence of each disease varied from one agro-ecological region to another. Rust and anthracnose were the most prevalent among fungal diseases in all the study regions. Phomopsis leaf spot (*P. vexans* f.sp. *vignae*); Dactuliophora leaf spot (*Dactuliophora* sp.) and southern blight (*Sclerotium* sp.) were new and emerging diseases on cowpea in Southern Dry Zone and Southern Transition Zone respectively. The pathogenecity test revealed the development of symptoms after 15-18 days of post inoculations. PCR amplification and sequence analysis of ITS rDNA regions confirmed the six fungal pathogens identity as *Phomopsis vexans* (99%), *Alternaria tenuissima* (99%), *Colletotrichum lindemuthianum* (99%), *Sclerotium rolfsii* (99%) and *Dactuliophora species* (100%) similarity with *Macrophomina phaseolina*.

L5

Bacterial wilt of solanaceous vegetables: Pathogen diversity and management options

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Bacterial wilt (BW) affects economically important solanaceous vegetables (eggplant, chilli, and tomato), potato and ginger. BW is caused by *Ralstonia solanacearum*, a soil bacterium which has broad host range and is distributed widely ranging from tropical, subtropical and warm temperate regions of the world. In India, BW is a major threat towards the production of solanaceous vegetables, potato and ginger. Though the pathogen is widely reported from varying agro-climatic and geographical regions of the country, genetic diversity among the Indian isolates is scarcely documented and is largely unknown. High genetic variation between isolates was used to define *R. solanacearum* as a 'species complex,' rather than a species. Early attempts to study the diversity present in the *R. solanacearum* species complex resulted in separate race and biovar systems. Later, four monophyletic clusters called phylotypes have been distinguished based on gene sequences and is in correlation to their geographical location. Our investigations using *R. solanacearum* collected during 2008 to 2011 from different states of India indicated that all our *R. solanacearum* isolates are phylotpe I and are most distinct from the other phylotypes. Within phylotype I, the isolates are grouped into two clusters. Sub group one consists majority of the isolates and the sequevars are unknown. In Sub group two, all the isolates were assigned sequevar numbers. This indicated the existence of *R. solanacearum* isolates with unknown/ unreported sequevars within phylotype I. MLST analysis of 20 isolates resulted in 15 haplotypes within Indian strains. All of which are new, different from the reference haplotypes. As Indian strains look clearly original, two strains were sequenced to understand and to study

various pathogenicity and virulence factors. Understanding of the genetic diversity of the pathogen is essential to develop strategies for the management of BW. Management of BW has been challenging because of the existence of vast genetic diversity of *R. solanacearum*. One of the promising management strategies is developing resistant varieties and our studies identified resistant donors and are used in developing segregating population. We identified a resistant, non-cultivated brinjal and the grafts were evaluated for BW management. Promising endophytic and rhizobacteria were identified for the suppression of BW in brinjal. The antagonistic bacteria reduced the incidence of BW through different mechanisms.

POSTERS

P1

Isolation, identification and characterization of chitinase producing *Trichoderma flavofuscum*

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At present, around 30% of all plant species have been destroyed by plant pathogens. Fungicides and other organic compounds are widely used to control plant disease in many countries. However, continues use of these chemicals has adverse effect on ecosystem and also leads to development of new strain of pathogens. *Trichoderma* sp have inhibitory action against pathogenic fungus like *Pythium, Fusarium* etc. either by secretion of certain enzymes like chitinase, or by enhancing the growth of crops by producing secondary metabolites. Chitinases are a group of enzymes that is responsible of hydrolyzing chitin polymer into its oligomers or monomers. we all knows that chitinase produce by *Trichoderma* has antifungal activity, Hence current study was carried out to confirm the fact that *Trichoderma* has caliber to produce chitinase enzymes. These enzymes are capable of degrading 4-N-acetyl- D glucosamine, the main cell wall component chitinase hydrolyzes chitin, a biopolymer of N-acetylglucosamine which is being widely used in biological and agricultural research. Optimization of chitinase production by *Trichoderma flavofuscum* was conducted in this experiment. *Trichoderma* was isolated from soil sample and identified on basis of morphological character studied under microscope and confirmation was done. The identified pure culture of *T. flavofuscum* was inoculated on Chitin rich CZ Broth and incubated for 10 days and filtrate was studied for extracellular chitinase activity by DNS method. During this experiment, peak chitinase activity was observed at 90 min after reaction time..

P2

Studies on characterization and pathogenicity of *Fusarium* sp. causing wilt of pomegranate

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Five different isolates (VKB- I to V) of *Fusarium* were isolated from pomegranate (*Punica granatum* L.) plants of Arakta and Ganesh varieties showing typical wilting symptoms. The fungal cultures were examined under microscope and found that mycelium was hyaline septate

and produce two types of spores i.e microconidia and macroconidia. The pathogen was found to produce pink colored pigmentation. Based on morphological characteristics the isolates were identified. The isolate VKB - III was identified as *Fusarium oxysporum*, which showed mycelium of whitish submerged growth, circular compact mycelium with oval straight to curved microconidia and thin hooked apex, sickle shaped, three to five septet macroconidia. The isolate VKB-IV was identified as *Fusarium moniliforme* showing compact mycelium of whitish pink colour, raised colonies with circular margin of pink colour forming simple microconidia in chain and fusoid, sharply curved, elongated macroconidia with 3 to 7 septa.The isolate VKB-V was identified as *Fusarium roseum* showing compact raised mycelium of whitish growth with pinkish center, forming abundant microconidia which are oval straight to curved and straight macroconidia with 3 to 5 septa. Whereas VKB-I and VKB –II were identified as *Fusarium* species on the basis of macro and microconidia. The pathogenicity test of five *Fusarium* isolates was carried out by artificial soil inoculation in pot culture. The artificially inoculated pomegranate air layers showed typical wilting symptoms after one month. The *Fusarium* sp. -I and Fusarium sp. -II showed partial wilting where as *Fusarium oxysporum* and *Fusarium moniliforme* showed drooping of branches after one month of inoculation. After two months of artificial inoculation *Fusarium oxysporum*, *Fusarium moniliforme* and *Fusarium* sp. -I showed complete wilting. The mixture of all the five isolates of *Fusarium* sp. showed complete wilting of pomegranate air layers of cultivar Ganesh.The investigation confirms the finding that there is wide variation in morphological characters and pathogenicity of *Fusarium* isolates associated with the wilt of pomegranate.

P3

Detection and quantification of sugarcane mosaic virus (ScMV) in diseased sugarcane using ELISA and RT-PCR technique

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Sugarcane mosaic virus (ScMV) causes yield losses cultivated sugarcane. Therefore, the detection of ScMV is very important for disease management. In present study, DAS-ELISA (Double Antibody Sandwich- Enzyme Linked Immunosorbant Assay) was optimized as a serological method to identify the virus using a monoclonal antibody. Leaf samples of sugarcane were collected from healthy and diseased plants. A total 80 cultivars of sugarcane were selected on the basis of morphological symptoms for ELISA test. Out of 80 cultivars, 57 represented the presence of ScMV infection through ELISA. RT-PCR (Reverse Transcriptase-Polymerase

Chain Reaction) was performed for detecting ScMV using a pair of primers design to amplify a fragment in the coding region of ScMV coat protein. Nine Cultivars showing presence of ScMV in ELISA were selected for molecular screening. Out of Nine cultivars four cultivars showed the presence of ScMV through one-step RT-PCR. The one step RT-PCR result depicted that four cultivars of sugarcane showed the presence of ScMV infection. This suggested that ELISA and RT-PCR can be routinely used for ScMV detection with high efficiency.

P4

Investigation on mosaic viruses infecting brinjal in Western Maharashtra

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Brinjal (*Solanum melongena*) is one of the most popular solanaceous vegetable crops in India and yields good profit in short period several viruses have been reported to infected brinjal naturally throughout the world causing enormous losses in respect of yield and quality of fruits. The virus infecting brinjal was readily sap transmissible by mechanical means from brinjal to brinjal and other plant species. The virus was not transmitted through the seeds of brinjal. The symptoms of mosaic disease were observed periodically in sap inoculated plant brinjal and cucumber. The virus was found to be transmissible by aphid species *viz., Myzus persicae, Aphis gossypii* and *A. craccivora* in non-persistant manner. *Myzus persicae* was found to be more efficient (90 %) than *Aphis gossyppi* (60 %) and *Aphis craccivora* (25%) in transmitting the virus. The virus infected 13 out of 25 plant species belonging to Chenopodiaceae, Cucurbitaceae,Leguminosae and Solanaceaefamilies. The host of virus included *Chenopodium amarnticolor, Cucumis melo, Cucumis sativus, Luffa acutangula, Glycin max, Capsicum annuum, Lycopersicon esculentum, Nicotiana glutinosa* and *Solanum melongena*. The non host of the virus included *Beta vulgaris, Helinanthus annus, Zea mays, A. hypogea, Cajanus cajana, Caccia tora, Cyumopsis tetragonoloba, Phaseouls vulgaris* and *Pisum sativum*. In DAC-ELISA (Direct antigen coating enzyme linked Immunsorbent assay) technique, the results revealed that virus isolates reacted positively to antiserum Cucumber Mosaic Virus. The result indicated that maximum value Pune 2.116, Satara 2-103, Nashik -2.204 and Ahmednagar -1.520 was shown by isolate. Brinjal virus isolates was positively related to antiserum CMV, which clearly indicated that the virus belonged to Cucumo virus group causing Cucumber Mosaic Virus (CMV).

P5

Studies on morphological variability and growth characteristics of different isolates of *Sclerotium rolfsii* on different media

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Sclerotium rolfsii is a most devastating soil borne plant pathogenic fungus, causes collar rot and root rot disease and occasionally causes leaf spot diseases. The stem rot affected groundnut samples were collected and reisolated, isolates were identified as *Sclerotium rolfsii*. The morphological characteristics of *S. rolfsii* on different media revealed that, there was significant variation in growth of all isolates on different media. Among 14 media tested, maximum colony growth was observed on oat meal extract agar, potato dextrose agar and sabouraud's agar followed by carrot agar. The least growth was observed in Elliot's agar and Rose Bengal agar. The colony colour of isolates were varies to dim, pure, white, dull, fluffy, cottony and fluffy cottony. The appearance of mycelial growth of isolates was also varies to irregular, clear, thick, tuft, full petriplate, thin, bread like, patchy and rare growth of mycelium. The radial growth and concentric circles were absent in all isolates. The shapes of sclerotial bodies were varies to round, ellipsoid, medium round, elongate, spherical, irregular and globose among isolates. The colour of sclerotial bodies were also recorded variation *viz.*, brown, light brown, chocolate brown, dark brown, yellowish brown, medium red and reddish brown. Among the isolates, the size of sclerotial bodies ranged from 0.15-1.96 mm and the numbers of sclerotial bodies per cm^2 varied from 6.07 to 1.51 cm^2. While, the test weight of sclerotia was varies from 261.33 to 53 mg. All these growth characters revealed that, there is existence of variability among the isolates of *S. rolfsii* on groundnut.

P6

Identification of *Cercospora* leaf spot resistance among fenugreek accessions and characterization of pathogen

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Fenugreek (*Trigonella foenum-graecum* L.) is a minor legume field crop, native of southeastern Europe and Africa. It is commercially cultivated in India. Fenugreeks have medicinal

properties used as spice and vegetable for humen consumption and forage for cattle. Diseases caused by *Cercospora traversiana* are one of the major constraints to the production of fenugreek in field condition and it is destructive and wide spread disease in warm humid climate. To identify the resistant germplasm and morphological characters of the pathogen under field condition on the fenugreek plants, the 20 promising accessions collected from different part of Uttar Pradesh and maintained at department of Vegetable science, NDUA & T, Kumarganj, Faizabad, UP, India were selected for the investigation during Rabi 20112-13 and 2013-14 where disease occurring year to year. The field trials were conducted in RBD using plot size 2.4 × 2.0 m. Cercospora leaf spot disease incidences were recorded from 18 randomly selected plants of each plots by using 0-5 scale at the prematurity of pods. Out of 20 accessions two accessions were characterized resistant, four moderate resistant ant rest were found susceptible in both experimental year. Percent disease incidence was observed lowest in 7.63 (NDM-96), 8.10 (NDM-93), 11.4 (NDM-95), 12.2 (NDM-99) and highest 63.63 (NDM-86), 62.3 (NDM-88), 51.7 (NDM-90) in 2012-13, whereas in 2013-14, PDI was minimum in NDM-93 (7.86), NDM-96 (8.43), NDM-95 (12.0) and maximum in NDM-87 (64.0), NDM-86 (43.33), NDM-84 (43.22). The field resistant accessions NDM-93 and NDM-96 were produced highest seed and biomass yield. Although, artificial inoculation studies are needed to verify the finding of resistant accessions.

P7

Molecular and biochemical characterization of potential isolates of *Trichoderma* species effective against soil-borne pathogens

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Trichoderma species can act as biocontrol agents through different bio-control mechanisms. Characterization and exact identification of strains is the first step in utilizing the potential isolates against plant pathogens. A total of 20 morphologically characterized high potential isolates (8 from *T. virens* and 12 from *T. harzianum*) and 4 low potential isolates (2 from *T. virens* and 2 from *T. harzianum*) were molecularly (based on ITS region and tef-1gene region) and biochemically characterised (based on hydrolytic enzymes). tef-1was found better thanITS to distinguish the *Trichoderma* isolates into 2 different species *viz.,T. virens* and *T. harzianum* based on maximum parsimony sequence analysis and were molecularly confirmed. The scan over the entire results of specific activity of the enzymes revealed that there was a clear cut difference between the two species of *Trichoderma viz.,T.harzianum* and *T. virens* against the pathogens tested (*Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii*). It appeared that the *Trichoderma* isolates alone showed less production of enzymes as compared to during

their interaction with soil-borne pathogens. Most of the isolates were found with moderate (10-20 IU/mg) or low (0-10 IU/mg) specific activity and very few isolates showed high (>20IU/mg) specific activity of enzymes in both with or without pathogen interactions. Among the potential isolates tested for enzyme assay, 3 isolates from *T. virens* (V-7, V-19 and V-21) and 3 isolates from *T. harzianum* (H-10, H-12 and H-21) were found as highly potential isolates based on the specific activity of the enzymes. Therefore, these morphologically and molecularly confirmed isolates having high specific activity of hydrolytic enzymes can be effectively used for biological control against soil-borne plant diseases.

P8

Association of seed borne pathogens with groundnut seeds and screening of *Aspergillus* flavus isolates for aflatoxin production

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Groundnut (*Arachis hypogea* Link) is an important oil seed crop and world's second largest source of edible oil. Groundnut contains 46-52% of oil. The high oil and protein content makes it an important food ingredient. It is also a valuable source of vitamin E, K and B. Seed borne diseases are commonly observed in cereals and pulses and the reduction in the yield of peanut is dependent upon several factors, among which the seed borne diseases have major share. The study was aimed to see the association of seed borne pathogens with groundnut seeds and screening of *Aspergillus flavus* isolates for aflatoxin production. Seven varieties of groundnut *viz.*, SB IX, JL-24, TAG-24, TG-26, JL-220, ICGS-11 and JL-501 were collected from Groundnut Breeder, AICRP on Groundnut, MPKV, Rahuri. The association of mycoflora was studied by standard blotter method as recommended by ISTA and screening of *A. flavus* was done with the help of simple fluorescence method. The fungi associated with these groundnut varieties were *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Phoma* spp. *Aspergillus fumigatus, Alternaria alternata* and *Macrophomina phaseolina*, The variety TG-26 showed the highest association of seed mycoflora (40%) while variety JL-501 showed the least mycoflora i.e. 25%. The variety TAG-24 showed the highest number of *A. flavus* isolates i.e. 22 of which 18 were found positive for aflatoxin production while the variety ICGS-11 showed the lowest number of *A. flavus* isolates i.e. 12 of which 3 were found positive for aflatoxin production.

P9

Variability and cross-infectivity potential of *Colletotrichum gloeosporioides causing* anthracnose and fruit rot of Annona in Maharashtra

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Fruit rot/anthracnose caused by *Colletotrichum gloeosporioides* has become an increasingly serious disease on green, unripe custard apple fruit in Maharashtra. Strains of *Colletotrichum* were isolated from the fruits of different *Annona* spp. collected from orchards in and around Pune district in Maharashtra. Isolates were identified using morphological characters, colony growth rate, and confirmed with microscopy. Five isolates from five *Annona* spp. *viz.*, *A. squamosa, A. atemoya, A. cherimola, A. glabra* and *A. reticulate* were designated as CgAs, CgAa, CgAc, CgAg and AgAr, respectively. Pathogenicity testing of these strains was carried out on their respective host. The potential for cross infectivity of five isolates of *C. gloeosporioides* was investigated on five *Annona* spp. by attached leaf culture and detached fruit techniques using mycelial bit inoculation method. Anthracnose lesions formed readily on wound-inoculated leaves and detached fruits of all hosts. Five isolates of *C. gloeosporioides* from *Annona* spp. differed from each other on the basis of leaf spot size, leaf area infected, disease severity and virulence index (VI) on leaves as well as fruits of five *Annona* spp. On the basis of virulence index, five isolates were grouped into two virulence categories by attached leaf culture. The isolates CgAs, CgAc, CgAg and AgAr were virulent, whereas, CgAa was moderately virulent. On the contrary, by detached fruit technique the trend was quite different wherein the isolate CgAs was highly virulent; CgAa, CgAg and CgAr were virulent while CgAc as moderately virulent. This revealed that the isolates showed variable reactions on leaves and fruits across five *Annona* spp. Similarly, the isolates on their respective host spp. showed maximum virulence index and produced all typical symptoms of disease as compared to other *Annona* spp. indicating that five *C. gloeosporioides* isolates from five *Annona* spp. were different.

P10

Recurrence of crazy top or green ear of finger millet

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Survey during 2014 rainy season 'crazy top' or 'green ear' of ragi was rampant at two villages Pemmanahalli and Galaga of Gubbi taluk in Tumkur district, Karnataka the disease showing up to 50% incidence both on the popular and blast resistant GPU 28 and susceptible PR 202 varieties. After its first report in 1947 by Venkatarayan from the erstwhile state of Mysore as well as from Tamil Nadu; and detailed studies by Safeeulla at the Mysore University, the disease was almost forgotten. However during kharif 2006 it was reported from Uttarakhand hills. Though the characteristic 'downy mildew' symptoms were absent the affected plants were stunted with short internodes and profuse tillering assuming a bunchy and bushy appearance. The green ear manifestation at the time of grain formation completely converted the ears into green narrow leafy structures causing complete sterility. Partial or whole ear including lemma, palea and glumes were converted into narrow leafy structures giving a 'bush-like' appearance. Enquiries revealed that the seeds of GPU 28 were certified and purchased from Raitha Samparka Kendra (RSK), whereas those of PR 202 were of farmer's own. The fields on which ragi was grown had paddy crop during three to four preceding seasons. However, heavy rains during the crop growth, water stagnation, prevalence of high humidity due to surrounding plantations; poor drainage etc. might have been responsible for such an outbreak. Laboratory studies of the affected samples revealed the presence of numerous thick walled oospores in the malformed portion of ears.

P11

Survey for Cercospora leaf spot disease of finger millet in the hills of Uttarakhand

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Finger millet [*Eleusine coracana* (L) Gaertn.] crop is very adaptable to higher altitudes and is grown in the Himalayas up to 2,300 m. It is widely cultivated by the farmers of Uttara-

khand over an area of 1.25 lakh ha both in the Garhwal as well as Kumaon regions. Cercospora leaf spot caused by *Cercospora eleusinis* is second only to blast in affecting production and productivity of finger millet in Uttarakhand. Hill campus, Ranichauri has reported that the resistance to the disease is not available in the improved lines. A rowing survey carried out during the rainy season of 2013 to know the occurrence and severity of the disease *inter-alia* to look for resistance sources in the local cultivars revealed that, the disease incidence ranged from 2-5 grade and was altitude dependent. No cercospora leaf spot was observed at lower altitudes (< 1000 m). Some local material (PRM 1012-1) appeared free from cercospora infection. The incidence of cercospora was much lower in Kumaon compared to Garhwal region. The incidence of cercospora leaf spot and brown spot was lower in the fields situated on the sunny side, but high in shady and moist areas. The symptoms of brown spot and cercospora leaf spot were initially alike as the ashy grey centre characteristic to cercospora was missing (e.g. Tal, Gwaldam, Chamoli) and needs to be thoroughly checked for combined infection. Cross sections through the infected lesions revealed presence of slender, elongated whip like conidia with many septa. Isolation of the pathogen in V8 juice agar revealed creamy white colonies.

P12

Longevitiy of *Fusarium moniliforme* in different parts of rice grains

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The survival of *Fusarium moniliform* incitant of foot rot and bakanae of rice (*Oryza sativa* L.) was studied in different part of grains of two commenrically cultivated Basmati rice varieties at CCS HAU Rice Research Station, Kaul during December, 2013 to August, 2014. The recovery of the fungus was more from the infected grains of Basmati CSR 30 (96.25%) than Pusa Basmati 1121 (90.00%) at the time of commencement of the experiment. Among different seed components, the maximum infection/recovery of *F. moniliforme* was recorded in lemma (91.25%) followed by palea (78.75%), endosperm (58.75%) and embryo (35.00%) in Basmati CSR 30 during December, 2013. It declined gradually with storage period in all the seed components and reached to 37.50%, 31.25%, 16.25%, 3.75% and 0.00% in whole grain, lemma, palea, endosperm and embryo, respectively in the month of August, 2014. On mean basis, the survival of pathogen varied significantly in different seed components. A similar trend in recovery of pathogen from the whole grain and their components was observed in Pusa Basmati 1121. The fungus survived in the embryo to the extent of 35.00% and 21.25% in Basmati CSR 30 and Pusa Basmati 1121, respectively in December, 2013, which reduced to 2.50% and 1.25% in month of July and to 0.00% in August, 2014 in both cultivars. These studies have clearly elucidated that the pathogen is both internally as well as externally seed borne.

P13

Identification of molecular markers for arbuscular mycorrhizal responsiveness in upland rice

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Mycorrhizal responsiveness is the change in plant biomass or phosphorus (P) uptake that results from arbuscular mycorrhizae (AM) colonization. Inter- and intra-specific variation in rice suggests it to be a genetic trait opening up the possibility of utilizing this genetic variability to select/breed high AM-responsive rice varieties for exploitation of the biological potentials of AM fungi in improving P nutrition of crops. For this, attempts were made to identify molecular markers linked to the AM-responsiveness trait in rice, a pre-requisite for developing AM-responsive varieties through marker-assisted selection (MAS). Two differentially responsive varieties were selected- Sathi 34-36 (extremely AM-responsive and Jonga (highly AM-non-responsive) and crossed. F1 hybrids were raised to obtain the F2 seeds. Parental polymorphism survey with 370 SSR markers revealed that 80 were polymorphic between the two genotypes. F2 plants were further phenotyped for AM-responsiveness. Bulk Segregant analysis was performed with two DNA bulks each consisting of pooled DNA from extremely opposing (AM-responsive and non-responsive) F2 phenotypes. RM 437 was found to be polymorphic between the two bulks on screening with the 80 polymorphic SSR markers and identified to be putatively linked to the AM-responsiveness trait.

P14

Molecular characterization of Zucchini yellow mosaic virus isolates infecting cucurbitaceous plants in Manipur region of North-East Hill region of India

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Cucurbitaceous vegetables (bottle gourd, cucumber, pumpkin and chow-chow) grown in the different districts of Manipur were surveyed during the year 2014. Symptoms of viral disease ranging from mosaic, blistering, veinal chlorosis, blistering, leaf deformation and shoe-string of leaves were observed under field conditions. A disease incidence of 38-48% was recorded on different cucurbitaceous vegetables surveyed. Flexuous virus particles of size 750-800 x 12 nm were recorded under electron microscope from the symptomatic bottle gourd, cucumber and pumpkin plants. Overall 42% of samples were tested positive in double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) using poty-group specific antisera, which confirmed the association of a Potyvirus. 1.7 kb region of viral genome (partial NIb, full coat protein (CP) and 3' un-translated region) of four isolates were amplified in reverse transcription-PCR (RT-PCR), cloned and sequenced. Basic local alignment search tool (BLAST) showed that sequenced isolates shared maximum identity with Zucchini yellow mosaic virus (ZYMV) and referred as ZYMV-Btg (isolated from bottle gourd), ZYMV-Cmb (isolated from cucumber) and ZYMV-Pmp (isolated from pumpkin). Isolates ZYMV-Btg, ZYMV-Cmb and ZYMV-Pmp shared maximum identity of 95-97% with ZYMV isolate from Southeastern Spain and Florida (97-99% query coverage). Phylogenetically all three isolates were in ZYMV cluster. Based on the combined results of symptomatology, electron microscopy, DAS-ELISA and RT-PCR, present study reports high incidence of ZYMV on cucurbitaceous plants grown in Manipur.

P15

Studies on variability of finger millet blast pathogen *Pyricularia grisea* (Cke.) sacc. in different cultivars

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Finger millet (*Eleusine coracana* (L.) Gaertn.) locally known as Ragi, Chodi, Tydalu, Mandua, Nagli, Kapai and Marwa occupies a special position in the hill agriculture of Andhra Pradesh occupying the largest area next only to Rice. Although, finger millet is known to cope with abiotic and biotic stresses, however, under vulnerable conditions some of the diseases cause enormous losses and can damage entire crop. Of the several fungal diseases that affect finger millet crop, blast incited by *Pyricularia grisea* (Cke.) sacc. is the most devastating and economically important disease of finger millet growing areas of Andhra Pradesh. The disease occurs regularly and causes heavy losses in grain yield. Due to extreme variations in the pathogen in different cultivars of the finger millet an experiment was conducted at Agricultural Research Station, Vizianagaram, Andhra Pradesh with recommended agronomic practices on the variability of the blast (leaf, neck and finger blast) in different entries of finger millet nursery. Total 12 entries were tested for blast variability. Among them GE 4449 (Leaf blast (Grade)-2, neck blast (%) 9.1, finger blast (%) 9.1) and GE 4440 (Leaf blast (Grade)-2, neck blast (%) 9.3, finger blast (%) 8.9) showed less incidence of blast compared to local check VR-708 (Leaf blast (Grade)-9, neck blast (%) 87.2, finger blast (%) 85.1). For assessing leaf blast incidence, 0-9 scale is used, neck blast percent was calculated based on the number of ears showing infection on peduncle/neck divided by total number of ears in a unit area multiplied with hundred whereas finer blast percent was calculated based on the number of infected fingers divided by number of healthy fingers multiplied with hundred.

P16

Variability among the isolates of *Alternaria solani* causing early blight of tomato

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Early blight caused by *Alternaria solani* is one of the major biotic constraints in the prof-

itable production of tomato (*Lycopersicon esculentum* L.). A high degree of diversity in respect of symptoms induced, cultural and morphological characteristics and molecular variability among the isolates of *A. solani* causing early blight in tomato/potato were reported elsewhere in India. However, from the states of Maharashtra very meagre work has been done in this respect. Therefore, present studies were undertaken at the Department of Plant Pathology, V.N.M.K.V., Parbhani during Kharif, 2011-12 and 2012-13. A total of eight isolates of *A. solani* were obtained from the early blight symptomatic disease samples collected during survey of various agro-climatic zones of Marathwada region of the state of Maharashtra. All the test isolates were assessed for their cultural, morphological and molecular variability by applying standard procedures.The results revealed that, on the basis of cultural characteristics all the 8 test isolates of *A. solani* were found distinct from each other. Further, these test isolates were also found to be varied in respect of their morphological characteristics *viz.*, mycelial width, conidial size, beak length and septation of conidia. Based on pathogenicity test, cultural and morphological variability, only four most virulent isolates *viz.*, AsLt (Latur), AsBd (Beed), AsJl (Jalna) and AsHl (Hingoli) of *A. solani* were further subjected to ascertain their molecular variability. The results on DNA finger printing pattern revealed that the isolates of AsHl (Hingoli) and AsJl (Jalna) were distinct from each other; whereas, that of AsBd (Beed) and AsLt (Latur) were closely related to each other. The dendrogram analysis revealed genetic similarity of about 85% in between the isolates of Beed and Latur; whereas, it was about 50% in between the isolates of Jalna and Hingoli.

P17

Variability among the isolates of *Xanthomonas axonopodis* pv. *citri* by using RAPD marker

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Citrus canker is one of the most important disease of acid lime caused by *Xanthomonas axonopodis* pv. *citri*. The disease is characterized by conspicuous raised corky necrotic lesions, surrounded by diagnostic yellowish halo on leaves. The isolations were made from the symptomatic samples collected from different districts of Maharashtra *viz.*, Akola (Xac1), Warora (Dist. Chandrapur, Xac2), Nagpur (Xac3), Parbhani (Xac4), Sangli (Xac5), Rahuri (A'nagar, Xac6), Pune (Xac7). The present investigation describes variability among the isolates of Xac using RAPD primer. The primers OPA, OPB, OPF, ERIC1R, ERIC2, REP, BOX, 211, 220, 230 and 232 were found to be most significant and polymorphic. Percent polymorphism was

98.10% and average number of polymorphic bands per primer is 8. The dendrogram analysis based on RAPD data revealed 2 major groups among 7 isolates of this pathogen. Xac 3 (Nagpur) had higher value of similarity coefficient (0.7643) wheras Xac 2 (Warora) had lower value of similarity coefficient (0.5350).

P18

Occurrence of diplodia ear rot of maize in Karnataka

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Maize is an important cereal crop and widely grown in various climatic conditions across the country. One of the vital factors responsible for very low yield is due to ubiquitous incidence of various diseases. Climate change influenced the shift in the pattern of disease spectrum. Since Karnataka state has a considerably large area under commercial cultivation and seed production programme of both public and private sector companies, disease occurrence, incidence and resultant losses assumes greater significance. Few commercially grown maize hybrids were found severely affected with Diplodia ear rot disease during kharif seasons of 2013 and 2014 in different part of Karnataka *viz.*, Kalghatagi (Dharwad district), Haveri and Davanagere districts. The incidence of disease varied from 5.50 to 30.50%. The characteristic symptoms initially appeared as white to grayish mold on and between the kernels on part of the ear. The disease starts at the base of the ear and progresses towards the tip. The ear leaf and husks on the ear prematurely bleached and become straw coloured. In case of severe infections, ears become grayish brown, shrunken, very light weight and completely rotten. The disease was favoured by wet weather after silking and more severe in monocropping system. The infection was prominent between three to four weeks after silking. Small black fruiting bodies (pycnidia) found on husks, cobs and sides of kernels. The fungus associated with the ear rot disease was identified as *Diplodia maydis* (Berk.) Sacc [Syn: *Stenocarpella maydis* (Berk.)] Sutton based on morphological characters. The association of *Stenocarpella maydis* (Berk.) Sutton with diplodia ear rot disease of maize constitutes a new record from Karnataka state. Highest incidence (35.80%) was noticed in Kalaghtagi taluka (Dharwad district). Haveri and Davanagere districts recorded incidence in the range of 5.5 to 26.0%. Losses to the tune of 23.35 to 47.89% was noticed in cob weight. The monitory losses ranged from Rs.400-460 per quintal of grains over healthy grains.

P19

Monitoring of maize diseases in Karnataka

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Maize is one of the major cereals grown in Karnataka which has come up on large area in different districts both under assured rainfed and irrigated command areas. The area under maize is increasing and the crop has attained its multifold dynamism due to its cultivation through out the year in all seasons. This has mainly responsible for increased severity of many diseases due to continuous availability of host crop for pathogen survival. Hence, extensive surveys were carried out to assess the prevalence and severity of maize diseases in the major maize growing areas of northern Karnataka. Studies revealed that, there is a major shift in the disease pattern during the recent past. Diseases of minor importance *viz.*, Curuvularia leaf spot, Diplodia ear rot and Banded leaf and sheath blight become increasingly severe due to change in weather conditions and introduction of many susceptible hybrids. Turcicum leaf blight and maydis leaf blight were noticed during second fortnight of July and severity progressed later on and reached maximum during September in Dharwad, Haveri, Gadag and Belgaum districts. Polysora rust and common rust diseases appeared during second fortnight of August and reached maximum during first fortnight of October in Dharwad, Haveri and Belgaum districts. Moderate to severe incidence of Diplodia ear rot and banded leaf and sheath blight diseases were noticed during second fortnight of September and attained severe form at grain filling stage in Dharwad and Uttar Kannada districts. Dry weather prior to silking followed by wet weather conditions during silking favoured Diplodia ear rot infection. Curvularia leaf spot severity observed in moderate to high level at flowering to grain filling stage in Dharwad, Haveri and Belgaum district. The late sown crop i.e., August – September was subjected to severe incidence of foliar diseases. Post flowering stalk rots incidence was severe at flowering to grain filling stage in Bagalkot, Bellary, Koppal, Belgaum and Gadag districts.

P20

Variability of colony characters, productivity and quality parameters of different strains of *Agaricus bisporus* (Lange) Sing

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Button mushroom is most popular mushroom in northern plains of India, generally grown by the farmers seasonally. At different locations different strains of *Agaricus bisporus*

are preferred which varies in their quality and yield potentiality. In present study, 33 strains of button mushroom (*A. bisporus*) were evaluated for their cultural characteristics, fruit body traits and yield potentiality. Button mushroom strains were procured from Indian Type Culture Collection (ITCC), IARI, New Delhi, deposited during 1975 to 1992 were used for the study. On the basis of cultural studies, the strains were broadly grouped in to fast, slow and medium growing. The fast growing colonies had aerial mycelium and some of them were showing appressed, dendric and strandy growth (ITCC-3697, 3614 etc.). Most of the slow growing colonies had appressed thick and patchy growth (ITCC-4291, 3502 etc.). The color of the colonies of different strains varied from cottony, snowy to milky white. Sectoring and zone formation were recorded in ITCC-1926, 1927, 3613, 3615, 3741 strains and in some slow growing colonies, where as pigment and exudation formation was observed in few strains *viz.*, ITCC-3607, 3708, 3613, 3741, 4822 and most of the slow growing strains. Fruit bodies color also varied creamy, snowy to pinkish white in different strains. On the basis of yield potentiality, the strains were grouped in to high yielder, medium and poor yielder. The yield evaluation data revealed that potentiality of yield in different strains varied from 2050 g (ITCC-4289) to15360 g (ITCC-3741) per 100 kg compost. The quality of the mushrooms was evaluated as average, good and very good on the basis of shelf life and compactness of fruit bodies. Among 33 strains evaluated best quality mushrooms was obtained in 13 strains, good quality in 10 strains and 9 strains had average quality mushrooms respectively. It has been observed that fast and medium growing strains with appressed, strandy and dendric mycelium yielded higher productivity and good quality mushroom than the strains showing slow and patchy growth. In *A. bisporus*, degeneration of hyphae, their genetic make-up and physiological characteristics may alter the normal behaviour under prolonged storage and could not provide proper results.

P21

Detection and characterization of Tobacco streak virus infecting okra in Tamil Nadu

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Tobacco streak virus (TSV) is the most economically important virus infecting okra (*Abelmoschus esculentus* L.) and the samples collected from the field was detected by RT-PCR using coat protein and movement protein genes of TSV specific primers. They were also serologically positive in direct antigen coating enzyme linked immunosorbant assay (DAC-ELISA). Sap inoculation of the okra strain induced local as well systemic infection on cowpea plants cv. C 152 and resulted in the production of circular necrotic lesions and death of plants. The coat protein and movement protein genes were amplified with a size of 929 bp and 1.2 kb re-

spectively including the UTR region as part of RNA3 of TSV. Sequence analysis of the coat protein gene had nucleotide similarity of 98.3 to 99.4% with known strains of TSV. The multiple sequence alignment revealed that the sequence had two unique variations at the position 15 where cytosine was substituted with adenine and it produced unique variation at the position 526 where cytosine was substituted with thiamine. There was no deletion and addition between nucleotide sequences in the group, further confirms the placement of the okra strain of TSV in a single subgroup. The nucleotide sequence of movement protein okra strain had single unique variation at position 438, where thiamine was substituted with cytosine. Phylogenetic analysis of the amino acid confirms that the okra strain of TSV forms single subgroup with other crop of Indian isolates.

P22

Phylogenetic analysis and identification of plant pathogenic Hymenochaetaceae members

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The Hymenochaetaceae family of Phylum Basidiomycotina is characterized by 28 genera and 400 species to date. Phylogenetic analysis and identification of various members of this family was attempted using morphological and molecular traits. Pathogenic 30 samples belonging to Hymenochaetaceae occurring on members of Fabaceae (Angiosperms) were collected from Pune region. Morphological studies were carried out using 14 quantitative and 70 qualitative characters. Out of these, 8 quantitative characters of the 30 samples along with the same characters from previously reported genera of this family were used in Principle Component Analysis (PCA) in an attempt to group the samples in different clusters. In addition DNA sequence data corresponding to 80S ribosomal large subunit rRNA (28S) and 70S (mitochondrial) ribosomal small subunit rRNA (16S) was collected for the same 30 samples. Sequence data for the same rRNA molecules was also downloaded from NCBI site for 12 reported genera of Hymenochataceae. The sequences were aligned using MEGA(version4) software and a phylogenetic tree was constructed using maximum parsimony (PAUP 4.0). Results indicated that

the Hymenochaetaceae genera studied did not appear to show host specificity; Mitochondrial 16S rDNA – based clustering represented the genetic relatedness between the genera better than rDNA sequences from 80S ribosomal genes and the temperate specimens of some genera did not co-segregate with the tropical specimens, indicating different paths of genetic diversification between tropical and temperate specimens of this genus.

P23

Diagnostics and management of fusarium wilt infecting tomato and chilli

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Diversified *Fusarium* cultures were isolated from samples collected from major tomato and chilli areas of India. PCR amplification of genomic DNA of the *Fusarium* isolates with ITS primersand sequencing indicated that out of 28 isolates of tomato, 20 were *Fusarium oxysporum* f.sp. *lycopersici* and 8 were *Fusarium solani* while in chilli out of 17 *Fusarium isolates* 12 were *Fusarium solani* and 5 were *Fusarium oxysporum* denoting that *Fusarium solani* is infecting both tomato and chilli. In *Fusarium*, chitin synthase gene plays a major role in pathogenesis. To know the relation between chitin synthase gene and virulence of *Fusarium* spp, in the present study different isolates of *Fusarium* were amplified with chitin synthase primers and phylogenic relationship was studied. Total DNA of F. solani (17 chilli isolates)and *F. oxysporum* (7 tomato isolates) were amplified by PCR using chitin synthase specific primers, CHS79-F (5-TGG GGC AAG GAT GCI TGG AAG AAG-3) CHS354-R (5-TGG AAG AAC CAT CTG TGA GAC TTG-3). The expected PCR amplicons of 300bp in size were amplified in all *F. solani* and *F. oxysporum* isolates. The amplified PCR products were cloned, sequenced and submitted to NCBI. The sequences are available under accession number of KF918427 to KF918449. The chitin synthase gene sequence of *F. solani* and *F. oxysporum* infecting chilli and tomato were compared with other plant infecting fungi available in the database. The nucleotide similarities in chitin synthase gene of *F. solani* and *F. oxysporum* isolates with *F. oxysporum*. f.sp. *lycopersici* (AY572421) ranged from 66.8 to 72.0% and 65.1 to 72.5%, respectively. The phylogentic tree was generated by MEGA 5.0 SOFTWARE using neighbor joining method with 1000 bootstrap replications to estimate evolutionary distance between all pairs of sequences simultaneously. The results of phylogenetic clustering were matching with results of pathogenic variability with

F. solani and *F. oxysporum in vivo*. Hence, it indicates that to study the pathogenic variability in higher order pathogenic fungi, Chitin synthase gene can be used as a tool.In view of identifying potential biocontrol agents,89 antagonist, microbes (fungal and bacterial) were tested against the wilt pathogen *in vitro*, greenhouse and field conditions, and identified two *Trichoderma* isolates *viz.*, BATF-39-1 and BATF-43-1 as potential isolates against wilt of chilli and tomato which showed >50-70% disease control as well as >40% yield enhancement. Apart from that a grafting technique to establish seedling with brinjal as root stock and tomato (cv. Kashiaman) as scion was standardized to develop resistance for *Fusarium* wilt, bacterial wilt and root knot nematode. This technique is more suitable for open field as well as protected cultivations.

P24

Survival of *Cerotelium fici* causing rust of fig

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Rust disease caused by *Cerotelium fici* (Cast.) Arth. is potentially serious threat to fig cultivation that occurs universally, wherever it is grown. About 50% loss have been reported indicating the seriousness of rust disease in fig. Survival of any pathogen plays very important role in recurrence of the disease. This gives idea about the perpetuation of the pathogen during off season. Very limited information is available on survival of *C. fici* and recurrence of disease. Considering the economic importance of fig rust, present investigation on viability of urediospores of *C. fici* in diseased plant parts was undertaken at National Agricultural Research Project, Ganeshkhind, Pune. Viability of *C. fici* urediospores under different field conditions was studied by collecting spores from fallen as well as attached rusted leaves and shoots.The detached rusted leaves below infected plants and under tree shade condition as well as attached rusted leaves and shoots were the four main spore sources for study. Spore viability was studied by hanging drop technique wherein spore germination count was recorded. The urediospores remained viable up to 25 and 30 days in detached leaves below plant as well as those stored under plant shade, respectively. In attached infected leaves the spores were found viable up to 55 days. However, at onset of favorable conditions, the pustules on attached infected leaves yielded spores. It clearly indicated that the rust inoculum on attached leaves of fig survived during aberrant weather conditions up to the resume of favourable weather conditions. On the contrary, the detached infected leaves failed to provide viable inoculum for the new flush of fig.

P25

Effect of relative humidity on conidial germination of *Colletotrichum capsici* and *Leveilula taurica* and disease development in chilli

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Laboratory experiments were conducted to study the effect of relative humidity levels on conidial germination of *C. capsici* and *L. taurica* and disease development in chilli (var. Parbhani Tejas) *in- vitro*. Results indicated that conidia of *C. capsici* and *L. taurica* could not germinate at 10% RH up to 48 h of incubation. Maximum conidial germination of both these species took place at 100% RH Followed by 75, 50 and 25% RH. Symptoms of *C. capsici* on leaves were not observed at 10% RH and on fruits at 10 and 25% RH up to a fortnight. Incubation period was minimum at 100% RH and steadily increased as humidity levels decreased. Powdery mildew symptoms on leaves up to a fortnight were not observed at 10, 25 and 100% RH. The symptoms developed within a week's period at 50 and 75% RH.

P26

Loop-mediated isothermal amplification (LAMP) for detection of *Ganoderma lucidum* causing basal stem rot of coconut

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The basal stem rot disease of coconut caused by *Ganoderma lucidum* is one of the devastating diseases of coconut in India and in severely infected areas, incidence as high as 80% was recorded. The disease can be managed effectively by an integrated disease management package, if the disease is diagnosed at the early stage. However, by the time visible external symptoms like yellowing, drying and drooping of the outer whorl leaves appear, the damage to the palms would have been done by the pathogen. Hence, diagnosis of the disease before the external symptoms appear is very much essential. In the present study, a loop-mediated isothermal

amplification (LAMP) protocol for real-time detection of *Ganoderma lucidum* associated with the basal stem rot disease of coconut was developed using the 'Genei® II' real-time LAMP reader (OptiGene Ltd. UK). The LAMP primers targeted the small subunit ribosomal RNA gene of Ganoderma. Isothermal amplification was carried out at 65°C. In the end of amplification, an annealing curve analysis was performed to check the fidelity of the amplicons. The sequence confirmation of the amplicons was also carried out by PCR amplification with external primers of LAMP and sequencing. The specificity of the assay was also tested by including other fungi namely *Trichoderma harzianum* and *Phytophthora palmivora*. The total DNA isolated from *G.lucidum* and root samples of basal stem rot affected coconut tested in the assay produced unique annealing peak at 84±0.5°C. Further sequencing and nucleotide BLAST (BLASTn) analysis confirmed that the sequences corresponded to Ganoderma small subunit ribosomal RNA gene. No amplification was observed for DNA isolated from *T. harzianum* and *P. palmivora*.

P27

Characterization and virulence determination of *M. phaseolina* causing root rot disease in coleus

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Coleus (*Coleus forskohlii* Briq.), is a subtropical and warm temperate medicinal plant and farmers have started raising this crop because of its economic potential. Among several limiting factors for successful cultivation, susceptibility to diseases is one of the major constraints. The variety *C. forskohlii* is affected by various diseases. Among them, fungal diseases in coleus, root rot caused by *M. phaseolina* is the most serious one which affects the crop in the later stages of growth in tuber formation, though the infection of the pathogen is observed throughout the growing period. Characterization of this pathogen helps to develop successful disease management strategies. In this connection, totally eight isolates of *M. phaseolina* causing root rot disease in coleus were isolated from different regions of Tamil Nadu. All the isolates were characterized based on cultural characteristic, growth, mycelia dry weight and their virulence. The results of the experiment revealed that, the Athur (MP6) produced more fluffy and cottony white mycelial growth on PDA and produced maximum mean dry weight of 1.04 g when compared to other isolates. Similarly, Athur (MP6) was found to be highly virulent recording a maximum root rot incidence of 78.29% followed by isolates of Sathiyamangalam (MP8) and Coimbatore (MP7) which recorded 74.27% and 72.43%, respectively. Hence, these results suggest that characterization of pathogens might be useful to adopt successful management strategies for sustainable production of coleus.

P28

Characterization, identification and detection of multiple *Phytophthora spp.* associated with citrus decline in India

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Phytophthora spp.cause serious diseases like root rot, crown rot, foot rot, gummosis and brown rot of fruits in citrus inflicting decline and yield losses in India.A total of 196 isolates belonging to 7 different *Phytophthora* spp (141 isolates of *P. nicotianae*, 36 isolates of *P. palmivora*, 6 isolates each of *P. citrophthora* and *P. insolita*, 4 isolates of *P. boehmeriae*, 2 isolates of *P. tropicalis* and 1 isolate of *P. lacustris*) were isolated from rhizosphere soil and water, citrus root, leaf, bark and fruit samples collected from 12 major citrus growing states of India. Occurrence of *P. insolita* and *P. lacustris* were reported for the first time in India. Morphological characterization coupled with PCR-RFLP based system of the ITS region of the genomic rDNA was used as a tool to identify all the seven *Phytophthora* spp. Sequencing and phylogenetic analyses of the ITS region, beta tubulin gene, translation Elongation factor 1 alpha and the region containing the mitochondrial cytochrome c oxidase subunit 1 and 2 gene fragments also confirmed the identity of these species. Species-specific primer pairs NIC1/ NIC2 and Pal1s / Pal2a were successfully tested for specific detection of *P. nicotianae* and *P. palmivora* isolates, respectively. These two species were also detected in citrus roots and rhizosphere soils using nested PCR and PCR RFLP techniques.The findings relative to morphological and molecular characterization of seven *Phytophthora* spp. obtained from Indian citrus ecosystem and its implications for pathogen identification and disease detection would be discussed.

P29

Characterization of phytoplasma associated with little leaf of brinjal in Kerala and Karnataka States

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Brinjal is one of the important vegetable crops grown in all states of India. In recent years, Phytoplasma associated with brinjal causing little leaf or phyllody disease responsible for considerable yield loss in all brinjal growing regions and the disease incidence is increasing

alarmingly year after year. In view of this, a study was conducted to identify and characterize the phytoplasma associated with brinjal gown in fields and home gardens from Kerala and Karnataka. A total of 8 samples (6 samples from Karnataka; 2 samples from Kerala) were collected along with representative asymptomatic brinjal leaf samples from Kerala and Karnataka and subjected to molecular identification. The genomic DNA of phytoplasma was isolated and 16SrRNA gene was amplified using P1/P7 and R16F2n/R16R2 primers (nested PCR). Nested PCR products were separated on 1.5% agarose gel and expected amplicon size of ~1.25kb were obtained. All the nested PCR products were purified and sequenced from both the directions, aligned and consensus sequences were subjected to n-BLAST for identification. Sequence analysis indicated that all the samples collected from home gardens of Kerala and fields of Karnataka were genetically identical. Virtual RFLP pattern obtained using iPhyClassifier online tool also validated the phytoplasmal association with little leaf disease of brinjal in two states. Phylogenetic tree constructed using Neighbour joining methods placed all the phytoplasma sequences with *Candidatus phytoplasma trifolii* group VI and subgroup D and the tree was rooted with *Acholeplasma laidlawii*. The study indicated that wide distribution and occurrence of *Candidatus phytoplasma* VI on brinjal plants grown in fields of Karnataka and home gardens of Kerala were genetically similar and insect vectors must have played a key role in spreading of phytopathogen.

P30

Multilocus gene characterization of phytoplasmas associated with brinjal little leaf in India

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Brinjal little leaf is a phytoplasma associated disease and widespread in India that induces severe economic losses. Survey was made in 12 states of India and 60 symptomatic samples of brinjal were collected showing, little leaf, phyllody and witche's broom symptom for phytoplasma identification with universal phytoplasma specific primers. Presence of phytoplasma was confirmed in all the test samples using polymerase chain reaction with first round primer pairs (P1/P6) and second round nested primer pair (R16F2n/R16R2n) which yielded amplified products of 1.5kb and 1.2kb, respectively. Further semi-nested PCR assays using universal phytoplasma specific primer pair secA-For1/secA Rev3 followed by secA For2/secA Rev3 yielded ~480bp product of secA gene with all the symptomatic brinjal plants. BLAST analysis and phylogenetic analysis of 16Sr RNA and secA gene sequences revealed that the phytoplasma strains associated with BLL disease belonged to 'Candidatus *Phytoplasma trifolii*' (16SrVI) and '*Ca. P. aurantifolia* (16Sr II) groups.

P31

A reverse transcription loop mediated isothermal amplification assay for rapid detection of Dasheen mosaic virus in *Amorphophallus paeoniifolius*

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Amorphophallus paeoniifolius (Dennst.) Nicolson or Elephant foot yam is a tropical edible tuber crop belonging to Araceae. Because of its high production potential and nutritional values, the crop plays a very important role in the socio-economic development of the country. One of the major diseases affecting the crop yield is mosaic disease caused by Dasheen mosaic virus (DsMV). DsMV is a single stranded positive sense RNA virus belonging to Potyviridae. For rapid detection of DsMV infection in *A. paeoniifolius*, a reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay has been developed. Species specific primers to amplify a conserved region in the coat protein gene were designed using the LAMP primer design software, Primer Explorer V4 (Eiken Chemical Co. Ltd., Japan). For getting characteristic ladder like bands of RT-LAMP product in agarose gel, a final concentration of 5.4 mM Magnesium sulphate and 0.7 M Betaine in the reaction mixture was found to be essential. The reaction was set at 65°Cfor 50 min and 86°C for 5 min in water bath circumventing the requirement of a thermal cycler. In tube detection of the product was carried out using fluorescence detection reagents as well as ethidium bromide. The assay was found to be 100 times more sensitive than RT-PCR. Upon validation, the assay proved to be effective in the rapid detection of DsMV.

P32

Development of molecular markers to investigate *Rhizoctonia solani* using conventional and real-time PCR

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Forty isolates of *Rhizoctonia solani* causing web blight and wet root rot in mungbean representing 7 anastomosis groups (AGs) from 11 states of India were analysed for genetic diversity

using internal transcribed spacer (ITS) region. The isolates gave ≈700 bp amplicon using ITS1 and ITS4 universal primers. The phylogenetic analysis of ITS sequences of 10 representative isolates included in the present study along with the sequences of 9 other isolates originated from different parts of the world, showed high level of similarity but the northern and southern Indian populations were clustered separately. Northern India populations showed affinity with the isolates of Asian origin. *R. solani* specific markers BKF1 and BKR1 were designed from these sequences. SCAR markers BKF2 and BKR2 were also developed from a specific RAPD fragment for detection of the pathogen. The ITS based (BKF1 and BKR1) and SCAR (BKF2 and BKR2) markers provided specific and sensitive detection of *R. solani* DNA up to the level of 100 pg through conventional PCR. The markers BKF1 and BKR1 detected 1pg while BKF2 and BKR2 detected 5 pg of genomic DNA of *R. solani* using real-time PCR assay. Hence, both set of markers proved to be reliable for detection of various AGs of *R. solani* in mungbean.

P33

Molecular characterization of *Anastomosis* group, AG1-IA of *Rhizoctonia solani*, incitant of rice sheath blight

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Rice sheath blight, caused by *Rhizoctonia solani* (Teleomorph: *Thanatephorus cucumeris*), is one of the most important rice pathogens worldwide causing huge economical loss to the farmers. Variation within *R. solani* exists in nature and hence it is important for better understanding of disease development and for the prediction of future disease outbreak. In this study, 25 isolates of *R. solani* were collected from different rice growing areas of Tamil Nadu and studied for colony morphology, anastomosis grouping and genetic diversity. Studies on morphological characterization of *R. solani* isolates showed that isolates were highly variable both in mycelial and sclerotial parameters, but these cannot be directly related to virulence or geographic origin. Hence, pathogenicity of *R. solani* on susceptible rice cv. ASD 16 and BPT 5204 were evaluated and based on their symptom expression, the isolates were classified into different virulent groups. With the advent of various molecular marker technologies, the studies of genetic diversity in plant pathogens have become feasible. In the present investigation attempts were made for identification of anastomosis groups of *R. solani* based on ITS- RFLP. Besides, anastomosis group of the isolates was determined using microscopic hyphal fusion examination with standard AG1-IA tester and also confirmed using AG1-IA-specific primers in 20 selected isolates which amplified the AG1-IA specific gene of 265 bp.

P34

Detection of acylated homoserine lactones produced by *Ralstonia solanacearum* isolated from diseased tomato plants

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Cell-to-cell communication in many Gram negative bacteria is mediated by signal molecules such as acylated homoserine lactones (AHL). Research in AHL quorum sensing has been considerably aided by simple methods devised to detect AHLs using bacterial biosensors that phenotypically respond when exposed to exogenous AHLs. Isolates of *Ralstonia solanacearum* were investigated for the production of AHLs. The production of signal molecules was detected using *Chromobacterium violaceum* CV026 and *Agrobacterium tumefaciens* NT1 (pZLR4) biosensor systems. *C. violaceum* CV026 only produces the pigment violacein in the presence of exogenous AHL. Similarly the *A. tumefaciens* NT1 (pZLR4) sensor strain has the lacZ gene fused to the promoter of the traI gene, which is regulated by auto-induction. The strain produces a blue pigment in response to AHL when the medium is supplemented with X-gal, which is the indicator of a positive result. All bacterial isolates from various bacterial wilts that affected tomato plants produced AHL molecules that induced the biosensor. Although it cannot provide the exact AHL structure, using a combination of different sensor strains is a simple method for the initial characterization of AHL molecules, and determining the activation pattern of different biosensors can reflect different AHL production profiles in different strains. We have developed an assay for these signals that couples separation by thin-layer chromatography with detection using *C. violaceum* CV026. An agar well-diffusion assay based on *C. violaceum* CV026 was used for quantifying AHLs from bacterial supernatants. The assay can be used to screen cultures of bacteria for AHLs and for quantifying the amounts of these molecules produced.

P35

Detection of food-borne pathogens using DNA based technique

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Food-borne diseases are mainly caused by fungal and bacterial pathogens, which are transmitted to humans through animal source via food or contaminates the food on the pro-

cessing line to cause serious health problem. The detection of the pathogenic food-borne c stituents a challenge, because they are present in low numbers and hindered by food mat and enumeration of microorganisms in food are an essential part of any quality control or foo safety plan. So, quick screening methods for food-borne pathogen are the hot research topic in scientific field. Traditional detection methods had limitations because, they need culture media, and growth followed by isolation, biochemical and/or serological identification, and in some cases, subs specific characterization. One of the most challenging problems to circumvent with these assays is sample preparation. Advances in technology have made detection and identification faster, more sensitive, more specific, and more convenient than traditional assays. The possibilities of combining different methods, including improved technologies for separation and concentration of specific bacteria, and for DNA extraction and purification, will facilitate the direct detection of pathogens in food. We have collected different food samples from different places and isolated both fungi, bacterial pathogens *viz., Aspergillus niger, Fusarium oxysporum, Trichoderma* and *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* were identified by polymerase chain reaction (PCR). Amplifying the region of rDNA using ITS1, ITS4 and 16S rRNA primers. The results showed a high degree of primer specificity to the target pathogens. The use of these PCR based methods in isolation and characterizations of food-borne pathogens were discussed in the present paper.

P36

Detection and diagnosis of *Sclerotinia sclerotiorum* in carnation

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Carnation is a cut flower that tyrannizes the global cut flower market, followed by rose. However, carnation cultivation is ravaged by various fungal diseases. Among them stem rot is highly torpedoing. The causal organism for stem rot was identified as *Sclerotinia sclerotiorum* through morphological and molecular characterization. In order to study the morphological character, carpogenic germination was induced artificially. The apothecia were cup like, ochraceous and produced monomorphic, ellipsoidal ascospores. The layers of apothecia were studied. Associated structures of apothecia *viz.*, crozier, spermatia and paraphyses were also studied. The pathogen was further confirmed by molecular characterization of ITS 1 and ITS 4 regions and the PCR product was sequenced and submitted in NCBI with Genbank accession no. KP6452. The sequence (KP6452) showed 99% similarity with *S. sclerotiorum* isolated from soybean (KM272350), available at NCBI database.

P37

Real time loop mediated isothermal amplification – an advanced tool for the field diagnosis of *Ralstonia solanacearum* biovar 3 infecting ginger

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Ralstonia solanacearum biovar 3 infecting ginger is a devastating pathogen, which causes great economic loss to the farmers. Early detection of the pathogen in rhizomes and in soil is essential for disease management and to prevent the crop loss due to further spread of the pathogen. The present study aims for specific and sensitive detection of *R.solanacearum* infecting ginger using a modern tool in the diagnostic field, Real time LAMP. An initial cross infectivity study confirmed that *R.solanacearum* strains from ginger could infect both ginger and tomato. But none of the *R.solanacearum* strains from solanaceous hosts like tomato, potato or brinjal could infect ginger. In order to detect the specific strain, a set of six primers were designed from gyr B gene sequence of ginger *R.solanacearum* using the software LAMP Designer 1.12. The primer set was first validated using LAMP, in which the primer set could specifically amplify and produce ladder like amplification only with DNA of *R.solanacearum* from ginger. The positive amplification was also obtained using soil DNA extracted from *R.solanacearum* inoculated soil. This primer set was used further in real time LAMP (Genei II, Optigene Ltd. UK) in which only *R.solanacearum* isolates from ginger could produce a sigmoid amplification curve. Analyzing the annealing curve and Ta value (92±1°C) confirmed the amplification of the correct product. The detection limit was found to be 5pg of the genomic DNA. When *R.solanacearum* inoculated water was used, the detection limit was found to be 104 CFU/ml. The method was also standardized with soil contaminated with *R.solanacearum* and found that even the soil supernatant can be used to detect the pathogen. Hence real time LAMP can be a suitable diagnostic tool for on farm detection of *R.solanacearum* infecting ginger either in soil or in planting material without any cumbersome sample processing.

P38

Characterization of *Pythium vexans* de Bary: the rhizome rot pathogen of cardamom

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Among the diseases of cardamom (*Elettaria cardamomum* Maton), rhizome rot disease caused by the Oomycete *Pythium* spp. is a serious threat in the nursery and in plantations. In the

present study, a detailed survey was conducted for rhizome rot incidence in various cardamom growing tracts of Kerala, Karnataka and Tamil Nadu during the monsoon period of 2012–2013. A total of 36 pythium isolates were obtained from the collected samples. Based on the morphological characteristics *viz.*, sporangial shape, shape of oogonia and number of antheridia all the isolates were identified as *P. vexans,* even though they showed variations in growth pattern and colony morphology on different media *viz.*, potato dextrose agar, corn meal agar, potato carrot agar and V8 vegetable juice agar. The molecular characterization of all the isolates by ITS RFLP using the restriction enzymes Msp1 revealed both intra specific and inter specific variability within the isolates. However, the isolates showed identical banding patterns with the restriction enzyme Alu 1.The variability among the *P. vexans* isolates needs to be further confirmed.

P39

Nature of false smut disease of rice in India

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False smut disease of rice caused by *Ustilaginoidea virens* is emerging threat to rice production in India. Although role of sclerotia, chlamydospores and conidia of *U. virens* is highly assumptive in causing the primary infection and initiation of the disease as experimental evidence is still lacking. Experimental trials were conducted at IARI to standardize the isolation technique of the pathogen. Artificial inoculation of the *U.virens* on rice plant was successfully done in rice hybrid PRH-10 through injecting conidial and mycelial bit suspension @22 x 10^5 cfu/mL at booting stage in rice plants. Soil inoculation with sclerotia, nursery raised from chlamydospores treated seeds and seedlings root dip method with conidial/mycelial bit suspension @2 x 10^5 cfu/mL as well as its spray inoculation at booting and panicle emergence stage of the plant failed to produce the typical false smut symptoms in rice hybrid PRH-10. Histopathological studies of false smut balls on rice spikelet were conducted through SEM and microtomy of the infected samples. The identity of the fungal culture of *U. virens* and detection of the fungus at differeny stages of the inoculated plant was done through polymerase chain reaction (PCR) analysis using *U. virens* specific internal transcribed spacer (ITS) based primer. To confirm the systemic nature of the disease, pot experiment was planned. Rice seedlings raised from seeds of rice hybrid PRH-10 treated with chlamydospores were grown in soil with sclerotia of *U. virens* in glass house. *U. virens* was detected in host plant only up-to four weeks and later on at panicle emergence stage it disappeared. Presence of fungus was validated through microtomy clubbed with SEM and *U. virens* specific marker. Panicle of the infected ear head (field sample) showing typical false smut symptoms also did not reveal the presence of the fungus. These observations showed that the false smut pathogen was not able to survive in the host plant for more than one month when growing in upward direction from root to panicle. Along with the data of artificial inoculation studies, it may be assumed that the false smut disease may not be systemic in nature.

P40

Phenotypic and molecular characterization of chrysanthemum white rust caused by *Puccinia horiana* (Henn)

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Chrysanthemum (*Dendranthema grandiflora*) being an important export oriented cut flower crop, the cultivable area is going on increasing in India and Tamil Nadu. Diseased cut flower chrysanthemum with symptoms of white rust was observed during 2012-2013 in Tamil Nadu in India. Chrysanthemum varieties were surveyed for the occurrence of white rust in Kothagiri hills of Nilgiris district and Yercaud hills of Salem district in India. Historically, identification protocols for white rust relied upon macroscopic symptom development and microscopic examination of infected leaves for teliospores. Symptoms become visible 7 to 10 days after initial infection under favorable conditions followed by the production of telia. Infected plants can therefore evade detection before symptoms and fruiting bodies are evident. White rust were detected in symptomatic leaves of two varieties using PCR with *P. horiana* genus specific primer (Ph-F1 and Ph-R1') amplified a fragment of approximately 240bp. Yet another *P. horiana* genus specific primer (Ph-F2 and Ph-R1) amplified a fragment of approximately 340 bp corresponding to the region of the 16S-23S rDNA intervening sequence, specific for *P. horiana*. The nucleotide sequence analysis of a 240-bp and 340-bp fragment had 100% identity. Amplified DNA fragments of 240bp and 340bp were ligated to the pBS based T/A plasmid vectors using T4 DNA ligase separately. The *P. horiana* primers did not amplify the rDNA target using DNA isolated from leaf tissue infected with *P. chrysanthemi*. The partial sequence of *P. horiana* isolates were submitted to the NCBI, Genbank, New York, USA. The isolates were assigned with accession numbers KC291657, KC291658, KC291659 and KC291660. Phylogenetic analyses of *P. horiana* based on 16S-rDNA sequences were grouped in cluster-I.

P41

Indicator plants for bitter gourd viruses

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Mosaic is a serious problem of bitter gourd cultivation in Kerala and it is mainly caused by three viruses *viz.*, cucumber mosaic virus (CMV), poty virus and bitter gourd distortion

mosaic virus (BDMV). Combined infection of these viruses is very common in the field condition. Isolation of individual viruses from the mixed infected field samples is a prerequisite for research purpose. Of the three viruses of bitter gourd, BDMV is a whitefly transmitted gemini virus, where as CMV and poty virus have similar mode of transmission i.e, by sap and aphids. Hence, it is difficult to isolate individual viruses from the mixed infected field samples. So a present study was conducted to isolate CMV and poty viruses from the mixed field samples using indicator host plants. Cosmos (*Cosmos sulphurous*) and papaya (*Carica papaya*) were used as indicator plants for CMV and poty virus respectively. Infected bitter gourd leaf samples from field were mechanically inoculated to the seedlings of indicator plants and kept under insect proof condition for symptom development. The symptoms appeared in cosmos were, vein clearing, mosaic mottling, leaf size reduction and shoe string. Puckering, yellowing and leaf distortion were the symptoms appeared in papaya. The infected leaves of these indicator plants were extracted in potassium phosphate buffer and were mechanically inoculated to bitter gourd seedling. This technique resulted in the separation of individual viruses from CMV and poty virus infected bitter gourd samples.

P42

Isolation and molecular characterization of *Xanthomonas axonopodis* pv. *dieffenbachiae* causing bacterial blight in anthurium

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Detection of harmful bacteria in plant material is essential to ensure safe and sustainable agriculture. Reliable and rapid detection techniques were evolved in the last few years. One of the important techniques is molecular characterisation. Anthurium (*Anthirum andreanum* Linden ex Andre) is an important export oriented cut flower crop. Bacterial blight of anthurium was caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* pathogen which causing bacterial blight was identified by polymerized chain reaction using specific primers amplified at 750bp. DNA sequencing and nucleotide blast results confirmed two isolates of *X. axonopodis* pv. *dieffenbachiae* designated as XAD1 and XAD2 were submitted in the NCBI bearing the accession numbers KJ603434 and KJ637328, respectively.

P43

Transmission study on Sri Lankan cassava mosaic virus in cassava by whitefly (*Bemisia tabaci*) and SLCMV infectious clones

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Bemisia tabaci is one of the most devastating tropical and subtropical agricultural pests, affecting the yield of a broad ranges of agricultural, fiber, vegetables and ornamental crops. Both immature and adult stages ingest phloem sap and can cause damage directly as a result of feeding, and indirectly from excretion of honeydew onto the surfaces of leaves and fruit. Virus transmission of cassava is by the whitefly vector *B. tabaci* Gennadius (Brown et al. 1995). Cassava mosaic disease was first shown to be transmitted by *Bemisia* species by Kufferath & Ghesquiere (1932) in the Congo and they subsequently named it *Bemisia mosaicivectura* Ghesq. Cassava mosaic disease (CMD) transmitted by *B.tabaci* have been reported from Africa and the Indian subcontinent (Fauquet & Stanley 2003). Geminivirus transmission is persistent and circulative in the whitefly vector, requiring an average latent period of 6-12 h prior to transmission. The geminiviruses are considered as non replicative in their vector, and they are not passed transovarially. The virus acquiring ability was studied for both male and female whiteflies each with 10 individuals. The circulative nature of whitefly was studied by organ PCR where different parts of the viruliferous whiteflies were dissected and used for PCR with specific primers. The virus SLCMV was detected in both stylet, abdomen and body fluid which reveals that the virus transmission was circulative in nature. SLCMV clones were inoculated into different laboratory plants, all the inoculated plants showed the presence of virus whereas the mock inoculated plants did not show any result.

P44

Diversity of whitefly transmitted begomoviruses in West Bengal

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Extensive survey, diagnostics and molecular studies on the genomic structures of various begomovirus species infecting different plants species in West Bengal have been doing to

get the detail insight of the viral genome and possible association of sub-viral agents. Here we will report the detection, characterization and phylogenetic analysis of the begomoviruses predominantly occurring in West Bengal. The presence of several begomoviruses in different plant species were confirmed by nucleotide hybridization technique and PCR based method. Many of the isolates of begomoviruses infecting different crops were found distantly related with isolates of the respective viruses reported in the world. The gene bank accession number *viz.* FN432356, FN691429, HM035545, HF545019, HF548665, HF679120, HF912381, HF936708, HF922628, HF679119, HG969195 and HG969194 of Sweet potato leaf curl virus, Bhendi yellow vein mosaic, Chilli leaf curl virus, Tomato leaf curl virus (ToLCV) in ridge gourd, Tobacco curly top virus in tomato, Bhendi yellow vein mosaic, Mungbean yellow mosaic virus, Tomato leaf curl virus, Dolichos yellow mosaic virus, Chilli leaf curl virus, respectively. Begomo viruses have been emerging in most threatening way in several economically important crops including cucurbits and trend in shifting new host like severe incidence of ToLCV in ridge gourd supply precise information about the frequency and distribution of distinct begomovirus isolates and its possible recombination breakpoints, which can shed light on the mechanistic processes underlying recombination.

P45

Influence of physiological parameters on growth and sporulation of *Colletotrichum truncatum* (Schw.) Andrus and Moore causing anthracnose of mungbean

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Anthracnose caused by *Colletotrichum truncatum* (Schw.) Andrus and Mooreis one of the major diseases of mungbean (*Vigna radiata* L. Wilczek). The disease has become one of the major constraints for mungbean cultivation. An experiment were carried out to study effect of different temperature, pH level and light intensity on the growth and sporulation of *C. truncatum* under *in vitro* at Department of Plant Pathology, Narendra Deva University of Agriculture and Technology, Faizabad, Uttar Pradesh. Results of the experiment indicated that the growth and sporulation of *C. truncatum* were recorded at 30°C followed by 25°C while it was minimum at 40°C. Maximum dry weight of mycelium and sporulation was recorded at 6.5 pH. However, optimum growth of fungus was also observed at pH of 5.5 to 7.0. Exposure of the fungus to alternate cycles of 12 h light and 12 h darkness resulted in the maximum mycelial growth (81.78 mm) of *C. truncatum* compared to the 24 h exposure to either continuous light dark (67.35 mm) or (60.58 mm).

P46

Pathogenic behaviour of *Alternaria alternata* and phytotoxicity of its culture filtrates on *Lepidium sativum*

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Alternaria alternata causing leaf spot in *Lepidium sativum* was isolated and purified from diseased leaf tissues collected from collected from the Medicinal and Aromatic plant garden, Department of Crop and Herbal Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.) isolated and purified on potato dextrose agar media. Microscopic examination of a seven days old culture revealed hyaline, septate and branched mycelia, conidiophores with 30.0-80.2 µ length and 3-6 µ width and obclavate to obpyriform conidia (23-30 × 9.2-12.7 µ) with short conical beak arranged in acropetal fashion. The isolated culture and its culture filtrates were inoculated to germinated seedlings of chandrasur and also incubated with healthy leaves in a growth chamber. Typical symptoms of Alternaria leaf spot was observed both in *in vivo* and *in vitro* inoculated plantlets and detached leaves respectively. Chlorosis on the hypocotyls and leaves were observed. *A. alternata* was consistently reisolated from symptomatic leaf tissues on PDA. Thus, an efficient and reliable screening method wherein the effect of the selection agent (pathogen culture, culture filtrate/phytotoxin) was demonstrated providing sound pharmacological rational in terms of micro propagation and development of *Alternaria* resistant *L. sativum*, an important medicinal herb.

P47

Biodiversity of fungal endophyte communities inhabiting the *Ventilago denticulata*

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Ventilago denticulata is known to possess medicinal properties. Medicinal plants harbour endophytic mycoflora. *V. denticulata* has been studied for its endophyte biodiversity and their potential to produce bioactive secondary metabolites. There is a need to understand the

Session II
PATHOGENOMICS, PROTEOMICS AND HOST-PATHOGEN INTERACTION

LEAD LECTURES

L6

Omics as a tool for understanding and managing the late blight disease of potato

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The stress due to fungi is one of the most crucial factor that affect plant growth and development and consequently the crop productivity. The *Phytophthora infestans* is one of the most confounding pathogen and model organism. It exhibits high evolutionary potential and rapidly adapts to changing environment responsible for global annual crop loss of US$12 billion. Despite over a century of potato resistance breeding, fungicide use, and other control measures, it still continues to be a major threat to sustainable potato production worldwide. *P. infestans* population has undergone drastic change during the last two decade. New population has emerged which is more diverse having more pathotypes, carrying the new mating type (A2), haplotype etc. The whole genome of *P. infestans* (240Mb) has been sequenced which is several fold larger than those of related species like *P. sojae* (95 Mb) and *P. ramorum* (65 Mb). It has been proved beyond doubt that, the dynamism in *P. infestans* is mainly due to the movement of transposable elements and covers 74% of the genome. During interactions, both host and pathogen secrete complex proteins for surveillance, assault, and defence and counter defence activities. Similarly, the potato genome (845Mb) containing 39045 protein coding genes and more than 700 R-genes have been identified which play crucial role in understanding host-pathogen interaction. Advances in science of molecular biology, high-throughput analytical disciplines like genomics, transcriptomics, proteomics etc. have emerged to gain deep knowledge about the secretome in understanding and managing the pathogen and the disease. Several hypotheses demonstrated that *P. infestans* RXLR effectors are candidate Avr genes and are functionally profiled on Solanum to detect corresponding R genes. Potato-Phytophthora interaction studies demonstrated that *P. infestans* secretes necrosis like proteins (PiNPP1, INF1 and Elicitin) which play a role in inducing hypersensitive response through interacting with SGT1 and R genes. Recently, European groups have identified the RXLR (520) and CRINKLER (419) effectors and 19 novel pathogenicity genes including unexpected hypothetical genes from the whole 240 Mb genome architecture through microarray. This has opened new vistas for developing resistant varieties through molecular approach. In Indian Scenario, CPRI has identified and cloned 10 defense genes from the LB resistant Kufri Girdhari cultivar and five pathogenicity genes from LB susceptible cultivar through cDNA microarray analysis. RNA sequencing studies have shown expression of defence genes in the background of RB transgenics. Similarly, CPRI demonstrated RB regulated defense system in Kufri Jyoti RB transgenic hybrids and identified R and R like genes in leaf tissues through cDNA microarray. Based on transcriptomic analysis, we have

demonstrated the pathogenicity of effector genes through dsRNA transfection studies which would be used for development of RNAi based fungicide against *P.infestans*. Based on comprehensive analysis, RNAi transgenics have been developed by using *P. infestans* Avr 3a gene. It is now evident that knowledge of omics is essential to understand the secretome of both host and pathogen to develop suitable management strategies.

L7

Pathogenomics for plant disease management with special reference to potato late blight

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Microbes and plants have co-evolved in divergent ways ever since the first appearance of vascular land plants. However, the destructive nature of plant-microbe interaction became evident only after the beginning of organized agriculture about 10,000 years ago. Ravages of plant disease are documented in almost all ancient literatures and presently about 12% of the global crop produce is lost every year due to diseases. This loss is more acute in developing countries where food security is already in a desperate situation. It is necessary to reduce this loss as much as possible to facilitate and supplement food security. Since disease is a manifestation of deviation from normal physiology, concerted efforts are being made to understand its molecular biology so as to get a handle over it. It was, however, a daunting task to understand the complex and illusive nature of host-pathogen interaction before the era of pathogenomics that attempts to utilize genomic and metagenomics data to understand microbe diversity and interaction as well as host-microbe interactions. Plants have evolved two complementary strategies for defending parasitic colonization. The basal immunity recognizes the pathogen associated molecular patterns (PAMPS) that triggers PAMP triggered immunity (PTI). Pathogens also evolved a mechanism to suppress the PTI using a cache of effectors. In the next layer, resistant plants evolved elaborate effector triggered immunity (ETI) that recognizes the effector molecules of the pathogens through R-proteins. This situation led to a coevolutionary arms race between pathogens and plants resulting in generation of large repertoires of cytoplasmic effectors that are collectively essential but individually dispensable and plant defenses that possess parallel redundancies. Pathogenomics facilitated better understanding of this coevolutionary arm race. It has been demonstrated that the 240 Mb *Phytophthora infestans* genome encodes a complex families of effector proteins that are grouped into two broad categories: apoplastic effectors that accumulate in the plant intercellular space (apoplast) and cytoplasmic effectors that are translocated directly into the plant cell by a specialized infection structure called the haustorium. Apoplastic effectors include secreted hydrolytic enzymes such as proteases, lipases and glycosylases

that probably degrade plant tissue; enzyme inhibitors to protect against host defence enzymes; and necrotizing toxins such as the Nep1-like proteins (NLPs) and PcF-like small cysteine-rich proteins (SCRs). The cytoplasmic effectors belong to two major classes, i.e. RXLR and Crinkler (CRN). At least 563 RXLR and 196 CRN genes have been predicted in the *P. infestans* genome. Both CRN and RXLR genes typically occur in repeat-rich, gene-sparse regions of the genome. On the other hand, 408 NBS-LRR class disease resistance related proteins have been predicted in 844 Mb potato genome. With availability of genome information of *P. infestans* as well as potato, ongoing advances in bioinformatics would facilitate innovative strategies for effective management of late blight. Role of pathogenomics in effective plant disease management will be discussed in this presentation taking the example of potato late blight.

L8

Pathogenomics: how can it help in understanding host specificity and pathogen evolution?

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The next-generation sequencing technologies provide new opportunities to study pathogens and the hosts they infect. The increasing availability of crop and pathogen genomes is providing new insights into pathogen biology, population structure and pathogenesis which in turn provide new opportunities for disease management. *Puccinia* spp. and *Cochliobolus* spp. are highly aggressive biotrophs and necrotrophs respectively causing enormous losses to cereal crops. All the three wheat rusts, yellow rust (*Puccinia striiformis*), brown rust (*P. triticina*) and stem rust (*P. graminis tritici*) affect wheat crop in India and importance of each rust is region based. A number of pathotypes exist in each rust pathogen and there is coevolution happening in pathotypes parallel with resistance genes. The full genome sequences of a number of pathotypes are available globally. The comparative genome sequence analysis together with transcriptome data analysis offers possibilities for understanding the evolution of races and detection of expressed genes in the host. Similarly, many *Cochliobolus* species have emerged rapidly as devastating pathogens due to HSTs. The genome sequences of *Cochliobolus* and related pathogens that differ in host preference, host specificity, and virulence strategies are available. The comparative analysis, at the whole-genome level with emphasis on genes for secondary metabolism and small secreted proteins highlight how pathogens develop and utilize these tools to adapt to a particular host or lifestyle. Necrotrophs and hemibiotrophs employ contrasting mechanisms of promoting the disease, but utilization of HSTs and protein effectors still overlaps. The diversity in secondary metabolite and SSP genes in each pathogen reflects enormous diversity among these species. But these genes are remarkably conserved among isolates of the same species. The

gene products, particularly those associated with unique genomic regions, are candidates for pathogenic lifestyle differences. Therefore, key component is understanding determinants of virulence.

L9

Host-pathogen interaction in sugarcane - red rot pathogen: role of 3-deoxyanthocyanidin biosynthesis pathway enzymes

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Among the various biotic and abiotic stresses faced by sugarcane, red rot caused by the fungus *Colletotrichum falcatum* is the major constraint, leading to severe yield losses. Sugarcane produces reddish pigment during the host pathogen interaction which probably contains 3-deoxyanthocyanidin phytoalexins synthesized *de nova*. To understand the phytoalexins role in red rot resistance, detailed studies were conducted through HPLC assays and genomic approach, with a set of sugarcane varieties varying in red rot resistance. Our results clearly established that resistant varieties accumulated greater levels of phytoalexins as compared to the susceptible ones during pathogenesis. Induction of 3-deoxyanthocyanidin compounds *viz.*, apigeninidin, luteolinidin and cyanidin along with uncharacterized compounds of varying concentrations related to category of disease reaction on resistant (R) and susceptible (S) types was found. The differential accumulation of phytoalexin compounds against the pathogen colonization in compatible and incompatible interaction evidenced their active role in disease resistance by restricting pathogen progression in the latter. Subsequently studies were carried out to assess expression of transcripts involved in flavonoid biosynthesis pathway through RT-PCR revealed differential transcript expression of chalcone synthase, chalcone reductase, flavanoid 3'5' hydroxylase, dihydrofolate reductase and 4-coumarate Coenzyme A ligase among resistant and susceptible varieties after pathogen inoculation. However, phenylalanine ammonia lyase, coumarate-4hydroxylase, isoflavone reductase and chalcone isomerase transcriptswere found to be constitutively expressed in both varieties. The results signify specific regulation of transcripts in resistant varieties as compared to the susceptible, as active phytoalexin induction and accumulation during pathogen intrusion which leads to restricting pathogen invasion in resistant plants.

POSTERS

P48

Effect of mycotoxins on pigment synthesis in mustard seeds

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Mycotoxins are toxic secondary metabolites produced by fungi that usually belong to the genera like *Aspergillus, Penicillium* and *Fusarium*. Some mycotoxins particularly aflatoxin, citrinin and zearalenone have been analysed as natural contaminants of various crops including mustard (*Brassica juncea* L.) from Bihar state at different stages of the crop development, harvesting and storage. In this investigation, effects of five different concentrations (*viz.*, 100, 250, 500, 1000 and 2000 µg/L) of aflatoxin B1, citrinin and zearalenone were evaluated against synthesis of different pigments (*viz.*, chlorophyll and carotenoids) in the emerging leaves of mustard seeds (var. Pusa bold) during seedling growth. The inhibitory effects of those myctoxins were observed at all concentrations which were directly correlated with the concentration of the treated toxins. Maximum inhibitions in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were 78.5, 56.7 67.6 and 84.9% in aflatoxin treated seeds whereas 57.8, 38.3, 61.9 and 68.7% and 64.4, 42.7, 66.5 and 76.1% inhibitions were recorded by citrinin and zearalenone, respectively.

P49

Pathogen modulation on host machinery for gaining virulence

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Plants live in a very complex environment where they are continuously under threat of pathogen infection. To combat these challenges plants undergo a huge reprogramming in gene expression profile especially, stress responsive genes. In our work, we have characterized role of a Polycomb Repressive Complex 2 (PRC2) member MEDEA, in pathogenesis. PRC2 complex function as a chromatin remodeler and repress transcription of target genes. MEDEA, a histone methyl transferase, has established role in embryo development and express only in female gametophyte and early embryo. We found this gene to be highly induced during pathogen

challenge in leaves. P35S:MEDEA plants are susceptible for bacterial pathogen as well as over expression plants are defective in SA, JA and ABA defence signalling. It has lower expression of various SA and JA defence signaling marker genes as well as lesser accumulation of SA in infected tissues. By using Yeast two hybrid assay and Bimolecular Fluorescence Complementation (BiFC), we have identified MEDEA interactor proteins, which are likely to influence immune response. Interaction of MEDEA protein with transcription factors as well as nuclear subcellular localization supports its role as a chromatin remodeler and a key protein in regulating the expression of target genes. The results uggested that MEDEA gene is an important regulator of disease defence.

P50

Expression analysis of defense related genes in resistant and susceptible cultivars of tomato against *Ralstonia solanacearum* treated with chemical and bio-inducer

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Ralstonia solanacearum, one of the most important bacterial pathogen of tomato, is a constant threat to this crop. In present study, expression pattern analysis of specific defense-related gene transcripts like PR-1a & GluA (salicylic acid pathway), Lox A (Jasmonic acid pathway) and PR-1b & Osmotin-like (Ethylene pathway) in resistant (Hawaii-7996) and susceptible (Pusa Ruby) tomato cultivars was assessed by using quantitative RT-PCR against the *R. solanacearum* strain UTT-25 (Biovar 3, race 1). The plants were treatment with chemical inducers of defense like Jasmonic acid (100 µM), and Salicylic acid (100 µM) with and without *Bacillus subtilis* (OD at 600 nm = 0.1) as bio-inducer prior to inoculation of pathogen i.e. *R. solanacearum* (10^9 cfu/mL). The expression analysis of all the genes was done at intervals of 6, 24, 48 and 96 (h.p.i). A combination of Jasmonic acid + *Bacillus subtilis* showed the high level of expression in all over defense related genes in resistant (Hawaii-7996) and susceptible (Pusa Ruby) cultivar of tomato plant. The overall differential expression pattern of PR-1a, PR-1b and Glucanase A gene showed up-regulation in susceptible (Pusa Ruby), whereas down-regulation in resistant (Hawaii-7996) cultivars of tomato. While, Lox A and Osmotin-like genes showed the up-regulation in resistant as compared to susceptible cultivar of tomato plant. Hawaii-7996 activated expression of these defense genes faster with a greater degree of responses to *R. solanacearum* infection than Pusa Ruby. Tomato plants infected with *R. solanacearum* was up-regulated in both ethylene (ET) and salicylic acid (SA) defense-related pathways interactions. In addition, Pusa Ruby triggered noticeably less production of defense-associated reactive oxygen species

(ROS) in stems and leaves than resistant cultivar, despite attaining similar cell densities in th plant. ROS activity was found more inresistant as compared susceptible cultivar of tomato after 24 h of inoculation, while, less production of ROS was found in untreated with pathogen as control. Collectively, these data suggested that the bacterial wilt-resistant plants can specifically recognize induction from *R. solanacearum*.

P51

Cloning and characterization of two detoxifying enzymes, glutathione s-transferase and carboxylesterase, from burrowing nematode (*Radopholus similis*)

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Radopholus similis Cobb Thorne is a migratory endoparasitic nematode infesting several tropical and sub-tropical plant species. Computational screening and conserved domain annotation of assembled EST sequences from the burrowing nematode *R. similis* revealed in seven contigs similar to glutathione S-transferase (GST) and four contigs for carboxylesterase (CES). One each of the contigs corresponded to each genes were cloned from *R. similis* cDNA (KM670018, KP027005) and characterized by phylogeny and structural motif comparison. Glutathione S-transferase is critical antioxidant and detoxification enzymes to degrade toxic substance while carboxylesterase is responsible for controlling the nerve impulse, detoxification, various developmental functions. This makes both the proteins as major target of pesticides and chemical warfare agents for management of plant parasitic nematode. The comparative structural analysis and molecular studies of these target enzymes that are broadly distributed in plant parasitic nematodes, will pave the way to new control regimes for nematode parasites specific to plants. We report for the first time the presence and amplification of two novel target genes (GST and CES) from *R. similis*. The two genes were compared with corresponding genes reported in other nematodes through structural motif characterization indicates the structural diversity of the conserved motifs present in RsGST and RsCES proteins. The search for protein signature motifs through InterProScan analysis confirmed the presence of thioredoxin-like fold (IPR012336), glutathione S-transferase, N-terminal and C-terminal (IPR004045, IPR010987), and glutathione S-transferase domain (PF00043) for RsGST and Carboxylesterase, type B (IPR002018), Alpha/Beta hydrolase fold (IPR029058) and Carboxylesterase domain (PF00135) for RsCES. The 3D protein models of each protein were developed through homology modeling and active-sites constituting the pharmacophore residues were predicted for future study.

P52

Analysis of potato genes involved during ToLCNDV-potato infection using transcriptome sequencing approach

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Apical leaf curl disease caused by tomato leaf curl New Delhi virus-potato (ToLCNDV-potato) is one of the most important viral diseases of potato. Identification of resistance source of this virus is critical in order to develop resistant varieties. Earlier reports suggest that under high vector population pressure, continuous use of same seed stock leads to lowest seed generation in Kufri Bahar while other varieties showed faster degeneration under field conditions. The variety Kufri Bahar also showed a delay in symptom expression by 15-20 days and developed no or only mild symptoms with very low virus load under artificial inoculation conditions. Hence, to know the mechanism behind the resistance, an experiment was carried out to study the genes expressed during ToLCNDV-potato infection in susceptible cultivar, Kufri Pukhraj along with Kufri Bahar using transcriptome sequencing approach. These cultivars were artificially inoculated with ToLCNDV-potato and samples were collected at different time intervals. Symptom expression was started 15 days after inoculation in Kufri Pukhraj and severe thereafter, whereas it was delayed up to 35 days and only mild symptom was observed in Kufri Bahar. Transcriptome libraries were prepared and sequenced using the Ion ProtonIM System. Analysis using potato genome from PGSC showed that genes were differentially regulated during ToLCNDV-potato infection in susceptible and resistant cultivars. This study provides genome-wide transcriptional analysis details of two potato cultivars differing in resistance level to ToLCNDV-potato.

P53

Understanding the effector proteins associated with Potato-*Phytophthora infestans* interactions through transcriptional analysis

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Late blight (*Phytophthora infestans*) is one of the dreaded diseases of potato throughout the world. To understand the nature of effector proteins that subvert the plant defense and

promote the pathogenicity, plants of a highly resistant (Kufri Girdhari) and susceptible (Kufri Bahar) cultivars were challenge inoculated with *P. infestans* and sampled at 0, 24 and 72 h after inoculation. To analyse the transcript expression, we performed a comprehensive transcriptional analysis using cDNA microarrays, containing 39,083 potato protein coding genes as well as 18,034 oligo probes of *P. infestans* in a single 12X135K Nimblegen array slide. Data revealed that out of 39,083 potato protein coding genes, 5,308 genes were up-regulated upon *P. infestans* contact in resistant cultivar. Out of 5,308 genes,11 genes (>5 fold expression) and 30 genes (>5 fold) were highly up-regulated at 24 h and 72 h after pathogen interaction, respectively. On the other hand, in susceptible cultivar only *P. infestans* effector genes expression was observed at higher level. Real time PCR and dsRNA transfection assay demonstrated quantification and validation of defence and pathogenicity nature of the potato and pathogen genes, respectively. Pathway maps were constructed to graphically represent the gene expression patterns which showed association of several events involved in biotic and abiotic stresses. This comprehensive transcript analysis provided useful information on nature of host and pathogen effector proteins in defense and counter-defense activities and provides a framework in developing resistant varieties.

P54

Effect of Trichoderma-treatment on the resistance of Castor bean against *Fusarium oxysporum ricini*

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Vascular wilt disease, caused by *Fusarium oxysporum* fsp *ricini*, is one of the major diseases of castor that results in yield reduction of 39 to 77%. Trichoderma is known to exert biocontrol activity against phytopathogens by producing antibiotics, siderophores, hydrolytic enzymes etc. Different *Trichoderma* species, [*Trichoderma harzianum* (Th4d), *T. asperellum* (N13, TV5, 7316)] were screened for the management of *Fusarium* wilt in castor. Experimentation was carried out using comparable concentrations of *Trichoderma* and *Fusarium* using a susceptible castor genotype JI-35. Germination percentage and wilt incidence was recorded regularly. Soil samples were collected at different time points to quantify the load of *Trichoderma* and *Fusarium*, on respective selective medium. Wilt incidence was reduced due to Trichoderma Th4d treatment (21%) with differential response to the strains: N13 (36%), TV5 (43%) and 7316 (31%) while "Fusarium alone" disease severity was 73% in treated pots. Further, fewer Fusarium colonies (190000 CFU/g of soil) were observed in Th4d-treatment vs other strains of Trichoderma against F.o.r alone treated pots (540000 CFU/g of soil) indicative of the induced systemic reistance (ISR) by Trichoderma. Molecular analysis of the ISR in both Th4d treated and un-

treated castor seedlings further challenged with *Fusarium* was done by studying the differential gene expression using candidate genes OPR3, PR1 and PR2 by RT-PCR. The expression of these genes was seen to be highly elevated in Th4D alone and Th4D + F.o.r treated seedlings 24 hpi vs untreated and F.o.r alone treated seedlings, respectively. These results indicated the direct relationship of the molecular pathways involving ISR with the reduction of wilt in *Fusarium* infected castor seedlings treated with Trichoderma.

P55

Phytotoxin produced by *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* – A tool for pathogenesis in mango

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Toxic compounds produced by the pathogens *viz.,C. gloeosporioides* and *L. theobromae* were extracted and their role in pathogenicity was tested by inoculation of crude culture filtrates and differentially diluted filtrates on mango leaves. Crude toxins extracted separately by solvent extraction method were tested with mango leaves and fruits at different dilutions to confirm their role in pathogenecity. Non-host selective nature of the toxin was determined by electrolyte leakage, seedling and seed germination bioassay using nine non-host plants and three cereal seeds. The toxic compounds were extracted from the yeast extract and potato dextrose broths inoculated with *C. gloeosporioides* and *L. theobromae* respectively, by solvent diffusion method and partially purified by Thin Layer Chromatography (TLC) using standardized diluents di-methyl sulfoxide and mobile phase of chloroform: glacial acetic acid: ethanol (3:1:1) for *C.gloeosporioides* and butanol: water: glacial acetic acid (5:3:2) for *L. theobromae*. The toxic compounds partially purified by TLC were directly identified through Gas Chromatography / Mass Spectrometry (GC/MS). GC/MS analysis revealed that the presence of various toxic metabolites from the partial purified samples. Among them, spiro-1-(cyclohex-2-ene)-2'-(5'-oxabicyclo[2.1.0]pentane),1',4',2,6,6-pentamethyl-and oxalic acid were detected at higher relative abundance in all the replications from the *C. gloeosporioides* and *L. theobromae* respectively. These results suggest that the toxic compounds produced by pathogens were playing a major role in pathogenesis and symptom expression.

P56

Viral genome methylation is triggered by tomato *Ty-2* gene and restricted by *viral ac4*

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Cytosine methylation is considered as one of the epigenetic antiviral defense. Host factors having role in defense contribute towards activating methylation machinery and viral factors antagonizing this effect determines resistance and susceptibility. Tomato leaf curl viral genome gets methylated during infection. Here we show that cytosine methylation levels get triggered in presence of tomato resistant gene Ty-2. Transgenic expression of viral protein ToLCNDV-AC4, limits viral genome methylation in favor of virus. On the contrary enrichment of viral derived siRNAs in tomato transgenic, stimulate viral genome methylation suggesting their role in de novo methylation pathway. Constitutive expression of ToLCNDV-AC4 in tomato resulted in aberrations in phenotype, possibly due to AC4 interference in host DNA methylation. The possible role of tomato Ty-2 gene and ToLCNDV- AC4 gene in regulation of *de novo* methylation machinery has been discussed.

P57

Effect of *Trichoderma* mediated induced systemic resistance in castor bean against *Phytophthora parasitica* var *nicotianae*

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Castorbean (*Ricinus communis* L.) is a non-edible oil seed crop belonging to Euphorbiaceae family. Seedling blight disease, caused by *Phytophthora parasitica* var *nicoitiane*, is one of the major diseases of castorbean at the initial stages of growth of the plant. It results in 30 to 40% of seedling mortality, which may lead to severe crop loss under highly favorable conditions. *Trichoderma*, a mycoparasitic fungus belonging to Ascomycetes, is known biocontrol agent and its efficacy in inducing systemic resistance against phytophthora disease of castor-

bean was studied. Seeds of DCS107, a susceptible genotype for seedling blight, were used for the experiment. Seed treatment was given, independently with three *Trichoderma asperellum* strains (TaDOR-N13, -TV5, -7316) and one *Trichoderma harzianum* strain (Th4d). After the appearance of cotyledonary leaves, 10 day old discs of *Phytophthora parasitica* was placed on the abaxial side, of one of the two leaves. Leaf wetness, temperature (25o C) and humidity (about 70%) were maintained. Necrosis caused by the pathogen was measured at 48, 72 and 96 h post infection in three replicates each. When compared with untreated seedlings disease severity was reduced by 85.7% in Th4d-treated seedlings. TaDOR-7316 and -N13 showed 50% and 42.9% of disease reduction, respectively, over check.

P58

Critical balance between virus induced vacuolar processing enzymes and mitochondrial alternative oxidase regulates programmed cell death

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Programmed cell death (PCD), through hypersensitive response (HR) is an important defense mechanism in response to pathogen attack and its initiation begins with the recognition of the pathogen by the plant. Various factors like ROS, oxidative burst and stress signaling contribute to cell death in the form of necrosis. Role of mitochondrial oxidases are important in regulating oxidative burst and Vacuolar Processing enzyme (VPE), cysteine proteases are responsible for virus-induced hypersensitive cell death. In the present study, we have showed that the critical balance between the levels of AOX and VPEs during Groundnut bud necrosis virus (GBNV) in tomato governs virus tolerance. GBNV-NSs, a potent RNAi suppressor has a role in inducing VPEs through its localization in vacuoles. Transient expression of GBNV-NSs in *Nicotiana benthmiana* induces localized HR response similar to viral infection by stimulating VPE levels at infection site, although the systemic basal defense in the form of higher expression

of AOX was observed at systemic site. Overall our results indicated that the tomato genotypes with higher levels of AOX expression during viral infections are more tolerant and susceptible genotypes showed higher expression of VPE, leading to HR response. The possible resistance mechanism for GBNV in tomato through a delicate balance of VPEs and AOX at infection and systemic site has been discussed.

P59

Secretomics-based investigations to unravel plant-pathogen interactions in sugarcane

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Secretomics is an offshoot of Proteomics, which addresses specifically the secreted proteins under distinct physiological conditions on a spatio-temporal manner. Most of the fungi secrete an orchestration of effectors against the multitude of defense engineered by the host. Effectors can also elicit host defense, when it is recognized by certain intracellular receptors (R proteins), resulting in Effector Triggered Immunity (ETI). Given the importance of fungal effectors and secretory proteins in host-pathogen interaction, we have employed 2-DE-MALDITOF/TOF to analyze the secretome of *Sporisorium scitamineum* (the sugarcane smut fungus) in response to synthetic and host signals incorporated in a growth medium. Differential secretion of proteins indicated that the fungus has perceived the host-signals and altered its secretome accordingly. Protein spots identified in this secretome analysis *viz.*, α-1, 2-Mannosidase, Phosphatidylethanolamine-binding protein (PEBP), Glycoside hydrolase, Glucose oxidase, etc might be core effectors of this fungus, playing crucial roles in modulating host metabolism during its interaction with sugarcane. Similarly secreted molecular signatures of the sugarcane red rot pathogen- *Colletotrichum falcatum* has been decoded to act as pathogen associated molecular patterns (PAMPs). MS/MS analysis of the secreted PAMPs identified them as Eliciting plant response-like protein (EPL1), Blastomyces yeast phase-specific protein (BYS1) and three other novel proteins. Full length gene sequences encoding EPL1, BYS1 and a novel protein has been deduced and further characterization is under progress. Deciphering secretome during sugarcane-pathogen interaction is expected to answer critical questions in pathogen biology during their dynamic interaction with sugarcane.

P60

Microarray analysis of *Ralstonia solanacearum* induced gene expression in *Arabidopsis thaliana*

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Bacterial wilt caused by the soil-borne bacterium *R.solanacearum* race 4 is a lethal disease of plant belongs to Zingiberaceae family. We used Arabidopsis as a model system to decipher plant-bacterial interaction. The bacterium typically wilted the plantlets in a density dependent manner. Microarray based gene expression analysis was conducted to profile plant gene expression during bacterial colonization vis-à-vis wilt development due to pathogen colonization. In the first experiment, plantlets emerged from seeds of Col-0 inoculated with pathogen was used for gene expression studies. In another experiment, the apparently healthy 21 days old plantlets inoculated with decimal dilutions of bacterium in the root tip was sampled for gene expression analysis. Gene expression analysis was carried out in bacterized Col-0 ecotypes using ATH1 Affymetrix gene chips representing over 22,500 probe sets representing approximately 24000 genes of *A. thaliana*. Over 1080 Arabidopsis genes were found differentially expressed when roots were inoculated with *R. solanacearum* at 10^9 cfu of bacterial suspension, whereas 443 were differentially expressed at 10^8 cfu of bacterial suspension. For seedling emerged from the seeds inoculated with bacterium at 10^8 cfu, 85 genes were found differentially expressed. The up-regulated genes included genes related to abscission (MYB112, DOX1, SRG1), aging (YLS9, SRG1, TET6), defense and immune response (like ACO1, LOX1, PAD3, PBS3, PDF2.1, PDF2.5, PROPEP2, PR-1-LIKE, PR4, YLS5), auxin stimulus (ABCB4, AIR3, AIR1, PDR9, LBD29, IAA30, ASA1, HB53, LRP1, GH3.3), ethylene stimulus, jasmonic acid and salicylic acid biosynthesis and signalling, water deprivation (MAPKKK19, WRKY75, SUS1, ATDI21, WNK4, SUS3), tap and lateral root development process (ATEXT3, RALFL27, LBD29, IAA30, MAP18, ATMYB66, AHK5, LRP1, LBD18). The down regulated genes were defense related genes (WRKY18, LOX2, FMA, FLS2, LOX3, BGLU18, WRR4, MEKK1, RPM1, RPP4, RPP5, RLM3, MLO12, NSL1), ethylene biosynthesis (LOX3, ACS11, SCL13), salicylic acid (MYB4, MYB7, PIF4, MYB60, MYB95, ATMPK5, PRK, SBPASE, LCL1) and jasmonic acid signaling (JAZ7, JAZ5, JAZ8, JAZ10, OPR3, MYB60, SNG1, LOX3, AOC1, MEKK1, MYB4, LOX2, CYP94B3, SBPASE, VSP1), Shoot system development and morphogenesis (RPT2, LIN2, FT, KCS5, PRK, WRKY22, ATML1, SBPASE), programmed cell death (RABG3B, XCP2, NSL1, CAD1, XCP1, DND1). Genome wide expression data from our microarray analysis clearly indicated that the symptom expression was a consequence of altered expression of hundreds of plant development

and defense related genes. The expression data further highlighted the existence of distinctive as well as shared metabolic and defense pathways between the seed and root mediated inoculations. The expression patterns of a few selected genes were further confirmed by RT-PCR.

P61

Proteomic approach to identify the differentially expressed proteins in rice plants treated with fungicide premixture (Fenoxanil 5% and Isoprothiolane 30% EC) against blast disease

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A field experiment was conducted for two seasons in the year 2012 and 2013 to evaluate the newer premixture fungicide (Fenoxanil 5% and Isoprothiolane 30% EC), Fenoxil 20% SC, Isoprothiolane 40% SC and Tricylazole 75% WP with different concentrations against rice blast disease. Among these fungicides, application of premixture molecule 35% EC at the concentration of 1000 mL/ha significantly reduced blast incidence and increased the grain yield followed by the treatments tricylazole under glass house and field conditions. Analysis of defense molecules revealed that more accumulation of phenylalanine ammonia-lyase (PAL), Peroxidase (PO) and Poly Phenol Oxidase (PPO) were observed in premixture fungicide 35% EC (Fenoxanil 5% and Isoprothiolane 30% EC) treated rice plants challenged with blast pathogen. Two-dimensional polyacrylamide gel electrophoresis strategy was adopted to identify the fungicide mediated differentially expressed proteins and elucidate the molecular responses in three way interaction of fungicide-host-*M. oryzae* through protein profiling. The result revealed that totally 25 proteins spots were differentially expressed in rice plants under various treatments. Among the 25 proteins, eleven proteins were altered by the pathogen for successful disease development but it was arrested upon treatment with interaction of fungicide premixture+-pathogen treatments. In additions 14 proteins were up regulated in fungicide treated rice plants. However three proteins were newly expressed in fungicide alone applied plants. These results suggested that application of fungicide premixture significantly expressed the proteins in rice plants there by induction the resistance were occurred against blast disease. Hence, this study revealed that the performance of fungicide premixture 35% EC (Fenoxanil 5% and Isoprothiolane 30% EC) were highly effective against blast diseases and enhanced rice grain yield. From this study, proteomic strategy unravels the fungicide mediated mechanism of resistance against rice blast disease.

P62

Expression analysis of defense associated transcription factors expressed during *Piper colubrinum-Phytophthora capsici* interaction

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Transcription factors are master regulators of gene expression at the transcriptional level and are also implicated in plant stress response. PAMP triggered immunity (PTI) and effector triggered immunity (ETI) leads to biotic stress signaling involving induction of defence- related transcription factors like MYB, MYC and WRKY etc. Study on expression analysis of these transcription factors is very important to understand their importance during host pathogen interaction. In the present study quantitative RT-PCR analysis was done for three transcription factors *viz*, MYB, MYC and WRKY, to assess the transcriptional activity of these genes during *Piper colubrinum-Phytophthora capsici* interaction. Quantitative RT-PCR analysis revealed higher folds of expression of these genes at initial stages of infection when compared to later stages. MYB gene showed upto 1.25 fold expression at 4hpi, MYC gene showed 2.6 fold expression at 8 hpi and expression of WRKY gene was 1.65 fold at 4 hpi. The higher expression level of these transcription factors during early stages of infection highlights the important role played by them in the early signaling events during defence gene activation.

P63

Influence of temperature on symptom expression, detection of host factors in virus affected black pepper

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Expression of symptoms in black pepper (*Piper nigrum* L.) plants infected with Piper yellow mottle virus vary depending on the season, being high during summer months. Based on this observation we made this attempt to address the influence of temperature on symptom expression. Our controlled environment study revealed increase in virus titer, total proteins,

IAA and reducing sugars when exposed to temperature stress. There was change in the 2D separated protein before and after exposure to temperature stress. The 2D-proteomics-LC MS identified host and viral proteins suggesting virus- host interaction during symptom expression. The analysis/detection of host biochemical compounds, proteins and the information from this study will help further our understanding of the detailed mechanisms underlying the viral replication and damage to the crop to possibly develop management strategies.

P64

Selection of reference gene for transcript normalization and expression analysis of defense related gene in *Piper nigrum* - *Phytophthora capsici* pathosystem

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A systematic validation of reference genes is a prerequisite for any gene expression studies and validated reference gene for gene expression analysis in *Piper nigrum* is lacking. In the present study, the leaf transcriptome available for a moderately resistant variety to *Phytophthora capsici* was used and the annotated reference genes namely Actin, Glyceraldehyde phosphate dehydrogenase, β-Tubulin, Ubiquitin conjugating enzyme (UbCE), 18s rRNA and Elongation factor-1-α were used to identify the stable reference gene for qRT-PCR based gene expression analysis of identified transcripts of *P. nigrum- P. capsici* pathosystem. A virulent isolate of *P. capsici* (05-06) was used to challenge inoculate moderately resistant and susceptible varieties of black pepper and cDNA synthesized from total RNA extracted at different time courses were used as template. The selection of stable reference gene was done with different algorithms (geNorm, NormFinder and Bestkeeper) along with Reffinder. PnGAPDH/ Ubiquitin conjugating enzyme (PnUbCE) was optimised as set of genes that can be used as internal control for expression in leaf tissue upon *P. nigrum- P. capsici* interaction. The expression patterns of locus_3839 which codes for 1, 3 beta glucanase (PnBGlu) was analysed with most stable reference gene combination PnGAPDH and PnUbCE at different time courses ranging from 0.5 hpi to 72 hpi. Constitutive expression of β 1, 3-glucanase was observed in IISR Shakthi and the maximum transcript abundance of PnBGlu occurs at late hours of infection (72 hpi). In contrast in the susceptible variety, the constitutive expression was absent and only downregulation of PnBGlu was observed. The possibility to identify excellent reference genes for a non model crop *P. nigrum* from the transcriptome data was elucidated. The differential expression of 1,3 beta glucanase in two test genotypes provides the underlying mechanism of defense against the oomycete pathogen, *P. capsici* in black pepper.

P65

Elucidating disease resistance response associated transcriptome of tomato against bacterial wilt

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Ralstonia solanacearum is a phytopathogenic bacterium that causes wilt disease of tomato (*Lycopersicon esculentum* Miller). During bacterial wilt pathogenesis, *R. solanacearum* infects roots and effectively colonizes the host plant xylem tissue, causing wilting of the whole plant. Because of its wide host range and soil borne nature, sustainable control of the disease is difficult. Plant resistance or susceptibility is dictated by the genetic backgrounds of both the host plant and the invading pathogen.Therefore, a better understanding of the mechanism of the resistance responses in the host is essential to combat the disease. An attempt has been taken to identify various host functions which are important for either providing or inducing resistance against this bacterium by transcriptome analysis. However, for this bacterial disease of tomato, genes imparting resistance to this pathogen has not been reported till date. Tomato germplasm have been collected from north eastern states and also from other parts of the country. We have characterized the resistant and susceptible genotypes by following standard methods of pathogenicity assays on tomato plants followed by studying the changes in antioxidative enzymes and increase in proline content after challenging with *R. solanacearum*.To understand the mechanism of resistance, we will perform tomato gene expression profiling following *R. solanacearum* infection in susceptible and resistant accessions to identify tomato genes expression profiling after challenging with the bacterium. Functional studies of some important up or down regulated genes by overexpressing and underexpressing would shed more light on mechanisms of resistance against this pathogen.

P66

Exploring microRNA-like small RNA in yellow stripe rust *Puccinia striiformis tritici*

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The advancement in sequencing techniques has made it possible to study small RNAs like miRNA, siRNA etc. comprehensively and efficiently. MicroRNAs are short (~20-24 nucle-

otides) non-coding RNAs that negatively regulate the gene expression post-transcriptionally in animals and plants. This negative regulation can be either by degradation of messenger RNA (mRNA) or by inhibition of protein translation. The existence of miRNAs is well studied in plants and animals but in fungi, the information is limited. Here, we report the prediction of miRNAs in fungus *Puccinia striiformis tritici* (Pst) using EST data available in NCBI. *In silico* approach has been used to predict the miRNAs and their hairpin precursors in this fungal pathogen. A total of 7550 ESTs were available in NCBI for *P. striformis* which were pre-processed using a perl program named 'ESTtrimmer' and then assembled using CAP3 assembly program and resulted into 6106 assembled ESTs.Of 6106 ESTs, 1215 precursor miRNAS were predicted that resulted into 120 miRNAs of which 71 were unique sequences. We identified targets of 120 predicted miRNAs. For target identification, 6106 initial EST sequences of *P. striformis* were used. Of these, 3167 ESTs were scanned for sequence complementary sites on hit sequences from BLAST (allowed 4 mismatches). These complementary targets were allowed to fold and their secondary structures were analysed for efficient folding that results into stable complex with lower MFE (minimal free energy). Of these, 232 potential targets were annotated successfully. The targets identified were mostly from fatty acid metabolism and its biosynthesis, signaling pathways, amino acid metabolism, purine metabolism, basal transcription factors, etc. It is well known that PAMP-triggered immunity (PTI) plays a vital role in the resistance of plants to numerous potential pathogens. Our further study will include experimental validation of these predicted miRNAs to evaluate the effectiveness of our method. Our findings will improve the understanding towards the role of these small RNAs in fungal kingdom and pathogenicity of Pst.

P67

Sequential events in the infection process of *Colletotrichum gloeosporioides* in black pepper

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Colletotrichum gloeosporioides (Penz.) Penz. and Sacc., the ascomycetous fungus which incites anthracnose/ fungal pollu disease is a major challenge to black pepper production, especially under misty weather conditions. It is reported that, related species of *Colletotrichum* adopts a hemibiotrophic strategy to establish compatible parasitic relationship with several crops. However, informations on sequential events involved in the infection process of *C. gloe-*

osporoiodes in black pepper are scanty and hence, the present study was devised. The conidial suspension (1×10^6 spores/mL) prepared from sporulating colony were sprayed on young leaves of the variety, Panniyur-1 and incubated under high humidity conditions. The leaf samples collected at 2 h period till 24 h and later at an interval of 24 h upto 168 h after inoculation were subjected to staining, destaining and microscopically examined. Conidial germination was observed 4 h after inoculation. The germinating conidia were found congregating more towards stomatal region and 75% of conidia germinated either with one (most cases) or two germtubes after 12–14 h. Higher percentage of germination was noticed, when the conidia were in disaggregated condition. The melanized clavate/spherical shaped appressoria differentiated from germ tubes had more affinity towards guard cells. The infection hyphae originating from appressoria entered through stomata and subsequent intra/intercellular invasion was observed. Invading hyphae in the mesophyll cells and localized tissue death were noticed after 22 h. Acervulus initials were formed and mature acervuli were observed after 120 h and 144 h, respectively. Acervuli with setae were observed after 144 h and 168 h after inoculation. Several localized necrotic spots manifested on leaf surface after 72 h and invaded epidermal cells turned brown, resulting in rapid collapse and cell death. The present investigation shed light into the sequential events involved in the infection process in black pepper-*C. gloeosporoiodes* pathosystem, which might facilitate in elucidating the mechanism of host plant resistance, physiological specialization of the pathogen and helps to decipher cascade of events associated with host-pathogen interaction.

P68

Host pathogen interaction in bacterial wilt pathogenesis

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Ralstonia solanacearum causes bacterial wilt, which is one of the most important and widely spread bacterial diseases of solanaceous crops in the tropics, subtropics, and warm temperate regions of the world. Extracellular polysaccharides are specific elicitors which have been used in our study of differential expression and some experiments have suggested that certain plants can recognize EPS from specific bacteria which acts as specific elicitor to prevent bacterial diseases by activation of Induced systemic resistance. Extracellular polysaccharide (EPS) were extracted from *R. solanacearum* strain DOB-R1, the partially purified EPS was air-dried. EPS was quantified by the Elson-Morgan assay for hexosamine sugars using N-acetyl-galactosamine as the standard. The chemical characterization of the different EPSs was carried out using spectroscopic methods, purified through HPLC and mass of the compounds were determined by LC-MS. Complete structural variation of isolated compound, in NMR (H1, C13, COSY, TOSY, NOSEY and HMBC) was also elucidated. The concentration of H_2O_2 was two

folds higher at 12 hpi than in control when treated with EPS and antioxidant enzymes *viz.*, APX, GR, MDHAR and DHAR were rapidly increased in susceptible and highly susceptible (HS) cultivars upon EPS treatment. The growth of *Ralstonia solanacearum* in plant tissues, particularly during disease development, probably leads to the formation of colonies that can be linked to biofilms. Very little is known about the factors required for biofilm formation in *R. solanacearum*. Isolates were tested for biofilm formation using a microtiter plate method. The differences in the formation of biofilm among the *R. solanacearum* isolates were studied using cell free extracts. Structure elucidation of the compound responsible for these differences was achieved using NMR and MS techniques. Proteomic approach was employed to identify the differentially expressed defense related proteins between the bacterial wilt resistant and susceptible chilli (*Capsicum annuum*) cultivars when subjected to biotic stress. Anugraha and Pusajwala cultivars were considered as resistant and susceptible cultivars, respectively. Proteins were extracted from leaves of 3-week-old seedlings of the resistant and susceptible cultivars and separated by 2-DE (Two dimensional electrophoresis). Our results indicate that a total of 400 protein spots are successfully identified under biotic-stress conditions (by PDQuest software analysis), out of this 70 protein spots are differentially expressed between the resistant and susceptible cultivars.

Session III

HOST PLANT RESISTANCE AND BIOTECHNOLOGICAL APPROACHES

LEAD LECTURES

L10

Role of microRNA in the pathogenesis of tomato leaf curl New Delhi virus

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Increasing evidences in recent years indicate that the symptom development in viral pathogenicity is due to altered host gene expressions which in turn are regulated by microRNA. miRNAs regulate various aspects of plant development such as leaf, root and flower development *via* post transcriptional control of many transcription factors. Whether begomovirus pathogenicity involves microRNA and whether the levels of selected microRNA are altered under begomovirus pathogenicity were examined .To determine altered expression of miRNA and to ascertain their role in symptom development, we had selected the system of *Nicotiana benthamiana*/Tomato leaf curl New Delhi virus (ToLCNDV)-[Luffa] and Luffa leaf distortion betasatellite (LuLDB) which is constantly associated with it.This system was chosen as the inoculation of the ToLCNDV-[Luffa] isolate produces clear symptoms in 10dpi. The PTR constructs were found to be highly infectious (100% infectivity) in *N. benthamiana*. However, by 21 dpi, newly developed leaves of systemically infected plants exhibit reduction in symptom severity (recovery). Therefore, in this system, recovery from symptoms due to RNAi and re-appearance of symptoms due to suppressor activity (silencing of RNAi) and its correlation with microRNA can be studied. In this study, we have done microRNA profiling of the host to assess the transcriptional variation in healthy (mock), symptomatic, recovered and symptom re-appearance stage leaves following betasatellite inoculation.ToLCNDV-[Luffa] DNA A alone infected plants developed only mild downward leaf curl in newly emerged leaves within 4 dpi, while DNA A and DNA B developed downward leaf curling. By 14 dpi, the symptoms developed into downward leaf curling, petiole curvature and stunted. By 21 dpi, these symptoms had become considerably attenuated in newly emerging leaves. It remained attenuated and, by the end of the experiment (30 dpi), leaves were essentially symptomless. In contrast to the plants inoculated with DNA A alone or DNA A and DNA B, the plants co-inoculated with LuLDB either the combination (DNA A and LuLDB or DNA A, DNA B and LuLDB) remained symptomatic ever since the initiation of symptoms for the duration of the experiment and led to the death of the plant. The leaves become extremely curling, reduced leaf lamina, and interveinal chlorosis intensified completely occupied the entire leaf lamina and the leaves became bleached. There was no recovery in the plants where there was infection with betasatellite, LuLDB.Alteration in the levels of all the miRNAs were analyzed in the study; one important observation of our experiment was with ToLCNDV in DNA A alone inoculated plants. When compared to the mock inoculated plants increase in the levels of miR159, miR164, miR167, miR171, miR319 and miR393

(varying from 3–10 folds) at symptom initiation, severe symptom expression and also at the recovery stage of infection. At symptom re-appearance stage when betasatellite was inoculated, the magnitude of increase was brought down in all miRNA except miR164. Co-inoculation of betasatellite with DNA A or DNA A and DNA B resulted in up- regulation of the five miRNA, miR159, miR167, miR171, miR319 and miR393.Study on the regulatory pathways of the miR159, miR319 and miR393 might help to elucidate the molecular details of some of the signal interaction events between the viral pathogenicity and phytohormones as miR159,miR319 and miR393 are involved in ABA and auxin signalling.The results on micro RNA assume greater importance in the context of recent finding that accumulation of miR393 contributes to plant resistance against bacteria by negatively regulating the mRNA level of F-box auxin receptors TIR1, AFB2 and AFB3.

POSTERS

P69

Chickpea wilt and its management through resistant breeding

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Chickpea (*Cicer arietinum* L.) is the world's third most important pulse crop. It is well known that pulses are the best and a cheapest source of protein in the human diet and especially for vegetarian. Among the important diseases of chickpea wilt is the severe in the central zone of India. The yield loses due to the disease are ranges from 10-50%.To develop *Fusarium* wilt resistant the genotypes, crosses have been made using Line ×tester design by using four lines *viz.*, BDN 9-3, BDNG 797, Vijay and Digvijay and six tester donor parents for *Fusarium* wilt *viz.*, BCP 23, BCP 59, BCP 27, BCP 45, BCP 26 and BCP 69 during Rabi 2013-14. Twenty -four F1's along with the susceptible check JG 62 were screened in the wilt sick plot. Among F1's Digvijay × BCP 27 (0.0%), BDBG 797 × BCP 59 (1.65%), Vijay × BCP 45 (1.78%), BDN 9-3 × BCP 26 (1.92%), Vijay × BCP 27 (2.63%) and BDNG 797 × BCP 27 (2.75%) were resistant for *Fusarium* wilt. The wilt incidence of susceptible check was 100%. The back cross breeding program is being implemented to develop back cross progenies during Rabi 2014-15 for further studies on the inheritance pattern of *Fusarium* wilt of Chickpea.

P70

Identification of diploid cotton genotypes against *Fusarium oxysporum* f.sp. *vasinfectum*

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Cotton wilt incited by *F. oxysporum* f.sp. *vasinfectum* is one of the most serious disease inflicting unaccountable quantitative as well as qualitative losses. A total of 240 diploid cotton genotypes were evaluated under glasshouse and field condition by Pune technique. In seedling Resistance Test (SRT), the earthen pot of 15 × 15 cm size were filled with wilt sick soil and dibbled with 10 seeds /pot. The germination count was recorded after 7 day after sowing and the seedling were observed for wilting upto 6 weeks. The virulence of the *Fusarium* organism determined by "Pilot Test" in which highly susceptible cultivar of DH2 was sown. When cotton

seedling of susceptible cultivars showed more than 80 per cent wilting within 6 weeks, wilting per cent of seedling was worked out. In Adult Plant Resistant Test (APRT), Seedling showing resistant reaction (< 15 per cent wilt incidence) in glasshouse at SRT was transplanted in wilt sick plot. Maximum 20 plants were maintained and wilting was observed and recorded till plants attain maturity. Further the plants which do not show wilting up to maturity are uprooted and cut opened longitudinally for recording vascular discoloration. The discoloration is graded as 1) resistant: >50% plants showing hyaline reaction. 2) Susceptible : <50% plants showing hyaline reaction. Result showed that all the genotypes screened, exhibited varied degree of reactions against *F. oxysporum* f.sp. *vasinfectum*. Among the 240 diploid cotton genotypes, only ten genotypes showed resistant reaction and 16 genotypes showed moderately resistant reaction in seedling resistant reaction. Out of these 26 cotton genotypes, 24 genotypes *viz.*, JLA 0606, JLA 0715, Das 385, GBhv 229, GBhv 253, GBhv 255, GBhv 277, Digvijay, GVhv 655, GVhv 637, G. Cot 19, AKA 07, JLA 794, JLA 802, F1-11-9, F1-11-17, F1-11-18, F1-11-20, F1-11-21, F1-11-23, PA 11, RAC 024, BN 1, RCH 2, NHH 44, Mallika shows resistant reaction in Adult Plant Resistant Test.The findings of present investigation are the identification of resistant sources of diploid cotton genotypes against *Fusarium oxysporum* f.sp. *vasinfectum*.

P71

Assessment of partial resistance in Indian wheat cultivars and postulation of stripe rust resistance genes

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Stripe (or yellow) rust, caused by *Puccinia striiformis* Westend. f.sp. *tritici* Eriks. & Henn. is one of the most damaging diseases of wheat and dominant factor limiting yield potential in wheat worldwide. Several Yr-genes that confer resistance to stripe rust in wheat have been identified and deployed into commercial wheat cultivars. Experiments were conducted to assess the level of partial resistance in Indian wheat genotypes and also postulation of yellow rust resistance genes. Thirty three wheat cultivars including susceptible checks and four isogenic/ differential lines with known resistance genes were screened against different pathotypes of *Puccinia striiformis* for yellow rust resistance both at seedling in temperature controlled growth chamber as well as adult plant stage under field conditions. By applying the gene matching technique, different combinations of three yellow rust resistance genes, *viz.*, Yr2, Yr9 and Yr18 were characterized. Partial resistance (adult plant resistance) was assessed under field condition through host response and epidemiological parameters estimates *i.e.,* AUDPC, rAUDPC, FRS, infection rate and coefficient of infection. On the basis of these parameters, promising adult plant resistance was observed on the cultivars DBW 71, PDW 314, HS 277, HS 507, VL 804, VL 829, VL 907, HPW 349, HD 2967, HD 3043, NIAW 34, C 306, HI 1563 and WH 1080 consistently during rabi seasons of 2012-14. All these promising yellow rust resistant varieties at adult

plant stage were susceptible at seedling stage to one or more pathotype/s of yellow rust, which indicated presence of adult plant resistance. At seedling stage, adult plant resistance gene Yr18 was postulated in eight varieties namely HS 277, VL 804, VL 829, HD 2733, NI 5439, NIAW 34, PBW 175 and C 306. All these varieties exhibited effective adult plant resistance.

P72

Influence of inoculum density of *Fusarium oxysporum* f.sp. *ciceri* isolates on chickpea wilt

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Both inoculum densities and isolates of *F. oxysporum* f.sp. *ciceri*(weakly pathogenic or highly pathogenic isolate) significantly influenced quantitative development of *Fusarium* wilt in three chickpea cultivars, with significant increase in final cfu count over initial cfu count. The inoculum levels of 50 and 100 g per kg soil of WPI (F5) leads to 7.0 and 13.3% wilt incidence in JG-62. But with increase in inoculums levels of HPI (F22) cultivar JG-62 showed progressive increase in wilt incidence and attend 100% wilting at 25, 50 and 100 g/kg soil inoculum levels. Also, the rate of multiplication of cfu in cv. JG-62 is more, ranged from 12.19 to 1.89 per 50 mg soil than AKG-96 (moderately susceptible) and SAKI-9516 (resistant). Evaluation of 65 genotypes against *Fusarium* wilt under pot culture and water culture showed that, only three (PG-05112, AKG-84 and AKG-88) and two genotypes *viz.*, PG-05112 and AKG-84 were found disease free under pot culture and water culture respectively. However, 15 and 17 chickpea genotypes showed a highly susceptible reaction in pot culture and water culture.

P73

Monitoring virulence of *Xanthomonas oryzae* pv. *oryzae* and identification of effective R gene/s for its management in Hyderabad Karnataka

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Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is an important limiting factor of rice productivity across the globe including in India. To develop a strong

management strategy requires the clear knowledge of the virulence structure of the pathogen population, but, which changes regularly, and hence, monitoring the virulence pattern is inevitable. The trial on virulence monitoring of *Xanthomonas oryzae* pv. *oryzae* was conducted at Agricultural Research Station, Gangavathi during kharif 2011 to 2014. The trial consisted of twenty two near isogenic lines (IRBB lines) possessing different BLB resistant genes (singly) or in various combination in the background of rice cultivar IR 24. The differentials like DV 85, Ajaya (IET 8585) and TN1 were also included in the trial. The experiment was conducted for four consecutive years from 2011 to 2014 during kharif under natural epiphytotic condition and evaluated based on Standard evaluation scale 0-9 for BLB.Most of the single gene differentials, except xa13 (IRBB13) and Xa21 (IRBB21)showed susceptible reactions. All the differentials with two gene combinations showed resistant reaction. One differentials with three gene combination IRBB59 (xa5 + xa13 + Xa21) showed susceptibility, whereas, most of the lines with three gene such as, IRBB 56 (Xa4 + xa5 + xa13), IRBB57 (Xa4 + xa5 + Xa21), IRBB58 (Xa4 + xa13 + Xa21), and four genes combinations IRBB60 (Xa4 + xa5 + xa13 + Xa21) showed resistant reactions. The results observed from 2011-2014 were consistence and revealed the stability in the virulence pattern during the trial period. The study has also identified the effective R gene or their combinations to be used for varietal improvement for successful management of this disease in HK region.

P74

Collection and evaluation of natural escapes for resistance to *katte* disease of cardamom

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Katte disease of small cardamom(*Elettaria cardamomum* Maton) caused by Cardamom mosaic virus (CdMV) is a severe problem in many cardamom growing pockets in Karnataka. Natural escapes from hot spots are potential sources of resistance that could be developed into new varieties through breeding programme. A total of 18 *katte* escapes collected during 1985-2010 and maintained as germplasm were clonally planted (September 2012) in poly bags for screening for resistance to the disease. Ten plants of each collection were inoculated with viruliferous aphids three times at 45-50 days interval during December - March (2013-14). One leaf funnel each was made on two tillers in a clump and labeled for inoculation with aphids and fur-

ther monitoring. Aphids were subjected to 2 h of fasting, 10 minutes of acquisition feeding and 12 h of inoculation feeding. Five aphids were released per leaf funnel (i.e. 10 per plant) consisting young and adult individuals. After inoculation feeding, the plants were sprayed with insecticide (Quinalphos 0.02%). The inoculated tillers were monitored for symptoms expression on newly emerging leaves and recorded 45 days after each round of inoculation. A susceptible local Malabar cultivar served as control. Eleven plants (SKP 75, 104, 187, 202, 368, 369, 370, 373, 374, 375 & control) showed *katte* symptoms after 3rd round of inoculation (as on 30-04-2014) and another one (SKP 371) showed natural infections of Banana bract mosaic virus (BBrMV). On incubation for another 120 days, three more accessions (SKP 74, 164 & 184) were expressed symptoms of *katte* (as on 31-08-2014). The remaining four accessions (SKP 72, 80, 81 & 182) free of *katte* symptoms were identified as tolerant to the disease and are under field evaluation in hot spots since September 2014.

P75

Induction of systemic resistance by rhizobacteria in tomato against bacterial wilt caused by *Ralstonia solanacearum* E.F. Smith

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The potential rhizobacteria of *Ralstonia solanacearum* (Rs), *viz., Pseudomonas fluorescens* (Pf) and *Bacillus subtilis* (Bs) were used alone and in combination in the induction of defense reaction in tomato in the pot culture experiment carried out under glasshouse condition. Six treatments, *viz.*, (i) seeds treated with Bs, (ii) seeds treated with Pf, (iii) seeds treated with Bs and challenge inoculation with *R. solanacearum* (Rs) 15 days after sowing (DAS), (iv) seeds treated with Pf and challenge inoculation with Rs 15 DAS, (v) plants inoculated with the pathogen 15 DAS and (vi) untreated control were imposed in a controlled randomized block design with four replications each. For sampling, plants were carefully uprooted without causing any damage to root tissues at different time intervals (0, 1, 2, 3, 4, 5, 7 and 10 days after the pathogen inoculation) to study the induction of defense enzymes in response to pathogen attack in tomato seedlings. PAL activity was determined as the rate of conversion of l-phenylalanine to transcinnamic acid at 290 nm, PO activity was determined as changes in the absorbance/min mg/protein, PPO activity was determined as changes in absorbance at 495 nm/min mg/protein and the phenolic content was determined as µg catechol mg/protein. Results indicated that seed treatment with Pf induced the plants to synthesize PAL, whereas an additional increase in the synthesis was observed in Pf pretreated plants challenge inoculated with Rs. The activity reached the maximum level on the third day after pathogen challenge and thereafter the activ-

ity remained at higher levels throughout the experimental period of 10 days. In plants treated with the pathogen alone, increased activity of PAL was observed for a period of 2–4 days and thereafter declined drastically. Increased activities of PPO and PO were observed in Pf pretreated tomato plants challenge inoculated with the pathogen, which remained at higher levels throughout the experimental period. The activity of PPO and PO reached maximum levels of 3.12 and 2.58 OD/min/mg of protein, respectively, on third day of challenge inoculation with the pathogen. The maximum phenolic content was observed in Pf pretreated plants challenge inoculated with the pathogen and the higher amounts of Phenolics (88.0 µg of catecol/mg of protein) were noticed even on the 10th day after challenge inoculation. In plants inoculated with pathogen alone, the phenolic content declined to the initial level on the 10th day after inoculation. Plants treated with *P. fluorescens* alone also increased phenolic contents as compared to untreated control plants.

P76

Identification of different genotypes of barnyard millet against sheath blight disease incited by *Rhizoctonia solani* Kuhn

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Barnyard millet (*Echinochloa frumentacea*) locally known as Shyama, Sanwa, Oodalu, Khira, Swank, Kuthiraivolly Udalu and Kodisama is cultivated throughout India in the states of Tamil Nadu, Orissa, Punjab, Madhya Pradesh, Chhattisgarh, Andhra Pradesh and Karnataka, as cereal crop under extreme soil and climatic conditions of agriculture. The crop was found infected with sheath blight caused by *Rhizoctonia solani* kuhn. leading to considerable losses in grain yield under favorable environmental conditions. An experiment was conducted at Agricultural Research Station, Vizianagaram, Andhra Pradesh during Kharif 2014 with recommended agronomical practices with basal 25N: 40P: 25K and top 25N in kg/ha. The percentage disease intensity was calculated as number of infected nodes divided by total number of nodes, multiplied with hundred. Twenty five genotypes of different maturity groups of Barnyard millet were screened for sheath blight susceptibility under natural field conditions using susceptible check VMBC-331. Among the 25 entries of barnyard millet, screened against sheath blight BAVT-18, 23 and 4 was recorded as resistant genotypes and BAVT-2, 8 and 21 was recorded as susceptible genotypes and the pathogen was isolated, purified and identified at Indian Type Culture Collection Centre (ITCC). The resistant genotypes can be further exploited in breeding programmes.

P77

Evaluation of national elite germplasm genotypes of finger millet for their reaction to blast incited by *Pyricularia grisea* (Cke.) sacc.

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Finger millet (*Eleusine coracana* (L.) Gaertn) locally known as Ragi, Chodi, Tydalu, Mandua, Nagli, Kapai and Marwa is cultivated throughout India in the states of Tamil Nadu, Orissa, Jharkhand, Maharashtra, Uttarakhand, Andhra Pradesh and Karnataka as a cereal crop under extreme soil and climatic conditions of agriculture. The crop does not suffer much from diseases yet blast incited by *Pyricularia grisea* (Cke.) sacc. is a major production constraint at times causing heavy yield losses both in quantity and quality. An experiment was conducted at Agricultural Research Station, Vizianagaram, Andhra Pradesh with recommended agronomic practices on the finger millet national elite germplasm genotypes for reaction to blast. The percentage of neck/finger blast was calculated as number of infected neck/fingers divided by total number of necks/fingers multiplied with hundred. Total 54 genotypes were tested, among them PCGF18 (6.5% neck blast (NB) and 7.2% finger blast (FB), PCGF42 (7.9% NB and 8.4% FB), GPU-28 (7.9% NB and 8.9% FB), PCGF26 (8.5% NB and 9.3% FB), PCGF17 (8.7% NB and 9.8% FB), PCGF13 (9.5% NB and 10.2% FB) and PCGF46 (9.8% NB and 10.9% FB) showed lesser incidence of neck and finger blast compared to check VR-708 (52.3% NB and 54.7% FB).

P78

Development of blast resistant lines in 360 finger millet genotypes of PR 202 × GPU 48

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Vizianagaram, being the hotspot for blast disease in finger millet, it is essential to identify the blast resistant gene mapping population and incorporating it into early generation segregating material for developing resistant varieties. Hence, an investigation was carried out on finger millet varieties in Agricultural Research Station, Vizianagaram with 360 genotypes de-

rived from the cross between a highly blast susceptible variety PR 202 and blast resistant GPU 48 and the parents act as checks, sown in a single seed progeny method. All the quantitative and qualitative characters were recorded along with scoring of leaf blast, neck blast and finger blast. It was concluded that the leaf blast ranged from 1.00 to 3.50 with a mean of 1.94, the neck blast ranged from 7.35 to 38.00 with a mean of 21.93 and the finger blast ranged from 8.75 to 45.20 with a mean of 22.64. Further, 89 lines were found resistance for leaf blast, one line for neck blast and no lines for finger blast showed better/equal ability to resistant parent. So the identified resistant lines may serve as potential parental genotypes for future breeding programmes to develop desirable stable segregants for finger millet crop improvement programmes.

P79

Evaluation of sorghum germplasm for resistance to anthracnose (*Colletotrichum graminicolum*) and leaf blight (*Exserohilum turcicum*)

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One hundred sorghum germplasm lines were evaluated for their reaction to sorghum anthracnose caused by *Colletotrichum graminicola* P. Henn., Kabat & Bubak and leaf blight caused by *Exserohilum turcicum* at CRS Solapur during Kharif 2014 along with eight checks (seven resistant and one susceptible check). Each entry was planted in rows of 3 meters at spacing of 45 × 15 cm. The design of experiment was augmented design with single replication for each entry. A fertilizer dose consisting of 80:40 NP in kg/ha was applied. These germplasm lines were collected from Gujarat, Madhya Pradesh, Rajasthan and Tamil Nadu states. The incidence of sorghum anthracnose was recorded on 1-9 scale at physiological maturity of the crop. The midrib infection due to sorghum anthracnose was recorded in cm on the leaves, while leaf spot infection was assessed on 1-9 scale. The inoculum of sorghum anthracnose was multiplied on autoclaved sorghum grain medium. Ten observations were recorded for each entry and average was worked. The incidence of anthracnose was observed on all the entries. Of the hundred germplasm lines evaluated, 20 accessions have recorded resistance reaction with an incidence of less than 3, while 33 accessions were moderately resistant, while all others were highly susceptible with an incidence of more than 5 on 1-9 scale. The susceptible entry Khaddar recorded an incidence of 8, while a resistant check *viz.*, IS 1107 and IS9627 recorded 2.5 on 1-9 scale. The mean midrib index was found to be 3.7, while leaf spot index was 4.8. All the above lines were screened for incidence of leaf blight caused by *Exserohilum turcicum*, and high level of incidence was observed on all the entries. Of the 100 germplasm lines evaluated for the incidence

of leaf blight only four entries *viz.*, E-1, EG-11,ERS-16 and GGUB-25 were found to be highly resistant with a reaction of <3 on 1-9 scale, while all other entries were highly susceptible with a reaction of >7. The susceptible check Khatar Khatar recorded an incidence of 9.0.

P80

Identification of neck blast resistance sources from local rice germplasm collected from Northeastern Hill Region of India

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Rice blast disease caused by *Magnaporthe oryzae* is a serious threat to rice production in India. In present study a total of 450 local rice germplasm collected from different North-eastern states (Manipur, Meghalaya, Nagaland and Arunachal Pradesh) were screened for rice blast disease under natural disease pressure for three consecutive years (Kharif 2012, 2013 and 2014). Entries were screened for leaf blast under uniform blast nursery conditions and for neck blast under natural disease pressure with a local variety Punshi as susceptible check. Based on the combined results of three consecutive years of screening 146 entries showed low reaction to neck blast disease (score 0-3) and 84 entries showed low reaction to leaf blast disease (score 0-3). The entries showing resistant reaction neck blast is the putative sources of resistance harbouring uncharacterized novel resistant genes. Present work will have practical implications in further resistance breeding against blast disease and cloning as well as functional analysis of novel blast resistance genes.

P81

Screening of groundnut germplasms for rust disease against *Puccinia arachidis*

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Groundnut (*Arachis hypogaea* L.) is one of the important legume oilseed crops of the world. India is the second largest producer of groundnut after China with area and production and productivity. Groundnut is susceptible to many diseases caused by fungi, bacteria, viruses

and nematodes. Out of these the important fungal foliar diseases is rust caused by *Puccinia arachidis* Spegazinni. Occurrence of these diseases result in reduction of quality and hamper yield up to 50-70%. One hundred fourteen groundnut genotypes from various *Arachis* spp. were screened in the field condition against rust during Kharif 2013-14. Out of these 114 groundnut genotypes, 13 genotypes showed the resistant reaction and 46 entries showed moderately resistant reaction. The 51 genotypes recorded susceptible reaction and 4 entries recorded highly susceptible reaction to the rust disease. Fourteen genotypes from various cultivated Arachis spp. and wild species were screened in the glasshouse condition by artificial inoculation. Out of these groundnut genotypes, four wild species (*Arachis diogoi, A. valida, A. batizicoi* and *A. cardenasi*) showed resistant reaction to rust disease, while, eight cultivated groundnut genotypes also showed resistant reaction (RHRG-6083, GPBD-4, KDG-128, ICG-11426, ICG-12672, ICGV-86590, ICGV-94118, ICGV-96283), and (SB- XI and JL- 24) were recorded highly susceptible reaction to rust disease.

P82

Screening *Piper* germplasm accessions against *Piper* yellow mottle virus

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Piper yellow mottle virus (PYMoV) (genus: Badnavirus) is an important production constraint of black pepper in India and other parts of the world. The virus is transmitted primarily through vegetative means and seeds while secondary spread occur through different species of mealybug. None of the currently grown varieties/cultivars of black pepper are resistant to the virus. In order to find source of resistance to the virus, cultivated accessions of black pepper and related species were screened. Each germplasm accessions was propagated vegetatively through cuttings in insect proof glass house and inoculated with PYMoV through mealybug, *Ferrisia virgata*. The non-viruliferous young adult mealybugs were given a 24 h acquisition access on infected black pepper leaves. Fifteen mealybugs each were then transferred onto test accessions and allowed an inoculation access period of 24 h after which plants were sprayed with insecticide and kept for observation in the insect proof glasshouse. After 90 days of incubation, the plants were scored both visually based on symptoms and through PCR test using PYMoV specific primers. Results of screening of 2300 accessions that included 1334 of cultivated accessions of black pepper and 966 accessions of wild Piper (belonging to *P. argyrophyllum, P. attenuatum, P. bababudani, P. barberi, P. betle, P. chaba, P. colubrinum, P. galeatum, P. hapnium, P.hymenophyllum,P. longum, P. mullesua, P. nigrum, P. peepuloides, P. sarmentosum, P. silentvalleyense, P. sugandhi, P. sylvaticum, P. thomsonii* and *P. trichostachyon*), none showed resistance to PYMoV.

P83

Salicylic acid induced defence responses in *Curcuma longa* (L.) against *Pythium aphanidermatum* infection

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The soilborne Oomycete, *Pythium aphanidermatum* is the causal agent of rhizome rot disease, one of the most serious threats to turmeric crops in India. At present, effective fungicides are not available. Here, we report the enhanced resistance response induced by salicylic acid (SA) in susceptible turmeric plants. The enzymatic activities of pathogenesis related (PR) proteins in control and SA treated turmeric plants were measured. SA pretreatment elicited marked increases in the activity levels of protease, trypsin and chymotrypsin inhibitors, soluble and ionically bound peroxidase activity. Such an increase in enzyme activities and protease inhibitors was enhanced and occurred much more rapidly in *P. aphanidermatum* infected rhizomes than those that were previously treated with SA (0.5 mM) suggesting that increased activities of peroxidases and protease inhibitors may play a role in restricting the development of disease symptoms in the rhizomes infected with *P. aphanidermatum* as evidenced by reduction in cell death. SA also induced new polypeptides in turmeric rhizomes corresponding to 19.0 and 41.0 kDa. The results demonstrated that SA is an effective resistance activator in turmeric, and also a potentially useful agent for the control of rhizome rot disease.

P84

Nursery evaluation of indigenous and exotic apple cultivars against alternaria blight in Kumaun region of Uttarakhand

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Alternaria leaf spot caused by fungus (*Alternaria* sp.) is an emerging disease of apple nursery field. *Alternaria* leaf spot causes unsightly necrotic spots or blotches on the leaves which

when viewed under magnification often have concentric rings of darker brown within the lesion. A study was conducted during 2012, 2013/14 to evaluate 31 varieties of apple (*Malus domestica* Borkh.) propagated in MM-rootstock series and seedling rootstock for alternaria blight disease in CITH RS Mukteshwar recording the total leaf area affected spot per plant. The disease severity on plant leafs recorded using a five point rating scale which was recommended by AICRP on sub tropical fruit crops based on the percentage of leaf area affected by the disease. The apple variety CITH Apple Lodh-1 in seedling rootstock, CITH-Apple Lodh-1, Chaubatiya Princess on MM-106, MM111 have been found 20-25% disease susceptible for alternaria blight, respectively. The Skyline Supreme, Oregon Spur, Red Spur, Red Delicious, Vance Delicious have been recorded the 10-15% susceptibility upon seedling rootstock. The variety Gala Mast, Mollis Delicious, Golden Delicious, Chaubatiya Anupam on MM111 and Prima, Mayan on seedling rootstock have been found resistance to alternaria blight. The present study showed that the cultivars Gala Mast, Mollis Delicious, Golden Delicious, Chaubatiya Anupam were showed highly resistance to alternaria blight disease.

P85

ISSR marker analysis of groundnut germplasm resistant to rust disease

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Groundnut (*Arachis hypogaea* L.) is presently cultivated in more than 80 countries in tropical and warm temperate regions of the world. The rust of groundnut is caused by *Puccinia arachidis* Speg. The genomic DNAs of ten resistant and two susceptible groundnut genotypes were subjected to PCR amplification using 32 ISSR markers. It was observed that, 22 ISSR markers showed PCR amplification, while 10 primers (ISSR 3, ISSR 5, ISSR 7, ISSR 8, ISSR 9, ISSR 11, ISSR 12, ISSR 13, ISSR 18 and ISSR 25) did not yield any amplification. Twenty two ISSR markers amplified a total 206 bands out of which maximum no. of bands (18) were amplified by marker ISSR 1 followed by ISSR 30, ISSR 31 and ISSR 17 amplified 14, 13, 13 bands, respectively. Whereas ISSR 15 and ISSR 24 primer yielded only two bands. Out of 206 bands, 124 bands were polymorphic with an average polymorphism of 57.17%. Fifty four bands were unique and 28 were monomorphic. Each primer thus produced on an avg.5.63 polymorphic bands. The sizes of amplification product ranged from 177.4 to 2342.5 bp. These 22 primers produced 54 unique bands which may be species specific bands were unique with 44.44% polymorphism. 1874.2 bp band was present in resistant genotypes while it was absent in susceptible genotypesviz., SB-XI and JL-24, respectively. The ISSR 30 amplified 14 bands out of which 11 were polymorphic and 3 were unique bands with 78.57% polymorphism. 1225.3 bp band was

only present in ICG 13160 and the band 1122.9 bp and 264.4 bp were only present in ICG 13165. These unique bands may be species specific.

P86

Biochemical mechanism of resistance in slow and fast rusting pearl millet genotypes

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Rust caused by the fungus *Puccinia substriata* Ell. and Brath.var. *indica* (Ramchar and Cumm.) is one of the major diseases affecting both forage and grain production in pearl millet. In order to understand the nature of biochemical changes due to rust disease,nine pearlmillet genotypes(ADMR17, ADMR49, ADMR 10, ADMR 16, ADMR 27, J 2510, J 2496, J 2526 and J 2517) have been subjected for biochemical analysis both under healthy and diseased condition. Field trial was conducted during kharif 2013-14 at Regional Agricultural Research Station, Bijapur, while glass house experiments were undertaken at Department of Plant Pathology, College of Agriculture, Bijapur, University of Agricultural Sciences, Dharwad, Karnataka. All the nine genotypes were sown in field in randomised block design with three replications and simultaneously the same genotypes were also raised in the pots in glass house to get the healthy plants for biochemical analysis and three pots were maintained for each genotype. Irrespective of the genotypes, total sugar content was less in diseased leaves compared to healthy leaves at 60 DAS.However, at 80 DAS, the total sugar content was more in diseased leaves compared to healthy leaves, due to synthesis of sugars by the pathogen interaction in the amylolytic activity. Total sugar content, reducing sugar and non reducing sugar content was increased in diseased leaves from 60 to 80 DAS. At 60 DAS they were more in healthy leaves as compared to diseased leaves. However, at 80 DAS, their content was more in diseased leaves compared to healthy leaves. Total free phenol and amino acid content was decreased in both healthy and diseased leaves from 60 to 80 DAS. At both 60 and 80DAS total free phenol and amino acid contents were more in healthy leaves as compared to diseased leaves irrespective of the genotypes, due to decrease in enzymatic activity in diseased leaves and also utilisation of amino acids and synthesis of phenolics by the pathogen.

P87

Screening of genotypes and varieties against fusarium wilt of pigeon pea

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Screening of selected genotypes/varieties/cultivars against fusarium wilt of pigeon pea was carried out during 2009-10 at research field of College of Agriculture, Marathwada Krishi Vidyapeeth, Parbhani. Thirty four genotypes/varieties/cultivars were screened. Results revealed that of the 34 pigeon pea genotypes/varieties/cultivars evaluated under natural epiphysis of wilt (*F. oxysporum* f.sp. *udum*) sick plot, eight (PT-02-5, PT-02-23,PT-02-134, PT-1034, PT-8208-1, PT-8208-1/SMVT-1, BSMR-846 and ICP-8858) were found resistance with wilt incidence in the range of 3.33 to 9.00%. Seven (PT-02-8, PT-02-27, PT-02-128, PT-02-131, PT-02-7, ICP-8862 and ICP-9174) were moderately resistance with wilt incidence to the tune of 12.69 to 19.86%. Twelve (PT-02-3, PT-007, PT-02-28, PT-02-29, PT-02-30, PT-02-126, Pusa-2003-2, BDN-708, BDN-2001-6, BSMR-736 and ICP-8803) were found susceptible with wilt incidence to the tune of 21.52 to 46.87%. Whereas, seven *viz.*, PT-002, (SMVT-II), PT-99021 (SMVT-II), PT-2-32-1, PT-99021 (RTV-1), Bahar, Pant-A-226 and Pusa-2003-1 were found highly susceptible with wilt incidence in the range of 50.26 to 65.00%.

P88

Phenotypic responses of *Arabidopsis thaliana* ecotypes to *Ralstonia solanacearum* race 4/biovar 3/phylotype I

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Bacterial wilt caused by the soil-borne bacterium *Ralstonia solanacearum* race 4 is a lethal disease of several vegetables and spices in the family Solanaceae and Zingiberaceae respectively. Edible ginger, among other Zingiberaceae plants such as small cardamom and turmeric, is reported as host for race 4 strains of *R. solanacearum*. We used *Arabidopsis* as a model system for studying plant-bacterial interactions, because it is one of the best characterized plants. In

order to understand the phenotypic response of *Arabidopsis thaliana* Col-0 to *R.solanacearum* isolate, CaRs-Mep, decimal dilution of bacterial suspension were inoculated on seeds and roots of plants. The bacterium typically wilted the plantlets in a density dependent manner where the yellowing and wilting was observed within 7 days and 14 days at 1010 and 109 cfu respectively. At lower concentrations of 108 cfuand below the seedling survived, but showed significant root growth inhibition as compared to mock. Besides, there was a significant increase in the length and number of root hairs due to bacterial colonization. Interestingly the plantlets succumb to wilt within 6 days post inoculation when the bacterium was inoculated onto roots. We also screened other ecotypes of *Arabidopsis thaliana* against CaRs-Mep. All ecotypes except Landsbreg erecta (Ler) were highly susceptible to race 4 strain of *R. solanacearum*. Scanning electron microscopic and light microscopic analysis clearly localized the bacterial cells on the rhizoplane, root interiors of the plantlets. Population estimation by dilution plate experiment clearly revealed that there was a progressive multiplication of *R. solanacearum* on rhizoplane and endorhizosphere. There was a 10 order increase in the population of bacterium from 6-logCFU/g to 7.7-logCFU/g. This study would pave way for utilizing Ler ecotype as a potential resistance source for R- genes against *R. solanacearum* as R- genes are scanty for race 4 strains.

P89

Performance of Fluxapyroxad 250g/l+Pyraclostrobin 250g/l500SC (BAS70301F) against powdery mildew disease of mango

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Mango is the most important commercially grown fruit crop of the country. It is called the king of fruits. The study on powdery mildew revealed that, Minimum PDI on fruits (Per cent Disease Index) was observed in the treatment of Fluxapyroxad 250g/L +Pyraclostobin 250g/L 500SC @200mL/ha(PDI 18.60%) and the next most effective treatment was Fluxapyroxad250g/L +Pyraclostobin 250g/L 500SC @150mL/ha (20.30%). However, the maximum powdery mildew disease severity (PDI) was recorded in untreated control 54.40%.Overall, Fluxapyroxad250g/L +Pyraclostobin 250g/L 500SC @200mL/ha was found most effective treatment which recorded 66.91% disease reduction followed by Fluxapyroxad250g/L +Pyraclostobin 250g/L 500SC @150mL/ha recorded 62.68% reduction over control. The highest yield was recorded in Fluxapyroxad 250g/L +Pyraclostobin 250g/L 500SC @200mL/ha (30.30t/ha) followed by Fluxapyroxad 250g/L +Pyraclostobin 250g/L 500SC @150mL/ha (28.20t/ha). The least yields were recorded in untreated control(14.80t/ha).

P90

Changes in grain quality and grain yield of slow leaf rusting Indian bread wheat genotypes by leaf rust

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Leaf rust of wheat (*Triticum aestivum* L. em. Thell)caused by *Puccinia triticina* Eriks. is a most important disease across the world. We assessed the effectiveness of leaf rust among slow rusting genotypes in comparison with susceptible and resistant Indian bread wheat genotypes for grain yield, thousand grain weight, grain protein content, sedimentation value and their losses. In total 40 genotypes including, 19 resistant, 11 slow-rusting and 10 susceptible genotypes were used in two year (rabi 2011-12 and 2012-13) experiment at All India Co-ordinated Wheat Improvement Project, Main Agricultural Research Station (MARS), University of Agricultural Sciences (UAS), Dharwad (Karnataka) under fungicide-protected and unprotected treatments with high disease severity. Twelve genotypes with resistance were immune to leaf rust remaining 7 genotypes showed slight infection. Slow rusting genotypes showed varied range of disease severity and susceptible checks displayed 100% disease severity. Mean yield losses for resistance, slow-rusting and susceptible genotypes were 1.57, 10.62 and 25.80%, respectively. The average mean loss of thousand grain weights was showed significant reduction for resistance genotype (4.55%), slow rusting (6.75%) and susceptible genotypes (11.62%). The grain quality parameters were either increased or decreased or no significant changes obtained when compared with protected and unprotected conditions among all the three group of genotypes. High levels of disease severity increased grain protein content and sedimentation value of the susceptible genotypes, Lal Bahadur (8.87 and 5.71%, respectively) and Agra local (1.40 and 4.85%, respectively). In the genotypic correlation protein content was positively non significantly correlated with sedimentation value. Majority of slow-rusting bread wheat genotypes showed reduction in grain protein content varied from 1.09 to 10.26% with low disease levels and low yield losses. Such genotypes can be used for wheat breeding programme with slow disease development allowing less selection pressure on leaf rust pathogen. The results confirmed the economic importance of leaf rust for its effect on yield loss and grain quality of Indian bread wheat genotypes.

P91

A high-throughput protocol for screening against Bakanae disease of rice

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Rice is one of the most important staple food crops grown in diverse ecological conditions cross the world. Its productivity is limited by various biotic and abiotic stresses. Among the biotic stresses, Bakanae or foot rot disease, caused by *Fusarium fujikuroi* (teleomorph: *Gibberella fujikuroi*, Sawada, Wollenweber) is one of the emerging diseases limiting productivity and quality. The infection leads to complete mortality of plants and also causes secondary spread affecting the productivity. In the present study, a high-throughput, reliable bioassay for screening rice germplasm against bakanae disease was developed and compared with other conventional techniques. This technique involves soaking of rice seeds in fungal spore suspension of 1.0×106 ml−1 for 24 h at room temperature which produces consistent and reproducible symptoms of bakanae disease, namely, seedling elongation and mortality. Raising seedlings at 30°/25°(±3)°C day/night temperature and 60/80 (±10)% day/night relative humidity in glasshouse were found to be ideal for screening. The new method described here would enable screening several hundreds of rice germplasm within a short span of 15 days, without any loss of precision in the previously used methods described for screening against bakanae disease. The new rapid bioassay developed here may find wider application in both pathogen and host studies, including the identification of sources of resistance for bakanae, mapping QTLs/ genes governing resistance and to develop resistant varieties with inbuilt resistance and to manage the threat that the pathogen poses to rice production especially in Basmati rice.

P92

Breeding for resistance to mungbean yellow mosaic disease

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Mungbean [*Vigna radiata*(L.) R. Wilczek] is an important pulse crops in India. Yellow Mosaic Disease (YMD) is a major threat to mungbean cultivation. It is caused by different virus species (MYMV, MYMIV and HYMV) of geminivirus. An effort was initiated to improve

mungbean for resistance to YMD in Eastern and North-eastern India. MYMIV has been reported to occur in the lower Gangetic alluvial zone (West Bengal). Two RIL populations of mungbean have been derived from the crosses between B1 (susceptible) x Sub2 (resistant) and PM5 (resistant) x Sub2 (resistant). One hundred forty seven lines of the RIL population of B1x-Sub2 and sixty-nine lines of PM5xSub2 along with parents B1, PM5 and Sub2 were screened and evaluated against MYMIV. Scoring of disease severity in the RIL population (B1xSub2) revealed approximately 3:1 (susceptible: resistance) segregation. In another RIL (PM5xSub2), almost 88% lines showed resistance and 12% showed either moderately resistance or susceptible (as both the parents were resistant to MYMV), suggesting that the resistance gene present in both parents are probably allelic with some modifier genes. Thus, RIL population derived from B1xSub2 is the ideal population for mapping major gene and PM5xSub2 for mapping the modifiers. Moreover, co-existence of both MYMV and MYMIV has been detected in North-eastern India (Meghalaya) through PCR assay using species-specific primers. Full genome characterization of the virus species is underway for further confirmation. Screening of the RILs under both conditions may result in identification of lines having resistance to both virus species.

P93

GRG-811;A early medium duration pigeonpea variety resistant to wilt and SMD

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Pigeonpea (*Cajanus cajan* (L.) Millsp) is an important pulse crop in Karnataka. Wilt caused by *Fusarium udum* Butler and sterility mosaic disease (SMD) caused by pigeonpea sterility mosaic virus (PPSMV) and transmitted by an eriophyid mite *Aceria cajani* are the major biotic constraints to pigeonpea production. Most of the pigeonpea popular genotypes/varieties being grown by farmers are highly susceptible to either of the diseases. The popular wilt resistant varieties ICP-8863 and TS-3R are highly susceptible to SMD. Keeping this in view efforts were made to identify multiple disease resistant (wilt and SMD) varieties by screening a number of entries in wilt and SMD sick plots. Most of the genotypes showing resistant reactions to wilt were showing susceptible reaction to SMD and vice-versa. The only one early medium duration (165 days) genotype i.e. GRG-811 was showing promising reaction for both the diseases *viz.*, resistant for *Fusarium* wilt (6.94%) and moderately resistant (15.68%) for SMD with good yield (13q/ha), Looking to these advantages this variety has been accepted for farm trial (for two years) for growing in wilt and SMD endemic areas of northern Karnataka.

P94

Evaluation of pigeonpea minicore collections against sterility mosaic disease

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Sterility mosaic disease (SMD) considered as "green plague of pigeonpea" caused by pigeonpea sterility mosaic virus (PPSMV) and the virus is transmitted by the vector eriophyid mite, *Aceria cajani* Channabasavanna is one of the major biotic factors which leads to heavy losses and hence pose a big challenge for pigeonpea production in Indian sub continent. Most of the popular pigeon pea varieties cultivated by farmers are highly susceptible to SMD due to prevalence of highly virulent isolate (Bangalore (B) isolate) in northern Karnataka. Keeping this in view 196 pigeonpea minicore collections were evaluated in SMD sick plot maintained at ARS, Bidar for two years during kharif 2012-13 and 2013-14 for their reaction to SMD. Only 3 genotypes *viz.*, ICP-7035, Bahar and ICP-11910 were showing resistant reaction by recording less than 10% of disease incidence, six genotypes *viz.*, ICP-16264, ICP-14976, ICP-3451, MA-6, ICPL-129808 and GRG-811 were showing moderately resistant reaction by recording disease incidence from 10-30%. Remaining 187 genotypes were showing highly susceptible (>30%) reaction.

P95

Molecular detection and role of extracellular polysaccharide in host resistance of bacterial wilt pathogenesis of eggplant

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Single-strand conformation polymorphism (SSCP) and Low stringency single specific primer (LSSP) PCR were assessed for genetic typing and molecular conformation of pathogens. Extracellular polysaccharides are specific elicitors which have been used in study of differential expression and some experiments have suggested that certain plant can recognize EPS from

specific bacteria which acts as specific elicitor to prevent bacterial diseases by activation of Induced systemic resistance. SSCP and LSSP were developed to molecular characterization of *Ralstonia solanacearum* DOB-R1 strain from suspicious bacterial wilt fields. Extracellular polysaccharide (EPS) were extracted from *R. solanacearum* strain DOB-R1, the partially purified EPS was air-dried. EPS was quantified by the Elson-Morgan assay for hexosamine sugars using N-acetyl-galactosamine as the standard. The morphological and structural characterization was assessed by SEM and AFM. Chemical characterization of the different EPSs was carried out using spectroscopic methods, purified through HPLC and mass of the compounds were determined by LC-MS. Complete structural elucidated through IR and NMR analysis. Seed germination was increased and disease incidence was decreased upon EPS treatment. The concentration of H_2O_2 was two folds higher at 12 hpi than in control when treated with EPS and antioxidant enzymes *viz.*, APX and GPOD were rapidly increased in highly susceptible (HS) cultivars upon EPS treatment. The enzymatic activities of these enzymes were confirmed by amplification of APX and GPOD defence genes. The developed molecular detection and elicitor which are valuable tools and eco-friendly approach to manage plant diseases.

P96

Differential accumulation of SA responsive proteins in bacterial wilt susceptible and resistant chilli cultivars

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To investigate the molecular mechanisms of bacterial disease resistance in chilli plants, a proteomic approach was adopted. Two cultivars of chilli were selected on the basis of their response to bacterial wilt disease caused by *Ralstonia solanacearum* inoculation *viz.*, cv *Anugraha* as Resistant and cv *Pusajwala* as Susceptible varieties. Proteins were extracted from leaves of 3 week-old-seedlings of the two chilli cultivars from different treatments which include SA treated, Pathogen treated followed by challenge inoculation with SA and separated by 2-DE, proteins were separated based on their individual pH on immobilzed pH gradient strips by first dimension (Isoelectric focusing) followed by Mwt on SDS-PAGE by second dimension. These differentially expressed protein spots between the resistant and susceptible chilli cultivars were analyzed using PDQuest analysis software. Our results indicate that a total of 400 protein spots were successfully identified, out of this 70 protein spots were differentially expressed between the resistant and susceptible cultivars, among them 52 spots were upregulated and 18 were downregulated. These SA responsive proteins were expected to be involved in signal transduction, stress/defence/detoxification, protein metabolism (i.e., translation, processing, and degradation), photosynthesis, amino acid metabolism, carbohydrate metabolism, and energy

pathways, and may therefore be functionally relevant for many biological processes. The present study provides important information with regards to proteomic methods aimed to study protein regulations of the *Capsicum annuum–R.solanacearum* pathosystem, especially in terms of host resistance to this pathogen.

P97

Differential response of black pepper genotypes to *Phytophthora* isolates

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The drastic drop in the black pepper production in all growing countries has been attributed mainly for pronounced death of vines by the dreaded disease foot rot caused by *Phytophthora capsici* Tsao. Developing varieties resistant to *Phytophthora* foot rot is significant in this context. Fifty seven genotypes of black pepper including 28 cultivars and 29 hybrids were phenotyped for *Phytophthora* resistance with leaf and stem inoculations with two distinct black pepper *Phytophthora* isolates namely 05-06 and 98-93. Only one genotype Acc. No. 1324 was found to be in the resistance level with ~5mm leaf lesion and < 10mm stem lesion size. However, 50% of the genotypes exhibited differential response on inoculation with the two isolates with more than 7mm difference in lesion size and the rest of them were equally susceptible. Among the differentially reacted genotypes 79% of them showed more affinity towards the isolate 98-93.

P98

Biotechnological approaches and R-Gene mediated resistance in banana for *Fusarium* wilt

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Many commercial banana varieties lack sources of resistance to pests and diseases, as a consequence of sterility and narrow genetic background. Fertile wild relatives, by contrast, possess greater variability and represent potential sources of disease resistance genes (R-genes). The largest known family of plant R-genes encode proteins with nucleotide-binding site (NBS) and

C-terminal leucine-rich repeat (LRR) domains. Conserved motifs in such genes in diverse plant species offer a means for isolation of candidate genes in banana which may be involved in plant defense. Hence, investigations are carried out at College of Horticulture Thrissur to characterize R-gene mediated resistance gene analogs in banana for *Fusarium* wilt.The aim of this study is to characterize the *Fusarium* wilt resistance gene analogs. In this study we used two banana varieties Palayankodan (Resistance to *Fusarium* wilt) and Poovan (Susceptible to *Fusarium* wilt). Artificial screening has done to confirm resistance/susceptibility to *Fusarium* wilt.Based on the conservative regions of the nucleotide-binding site and the leucine-rich repeat (NBS-LRR) in cloned wilt resistance genes, the polymerase chain reaction with degenerate primers was employed to isolate resistance gene analogues (RGAs) from the genomic DNA of wilt resistance germplasm 'Palayankodan' banana. As a result, fragments of RGAs were isolated, which were of expected size (about 530 bp). Analysis of the deduced amino acids of these RGAs show that they share the NB-ARC domain and containing 4 conservative amino acid domains and hydrophobic amino. Other results reveal that sequence identity of the RGAs range from 41.1% to 99.3%, while identity of the deduced amino acid sequences range from 33.2% to 96.3%.The Foc resistant cultivar 'Palayankodan' has 'R' genes of class NBS type and can serve as source for this type of genes for molecular breeding. Study will be useful for development of molecular markers for marker assisted selection and cloning of full length R" genes for *Fusarium* wilt resistance. Technically, these RGAs isolated in the present study would lay a base for the further cloning of wilt resistance genes in banana, which could also be used as molecular markers for screening candidate wilt resistance genes in banana.

P99

Identification of stable reference genes for quantitative real time PCR studies and expression analysis of resistance genes under *Piper colubrinum- Phytophthora* interaction

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Piper colubrinum Link.is a distant relative of cultivated black pepper highly resistant to the most destructive pathogen, *Phythophthora capsici*, the causative organism of foot rot disease. Investigating the expression of defense genes and R genes in the plant can be of utmost importance in understanding the resistance mechanism. Relative gene expression studies using qRT-PCR is gaining importance in plant biological research and identification of stable internal reference gene is essential for valid qPCR analysis. A study was undertaken for investigation on the stable reference genes and the expression analysis of selected R genes in *Piper colubrinum*

under *Phytophthora* interaction. Expression profiles of six commonly used housekeeping genes (ACT, GAPD, SKP1, α TUB, FLAP andEF1α) were evaluated for transcript stability analysis using mock inoculated and pathogen inoculated (using *P. capsici* 05-06 and P. capsici 98-93 isolates) *Piper colubrinum* leaf tissue samples at 1hpi, 2hpi, 8hpi and 16hpi along with uninoculated sample. The expression pattern was analyzed for the reference genes using the online tool RefFinder which incorporates four different computational algorithms. SKP1 was revealed to be the most stable reference gene under the given experimental condition followed by eF1α and ACT and TUB was found to be the least stable. Using SKP1as reference gene, we analyzed the expression pattern of four different R genes (PCR07, LR2277, LR2905 and lNR1990) in the leaves of *P. colubrinum* challenge inoculated with two strains of *Phytophthora* at different hours post inoculation (hpi). All the genes showed a maximum expression at 16hpi with both *Phytophthora* strains except for PCR07 which showed maximum expression at 8hpi when infected with *Phytophthora* 05-06.

P100

RNAi vector construction using 2b gene from black pepper isolate of Cucumber mosaic virus

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Cucumber mosaic virus (CMV) is a major production constraint in 1200 crops including black pepper. None of the currently grown varieties/cultivars of black pepper are resistant to this virus. 2b gene of CMV is known to suppress post transcriptional gene silencing in the host. Hence the study was done to prepare a hairpin construct using 2b gene which can provide RNAi mediated resistance in black pepper. Initially 2b gene was amplified from CMV infected black pepper leaves using gene specific primers, cloned and sequenced. 2b gene comprised of 337 nucleotides potentially coding for 111 amino acids. Also on sequence comparison it was found that this gene showed at the nucleotide level 82 to 95% and 65% similarity to subgroup I and II strains respectively indicating that the resistance conferred by this gene will be highly host specific. Hairpin construct was assembled with Phytoene desaturase, 6th intron of size 236 bp from Solanum lycopersicum in between the 330bp sense and antisense strands of 2b gene in pBSK vector. This was then mobilized into the binary vector pBI121. Confirmation of construct was done by PCR, restriction digestion and sequencing. Finally the construct was transferred to *Agrobacterium tumefaciens*. This will be used for transformation of black pepper.

P101

Marker assisted pyramiding of three genes governing blast resistance (Pi54, Pi1, Pita) into a popular indica rice variety "Samba Mahsuri"

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Blast disease caused by *Magnaporthe grisea* is one of the most dreaded diseases of rice causing yield losses upto 90%. The rice variety BPT5204, popularly known as "Samba Mahsuri" is one of the most popular rice varieties among both farmers and consumers, owing to its medium slender grain, excellent grain and cooking quality characteristics. It is highly susceptible to blast disease which not affects its productivity but also its grain quality. Therefore, the present study was conducted with objective of pyramiding three genes governing blast resistance, namely Pi54, Pi1 and Pita into BPT5204 using DHMASQ164-2b as the donor through marker assisted backcross breeding. Foreground selection using gene based markers for target gene(s) and background selection augmented with phenotypic selection for grain and cooking quality was employed for accelerated recovery of recurrent parent genome and phenome. A set of 30 elite lines including 17 monogenic lines, one two gene and 9 three gene pyramided lines were developed and evaluated for agronomic performance at three locations namely New Delhi, Pusa and Aduthurai for two seasons. The improved lines showed resistance to blast disease both under under artificial inoculation as well as under three hotspot locations as compared to susceptible reaction shown by BPT5204. Based on the phenotypic performance as well as the disease reaction three elite pyramided genotypes namely BPL16 (Pi54+Pi1), two three gene pyramids (BPL 27 and BPL 33) were identified as promising, which are being multiplied for further evaluation in the national trials. The evaluation and deployment of these pyramided lines will help in effective management of rice blast in Samba Mahsuri growing regions of India. Additionally, since these lines with improved agronomic performance, grain and cooking quality will also serve as useful donors for blast resistance, particularly in improvement of medium slender rice grain varieties.

P102

In vitro selection of resistant candrasur (*Lepidium sativum* L) plants against leaf spot caused by *Alternaria alternata via* tissue culture technique

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Disease resistance in callus cultures of chandrasur (*Lepidium sativum*) against leaf spot disease caused by *Alternaria alternata* was induced by subjecting the calli raised and maintained on semi-solid Murashige amd Skoog (MS) medium, to various concentrations of A. alternata culture filtrates (0%, 5.5%, 6%, 6.5%, 7%, 7.5% and 8%). At higher concentrations (from 8.0 to 10.0 ml/L) almost all the calli died and total cell mortality was observed. At next lower concentration of 7.5 ml/L 13% calli survived. At lower levels of toxic culture filtrates (5.5 ml/L andbelow to 5.5 ml/L) and also in the uninoculated callus no effect on growth of calli was observed. LD50 value was calculated on the basis of fresh weight of callus when subjected to toxic medium and it was found that at 6.0 ml/L, 50% callus survived. The leaves of putative resistant clones remained green and viable in the presence of toxin and regenerated shoots directly on the toxin-free regeneration medium.

Session IV
INTEGRATED DISEASE MANAGEMENT

LEAD LECTURES

L11

Plant quarantine as a biosecurity tool for exchange of plant genetic resources under SPS regime

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Introduction of valuable planting materials from other countries has played an important role in crop improvement in India. However, such introductions without proper examination for associated pests may prove disastrous as is evident from several glaring examples of pest outbreaks in the past. These introductions highlighted the fact that increased international travel and trade under WTO had exposed the country to the danger of infiltration of exotic pests harmful to our agriculture. Therefore, plant quarantine assumes utmost importance in safe exchange of plant genetic resources (PGR). Plant quarantine is a government endeavour enforced through legislative measures to regulate the introduction of planting materials, plant products, soil and living organisms, etc. in order to prevent inadvertent introduction of pests harmful to the agriculture of a country/state/region and if introduced, prevent their establishment and further spread. NBPGR is the nodal institution for exchange of PGR and has been empowered under Plant Quarantine (Regulation of Import into India) Order 2003 to handle quarantine processing of germplasm and transgenic planting material being imported for research purposes in the country by both public and private sectors. Every year around 80,000 samples (true seeds, rooted plants, cuttings, rhizomes, suckers, bulbs, nuts, tissue cultured plantlets etc.) are processed which involves visual examination for the presence of unwanted pests and stages thereof, plant debris, weed seeds, soil clods and other contaminants followed by detailed testing using specialized techniques for their diagnostics using modern facilities. At NBPGR, adopting a workable strategy, a number of pests of great economic and quarantine significance have been intercepted in exotic material, many of which are yet not reported from India *viz.*, insects like *Acanthoscelides obtectus* in *Cajanus cajan*, *Anthonomus grandis* in *Gossypium* spp., Ephestisa elutella in Macadamia nuts and *Vigna* spp., *Quadrastichodella eucalytii* in Eucalyptus, nematodes like *Heterodera schachtii, Ditylenchus dipsaci, D. destructor, Rhadinaphelenchus cocophilus*, etc. in soil clods and plant debris, fungi like *Claviceps purpurea* in seeds of wheat and barley, *Peronospora manshurica* on soybean, *Fusarium nivale* on wheat, barley and Aegilops, Uromycesbetae on sugarbeet, bacteria like *Xanthomonas campestris* pv. *campestris* on *Brassica* spp. and viruses like Barley stripe mosaic virus in barley, Cherry leaf roll virus on French bean, Cowpea mottle virus in cowpea, Pea seed borne mosaic virus in broad bean, Tomato black ring virus on cowpea and French bean, etc. All attempts are made to salvage the infected/ infested material and only healthy material is released to the indentors. At NBPGR, priority has been given to identify critical gaps in plant quarantine and to re-orient research areas using modern

techniques in order to prepare us to meet the challenges posed by increasing international exchange.

L12

Wheat rust in peninsular zone: Past and present

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Presently, three wheat species namely *Triticcum aestivum* L. em. Thell (Bread Wheat) *T. durum* Desf (kothia, macroni wheat) and *T. dicoccum* (Schrank) Schubler are commercially grown in different parts of Peninsular Zone includes Karnataka, Maharashtra, part of Andhra Pradesh and Gujarat. Among the rusts, the leaf rust (*Pucccinia triticina* Eriks.) is known to occur in all the wheat growing areas of Peninsular Zone in general and Karnataka in particular plays an important role in wheat rust epidemiology. It serves as donor of stem and leaf rust inoculums to different parts of the country. First ten years (1986 to 1995-96) totally seven different leaf rust races detected out of which race 77 group was most predominant. However, as high as 10 leaf rust races reported. Eight different stem rust races were detected in this period out of which first five years 117 was most predominant subsequently this race not detected. Totally, as high as six races were detected. Next ten years (1996-97 to 2005-06) only three leaf rust races were detected out of which race 77 and 104 group was most predominant. As for as stem rust races concerned only one race (40A) detected during 1996, 2000 and 2005. From 19986 to 2007 stem rust races detected and after that it was not noticed in the farmer's field of Karnataka. However, leaf rust continued to appear till date. Recent three years survey has been done both in Karnataka and Maharashtra. In total, among all the year survey showed the presence of 20 different races in wheat growing areas of Karnataka. During 2010-11 and 2011-12 pathotype 121 R 63-1 (77-5) showed highest frequency in the surveyed area (35.71 and 58.90% respectively) whereas, during 2012-13 pathotype 121R60-1 (77-9) was found in highest frequency (29.89%). DNA profiling was done to know the extent of genetic diversity. Twenty five isolates were grouped into 14 clusters. The cluster XIV was largest with 12 isolates including phenotypically known isolates (12-4, 77-6 and 77-5). Remaining 13 isolates formed individual cluster including phenotypically known isolates (104-2 and 162-2). This clearly reveals that, these 13 individual clusters differed 100% with each other and also with cluster XIV. In the cluster XIV, twelve isolates showed similar as they were grouped in the same cluster and these isolates were 100 per cent dissimilar from that of all other 13 isolates.Survey of leaf and stem rust in Maharashtra during 2010-11, 2011-12 and 2012-13 in Maharashtra districts like Pune, Nasik, Satara, Ahamadanagar and Kollapur

showed the highest severity of the disease during 2010-11 as compare to 2011-12 and 2012-13. Out of three years survey totally 15 races of leaf rust were detected. The race analysis showed the presence of 11 different pathotypes during 2010-11 belonging to four groups (77, 104, 162 & 12). Among the four groups highest per cent was observed in group 77 and race 77-5 was found highest with 61.77% followed by 104-2 with 12.22%. During 2011-12, a race 104-2 was showed dominant with 41.67% and during 2012-13 races 77-9 and 12 was found equally dominant with 26.32%. In comparison of all the surveyed years, race 77-5 was found highest with mean of 32.21% followed by 104-2 with mean of 21.47%. The survey of stem rust in Maharashtra showed the presence of five important races during 2010-11 and 2011-12 but disease occurrence was not noticed during 2012-13. The highest severity was observed during 2011-12 with four races, among them race 40A was found dominant with 66.67 and 58.33% during 2010-11 and 2011-12, respectively.

L13

Molecular characterization of non-aflatoxigenic fungi and their application in aflatoxin management in groundnut

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In nature all the *Aspergillus flavus* isolates are not aflatoxin producers. Because of mutation in the genes involved in the biosynthesis pathway of aflatoxin, several strains exist as non-aflatoxigenic. In India, the studies and research regarding isolation and characterization of non-aflatoxigenic *A.flavus* and its use in managing aflatoxin and aflatoxigenic fungi in agricultural crops is generally ignored. An attempt has been carried out where 78 *A. flavus* isolates were isolated from groundnut seed samples belonging to different agro-climatic zones of India. They were differentiated based on their aflatoxigenic and toxigenic nature based on cultural, analytical, immunological methods and finally nine isolates were categorized as non-aflatoxin producers. But, dendrogram obtained from Random-amplified polymorphic DNA (RAPD) and ISSR-PCR failed to discriminate the *A. flavus* strains either on their toxicity or their geographical origin. Genetic characterization of these isolates using 13 primer pairs encoding the specific genes involved in aflatoxin biosynthesis pathway revealed the presence of six different deletion patterns (B-G). Among the 13 genes tested aflR was common deletion found in five deletion patterns (C-G) in controlling toxigenic strains. In the further study, the management of aflatoxigenic fungi using non-aflatoxigenic *A. flavus* strains in comparison with rhizobacteria, *Trichoderma* isolates and plant extracts were evaluated for their potential to reduce aflatoxigenic fungal infection and aflatoxin contamination in groundnut. Two rhizobacterial strains and one *Trichoderma* isolate found effective under in-vitro as well as *in-vivo* studies. Vanillin from

Decalepis hamiltonii was recorded its effective in reducing *A. flavus* incidence and aflatoxin production. In addition, among the nine non-aflatoxigenic strains, AFGS5 and AFGS12 significantly reduced the population of aflatoxigenic fungi and total aflatoxin in rhizosphere/ geocarposphere soil samples under greenhouse conditions. Quantification of aflatoxin by ELISA analysis in seeds revealed that AFGS5 and AFGS12 strains significantly reduced aflatoxin level and was well correlated with reduced seed-borne infection of aflatoxigenic fungi, determined by standard blotter method. The results demonstrated the biocontrol potential of native non-aflatoxigenic strains AFGS5 and AFGS12. Determination of other toxins in non-aflatoxigenic A. flavus and its bio-formulation for field application is under progress.

L14

Immigration of plant pathogens and their need for regulation to protect cut flower industries

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Cut flowers, being a high valued crop, most of the entrepreneurs venture in to the cultivation of cutflowers like carnation, orchids, anthurium, gerbera, lillium, alstomeria, bird of paradise, chrysanthemum and rose. The import of the propagative materials results in the introduction of unknown diseases of National significance, which are to be quarantined and regulated. Based on our experience with various cut flower growers, we came to detect the entry of quarantine pathogens like tomato spotted wilt virus from cut chrysanthemum, which is not known to exist earlier. The TSWV was introduced through chrysanthemum stem cuttings (*Dendranthema grandiflora* Tzvelev). RT-PCR was performed using the tospovirus degenerate primers gl 3617/ F-5'CCTTTAACAGTDGAAACAT 3'; gl4435c/R– 5'CATDGCRCAAGARTGRTARACAGA3') corresponding to the RNA–dependent RNA polymerase (RdRp) gene of tospoviruses. An amplicon of approximately 750 bp (KJ013534) was amplified from the symptomatic samples and had 98% identity with the RdRp gene of Tomato spotted wilt virus(TSWV) (GenBank Accession numbers, KC261950, KC261956, KC261959, KC261962). Further it was confirmed with two sets of primer pairs specific to the TSWV nucleocapsid gene (GK TSWV CP F-5'CAAGCAATAAAGATAAAGAAAGC3';GKTSWV CPR- 5'AGCATATAACAACTTCTACGATC 3'), and movement protein (MP) gene (GK TSWV MP F-5'ACCTATTATACACTTTGCTAAGAA3'; GK TSWV MPR 5'AATGCAAAATWRACAGAAATT 3') of TSWV (GeneBank Accession numbers for N gene-KJ494928; MP gene - KJ494927) exhibited the highest nucleotide identity of 98% with corresponding regions of TSWV isolates from different hosts and countries. Chrysanthemum was also infected by white rust, the quarantine pathogen *Puccinia horiana*. The *P. horiana* species specific primer (Ph-F1 5'- TGCATGAAT-

TTTTGAAAGGT-3' and Ph-R1-5'-CAAAAATTATTTTGTGAGAGGG-3') amplified a fragment of approximately 240bp. Yet another, *P. horiana* species specific primer for the region of 18S to 28S rDNA intervening sequence (Ph-F2 5' CCCCTTTTTTATTATATAACACAAG-3' and Ph-R1-5'-CAAAAATTATTTTGTGAGAGGG-3') amplified a fragment of approximately 340 bp. DNA fragments amplified with species specific primer pairs of *P. horiana* were cloned and sequenced for the K5 rust isolate from the variety (punch white) and the isolate HP2 from the variety (Saffin Pink). In the BLAST analysis sequences f K5 and HP2 isolates (Accession no: KC291657, KC291658, KC291659 and KC291660 respectively) had a nucleotide sequence identity of 100% with the *P. horiana* (Accession no: EU816916.1, HQ201326.1 EU816916.1 and EU816924.1) isolates.The pathogen causing bacterial blight was identified up to genus level through MALDI TOF-MS as *Xanthomonas.* It was further confirmed as *Xanthomonas axonopodis* pv. *dieffenbachiae* through FAME analysis, BIOLOG and by polymerized chain reaction using pathogen specific primers. DNA sequence of *X. axonopodis* pv. *dieffenbachiae* isolates designated as XAD1 and XAD2 were submitted in the NCBI Gen Bank bearing the accession numbers KJ603434 and KJ637328 respectively.House keeping genes also confirmed the identity of *X. axonopodis* pv. *dieffenbachiae* (KJ603435, KJ603436, KJ603437,KJ603438, KJ637329 and KJ637330).Survey was conducted in carnation growing areas of Tamil Nadu revealed the occurrence of stem rot of carnation seedlings. The pathogen was confirmed as *Sclerotinia sclerotiorum* through morphogenetic and molecular means. The genomic DNA was extracted from the fungus by CTAB method and the region between ITS 1 and ITS 4 primers were amplified with the primer pairs, ITS 1 (5'-TCTGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3'). The amplicon of ~ 600bp obtained were sequenced. The nucleotide sequence (GenBank accession number KP676452) had maximum identity of 99% with *S. sclerotiorum* (KM272350).

POSTERS

P103

Investigation of black cells and black spots of pomegranate during sea transportation and cold storage

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A laboratory and field experiment conducted at Department of Horticulture, Mahatma Phule Agricultural University, Rahuri during 2009-10 to find out the reasons for development of blackening of seed arils (seed) as well as the black spots ofpomegranate during sea transportation and cold storage. The laboratory study revealed that inhibitors *viz.*, Potassium metabisulphate (5 mM) inhibited the enzyme activity about 98% when pyregallol and catechol were acted as substrate. The pathogens *viz., Aspergillus niger, Microphamina* sp. and *Penicillium* sp. were responsible for spoilage of fruits; however fruits treated with Waxol + Carbendazim (0.1%) controlled the attack of spoilage of microorganisms. In field conditions the pathogen responsible for black spots were *Cladosporium cladosporiodes, Colletotrichum gloecisporiodes, Alternaria alternata* and *Alternaria tennuissima*. The fungicides *viz.*, Difenconazole @ 0.1%, Propiconazole @ 0.1% and Carbendazim @ 0.1% were found effective for the both leaf and fruit spots diseases in Mrig bahar (June-November) conditions. The levels of residues of fungicides were within the maximum residue limits.

P104

Bio-efficacy activity of fluorescent *Pseudomonas* against *Fusarium* sp. causing wilt of pomegranate

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Twenty four isolates of rhizobacteria isolated from four different locations were screened for *in vitro* antagonism by using co-inoculation technique against five different isolates of *Fusarium* sp. pathogenic to pomegranate (*Punica granatum* L.) plants. Among the twenty four isolates, twelve fluorescent rhizobacteria *viz.*, KHFR-1, 2, 3, 6, 7, RHFR-9, 10, 14 and NHFR-17, 18, 19, 20 identified as *Pseudomonas* sp. were found effective in inhibiting the growth of five *Fusarium* sp. Fluorescent *Pseudomonas* isolate KHFR-6, 7, RHFR-14 and NHFR-18 exerted maximum inhibition (83.52% each) of *Fusarium* sp-I and isolate RHFR-14, and NHFR-19 ex-

erted maximum inhibition on radial vegetative growth of *Fusarium* sp. –II. The rhizobacterial isolate NHFR-18 showed the maximum inhibition of radial vegetative growth of *Fusarium roseum* (85.00%) and *Fusarium oxysporum* (83.53%) followed by KHFR –7 RHFR-10 (each with 82.35% and 81.25, respectively). The isolate KHFR-7 exerted maximum inhibition of mycelia growth of *Fusarium moniliforme* (86.36%) followed by NHFR-18 (85.22%) and RHFR-10 (84.09%). This can be attributed to the release of antagonistic substances like siderophores or antibiotics in advance in culture media. The siderophores enhance the microbial acquisition of iron and make it unavailable to *Fusarium* suppressing its growth. The investigation confirms the finding that fluorescent *Pseudomonas* are involved in the suppression of all *Fusarium* species under study.

P105

Effect of different agrochemicals on intensity of Alternaria leaf spot/blight on cluster bean in arid region of Rajasthan

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Cluster bean or Guar (*Cymposidis tetragonoloba*) is a drought-tolerant legume crop. It is an important source of nutrition to animals and human and is consumed as a vegetable and cattle feed. Extracts from guar seed accounts for guar split/gum of about 29%. India earned Rs. 11735 crore from export of guar seed and its products to various countries in year 2013-14. *Alternaria* leafspot/blight is an (*Alternaria cucumerina* var. *cyamopsidis*) is an important disease of cluster bean and frequently causes considerable losses in both seed yield and quality. Therefore, a field experiment was conducted in Kharif seasons of 2013 and 2014 to find out the efficacy of different agrochemicals for the management of this disease at Agricultural Research Station, Mandor, Jodhpur on a susceptible cluster bean cv RGC 936. The randomized block design was followed with three replications maintaining a plot size of 4 × 3 m. Pooled data of two years revealed that minimum disease intensity (5.17%) and maximum seed yield (13 q/ha), fodder yield (27.63 q/ha), pods (52.3 pods/plant), net return (Rs. 49066/ha) and cost benefit ratio (Rs. 2.42/Rs. invested) respectively, recorded in the plots that were sprayed with difenconazole (@ 0.05%) as compared to maximum disease intensity (52.5%), and minimum seed yield (5.96 q/ha), fodder yield (13.05 q/ha), pods (28.0 pods/plant), net return (Rs. 15631/ha) and cost benefit ratio (Rs. 0.86/Rs. invested) recorded in control.

P106

Biological management of *Stemphylium* blight of Onion (*Stemphylium vesicarium*)

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Stemphylium blight caused by *Stemphylium vesicarium* (Wallr.) E. Simmons is one of the most destructive diseases, cause yield losses of about 90% and appearing in severe form and posing a threat to the cultivation of onion. Considering the economic importance of the disease the present investigations were undertaken. An experiment was carried out to find out the biological management of *Stemphylium* blight with botanicals/plant extracts and bioagents. All the 10 botanicals/plant extracts evaluated *in vitro* (@10, 15 and 20% each) were found fungistatic/fungicidal against *S. vesicarium*. However, significantly least mean radial mycelial growth (13.44 mm) and significantly highest mean mycelial inhibition (85.05%) was recorded with *A. indica* followed by *Z. officinale* (75.03%) and *A. sativum* (65.98%), respectively. All the six fungal and two bacterial bioagent/antagonists evaluated *in vitro* against *S. vesicarium* were found antifungal against the test pathogen. However, *T. viride* was found most effective and recorded least linear mycelial inhibition (78.51%) of the test pathogen followed by *T. harzianum* (71.85%) and *T. hamatum* (66.29%), respectively. The results obtained on the field efficacy of three fungal and one bacterial antagonists *viz., T. viride, T. harzianum, T. koningii* and *P. fluorescens*; and three botanicals *viz.,* Neem (*A. indica*), Noni (*M. citrifolia*) and Nirgudi (*V. negundo*), revealed that, all the spray treatments significantly reduced the Stemphylium blight disease incidence, intensity and per cent disease control significantly increased the bulb yield in onion cv. AFLR. Among the botanicals tested, *A.indica* (@10%)wasfound most effective and recorded significantly least mean disease incidence (23.75%), intensity (15.00%) and per cent disease control (64.89%) with corresponding significantly increased bulb yield (264.16 q/ha) with highest ICBR of 1:51.33. The second and third best bioagents found were *T. viride* (@ 20 ml/L) and *T. harzianum* (@20 ml/L), which recorded significantly least mean disease incidence, respectively of 25.00 and 28.91%, intensity respectively of 16.48 and 20.36% and per cent disease control respectively, 61.62 and 51.57% gave corresponding maximum bulb yield, respectively of 232.50 q/ha and 223.41 q/ha with maximum ICBR (1:12.69 and 1:11.05, respectively).

P107

The bioefficacy of *Pseudomonas* strains isolated from chilli rhizophere of TBP area of Karnataka against plant pathogen *Fusarium oxysporum* under *in vitro* condition

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The bioefficacy of *Pseudomonas* strains isolated from chilli rhizophere of TBP area of Karnataka against plant pathogen *Fusarium oxysporum* under *in vitro* condition. Total 40 isolates of *Pseudomonas* were screened for their biocontrol activity *in vitro* against fungal pathogen *i.e.*, *Fusarium* oxysporum on PDA medium by the dual culture technique. Qualititatively out of 40 isolates, 21 isolates showed inhibitory activity against *Fusarium oxysporum*. The isolates of *Pseudomonas* which showed inhibitory action against fungal pathogen, further examined for quantitative estimation. The isolate PS-5 isolated from Manvi taluq of TBP area was found to be most efficient strain which reduced the radial mycellium growth of *F. oxysporum* up to 6.69 with inhibition of 25.66% after 3 days of incubation and the least reduced mycelium growth was recorded in PS-21 isolate i.e., 8.80 with inhibition of 2.22% in terms of plant pathogenicity against plant pathogen.

P108

Management of foliar and basal rot diseases of *Aloe vera* (L.) burm by fungicides

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Aloe vera has medicinal history around world. The foliar diseases caused by *Alternaria alternata* and *Phoma* sp. The basal rot disease of Aloe caused due to *Fusarium oxysporum* and *Sclerotium rolfsii*. Percent disease incidence of foliar diseases was observed in different *Aloe vera* genotypes collected from different locations. For management of foliar and basal rot diseases spraying with Carbendazim + Dithane M-45, Tebuconazole + Dithane M-45, Carbendazim + Propiconazole 70 WP, Tebuconazole + Propiconazole 70 WP, Carbendazim + Tebuconazole and Dithane M-45 + Propiconazole 70 WP was taken. The fungicides *viz.*, Propiconazole, Tebuconazole, Hexaconazole, Carbendazim and Dithane M-45 were used. Lowest disease incidence

of foliar disease was observed in treatment with spraying of Carbendazim + Dithane M-45. Lower disease incidence of basal rot disease was observed in treatment with spraying of Tebuconazole + Dithane M-45. Lowest disease incidence was observed on top leaves and highest disease incidence was observed on lower leaves in all genotypes collected from different places *viz.*, Genotype IC112527 collected from Anand, Gujarat and genotype IC112532. Maximum disease incidence was observed in genotype AKAV04 collected from Akola. *In vitro* and *in vivo* the highest disease intensity of foliar diseases observed in control. All fungicides Carbendazim (0.2%), Dithane M-45 (0.3%), Hexaconazole (0.1%), Propiconazole (0.2%) and Tebuconazole (0.2%) showed cent per cent inhibition for all pathogens except *Sclerotium rolfsii* showed maximum growth in Carbendazim and *Fusarium oxysporum* showed growth in Dithane M-45.

P109

Integrated management of gummosis of mandarin orange caused by *Phytophthora citrophthora* in Marathwada region of Maharashtra

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Gummosis of Mandarin orange caused by *Phytophthora citrophthora* caused considerable losses in Marathwada region of Maharashtra. Therefore, an extensive roving survey in 115 fields was conducted to study the incidence of gummosis in the region. The average disease incidence was observed in the range of 10-65%. The highest disease severity and incidence was observed in Nanded district followed by Jalna, Parbhani, Hingoli, Aurangabad, Latur and Osmanabad districts. To manage the disease the bio efficacy of ten fungicides viz.,propineb (Antracol 70WP), hexaconazole (Contaf 5HC), iprodione 25% + carbendazim 25% (Quintal 25WP), fosetyl Al (Aliette 80WP), tridemefon (Balyton 25WP), dimethomorph (Acrobat 50WP), copper oxy chloride (Blue copper 50%), copper hydroxide (Kocide 77WP) and propiconazole (Tilt 25EC) and aqueous extract of seven botanicals i.e. leaf extracts *viz.,* mehendi, dhatura, acacia, eucalyptus, lantana, glyricidia and neem (@ 5% conc.) were tested in vitro against *P. citrophthora* using poison food technique.All treatments were effective against the *Phytophthora citrophthora* over untreated control. Among fungicides, the dimethomorph showed the least mean colony diameter (19.02 mm), and maximum per cent inhibition of mycelial growth of (47.97%), which was at par with iprodione + carbendazim (20.45 mm & 43.95% inhibition) and copper hydroxide (23.10 mm & 37.05% inhibition). These were followed by fosetyl-Al, propineb, copper oxychloride, propiconazole, tridemefon, hexaconazole and captan. All treatments are statistically at par with each other.Among botanicals, the treatment of Lantana was significantly superior over other treatments and recorded minimum mean colony diameter (20.85 mm) and highest mean mycelial growth inhibition (45.02%). Glyricidia ranks second, followed by mehendi, dhatura,

acacia, neem and eucalyptus in inhibiting the *P. citrophthora*.

P110

Efficacy of antimicrobial peptides against *Xanthomonas axonopodis* pv. *punicae*

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Bacterial blight disease of pomegranate, is a economically important diseases causing huge devastating losses. Bacterial blight or Oily spot disease of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae (Xap)* attack all above ground parts of the pomegranate. The Xap not only developed resistance to different commonly sprayed fungicides and antibiotics but also caused a heavy cytotoxicity to human and animals and also have a negative environmental impact. Therefore, the *in vitro* studies on the efficacy of nine different antimicrobial peptides (AMPs) against the *Xanthomonas* spp. were conducted. Filter paper disc and micro-dilution broth methods were used for *in vitro* evaluation of the anti-microbial peptides (AMPs) viz., D4E1, PEP11, ESF1, ESF4, ESF5, ESF6, ESF12, ESF13 and ESF17 against xap. The Dd4E1 was the most effective AMP against xap, which followed by PEP11, ESF1 and ESF17. While ESF12 was the least effective amongst all. Whereas, ESF4, ESF6, and ESF13 was not effective against Xap. The micro-dilution broth method through ELISA reader showed that the inhibition of growth of Xap was greater than 90% (MIC90) in D4E1, PEP11, ESF17, ESF5 and ESF12at 500 ppm concentration. Percent growth inhibition observed in D4E1, PEP11, ESF17 and ESF12 were comparable to that of Streptocycline. Thus, the peptides *viz.*, D4E1, PEP11, ESF1, ESF17, ESF5, ESF12can be used effectively for improvement of Pomegranate.

P111

Trichoderma spp. mediated inducted systemic resistance against bacterial leaf spots of tomato

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Twelve species of *Trichoderma* species were isolated from different districts of western Maharashtra. The *in vitro* efficiency of *Trichoderma* isolates in inhibiting the growth of *Xanthomonas campestris* expressed inhibition method. Among the 12 *Trichoderma* isolates Th-3

produce a highest inhibition zone of 24.44 mm followed by Th- 5 (22.00 mm). Observation on disease incidence of tomato recorded that the Th-3 showed the resistance response to challenge inoculation of *X. campestris* at 5, 10 and 15 days after inoculation followed by Th-5. While the inoculated control showed maximum disease intensity towards bacterial leaf spot of tomato. The lowest disease incidence of 26.67%, 28.33% and 33.00% was recorded by the challenge inoculation of Th-3 isolate in soil and seedling treatment after five, ten and fifteen days of inoculation respectively. The highest disease incidence was observed in the uninoculated control treatment. The significant increase in the biochemical constituents like protein content (94.50%), total phenol (69.03%), reducing sugar (18.18%), Phenyl alanine ammonia lyase activity(34.66%), Peroxidase activity (51.28%) of tomato due to soil and seedling treatment of Th-5 isolate of *Trichoderma* sp. were observed after 15 days of inoculation. Therefore it is found that *Trichoderma* spp induce systemic resistance in tomato plant against bacterial blight disease.

P112

Natural occurrence of entomopathogenic microorganisms and efficacy of *B. bassiana* and *Metarhizium anisopliae* against white grub infesting soybean

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White grub (Coleoptera: Scarabaeidae) is a major destructive pest in western Himalayan region. Survey was conducted in 14 villages, representing white grub endemic pocket of Haridwar, Pauri and Tehri districts from August to September during 2009-11 to quantify the mortality of whitegrub through natural infection of entomopathogenic microorganisms. For the observation, one square meter area from different crops were excavated and all the available grubs were collected in buckets having same soil and host plant for feeding of grubs. The diseased or dead cadaver were also collected and brought in the laboratory and identified the causal organisms. Field experiments were also conducted at Crop Research Center, College of Forestry and Hill Agriculture, GBPUA&T, Ranichauri during 2009-10 to evaluate the effectiveness of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* with few insecticides against white grub, *Holotrichia longipennis* damaging soybean. The study revealed that microorganisms like *B. cereus, B. bassiana, M. anisopliae, Steinernema, Heterorhabditis* are major disease causing agents in whitegrub under rainfed condition of Uttarakhand which caused 15.55 to 21.63% natural mortality of grubs with an average of 18.91%. However, among the microorganisms, *B. cereus* found to be significantly more efficient (7.03% mortality) than

the entomopathogenic fungi (3.80% mortality) and nematodes (3.20% mortality). On the basis of average cumulative plant mortality under field condition, *M. anisopliae* (5.0×10^{13} spores/g) was found to be superior compared by recording lowest plant mortality (21.83%) and 61.58% reduction in grub population followed by *B. bassiana* (5.0×10^{13} spores/g) where the plant mortality and reduction of grub population was 25.18% and 54.75%, respectively, compared to control. Highest percentage of yield increase (113.41%) was also recorded with *M. anisopliae*. However, among all the treatments, chemical insecticides i.e. imidacloprid was found to be the most effective in reducing the grub population (82.28%) and thereby increasing the yield 206.86% over control.

P113

Investigation of bacterial soft rot of *Aloe vera* caused by *Pectobacterium chrysanthemi*

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Soft rot of *Aloe vera* has a long botanical and medicinal history around world. The soft rot disease of Aloe caused due to *Pectobacterium chrysanthemi* which was confirmed by different parameters *i.e.* morphological and biochemical characterization and pathogenicity test. It is difficult to control by fungicides, bactericides treatments or cultural practices. In present investigation studies were undertaken to control this disease through the use of chemicals as well as bioagents. *In vitro* control of *Pectobacterium chrysanthemi* treatment with streptomycin sulphate at 100 ppm, 200 ppm, 300 ppm and copper oxychloride at 0.1 %, 0.2 %, 0.3% were-used. The results indicated that the growth of *Pectobacterium chrysanthemi* observed in control plate. The fungicide copper oxychloride @ 0.1%, 0.2%, 0.3% showed 14.50 mm, 16.00 mm and 17.50 mm resp. zone of inhibition for *Pectobacterium chrysanthemi* pathogens. The bactericide streptomycine sulphate @ 100 ppm, 200 ppm, 300 ppm showed 15.80 mm, 17.60 mm and 18.50 mm resp. zone of inhibition for this pathogen. However, the highest colony count of *Pectobacterium chrysanthemi* was observed in control plate. The bioagents *viz., Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* were used for control of *Pectobacterium chrysanthemi.* Per cent growth inhibition of bacterium was observed with *Bacillus subtilis* 86.77% followed by *Pseudomonas fluorescens* 77.17% and minimum growth inhibition *i.e.* 60.90% was observed with *Trichoderma viride.*

P114

In vitro efficacy of different bioagents against major seed borne fungi of cotton

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Cotton (*Gossypium* spp.) is one of the important commercial fiber and cash crop in India. Seed samples of four varieties *viz.*, AKA-7, AKA-8, LRA-5166 and PKV Hybrid-2 were tested for seed mycoflora by standard blotter paper (ISTA, 1985) and agar plate method. Seed samples of cotton showed association of eight fungi *viz., Aspergillus flavus, Aspergillus niger, Chaetomium globosum, Cladosporium oxysporum, Colletotrichum dematium, Fusarium semitectum, Myrothecium roridum and Rhizoctonia bataticola* belonging to seven genera. Among two methods, agar plate method was found superior than standard blotter paper for detecting seed borne mycoflora of cotton. An attempt was made to see *in vitro* efficacy of different bioagents *i.e. Trichoderma viride, Pseudomonaas fluorescens* and *Bacillus subtilis* against major seed borne fungi of cotton by using dual culture technique. *Rhizoctonia bataticola, Myrothecium roridum* and *Colletotrichum dematium* are major seed borne fungi of cotton. Among these bioagents, *Trichoderma viride* was found effective which shows 66.00%, 84.80% and 88.25% growth inhibition of *R. bataticola, M. roridum* and *C. dematium,* respectively followed by *Pseudomonaas fluorescens* and *Bacillus subtilis.*

P115

Antifungal effect of cow urine extracts against *Colletotricum capsici* causing leaf spot disease of turmeric

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The present study was conducted to determine inhibitory effect of cow urine with five plants namely *Azadiracta indica, Allium cepa, Allium sativum, Calotropis procera* and *Datura stramonium* against *Colletotricum capsici* isolated from leaf spot of turmeric. Poisoned food technique was performed to investigate antifungal effect of plant extracts with cow urine, acetone and distilled water. All extracts were found inhibitory against *C. capsici* but to a varied extent. The effectiveness of cow urine based *Allium cepa* extract was 51.58% followed by *Azadiarcta indica* (48.63%)and *Datura stramonium* (46.58%). Acetone based plant extracts were

also effective against *C. capsici*. The highest inhibition was recorded in, *Allium cepa* (49.21%), followed by *Azadiracta indica* (45.79%) and *Allium sativum* (42.94%). Whereas distilled water based plant extract was found least effective. Hence, cow urine based plant extracts appears to be promising and can be used to control *Colletotricum leas* spot of turmeric.

P116

Efficacy of different fungicidal molecules against *C. gloeosporioides* causing leaf and fruit spot of pomegranate

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Six fungicides were tested in the laboratory for their efficacy against *C. gloeosporioides*-causing leafand fruit spot in pomegranate (*Punica granatum* L.)by poisoned food technique. All fungicidal treatments showed significantly least colony diameter as compared to control (8.63 cm). Among six fungicides tebuconazole (0.1%) was found to be most effective which inhibited 87.48% growth of the fungus at recommended concentration, having colony diameter 1.08 cm followed by difenconazole (0.1%) inhibited 85.39% growth of the fungus having colony diameter 1.27 cm and pyraclostrobin + metiram (0.1%) inhibited 83.66% growth of the fungus having colony diameter 1.42 cm at recommended concentrations. The inhibition was lowest in propineb (0.25%) inhibited only 69.17% having colony diameter 2.67 cm at recommended concentration. In case of sporulation, the result indicated that tebuconazole (0.1%), pyraclostrobin + metiram (0.1%), difenconazole (0.1%) fungicides completely inhibited the sporulation and the fungicides azoxystrobin (0.05%), propineb (0.25%), metiram (0.25%) showed poor sporulation.

P117

Management of stem/pod blight of soybean caused by *C. truncatum*

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Stem/pod blight of soybean (*Glycine max* L.), incited by *Colletotrichum truncatum* (Schw.) Andrus and Moore, is a serious disease in almost all soybean growing areas of the world. Since most of the cultivated varieties were found susceptible to the pathogen, hence the foremost aim was to find out the chemical control measures, which can reduce the losses. A

number of chemical fungicides were evaluated *in vitro* conditions using food poison method and the chemicals found effective were further evaluated in field conditions. As compared to control all the test fungicides were found effective and inhibited the mycelia growth of *C. truncatum*. However, the inhibition varied from 79% to 89%. Maximum inhibition was recorded in benlate followed by thiophanate methyl, carbendazim and propiconazole.A field experiment was conducted. As compared to control all the treatments were effective in reducing the disease incidence. The minimum disease incidence was recorded in case of T9 (St with vitavax + spraying of captan 0.1%) (5.1%) and the maximum in un treated control (14.4%). Except T1 (control) and T2 (St with vitavax only) all the treatments were at par. Seed treatment alone reduced the disease incidence (27%), while St + spraying of fungicides further reduced the disease incidence, which varied from 54% to 65%. The highest yield was recorded in case of T9 followed by T5 (St. vv + benlate sp @ 0.2% and T3 (St. vv + carbendazim sp @ 0.1%).

P118

Influence of planting time on sheath rot of paddy

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At present, the sheath rot of paddy (*Oryza sativa*) caused by *Sarocladium oryzaeisa* devastating disease causing about 20-50% losses in yield. The survey carried out during Kharif, 2010 to 2014 showed its high severity wherever the crop is grown in the state of Maharashtra including Konkan region. All cultivated varieties of rice were found to be highly susceptible to sheath rot. Similarly, the disease cannot be kept completely under check by the use of fungicides. Hence, under the circumstances of susceptibility of most of the popular cultivars to sheath rot as well as unavailability of most effective chemical for management of disease, it is very necessary to manage the disease by using alternative methods of disease management. Adjusting the planting time with timely spray of fungicides helps to reduce the severity of many diseases in several crops. Hence, the experiment was conducted consecutively for three years (kharif, 2011 to 2013) in factorial RBD having five planting dates *viz.*, 15th June, 30th June, 15th July, 30th July and 16th August with and without sprays of carbendazim (0.1%) + quinalphos 25 EC (0.075%) at Agricultural Research Station, Lonavala. Three years pooled results revealed that the crops of first and second planting dates with sprays recorded significantly least incidence of 42.38 & 48.05% and severity of 12.66 & 14.96% thus, highest disease reduction of 82.33 & 79.12% over absolute control, respectively. On the contrary, the crop of last planting date without spray had significantly maximum disease incidence of 99.70 and intensity of 71.65%. Further, the second planting date with sprays produced significantly maximum grain (57.40 q/ha) and straw (68.23 q/ha) yields thereby, highest yield increase of 239.45 and 159.73% over control,

respectively with maximum B:C ratio of 2.29. This was followed by first and third planting dates with sprays, which produced 214.80 & 206.28% higher grain and 142.12 & 135.48% more straw yields over control as well as had B:C ratio of 2.12 and 2.07, respectively. Thus, transplanting of paddy from 15 to 30th June with two sprays of carbendazim 50 WP (0.1%) + quinalphos 25 EC (0.075%) at sheath formation and panicle emergence stages were found to be most effective for management of sheath rot and thereby increasing the grain and straw yields with monitory returns.

P119

Efficacy of botanicals and microbial inoculants on the growth of pigeonpea in relation to plant pathogens

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Efficacy of botanical, *Lantana camara* and bioinoculants such as *Azospirillum brasilense* and *Trichoderma harzianum* was determined in field experiment conducted for three summer seasons of 2010-2013 at University Agricultural Research Farm singly and in various combinations along with different recommended doses of inorganic nitrogenous fertilizers on growth parameters of pigeonpea (*Cajanus cajan*) in relation to the eco-friendly management of plant-pathogens. A significant reduction was observed in the population of plant-parasitic nematodes as well as soil-pathogenic fungi due to the soil application of bio-inoculants and a botanical along with recommended doses of inorganic fertilizer as compared to untreated control. However, the frequency of saprophytic fungi such as *Aspergillus niger, A. flavus, Penicillium digitatum*, etc. increased significantly due to addition of organic bioinoculants and inorganic fertilizers. The maximum enhancement was noticed in the growth and yield parameters such as plant height, fresh as well as dry weights, number of pods/plant, fruit weights/plant, protein content and chlorophyll content when these bioinoculants added simultaneously along with *L. camara* and 100% recommended dose of nitrogen in various combination. Application of *Azospirillum brasilense* and *Trichoderma harzianum* showed significant improvement, however, *Azospirillum brasilense* was found more prominent than *Trichoderma harzianum*. Physiological parameters such as rate of photosynthesis, fluorescence variable (Fv/Fm) increased significantly while rate of transpiration and stomatal conductance were decreased reciprocally when plants were given with aforesaid individual as well as combined application. Productivity parameters such as N, P and K contents in plants as well as in soil increased significantly in all the combinations irrespective of the treatments but most effectively in concomitant inoculation of these bioinoculants. This field study suggests that the soil application of organic additives and microbial bioinoculants minimises the additional supplement of chemical fertilizers which continuously

and regularly changed the ecological balance among diverse group of microorganisms harbouring in soil ecosystem.

P120

Management of foliar diseases of cotton

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A field experiment was conducted during Kharif 2014-15 at Cotton Improvement Project, MPKV, Rahuri for evaluation of seven fungicides and two bioagents against foliar diseases of cotton. Among leaf spot diseases (Alternaria/Cercospora/Myrothecium/Bacterial blight) *Alternaria* blight was the major disease observed in the experimental plots ranged from 9.22 to 34.52%. The lowest disease intensity (9.22%) and maximum disease control (73.29%) was recorded in spray treatment of Nativo-75 @0.05% WG (Trifloxystrobin 25% + Tebuconazole 50% WG), which was at par with all the fungicide treatments and significantly superior over bioagent spray treatments and untreated control. The treatment sprays of bioagents *P. fluorescens* (0.5%) and *T. Viride* (0.5%) recorded 20.46 and 23.39 PDI, respectively and was significantly superior over untreated control treatment recorded maximum 34.52% disease intensity. Moreover, the maximum seed cotton yield of 19.99, 19.70 and 19.41 q/ha was recorded in treatment sprays of Nativo-75 @0.05% WG, Copper oxychloride 50 WP (0.3%) + Streptocyclin (0.01%) and Pyrachlostrobin 20 WG (0.1%), respectively and minimum seed cotton yield (15.18 q/ha) in control treatment.

P121

Management of root rot and damping off complex in French bean with *Trichoderma* and *Pseudomonas* based bio-formulations

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The experiments were conducted during 2012-13 and 2013-14 at ICAR-IARI, New Delhi to evaluate the performance of different seed dressing and soil application formulations of *Trichoderma* species and talc based formulation of *Pseudomonas fluorescens* (IBSD-P137) against root rot (*Rhizoctonia solani*) and damping off (*Pythiumultimum*) complex in French

bean. The seeds treated with seed dressing formulations of Trichoderma and Pseudomonas alone and in combinations increased the seed germination, root and shoots lengths, plant dry weight and pod yield and decreased the disease incidence. The seeds treated with Pusa 5 SD (*T. harzianum*-IARI P4) in combination with *P. fluorescens* gave the highest seed germination, shoot and root lengths, dry plant weight and pod yield along with the lowest disease incidence followed by Pusa 5 SD (*T. harzianum*-IARI P4) alone, Pusa 5SD (*T. viride*; IBSD T-20) in combination with IBSD isolate of *P. fluorescens* and Pusa 5SD (*T. viride*; IBSD T-20) alone. Seed germinations, root length and disease incidence recorded in these treatments did not differ significantly. Soil application of Pusa bio-pellets of *T. harzianum* (IARI P4) and *T. viride* (IBSD T-20) also enhanced the seed germination shoot and root lengths, dry plant weight and pod yield and reduced disease incidence. Seed germination, root lengths and pod yield recorded in these treatments were significantly higher over the controls, whereas shoot length and disease incidence did not differ significantly over the controls.

P122

Management of leaf blight of tuberose

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Tuberose (*Polianthes tuberosa* L.) is one of the most important bulbous ornamentals of tropical and subtropical areas. It is commercially cultivated for cut and loose flower trade and also for the extraction of highly valued natural flower oil. The tuberose crop is taken well with less protection measures, however due to change in climatic conditions the leaf blight diseases caused by *Alternaria polyanthi* accounting 15-20% losses in yield and quality of tuberose and becomes the major threat in Maharashtra state. A field trial was conducted at All India Co-ordinated Research Project on Floriculture, NARP, Ganeshkhind, Pune, during 2011-12 to 2013-14 for three years to find out the most suitable management measures against leaf blight of tuberose. The cultivar Phule Rajani was planted in randomized block design with three replications at 30 × 30 cm spacing in flat beds. The six sprays of six fungicides *viz.*, mancozeb 0.2%, chlorothalonil 0.2%, tricyclazole 0.1% iprodine + carbendazim 0.1%, difenaconazole 0.1%, azoxystrobin 0.1% and control at 10 days interval starting from first incidence of disease were given.The three years pooled results revealed that the treatment with azoxystrobin 0.1% showed the least disease intensity (2.64 PDI) with maximum disease reduction (87.0%) but it was at par with difenoconazole 0.1% (3.96 PDI and 80.50% PDR) and iprodine + carbendazim 0.1% (4.21 PDI and 79.3 PDR) and were found significantly superior over rest of the treatments. The maximum yield of flower stalks and salable bulbs were obtained in azoxystrobin 0.1% (8.40 lakh fls/ha and 23.6 lakh bulb/ha), iprodine + carbendazim 0.1% (8.37 lakh fls/ha and 24.47 lakh bulbs/ha) and difenoconazole 0.1% (8.28 lakh fls/ha and 23.55 bulbs/ha). The different treatments gave

monetary returns ranging from Rs. 18.44 lakh/ha to 20.60 lakh/ha as against Rs. 15.83 lakh/ha in control. The highest monetary returns of Rs. 20.60 lakh/ha with maximum benefit cost ratio 3.47 was obtained in sprays with iprodine + carbendazim 0.1%.

P123

Disease management through organic practices in groundnut

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The present investigation was conducted to evaluate the efficacy of application of organic amendment enriched with biological control agents biological control agents and bio-product as foliar spray in managing soil borne as well as foliar diseases (stem rot, collar rot, tikka and rust) of groundnut for three years. The highest income (Rs. 107,256) and ICBR (1:13.52) were obtained in treatment where mancozeb was applied as seed treatment and hexaconazole as spray followed by *Trichoderma viride* as seed, soil and spray treatments (Rs. 94463; ICBR 1:4.92). It was obvious from the data that fungicides were essential for managing foliar diseases of groundnut. Moreover, foliar spray of hexaconazole, a systemic fungicide was also effective for reducing stem rot at later stage. Though organic farming is eco-friendly and safe, the application of FYM was costlier than fertilizers. Likewise, hexaconazole was more effective for the management foliar diseases as compared to the neem extract and biological control agents. On the efficacy of fungicides, yield and ICBR, it is concluded that seed treatment with mancozeb followed by two sprays of hexaconazole along with the application of recommended fertilizers (12.5N:25P) was very effective and economical in managing groundnut diseases, giving better yield as compared to the application of FYM and biological control agents (organic farming).

P124

Fungicidal management of leaf spot of turmeric caused by *Colletotrichum capsici*

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Turmeric (*Curcuma longa* L.) is one of the major spices cultivated for its underground rhizome. In this crop several diseases occur among which *Colletotrichum* leaf spot is an important disease. This disease was first reported from Coimbatore district of Tamil Nadu as *Ver-*

micularia curcumae (Syd.) in 1917. Later, in 1947 concluded that *Colletotrichum capsici* and *C. curcuma* are the same species. Considering the potential of leaf spot disease to cause the yield damage and importance of the turmeric crop in Maharashtra and as well as in India.The present studies conducted (*in vitro* and *in vivo*) to test the efficacy of a eight fungicides *viz.*, Mancozeb, Trycyclazole, Copper hydroxide, Propiconazole, Carbendazim +Mancozeb combination, Hexaconazole, Carbendazim and Copper oxychloride. *In vitro* study revealed that Propiconazole was most effective in controlling *C. capsici*, followed by Hexaconazole, Carbendanzim + Mancozeb, Carbendanzim and Mancozeb. However, the mean per cent inhibition of Propiconazole was highest (100%), followed by Hexaconazole (88.14%), Carbendanzim + Mancozeb combination (83.70%), Carbendanzim (80%), and Mancozeb (73.33%). In *in vivo* experiments the minimum disease severity of leaf spot was recorded in Propiconazole(8.88%) which was at par with Hexaconazole (9.62%), Carbendanzim + Mancozeb combination (11.11%) followed by Carbendanzim (13.33%). Maximum leaf spot disease severity was observed in control (40.00%).

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Delonix regia nursery disease management in Arunachal Pradesh

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Forestry has the prime objective of developing and protecting forests for their maximum productivity in Arunachal Pradesh. Fungal pathogens are major biological determinants in nurseries. They cause heavy damage to seedlings and hence reduce both quantity and quality of planting stock. *Delonix regia* is an important forest tree; they are prone to nursery diseases. Further, the infected seedlings are weakened and unable to withstand the adverse field/plantation conditions. Thus, the economic loss resulting from nursery diseases are considerable. Therefore, studies were conducted to control the nursery diseases in the college. *Delonix regia* seeds were treated with biocontrol agents and chemicals. Bavistin 0.2% and Trichoderma 2.5% were used and treated seeds were sown in the nursery area. The nursery sites are sandy soils and beds are ill trained. Hence, experiments were conducted to control soil borne pathogens. Nursery area was treated with hot water, chemical and biological control agent. Result obtained from seed treatment shown that chemical (bavistin 0.2%) and biocontrol (0.5%) reduced the disease incidence by 23%. Further, soil treatment, Bordeaux mixture (0.1%) chemical reduce the disease incidence by 75% followed by biocontroal agent 55% and hot water treatment 18%. Thus treating the *Delonix regia* seed with bavistin and *Trichoderma* and Bordeaux mixture (0.1%) application 10 days after sowing have reduced disease incidence in nursery.

P126

Management of *Fusarium* wilt in gladiolus

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Gladiolus (*Gladiolus dracociphalus*) is an important cut flower growing mainly for cut flower purpose. The main biotic stress is wilt caused by *Fusarium oxysporium* f.sp. *gladioli* and may cause a crop loss up to 60-80%. A field trial was conducted at All India Co-ordinated Research Project on Floriculture, NARP, Ganeshkhind, Pune, during 2011-12 to 2013-14 for three years to find out the most suitable management measures against *Fusarium* wilt disease of gladiolus. The sansarre a susceptible variety was raised in randomized block design with three replications. A spacing of 60 × 10 cm. was adopted and 15 corms were planted for each treatment using ridges and furrow method. The inoculum of *Fusarium oxysporium* f. sp. *gladioli* @ 250 g/m^2 was mixed thoroughly with 5 kg of well pulverized field soil and the mixture was spread evenly in experimental plot prior to planting. The bulb treatment at the time of storage and planting was given with hot water treatment (50°C for 30 min.) coupled with two bio control agents namely *T. viride* and *P. fluorescens*, one fungicide namely caftaf @0.3% and bulb treatment before storage and after planting only with captaf @0.3% were used. The pooled results over three years revealed that the least disease incidence (3.58, 5.55 and 7.22 PDI) and maximum disease reduction (94.67%, 91.74% and 89.26%) was recorded in pre storage hot water treatment of corm (50o for 30 min.) followed by pre planting corm treatment with captan 0.2% + carbendazim 0.2% followed by corm treatment with *T. harzianum* 10 g/L for 30 min., pre storage hot water treatment of corm (50°C for 30 min.) followed by pre planting corm treatment with captan 0.2% + carbendazim 0.2% and pre storage and pre planting corm treatment with captan 0.3%, respectively and were found at par with each other. However, the pre storage and pre planting corm treatment with captan 0.3% gave maximum benefit cost (1.90) and maximum monetary returns per ha (Rs. 13.21 lakh) and was found cost effective for better management of *Fusarium* wilt of gladiolus.

P127

Effect of organic matters and *Trichoderma viride* on the growth and productivity of *Abelmoschus esculentus* in relation to the management of phytonematodes

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Efficacious nature of some oil-seed cakes such as sesamum cake and groundnut cake, a botanical *Thevetia peruviana* and antagonistic fungus *Trichoderma viride* were inoculated singly and in various combinations in field condition in terms of growth and productivity parameters of *A. esculentus* in relation to the management of plant-parasitic nematodes. The growth parameters such as fresh as well as dry weights, number of fruits, ascorbic acid content and chlorophyll content were found significantly increased in all these treatments as compared to untreated control. Combined applications were more effective than individual application. Among organic matters, seasmum cake was found better in enhancing plant growth than groundnut cake, followed by *T. peruviana* and *T. viride*. Rate of multiplication was greatly affected due to soil application of organic matter and *T. viride* because of its suppressive nature. Productivity parameters, NP and K in plants as well as in residual soil were found significantly increased in all the treatments but more prominently in combined inoculation. The highest improvement in growth parameters was observed when the okra plants treated in combination with oil-seed cakes, *T. peruviana* and *T. viride*. The study will be useful in organic agriculture system to enhance the production without the addition of chemical fertilizers and synthetic chemicals.

P128

Efficacy of UV irradiated *Trichoderma viride* mutants against *Sclerotium rolfsii*

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Trichoderma, a well-known fungal biocontrol agent, grouped within the sub-division Deuteromycotina, in which sexual stage (perfect stages) is not known or rarely found and repro-

duction is limited to the production of conidia. Therefore, UV mutagenesis was found to be one of the novel strategies for developing *Trichoderma* mutants with enhanced biocontrol abilities against predominant soilborne fungal pathogens, Four efficient mutant strains were obtained from the wild *T. viride* strain, after exposing to UV irradiation source i.e. UV- tube, wavelength (λ)-254 nm, power 15 watt for different time periods, ranging from 20 to 80 minutes. Selected *T. viride* mutants showed variation in morphological characters,better sporulation and highest growth on PDA plates than their wild type strain. All mutants could grew at temperature 35°C having linear extension rate (mm/day) in the range of 25.95 to 32.11. However, wild strain of *T. viride* (control) showed less growth rate at 35°C. *S. rolfsii* was used as soil borne fungal test pathogen, which was found very sensitive to *T. viride* mutants *in vitro*. In dual culture study, *T. viride* mutant TvM2-UV2/60 showed 89.87% mycelial inhibition of *S. rolfsii* over the control (wild strain of *T. viride*). *S. rolfsii* was also found to be more sensitive to the volatile compounds released by *T. viride* mutant TvM2-UV2/60 which showed 82.72% mycelial inhibition of *S. rolfsii* over the control. In pot culture experiment, *T. viride* mutants enhanced root growth than untreated control and thus helped to enhance growth of tomato seedlings. It can be inferred from the results that mutagenesis in *T. viride* by using UV-irradiation is the best way to create efficient strains with enhanced antagonistic potential for suppression of predominant soilborne fungal plant pathogens (PSFP) like *S. rolfsii*.

P129

Impact of organic manures on two different cultivars in little millet against sheath blight

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Little millet (*Panicum sumatrense* Roth ex Roemer and Schultes), locally known as kutki, kuri, samalu, samai, suan and sama is cultivated throughout India in more than half a million hectares in the states of Tamil Nadu, Orissa, Bihar, Jharkhand, Maharashtra, Madhya Pradesh, Chhattisgarh, Andhra Pradesh and Karnatakaas cereal crop under extreme soil and climatic conditions of tribal agriculture.The crop is hardy and provides reasonable harvest even in degraded soils and unfavorable weather conditions. Generally, little millet is known as a disease free crop, but under favourable conditions, found infected with sheath blight caused by *Rhizoctonia solani* kuhn leading considerable loss in grain yield under favorable environmental conditions. An experiment was conducted at Agricultural Research Station, Vizianagaram, Andhra Pradesh with different organic manures (Farmyard manure, vermicompost and neemcake) in two different cultivars of little millet (Peddasama and OLM-203) in split plot design. The per-

centage disease intensity was calculated as number of infected nodes divided by total number of nodes, multiplied with hundred. Highest percent disease intensity of sheath blight was recorded in Farmyard manure applied Peddasama (15.6%) cultivar and also lower yield (7.67 q/ha) compared to Farmyard manure applied OLM-203 (9.0%) and yield was (8.52 q/ha) cultivar followed by vermicompost and neemcake in respective cultivars.

P130

Biological control of chilli root rot complex with soil amendments

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During recent years, the root rot complex disease of chilli (*Capsicum annuum* L.) has attained its serious proportion under nursery as well as field conditions. The pathogens responsible for root rot complex identified were *Sclerotium rolfsii* and *Fusarium solani*, causing about 60-80% and 35-50% losses, respectively. Therefore, present studies on manangement of chilli root rot complex were undertaken at Department of Plant Pathology, V.N.M.K.V., Parbhani during Monsoon 2013. A total of 12 amendments were evaluated as pre-sowing soil application, employing the sick soil technique and sowing susceptible chilli Cv. Parbhani Local, in pot culture under screen house conditions. Two separate experiments (one each of S. rolfsii and F. solani) were planned with CRD and all the treatments replicated thrice. Observations on seed germination, pre-emergence seed rot and post emergence seedling mortality in both the experiments were recorded separately. The results revealed that all the treatments showed significant increase in per cent seed germination, over untreated control. However, neem seed cake followed by mustard seed cake and castor cake recorded 66.67, 66.00 and 63.83% increase in seed germination, respectively in *S. rolfsii* sick soil. While, in *F. solani* sick soil, the best soil amendments found were mustard seed cake, followed by neem seed cake and castor cake with 67.59, 66.22 and 64.23% increase in seed germination, respectively over untreated control. All the test soil amendments were found effective against both the pathogens (*S. rolfsii* and *F. solani*), which recorded significant reduction in pre-emergence seed rot, post-emergence seedling mortality and average mortality in chilli crop.

P131

Field evaluation of ecologically sustainable management technology for apple replant disease

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Apple is a commercial crop of NW Himalayan region of India including state of Himachal Pradesh. The area under apple plantation is increasing as farmers are introducing improved and highly productive varieties for plantation in their orchards. Because of limitation of land in hilly areas growers are compelled to plant most of the new seedlings stock on old orchard site. This practice has resulted emergence of replant disease in most of the replanted apple orchards. Hence, there was an urgent need to evolve an ecologically sustainable management strategy for replant disease. In this regard, different treatment combinations including cover crops, bio-fumigant crops, arbuscular mycorrhizal (AM) fungi, biocontrol agents (BCA), tolerant rootstock and specific fungicide were tested first under pot cultures and then under field conditions. Out of these five treatment combinations *viz.*, (1) formaldehyde soil fumigation + wheat as cover crop + soil application with AM fungi and BCA formulation + M-793 rootstock, (2) formaldehyde soil fumigation + wheat as cover crop + soil application with AM fungi and BCA formulation + MM-111 rootstock, (3) soil bio-fumigation with Brassicas + wheat as cover crop + soil application with AM fungi and BCA formulation + M-793 rootstock, (4) soil bio-fumigation with Brassicas + wheat as cover crop + soil application with AM fungi and BCA formulation + MM-111 rootstock, and (5) soil bio-fumigation with Brassicas + wheat as cover crop + MM-111 rootstock + Ridomil MZ (0.4%) soil drench did not show any symptoms of replant disease till one year of transplanting as compared to 60% incidence in untreated control. The annual shoot growth of the apple plants in these treatment combinations was measured to be 24 to 64 cm as compared to 14.8 cm in untreated control. The highest annual shoot growth of 64 cm was observed in the treatment combination (1) formaldehyde soil fumigation + wheat as cover crop + soil application with AM fungi and BCA formulation + M-793 rootstock followed by (4) soil bio-fumigation with Brassicas + wheat as cover crop + soil application with AM fungi and BCA formulation + MM-111 rootstock and (5) soil bio-fumigation with Brassicas + wheat as cover crop + MM-111 rootstock + Ridomil MZ (0.4%) soil drench. Any of these treatment combinations can therefore be used to check replant disease in affected apple orchards.

P132

Bioefficacy of fungicides against the fungi associated with root complex of chilli

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Among the fungal diseases of chilli (*Capsicum annuum* L.), root rot complex caused due to co-infection of the fungi *viz., Sclerotium rolfsii* and *Fusarium solani* is the major one, causing reduction in plant population in nursery as well as under field conditions. Therefore, present *in vitro* studies were conducted during Monsoon 2013 to evaluate the bioefficacy of ten fungicides (each @ 500, 1000, 2000, 2500 ppm), applying Poisoned food technique and using Potato dextrose agar as basal culture medium. The experiment was designed with CRD and all the treatments replicated thrice.The results revealed that 100% average mycelial growth inhibition of *S. rolfsii* was recorded with the fungicides *viz.,* Carboxin 37.5% + Thiram 37.5% and Mancozeb; whereas, that of in *F. solani* cent per cent average mycelial growth inhibition was caused with the fungicides *viz.,* carbendazim, carboxin 37.5% + Thirum 37.5% and carbendazim 12% + Mancozeb 63.0%. The second and third highest average mycelial growth inhibition in *S. rolfsii* was recorded with the fungicides *viz.,* metalaxyl 8% + Mancozeb 64% (94.30%) and propineb (87.72%). Whereas, in *F. solani* second and third best fungicides found were Mancozeb (85.12%) and metalaxyl 8% + Mancozeb 64% (83.39%). Rest of the test fungicides recorded average mycelial growth inhibition in the range of 41.65 to 85.65% and 70.95 to 81.91%, respectively in *S. rolfsii* and *F. solani*.Thus, the combi-fungicides *viz.,* Carboxin 37.5 % + Thiram 37.5%, followed by metalaxyl 8% + Mancozeb 64% and carbendazim 12% + Mancozeb 63.0% could be used for effective management of the fungi responsible for causing root rot complex in chilli.

P133

In vitro efficacy of fungicides botanicals bioagents against *Xanthomonas axonopodis* pv. *citri* by paper disc method

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Citrus canker disease of acid lime caused by *Xanthomonas axonopodis* pv. *citri* an important disease in many parts of MH region. The bacterium infects the twigs, petioles, fruit stalks and fruits, resulting in both qualitative and quantitative loss in acid lime. Efficacy of different

combination of chemicals, botanicals and bioagents against *Xanthomonas axonopodis* pv. *citri* was assessed *in vitro* by measuring the growth by paper disc method. Maximum zone of inhibition was recorded in chemical treatments. i.e. copper oxychloride (0.2%) + Streptomycin sulphate (200 ppm) with recorded 36.64 mm zone of inhibition which is at par with treatment copper oxychloride (0.2%) + Streptomycin sulphate (100 ppm) showed 30.00 mm zone of inhibition. Among the bioagents *P. fluorescence* (10.33 mm) exhibited maximum zone of inhibition followed by *B. subtilis* (10.13 mm) and in botanicals 5% neem extract showed 13.13 mm zone of inhibition.

P134

Prevalence, diagnosis and integrated management of collar rot in apple through eco-friendly Methods

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Incidence of collar rot in apple under nursery and orchard conditions of Himachal Pradesh varied between 2.5-24.5% and 0.2-77.5%, respectively. *Phytophthora cactorum* was identified the most common pathogen to cause collar rot with average frequency of occurrence ranging between 73-85%. *Pythium ultimum* was the next common pathogen with frequency ranging 12-22%. Other species of *Phytophthora viz., P. citricola* (2-4%), *P. citrophthora* (1.5-3%). *Psyringae* (1.0-2.5%) and *P. litorale*- a new record(0.5-1.0%) were also identified. Different individually highly effective eco-friendly inputs *viz.*, fungal (*Trichoderma harzianum*-5(TH5), *T. hamatum*-2 (THM2), bacterial (*Bacillus subtilis*-11 BS11, *Pseudomonas fluorescens* KB6), mycorrhizal (*Glomus mosseae* -2 GM2, *G. heterosporum* GH1) biocontrol agents (BCAs), soil amendments (mustard cakes, dried leaves of Eucalyptus, *Murraya koeningi*, seeds of *Melia azedarach*), bio-fumigation with mustard plants, resistant rootstocks and soil solarization against target disease in apple in the earlier studies (2008-11) were further evaluated in different combinations under nursery (sick plot) and orchard conditions during 2012-14 to develop an eco-friendly integrated management strategy. Under nursery conditions, different single combination of individually effective inputs with soil solarization (SS) provided much enhanced control (87.6-98.3%). Further, combination of SS and mustard cake with fungal and bacterial BCAs provided complete control of target disease. Addition of fungal BCAs *viz.*, TH5/ THM2 separately with KB6 and BS11 provided 89.4, 94.2, 90.4, 94.2 PDC, respectively. Applications of TH5, BS-11, and KB6 separately with mustard cake gave 96.4, 95.3 and 92.2 PDC, respectively whereas the combinations of mustard cake with TH5 and BS11 provided 99.1 PDC. Under orchard conditions, addition of TH5 in combination with BS-11 and mustard cake increased

average shoot growth to 31.6 cm as compared to 6.1 cm in untreated plants. Further, approach grafting and addition of TH5, BS11 and mustard cake increased the shoot length to 36.4 cm whereas three applications of botanical cow urine decoction prepared from Murraya leaf, Eucalyptus leaf and Melia azedarach seed (7.5% @10 L/plant) alone in the month of March, June and August alone as well as with approach grafting increased the shoot length to 32.3 cm and 39.9 cm against 6.3 cm in untreated one. Spreading of red soil (10 cm thick layer) in plant basin along with addition of cow urine decoction as above (increased shoot length 36.3 cm) and its combination with approach grafting were also effective (41.8 cm).

P135

Diagnosis and integrated management of mouldy core and core rot of apple fruits: A new emerging disease

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Mouldy core and core rot of apple fruits has been appearing in moderate to severe form leading to excessive pre-harvest fruit drop and post harvest fruit rot during storage since 2005. Amongst the different associated pathogens (16 No.) identified; *Alternaria mali* occurred in highest frequencies (54.0-81.5%) followed by *T. roseum* (14.5-37.50%). *Fusarium* (0.1-5.8%) and *Penicillium* (0.2-2.60%) species occurred in low frequencies and other were occasionally observed. In management studies, evaluation of 10 fungicide spray schedules each consisting of individually *in vitro* effective fungicide two consecutive years indicated that a schedule comprising three sprays starting with propineb (0.3%) at pink bud stage followed by another two sprays with difenoconazole (0.015%) and dodine (0.075%) at petal fall– pea and marble - walnut stage, respectively was highly effective (96.4%) and economic (CBR 1:9.2). Other schedules were effective up to an extent of 84.5-94.2%. Similarly appraisal of nine different combinations of plant water extracts showed that three sprays starting with garlic bulb extract (5.0%) followed by *M. koenigii* leaf extract (10.0%) and *M. azedarach* seed (10%) + aonla fruit (10%) at above said three critical stages of plant growth in order was highly effective (82.5%) and was at par with schedule wherein first two sprays as above schedule has been exchanged and third one is replaced with walnut leaf extract (10%). In development of integrated management approach, out of nine different schedules evaluated for three consecutive years (2011-13), a schedule consisting three sprays starting with garlic bulb extract (5%) + difenoconazole (0.012%) followed by another two sprays with *M. koenigii* leaf water extract (10%) + propiconazole (0.04%) - mancozeb (0.25%) + aonla fruit extract (10%) at above three stages of plant growth as above, respectively was most effective (96.2%) and quite economic (CBR 10.89).

P136

Somatic embryogenesis and testing somatic embryo derived plants of black pepper for Piper yellow mottle virus

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Matured berries obtained from black pepper plants infected by Piper yellow mottle virus (PYMoV) of nine varieties namely IISR Thevam, IISR Girimunda, IISR Shakthi, IISR Malabar Excel, Pournami, Subhakara, Sreekara, Karimunda and Panniyur-1 were used for somatic embryogenesis. Embryo along with surrounding micropylar tissue scooped out from the surface sterilized berries were used as starting material. Primary somatic embryo was induced from micropylar region. Secondary somatic embryos were visible from root pole region of primary embryos within 65 to 90 days in different black pepper varieties on growth regulator free Schenk and Hidebrandt (SH) medium. Secondary somatic embryo gave rise to cyclic secondary somatic embryos (proembryogenic mass) within 10-20 days in different varieties. Rate of multiplication of somatic embryogenic mass varied with sucrose concentration. Regeneration of embryogenic mass into plantlets was carried out in SH liquid medium with 3.5% sucrose. Well developed plantlets were transferred in Woody plant medium with 3.5% sucrose and well rooted plants were hardened. Genetic fidelity testing of somatic embryo derived plants with respective mother plants using five SSR markers, showed genetic uniformity. Out of 53 somatic embryo plants were tested for PYMoV using primers specific for four regions of virus (ORF I, ORF II, ORF III and ORF IV), nine plants showed freedom for virus, indication virus elimination.

P137

Sheath blight disease control by *Pseudomonas* strains PRS 3 & PRP 5 isolated from the acidic soil of Kuttanad, Kerala

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Rice the primary staple food in many countries, is subjected to many diseases that often place major biological constraints on production. Sheath blight of rice caused by *Rhizoctonia solani* is an important disease which curtails rice production. The *Pseudomonas strains* PRS3 & PRP5 were isolated from rhizosphere of rice seedlings collected from Kuttanad, Kerala, were tested for their *in vitro* antagonistic activity against *Rhizoctonia solani*. The crude metabolites from PRS3 & PRP5 were extracted with organic solvents such as ethyl acetate and petroleum

ether and these were tested against *Rhizoctonia solani*. Seed bacterization with PRS3 & PRP5 enhances plant growth in gnotobiotic as well as in green house condition. These two strains also showed their efficacy in plant growth promotion and disease suppression in herbicide contaminated soil. The present study confirms the antagonistic and herbicide resistant PGPR strains can be used in managing sheath blight disease along with or without herbicides.

P138

Epidemiology and management of foliar diseases of groundnut in Kharif season

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The epidemiological studies on early, late leaf spots and rust diseases in relation to weather parameters revealed that during kharif, 2014 the development of early leaf spot was highly influenced by mean temperature and crop age and for late leaf spot development, maximum temperature and crop age were highly favourable. Moreover, evaporation rate and crop age played predominant role in rust development in groundnut under Dapoli conditions. Management studies on early leaf spot, late leaf spot and rust, revealed that three sprays of Hexaconazole + zineb (0.1%) at an interval of 20 days was the most effective treatment in reducing ELS, LLS and rust of groundnut. It was followed by tebuconazole (0.3%), tebuconazole (0.15%), difenconazole (0.1%) and propiconazole (0.1%).

P139

Management of *Fusarium* wilt of tomato by cold-tolerant *Trichoderma* species

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Vascular wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* is highly destructive in all tomato-growing areas of the world. Biocontrol by use of *Trichoderma* represents a potentially attractive and alternative disease management approach to chemical control. How-

ever, most *Trichoderma* spp. being mesophilic, their biocontrol efficiency would be greatly affected by low temperatures in winter. Hence, cold-tolerant isolates of *Trichoderma* spp. have the potential of being harnessed for use in biocontrol and for exploring cold-adapted enzymes like psychrozymes.Cold-tolerant *Trichoderma* species capable of growing at 5°C were isolated from rhizosphere soils of western Himalayan region. Sequencing of Internal Transcribed Spacer region indicated the taxonomic affiliation of the isolates to *Trichoderma gamsii, Trichoderma velutinum* and *Hypocrea lixii*. One isolate PETX-Behli-1 (*T. gamsii*) showing maximum antagonism against *Fusarium oxysporum* f.sp *lycopersici* (FOL) was elucidated for its chitinolytic ability. The isolate displayed maximum chitinolysis on chitinase detection agar and recorded higher chitinase activities of 63.1 to 83.8-folds at 5 through 37°C in the extracellular proteins of minimal synthetic broth (MSB) amended with colloidal chitin over MSB alone. Extracellular proteins of cell free extracts of MSB amended with and without chitin contained polypeptides of 26-180 kDa. In plate assays, the crude extract containing chitinases displayed 46.2% inhibition of mycelial growth of the pathogenic fungus. Seed bacterization and soil application of chitin-supplemented talc-based formulation of PETX-Behli-1 challenge-inoculated with FOLresulted in significantly lowerincidence (61.9% reduction) relative to the non-bacterized pathogen control in a greenhouse (18-20°C). This was associated with an increase in the plant vigour index, fruit number and fruit weight of 64.8, 205.4 and 210.8%, respectively, relative to the pathogen control.In native gel activity assays, PETX-Behli-1 with challenge-inoculation, expressed high intensity or more isoforms of chitinase, peroxidase and polyphenol oxidase. The results suggest that the cold active PETX-Behli-1 may represent an important biocontrol agent to control *Fusarium* wilt of tomato plants grown in winter.

P140

Integrated disease management of citrus canker at the early stage in Kashi Mandarin

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Khasi mandarin farming is the main source of income for more than 60% of people of east siang district, Arunachal Pardesh. A large variety of *Citrus* sp including sweet oranges, mandarin, lemons, lime and grapefruit are grown in this region. Although Arunachal Pradesh posses second largest area in citrus but stands last in productivity. The limiting factor in production and productivity is mainly due to poor plant protection measure, negligence of orchard management, poor maintenance of seedling and trees and unawareness about organic manure application, storage and post harvest diseases. Khasi mandarin is generally propagated through budding and budded seedlings are more prone to bacterial blight, leaf miner and citrus butter-

fly. Nursery stage seedlings are more susceptible to bacterial canker caused by *Xanthomonas axonopodis* pv *citri* and leaf miner. Experiments were conducted at seedling stage to manage the citrus canker and leaf minor using chemical and biocontrol methods. To manage the leaf minor spraying of 0.05% monochorotophos proved to be effective one followed by 0.05% dimethoate and Spraying of entomo pathogenic nematode. Spraying of Bordeaux mixture 1% reduced the disease incidence by 88% followed by spraying of PGPR @ 0.5% reduced the disease incidence upto 55%.

P141

Management of leaf spot and rust of groundnut by difenconazole 25% EC in Northern Karnataka

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Groundnut (*Arachis hypogaea*) occupies one of the most important place among the oil seed crops in India and particularly in Karnataka causing considerably yield losses due to rust (*Puccinia arachidis*) and late leaf spot (*Phaeoisariopisis personata*) diseases. The present study was under taken at KVK Farm, Bagalakot for two growing seasons of 2012 and 2013 in order to test the efficacy of difenconazole 25% EC against late leaf spot and rust diseases. The results revealed thatspraying of difenconazole 25% EC reduced the incidence of late leaf spot and rust diseases considerably. The data of over two seasons clearly indicated that difenconazole 25% EC @0.1%, 0.15% and 0.2% were most effective for the management of both the diseases. The Bio-efficacy of difenconazole 25% EC was tested at different concentarations and the results indicated that three sprays with difenconazole 25% EC @ 0.2 and 0.1% at an interval of 12 days recorded minimum late leaf spot severity (14.07 PDI & 15.50 PDI) and minimum rust disease severity (7.33 PDI & 7.70 PDI) respectively. The maximum yield of 26.23q/ha in difenconazole 25% EC @ 200 mL/100 L followed by 25.50q/ha in difenconazole @0.1%. The maximum disease pressure (Leaf spot severity of 58.30 PDI, Rust severity of 48.50 PDI) was recorded in untreated control with minimum seed yield of (20.13 q/ha). There were no visual symptoms of phytotoxicity noticed in terms of epinasty, hyponasty, necrosis, vein clearing, wilting, leaf tips and surface injury on groundnut crop in all concentrations tested. Hence, the study identified difenconazole as an effective molecule at 0.2 and 0.1% for the management of late leaf spot and rust of groundnut is recommended as an effective management strategy to enhance the productivity of groundnut in Northern Karnataka.

P142

Azoxystrobin 23% SC a new molecule for the management of leaf spot and fruit rot of pomegranate caused by *Alternaria alternata* (Fr.) Keissler in Northern Karnataka

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Pomegranate (*Punica granatum* L.) a high value crop; is one of the important fruit crops in arid and semi-arid regions known for its drought tolerance which thrives well in dry tropical conditions with marginal soils of low fertility. There are several reasons for low productivity among them diseases are the major factors that take heavy toll.In recent years the crop is under threat due to number of serious diseases such as bacterial blight (*Xanthomonas axonopodis* pv. *punicae*), wilt due to *Ceratocystis fimbriata*, anthracnose (*Colletotrichum gloeosporioides*) and leaf spot and severe fruit rotting due to *Alternaria alternata, Cercospora* sp., *Pseudocercospora* sp., *Drechslera* sp. and *Sphaceloma* sp. etc. The present investigation was undertaken with new molecule azoxystrobin 23% SC during 2012-13 and 2013-14.The experiment was laid out in a randomized block design (RBD) in the farmer's field in Bagalkot district (South India) during Hastabahar seasons (2012 and 2013) with 8 years old pomegranate (Cv. Kesar) plants. The bio- efficacy of 0.06%, 0.08%, 0.10%, 0.12% and 0.24% concentration of azoxystrobin 23% SC with other two effective fungicides *i.e.* difenconazole 25 EC at 0.05% and mancozeb 75% WP at 0.26% in pomegranate leaf spot and fruit spot /rot was carried out. The pooled analysis over two years revealed that, spraying with azoxystrobin at 0.12% concentration recorded minimum disease intensity (2.50% and 4.67%) with 83.94 and 96.82% disease control of leaf spot disease and minimum fruit spot index of 5.53 and 9.07 with 92.68 and 86.76% disease control. The maximum disease severity of 15.57 and 75.53 leaf spot and fruit rot was observed in untreated control. The maximum fruit yield/plant with 25.75 t/ha and with 21.50 t/ha was observed in spraying with azoxystrobin at 0.12% during the year 2012-13 and 2013-14 respectively. The minimum fruit yield of 14.50 t/ha was recorded in untreated control. There was no phytotoxicity effects in any of concentrations tested. Hence, It can be recommended that azoxystrobin 23% SC (Amistar 25 SC) @ 0.028% g ai/ha would help in managing both leaf spot and fruit rot of pomegranate in northern Karnataka that would help in promotion of export potential.

P143

Effect of mulches on disease incidence and yield of ginger

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Rhizome rot caused by *Pythium* spp. is a serious disease throughout the ginger growing regions. Application of mulches is common in rainfed ginger production to conserve soil moisture and weed suppression. However, not much work has been done on the influence of mulches against disease incidence in ginger. Hence an experiment was conducted at at ICAR-IISR, Kozhikode to study the effect of mulches on rhizome rot incidence besides weed suppression. There were 15 treatments comprising organic and plastic mulches, evaluated in RBD with four replications. Among the mulches used, significantly lesser incidence of soft rot (8.7%) was observed with application of dried coconut leaves alone at the time of planting in ginger beds and maximum soft rot (33%) was found in the non mulched control. Mulches that have given higher yield in ginger were again evaluated in FLD during the year 2014-2015. Application of dried coconut leaves also increased nutrient availability of N (266 mg/kg), K (202.3 mg/kg), Mg(127.3 mg/kg), Mn (33.00 mg/kg, Zn (2.50 mg/kg) and Cu (2.0 mg/kg) in soil at 120 DAP. The FLD conducted at Kannur and Kozhikode also confirmed that less incidence of soft rot (5.0% and 3.0%, respectively) with maximum yield at Kannur (11.13 t/ha) and at Kozhikode (12.67 t/ha) due to application of dried coconut leaves.

P144

Integrated management of soybean rust caused by *Phakopsora pachyrhizi* Syd. in northern Karnataka

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"Yellow Revolution" was the result of enhanced pace in the development of Indian agriculture after Green Revolution which contributed remarkably due to newly introduced crops like soybean in the country. In recent years, soybean rust caused by *Phakopsora pachyrhizi* Syd. is more severe under assured rainfall/ irrigated conditions. Survey conducted for identification of hot revealed that soybean rust was prevalent in all the soybean growing areas of north Karnataka with the per cent disease index ranging from 20 to 95. Severity of the disease increased with delayed sowing and drastic reduction in grain yield and 100 seed weight. Among the 97

genotypes screened DSb 21 exhibited highly resistant reaction with high yield potential. Integrated disease management studies identified the effective biorationals and fungicides. Adaptive Module is the most preferred module over chemical and biointensive modules in integrated management of soybean rust. Two sprays of hexaconazole @1ml/liter recorded the highest yield than unsprayed treatment. DSb- 21 recorded the highest yield (19.05 q/ha) among all variety in protected (19.05 q/ha) and unprotected plot (16.80 q/ha) followed by VLS 63. Among the 12 treatments cow urine @10% + potassium phosphonate @0.3% recorded less disease severity with better yield compared to untreated control. Significantly lowest disease severity was recorded in hexaconazole @0.1% sprayed treatment. Among the three modules, seed yield was highest in chemical Module (32.20 q/ha) which was on par with adaptive Module (30.80 q/ha).

P145

Epidemiology and management of foliar diseases of groundnut in Kharif season

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The epidemiological studies on early, late leaf spots and rust diseases in relation to weather parameters revealed that during kharif, 2014 the development of early leaf spot was highly influenced by mean temperature and crop age and for late leaf spot development, maximum temperature and crop age were highly favourable. Moreover, evaporation rate and crop age played predominant role in rust development in groundnut under Dapoli conditions. Management studies on early leaf spot, late leaf spot and rust, revealed that three sprays of Hexaconazole + Zineb (0.1%) at an interval of 20 days was the most effective treatment in reducing ELS, LLS and rust of groundnut. It was followed by Tebuconazole (0.3%), Tebuconazole (0.15%), Difenconazole (0.1%) and Propiconazole (0.1%).

P146

In vitro evaluation of systemic and nonsystemic fungicides against *Alternaria alternata*, causing leaf spot of gerbera

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Gerbera (*Gerbera jamesonii* Hook), one of the important flower crops suffers from substantial damage with leaf spot disease incited by *Alternaria alternata* (Fries) Kessler. Results of

the in vitro evaluation revealed that all the test fungicides, botanicals and bioagents significantly inhibited mycelial growth of *A. alternata*, over untreated control. Of the systemic fungicides tested, highest average mycelial growth inhibition was recorded with Hexaconazole (94.44%), followed by Carbendazim (84.93%), Propiconazole (81.53%), Difenconazole (75.97%). Whereas, fungicide thiophanate methyl was found less effective with mycellial inhibition of 51.21%. Of the non- systemic fungicides tested, Mancozeb recorded highest average mycelial growth inhibition (92.21%), followed by Curzet (84.45%), Chlorothalonil (80.90%) and Propineb (78.89%). Whereas, fungicide copper oxychloride was found less effective with mycellial inhibition of 74.03%.

P147

Evaluation of botanical soil amendments against soil borne inoculum of early blight of tomato

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In the present study, botanical soil amendments such as composts, enriched composts (with *Trichoderma viride*) and dry biomass-powders of two botanicals *viz., Crotalaria trichotoma* and *Azadirachta indica* were tested in greenhouse condition against soil borne inoculum of early blight of tomato, caused by *Alternaria solani*. Inoculation was done by splash irrigation once a day. All the amendments at 0.5% rate (soil/amendment : w/w) were found to be effectively suppress the disease, with *Crotolaria* enriched compost, *Crotolaria* dry powder, *Azadirachta* enriched compost, *Azadirachta* compost and *Crotolaria* compost showing percent early blight index of 16.5%, 18.3%, 20.1%, 21.6% and 23.3%, respectively, as compared to 83.3% of negative control.

P148

Management of downy mildew of bitter gourd by chemicals and bio agents

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Downy mildew is a major disease of bitter gourd in Kerala. A field experiment was conducted to evaluate newer fungicides and biocontrol agents against the disease during June to

September 2013.The bitter gourd variety Preethi was raised as per the POP recommendation of KAU–2011, in the seed production plot of Central Nursery, KAU, Vellanikkara. The treatments used in the experiment were, azoxystrobin-1.5mL/L, pyraclostrobin-0.5g/L, propineb-3g/L, fenamidone+mancozeb-2g/L, cymoxanil+mancozeb-2g/L, famoxadane+cymoxanil-1mL/L, mancozeb-2g/L, *Pseudomonas fluorescens* (KAU)-20g/L, *Trichoderma viride* (KAU)-20g/L and untreated control. The treatments were applied as foliar spray on the appearance of disease and after 15 days of first spray. Disease severity was recorded before spraying and 10 days after each spraying using 0-5 scale and per cent disease severity was calculated. Fruits were harvested for seed purpose at ripening stage and yield was recorded after each harvest. The symptoms of the diseased were appeared at 60 days after sowing and cent per cent disease incidence was recorded in all treatments. After the first spray, *Pseudomonas fluorescens* was recorded the minimum disease severity (15.66) with maximum reduction of 44.05% over control followed by fenamidone+mancozeb (42.33%) and pyraclostrobin (41.15%). After second spray, pyraclostrobin showed maximum reduction of 69.64% with minimum disease severity of 16.47%. It was followed by the bio agent, *Pseudomonas fluorescens* and combination fungicides, famoxadane+cymoxanil and cymoxanil+mancozeb which were on par and recorded 68.79, 67.24 and 66.55% reduction over control. No significant difference was noticed among the treatments with respect to yield.

P149

Bioconsortial and fungicidial efficacy for managing the mulberry wilt pathogen (*Fusarium solani* (Mart.) Sacc.)

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Mulberry (*Morus* spp.) foliage is the only food for the silkworm (*Bombyx mori* L.) and is grown under various climatic conditions. The diseases have become more alarming because of its epidemic nature and propensity to kill the plant completely. Among them, root rot and wilt is considered one of the major disease, leading to death of entire plant. In mulberry, the wilt pathogen *Fusarium solani* was first reported in India by Siddaramaiah & Hegde (1990). Although fungicides and antagonistic microorganism's *viz., Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated individually and in combinations for their biocontrol potential against *F. solanito* test the efficacy of biocontrol agents (Pf1, Bs4, and Tv1) with seri bed waste, neem cake and fungicides (Carbandazim 0.1%, pre mixture fungicide Carbendazim + Mancozeb 0.1%, Tebuconazole 0.1%. The results showed that maximum reduction was recorded in Carbendazim (0.1%) (74.22%) treated plots followed by Consortia (Seri bed waste + Pf1 + Bs4 + Tv1+ Neem cake) 500 g/plant recorded (68.22%). The highest leaf yield was recorded in Consortia (Seri bed waste + Pf1 + Bs4 + Tv1+ Neem cake) applied plots recorded the maximum

leaf yield of 3.59 kg/plant and followed by Carbendazim applied plots recorded the leaf yield of 3.48 kg/plant respectively.

P150

Eco-friendly management of purple blotch of onion

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Purple blotch of onion caused by *Alternaria porri* (Ellis) is one of the most destructive disease of onion causing accountable losses of about 80 to 90%. Considering economical importance of disease the present investigation on eco-friendly management of disease with fourbio-agents, five botanicals and one fungicide, during Kharif 2013 at College of Agriculture, Latur (MS) was carried out.Among the treatments, the most effective found was bio-agent *Trichoderma harzianum* with lowest disease severity (34.48%), highest bulb yield (315 q/ha) and most economical C: B ratio (1:31.81), followed by *T. viride*, with disease severity of 40.32%, bulb yield of 302 q/ha and C: B ratio of 1:27.34.

P151

Evaluation of biocontrol agents and different fungicides against bhendi powdery mildew (*Erysiphe cichoracearum* DC)

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Among many fungal diseases of bhendi (*Abelmoschus esculentus* (L.) Moench.) the most important disease is Powdery mildew. The trials were laid out in Randomized Block Design with twelve treatments and three replications. The results from the field trials were indicated that foliar spraying of UPF 807 @0.36% recorded the minimum severity of the powdery mildew disease (PDI 5.59) with 67.44% reduction, which was on par with *P. fluorescens* (0.2%) + *B. subtilis* (0.2%) + UPF 807 (0.36%) (PD1 5.60) with 67.38% reduction, when compared to control with PDI of 17.17 and the maximum yield was recorded 15.92 t/ha. UPF 807 treatments at 0.21%, 0.27%, 0.36%, 0.42% and 0.71% (double of effective dose) doses were assessed for the phytotoxicity. It was safe to the crop and it didn't show any phytotoxicity when compared to other treatments.

P152

Detection of watermelon bud necrosis virus (wbnv) in watermelon and IPM strategies for its management

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Watermelon bud necrosis virus (WBNV) (genus: Tospovirus, family: Bunyaviridae) is a serious pathogen of Watermelon (*Citrullus lanatus* (Thumb) Mans) and is transmitted by thrips in a propagative manner. The yield loss due to WBNV infection in watermelon ranging from 60-100%. The field samples showing characteristics symptoms (necrotic spots and patches on leaves; necrosis of the buds; chlorotic ring spot and necrotic lesions on fruits) were collected from Kittampalayam village of Coimbatore district of Tamil Nadu and were artificially inoculated on cowpea cv. C152 plants through mechanical sap inoculation. WBNV was serologically detected using the polyclonal antiserum of GBNV by DAC-ELISA, DIBA, TIBA and western blot analysis. Molecular detection of WBNV was also done with RT-PCR using Tospovirus universal primers and the WBNV coat protein gene specific primer. An IPM module consists of seed treatment and soil application of *Pseudomonas fluorescens* along with neem cake; installation of yellow sticky traps; soil mulching with UV reflectant polythene sheets; growing maize as border crop and use of botanical pesticide (neem oil 3% spray) was evaluated under field conditions in two different locations and compared with farmers practice. In both the trials, the WBNV disease incidence and the vector population were found to be significantly reduced with an increased yield in the IPM plot. Thus, similar environmentally benign IPM strategies can be implemented as an alternation to pesticide-based measures for mitigating negative impacts of WBNV.

P153

In vitro bioefficacy of fungicides and bioagents against *Fusarium oxysporum* f. sp. *Udum* causing wilt disease of pigeonpea

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Fusarium wilt (*Fusarium oxysporum* f. sp. *udum*) is one of the major diseases of pigeonpea during kharif season. Study was conducted at research field of College of Agriculture, Marathwada Krishi Vidyapeeth, Parbhani (MS) India; during Kharif 2009-10 aimed to integrate the

fungicides and bioagents for the management of pigeon pea wilt. Results revealed that all five fungicides and six bioagents evaluated significantly reduced the mycelial growth over untreated control. However, Thiram + Captan was found most effective with the highest mycelial growth inhibition of 87.77%, was followed by MAU fungi (86.66%), Carbendazim (85.55%) and Thiram + Captan and *T. Harzianum* (81.11% each). Whereas, least mycelial growth inhibition was recorded with *T. hamatum* (65.55 %), followed by *P. fluorescens* (71.11%) and Captan (72.22%).

P154

Integrated management of wilt of pigeon pea caused by *Fusarium oxysporum* f. sp. *udum*

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Pigeon pea (*Cajanus cajan*), is one of the most popular legume crop grown throughout the world. Pigeon pea crop is affected many fungal, bacterial and viral diseases and among the fungal diseases, wilt caused by (*Fusarium oxysporum* f.sp. *udum*) is the most destructive disease causing yield losses of about 50 to100%. In the present study, five fungicides and six bioagents were assessed for integrated management of wiltof pigeon pea in pot culture. Results revealed that, all the seed treatments significantly reduced the disease incidence. Fungicide, Thiram + Carbendazim was found most effective with significantly least disease incidence of 11.23%, was followed by Carbendazim (21.40%), Thiram (35.17%), MAU fungi (39.83%), Thiram + Captan (43.82%), *T. harzianum* (49.23%) *T. koningii* (51.45%) and *T. viride* (58.15%). Whereas, *P. fluorescens* was found with highest wilt incidence of (63.45%), followed by *T. hamatum* (60.85%) and Captan (68.58%).

P155

Integrated management of sugarcane pineapple disease

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Pineapple disease caused by *Ceratocystis paradoxa* (De Seynes) Moreau. of sugarcane (*Saccharum officinarum*) is one of the most destructive disease of sugarcane, causing accountable losses to the tune of 100 % in severe infection.The pathogen/disease is very difficult to

manage with alone fungicide. Therefore, present study was attempted to manage pineapple disease with integration of effective fungicides, bioagents, botanicals and organic amendments. Results revealed that all the treatments attempted significantly enhance the setts germination and thereby reduce both the mortalities (Pre and post emergence) over untreated control. Significantly highest germination was recorded with combination Carbendazim+ *T. viride* +NSC (@1g/L+50mL+50g/kg soil) and Carbendazim + *T. viride* (@1g/L+50mL/kg soil) each 88.90%. This was followed by the treatments Carbendazim, *T. viride* and *T. viride* +NSC (each 83.34%), Propiconazole, *T. harzianum, A. sativum*, FYM+*Azotobacter chrococcum,* Neem seed cake and Carbendazim +NSC (each 77.79%) and *Z. officinale* (72.23%).The percentage reduction in mortality (Pre emergence setts rot + post emergence seedling mortality) recorded with all the treatment was ranged from 66.66 to 81.48% as against 0.00% in untreated control. However, significantly highest reduction in mortality (81.48%) was recorded with the combination of Carbendazim + *T. viride* +NSC (@1g/L+50mL+50g/kg soil). This was followed by the treatments Carbendazim + *T. viride* (79.44%) and Carbendazim (77.50%).

P156

Combinatorial efficacy of lipoxygenase hexanal and biocontrol agents in managing anthracnose and stem end rot diseases of mango

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Anthracnose and stem end rot are the major post harvest diseases of mango. Anthracnose is caused by *Colletotrichum gloeosporioides* (Penz.) (Penz. and Sacc) and stem end rot is caused by *Lasiodiplodia theobromae* (Pat.) (Griffon and Maubl). The efficacy of the hexanal on the mycelial growth and spore germination of *C. gloeosporioides* and *L. theobromae* was studied. The evaluation of different concentrations indicated that, hexanal 0.06% showed maximum inhibition in mycelial growth of *C. gloeosporioides* and *L. theobromae* (95.56 and 93.33%, respectively). Simultaneously *in vitro* efficacy of antagonists was tested against the pathogens. *Pseudomonas fluorescens* strain, Pf 1 showed effective reduction in the mean mycelial growth of *C. gloeosporioides* and *L. theobromae* (36.16 and 54.16 mm, respectively). *Bacillus subtilis* strain EPCO 16, significantly reduced the mean mycelial growth of *C. gloeosporioides* and *L. theobromae* (30.09 and 43.36 mm, respectively). Compatibility of potential antagonists *P. fluorescens* (Pf 1) and *B. subtilis* (EPCO 16) with hexanal was tested *in vitro* by poisoned food technique and turbidometric method. The compatible hexanal+antagonists formulation and carbendazim

was tested in the field and post-harvest conditions against the anthracnose and stem end rot diseases of mango. The results revealed that pre-harvest spraying of 2% hexanal + 0.5% *P. fluorescens* (Pf 1) at 30+15 days before harvesting followed by post-harvest dipping of fruits in 2% hexanal+0.5% *P. fluorescens* (Pf 1) stored under cold condition showed maximum inhibition in anthracnose incidence next to 0.1% carbendazim check fungicide.

P157

Plant resident microorganisms for disease management in tree spices

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Kerala considered as land of spices and wide range of spices are produced in large quantities. Among allspices, treespices like Nutmeg. Clove and Cinnamon are also has important role in production and productivity. These tree spices production facing a major constraint in the form of foliar diseases. Farmers are indiscriminately using the plant protection chemicals which creating the pesticide residue problem. The experiments had three fungus of nutmeg namely *Colletotrichum* sp, *Pestalotiopsis* sp, *Rhizoctonia* sp and one pathogen from clove *i.e Colletotrichum* sp. Epiphytous and endophytous fungus isolated from healthy leaves of nutmeg from 6 different locations of Kerala. The parameters taken for the observation are percent inhibition of plant resident microorganisms over the pathogen control. Monoculture of the pathogen taken as control. A total of 118 (74 Bacteria and 44 Fungi) was isolated from tree spices. The results showed highest percent inhibition in Nutmeg *colletotrichum* sp against epiphytic fungus (53.3) followed by Nutmeg *colletotrichum* sp against endophytic fungus (54.4) and clove *colletotrichum* sp *verses* nutmeg endophytic fungus (54.6). Clove pathogens also tested against its endophytic fungus it has showed 55% inhibition over control. Hence, plant disease management using plant resident microorganisms creats a new way in disease management towards ecofriendly green agriculture.

P158

Interaction of beneficial rhizosphere microorganisms and their effect on groundnut wilt [*Fusarium oxysporum* (Schlecht.)]

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Groundnut or peanut (*Arachis hyphogaea* L.) is an important leguminous oil seed crop. Groundnut is susceptible to many foliar and soil-borne fungal diseases. Among these the soil-borne diseases *viz.*, dry root rot, stem rot and wilt cause serious losses to the crop which is extensively grown under rain fed conditions. Evaluation of 13 isolates of bioagents like *Trichoderma, Bacillus* and *Pseudomonas* from rhizosphere soils were screened against *Fusarium oxysporum* under *in vitro* and glasshouse condition. Bioassay study and plant growth promotion activity were analysed.*Trichoderma viride* (Tv1), *Pseudomonas fluorescens* (Pf1) and *Bacillus subtilis* (Bs10) effectively inhibited the growth of *F. oxysporum* to an extent of 71.59, 54.13 and 49.54% over control respectively. Six oil cake extracts tested *in vitro*, mahua cake extract (10%) and neem cake extract (10%) were effective in reducing the growth of *F. oxysporum*. And evaluating the effect of six fungicides screened against *F. oxysporum* showed that carbendazim, benomyl and SAFF (carbendazim 12% + mancozeb 64%) inhibited the fungal growth completely. The efficacy of biocontrol agents, oil cake and fungicide effective *in vitro* was evaluated in pot culture experiment to manage *Fusarium* wilt of groundnut. Seed treatment followed by soil drenching of carbendazim 0.1% at 30 and 60 days after sowing was found to be effective in reducing the disease incidence up to 91.02%. Among the biocontrol agents, combined application of Tv1 + Pf1 + Bs10 with mahua cake reduced the wilt incidence up to 16.09 followed by the application of Tv1 + Pf1 and Bs10 with contributing to 78.49% disease reduction.

P159

Biological properties of potential *Streptomyces* spp. effective against black pepper pathogens

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The present study was aimed to unravel the growth promoting as well as biocontrol traits along with enzymatic activities of certain shortlisted *Streptomyces* spp. which really contribute

to growth promotion and biocontrol in black pepper. Growth promoting characteristics such as production IAA and siderophores and enzymes like high amylase, cellulase protease, lipase and L -Asparaginase were studied. Siderophore production was found in all the tested isolates while 63.6% of the isolates were able to utilize tryptophan with the production of IAA. The potential actinomycete isolates BP Act 1 showed high amylase and lipase activity along with production of IAA, siderophores and L-Asparaginase. BP Act 25 showed high cellulase and amylase activity while BP Act 42 showed high amylase, cellulase and protease activity. When culture filtrate of these potential actinomycetes were evaluated for their inhibitory effect on pathogens in plant system, 52-62% reduction in lesion development was observed against *Phytophthora capsici* and 50-100% reduction against *Sclerotium rolfsii*. The culture filtrate of the isolate IISRBP Act 1 and IISRBP Act 42 showed 100% inhibition of *S. rolfsii* while the isolate gave only 50-62% inhibition against *P. capsici*. This study attempts to highlight the potential biological traits of *Streptomyces* spp. and extends to signify Streptomyces as promising harbingers of biological control of diseases of black pepper.

P160

Bioactive mycomolecules of *Pisolithusalbus* (Cooke & Massee) against soil borne plant pathogens

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Pisolithusalbus (Cooke & Massee), an ectomycorrhizal fungi with hidden treasure of bioactive compounds and secondary metabolites having multifaceted use in health and agro-chemical industries. Studies were undertaken on the basis of biomass production, extraction and testing of its bioactive compounds and secondary metabolites against soil borne plant pathogens. The biomolecules composite of CFC (secondary metabolites) extracted from 45 days old culture grown in Modified Melin Norkrans (MMN) growth medium at a concentration of 1 mg/mL resulted the maximum inhibition of *F. oxysporum*. f. sp. *lycopersici* colony (920 mm2); *R. solani* (890 mm^2) and *M. phaseolina* (870 mm^2) also CFC condensate fraction showed the maximum inhibition respectively. GC-MS analysis of biomolecules composite of ethyl acetate fraction of CFC and whole fruiting bodies indicated the presence of 12 identified compounds that belonged to nature of fatty acids, aromatic alcohol and flavonoids. Prominent among the biomolecules were benzeneethanol; 2-allyl-5-t-butylhydroquinone; 1, 2-benzenedicarboxylicacid, diethyl ester; 1,4- diaza-2,5-dioxoisobutylbicyclo[4.3.0] nonane; Benz[j] aceanthrylene,1, 2, 6, 7, 8, 9, 10, 12b octahydro-3-methyl;3-benzyl-1, 4-diaza-2, 5, dioxo-bi - cyclo[4.3.0]nonane; Phthalicacidnonyl 4-octyl ester. Similarly, the biomolecules composite of sporocarps contained several organic compounds including hexadeconic acid; 2-penta-deuterio-isopropenyl-3-heptadeuterio-isopropylnaphthalene; tetrakis-dimethyl-silyl-carbodiimide),

Di-(2-ethylhexyl) phthalate and Hesperetin (1-[2, 4, 6-tris(trimethylsiloxy)phenyl] 3- [3-methoxy-4- (trimethylsiloxy) phenyl] - 2- propen – 1–one). Likewise, the diethyl ether fraction of mycelial mat contained cyclohexane, 1, 4-dimethyl-2-octadecyl-, methyl -2-(3', 3' – dimethyl- 1' – butyn-1' – yl)-1cyclohexenecarboxylate and 7, 9- di-tert-butyl-1oxaspiro(4, 5)deca-6, 9 – diene-2, 8-dione.

P161

Bio-efficacy of Difenconozole 25% EC in paddy against sheath blight disease

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Effect of Difenconozole 25% EC was evaluated against sheath blight of paddy in farmers fields of banavasi, Sirsi taluk, Uttara kannada during 2013-14.The results indicated that two sprays with Difenconazole 25 EC @ 1.0mL/L was effective in checking the incidence/severity (7.65%) followed by Difenconazole 25 EC @ 0.7 mL/L (8.15%). However, the maximum sheath blight disease severity (PDI) was recorded in untreated control 32.15. The per cent disease control after the second application was 71.41% in Difenconazole 25% EC @ 0.5mL/L, 74.65% in Difenconazole 25% EC @ 0.7mL/ L and 76.20% in Difenconazole 25% EC @1.0mL/L treated plots. The maximum highest yield wasrecorded in Difenconazole 25% EC @1.0mL/L (40.35 q/ha) followed by Difenconazole 25% EC @ 0.7mL/L (38.81 q/ha). The least yields were recorded in untreated control (24. 354 q/ha).

P162

Standardization of protoplast fusion technique in *Trichoderma harzianum*

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Trichoderma is one of the most important filamentous fungi used as a Biocontrol agent. Due to absence of sexual reproduction in this fungus, methods like protoplast fusion are devised for gene transfer between two organisms of same or different evolutionary origins and can be used as a tool for genetic improvisation. Based on the reported method (Fahmi et al. 2012), a modified protocol for isolation, intra fusion and regeneration of protoplast from *Trichoderma*

harzianum (Th3) strain was developed. Protoplasts were isolated using Sigma Lyticase (Source: *Arthrobacter luteus*) and 0.6 M KCl used as osmotic stabilizer. Intra-strain *T. harzianum* protoplast fusion had been carried out using STC (sorbitol, Tris – HCl, $CaCl_2$) buffer medium. The maximum number of protoplasts (2.0 x 10^3/mL) were obtained from 16 h mycelium at pH 6, 28°C for 2 h with 30% Poly Ethylene Glycol (PEG)used as a fusogen. The fused protoplasts were regenerated on 2% colloidal chitin agar selective medium.The fusant frequency was found to be 25 % with less survival rate of 5-10% but all were morphologically similar to the parent. Selected five self-fusant strains (Th3fu1, Th3fu2, Th3fu3, Th3fu4, and Th3fu5) showed antagonistic behavior in dual culture experiment against *Sclerotinia sclerotorium, Fusarium oxysporum, Altenaria brassicicola* and *R.solani.* Fusants were also tested for fungicidal sensitivity with a group of widely used fungicides. Genetic closeness of fusants with the parent strain was also established by using RAPD (OPA-8, OPA -11, (OPA -10, and OPA- 14) and ITS primers which generated polymorphic fragments ranging from 100bp to 600bp. However, this study can be be further extended for both inter and intra strain combinations.

P163

Bioprospecting of microorganisms from horticultural cropping systems for their antibiotic and phosphate solubilising activities

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The microbial composition of the soil has direct repercussions on the crop production and soil health. Microorganisms are involved in a number of biochemical processes that contribute to availability of plant nutrients and disease management. Enrichment of soil with desired microbes or their combinations can have a significant effect on the soil properties and plant growth besides minimizing biotic and abiotic stresses. Exploration for potential of the microbial diversity available in different agri-horticultural cropping systems is worthwhile for their benefits. The soil samples from different horticultural cropping system were collected. The total microbial load in the soil samples were screened for their phosphate solubilising activities and antibiosis against fungal pathogens like *Sclerotinia sclerotiorum, Colletotrichum* sp. etc. The isolated organisms were tested for their effect on the seed germination and seedling growth of test crops like tomato and brinjal. None of the isolates had any negative effect on the seed germination and seedling growth of test crops as tested in pot trays. The best two isolates have been selected for phosphate solubilisation and three isolates for *in vitro* antibiosis against fungal

pathogens. These isolates were sequenced for 16S rRNA region and based on online BLAST submission and subsequent matches with NCBI sequence database sequence homology, the phosphate solubilising isolates have been tentatively identified as *Brevibacillus borstelensis* and *Brevibacillus nealsonii*, while the antibiotic isolates have been identified as *Bacillus subtilis* (two isolates) and *Bacillus amyloliquefaciens* (one isolate).

P164

Integrated management of *Colletotrichum gloeosporioides* of mango

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Mango is prone to infection by pathogens, especially fungi and suffers heavy loss in terms of quality and quantity. Anthracnose, caused by the pathogen *Colletotrichum gloeosporioides* (Penz. and Sacc.) (teleomorph: *Glomerella cingulata*) is one of the most serious post-harvest diseases of mango. Since the fruits are consumed fresh, it is utmost necessary to reduce the use of hazardous chemicals and identify naturally available compounds with fungicidal properties, which, besides combating the disease, would also ensure safety of the consumer as well as the environment. The present study was undertaken to investigate the effect of hexanal in combination with biocontrol agents against *C. gloeosporioides,* the incitant of anthracnose disease of mango.Twenty isolates of *C. gloeosporioides* were collected from the major mango growing regions of Tamil Nadu, isolated on PDA medium and designated as Cg 1-20. Based on the morphological characters, pathogenicity tests and sequence analysis, the strains were identified as *C. gloeosporioides*. Among the twenty strains, Cg 4 was the most virulent, as tested on mango fruits, exhibiting a PDI of 83.33. The efficacy of hexanal was evaluated against the virulent strain of *C. gloeosporioides in vitro*. Among the different concentrations tested, 0.06% showed complete inhibition in mycelial growth. Further, the minimum inhibitory concentration of hexanal against *C. gloeosporioides* was determined by poisoned food technique and confirmed as 0.06%. Hexanal also effectively reduced the germination of spores of *C. gloeosporioides* after 24 h of incubation. The germination of control spores after 24 h was 88.64%, while that of spores incubated in hexanal was 21.76%. Scanning Electron Microscopic analysis revealed that hexanal caused severe distortions and breakages of the mycelial strands and also the spores were found to be deformed.In order to identify elite bacterial biocontrol agents, twenty strains each of *Pseudomonas fluorescens* and *Bacillus subtilis* were screened against *C. gloeosporioides in vitro*. Among the *P. fluorescens* strains, Pf-1 was the most effective, reducing the mean mycelial growth by 44.44%. Among the strains of *B. subtilis* screened, EPCO-16 was the most effective, inhibiting mycelial growth by 37.44%. Examination of the mycelial strands of the pathogen plated alongside the

biocontrol agents with an Image Analyser revealed that Pf-1 caused swellings in the mycelial strands, whilst EPCO-16 caused increased vacuolization. Glass house studies were undertaken in mango grafts with six treatments, comprising hexanal and the biocontrol agents in order to examine the expression patterns of the defense enzymes, *viz.*, peroxidase, polyphenol oxidase and catalase. Native PAGE analysis showed that hexanal was able to induce defense enzymes in the grafts, upon challenge inoculation with the pathogen. Two field trials were taken up in different locations in five cultivars of mango, namely, Alphonso, Himampasand, Banganapalli, Bangalora and Neelum. In the first trial, the treatments comprised of hexanal sprays in two different concentrations, *viz.*, 0.02 and 0.04%, given as pre-harvest sprays 30 and 15 days before harvest. Among the treatments, 0.04% was effective in reducing the post-harvest anthracnose incidence. In the second trial, six treatments were imposed, namely, 0.04% hexanal, spray, 0.5% Pf-1 spray, 0.5% EPCO-16 spray, combination spray of all the three, 0.1% carbendazim and control, given as pre-harvest sprays. Among these, combination sprays of hexanal and the biocontrol reduced the post-harvest incidence of anthracnose irrespective of the cultivar.

P165

Effect of novel fungicides against *Sclerotium rolfsii* Sacc., the incitant on groundnut stem rot *in vitro*

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Sclerotium rolfsii is a soil-borne fungal pathogen causing stem rot disease in groundnut crop. In vitro study was conducted with different fungicides namely Cymoxanil 8% + Mancozeb 64%, Captan 70% + Hexaconazole 5% WP, Hexaconazole 5% EC, Tebuconazole 50% + Trifloxystrobin 25% WG, Carbendazim 50% WP and Azoxystrobin 23% SC at concentration of 100, 200 and 500 ppm for their effectiveness against the *S. rolfsii* by poisoned food technique at Department of Plant Pathology, AC & RI Madurai. Among the different fungicides Hexaconazole 5% EC and Captan 70% + Hexaconazole 5% WP were found to be highly effective in inhibiting the growth of the pathogen at all the concentration when compared to other fungicides. The pathogen inhibition was recorded 100% at 100ppm, 200ppm and 500ppm in above fungicides. Other fungicides such as Tebuconazole 50% + Trifloxystrobin 25% WG inhibited 83.33% at 100ppm, 86.67% at 200 ppm and 88.89% at 500ppm. Azoxystrobin 23% SC inhibited 72.22% at 100ppm, 75.55% at 200ppm and 84.45% at 500ppm. Cymoxanil8% + Mancozeb64% inhibited 13.33 % at 100ppm, 17.77% at 200ppm, and 60.00%in 500ppm concentrations. Carbendazim 50% WP inhibited 7.77% at 100ppm, 12.22% at 200ppm and 60.00% inhibition was recorded in 500ppm.

P166

Studies on the effectiveness of oil cakes and biological control agents against *Sclerotium rolfsii* Sacc., stem rot disease of groundnut *in vitro*

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Groundnut is one of the major oil seed crop is susceptible to many foliar and soil borne diseases includes leaf spot, rust, dry root rot, stem rot, wilt. Among the soil borne diseases stem rot causes major yield loss to the crop which is grown in rainfed condition. An *in vitro* study was conducted to find the effectiveness of different organic amendments and biological control agents against *Sclerotium rolfsii* which cause stem rot disease in groundnut. Five different oil cakes were used namely Mahua cake, Neem cake, Karanj cake, Castor cake, Gingelly cake. Poisoned food technique was used at two different concentrations of 5% and 10%. Inhibition of radial growth of the pathogen by organic amendment was recorded. A plate without organic amendment served as control. Among the oil cakes tested, illupai cake exhibited maximum inhibition of mycelial growth and followed by neem cake which exhibited minimum inhibition in both concentrations of 5 and 10%. Gingelly cake exhibit the least inhibition at 5% and 10% concentration. Five different isolates of each *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* were used. The antagonistic organisms were isolated from the rhizospheric zone of groundnut crop from different regions of Tamil Nadu. *In vitro* study was conducted by adopting dual plate technique. A plate without antagonist pathogen alonewas used as control. Among the different isolates of *T. viride*, Tv1 exhibited the maximum control of the radial growth of the pathogen with 75.5% inhibition. In *Pseudomonas fluorescens*, Pf1 exhibited the maximum control with 71.1% inhibition. In *Bacillus subtilis*, Bs3 exhibited the maximum control with 67.7% inhibition.

P167

Investigation of molecular diagnostics, characterization and management of tomato *Fusarium* wilt by using *Pseudomonas fluorescens*

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The survey was taken from different tomato growing areas of Tamil Nadu for isolation, characterizing and managing the soil borne pathogen *Fusarium oxysporum* f. sp. *lycopersici*. *In*

vitro study was undertaken to exploit the antagonistic efficacy of the *Pseudomonas fluorescens* bacteria against *Fusarium* wilt. Totally, fifteen *P. fluorescens* isolates of were collected from rhizosphere soil at different regions of Tamil Nadu and the isolates were characterized by standard morphological and biochemical methods, they found to be gram-negative, rod shaped, showed typical fluorescence under UV light, produced siderophore on CAS agar medium and studied their efficacy by using the *in vitro* and *in vivo* screenings. Among, fifteen isolates of *P. fluorescens*, the isolate Karumathampatti exhibit high percentage of antagonistic efficacy against *Fusarium* wilt pathogen under lab and field condition. Molecular diagnostics and characterization of different *P. fluorescens* isolates were made by amplification of ITS1 and ITS2 regions of 16s rDNA. Totally, 15 potent isolates were examined for the amplification of ITS region and these isolates showed amplified product with size range of 560bp. It is thus suggested that *P. fluorescens* has potential to be a microbial control agent for this *Fusarium* wilt pathogen.

P168

Growth promotion of the edible fungus *Pleurotus eous, Pleurotus florida* and *Hypsizygus ulmarius* species by mycelium associated bacteria

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Bacteria were isolated from the mycelial surface and their role in fruiting body induction and mycelial growth of the edible mushroom *Pleurotus eous, P. florida and Hypsizygus ulmarius* was investigated. Analysis of the bacterial community that colonized the mycelium showed that the species composition and numbers of culturable bacteria differed according to the developmental stages of mushroom. In particular, the population size of fluorescent *Pseudomonas* increased during fruiting body induction. An experiment showed that inoculation of fluorescent *Pseudomonas* spp. strains isolated from the mycelial plane of commercially produced mushrooms promoted the mycelial growth of *P. florida* (82.3 mm), *H. ulmarius* (78.8 mm) and *P. eous* (68.6mm) when compared with control (63.8 mm) and enhanced the development of the basidiome of *P. eous, P. florida* and *H. ulmarius*. Results of this research strongly suggest that inoculation of the mycelium with specific bacteria may have beneficial applications for mushroom production.

P169

Efficacy of secondary metabolites of *C. indica* and *P. florida* against plant pathogens

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During the course of mushroom cultivation and processing, sometimes the cut wastes during packing and shapeless mushrooms are unutilized and wasted. Value addition and economic utilization of such wastes would certainly increase the revenue of mushroom farms. The present study was under taken to investigate the antifungal effect of secondary metabolite mixture of cultivated mushroom fungi, *Calocybe indica* P & C and *P. florida* (Kumm) against soil borne plant pathogens *viz., Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) Synder and Hansen; *Macrophomina phaseolina* (Goid); *Rhizoctonia solani* (Kuhn) and *Sclerotium rolfsii* (Sacc). Secondary metabolite composite of *C. indica* and *P. florida* in a crude form was extracted separately from the sporophores with different solvents such as ethyl acetate, hexane and methanol. The inhibitory effect of methanol extract (1500 ppm) in terms of area unoccupied by the test pathogen, traced and measured graphically exhibited 740.00 mm^2 in *R. solani* followed by 690.00 mm^2 in *S. rolfsii*, 610.00 mm^2 in *M. phaseolina* and 540 mm^2 in *F. oxysporum* f. sp. *lycopersici* inoculated plates. Hexane extract of *C. indica* inhibited *R. solani* only and recorded 390.00 mm^2 of unoccupied zone in the Petri plate.

P170

Need for integrated plant health knowledge management in India

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Scientific research comprises of a creative, meticulous work undertaken by the academia in a methodical manner to further enrich the stock of existing knowledge. Research may confirm facts, reaffirm results of previous work or solve new or existing problems generating knowledge and thus at some given point, it becomes an absolute requirement to acknowledge that knowledge management and dissemination should be brought about to identify the very sole purpose of its generation. Agricultural sustainability to a large extent is threatened by a vast number of plant pathogens ranging from viroids, which are of a few hundred nucleotides

to more serious pests and invasive species of plants causing diseases and voluminous harm to our crops globally which result in global food supply deficit. For every 1% of crop loss incurred globally, more than 25 million people around the world are deprived of food. Consequently, knowledge management related to the areas of plant health research and development demands a common platform for effective sharing. This would encompass diverse information, databases, research outputs and market intelligence, post-harvest incomes, biosecurity, supply chain etc. to be collected and collated on one platform for easy access and customisation as per needs of the stakeholders such as policy makers, farmers, donor agencies, researchers, exporters and importers. Besides this, knowledge management would facilitate early detection and development of rapid response and strategy for crisis management.

P171

Evaluation of bio-control agents for management of dry root rot of chickpea caused by *Rhizoctonia bataticola*

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Isolates of fluorescent *pseudomonas* (22 isolates) and *Trichoderma* spp.(15 isolates) were isolated from dry root rot infested areas. For isolation of *Trichoderma* and *Pseudomonas* soil was derived from chickpea rhizosplane. Both the all *R. bataticola* isolates were screened against collected *Pseudomonas* and *Trichoderma*. T40 (Bemetara) isolate was highly effective with significant mycoparasitic activity 67.65 % across all *R. bataticola* isolates taken in this study, this was followed by T36 (Dhamdha) 64.8% and T14 (Khadsiya) 64.07%. The average mortality of chickpea in sick pots after 30 days of challenge inoculation was minimum in T40 (Bemetara) and T 23 (Bilaspur) that is 20.83%. Diseases grade observed was 4.3 out of 9 in both the isolates whereas, maximum in T23 (Bilaspur) isolate that is 8.3. Fluorescent *Pseudomonas* were screened for confirmation with following biochemical test gelatin hydrolysis test, nitrate test, starch hydrolysis test, lypolytic test, casein hydrolysis test, growth test at 4°C and 42° C, hydrogen sulphide gas production, triple sugar iron test and antibiotic sensitivity test. Fluorescent *Pseudomonas* inhibited the mycelial growth by the antagonistic activity across all isolates of *R. bataticola*. The maximum growth inhibition was recorded by P33 (Lohara) isolate (63.21%) followed by P40 (Bemetara) isolate (62.35%) and P36 (Dhamdha) isolate (61.91%). The average mortality of chickpea in sick pots after 30 days of challenge inoculation with RB4 (Kawardha) was recorded minimum in P3 (Rajnandgoan), P8 (Raigarh) and P40 (Bemetara), which were significantly superior over control, with 12.5% mortality and disease grading on the basis of extent of root damage were 3.66, 5.66 and 5.66 respectively.

P172

Native antagonists from Wayanad in management of foot rot in black pepper nursery

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Black pepper (*Piper nigrum* L) is an important spice crop in Kerala. Wayanad dominated in pepper farming in the state about 20 years ago. Foot rot and yellowing are the most important diseases affecting black pepper production in Wayanad district. Foot rot is caused by the soil-borne fungal pathogen *Phytophthora capsici*. Continuous and abusive application of chemical fungicides, has become a threat to human health. Biological control has emerged as an important alternative, as it is more environmental friendly. Hence, the present study was focused on assessing *in vitro* and *in vivo* antagonism by native isolates from Wayanad in controlling foot rot disease. Two hundred and seven microbial isolates obtained from the rhizosphere of healthy vines were screened *in vitro* for their antagonistic activity against foot rot pathogen *P. capsici* by dual culture method. Among bacteria, *Paenibacillus polymyxa* recorded maximum inhibition (69.27%), among actinomycetes, *Streptomyces termitum* recorded maximum inhibiton of 66.66% and among the fungal isolates, maximum inhibition (75.17%) was recorded by *Trichoderma viride*. Further evaluation under *in planta* condition *T. viride* treated plants recorded minimum disease incidence of 6.23% and severity of 4.00%. This was followed by *S. termitum* with disease incidence of 13.20% and severity of 8.00%. In addition to biocontrol activity, *T. viride* also improved plant growth parameters such as length of vine, number of leaves and roots. *T. viride* exhibited hyphal coiling and lysis on pathogen and produced non-volatile metabolites, whereas both volatile and non-volatile metabolites were responsible for the antagonistic activity of *S. termitum*.

P173

Integrated management of *Phytophthora* root rot in Nagpur mandarin

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Phytophthora spp. are the causal agents of several various diseases of citrus in India. *Phytophthora parasitica, P. citrophthora* and *P. palmivora* have been mostly involved in causing damping off, collar rot and root rot in citrus nursery. The soil samples were collected from cit-

rus rhizosphere of different orchards and nurseries of Vidarbha region. *Phytophthora* spp. were found associated with all soil samples enumerated by soil spreading method on PARPH-CMA medium and it was in the range of 15.67 to 30.46 cfu/g of soil. The bioagent *Trichoderma viride* gave highest growth inhibition (75.33%) of *Phytophthora* by dual culture method. In poisoned food technique complete inhibition of *Phytophthora parasitica* was recorded where garlic extract was used @ 5%. *Trichoderma viride* was found most compatible with all plant extract @ 5% concentration except Garlic. Under green house experiment, combined application of *Trichoderma viride* @ 4 g/kg + Garlic clove extract @5% showed maximum seed germination (90.27%) with minimum pre and post emergence damping off (9.23%, 5.08%), collar rot (5.35%) and root rot incidence (11.32%). In addition to this similar treatment showed maximum shoot and root length (4.5 cm, 5.96 cm). In orchard the different treatments were imposed and observed that drenching of Metalaxyl + mancozeb 0.2% + Bordeaux Pasting 1:1:10 + *Trichoderma harzianum* 50 g/50 kg FYM +Neem seed cake 2 kg/tree was best for reducing the intensity of root rot (55.42%), gummosis (52.92 %) and *Phytophthora* propagules (62.01%). This treatment was also found best to increase in canopy volume (14.19%) and number of shoot (23.55) per branch upto 30 cm length.

P174

In vitro antagonism of *Trichoderma* isolates, their response towards enhancement of root growth and molecular mapping for root traits in rice

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Trichoderma species are one of the endophytic plant symbionts that are widely used as seed treatments to control diseases and to enhance plant growth particularly the root length and volume. Therefore, the attempt has been made to study the effect of seed treatment of different isolates on selected rice cultivars. Simultaneously we also performed molecular analysis study and identified the molecular markers linked to root traits using marker based approaches. In this study 83 elite rice population used for assessment of root pulling strength which showed the positive correlation with root length and other root parameters. The seeds of selected cultivars were treated with 8 different *Trichoderma* isolates and the plants roots were analyzed using Epson perfection scanner with WinRhizoReg software. This analysis revealed that the response of *Trichoderma* spp. varied with different rice cultivar. Amongst the 8 isolates tested, the antagonistic activity of *T. viride*, and other *Trichoderma* spp. (94a) isolates were found to be more efficient against *R. solani* than *S. rolfsii*, where isolate IRRI-1, IRRI-2 and IRRI-3 were highly

efficient in controlling the growth of *R. solani*. Isolate T14 showed high level of phosphate solubilisation and produced highest IAA. These all results reflects that the *Trichoderma* spp. affect the plant growth promoting activity that ultimately increase the biomass production giving higher yield. Molecular analysis for root traits revealed that for root length, the marker RM242 was statistically significant with p-value 0.008171 and percent of total phenotypic variation for a trait was 8.52. A total of nine significant marker trait associations were identified, the total phenotypic variation ranged from 5.72 to 27.23%.

P175

Identification of viruses associated with chilli mosaic in western Maharashtra by Transmission Electron Microscopy

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Identification of viruses associated with chilli mosaic disease was done through electron microscopy of virus particles in diseased samples. The symptoms of chilli mosaic disease in the PhuleJyoti variety in the farm of Post Graduate Institute of Mahatma Phule Krishi Vidyapeeth, Rahuri (Maharashtra) includes yellow mosaic, mottling of leaves with or without deformation, stunting and extremely deformed shoe string leaf in advanced stages of the disease. In Transmission Electron Microscopy, negatively stained preparation of the disease samples of chilli revealed the presence of two types of virus particles, one similar to cucumber mosaic cucumovirus (CMV) and other similar to papaya ring spot virus – potyvirus also reported on chilli mottle virus in India. The infection of these viruses were confirmed on the basis of particle shape and size. The chilli mosaic virus is spherical particles having 28 nm size and chilli mottle virus particles is flexuous shape having 850 nm size.

P176

Potential of fortified spent mushroom substrate against tomato damping off

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Spent mushroom substrate (SMS) is the by-product obtained after mushroom cultivation. SMS contains with many potential antagonists. A pot culture experiment was conducted at

Vellanikkara, Thrissur during 2014 – 2015 to evaluate the effect of fortified SMS on damping off in tomato caused by *Pythium aphanidermatum*. By *in vitro* evaluation the best antagonists were selected and compared with standard cultures of Kerala Agricultural University (KAU). The SMS was fortified separately as well as in combination withthe best fungal antagonist *Trichoderma hamatum* isolated from SMS and with the best bacterial antagonist *Pseudomonas fluorescens* standard culture of KAU, and applied with potting mixture in the ratio of 1:1.The fortification was done by adding 300 mL of the respective antagonist broth per kg of SMS. Soil drenching with the respective antagonists and copper hydroxide 2% were also evaluated.The plants grown in potting mixture (sand: soil: cowdung in the ratio of 1:1:1) was kept as control. The treatment with SMS fortified with microbial consortium was found highly effective to suppress the damping off of tomato. In addition, highest germination (84%), root length (15.77 cm), shoot length (35.73 cm) and vigour index (2701) was obtainedfrom the treatments with microbial consortium. So the fortified SMS can be used for the eco-friendly management of damping off of tomato.

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Evaluation of transgenic groundnut plants for resistance to peanut stem necrosis disease caused by *Tobacco streak virus*

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Virus diseases are serious constraints to groundnut (*Arachis hypogaea* L.) production worldwide and more than 20 viruses have been reported to infect this crop. Peanut stem necrosis disease (PSND) caused by *Tobacco streak virus* (TSV) is a major limiting factor to groundnut cultivation in India. We explored the possibility of controlling PSND by expressing double stranded (ds) RNA of the replicase (Rep) gene of TSV in groundnut through genetic engineering. We have successfully introduced a gene construct containing 535 bp sense and antisense TSV-Rep sequences flanking a 742 bp spacer sequence (Pdk Intron) under the control of the constitutive *Cauliflower mosaic virus* (CaMV) 35S promoter into groundnut (cv. TMV-7)through *Agrobacterium tumefaciens* mediated transformation method. The presence of the transgene in the transgenic lines was confirmed by PCR amplification of the 535 bp fragment of TSV Rep gene up to T3generation of the transformed lines.The bioassay results indicated that the transgenic lines showed high levels of resistance to PSND. ELISA results indicated that the wild- type plants inoculated with TSV recorded the highest virus concentration as compared to the transgenic lines. No significant differences in morphology and growth were observed between transgenic lines and non-transformed plants.

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Assessment of molecular diversity of *Colletotrichum gloeosporioides* using RAPD and ISSR markers

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Anthracnose caused by *Colletotrichum gloeosporioides* degrade fruit quality and can be troublesome for exported products. There is large variation among and within *Colletotrichum*-species in pathogenicity, culture appearance and uncertain relationships with host plants. The amount and distribution of genetic variation constitutes the first step in fungal population genetics. In this study, two marker systems *viz.*, Random Amplified Polymorphic DNA (RAPD) and Inter simple sequence repeat (ISSR) were used to characterize the genetic diversity of 26 *C. gloeosporioides* isolates from different places of Tamil Nadu. The similarity coefficient based on RAPD ranged from 65 to 88 %. Six out of 10 primers (OPB 07, OPF 14, OPF 07, OPL 05, OPD 07 and OPG 16) produced 100% polymorphism with the allele range of 100 – 2000 bp. The isolates could be grouped into two main clusters I and II with 65.00 % similarity and was not associated with exact geographical localities from which the isolates were obtained. Sixteen ISSR primers generated amplification products of 189 alleles, with the range of 100 to 2500 bp. Ten out of 16 primers *viz.*, (CAG)5, (TGTC)4, (AGG)5, (TCC)5, (CAG)3, (AG)8 T, (GA)8 T, (TG)8 A, (GA)8 YG, (GT)8YC were shown cent per cent polymorphism. Genetic diversity assessment through ISSR markers revealed that 26 isolates could be grouped into cluster I and cluster II with the similarity of 59.50 % and it was correlated with the virulent nature of *C. gloeosporioides*.

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Introgression of bacterial leaf blight resistance gene (xa5) in rice cultivar IR64 through marker assisted selection

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Bacterial leaf blight (BLB) is a severe rice disease throughout the world that is controlled primarily through use of resistant cultivars. Marker assisted selection (MAS) is essential for improvement of resistance in breeding programs because of they are co-dominant, based on PCR amplification, represent single loci and detect high levels of polymorphism. As on date,

30 major genes have been reported to confer resistance against *Xanthomonas oryzae* pv *oryzae* (Xoo), which included 21 dominant and 9 recessive R genes. In the present investigation variation in virulence spectrum was observed and speculated due to the presence or absence of avr genes in the pathogen.The behaviour of R genes in population indicated that faster the response rate is more likely a resistant phenotype is to arise. The recessive R genes i.e. xa5 (chromosome 5) which specifically confers resistance to BLB of rice only in recessive homozygous condition. The PCR based screening of selected 40 F4 individuals, each plants selected which derived from cross between IRBB5 x IR64. The F4 progenywere tested and inoculated artificially by *Xanthomonas oryzae* pv *oryzae* (Dhamtari) isolate. In this study microsatellite and sequence-tagged site (STS) were most practical markers used for marker assisted selection of the targeted BLB resistant genes xa5. tagged rice lines #1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19, 22, 24, 25, 26, 27, 28, 30, 31, 32, 33, 34, 35, 36, 37, 38 & 39 derived from IRBB5 x IR64 were resistant phenotypically and also showed co-segregation with linked SSR marker RM610.

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