

Special Symposium
on
**Microbial Antagonists and Their Role in
Biological Control of Plant Diseases**

&
West Zone Meet of IPS (2017)

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Souvenir
&
Abstracts

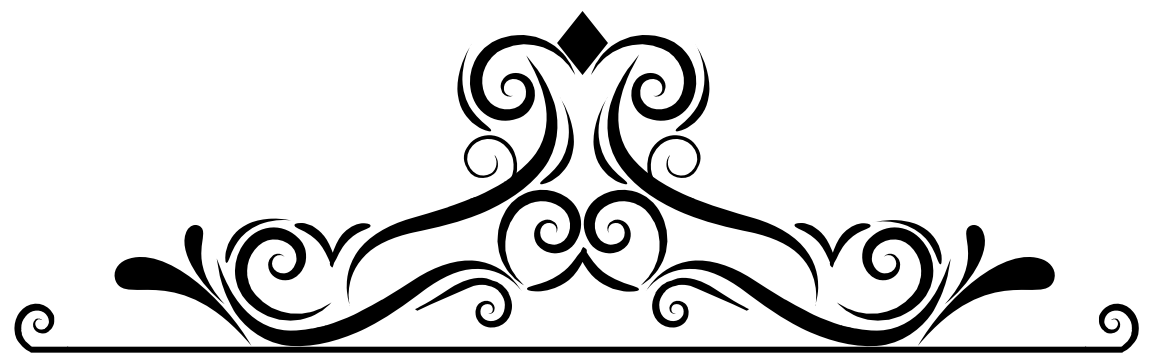
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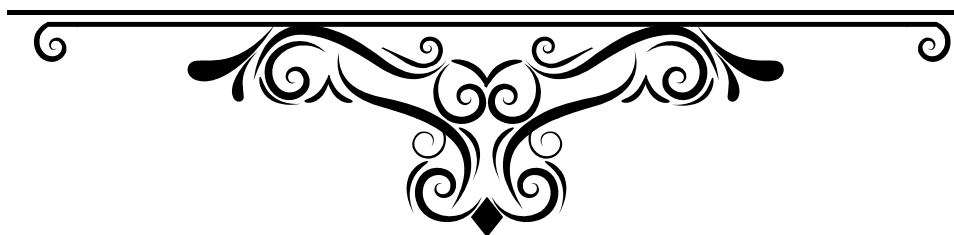
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Indian Phytopathological Society
New Delhi





PLENARY LECTURES



Plenary Lectures

PL 01

Biopesticides for biocontrol of crop diseases: problems and prospects

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In the current intensive agricultural practices where the adverse side effects of mismanaged chemical agriculture are weighing more than their benefits, biocontrol systems of pests and diseases are assuming an important component of the modern agriculture for enhancing the productivity within the framework of Integrated Pest Management (IPM) without affecting the ecosystem. Biological control of diseases involves the use of Bio pesticides which by definition are a form of pesticide based on microorganisms or natural products. By and large three categories of bio pesticides are recognized. 1) living organisms i.e. natural enemies which include invertebrates or predatory insects, nematodes, fungi, bacteria, viruses etc., 2) naturally occurring substances which include plant extracts and semi-chemicals such as; insect pheromones, and 3) genetically modified plants that express genes that confer protection as in case of Bt cotton or Bt brinjal. Except for the GM category, other forms of bio pesticides have been accepted in some organic agriculture but not by all organic movers and shakers.

Although they have been demonstrated to give better yield at low cost, their full exploitation is still awaited mainly because of the unease to handle them by users with inadequate scientific knowledge of biology, difficulty in commerce and also intrinsic issues of marketing. In India, the market is still miniscule but dominated by antagonistic fungi and bacteria such as; *Trichoderma* spp., *Pseudomonas fluorescens*. Government of India has offered series of promotional programs for the use of these products in the IPM strategy as a core policy issue of plant protection. There are excellent opportunities to enhance the use of bio pesticides in India provided we overcome some of the long standing challenges and constraints. At the user level, it is highly necessary to have basic scientific knowledge on its use. Farmers rarely see the short expiry of these pesticides and are comparing them with the chemical pesticides. Application technologies also need a relook. In marketing, these products shelf life is major limitation and the production is hampered by demand. Since storability is a problem there are production limitations and in many cases suitable mass production techniques do not exist. In spite of relaxed guidelines for the registration, a lot of unregistered products are floating in the market. The most serious issue is of quality. Many producers sell their products in the name of 'BIOPRODUCT' to avoid registration which is not including in the Insecticide Act 1968. Several of the spurious bio-products have been shown to contain illegal insecticides or other chemicals. They are posing a real threat to the entire pesticide industry and spoiling the name of organic movement. Such fraudulent activities are indirectly getting encouraged because of the blind policies of Government to offer subsidies without checking the quality. Bio pesticides are certainly a useful component of IPM but the problem of quality is taking a heavy toll. In this regard, there are several suggestions which will go a long way to convert the problems in to solutions.

PL 02

Biological control of fungal diseases of plants with chitinolytic bacteria and induction of immunity by chitooligosaccharides

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Biological control of fungal diseases of plants using antagonistic bacteria has become a reality with the available of several native antagonists. The methods for preparation of a variety of formulations of these antagonists has made the use of biocontrol agents even more effective in the past two decades. Use of chitinolytic bacteria as biocontrol agents has the dual advantage of directly attaching the cell wall of most of the fungal pathogens and indirectly activating a broad spectrum of defense system of the plants against the fungal pathogens. We have been working with chitinolytic bacteria as biocontrol agents and a brief overview of our work will be discussed.

Plants interact with a wide range of pathogens and have evolved mechanisms to recognize pathogen-derived molecules to elicit induced resistance. The plants are able to sense evolutionarily conserved general elicitors of pathogens called pathogen-associated molecular patterns (PAMPs), and activate immune responses, a process that is often referred to as pathogen-triggered immunity (PTI). Most commonly known PAMPs from fungi include fungal cell wall-derived glucans and mannans, glycoproteins from oomycetes, fungal cell wall chitin etc., Pattern recognition receptor (PRR) proteins located on cell surfaces are mostly kinases or leucine-rich repeat proteins. Chitooligosaccharides (COS), released during plant fungal interaction, and elicit plant defense upon recognition. In this talk the progress made towards understanding the mechanism of COS sensing in plants and on the application of chitin/chitosan/COS in disease management would be discussed.

The large scale production of long chain length COS is a daunting task, as the available methods including chemical hydrolysis, direct synthesis, and enzyme-catalyzed processes result in COS with low degree of polymerization (DP), while high DP COS ($DP \geq 6$) are required for the biological activity. Elicitor activity of COS also varies depending on the plant. Nevertheless, biological activity of COS increases with the increase in DP 4 to 7. We work on transglycosylation (TG) of low DP COS to generate long DP COS by chitinases and to develop a suitable bioprocess to generate high DP COS for large scale application of COS for biological applications that include induction of innate immunity in plants.

PL 03

The concept and formulation of Kunapajala, World's oldest fermented liquid organic manure

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Beginning in 1990s, researchers and farm policy makers in India and rest of the world, focused their attention to "organic farming" in order to better the sustainability. I had recently described and discussed most of the methods currently recommended in different parts of India. These methods are: (i) The natural way of farming or "Do Nothing" farming by Fukuoka, (ii) Biodynamic agriculture by Steiner – introduced in India, (iii) Vermi-culture developed by Appelhof– introduced in India, (iv) "Natueco" culture by Dabholkar, (v) Zero Budget Natural Farming [ZBNF] by Palekar, (vi) Rishi-Krishi by Deshpande, (vii) Agnihotra by disciples of Gajanan Maharaj of Akkalkot, (viii) Panchagavya by Natarajan, (ix) Krishi-suktis and Vrikshayurvedas [Surapala, Sarangadhara, and others] by sages and scholars of ancient and medieval India, (x) Compost tea by Ingham– introduced in India, and (xi) Bokashi tea by Higa– introduced in India. Of these methods, Dabholkar's "Natueco", Palekar's ZBNF, Natarajan's Panchagavya, Ingham's Compost tea, and Bokashi EM of Higa are related to Kunapajala, which is produced by adopting the liquid fermentation technology as documented by Surapala [c.1000 CE; Sadhale, 1996] in the first ever compilation of Vrikshayurveda methods. Thus, the ancient Kunapajala, fermented liquid manure was a stupendous innovation. This went un-noticed and currently the agronomists, all over the world, believe that the innovation of fermented liquid manure was done by farmers in Japan, Korea, China, or medieval Europe, but not by farmers of India. In the following paragraphs, I have attempted to share evidence to support my viewpoint that the Indians pioneered the innovation and use of fermented liquid manure almost 1000 years ago.

PL 04

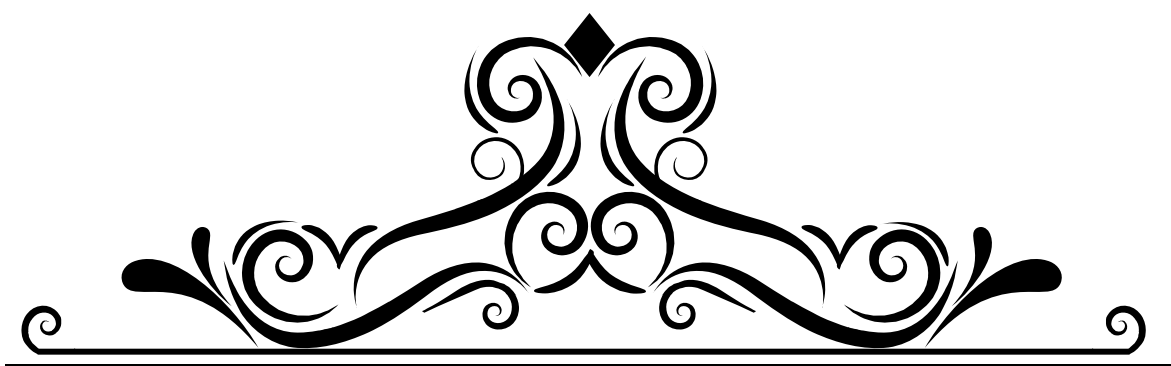
“New Developments in Microbial and Organisms and their Role in Eco Agri Revolution”

M.H. Mehta

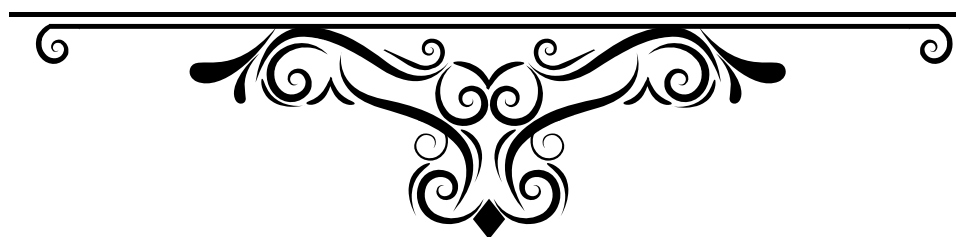
President – The Science Ashram

Ex. Vice Chancellor – Gujarat Agriculture University

In the coming Eco Agri Revolution – the eco-friendly Agri Bio inputs like Bio fertilizers, Bio pesticides, Bio composts, stimulants etc. will play key role. Bio pesticides and Biocontrol Industries are growing globally at more than 16% annual rate and offer great opportunity for a balanced and sustainable development. Global experience of working in a triangular arrangement between Farmers, Agri Universities and Industries emphasizes and the evaluation of 20-20 model for sustainable Eco Agri Revolution for the world are explained. A future plan and the road map is discussed.



ORAL PAPERS



Ecology, biodiversity and taxonomy of antagonists/ bio-agents

Lead Papers

LP (S1) 01

Development of *Trichoderma*-based means of biological control: from the field to the laboratory and back again

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The filamentous fungal genus *Trichoderma* has been a popular subject of basic and applied mycology research for a long time, which is basically due to the fact, that *Trichoderma* species play important roles in various agricultural environments. Several members of the genus have the potential to biologically control plant pathogenic fungi and nematodes by antagonistic action based on competition, antibiosis and/or parasitism. The ability of certain *Trichoderma* species to promote plant growth and induce systemic resistance in plants can also be exploited within the frames of environmentally friendly agricultural practices.

The first step of the development of biocontrol means based on *Trichoderma* is generally the isolation of *Trichoderma* strains from soil samples derived from agricultural fields of the crop plants to be protected. The isolated strains are then screened in the laboratory for their antagonistic abilities towards the target pathogens and properly characterized to select potential biocontrol agents (BCAs). An exact, species-level identification of the selected BCA candidate *Trichoderma* strains, preferably performed by sequence-based molecular methods, is also suggested as a crucial step of the process. The main reason for this is that besides the positive implications of the genus in biocontrol, *Trichoderma* may also be harmful for agriculture, like in the case of mushroom production where certain *Trichoderma* species occur as the causal agents of green mould infections affecting cultivated mushrooms. Furthermore, although rarely, but some *Trichoderma* species are also able to cause opportunistic infections in humans. An exact and reliable species-level identification of the BCA candidates makes possible to avoid the development of potentially harmful members of the genus to biocontrol products, thereby lowering the risks of *Trichoderma* biocontrol. Strain improvement by classical mutagenesis, protoplast fusion or genetic transformation can be performed to enhance the beneficial properties of the selected BCA candidate *Trichoderma* strains; However, the improvement by genetic manipulation may restrict the applicability of the resulting BCAs in several countries with strict GMO regulations. The subsequent steps are the optimization of fermentation conditions for the selected BCA as well as the selection of proper means of formulation and delivery. If the beneficial effects of the BCA candidate *Trichoderma* can be confirmed by greenhouse and field trials, the process can continue with registration and commercialization. In addition, the development of proper, specific monitoring tools for the applied *Trichoderma* strains may provide us important information about their population dynamics and performance in various agricultural systems.

The aim of this lecture is to give an overview of the above-mentioned procedure, illustrated with examples deriving from my own experience in *Trichoderma* product development, including the *Trichoderma*-based biocontrol of rice sheath blight and the inclusion of multiple *Trichoderma* strains in the multicomponent soil biofertilizer product BioeGO.

The preparation of this lecture was supported by the Hungarian Government and the European Union within the frames of the Széchenyi 2020 Programme (GINOP-2.3.2-15-2016-00052).

LP (S1) 02

Exploring root microbial diversity vis-a-vis PGPR ecogenomics for holistic crop health management

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The biodiversity of earth is astounding. Our planet supports between 3 and 30 million species of plants, animals, fungi, protozoa, nematodes, bacteria, viruses, etc. Despite two centuries of research, systematics has described only about 1.4 million species. The ecology or role of these species in ecosystems, has been studied far less than one per cent. We know more about large, economically important plants and animals than we do about fungi and bacteria, despite their important ecological roles. Tangled dirty and buried underfoot, roots are a mess to study. Digging them up is a time consuming and sometimes back-breaking process. The shovel be wielded with care to preserve the roots' delicate branching patterns, the root hairs and the microbes that cling to them. All of this explains why roots have largely been out of mind, as well as out of sight, for agricultural researchers-until now. Many scientists are starting to see roots as central to their efforts to produce crops with a better yield. And as the keys to a second green (or grain) revolution –one that doesn't rely on expensive inputs. Roots are most efficient when their architecture is tailored to their environment providing large labyrinth to swallow friendly microbes. What we want is a greedier plant that takes up nitrogen early in the season and better performers in the conjunction of dear fellow microbes. A groups of root aficionados wants to improve crop yields by harnessing microbes that grow on and around the rhizosphere –the narrow band of soil that surrounds roots. The designer roots can obey the follow ups of genetic directions of its host faithfully.

What lies beneath? Understanding soil is a matter of rising urgency. Soil quality is the biggest barrier to higher crop yields for farmers all over the world. Knowing what myriad organisms live in the soil, and how they interact, is crucial to creating a healthy ecosystem. For all those scientists who are willing to crouch down and dig, the diversity of soil denizens beat any above-ground system, even that of a tropical rainforest. A handful of soil from one spot may house a different community from soil just a meter away, because of variations in the availability of water or nutrients. For example, the ground under a decaying plant or animal is a different environment from soil lacking such enrichment. And around plant

roots, specialized organisms inhabit the rhizosphere, a thin layer where roots and soil organisms interact in myriad ways. Large animals such as moles contribute, changing and aerating the underground landscape by tunneling. Even a small clump of soil has a gradient of oxygen from its edges to the centre, and each oxygen concentration may make the perfect habitat for different kinds of creatures. "It is the most incredible zoo"- Diana Wall (Soil Ecologist) at Colorado State University, Fort Collins, exclaimed.

Wall studied two different samples of soil at 400 km distance apart and found that only 18 invertebrate taxa out of an estimated 1,300 appeared in both locations. "That is mind blowing", says Wall. PGPR, *Bacillus* spp, *Pseudomonas* spp etc have been well elucidated in plant disease control and sustaining crop health by stimulating resistance and growth in plants.

It is a disturbing reality that we have only fragmentary understanding of the enormous microbial diversity that exists on our planet: This applies not merely to microbes living in extreme environments and which would be expected to possess unusual and perhaps not yet fully characterized properties, but also those in mundane habitats- a gram of soil, a ml of seawater or a μ l (micro liter) of earth worm gut environment. The microbial catalyzed, anaerobic oxidants of ammonia and methane have also expanded the types of microbial metabolisms in anoxic environments that contribute to global elemental cycles. It is in terms of their metabolism that microbes have revealed incredible diversity as compared to the uniform types of metabolism found throughout the plant and animal kingdoms.

Ecological theory will be increasingly concerned with the often subtle biological details of organisms, as well as the implications of evolutionary dynamics. Microbial ecology will become main stream. At the same time, it will be essential to look at how species and communities fit into Earth's history. In a decade's time, ecology will be viewed both as a core part of biology, and increasingly as an essential dimension of Earth sciences.

Plant diseases have played a great havoc since time immemorial causing severe epidemics resulting into famines affecting the lives of several million people be it potato devastation (Irish famine) or destruction of rice (Bengal famine). The diseases have always posed a big challenge for the scientists in respect of yield and quality of the food production. In the wake of burgeoning population which is expected to be 10 billion in next few years the challenges of achieving food security becomes a distant dream particularly in view of climate change, losses of biodiversity because of environmental hazards posed by pesticides use, emergence of new diseases, shrinking farming area etc. Under such circumstances efforts have to be made to search for the holistic approaches for management of diseases using ITK, application of bio-control strategies including botanicals, PGPRs, Mycorrhizal organisms, biotechnological approaches, nanotechnology, resistance sources *etc* to prevent losses to the tune of more than 36 per cent caused by biotic stress alone. The microbial diversity and PGPR may play a greater role in crop health management and in resilience of ecological balances.

Nevertheless, atop list of recommendations made in a recent report by the American Academy of Microbiology was a need to develop training opportunities to advance the emerging new field of "ecogenomics" (the intersection of ecology and genome science) as a means to explore the many levels of biological organisms that sustain the biosphere. Such training programmes will require an integration of

knowledge from a number of disciplines, including microbiology, ecology, evolutionary biology, genomic science, biogeochemistry, mathematics and bioinformatics.

LP (S1) 03

Endophytism in *Trichoderma* spp.: An overview

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Endophytes are microorganisms that colonize internal tissues of plants. The field of endophytic research have gained importance in the scientific realm especially in the area of biological control of plant pests. It is believed that the use of various innovative biotechnological tools will shed more light on plant-endophyte interactions and mechanism. Fungal endophytes occur in stems, leaves and roots of monocots and dicots. They are vital for their beneficial impact on human beings and plants. Various studies done on *Trichoderma* spp. have reported that it occurs as endophytes. Endophytic *Trichoderma* spp. has shown to produce secondary metabolites and enzymes that are antimicrobial; they also promote plant growth and antagonize various phytopathogens. This chapter serves to give an overview on work done on endophytic *Trichoderma* spp.

LP (S1) 04

Managing soil borne plant pathogens by native bio agents in Indian arid region

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In Indian arid region, the principle rain fed crops include pearl millet, clusterbean, moth bean, mung bean, cowpea, sesame, sorghum, etc. In the irrigated pockets, farmers grow wheat, mustard, cumin, Blond psyllum or Isabgol, onion, garlic, etc. These crops suffer heavily due to attack of many soil borne plant pathogens. An effort has been made for the last two decades to isolate, identify and investigate role of native bio-agents in managing these soil borne plant pathogens. A native heat tolerant strain of *Trichoderma* has been isolated from arid soils, which was found effective against pathogens like *Macrophomina phaseolina* causing charcoal root rot in legumes and oilseed, *Fusarium oxysporum* f. sp. *cumini* (wilt of cumin)

and *Ganoderma lucidum* inciting root rot mortality in trees and shrubs in studies conducted at CAZRI. Subsequently, detailed studies were initiated to confirm the bio-control potential of another bio agent, identified as *Aspergillus versicolor*. Studies on thermal resistance showed that it was able to survive even at 65°C. Once a bio agent has shown its potential towards a pathogen, it becomes important to know its survival and shelf life. Therefore, to achieve this target, a bioformulation of *A. versicolor* in a mixture of sterilized neem compost and talc was prepared, which was able to maintain viable propagules of *A. versicolor* for more than 120 days. The bioformulated product was distributed to selected farmer, as Maru sena-2. A bacterium was found to be antagonistic to *M. phaseolina*, which was identified as *Bacillus firmus* by IMT, Chandigarh, India. The bacterium has already been established as a phosphate solubilizing bacterium. Separate tests were performed to ascertain the activity of antagonistic bacterium against prevalent soil fungi viz., *Aspergillus terreus*, *A. niger*, *A. fumigatus*, *A. regulosus*, *A. awamori*, *Paecilomyces* spp. and *F. o. f. sp. cumini* and a native isolate of *T. harzianum*. *B. firmus* failed to inhibit mycelial growth of all the soil fungi including *T. harzianum* and *Fusarium* tested. Bioformulation of *B. firmus* was developed in locally available food substrate and lignite for maintaining adequate soil moisture for a period of 120 days. This product was coined as Marusena 3. The process and the development of the product were submitted for patenting and have been published on Indian Patent site. Bioformulated product can be safely used for treating the seeds of legumes and oilseeds to reduce charcoal rot incidence. In addition to fungal and bacterial antagonists, there is ample scope of utilizing native strains of actinomycete in biological control of soil borne plant pathogens. Strong correlation was found between increase in actinomycetes population and decrease in *M. phaseolina* counts. In sequel, an effort was made integrate native bio agents in compost to improve pathogen control. In composts prepared from residues of *Prosopis juliflora*, neem, Indian mustard and weeds, population of antagonistic actinomycetes to *M. phaseolina* was maximum (37% increase) in mustard + weed residue compost. In this presentation salient findings of these aspects are discussed in detail.

Oral Papers

OP (S1) 01

Exploration of biocontrol potential of nematophagous fungi: status and opportunities

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In recent past, chronic occurrence and sporadic emergence of plant parasitic nematode is main stumbling block in achieving food security. To combat such problem we mostly depend on chemical pesticides that are toxic to human and animal health. Due to environmental hazards, use of nematicides is being restricted in many countries and focus is now shifted towards the promotion of non-chemical

strategies, such as bio-pesticides. Use of microbial inoculants for pest management is always encouraging. Among microbial antagonists, *Bacillus*, *Pasteuria*, *Pseudomonas*, and *Trichoderma* have good potential, but have limited success in field for providing nematode protection. Hence, exploration the biocontrol potentiality of nematophagous fungi would be novel alternative.

These nematophagous fungi represent a diverse group of micro-fungi with carnivorous ability. They are ubiquitous, and well distributed in diverse ecological habitats, from terrestrial, agricultural, forest to aquatic, marine ecosystem. They were mostly studied for their unique ability to capture, kill and digest nematodes through diverse mechanism. These include nematode trapping fungi, endoparasitic fungi, egg and female parasitic fungi, and nematotoxin producing fungi. Generally, predacious fungi trap nematode directly by hyphal coiling and kill them. Among predacious species, *Arthrobotrys oligospora* is the most common and abundant species, and have good biocontrol potentiality against root-knot nematode infecting cereals and vegetables in field. Within egg and female parasitic group, the biocontrol potentiality of *Verticillium chlamydosporium*, *Poochonia* sp. against *Meloidogyne arenaria* and that of *Paecilomyces lilacinus*, *P. fumosoroseus* against *M. incognita*, *Heterodera schachtii*, respectively was evaluated. Bioformulation of such fungal bioagents is available in European market. Whereas, very few bioagents have been commercialized in Indian market, and *Paecilomyces lilacinus* is only example. But opportunistic human pathogenic nature of certain *Paecilomyces lilacinus* strains raises the biosafety concern. Moreover, failure of *P. lilacinus* in reducing root gall is already become a concern for large scale application. Hence, there is urgent need to search for other antagonists, especially nematode trapping fungi. Biological control potential of trapping fungi against root knot nematodes (*Meloidogyne* sp.) is already established. Of them, *Arthrobotrys* sp, *Dactyleria* sp., is very promising, but not yet exploited. They perform very efficiently against phytonematodes at field. Besides their nematode trapping nature, they also colonize as root endophytes and enhance the plant growth and defence. Due to different mode of nematode parasitism, their co-inoculation reduces the population density and disease dynamics of phytonematodes at significant level. Genomics and proteomics of their nematode parasitism were already explored. In spite of their immense potentiality, these fungal biocontrol agents still remain under utilize in large scale. Hence, there is ample scope for generating bioformulation of this fungal bioagents, with the aim of their exploitation and popularisation for nematode management. Hence, exploration and promotion of nematophagous fungi as biocontrol agent would open up scope of sustainable nematode management.

Molecular applications in characterization of bio-agents

Lead Papers

LP (S2) 05

***Agrobacterium radiobacter*: A potential biocontrol agent for management of crown gall disease of plants**

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Crown gall caused by *Agrobacterium tumefaciens* is the major limiting factor in raising healthy stone fruit plants in nurseries. The incidence of crown gall varies from 4 to 97.5 per cent at different locations resulting in out-right rejection of an average of 30 per cent stone fruit plants in nurseries. A *radiobacter* strain K-84 has been reported to provide nearly complete control of this disease, however this strain is not available in India. Therefore, the present work was aimed to evaluate the antagonistic activity of native potent *A. radiobacter* strain(s) effective against the crown gall in stone fruits, three hundred fifty *Agrobacteria* were collected from a variety of stone fruit nursery soils covering entire state of Himachal Pradesh in India. Different *Agrobacteria* were segregated in two categories virulent and avirulent by following a standard protocol. PCR based virulence assay were carried out based on *virD2* and *ipt* genes and finally isolated non-pathogenic bacteriocin producing native bacteriocin producing non-pathogenic *A. radiobacter* strains were molecularly analysed and compared with strain K84. A PCR based comparative analysis between non-pathogenic *A. radiobacter* strain UHFBA 218 and reference strain K 84 was done using *ahc Y* gene amplification. Results revealed that the native population of *A. radiobacter* does not produce the same type of agrocin as earlier observed in strain K-84, however, the growth of all pathogenic *A. tumefaciens* isolates were inhibited by strain K-84 which showed that all the *A. tumefaciens* are harbouring nopaline utilizing Ti plasmid and able to utilize nopaline and agrocinopine *A. In vitro* tested, most effective *A. radiobacter* strain UHFBA-218 (MCC 2101, National Centre for Cell Science, Pune, India; NCBI: KC488176) were also tested in planta. Different doses of talc based formulation of *A. radiobacter* strain UHFBA-218 having initial count of (267x10⁸ cfu/g) were evaluated to minimize the crown gall incidence and it was 0.1% which resulted in minimum disease incidence. This particular dose is currently being used for minimizing the incidence of crown gall in commercial stone fruit nurseries grown by farmers.

LP (S2) 06

Biotechnological approaches for enhancing quality parameters of *Trichoderma* for plant disease management

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Plant diseases caused by bacteria and fungi are the major hindrances worldwide in successful cultivation of crops for good yield. There is growing need to control these diseases, so as to ensure continuous supply of food to ever increasing population. The need for increasing agricultural production, productivity and quality has led to excessive use of chemical pesticides, and fungicides creating serious environmental problems. The use of bio-fertilizers and bio-pesticides are some of the alternative approaches for achieving high production with low ecological impact.

Control of soil borne pathogens by the addition of some specialized strains of bacteria and fungi, to the infested soil is a potential non-chemical approach to control plant diseases. Various *Trichoderma* species are among the most frequently isolated soil fungi present in plant root ecosystems for use as bio-control agent. Their potent ability to survive under diverse unfavorable conditions by regulating their growth, reproduction and producing enzymes for promoting plant growth and defense mechanism, has led to their commercial success as bio-control agents. A considerable knowledge of the biological principles and their mode of action are essentially required for improvement of existing strains as well as development of new strains of *Trichoderma* for their use in agriculture as biocontrol agents. This has encouraged the plant biotechnologist, microbiologist and pathologist to explore new biotechnological approaches to enhance the bio-control efficacy and efficiency of *Trichoderma* species.

Genome sequencing of major strains of *Trichoderma* species has been carried out since 2008, which has facilitated the comparative transcriptome analysis among different species, whole-genome expression studies, and provide unique data for phylogenetic and bio-informatics analyses for better understanding of the role of *Trichoderma* in ecosystem. In addition, several molecular approaches involving genetic manipulations have been employed for the development of superior *Trichoderma* strains, such as protoplast fusion, chemically and physically induced mutations, *Agrobacterium*-mediated genetic transformation, biolistic transformation and transposon mediated insertion mutagenesis.

Oral Papers

OP (S2) 02

Characterization of rhizosphere microflora isolated from chickpea and its antagonist effect against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt

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India being largest producer of chickpea although is unable to meet the demands of its own. Chickpea (*Cicer arietinum* L.) is one of the most important *rabi* pulse crop and is prone to attack by several diseases. Chickpea wilt is soil borne in nature and caused considerable damage in the field of Ghed area of Saurashtra region. The biocontrol agents such as *Trichoderma* species are potential and well known biological control agents for soil borne fungi including *Fusarium* spp. In the light of certain constraints on management of soil borne diseases, biological control is increasingly gaining importance as a possible practical and safe approach. Biological control of plant pathogens has been considered as a potential control strategy in recent years and the search for these biological control systems is in its infancy, having largely taken place in the last 30 years. Antagonistic microflora from rhizosphere soil of partially and completely wilted chickpea plants were isolated by serial dilution as per standard procedure. The fungal antagonists were purified by single spore method and were maintained on PDA medium, while bacteria were purified by streak plate method and maintained on Nutrient agar medium. Fungal and bacterial bio agents isolated from rhizosphere of chickpea plant were identified by molecular tools i.e. 18S for fungi and 16S for bacteria. Bio-agents which showed antagonistic effect against *F. oxysporum* f. sp. *ciceri* were identified and their sequences were matched with database in BLAST and identical per cent presented. Efficacy of antagonistic potential of mycoflora and bacteria against *F. oxysporum* f. sp. *ciceri* was evaluated using dual culture technique. The data revealed that *Trichoderma viride* showed maximum inhibition of growth of *F. oxysporum* f. sp. *ciceri* (56.36%) followed by *Stenotrophomonas maltophilia* (44.31%) and *T. longibrachiatum*-I (34.09%). Inhibition of mycelial growth of *F. oxysporum* f. sp. *ciceri* was minimum in case of *Aspergillus* spp. (12.5%).

OP (S2) 03

Oligonucleotide Barcoding in identification of *Trichoderma* species

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Trichoderma species are all well known for their biocontrol properties in the whole world. This biocontrol activity is well known by the characterization and isolation of the specific genes which are

involved in this mechanism. Although the genus *Trichoderma* has been known since 19th century. Its association with teleomorphs in *Hypocrea* Fr. was recognized by the Tulasne brothers in 1865, its taxonomy has remained obscure until recent decades. The first serious attempt to morphologically distinguish species, or "species aggregates", was made by Rifai. Other taxonomic methods supplementary to morphology include studies of secondary metabolites, Physiological features, iso-enzyme profiling have been used as an effective taxonomic tool. Molecular data, particularly sequences of the ITS region of ribosomal DNA and fingerprinting techniques, in recent years allowed the finest resolution of taxonomic entities. The estimated genome sizes and chromosome numbers of *Trichoderma* spp. range from 31 to 39 Mb and from 3 to 7, respectively. The size of chromosome differs among the species. Once the strains are isolated in wet lab, the identification of isolated strains can be done and validated at the ISTH website. As ISTH is solely dedicated for the identification of different strains of *Trichoderma* and *Hypocrea* species based on ITS sequences. ISTH (International Sub-commission on *Trichoderma* and *Hypocrea* Taxonomy), a Sub-commission of ICTF (International Commission on the Taxonomy of Fungi), hosts an online method for the quick molecular identification of *Hypocrea* /*Trichoderma* species based on an oligonucleotide barcode. It is a diagnostic combination of several oligonucleotides specifically allocated within the Internal Transcribed Spacer 1 and 2 (ITS1 and 2) sequences of rDNA repeat. A set of 5 oligonucleotide sequences which are present in all known *Hypocrea* / *Trichoderma* ITS1 - 5.8S RNA - ITS2 sequences, is used in combinations to identify the species at generic level. It helps in identifying specific strains of *Trichoderma* by comparing the sequence with the database by locating Genus Specific Hallmarks (GSH). The nucleotide sequences (submitted and retrieved from NCBI) of all seven strains of *Trichoderma* species can be analyzed through TrichOKEY 2 program for their validation post molecular identification.

OP (S2) 04

Cloning of important antifungal genes in *Trichoderma*

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Crop husbandry is basis of human livelihood. But this crop husbandry is widely threatened by various microbes like fungus, bacteria, viruses, nematodes, etc. These microbes can be controlled by chemicals means effectively but same time these chemicals negatively affects ecosystem. The devastating impact of chemical can be reduced and or mitigated through biological control of phytopathogens. Biological control with *Trichoderma* has special significance being an eco-friendly and economical strategy for disease management. The various species of *Trichoderma* produces diverse enzymes which are important players of biocontrol activity. Cloning and expression of genes encoding antimicrobial enzymes and proteins provides a better mean to their functional characterization. In present era of molecular biology and different omics especially genomics and transcriptomics many antifungal genes from *Trichoderma* has been isolated

and cloned. Cloning and expression of genes encoding antimicrobial proteins is an important mean for functional characterization of genes which helps in enhancing the biocontrol activity of *Trichoderma*. A large number of genes producing biocontrolling proteins in different *Trichoderma* species have cloned and characterized; and some of them are chitinase, glucanase, peptaibol synthetase, protease and galacturonase. The reports of various researchers demonstrated that *Trichoderma* a reservoir of antimicrobial genes- can be exploited to control a range of phytopathogens in a very economic manner.

OP (S2) 05

Protein profiling of fungicide tolerant efficient isolates of *Trichoderma* spp.

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In vitro studies on sensitivity of fungicides on radial growth of *T. viride*, *T. harzianum*, *T. virens* and *T. asperellum* revealed maximum tolerance against metalaxyl followed by metiram, mancozeb, thiram and copper oxychloride, respectively. Studies on generation series and tolerance induction in *T. viride*, *T. harzianum*, *T. virens* and *T. asperellum* subjected to increasing gradient of fungicides showed that by gradual transfers of the bioagent from lower to higher concentrations of the fungicides, the bioagents acquired tolerance to the higher doses of fungicides viz., metalaxyl, thiram, mancozeb, chlorothalonil and copper oxychloride.

Protein profile of *T. viride*, *T. harzianum*, *T. virens* and *T. asperellum* exposed to higher concentration of fungicides analysed through SDS-PAGE indicated an increase in the protein content in the form of more number of protein bands of higher molecular weight as compared to non-tolerant isolates which indicated its association with presence of more number of extracellular cell wall degrading hydrolytic enzymes responsible for the antibiosis mechanism involved in biocontrol activity of the bioagent. *T. viride*, *T. harzianum*, *T. virens* and *T. asperellum* showed maximum protein banding pattern with seven, six, eight and six bands against metalaxyl exposed at higher concentration of 1000 ppm; followed by mancozeb (100 ppm) showing six, five, seven and three bands and copper oxychloride (600 ppm) with seven, seven and five bands while lowest number of protein bands i.e. four, three, three and three were observed with thiram at concentration of 100 ppm. The *Trichoderma* spp. viz., *T. viride*, *T. harzianum*, *T. virens* and *T. asperellum* tolerant to commonly used fungicides can be mass multiplied, formulated and recommended for efficient management of major soil and seed borne plant diseases.

Session 3

Secondary metabolism of the bioagents in crop growth promotion through host interactions

Lead Papers

LP (S3) 07

Sensing of plants, microbes and nutrients by *Trichoderma*

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The natural environment of the potent cellulase producer *Trichoderma reesei* (syn. *Hypocrea jecorina*) is a tropical forest, where it degrades cellulosic plant biomass. However, related *Trichoderma* spp. are also known as efficient plant protection agents, antagonizing fungal pathogens.

In the plant pathogen *Fusarium oxysporum*, plant sensing is accomplished by pheromone receptors responding to secreted plant peroxidases. Here we investigated the relationship between plant sensing, sexual development and nutrient detection in *T. reesei*.

We found that *T. reesei* is able to sense plant root exudates and peroxidases via pheromone receptors, suggesting that this mechanism is conserved between *Trichoderma* and *Fusarium*. Moreover, *T. reesei* also showed a concentration dependent chemotropic response to glucose and signals related to sexual development and plant sensing override nutrient regulated germination. Accordingly, we found that the presence of soybean germlings promotes sexual development of *T. reesei*.

We further tested whether *Trichoderma* spp. can sense and cooperate with plant beneficial *Bacillus* spp., which are suitable for combined application in agriculture for plant protection. For biocontrol assays, we isolated *Diaporthe sojae* from contaminated soybean seeds with deleterious effect on seed germination.

We propose that *T. reesei* is attracted to plants by a mechanism that integrates directed hyphal growth and sexual development for efficient adaptation to the host and potentially also for optimized antagonism of competitors and plant pathogens. Thereby, association with a plant has higher priority than availability of nutrients.

LP (S3) 08

Changing paradigms on the mode of action of fungal bio-control agents

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Use of bio-control fungi (BCF) are often a preferred means of managing plant diseases in the recent years to avoid growing pesticide residue problems in our food chain as well as for the environment safety. These BCF are mainly ubiquitous *Trichoderma* spp and recently described *Sebacinales* spp. (Wess *et al* 2011). They have the vast ability to control numerous foliar, root and fruit pathogens and even certain invertebrate pests like nematodes. However, disease control is only a subset of their ability to immensely influence plant eco-system. It is now explicitly demonstrated that they have ability to enhance plant growth and to increase resistance of their host plants against a wide range of abiotic stress factors and some of them can also alleviate physiological stresses such as seed-aging in addition to fungal pathogens (Bae *et al* 2009). They also can enhance nutrients uptake in host plants and significantly increase nitrogen use efficiency (NUE) for increasing crop yield (Harman, 2000; Sherameti *et al* 2005). Some biotypes/strains do have abilities to improve photosynthetic efficiency and probably alter respiratory activities in plants (Harman, 2000, 2006). All these capabilities are an outcome of their abilities to alter plant expression genes by reprogramming through activation of a limited number of common pathways in plants (Korolev *et al* 2008; Segarra *et al.* 2007). These abilities are added importance in agriculture than disease management alone. I believe that the abilities of these BCF to induce resistance to biotic (Ahmed *et al* 2000; Bigirimana *et al* 1997; Harman *et al* 2004; Yedidia *et al* 2003) and abiotic stresses (Waller *et al* 2005, 2008) and increase NUE make them extremely useful tools for increasing crop productivity and improving food security as well as environment.

LP (S3) 09

Microbial antagonists and their role in biological control of plant diseases

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Continuous mono cropping and indiscriminate use of fungicides have lead to the development of resurgence among the pathogens. Pathogens infecting various crops, has been reported to exhibit resistance against various fungicides including mancozeb, propineb, and carbendazim. Moreover, application of pesticides to soil reduces the population of *Azotobacter*, *Azospirillum*, phosphate solubilizing bacteria, and

other beneficial bacteria and fungi. Under these situations, an alternative approach is essential. Biological control of plant diseases with antagonistic fungi and bacteria have been demonstrated successfully with various crop plants. Among them *Bacillus* and *Trichoderma* species have been widely exploited.

Bacillus species exploited includes, *B. subtilis*, *B. amyloliquefaciens*, *B. pumilus*, *B. methylotrophicus*, *B. licheniformis*, *B. megeyeri*, and *B. tequilensis*. *Bacillus* sp, handle an array of mode of actions against plant pathogens, including competition, secretion of siderophores, mycoparasitism, induction of plant defense. Among them antibiosis remains the most crucial. *Bacillus* species contains antimicrobial peptide genes responsible for the biosynthesis of antibiotics like iturin, bacilysin, bacillomycin, fengycin, surfactin, mersacidin, ericin, subtilin, subtilosin, and mycosubtilin (Isabel Mora *et al.*, 2011, Vinodkumar *et al.*, 2017b). Antibiotics produced by the bacteria have specific modes of actions. However, the common action includes osmotic dysregulation of the pathogen cell and finally death. Apart from anti microbial peptides, *Bacillus* sp. secrete volatile and non-volatile antimicrobial compounds that synergistically aid in curtailing plant diseases. GC/MS analysis of the crude secretions of *Bacillus* species revealed the presence antifungal and antibacterial compounds including, chloroxyleneol, pentadecenoic acid, heptadecenoic acid, octadecenoic acid, pyrrolo, and hexadecenoic acid (Vinodkumar *et al.*, 2017b).

Among the fungal antagonists *Trichoderma* species are widely exploited. The most commonly utilized *Trichoderma* species are *T. asperellum*, *T. asperelloides*, *T. harzianum*, *T. viride*, *T. virens*, *T. hamatum*, *T. koningii*, *T. pseudokoningii*. *Trichoderma* species have been widely exploited due to their high reproductive capacity, survivability under unfavorable conditions, efficiency in the utilization of nutrients, rhizosphere competence, efficiency in promoting plant growth and induction of defense mechanisms. Modes of action of *Trichoderma* spp. includes, competition for nutrients and space, mycoparasitism by secretion of diverse hydrolytic enzymes, exudation of antimicrobial secondary metabolites, induction of systemic resistance, and plant growth promotion. Among all mycoparasitism by the secretion of hydrolytic enzymes was correlated with the efficiency of the antagonist. *Trichoderma* spp. are known to possess genes encoding hydrolytic enzymes such as cellobiohydrolase, chitinase, glucanase, xylanase and protease. *Trichoderma* species encoding *ech42* (endochitinase), *egl4* (endoglucanase) *cbh1* (cellobiohydrolase) gene was found to be more effective in the suppression of various fungal diseases compared to those strains lacking the genes. Apart from the secretion of hydrolytic enzymes, *Trichoderma* sp secrete wide range of antifungal metabolites including, hexadecane, dodecene, heptadecene, eicosane, phenyl ethyl alcohol, benzene dicarboxylic acid, heptacosane, octacosane, nonacosane (Vinodkumar *et al.*, 2017a).

Application of *Bacillus* species and *Trichoderma* species have resulted in reduced disease in various crop plants. Delivering of *B. subtilis* isolate BS2 through root dipping and soil drenching @ 5ml/litre recorded only 1% fusarial wilt incidence in carnation. Soil application of liquid formulation of *B. amyloliquefaciens* (VB7) - (2.5×10^{10} cfu/ml) by root dip followed by soil drenching (5ml/l) at monthly intervals resulted in a minimal stem rot incidence up to 87.9% relative to the control. Foliar application of *B. subtilis* (BS2) and *B. amyloliquefaciens* (BSC7) in chrysanthemum recorded the minimum of 3.88 white rust spots per leaf as against 26.79 white rust spots in untreated control (Dheepa *et al.*, 2016). Root dip, followed by soil drenching of *T. asperellum* (NVTA2) at 0.5% (5 ml/l) reduced percent stem rot incidence in carnation up to 11.8 compared to the control (37.9 PDI), which was approximately 69% reduction in stem rot incidence compared to the untreated control.

Further, *Trichoderma* species have been reported to control fusarial wilt and *Pythium* rot in cucumber (Vasumathi *et al.*, 2016; Vasumathi *et al.*, 2017). Moreover, the antagonists have also been reported to promote plant growth and yield in carnation, chrysanthemum, rose, liliium and cucumber. In this constraint, future studies in the exploitation of *Bacillus* species with diverse anti microbial peptide genes and *Trichoderma* species would be a scope for the management of various fungal and bacterial diseases in crop plants.

LP (S3) 10

A novel bio-control agent *Bacillus subtilis* which induces systemic resistance in plants

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Plant pathogens are responsible for many crop plant diseases, resulting in economic losses. The use of bacterial agents is an excellent option to fight against plant pathogens and an excellent alternative to the use of chemicals, which are offensive to the environment and to human health. The one of the most common biocontrol agent are members of the *Bacillus* genera. *Bacillus* genera have important traits such as plant growth-promoting (PGP) properties. This review analyzes pioneering and recent works and the mechanisms used by *Bacillus* in their behavior as biocontrol and PGP agent and discussing their mode of action. Undoubtedly, future integrated research strategies for biocontrol and PGP will require the help of known and novel species of *Bacillus* genera.

LP (S3) 11

Green nanotechnology for plant diseases management

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Green nanotechnology is one of the promising research areas in the biological fields including agriculture and allied areas. Application of nanotechnology in plant pathology helps in detection, management of plant pathogens and to study the host-pathogen interactions. Only few researchers were worked on synthesis of nanoparticles and their efficacy against plant pathogens. Synthesis of nanomaterials by a simple, low cost and high yield has been a great challenge since the early development of nanoscience. The methods for designing of nanoparticles (1-100 nm) generally involve top-down or bottom-up approach. Various nanoparticles were synthesized from different methods based on their application from metals /nonmetals or their salts such as silver, copper, iron, zinc, sulphur, phosphorus, titanium dioxide,

silica, cobalt, nickel etc. Green synthesis of silver, copper, zinc, iron and sulphur from plant/microbe/chitosan were attempted and successfully synthesized. Synthesized nanoparticles were analyzed by UV-Vis spectrophotometer, Particle Size Analyzer (PSA), Atomic Force Microscope (AFM) and Scanning Electron Microscope (SEM) with EDAX. Confirmed nanoparticles (<100 nm) were evaluated against major plant fungal pathogens viz., *Pythium aphanidermatum*, *Sclerotium rolfsii*, *Exserohilum turcicum*, *Golovomyces cichoracearum*, *Ustilago nuda tritici*, *Puccinia triticina* and bacterial pathogens viz., *Xanthomonas* spp. *Ralstonia solanacearum*. Nanoparticles did not show any phytotoxicity on cowpea, chilli and tomato seedlings under glasshouse studies by spray inoculation method. Green nanoparticles were found to be effective against major plant pathogens at very lowest concentration. In future green nanoparticles will be used as alternative to presently available commercial fungicides.

Oral Papers

OP (S3) 06

Evaluation of abiotic and biotic elicitors for induction of defense related enzymes in pea against *Peronospora viciae*

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Resistance in plants against pathogens are induced by several natural and synthetic compounds. Induction of systemic resistance is associated with activation of a wide range of resistance mechanisms and the production of a wide range of defense compounds. It is race non-specific and is often effective against a broad spectrum of pathogenic agents. SAR activation has been successfully used in controlling fungal and bacterial plant pathogens by application of both abiotic and biotic inducers. Therefore, an attempt has been made to evaluate defense related enzymes viz. phenyl ammonia lyase (PAL), polyphenol oxidase (PPO), peroxidase (PO) and total phenol in pea against *Peronospora viciae* Syd. (Oomycete) through abiotic and biotic elicitors. A total of eight elicitors were tested alone/or in combination for induction of defense related enzymes in pea against *Peronospora viciae* Syd. Pea seeds after treatment with different elicitors were sown in different pots. After 30 days of sowing samples were collected from each treatments and same treatments were applied as foliar application with the help of a atomizer followed by challenge inoculation. Five leaves were collected from all the treatments 24, 48, 72 and 96 hr after challenge inoculation. Among all treatments maximum PAL activity (28.28 mg/g of fresh weight) was found in case *Pseudomonas fluorescens* followed by oxalic acid (16.24 mg/g of fresh weight). Foliar application followed by challenge inoculation lead to increase in PAL activity. Maximum induction was observed 48 hrs after the challenge inoculation (47 mg/g of fresh weight) in case of *Pseudomonas fluorescens* treated plants. Among all treatments maximum POP activity (4.40 $\mu\text{mol}/\text{min}/\text{mg}/\text{protein}$) was found in case of chitosan + *Trichoderma harzianum* treated plants followed by chitosan (3.53 $\mu\text{mol}/\text{min}/\text{mg}/\text{protein}$). Foliar application followed

by challenge inoculation lead to increase in PPO activity and maximum induction was observed 48 hrs after the challenge inoculation (6.05 $\mu\text{mol}/\text{min}/\text{mg}/\text{protein}$) in case of chitosan + *Trichoderma harzianum* treated plants. Among all treatments maximum PO activity (26.55 $\mu\text{mol}/\text{min}/\text{mg}/\text{protein}$) was found in case of chitosan + *Trichoderma harzianum* treated plants followed by chitosan (25.13 $\mu\text{mol}/\text{min}/\text{mg}/\text{protein}$). Foliar application followed by challenge inoculation lead to increase in PO activity. Maximum induction was observed 96 hrs after the challenge inoculation (63.72 $\mu\text{mol}/\text{min}/\text{mg}/\text{protein}$) in case of chitosan + *Trichoderma harzianum* treated plants. Among all treatments maximum phenolics (11.23 mg/gm of fresh weight) was found in case of chitosan + *Pseudomonas fluorescens* treated plants followed by oxalic acid (10.75 mg/gm of fresh weight). Foliar application followed by challenge inoculation lead to increase in phenolics concentration. Maximum induction was observed 72 hrs after the challenge inoculation (24.85 mg/gm of fresh weight) in case of chitosan + *Pseudomonas fluorescens* treated plants. Activation of SAR by exogenous application of elicitors can protect from a broad range of pathogens. Therefore, the above mentioned treatments might be an alternative for the conventional pesticides in pea crop protection, with the advantage of a low environmental impact.

OP (S3) 07

Plant growth promotion and induction of systemic resistance by *Bacillus* sp.

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PGPR (Plant Growth Promoting Rhizobacteria) plays a significant role in maintaining plant health and growth by showing a plethora of mechanisms in the root of plant. In the present study some PGPR isolated from rhizosphere of various crops and screened them for various plant growth promoting activities such as phyto-hormone production, beneficial enzyme production, secondary metabolite production, antifungal compound production *etc.* In the present work, among isolates a *Bacillus* sp. was selected for ISR (Induced Systemic Resistance) study on chickpea and groundnut. During screening, various plant growth promoting traits like production of various enzyme *i.e.* chitinase, phytase, lipase, cellulase, protease, Siderophore production, organic acid production, phosphate-zinc and potassium solubilisation, phyto-hormone production *etc.* were observed.

Induced systemic resistance in chickpea (*Cicer arietinum*) against *F. oxysporum* and in groundnut (*Arachis hypogaea*) against *A. niger* by pot experiments were studied for the management of fusarium wilt and collar rot disease, respectively. Coating of seeds with *Bacillus* sp. showed an increase in seed germination, shoot length, shoot fresh and dry weight, root length, root fresh and dry weight of plant as well as induced the enzyme activity *viz.*, peroxidase (POX), polyphenol oxidase (PPO), phenyl alanine ammonia lyase (PAL), catalase, chlorophyll content, protein content, phenol content, *etc.* in comparison to control. The disease suppression and induction of systemic resistance along with plant growth enhancement indicate its possible use as PGPR/ bio-control agents against both diseases.

Session 4

**Bioagents/ antagonists in integrated management of
biotic and abiotic stresses of crops**

Lead Papers

LP (S4) 12

***Bacillus*: A potential biocontrol agent for management of crop diseases**

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Bacillus is characterized by having a rod shape within the group of Gram positive, and is therefore classified as strict aerobes or facultative anaerobes and integrated by 88 species. The use and number of antagonistically important *Bacillus* species is increasing very rapidly. They have a unique ability to replicate rapidly, resistant to adverse environmental conditions as well as have broad spectrum of biocontrol ability. They are recognized as one of the most effective biological control agent because of their properties on pathogens growth inhibition. Generally, the mode of action of *Bacillus* is antibiosis by producing extracellular hydrolytic enzymes which decompose polysaccharides, nucleic acids, other way are: production of antibiotics (Subtilolysin, Surfactin, Mycosubtilin, Iturin, Fengycin, Bacillomycin, Difficidin, Bacillaene, Macrolactin, Cerein, Zwittermicin, Bacillibactin, polymyxin, gramicidin and polyketides), competition for nutrients and induced resistance. Several products based on *Bacillus* spp. are available in the market such as Avogreen, Biosafe, Bio subtilin, Cease, Companion, Ecoshot, FZB24 WG Ji and TB, HiStick N/T, Subtilex/Pro-Mix, Kodiak, Rhizo Pus, Serenade, Sonata, Yield shield, Balada, Botrybel and RhizoVital42TB. These product can be used against various plant diseases including post harvest diseases. In conclusion, we can say that bacillus based bioformulations have great potential to control various plant diseases. Thus it should be even more used to make the future agriculture more sustainable. Much work has been done but a lot is still to do for scientists and industrials, to improve reliability and efficacy of these products and keep gaining an increasing market share.

LP (S4) 13

Role of microbes and fungi in plant disease management

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Nature harbours number of microbes and fungi, which have potential use in agriculture, industry and medicine. Diversified groups of above organisms play an important role in natural cycling of elements, biotransformation, bioremediation and in other biological processes. Some of the microorganisms like bacteria, viruses, mycoplasma, phytoplasma, viroids and fungi have been causing diseases on crop plants and forest plants. These diseases caused huge losses affecting the economy of the country, shifting of crop cultivation, change in food habits and some pathogens like late blight of potato have created history by causing famine and disease epidemics. Further, the growing population made the scientists to realize about food security. These conflicts have made the farmers to realize the importance of diseases and make an effort to control. Many researchers have come out successfully to control the disease by applying chemicals, biological agents, cultural practices, use of healthy seeds, breeding for disease resistance and by initiating transgenics. The extensive and non-judicious use of chemicals contributed for increase in toxic levels of chemicals in the products such as grains, seeds, tissues etc. Further, many of the plant pathogens have also created resistance against chemicals creating serious problem for the growers. All these developments have led to find out an alternate mechanism for the control of plant diseases caused by fungi, bacteria, viruses, insects, nematodes, viroids, mycoplasma, phytoplasma and others. Use of living biological organisms such as microbes, fungi, predators and other biological agents have given a new hope in combating several disease causing agents as biological control agents. In this review a detailed account of plant diseases, fungicidal control, biological control of plant disease mechanisms, integrated disease control, role of PGPR and endophytes, microbial nanoparticles as biocontrol agents, biocontrol of insect pests, mycobiocontrol, use of *Trichoderma* as potential biocontrol agent and also future strategies on the role of microbes and fungi in plant disease management have been discussed in depth.

LP (S4) 14

Use of bio agents for management of nematodes in horticultural crops

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Phytonematodes are microscopic in nature and ubiquitous browsers to the crop roots. They attack almost all agricultural and horticultural crops such as vegetables, pulses, oil seeds, cash crops such as tobacco, cotton, sugarcane, spices; cereals, all tropical and subtropical fruits crops, plantation crops, etc. in the world. Nematodes cause 12-13% yield losses in crop production worldwide. In India, statistically estimated monetary field loss comes to about Rs. 2100 crores only in 24 crops covered under All India Co-ordinated Research Project on Nematodes. Chemical control has been most effective methods for management of PPNs but only few nematicides *i.e.*, carbofuran, phorate and carbosulfan are officially registered by Government of India and are available to the farmers. However, they have not remained much effective under severe infection of nematodes in crops in open and protected cultivation. In developed countries, chemical nematicides/ fumigants are well managed and utilized with high expertise to achieve considerable results but are not available as such in India. However, because of the environmental concerns, health conscious attitude of human beings and other hazards associated with the use of chemicals, use of bio agents to suppress plant parasitic nematodes population is gaining importance. In this path, a number of commercial products have been registered both at national and inter-national levels based on different fungal and bacterial antagonists. Good qualities of bio-nematicides are now available in the country. Since last one decade or so, bio-nematicides such as *Trichoderma viride* (Tv) *Purpureocillium lilacinum* (Pl) and *Pseudomonas fluorescens* (Pf) of good quality (cfu 2×10^9) are being extensively used for management of nematodes by farmers.

Two seasons pooled data showed that the application of Carbofuran@1Kg (Furadan 3G@33 kg/ha) + 5 kg Pl/ha under the crop row a day before potato seeding checked 94% root-knot infection on potato tubers and enhanced 45% production of healthy tubers. Results of three years pooled data showed that application of Pl @ 25 kg spore dust with carrier/ha (10^9 cfu/g) either with poultry manure @ 10 t/ha or mustard cake or neem cake @ 2 t/ha improved plant growth and considerably reduced gall index and gave higher brinjal fruit yield over control (ICBR 1:5.8 to 1:18.5). Field application of Pl (2×10^6 cfu/g) and Pf (2×10^6 cfu/g) each @ 2.5 kg/ha along with 2.5 t FYM/ha reduced root-knot nematode population by 32.01 and 35.67% and increased okra fresh pod yield by 92.97 and 71.24% over control. Application of bio-nematicides Neemate 10 G @ 2 g a.i./plant and Samarat (FYM enriched with Pl) @ 750 g a. i./plant decreased 28.74 & 27.15 % and 22.69 & 18.21 % no. of root galls and nema population/ 5 g roots and thereby increased pomegranate fruit yield by 25.38 & 21.91 %, respectively. In banana also, Neemate 10 G@ 2 g a. i./plant and Samarat @ 750 g a.i./plant could reduce 49.14 & 61.48 % lesions on rhizomes, 31.52 & 37.32 % root-knot index, 63.59 & 61.07 % nema population/ 200 g soil and 91.26 & 90.50 % nema population/ 5 g roots and thereby increased banana fruit yield by 37.82 & 46.98 %, respectively.

Root- knot nematodes (*M. incognita*) on grape was effectively checked by application of Pf @ 20 kg/ha (ICBR 1:32.10) and Tv plus @ 20 kg /ha (ICBR 1: 26.64) in Maharashtra. Application of PI multiplied on neem cake (7 X 10⁸cfu/g) @ 10 kg/ha and on talc (15X10⁸ cfu /g) reduced population of PCN and increased potato tuber yield by 88.2 and 76.2 %, respectively over control. Use of *P. penetrans* and Tv with neem or castor cake increased plant growth and managed *M. incognita* in tomato. Application of these bio-agents enriched with good quality organic manures has given very interesting results. Hence, there is a need to popularize use of good quality bio agents for effective and economic management of phytonematodes in integrated management programs to enhance crop production and productivity by farmers. They are eco-friendly and good component of organic farming as well.

LP (S4) 15

Management of guava wilt through microbial antagonists

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Guava (*Psidium guajava* Linn.) is one of the important commercial fruits in India but it suffers badly with wilt disease. The average productivity can at least be doubled, if wilt disease is managed effectively. Work in last two decades at CISH, Lucknow revealed that microbial intervention is the correct approach for the management of this soil borne disease, as microbes propagates itself in the soil, if suitable substrates and conditions are provided. In recent past efforts were made to understand the disease at ICAR-CISH, Lucknow and information of guava wilt was generated on the understanding and practical aspects of the disease for disease management and major emphasis was given on management through microbial intervention.

Wilt affected plants show varied symptoms, but it can be grouped into two major symptoms i.e., slow and quick wilt. Various pathogens are reported by different workers for the cause of wilt. Major pathogens are *Fusarium oxysporum* f. sp. *psidii*, *F. solani*, *F. chlaymydosporum*, *Gliocladium roseum*, etc. It was found that *F. oxysporum* f. sp. *psidii* and the *F. solani* are most frequently isolated pathogens and can be considered as the 2 most important pathogens of wilt disease of guava. In a lab and field studies it was found that *F. oxysporum* f. sp. *psidii* and *F. solani* are highly variable pathogens. Variation was recorded in cultural, physiological, pathogenic and molecular characters. As wilt is a soil borne disease and great mass of soil in different depth is to be tackled, it is obvious that some dynamic system is required, which itself flourish in the soil and hence microbial intervention seems practical. At ICAR-CISH, Lucknow, three bio-agents were found effective for the management of the wilt disease i.e. *Aspergillus niger*, *Trichoderma* sp. and *Penicillium citrinum*. Besides fungal bioagents, bacterial antagonists also showed potential. In a test of isolates, isolate AN-9 of *Aspergillus niger* was identified as potential, while all the tested isolates of *P. citrinum* and TD 1 of *T. virence* were found better effective. When direct use of bioagents (*A. niger*,

P. citrinum and *Trichoderma* spp.), their culture filtrates and released volatile compounds were tested for inhibition of isolates of guava wilt pathogens, direct inhibition by culture was more pronounced. The bacterial antagonistic isolates AB1, AB3 and AB5 were very effective. When relative growth of three bio-agents viz., *A. niger*, *P. citrinum* and *Trichoderma* spp. were studied, it was found that the *A. niger* is the fastest growing pathogen as compared to other two bio-agents. Multiplication of bioagents in grains is the general practice but it can also be multiplied on cheap substrate like guava drooped leaves or grasses. For medium quantity propagation bioagents can successfully be grown in containers like bottles, polypropalin bags or even in earthen pots. For mass multiplication, it can be multiplied in FYM and directly be applied in the field. At planting 5 kg of enriched FYM with bioagents should be filled in pits with soil in the month of June. By this application, while bioagent managed the disease, it was also good for the initial health of the plants, which was clearly indicated by the girth and height of plants. For number of application during a year, it was found that double application in a year give better result compared to single application. In full grown plants, 10 kg of enriched FYM with bioagents should be applied in basin soil in the month of June and October. This practice should be repeated every year as preventive measure. It was also seen that the organic mulch help in the better establishment of bioagents. Mulch by defoliated guava leaves or Saccharum grass may be used, which are generally available in the guava orchard in plenty. With the microbial management, some of the culture practices needs to be followed, which include more organic matter in the soil, no damage of root system during planting, less tillage and avoidance of flood irrigation. Separate basin irrigation or drip irrigation is better. Use of resistant root stock, F1 population of *Psidium molle* X *Psidium guajava* needs to be encouraged. For effective management either the consortium should be used or the effective bioagents should be used one after another for getting desired better result.

LP (S4) 16

***Sclerotinia* stem rot - An emerging threat and ecofriendly management in rapeseed-mustard**

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Sclerotinia is recognized as an important plant pathogen due to its worldwide distribution, wide host range and the difficulties encountered in controlling the diseases it causes. Three species of *Sclerotinia* viz., *S. sclerotiorum*, *S. minor* and *S. trifoliorum* are considered important on the basis of wide distribution, host range and heavy yield losses caused in several crops of economic importance. *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most devastating and cosmopolitan plant pathogens. The fungus infects over 400 species of plants worldwide including important crops and numerous weeds. The disease causes serious and unpredictable yield losses in field which ranges from a trace to 100 per cent. *Sclerotinia* is generally considered to be a simple interest pathogen utilizing ascospores as the primary inoculum and plant infection by mycelial growth between stem bases or by contact between infected plants with neighboring healthy plants late in the growing season as secondary inoculum. The epidemiology of *Sclerotinia* diseases is

dependent on several factors viz., soil inoculum; soil type; soil moisture; rainfall; soil and environmental temperature; host susceptibility; plant density and cultural practices adopted. None of the variety or genotypes has been found resistant or tolerant to this disease when evaluated by artificial inoculation against *Sclerotinia sclerotiorum*. The role of frequency and quantity of irrigation in the germination of sclerotia and development of apothecium in different soil types, were assessed in field. It was observed that amount and duration of irrigation affect the carpogenic germination and severity of the disease. Optimum irrigation once in 7 days and flooding once in a week resulted in less pre-emergence damping-off compared to controls. Minimum pre-emergence damping-off was observed in sandy soil followed by clay and sandy loam soils. Post-emergence damping off was minimum where optimum irrigation was given once in 3 days and it was in sandy soil followed by clay and sandy loam soils. The production of apothecia in relation to irrigation levels showed that flooding for two consecutive days once in a week and optimum irrigation once in 7 days were most effective in reducing the apothecial population as compared to control. Maximum apothecia production was recorded in treatment with optimum irrigation once in 10 days. Amongst different soil types, the least number of apothecia production was recorded in sandy soil whereas, sandy loam soil resulted in maximum number of apothecia production. Prior to sowing, flooding of inoculated pots once in a week resulted in the least disease incidence and minimum lesion length whereas maximum disease incidence was recorded where daily irrigation was applied as compared to control treatments. The plants raised in sandy soil had the least lesion length and disease intensity while those raised in sandy loam soil produced maximum lesion length. Disease intensity was less in the treatments where flooding once in a week and optimum irrigation applied once in 3 or 7 days as compared to control pots. Evaluation of plants extract against this pathogen revealed that out of 13 leaf extracts tested, the extracts of *Bougainvillea spectabilis* (Bougainvillea), *Azadirachta indica* (Neem) and *Allium sativum* (Garlic) were significantly effective in checking mycelial growth and sclerotia formation at 50 per cent concentration. Soil amendment with Bougainvillea, Jamun and Mehndi leaf were able to reduce the number of apothecia appearance, lesion length and disease intensity. *Lawsonia inermis* (Mehndi), *Canabis sativa* (Bhang) and *Clerodendrum inerme* (Clerodendron) were as next best leaf extracts in reducing linear mycelium growth and sclerotial formation. There was positive and high degree of correlation between number and total weight of sclerotia with regression equation $Y = 12.742x - 4.2905$ and $R^2 = 0.8758$. Organic amendments with the use of cakes/organic manures have also been found significantly reduced number of apothecia production in pots. Poultry manure was most effective and significantly better in reducing lesion length and disease intensity. Biological control of *Sclerotinia* as an alternative disease control strategy. Isolates of *Trichoderma harzianum*-3, *T. harzianum*-4 and *Bacillus subtilis* have been observed potent in decreasing the linear growth and number of apothecia production in *in vitro* conditions. *T. harzianum* was quite effective in reducing lesion length and disease intensity when applied simultaneously and seven days prior to the pathogen. Systemic acquired resistance chemicals like INA, SA as seed treatment or spray @ 100 ppm could reduce seedling mortality, lesion length, disease intensity and disease incidence. Seed treatments with fungicides like carbendazim, thiophanate methyl, benomyl, thiram, mancozeb and organo-mercurials have been reported to be effective for the control disease. Foliar application to control this disease must be applied before infection occurs. Proper timing of spray and method of application have a great impact on the management of this disease. The regression equation developed revealed positive and perfect correlation ($R^2 = 1$) between number of apothecia formation and disease intensity. Studies were also carried out to investigate the callogenic

response of rapeseed-mustard species to culture filtrates of *Sclerotinia sclerotiorum* responsible for white stem rot and their biochemical differences were evaluated through tissue culture technique. Different concentrations of culture filtrate were added in MS medium supplemented with NAA 1 mg⁻¹+ BAP 1 mg⁻¹. Percent callus formation in response to fungal culture filtrate was more in *B. carinata* cv. HC-9002 than other *Brassica* species. *In vitro*, callus screening with *S. sclerotiorum* culture filtrate revealed a similar behavior with field reactions of the *Brassica* species to the disease. Since twenty per cent concentration of culture filtrate was lethal to some genotypes except *B. napus* cv. GSH-1 and *B. carinata* cv. HC-9002, 15% concentration of culture filtrate can be used for *in vitro* screening for selection of tolerant species. Increase in concentration of culture filtrate resulted in decrease of fresh as well as dry weights of calli derived from all the species. Investigations on biochemical characterization of calli tolerance to culture filtrate revealed that total soluble sugars and reducing sugars were high after selection with fungal culture filtrate in calli of *B. napus* cv. GSH-1 and *B. carinata* cv. HC-9002 but other species showed less sugar contents. Increase in level of culture filtrate in callus induction medium increased total phenol, flavanol and total soluble protein contents in calli of all the species. Maximum being in calli of *B. carinata* cv. HC-9002. Activity of all the three enzymes viz., polyphenol oxidase (PPO), peroxidase and catalase increased with response to increase in concentration of culture filtrate. PPO and peroxidase activity was maximum in *B. carinata* cv. HC-9002 which was less susceptible than other species, while catalase activity was maximum in *B. juncea* cv. RH-30 which was highly susceptible to disease.

LP (S4) 17

Biological control agents based management of major diseases of chickpea

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Chickpea is an important pulse crop of India suffers from number of fungal diseases, mainly, wilt [*Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo and K. Sato], wet root-rot [*Rhizoctonia solani* Kuhn], dry root-rot [*Rhizoctonia bataticola* (Taub.) Butler Goid], stem rot [*Sclerotinia sclerotiorum* (Lib.) de Bary], Ascochyta blight [*Ascochyta rabiei* (Pass.) Labr.], grey mould (*Botrytis cinerea* Per. ex. Fr.) and collar rot (*Sclerotium rolfsii* Sacc.). *Trichoderma* has received a lot of attention as biocontrol agent. The success of *Trichoderma* is due to their high reproductive capacity, ability to survive adverse conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against plant pathogens and efficiency in promoting plant growth and defense mechanism. *Trichoderma* spp. may exist as direct biocontrol by parasitizing a range of fungi. They are compatible with other beneficial microorganisms and suitable for various modes of application. The *Trichoderma* spp. alone and in combination with fungicides proved effective against several seed and soil borne diseases of crop plants and is considered as a major component of integrated disease management. Various seed dressing and soil application formulations were developed from *Trichoderma viride* (IARI P1; MTCC 5369), *T. virens* (IARI P3; MTCC 5370) and

T. harzianum (IARI P4; MTCC 5371) to increase the shelf life of bio-formulations used to manage plant diseases. The shelf life of the formulations developed was monitored by counting colony forming units (cfu) up to 25 months of storage at room temperature ($26\pm 8^{\circ}\text{C}$). A newly developed seed dressing formulation, Pusa 5SD soil application formulation, Pusa Biopellet (PBP) exhibited longer shelf life. Pusa 5SD could be used up to 25 months of storage while PBP 10G and PBG 5 could be used up to 15 months of storage ($>10^5$ cfu). The formulations were evaluated against the diseases of chickpea in different mode of application as seed treatment and soil application alone and in combination under pot and field conditions. PBP 4G, PBP 10G and PBP 16G for soil application and Pusa 5SD for seed treatment were found effective under pot conditions. A combination of PBP 10G (*T. harzianum*)/ PBP 4G (*T. viride*) for soil application and Pusa 5SD (*T. harzianum*) for seed treatment together with a fungicide, carboxin provided the highest seed germination, shoot and root lengths and grain yield with the lowest disease incidence in chickpea under field conditions. *T. harzianum* based formulations proved to be more effective as compared to others against wilt and dry root rot whereas, *T. virens* based formulations against wet root rot. The efficacy of Pusa 5SD as seed treatment has been validated at different locations in India. Further, a combination of seeds treated with Pusa 5SD developed from *T. harzianum* + *Pseudomonas fluorescens* 80 + *Mesorhizobium ciceri* + Vitavax power also provided the highest germination, grain yield and the lowest wilt incidence.

LP (S4) 18

Organic production of crops using biocontrol agents: scope and future perspectives

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Biocontrol agents and biofertilizers, the naturally occurring formulations made from the substances that control pests by non-toxic mechanisms and in ecofriendly manner, are not newer technologies. They have been used in various forms since human civilization. Biocontrol agents being living organisms (natural enemies) or products there of pose less threat to the environment and to human health, hence can be used for the management of pests. Biocontrol agent is gaining interest because of its advantages associated with the environmental safety, target-specificity, efficacy, biodegradability and suitability in the integrated pest management (IPM) programs. Thus, biocontrol agent is one of the promising alternatives to manage environmental pollutions. Biocontrol agents are attracting global attention as safer strategy to manage pest populations such as weeds, plant pathogens and insects while posing less risk to human being and the environment. Biocontrol agents also produce growth hormones like, auxins, cytokinin, gibberellins, etc. These hormones suppress the deleterious pathogens and promote the growth of plants and simultaneously increase the yield. In recent years considerable attention has been paid to PGPR to replace agrochemicals. Plant growth promoting rhizobacterial bioformulation refers to preparations of microorganism that may be partial or complete substitute for chemical fertilization, pesticides, offer an environmentally sustainable approach to increase crop production and health. Presently, biocontrol agents cover only 2% of the plant protectants used globally; however, its growth rate shows an increasing trend in past two decades. Globally,

the use of biocontrol agents is increasing steadily by 10% every year. Policy measures need to be strengthened in order to reduce excessive use of chemical pesticides and to promote the use of biocontrol agents. This review provides environment friendly approach to increase crop production and health, development of sustainable agriculture and commercialization by using of bioagents with global applicability. Most of the countries have amended their policies to minimize the use of chemical pesticides and promote the use of biocontrol agents; however, biocontrol agents are still largely regulated by the system originally designed for chemical pesticides.

LP (S4) 19

Status of biological control of bacterial wilt of solanaceous vegetables

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Bacterial wilt is one of the important constraints in the cultivation of solanaceous vegetables in India. The disease is caused by *Ralstonia solanacearum*, a soil bacterium. In the vegetable growing regions of India, bacterial wilt poses a major threat towards the production of solanaceous vegetables viz. brinjal, tomato and chilli. Management of bacterial wilt in these crops has been difficult due to the diversity in *R. solanacearum* strains, ability of the bacterium to survive in adverse soil conditions, worldwide distribution, wide host range including asymptomatic hosts and efficient mechanism of invading host. Various strategies, including resistant varieties, soil amendments, crop rotation, soil solarization, use of bio-fumigants, etc. had been developed with limited success. Biological control has been accepted or emerged as one of the important methods in the management of plant diseases including bacterial wilt. In this article, brief account of the bacterial wilt disease, the causal agent, disease cycle including symptoms, scope of biological control and various biological control agents or strategies used to manage bacterial wilt disease are described. Mostly plant associated bacteria (rhizobacteria and endophytic bacteria) are used as biocontrol agent for the management of bacterial wilt. The suggested mechanisms of action for the disease suppression are antibiosis, niche exclusion by competition, induced resistance and inhibition of pathogen virulence factors. Bacteriophages and avirulent strains of *R. solanacearum* were also reported as biocontrol agents. Though lot of scientific progress is made in understanding the nature of pathogen, biocontrol agents, plant-microbe interactions at the molecular level which has yet to be translated into effective use in disease control. It is very important to realise that efficacy of biological control would be improved when integrated with cultural, host resistance and other management practices.

LP (S4) 20

Microbial antagonists in biological control of wheat diseases

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Wheat crop suffers from many biotic and abiotic stresses in all stages of growth that limit its production. Many biotic stresses interfere with normal functioning of physiological processes of plants and cause different foliar, seed, soil and air borne diseases. The fungal diseases of wheat *viz.*, rusts (stripe, leaf & stem), Karnal bunt, spot blotch, Fusarium head blight, powdery mildew, Septoria blotches, tan spot, take-all, Rhizoctonia root rot and wheat blast are the most potentially damaging because they cause substantial reduction in yield as well as deterioration in quality of grain. These diseases are considered as a serious constraint inflicting high economic yield losses in most of the wheat growing areas of the world. Control of wheat diseases has relied on a combined approach of growing resistant cultivars and applying fungicides. However, the potential risk of the pathogen becoming resistant to fungicides has been increasing because triazole compounds as fungicides, especially triadimefon, have been widely used for a long period of time. Meanwhile, the widespread use of fungicide can cause environmental pollution. Resistance of cultivars to diseases could be easily overcome by new virulence of pathogens. Therefore, it is better to develop a non-chemical alternative approach to manage diseases. Use of beneficial microbes may provide an environmental friendly option for control of diseases and reduce the potential risk of fungicide-resistant strains in the fungal population. There are various naturally occurring soil microbes that aggressively attack on plant pathogens and benefit plants by disease suppression and hence referred to as biocontrol agents. It has the potential to be more stable and long lasting than other controls and is comparative with the concepts and goals of integrated pest management and sustainable agriculture. Biological control, offers an additional strategy and can be used as part of an integrated management of wheat diseases for a greater level of protection with sustained yield.

Among the microorganisms with high potential for the development of biocontrol agents, using *in vitro* production systems, are bacteria and fungi. In combating plant diseases, microbial antagonists belong to the genus *Bacillus*, *Pseudomonas* and *Trichoderma* are among the most widely used bacteria and fungus respectively, and a good candidate for biological control. The *Bacillus* spp. is among the highly potent bacterial biocontrol agents used for controlling principally rhizosphere and to lesser extent foliar diseases of plants. These bacteria are germ-positive, aerobic, and found to colonize the root surface which increase the plant growth and cause the lysis of fungal mycelia. The capacity of *Bacilli* to produce spores which are extremely resistant to high temperatures, unfavorable pH, and lack of nutrients or water are a very good feature required for field application. The pseudomonads are common gram-negative, rods-shaped bacteria, has simple nutritional requirements and grows well in mineral salts media supplemented with any of a large number of carbon sources. Certain members of the *Pseudomonas fluorescens* have been shown to

be potential agents for the biocontrol which suppress plant diseases by protecting the seeds and roots from fungal infection. They are known to enhance plant growth promotion and reduce severity of many fungal diseases through the production of a number of secondary metabolites including antibiotics, siderophores and hydrogen cyanide. A number of antagonistic bacteria belongs to pseudomonads, possess antifungal and growth promoting activities, and widely associated with wheat rhizosphere have been found effective against various wheat diseases under *in vitro*, greenhouse and the field conditions. As several bacterial antagonists belonging to the genera *Bacillus* and *Pseudomonas*, have been found to inhibit mycelial growth of wheat pathogens, *Fusarium graminearum* (Fusarium head blight), *Neovossia indica* (Karnal bunt) and *Blumeria graminis* f.sp. *tritici* (Powdery mildew), while a few of them also inhibit growth of other fungal pathogens like *Puccinia striiformis* f.sp. *tritici* (stripe rust), *Puccinia recondite* f.sp. *tritici* (leaf rust) and *Septoria tritici* (Septoria leaf blotch). Laboratory studies also revealed that a large number of bacterial antagonists possess the ability to protect wheat plants from Karnal bunt, spot blotch, rusts, powdery mildew, take-all, Rhizoctonia root rot and Fusarium head blight. Biocontrol potential of some of the important fungal antagonists belongs to genera *Trichoderma*, *Chaetomium*, *Cladosporium*, *Talaromyces*, *Verticillium*, *Paecilomyces*, *Beauveria*, *Gliocladium*, *Trichothecium*, *Curvularia*, *Stachybotrys*, *Cylindrocarpon* and *Nigrospora* has been proved to efficiently manage several wheat diseases/pathogens.

LP (S4) 21

Management of *Sclerotium rolfsii* through *Trichoderma harzianum*

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The *Sclerotium rolfsii* Sacc., (teleomorph *Athelia rolfsii* (Curzi) Tu & Kimbrough) is soil borne pathogen causing root rot, stem rot, wilt and foot rot in more than 500 plant species. It is very difficult to manage this pathogen because of its diverse nature of survival, large number of sclerotia produced and their ability to persist in the soil for several years. The loss of yield caused by the pathogen is 25 per cent, but sometimes it reaches 80 - 90 per cent. The management of disease through chemicals is not effective and economically viable. Hence, management of disease through biocontrol agent is better approach to reduce the loss incurred by pathogen. The biocontrol agent *Trichoderma harzianum* found potential antagonistic against *S. rolfsii* through synthesis of antibiotic and enzymes like glucanase, cellulase, chitinase, protease etc. The furrow application of *T. harzianum* grown in wheat husk, or mix with farm yard manure and castor cake found most effective for control of pathogen in various crops. The seed treatment of *T. harzianum* also found effective against *S. rolfsii* and to improve the yield.

LP (S4) 22

Bioagents in the management of diseases of spice crops

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India is the largest producer, consumer and exporter of spices in the world. Of 109, around 60 spices are blessed in India as listed by International Organization for Standardization (ISO). India is producing 7.08 Million MT of spices per year while exporting around 180 Spices /Spice based products to over 160 countries. Indian spices are enriched with a variety of species from seed spices to condiments, grown in varied agro-climatic zones. Among these, arid and semi-arid regions of Gujarat and Rajasthan is very conducive for wide range of seed spices, including cumin, coriander, fennel, fenugreek, ajwain, dill seed, etc. and contributes 70% of the total seed spices production of the country. Thus, it is popularly called as the 'Bowl of Seed Spices'. But, with changing climate and intensification of agricultural practices, constrains of production is increasing day by day. Especially biotic stress becomes the major concern. Growing problems of blight, powdery mildew and wilt of cumin, stem gall and powdery mildew of coriander, blight and stem/root rot of fennel and powdery mildew of fenugreek, are evidence of this. Apart from these fungal diseases, plant parasitic nematodes, and insect vector borne phytoplasma are the major drawbacks. To protect these high value crops, farmers make excessive use of pesticides which increases the residual toxicity. In this WTO era, pesticide residue hazards in major seed spices become the major concern due to rejection of consignment for international export purpose. To reduce pesticide residue load, adoption of alternative management strategy which are ecofriendly as well as effective, should be prioritized.

So far, 'biological control' is found to be very promising option for pest management in various crops. It is free from any residual toxicity, and also very encouraging to reduce pesticide hazards, environmental pollution. Moreover, bioagents are effective against broad range of plant pathogens with multiple antagonistic functions, hence adopt in various crops along with many spices. Now days, antagonistic *Trichoderma* spp. is widely applied against soil-borne pathogens of cardamom, cumin, ginger, pepper, turmeric, etc. and *T. harzianum* is the promising solution for the management of foot rot in black pepper. It is also recommended as potential bioagents for managing capsule rot, rhizome and root rot diseases of other spices. *Trichoderma* spp. is also equally effective against wilt-root rot complex of cumin, fennel and stem gall of coriander. Besides *Trichoderma*, other fungal bioagents like *Gliocladium virens*, *Pochonia chlamydosporia* are also popularized for bio-management of diseases in spice crops. Other than fungal bioagents, some bacterial antagonists, viz., *Pseudomonas fluorescens*, *P. putida*, *Bacillus subtilis* are providing substantial management of foliar disease like blight of cumin, fennel, etc. The rhizospheric isolates of these bioagents also promote the plant growth. Hence, their application in combination with fungal bioagents forms microbial consortia for the improvement of biocontrol efficacy. Better rhizospheric competence, good antagonistic potential and wider ability for producing antibiotics, lytic enzymes and

toxins makes them a great promise for rhizospheric engineering of various spices crop. Although researches have been conducted on various aspects of biological control of phytopathogens in spices crops, but opportunities still exist for exploration of bioagents for managing insect vector borne plant viruses and phytoplasma of spices. Potentiality of such bioagents should be exploited in commercial scale. We need to look still forward to carry out new researches to facilitate new biocontrol technologies and delivery system for improving the efficacy of biocontrol agents and sustainable management of plant diseases in spices.

LP (S4) 23

The diversity of *Ralstonia solanacearum* causing bacterial wilt of brinjal in Karnataka and its management by biological ways

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The current scenario with ominous disease, bacterial wilt of brinjal caused by *Ralstonia solanacearum* demands high cost-antibiotics and other synthetics having lethal repercussions on symbiotic microbial population and human health. Hence, the survey for the incidence and prevalence of bacterial wilt of brinjal has been carried out from different agro climatic zones of Karnataka. Totally forty isolates of *R. solanacearum* collected from all the zones and finally selected nine isolates *i.e.*, one each from the zone. The nine isolates were identified as *R. solanacearum* on the basis of colony morphology and confirmed by standard physiological and biochemical tests. Minimizing the global dependence on hazardous agricultural plant protectant chemicals threatening the agro- ecosystems is a very challenging for plant pathologists. Further, using virulent strain collected from the diversified areas of Karnataka has carried out screening of advanced breeding lines of brinjal to bacterial wilt disease. Among all the lines screened against bacterial wilt of brinjal, the advanced line COHBB 16-1-12 and COHBB10-1-25 has showed resistant reaction to bacterial wilt. Among the individual application of bio agents *Pseudomonas fluorescens* showed lowest wilt incidence (26.66%), compared to control (88.00%) and suppressed the rhizosphere soil population (0.66×10^6) compared to initial rhizosphere soil population at 30 days (103.33×10^6).

LP (S4) 24

***Trichoderma* mediated abiotic stress management - A novel approach to sustain crop productivity**

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Plant-microbe interactions are inevitable and role of beneficial microorganisms in crop health management is well established. Fungal bioinoculants for plant disease management has been well known and many commercial formulations are in use for management of plant pathogens especially soil-borne. In recent decades, with the advent of modern research tools, it has been possible to understand the plant-microbe interactions up to a minute detail and thereby overcoming the uncertainties for commercialization of bioinoculant technology for crop productivity enhancement. The recent literature suggests that fungi have been shown to play a role in management of abiotic stresses and also nutrient mobilization. Isolates of *Trichoderma* have been shown to promote plant growth, mobilize nutrients and impart tolerance towards abiotic stresses. The genetic and molecular basis of imparting these resistances has also been unraveled. Different mechanisms understood in various plant-*Trichoderma* interaction systems call for an integrated approach through consolidation and systems biology approach as plant metabolic responses are interlinked. Further, in many abiotic stresses, the resistance mechanisms are quantitative in nature and hence the overall effect in terms of plant's response is of utmost significance to draw meaningful conclusions. In this review, the current status of role of fungi in abiotic stress management in crops and future prospects is discussed

LP (S4) 25

Biological control of powdery mildew disease in grapes

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Grape is one of the popular horticultural crops of India. It is grown on an area of 1,36 thousand ha most of which lies in the state of Maharashtra. The annual production is about 2683 thousand MT, out of which about 232 thousand MT is exported to EU and rest is used for domestic consumption and preparing raisins. Wine grape cultivation is restricted to a small area. The commercial varieties belong to *Vitis vinifera* and are highly susceptible to powdery mildew caused by *Erysiphe necator*. The disease occurs throughout the year, except during the hot and dry summer months, on young, green vine parts affecting vine growth, yield and quality of fruits. Disease management is quite challenging and largely dependent on chemical

fungicides which sometimes results in detection of fungicide residues above the permissible maximum residue limit (MRL) at harvest time, compromising food safety. Furthermore, the selection pressure exerted by the continued applications of single site mode of action fungicides has led to the development of fungicide resistant pathogen populations in commercial vineyards. The stakeholders are looking for alternate safer options which can help to reduce the total number of pesticide applications.

This Center has attempted control of powdery mildew by using antagonistic microorganisms like *Trichoderma* and *Bacillus* spp. Thirty four isolates of *Trichoderma* were screened and a *T. afroharzianum* isolate was selected, which could provide about fifty percent reduction in disease severity. *Trichoderma* hyphae typically grew towards and coiled around *E. necator* conidiophores and caused distortion of conidial structure. Similar level of control could also be obtained by using a *Bacillus licheniformis* TL-171 or a combination of two *Bacillus* spp. Replacing two late season sulphur applications with those of *T. afroharzianum* or TL-171 in a fungicide spray schedule, enhanced powdery mildew control by thirty to thirty-five percent as compared to the only fungicide schedule. The identity of these microorganisms was determined by multigene analysis. Application of these microorganisms along with fungicides can help in getting desired level of disease while reducing total number of fungicide applications.

Microorganisms have a complex bio-control mechanism which will not be affected by the mutation in the fungicide resistant isolates and they are expected to be equally antagonistic to the fungicide resistant isolates as they are to the fungicide sensitive isolates. Use of biocontrol agents in vineyards with undesirable population of fungicide resistant strains can help in curtailing the fungicide resistance problem.

Attempts were also made to induce systemic resistance in grapevines against powdery mildew disease. In a vineyard maintained with four sulphur and one potassium spray for control of powdery mildew during the season, soil application of a mixture of *T. asperelloides* strains 5R and NAIMCC-F-01812 or a mixture of *Bacillus* strains TP-232 + TL-171 enhanced disease as compared to the vines which were not given microbial treatments. The activities of chitinase, β -1, 3-glucanase, peroxidase, polyphenol oxidase enzymes and total phenols were higher in leaves treated with *Trichoderma* or *Bacillus* and may have been responsible for imparting disease resistance in treated vines.

LP (S4) 26

Role of Bacterial Antagonists in Management of Plant Diseases

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In the recent years, scientists have diverted their attention towards exploring the potential of beneficial microbes for plant protection measures. Biocontrol agents are easy to deliver, improve plant growth, activate resistance mechanisms in the host, increase biomass production and yield. Among them bacterial antagonists act through antibiosis, secretion of volatile toxic metabolites, lytic enzymes, parasitism and through competition for space as well as nutrients. Though biocontrol with PGPR is an acceptable green approach, the proportion and registration of bacterial biocontrol agents for commercial availability is very low. Bacterial biocontrol agents are known to effective for management of bacterial, fungal and nematode diseases. Development of bacterial formulations with increased shelf life and broad spectrum action with consistent performance under field conditions could pave the way for acceptance of the technology at a faster rate.

Oral Papers

OP (S4) 08

Assessment of synergism in consortial application of biocontrol agents for management of crop disease

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Biocontrol agents applied in combinations to exploit potential synergistic effect among them. Individual BCA is not likely to perform consistently against all pathogens of a particular crop. Combining two BCAs in a single bioformulation or their simultaneous dual application may overcome this inconsistent performance. We investigated management of rice blast, caused by *Magnaporthe oryzae* and bacterial leaf blight (BLB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) with the combined application of *T. harzianum* Th3 and *P. fluorescens* RRb-11 and assessed the synergistic effect of the two BCAs using numerical hypothesis of 'Bliss Independence'. Combined application of *T. harzianum* Th3 and *P. fluorescens* RRb11 allowed to reduce the disease severity by 69.5% in compared to untreated control plants. And it showed a synergy factor value of 1.29. The mixture of biocontrol agents was less efficacious in reducing BLB (50-53%) than application of *P. fluorescens* RRb11 alone (57.9%). This combined application of *T. harzianum* Th3 and *P. fluorescens* RRb-11 was also able to enhance rice plant growth promoting parameters including plant height, root length, tiller number, number of grains per plant, and 1000 grain weight. These results showed overall increased efficacy and consistency of combined application of biocontrol agents against blast and BLB of rice. Combined application of *T. harzianum* Th3, *P. fluorescens* RRb-11 along with Carbendazim reduced disease severity over the control but showed antagonistic effect as observed efficiency of disease control is less than expected efficiency (SF=0.77) against rice blast. This may be due to moderately suppressive nature of Carbendazim against *Trichoderma*. Application of *P. fluorescens* RRb11 formulation alone against BLB of rice pathogen, gave significant control of the disease but the combination of *P. fluorescens* RRb 11 with *T. harzianum* Th3 and/or Carbendazim which is expected to perform better, falls short to treatment with *P. fluorescens* RRb11 alone. Nevertheless, combined use of two BCAs resulted in a slightly longer delay in epidemic development than did individual use of BCAs. However, for high BCA activities, combined use with at least one competitive BCA resulted in better control than combined use of two mycoparasitic BCAs. The results suggested that combined use of mycoparasitic BCA with competitive one is unlikely to result in synergy against some diseases.

OP (S4) 09

Integration of species of *Trichoderma* and fungicides for management of root rot of chilli caused by *Macrophomina phaseolina*

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Chilli (*Capsicum annum* L.) is an important spice crop having commercial and therapeutic value. *Macrophomina* root rot is an important disease which attacks at any stage of the growth of chilli. In nursery, it kills the seedlings by causing the rot of primary and secondary roots. The disease occurs in severe form during moisture stress conditions in the field along with high temperature (>35°C). *Trichoderma* is an exceptionally good model of biocontrol agent as it is ubiquitous, easy to isolate and culture, multiplies rapidly on many substrates, acts as a mycoparasite, competes well for food, site and oxygen, produces antibiotics like trichodermin, viridin and has the enzymes chitinases, chitosanases, cellulase, pectinase, cutinase and endonuclease which are *modus apreudi* to act as mycoparasite (Mukherji *et al.*, 1997). Present investigation on integration of *Trichoderma* species and fungicides for the management of root rot of chilli under pot conditions was conducted at the Department of Plant Pathology, AAU, Anand during the year 2008-09. On the basis of the earlier study conducted the fungicides which were found toxic against *M. phaseolina* and safer to *Trichoderma* spp were included for further investigation to find out their efficacy in controlling root rot of chilli *in vivo*.

OP (S4) 10

Biological control of charcoal rot of cluster bean

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Cluster bean (*Cyamopsis tetragonoloba* syn. *C. proraliodes*) commonly known as "Guar" is one of the most important commercial crops of arid and semi-arid regions of India. The area of crop is day by day increasing due to decrease in underground water table and uneven rainfall in different districts of Rajasthan. In spite of this the farmers are preferring its cultivation as a substitute of groundnut because groundnut requires more water, care and investment for its cultivation. Bacterial blight, charcoal rot, root rot, aphid, jassid and white fly are the major biotic stresses affecting the productivity of cluster bean. Among them charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid causes considerable losses in the productivity of cluster bean. Biological control works through several processes; therefore, it is more

stable than specific chemical control especially against soil borne diseases. The study was carried out for the biological control of charcoal rot of cluster bean through soil and seed treatment on farmers field in the adopted village Ghinoi of district Jaipur (Rajasthan) during *Kharif* 2015 and 2016. A total number of seven treatments including untreated control were taken. The results indicated that the minimum seedling mortality (4.96%) and maximum seed yield (13.85 q/ha) were recorded in the plot where soil and seed treated with *T. viride* at 2.5 kg/ha and 8.0 g/kg seed, respectively. The next best performance was recorded in the plot where soil and seed were treated with *Trichoderma harzianum* at 2.5 kg/ha and 8.0 g/kg seed, respectively, where the seedling mortality was 5.84 per cent and seed yield was 12.46 q/ha followed by soil treatment alone with *T. viride*, soil treatment alone with *T. harzianum*, seed treatment alone with *T. viride* and seed treatment alone with *T. harzianum*. Where as in untreated control the seedling mortality was 22.70 per cent and seed yield was 10.28 q/ha. It was also recorded during the study that soil treatment alone has given better performance than seed treatment alone in minimizing the charcoal rot incidence and maximizing the seed yield.

OP (S4) 11

Development of bio-control measures for the management of saprophytic stage and postharvest of apple scab pathogen *Venturia inaequalis* in Uttarakhand Himalayas

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Surveys for prevalence and severity of apple scab disease conducted in Kumaon and Garhwal Himalayas revealed severe disease incidence in the Harsil, Koti, Tuni, Joshimath and Gwaldam fruit belts. The incidence of scab ranged between 05 to 85 per cent on foliage and 5 to 62 per cent on fruits. Twenty five isolates of *Venturia inaequalis* were collected from different places of Uttarakhand hills. The decomposition rate for samples collected up to 15 November indicated 96% intact leaves compared with are 100% in untreated samples after month overwintering period, whereas on 25 February it was recorded that treated leaves had 7% complete decomposition, 31% were left with midrib portion, 40% partial and 22% intact leaves as compared to 97% intact leaves in checks. The pseudothecia continued to develop ascus from the end of February until April, and ascospores discharged between May to June in Harsil, Purola-Naugao, Koti-Kanasar, Gwaldam and Joshimath fruit belt. In other districts of Uttarakhand i.e. Tehri, Dehradun, Pauri, Nainital and Pithoragarh, pseudothecia were always found to have developed up to the late filamentous stage only during January to early March and did not develop any further. In fact, at most of the sites, there was no development beyond late filamentous stage until last week of March.

One hundred seventeen fungal/ bacterial organisms were isolated from apple phylloplane/ leaf litter and most of them colonized and antagonized *V. inaequalis* on naturally infected leaves. Nineteen isolates could inhibit radial growth of *V. inaequalis* on PDA. Distinct inhibition of *V. inaequalis* (> 80%) occurred by seven isolates. The conidial germination was significantly reduced by 50 % by the six antagonistic isolates (*Trichoderma* sp., *Epicoccum nigrum*, *Chaetomium* sp., *Myrothecium* sp. *Fusarium* sp. *Botrytis cinerea*) as compared to the control set. Profuse and rapid growth was observed in a new "modified potato dextrose agar amended with 20 g green apple leaves, and 20 µg/ ml of streptomycin sulfate medium" (MPDA). Two bacterial isolates completely inhibited germination of *V. inaequalis* conidia *in vitro*. The combined effect of urea and isolated fungi under study did not make any significant difference on the productivity of asci. Urea did not stimulate antagonists to colonize the leaf disk, and the antagonists and urea generally acted independently to inhibit pseudothecial development. The isolated fungi alone showed the significant effect over untreated check on the productivity of asci and ascospores.

A total of nine bacterial strains were isolated from the rhizosphere soil along with scab infected overwintered fallen leaves as well as pseudothecial suspensions made out of fallen leaves of apple. All the bacterial isolates (*Pseudomonas* spp. and Gram negative nonfluorescent bacteria) inhibited germ tube growth *in vitro*. Of these two, UHCH 53, UHCK 79, were most effective and inhibited complete germination of conidia. Isolate UHCH 53 was frequently obtained each year from organic orchard of Harsil. In repeated experiments only eighteen isolates suppressed scab by at least 46 percent of the disease that developed on nontreated seedlings. Of these, only three isolates that completely inhibited conidial germination and scab formation on leaf surface were, UHCR16, UHCR 22, and UHCR 47. These isolates provided maximum scab suppression in experiments and the inhibition was comparable to that provided by the fungicide, Flusilazole.

Three important commercial cultivars of apple namely, 'Red Delicious', 'Royal Delicious', and 'Golden Delicious' were screened against post-harvest rot causing fungi. Fruits of the cv Red Delicious developed more scab lesions than those of 'Royal Delicious' and 'Golden Delicious'. Scab lesions gradually increased in all the fruits of Delicious cultivars stored at ambient temperature. The rate of lesion expansion was inversely related to the number of lesions on the fruit. Scabbed fruits showed pronounced shriveling as compared to healthy fruits. Other fungal pathogens associated with scab lesion were identified as *Trichothecium roseum*, *Penicillium expansum*, *Glomerella cingulata*, *Botrytis cinerea* and *Monilinia* spp. which were responsible for fruit decay in storage. 'Red Delicious' was highly susceptible to most of the post-harvest pathogens. Preharvest sprays of flusilazole (0.015%) bitertanol (0.075%) and carbendazim (0.05 %) were effective in controlling storage scab and other rotting fungi. Biocontrol agents effective against *P. expansum* and *B. cinerea* were isolated from apple leaves, and a quantitative relation between antagonists and pathogen inoculum concentration was determined. Bitertanol (0.075%) and Penconazole (0.05%) were highly effective as pre-harvest sprays for inhibiting scab lesions after 30, 45 and 60 days of storage at room temperature. In protective spray programme, propineb (0.4%) was highly effective for storage rot even after 60 days. Thiophanate methyl (0.10%) was effective against development of storage scab and fruit rot. Aonla leaf extract, garlic extract, bitertanol and carbendazim completely inhibited the storage scab and fruit rot up to 60 days during post-harvest dip treatment. It could be suggested that antagonist, plant product and fungicides could make them an excellent treatment for controlling postharvest losses of apple fruits and increase the shelf-life of apple.

OP (S4) 12

**Strategies for management of dry rot of potato caused by
Fusarium solani (Mart.) Sacc.**

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Potato is fourth most important food crop in India after rice, wheat and maize. The annual production of potato in India for the year 2013-14 was 41,555 MT (Anon., 2014). India contributes about 15 per cent of total potato production of the world. India is the second largest producer of potato (45 million MT) in the world after China (85 million MT). *Fusarium* dry rot of potato (*Solanum tuberosum* L.) is devastating postharvest disease worldwide and is incited by several species of *Fusarium* (Boyd, 1972). Bhardwaj (2012) reported 25 to 60 per cent yield loss in India due to dry rot (*Fusarium solani* (Mart.) Sacc.). In the present investigation nine fungicides, eleven phytoextracts (10%) and six antagonists were screened to know their bio-efficacy in controlling the *Fusarium* dry rot of potato *in vitro* and *in vivo*. Among the fungicides screened *in vitro*, carbendazim (100 ppm) and carbendazim (12 %) + mancozeb (63 %) at both the concentrations (500 & 1000 ppm) completely inhibited the mycelial growth of *F. solani*. The result of *in vivo* study revealed that the dry rot severity was not observed in tubers treated with carbendazim (50 & 100 ppm) on 4th and 8th day in pre- and post- inoculation treatments. Red Kaner found significantly superior in inhibiting the mycelial growth (69.17%) of *F. solani*. Significantly, lowest *Fusarium* dry rot severity was noticed in tubers treated with Red Kaner (0.20 & 0.60%). *Trichoderma asperellum* found most efficient antagonist in inhibiting the mycelial growth of *F. solani* on 4th (89.95%) and 8th day (93.99%) in *in vitro* and also found most effective in reducing the *Fusarium* dry rot severity both in pre- (0.70 & 3.40%) and post-inoculation (0.73 & 5.60%) treatments.

OP (S4) 13

Activity of indigenous *Streptomyces* against fungal phytopathogens of *Cajanus cajan*

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Actinomycetes, the ubiquitously present filamentous bacteria, are extensively studied for antibiotics and related secondary metabolites. This group also plays a vital role in soil nutrient cycling and biocontrol activities. Pigeon pea [*Cajanus cajan* (L.) is a very important grain legume crop. However, its production is

affected by *Fusarium* wilt (*Fusarium udum* Butler), which causes up to 100% yield loss. The deployment of resistant varieties would be an economical way to manage the disease, and for this, more information is needed on farmers' preferences for local land races, how farmers and consumers can be involved in developing new varieties resistant to wilt disease, and the genetics of inheritance of resistance. This information would be used to devise a breeding strategy. Both primary and secondary screening methods were used to screen actinomycetes for antifungal activity. The best strain were found to be *Streptomyces* sp as they showed broad spectrum activity with big zone of inhibition, the strain *Streptomyces* sp showed increased antifungal activity against all the tested phytopathogenic fungus. Among best potent isolates S-180, S-107, S-280, S-4, S-221, S-18 gave the antifungal activity against *Fusarium* sp. These gave distinct antagonistic reaction on *F. udum* and *F. oxysporum*. Laboratory and greenhouse studies were performed to develop a new *Fusarium* wilt screening technique. In the presence study S-180 and S-280 were more effective for protecting Pigeon pea plants from damaged induced by *F. udum*. Incidence of disease remained low due to a shift in the biological equilibrium in favour of the antagonists in the soil. Thus the antagonist proved effective in reducing the population of pathogen in soil and ultimately minimizing the disease. Future studies may include scaling up of these isolates as potential bio-inoculants and bio-control agents in field study.

OP (S4) 14

Beneficial microorganisms as mediators of crop protection and their mechanisms of action

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Soil is a huge store house of microorganisms where continuous interactions occur among them as well as with the plants. The majority of such soil microorganisms are unculturable and the ones which have been isolated, identified and characterized are the tip of the iceberg. Hence, there is no doubt that microorganisms play a very important role in plants' health. In this work we report the isolation, identification and characterization of beneficial bacterial isolates from the rhizosphere of a large number of crops- plantation, cereals and legumes. All the isolated bacteria were tested both *in vitro* and *in vivo* for their ability to improve plant growth as well as reduce disease and analyses were conducted for determining the mechanisms of action of selected ones. The isolated bacteria were initially tested for their antifungal activities as also plant growth promoting traits such as production of siderophores, volatiles, HCN, IAA and phosphate solubilization. Based on these, the most promising ones were selected and identified as *Bacillus pumilus*, *B. altitudinus*, *B. amyloliquefaciens*, *B. megaterium*, *B. methylotrophicus*, *B. safensis*, *Ochrobactrum anthropi* and *O. pseudogrignonense*. When tested *in vivo* against crops such as *Glycine max*, *Vigna radiata*, *Cicer arietinum*, *Triticum aestivum*, *Sorghum bicolor*, *Oryza sativa* and *Camellia sinensis*, all these bacteria could promote growth of the crops and improve their overall health.

They were also tested for their efficiency in reducing diseases caused by pathogens such as *Sclerotium rolfsii*, *Thanetophorus cucumerensis*, *Phellinus noxius* and *Bipolaris sorokiniana* and proved to be highly efficient biocontrol agents. GC-MS analysis of culture filtrates of two of the bacteria – *B. safensis* and *O. pseudogregnonense* revealed the presence of a large number of compounds, of which, a few such as 1-Hexadecane, 1-Tetradecane, Pyrrol o[1,2a] pyrazine-1,4-dione, hexahydro-3(2-methylpro) have been reported to be antifungal. Besides directly affecting the pathogens by their antagonism, these could also induce systemic resistance in the plants as evidenced by enhanced activities of defense enzymes such as chitinase, glucanase, peroxidase and phenyl alanine ammonia lyase as well as increased accumulation of phenolics. This was further confirmed by Real-Time PCR where expression of genes in the defense pathway increased several fold in treated samples in comparison to untreated ones. HPLC analyses of treated and untreated samples revealed higher accumulation of different phenols in treated samples. Reduction of pathogen population in soil was also detected immunologically by ELISA and FITC using antibodies of the organisms. It was further observed that better results could be obtained by preparing consortia of 2 or more bacteria, as well as consortia with PGPR, PGPF and AM fungi. From the accrued results of all accumulated data it can be pointed that biopriming with beneficial microorganisms not only improve the growth of plants but also act as biocontrol agents and improve the crop health. Their mechanisms of action are multifold- directly attacking the pathogens and reducing their population as well as enhancing the defense mechanisms through ISR in the host which enable the plants to defend themselves.

OP (S4) 15

Biological control of nematode diseases of vegetables

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Vegetable crops are highly susceptible to several pests and diseases, among which the root-knot nematodes, *M. incognita* and *M. javanica* infest almost all vegetables and are prevalent throughout the country and in recent years have become serious pests in polyhouses. In India, the per cent estimated yield loss in brinjal, cucurbits, okra, and tomato due to *Meloidogyne* spp., is to the extent of 16.67, 18.20, 14.10, and 27.21, respectively, as reported by AICRP. The root-knot species are difficult to manage because of their wide host range, short life cycle and high reproductive rates. The application of nematicides like carbofuran may reduce the nematode densities, but on repeated use, fail to manage the pest due to build-up of pesticide degrading microbes in the soil. The root-knot resistant cultivars are limited and not easily available. The cultural practices like crop rotation are effective but not always feasible. In such a scenario, biological control of plant-parasitic nematodes is receiving increasing attention. Some of the bioagents like *Pseudomonas fluorescens*, *Trichoderma harzianum* and *Pochonia chlamydosporium* effectively reduce the nematode densities, are safe to the user, are not detrimental to the beneficial flora and fauna in the soil, and thus, maintain soil biodiversity and health. The hyphomycete *Purpureocillium lilacinum* which was a very

popular bionematicide is now no longer listed in the list of Pesticides Registered under section 9(3) of the Central Insecticides Board as on 30/06/2017. Data on product characterization, efficacy, safety, toxicology, and labelling is required for registration, and quality standards with reference to content, virulence of the organism in terms of LC50, moisture content, shelf life, and secondary non-pathogenic microbial load should be met, as per described protocols.

The factors that govern the efficacy of a bionematicide are the nematode species, stage, its rate of development and density in the soil, besides the host crop, its cultivar, abiotic factors and cultivation practices.

OP (S4) 16

Microbial consortium for plant growth promotion and disease management

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Microbes associated with plant's root are enormously diverse. The complex microbial communities associated with plant species is cited as the second genome of the plant which is considered to be highly important for the plant's health and development. The current trend is to mix biocontrol agents (BCAs) of diverse microbial species having plant growth-promoting activities to achieve desired agricultural outcomes. A microbial consortium is two or more microbial groups living symbiotically. Microbial consortia have various advantages over single species, or "superbugs", such as efficiency, robustness, and modularity. In biocontrol of plant diseases, the deployment of a single antagonist often results in unsuccessful field performance, mainly due to its poor stability at prevailing environments in different agricultural ecosystems. The stability and efficacy of biocontrol can therefore be improved by deploying bioagents selected for compatibility and exerting multiple modes of action. combinations of compatible bioagents may involve antagonistic bacteria or fungi or bacteria and fungi. Among Rhizobacteria, fluorescent pseudomonads and gram positive endospore forming *Bacillus* spp. are potential candidates by virtue of their abilities to promote crop growth and pathogen suppression. Likewise, the free living saprophytic *Trichoderma* sp. found in soil and root ecosystems form an inevitable group of fungal antagonists against several plant pathogens besides promoting root growth and shoot development. Microorganisms under natural habitats live in communities and some provide benefits to plants. It is unveiled that microbes in small consortia enhance defense signaling cascades leading to enhanced transcriptional activation of several metabolic pathways. In this optic, controlled root exudation or nutritional amendment could lead to more successful disease management. Bacteria with more than one beneficial effect are of great interest in biocontrol. By combining strains with different disease-suppressive mechanisms, the impact of field fluctuating biotic and abiotic conditions could be minimized, as some biocontrol mechanism could be effective even if others are unfunctional. In addition, such combinations could be effective against multiple phytopathogens. Application of microbial consortium consisting of efficient strains for biological control may be a superior technique compared

to application of individual microbes for managing plant diseases. Moreover, application of microbes in a consortium may improve efficacy, reliability and consistency of the microbes under diverse soil and environmental conditions. Therefore, applying microbes as a consortium has great potentiality particularly in modern agriculture where minimization of chemical fertilizers and pesticides is one of the priorities.

OP (S4) 17

Bioagents in management of seed borne diseases of cereals – an overview

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Seed is the basic input for crop production. About 90% of the world's food crops are grown using seeds. The major world food cereal crops are maize, millet, rice, sorghum, wheat and barley. These crops are attacked by a number of pathogens, a majority of which are seed borne in nature. Seeds are the most important means for perpetuation of plant pathogens. Many seed borne pathogens become active when seeds are sown, which may result in seed decay and/or pre- or post-emergence damping-off. This in turn results in a poor plant stand in field. Transmission of a pathogen through seeds is considered more important than other survival means. Pathogens remain viable in seeds than in vegetative plant parts or soil. The host-parasite relationship within seeds also favors the earliest possible infection in the field. Since pathogens are in direct contact with the seeds, the chances of seedling infection are enhanced; it also results in discoloration and shriveling, biochemical changes in seeds, such as color, odor, oil content, protein content and alteration in physical properties of seeds, such as shape, size, surface area, volume and weight. Seed treatment is a biological, chemical, mechanical or physical process designed to mitigate externally or internally seed- or soil borne microorganisms, resulting in the emergence of a healthy seedling and, subsequently, a healthy plant. Biological control leads to reduction in inoculum load or disease-producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host or antagonist or by mass introduction of one or more antagonists. *Trichoderma viride* was the first fungus demonstrated as an antagonist for control of soil borne pathogens, such as *Rhizoctonia solani*, Fungal Antagonists applied commonly to seeds are *Bacillus subtilis*, *Chaetomium globosum*, *Penicillium oxalicum* and *Trichoderma* spp. *Clonostachys rosea*, *Gliocladium* spp. are also used now a days. For bacterial pathogens bacterial bioagents like, *Serratia odorifera*, *Pseudomonas fluorescens*, *Propionibacterium freudenreichii*, *Shermanii* spp., *Lactobacillus rhamnosu* bacteriophages, strains of *Bacillus amyloliquefaciens*, *Pseudomonas brassicacearum*, *Pseudomonas chlororaphis*, *Pseudomonas aureofaciens* and *B. cereus* are being used now a days. Their application to seeds reduces seed borne fungi, bacteria and results in vigorous growth of seedlings.

OP (S4) 18

Evaluation of arbuscular mycorrhizal association in *Capsicum chinense* Jacq. and biofertilizer potential on its growth and yield enhancement in nursery conditions of Manipur

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Diversity and colonization patterns of arbuscular mycorrhizal fungi (AMF) were investigated in the rhizosphere soil and roots of Naga King chilli (*Capsicum chinense* Jacq.) at three different growth periods (pre-flowering -D1, flowering -D2, and fruit ripening -D3) under shifting cultivation system. Paris- type of AM morphology was observed in *C. chinense* with intracellular hyphal coils, arbusculate coils and vesicles. Root colonization by AM fungi and spore density varied significantly with different growth stages of chilli plants. The extent of AMF colonization was highest during flowering (D2) period. In contrast, spore density of AMF was higher at pre-flowering (D1) stage. A total of 11 AMF spore morphotypes belonging to 5 genera, i.e. *Funneliformis*, *Glomus*, *Rhizophagus*, *Sclerocystis* and *Scutellospora*, were isolated from the natural field soil and trap cultures of *C. chinense*. Further, we evaluated the inoculation effect of a dominant AM fungi i.e. *Funneliformis geosporum* alone and in combination with *Pseudomonas fluorescens* (PSB) and *Azotobacter chroococcum* (PGPR) on growth and yield performances of *C. chinense* in nursery conditions. Among different bioinoculant treatments, *F. geosporum* + PSB promoted maximum plant growth, flowering, and fruit production of chilli and tissue nutrient contents were significantly higher compared to control plants. The root colonization was significantly higher in biofertilizer-inoculated plants compared to control ones. Our finding suggests that the application of indigenous and host-specific AM fungi along with efficient biofertilizers during seedling transplantation can significantly increase the overall growth and yield of *C. chinense* and they may be considered as a sustainable substitute for P fertilizer.

OP (S4) 19

Prospects of biological control in the sustainable management of newly emerging pomegranate wilt caused by *Ceratocystis fimbriata*

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The world is entering a population cycle that will challenge agricultural production technology like never before. The programme of doubling the food production indeed a great challenge for the next generation of agricultural scientists. Indiscriminate use of chemicals for disease management has led to substantial pollution and harmful effects on human beings. The exploitation of beneficial microbes as biocontrol agents has become paramount importance in agricultural sector for their potential role in food safety and sustainable crop production. The ecologically safe approaches inspire a wide range of application of bio-agents which led to improved nutrient uptake, growth promotion, disease resistance and control against biotic stresses. In the recent years, there has been a increased interest in the search of antagonistic microbes for sustainable crop production. Pomegranate is an economically important fruit crop of India and it is regarded vital cash cop in many states. Wilt caused by *Ceratocystis fimbriata* is becoming a major threat leading to destruction of several pomegranate orchards by farmers. Wilt is prevalent in Maharastra (49.20%), Karnataka (61.11%) and Andhra Pradesh (8.69%). The pathogen produces septate and hyaline to dark greenish brown mycelium with asexual structures as hyaline endocoidia and olive brown aleurioconidia. The sexual stage of pathogen is long beaked perithecium with globose base producing hat shaped ascospores. The *Ceratocystis fimbriata* is characterized using universal primers (ITS-1 and ITS-4) which successfully amplified the entire ITS region and produced an amplicon of size 600-650 bp. The NCBI BLAST analyses revealed its homology with various other ITS gene sequences with maximum similarity (96-99%) with *Ceratocystis fimbriata* on pomegranate. In the present studies, thirty five native isolates of fluorescent pseudomonads and *Trichoderma* were isolated from the healthy rhizosphere soil of wilt affected pomegranate orchards and screened for their antagonistic activity through dual culture technique against the test pathogen. Among them, eleven isolates of fluorescent pseudomonads (PFP1 to PFP11) and *Trichoderma* (PT1 to PT11) which were found promising were further tested for their biocontrol potential *in vitro*. All the eleven isolates of fluorescent pseudomonads and *Trichoderma* showed significant antagonistic activity against the pathogen. Further, all eleven fluorescent pseudomonads isolates also showed positive response for siderophore production and plant growth promoting activities such as HCN and IAA production. Similarly, all eleven isolates of *Trichoderma* were positive for volatile compound production indicating their biocontrol activity. Two isolates of fluorescent pseudomonads namely PFP-11 and PFP-10 showed remarkable antifungal activity showing highest inhibition of 72.04% and 71.20%, respectively and they are identified as strong producers of hydrogen cyanide, indole acitic acid, siderophore and enzymes responsible for induced systemic resistance. The *Trichoderma* isolates (PT-11 and PT-6) also showed antagonistic activity with inhibition of 73.10% and 72.03%, respectively. The pot culture experiment also indicated that PFP-11 and PT-6 were also found promising not only for disease control but also growth promotion activities. Overall, our results indicated that there is lot of scope for exploitation of bio-agents in the sustainable management of newly emerging pomegranate wilt.

OP (S4) 20

Efficacy of bioagents against *Macrophomina phaseolina* (Tassi) Goid. causing dry root rot of soybean [*Glycine Max* (L.) Merr.]

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The *Macrophomina phaseolina* (Tassi) Goid. is a pathogen with an exceptionally broad host range. It is soil-borne root and stem infecting organism that causes damage by plugging or rotting of vascular tissues in root and lower stems and stalks. Disease incidence is often greatest when maturing plants are stressed by drought and high temperature. The experiment was conducted in the laboratory of Department of Plant Pathology, N. M. College of Agriculture, NAU, Navsari, during 2015 – 2016 to efficacy of bioagents against *M. phaseolina* (Tassi) Goid.) causing dry root rot of soybean. Seven antagonists viz. *T. viride*, *T. harzianum*, *Pseudomonas fluorescens*, *T. fasciculatum*, *T. longibrachyatum*, *T. koningii* and *Bacillus subtilis* were tested *in vitro* for their efficacy to *M. phaseolina* by dual culture method. Among them, *T. harzianum*, *Pseudomonas fluorescens* and *T. fasciculatum* with 68.39, 65.52 and 63.79 per cent growth inhibition, respectively, appeared as strong and potent antagonists against *M. phaseolina*. Next best in order were *T. viride*, *T. longibrachyatum*, *T. koningii* and *Bacillus subtilis*.

OP (S4) 21

Efficacy of biocontrol agents against *Didymella bryoniae* under field conditions

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Gummy stem blight of bottle gourd is an economic important disease and causing severe loss in quality and quantity yield of bottle gourd. Hence testing of six different bio-agents were evaluated for its field efficacy against gummy stem blight of bottle gourd caused by *Didymella bryoniae* during *Kharif* 2016 and *Rabi* 2016-17 at college farm, Navsari Agricultural University, Navsari. Three sprays of these bioagents were applied at the time of disease initiation and successive sprays were given at 14 days interval. All the tested bioagents have provided protection against gummy stem blight during *Kharif* 2016 and *Rabi* 2016-17 season. Among various bioagents both the species of *Trichoderma* viz. *Trichoderma viride* and *Trichoderma harzianum* provided better protection with minimum disease intensity of gummy stem blight and maximum yield. The pooled over season data found similar trend of effectiveness of various bio-agents. All the plots treated with bioagents found significantly higher yield compared to untreated check. The highest yield was found in *Trichoderma viride* and *Trichoderma harzianum* due to better efficacy

against gummy stem blight resulted in higher yield compared to other bioagents. The highest yield 20.33 t/ha during *Kharif* 2016 and 20.67 t/ha during *Rabi* 2016-17 season was observed in *Trichoderma viride* followed by *Trichoderma harzianum* 20.0 t/ha and 20.33 t/ha during *Kharif* and *Rabi* season, respectively and they were at par with each other but superior to rest of the bioagents treatments. The maximum fruit yield was recorded during *Kharif* 2016 was found in *Trichoderma viride* (20.33 t/ha) significantly followed by *Trichoderma harzianum* (20.00 t/ha), next in order was *Bacillus subtilis* (17.67 t/ha) and *Pseudomonas fluorescens* (15.67 t/ha). Same trends of bioagents were found during *Rabi* 2016-17. ICBR data suggested highest ICBR of 1:38.81 in the treatment of *Trichoderma viride* followed by *Trichoderma harzianum* (ICBR 1:36.57) and *Bacillus subtilis* (ICBR 1:20.32).

OP (S4) 22

Role of Bacterial endophytes in plant growth and disease management

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Plant growth-promoting bacterial endophytes colonize the internal tissues of plants. Some endophytes promote plant growth and also work as biocontrol agent. Mechanisms employed by bacterial endophytes for plant growth promotion are similar to the mechanisms of rhizospheric bacteria. The comparisons of genomic study between bacterial endophytes and rhizospheric plant growth-promoting bacteria revealed certain potential genetic factors involved to establish endophytic lifestyle facilitate a better knowledge about of the functioning of bacterial endophytes. Endophytic microorganisms are found in virtually every plant on earth. Endophytic bacteria exist within the living tissues of most plant species and perform a variety of relationships, from symbiotic to slightly pathogenic. Root endophytic bacteria are seen as promising alternatives to replace chemical pesticides and fertilizers in sustainable and organic agriculture. The ability to colonize inside the host tissues by bacterial endophytes made them important for agriculture as a new tool to improve crop productivity. This association may be mutualistic and endophytes provide the plants resistance against diseases. Bacterial endophytes usually have associated with novel secondary natural products and processes. In this chapter, we discussed the major areas about the control practices of plant diseases and plant-growth promotion by entophytes.

OP (S4) 23

Biological control of nematode diseases of medicinal and aromatic plants

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Plant parasitic nematodes (PPN) cause extensive yield losses of agricultural crops including medicinal and aromatic plants (MAPs) with a resultant worldwide cost of > \$125 billion per annum. MAPs are continued to be the source of important life saving herbal drugs even after significant progress in synthetic substitutes. However, the success of sustainable agricultural crop production of MAPs is severely hampered by the attack of various plant parasitic nematodes including root-knot nematode (*Meloidogyne* spp.) which causes severe economic losses to variety of MAPs. Plant growth promoting microorganisms (PGPMs) in sustainable agriculture has provided new insights to ecofriendly agro-economy and is being looked upon as a substitute strategy to lesser reliance on chemicals nematicides for the successful cultivation of MAPs. In recent years, the application of beneficial microbes have shown potential in enhancing production of plant secondary metabolites and significant nematode disease eradication through various mechanisms during interaction with plants *viz.*, induction of plant host-defense mechanisms, rhizosphere competence, and improvement of plant nutrition *via* antagonistic activity, ability to produce antibiotics, lytic enzymes and plant hormones production. In the present article, we have made an attempt to focus on the potential applications of beneficial rhizospheric microbes as "booster agents" to manage plant parasitic nematode in MAPs along with their application in enhancement of plant secondary metabolites production.

OP (S4) 24

Endophytic microorganisms: Prospects as biological control in family Brassicaceae

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Wide range of products (i.e vegetables, oil to fuel) from family Brassicaceae makes it worth considering for introducing various improvements. Members of Brassicaceae are known for different agronomically important traits including some imperative microbial population popularly known as endophyte as part of breeding programme. Presence of endophytes itself and/or their metabolites in Brassicaceae have been improved beneficial for plants in terms of promoting plant growth; increasing yield; reducing disease symptoms caused by plant pathogens; protection from insect pests; remediating soil; improving plant performance under extreme conditions of temperature and water availability; solubilizing

phosphate and provide assimilable nitrogen to their hosts. The endophytic fungi present in Brassicaceae might have some effects that are similar to mycorrhizal fungi in Brassicaceae members like *Arabidopsis* and *Microthlaspi*. The endophytic fungus *Acremonium alternatum* reported to suppress clubroot development in *Arabidopsis*. *Alyssum bertolonii* Desv. has been explored and isolated eighty-three endophytic bacteria from roots, stems, and leaves and classified into 23 different taxonomic groups by restriction analysis of 16S rDNA (ARDRA) and partial 16S rDNA sequencing. In this review, an overview of different genera of Brassicaceae is described carrying friendly microorganisms for better host plant growth.

OP (S4) 25

Biological control of postharvest diseases of citrus

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Postharvest disease causes severe losses to the citrus crop during its transportation and storage and thereby affects its trade. Although, there are abundant synthetic fungicidal formulations derived for successful control over postharvest rots and diseases, but the approach of using biocontrol agent(s) in its postharvest management is now getting very common nowadays. As the use of microbial antagonists is safe to health and environment, this leads to its popularity among the researchers, governmental organizations and farmers. Single and mixed cultures of antagonist microbe at both pre-harvest and postharvest stage has been found effective for major citrus postharvest disease *viz.* green and blue mould and sour rot etc., reducing postharvest losses. As microbial antagonist can be used in many ways such as solely, in combination, in addition with other treatments and with additives for successfully controlling citrus rots, which not only found effective but further reduces the risk of chemical residues. Commercial formulations such as Aspire, Biosave 10LP, 110, Boniprotect™, Candifruit™, Shemer™, Pantovital™ and Serenade™ have proven promising. Recent advances and findings in this field further open up gates for the successful exploration of this technology. By looking at the pace of the scientific work carried out in this aspect, it is expected that some more effective formulations will hit the market soon. This chapter presents an overview of control of the postharvest decay via use of biocontrol antagonist. Losses of citrus fruits can be controlled in an ecofriendly manner by using the emerging approach of biocontrol in postharvest chain of citrus fruit.

OP (S4) 26

Biological management of major citrus diseases in Central India - A Review

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Citrus occupies an important place in the horticultural wealth and economy of India as the third largest fruit industry after mango and banana. Citrus production in the country is 8.7 million tonnes at present with world ranking at sixth position after China, Brazil, USA, Spain and Mexico. Many fungal, bacterial and viral diseases that threaten citrus crops but some fungal disease caused by *Phytophthora* spp. and bacterial citrus canker caused by *Xanthomonas axonopodis* pv. *citri* are the most devastating in central India. The *Phytophthora* spp. includes *P. parasitica*, *P. citrophthora* and *P. palmivora* are responsible for root rot, gummosis and collar rot in citrus of central India. At least 3 distinct forms or types of citrus canker are recognized among these, Asiatic form (Canker A) is the most destructive and affects most of the major citrus cultivars in our region. Some potential biological agents investigated for control of these dangerous diseases. *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus subtilis* were found effective in management of these diseases.

OP (S4) 27

Consortium of plant growth promoting rhizobacteria for synergistic biocontrol mechanism

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Application of plant growth promoting rhizobacteria such as *Pseudomonas*, *Bacillus*, *Azospirillum*, *Agrobacterium*, *Azotobacter*, *Arthrobacter*, *Alcaligenes*, *Burkholderia*, *Beijerinckia*, *Clostridium*, *Serratia*, *Rhizobium*, *Enterobacter*, *Klebsiella*, *Vario-vovax*, *Xanthomonas* and *Phyllobacterium* in crops could have synergistic effect on growth promotion and biocontrol of plant pathogens. Microbial consortia exist at different levels of community and metabolic complexity. To assess consortial impact of interactions there

is need to statistical methodologies for investigating synergistic effects among PGPRs. Combined use of two PGPRs with different biocontrol mechanisms may be synergistic or antagonistic or additive. However, combined use of two PGPRs resulted in a slightly longer delay in epidemic development than did individual use of PGPRs. In this review we have presented the various mechanisms involved in the application of PGPRs and their consortial effect against crop diseases, nematode and effect on growth promotion. Later we discussed the numerical hypothesis of Bliss independence and Loewe's additivity to assess various statistical methodologies for investigating synergistic effect among microorganisms and to evaluate the currently available evidences for their presence. Research is needed to establish under what spatially heterogeneous conditions the combined use of biocontrol agents may lead to synergistic interactions and reduced crop losses.

OP (S4) 28

Phosphate solubilizing microorganisms and their effectiveness against phytonematodes

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Phosphate-solubilizing microorganisms (PSM) represent a potential group of beneficial microorganisms, which besides promoting plant growth, may also suppress phytonematodes. In view of multifacious action, the phosphate-solubilizing fungi and bacteria may contribute substantially in the management of plant parasitic nematodes. Soil application or seed treatment with the phosphate-solubilizing fungi such as *Aspergillus niger*, *Penicillium digitatum*, *P. anaticum*, *Purpureocillium lilacinum*, *Pochonia chlamydosporia*, *Trichoderma* spp. etc. may suppress the reproduction and soil population of ecto and endo-parasitic nematodes, and improve the crop yield. Culture filtrates of these fungi have been found to inhibit the hatching of eggs and induce mortality to nematode larvae. The use of phosphate solubilizing bacteria in nematode management has also been extensively evaluated. Studies have demonstrated that application of efficient strains of *Bacillus subtilis*, *B. polymyxa*, *Pseudomonas fluorescens*, *P. stutzeri*, *P. striata* etc. can control the nematodes and substantially improve the crop yields. Overall performance of PSMs against plant nematodes has been found to a level that ensures their commercial exploitation. This chapter present a detailed and critical analysis of the information on PSMs and their potential role in nematode management. The merits and bottlenecks have been identified so as to exploit full potential of PSMs in crop improvement and nematode management programmes in different agroclimates.

OP (S4) 29

2,4-Diacetylphloroglucinol biosynthesis in pseudomonads and its antagonistic efficacy against phytopathogens for sustainable agriculture systems

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The biocontrol ability of pseudomonads is directly correlated with production of potential antibiotics such as 2,4-diacetylphloroglucinol (2,4-DAPG) which exhibits broad antiviral, antibacterial, antifungal and anthelmintic properties and induces systemic resistance and systemic tolerance in the host plant. The antifungal metabolite 2,4-DAPG in *Pseudomonas* is synthesized through the expression of six genes *phlFACBDE* which forms the *phl* operon. The different functions of these genes have been well characterized of which *phlD* plays an important role in the biosynthesis of the precursor molecule for 2,4-DAPG production, *phlE* acts as an exporter of 2,4-DAPG metabolic (toxic) intermediates, *phlG* is involved in the degradation of 2,4-DAPG, *phlF* is a pathway-specific repressor and *phlH* acts as a regulator. Among these *phlD* is a key gene involved in the synthesis of monoacetylphloroglucinol (MAPG), the precursor of 2,4-DAPG from acetoacetyl CoA which encodes for a polyketide synthase. *Pseudomonas fluorescens* strains that produce the polyketide antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG) are the most effective biocontrol rhizobacteria that suppress a broad spectrum of root borne disease such as root and crown rot, wilt, and damping-off diseases in a variety of crops under field conditions. A number of commercial products of 2,4-DAPG *Pseudomonas* strains have been successfully employed in agriculture.

OP (S4) 30

Endophytic fungi: Application for plant disease management

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Endophytes are the microorganisms residing in the internal tissues of the plants without causing any detectable symptom of disease to their host. Endophytes are very diverse and almost plant species studied till date, are found to harbor one or more endophytes. Most of the studies mainly targeted endophytes for the production of novel bioactive compounds with varied activities like antibacterial, antifungal, anticancer, immunosuppressive, insecticidal, antioxidant, etc. Fungi are the most studied endophytic organisms that

produced plethora of biologically active compounds. In addition to bioactive compounds, some of them are known to produce toxic alkaloids which protect their hosts from grazing animals. Their role in crop protection and disease management gained a keen interest after some endophytes associated plants were found to show resistance for certain plant pathogens. This chapter discusses the roles and possibilities of endophytic fungi in the arena of phytopathogens control and management.

OP (S4) 31

Management of diseases of crops through bioagents under protected cultivation

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Protected cultivation is the concept of growing potential crops in the modified natural environment for ensuring optimum growth of the crop plants without any or least stress. Tomato, bell pepper, cucumber, lettuce, rose, carnation, gerbera, orchids and anthuriums are the most extensively grown vegetable and ornamental crops under greenhouse to achieve higher returns. Quality is a high priority for greenhouse crops, requiring much care in pest and disease management, not only to secure yields but also to obtain a high cosmetic standard. Pest and diseases are one of the major challenges to protected cultivation. Year-round warm temperatures and relative humidity and abundant food make the protected environment of greenhouses highly favourable to pests and diseases. Greenhouse provides the ideal environmental conditions not only for the optimum plant growth but also for the plant diseases. Fungi and nematodes are the most serious disease causing organisms of the various vegetables and fruits grown under protected cultivation. The ideal and stable environment with, humid and abundant food under greenhouse provides an excellent platform for the development of diseases often more than field conditions. The controlled environment of greenhouses, the high value of the crops and the limited number of fungicides offer a unique niche for the biological control of plant diseases. Of the commercial biocontrol products, over half have applications in nurseries or greenhouses and many were specifically developed against the soil borne pathogens *Pythium* and *Rhizoctonia*, which are major greenhouse pathogens. During the past ten years, over 100 biocontrol products have been marketed worldwide. Several formulations of either of the fungi *Gliocladium* and *Trichoderma* or the bacteria *Pseudomonas* and *Bacillus* have been widely used for biocontrol of soil borne pathogens. These products are not only registered as biofungicides but also used as plant strengtheners. The use of bioagents under protected cultivation for management of plant diseases are discussed.

OP (S4) 32

***Catenaria anguillulae* Sorokin: characteristics, biology and its traits as a potential biological control agent against plant parasitic nematodes**

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Catenaria anguillulae Sorokin is a wonderful gift of nature has all most all the attributes of a good biological control agent. The researchers around the world has been attracted after its first report by Sorokin in 1876 who reported its epidemic in nematode population eventually killing most of them. Although the fungus has enough attributes to take up it for studies pertaining to biological control of plant parasitic nematodes is still not so known and does not picked up as it should be, therefore, the efforts are made to describe historical remarks about its taxonomy, morphological characteristics, nature, biocontrol attributes, distribution, compatibility with agrochemicals, salt, wide range of pH and temperature, natural epidemics, techniques for its semi-quantification, rapid virulence test, selective isolation, preliminary solid state mass culture and purification and maintenance, varying degree of virulence with various nematodes, nutritional aspect, selection of substrates for its mass culture and performance test. Additionally, valid points pertaining to its future prospect to take up *C. anguillulae* at the field level around the world are also suggested for the management of plant parasitic nematodes.

OP (S4) 33

Potential of biocontrol agents against plant pathogens and their mechanism of action

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There are several naturally occurring soil microbes that aggressively attack on plant pathogens and benefit to plants by disease suppression and hence referred to as biocontrol agents and process is known as biological control. Understanding the mechanisms of biological control of plant diseases through the interactions between antagonists and pathogens may allow us to select and construct the more effective biocontrol agents and to manipulate the soil environment to create a conducive condition for successful biocontrol. In the past decade, the innovative applications of molecular techniques have broadened our insight into the basis of biological control of plant diseases. Particularly, molecular approaches are

useful in determining the relative contributions of different genetic traits in complicated processes. In so far as we know, the mechanisms of biocontrol may involve and be divided into antibiosis, competition, mycoparasitism, cell wall degrading enzymes, and induced resistance etc. Application of *Trichoderma* formulations with strain mixtures perform better than individual strains for the management of pest and diseases of crop plants, in addition to plant growth promotion. Commercialization of the bioproducts is primarily hindered due to the poor shelf life. Hence, research should be concentrated to increase the shelf life of the formulation by developing superior strains that support the increased shelf life, or the organic formulations that support the maximum shelf life with low level of contaminants must be standardized for making biocontrol as a commercial venture.

OP (S4) 34

Antagonistic microorganisms and their role in the management of post-harvest species of *Fusarium*

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The *Fusarium* link. with different species are known to attack variety of post-harvested food grain (seeds) and the results found to be responsible to deterioration, loss in seed germinability and also poisoning of the food grain (seeds). The species of *Fusarium* highly active potent for production of toxin when they are associated with the seeds in the field as well as during the storage condition. Some of the species of *Fusarium* which were isolated from the storage food grain (seeds).

They are post-harvest and one of the pathogenic and toxified to the plants, animals and human beings found to be studied phragmentary in the literature. The management of the species of *Fusarium* is one of the challenge before the traditional diseases management. To give the alternative to control of these species of *Fusarium* by adopting antagonistic microorganism is also called as biological control.

The biological control by using Botanical and bioagent is the phenomenon of the antagonism alternative is to traditional management of pathogenic micro-organism. Bio-agent and the use of Botanical which are economical, safe, biodegradable, easy to available and neither side effects to plants nor environment. Therefore, management of pathogenic *Fusarium* species by adopting bio-properties of Botanicals and Bio-agents are highly potent to prevention of pathogenic microbes.

This investigation to known and understand the antagonistic ability of microorganisms such as *Trichoderma viride*, *Spicaria violacea*, *Aspergillus sulphureus*, *Aspergillus terreus*, *Aspergillus niger*, *Alternaria alternata* and *Macrophomina* were screened against the *Fusarium oxysporum*, *Fusarium equisetum*, *Fusarium moniliforme*, *Fusarium avenaceum*, *Fusarium nivale*, *Fusarium roseum* for their management.

It is clear, from the results, the antagonistic micro-organisms have a ability to inhibit the growth of the tested Fusarium species. The results are promising. The Aspergillus sulphureus, Aspergillus terrus, Aspergillus niger, Trichoderma viride and Macrophomina are able to prove their antagonistic ability followed by other tested micro-organisms against the species of Fusarium.

OP (S4) 35

Influence of native plant growth promoting rhizobacteria for management of damping off and collar rot diseases of vegetables

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The regular use of fungicides can potentially pose a threat to the environment, predominantly if residues persist in the soil or migrate off-site and enter waterways. Plant growth promoting rhizobacteria (PGPR) are natural destroyer of the plant-disease causing organism(s) by controlling pathogens' populations in order to minimize their impact on economic and environmental practices. It is a very specific strategy and safe alternative to chemical pesticides. Bio-fungicides are established fact worldwide. It is produced in every continent in the world but the dilemma of popularity in the massive scale lies in the fact regarding its inefficacy in the field condition of end users. On the other hand cost of growth media, contamination, critical steps involved in mass production are the major constrains in the effectiveness and performance of the bio-fungicide in the field condition. In the present study native PGPR isolated from rhizosphere soil of different agro-ecological region of West Bengal were found to harbor antagonistic activity against several fungal pathogens as well as having unique plant growth promoting (PGP) traits e.g., IAA production, P- solubilisation, siderophore, salicylic acid production and different extracellular hydrolytic enzyme (protease, amylase, cellulase, lipase, pectinase, chitinase) production. Further they were tested for genetic and functional diversities by different culture dependent and independent approaches and to monitor their population dynamics based on various edaphic factors. The field application of this rhizobacteria in mono or in consortia through seed treatment and soil treatments under nursery bed and main field conditions were performed to test their efficacy on plant growth promotion (chili , tomato and cowpea) against damping off and collar rot disease. Thus, Knowledge and strategies generated from different aspects of the present study will be useful to design blue prints of novel ideas to use these strains as inoculants in sustainable and organic agriculture.

Bioagents in enrichment of organic substrates

Oral Papers

OP (S5) 36

Evaluation of bioagents for management of dry root rot [*Macrophomina phaseolina* (Tassi) Goid] in mungbean [*Vigna radiata* (L.) Wilczek] through seed treatment and soil application

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Pulses play an important role in improving livelihood and nutritional security of the people. In a country like India, where majority of the population is vegetarian, the cheap and best sources of protein are pulses, especially for the poor people. Keeping in view, many benefits of pulses for human health, United Nations has proclaimed 2016 as the "International Year of Pulses". Thus, due attention is required to enhance the production of pulses for not only meeting the dietary requirement of protein but also to raise the awareness about pulses for achieving nutritional, food security and environmental sustainability. Mung bean [*Vigna radiata* (L.) Wilczek] is one of the most important and extensively cultivated pulse crop belonging to the family *Fabaceae*. It is an excellent source of high quality protein. The crop is affected by a number of pests and diseases of which dry root rot incited by *Macrophomina phaseolina* is an economically important disease. Soil and seed borne nature of the pathogen creates difficulties for an effective disease management. Considering the importance of the disease, the field experiments were conducted at College Agronomy Farm, B. A. College of Agriculture, Anand Agricultural University, Anand during *kharif*: 2014 and 2015 in Randomized Block Design with fourteen treatments along with three replications. The pooled data of two years revealed that application of *Trichoderma harzianum* or *T. viride* (2×10^8 cfu/g – 1% WP) enriched FYM (10 kg bioagent/ ton FYM) in furrow @ 1 ton/ha, followed by seed treatment with *T. harzianum* or *T. viride* (2×10^8 cfu/g) @ 10 g/kg seeds, respectively at the time of sowing have been found effective and economical for the management of dry root rot of mungbean.

OP (S5) 37

Effect of organic practices and chemical fungicide in managing groundnut diseases

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To know the effect of organic practices compared with chemical fungicide in management of groundnut disease under field condition, ten treatments including a control were applied. Result revealed 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). Application of FYM (7.5 tonne/ha) in place of NPK and *Trichoderma* spp. as seed treatment @10 g/kg seed and in furrow @ 4.0 kg enriched in 250 kg FYM to realize higher income for the organic management of groundnut diseases. But, for the better management of foliar diseases, fungicide hexaconazole is essential as it reduces the development of inoculums and further spreading of the diseases. Therefore, it is concluded that organic farming could reduce the soil borne diseases to some extent.

OP (S5) 38

Integrated management of root rot disease [*Macrophomina phaseolina* (Tassi.) Goid] of castor through bio agents, FYM and chemicals under field conditions

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Castor (*Ricinus communis* L.) is one of the important oilseed crops of India. The oil extracted from castor seed is being widely used widely for various purposes. Occurrence of root rot disease has become a major constraint in recent years for successful and profitable cultivation of castor crop. The efficacy of various bio agents and chemical viz. *Trichoderma harzianum*, *Pseudomonas fluorescens*, FYM and carbendazim through seed treatment and soil application were evaluated against *Macrophomina phaseolina* (Tassi.) Goid causing root rot disease of castor. Integrated management study of root rot disease of castor under field condition showed that Seed treatment with combination of *P. fluorescens* Pf 2 WP (5g/kg) + *T. harzianum* Th4d WP@ 10g/kg and Seed treatment with carbendazim @ 2g/kg resulted higher germination (97.22 %) followed by Seed treatment @ 4g/kg and soil application of *T. harzianum* local isolate (2.5 kg. mixed with 100 kg FYM incubated for a week and applied while seed dibbling) (95.83). In case of disease incidence and yield of crop, Seed treatment with combination of *P. fluorescens* Pf 2 WP (5g/kg) + *T. harzianum* Th4d WP@ 10g/kg has lowest disease incidence (24.20 %) and gave highest yield (2099 kg/ha) followed by Seed treatment @ 4g/kg and soil application of *T. harzianum* local isolate (2.5 kg. mixed with 100 kg FYM incubated for a week and applied while seed dibbling) 25.55 % and 2007 kg/ha, respectively.

Development of formulations and delivery systems for bioagents/antagonists

Lead Papers

LP (S6) 27

Importance of delivery methods in the success of biological control of plant diseases

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India has been following a sustainable agricultural system by recycling the organic waste produced in the farming system. Farmers were initiated to high input intensive agriculture that helped to record food grain production that resulted in Green revolution. But excessive use of chemical inputs in some part of the country, has brought in deterioration of soil health and in turn plant and human health. The role of microbes in nutrition mobilization and crop productivity has been realized and followed by farmers for ages. Inoculation of rhizobia for legumes and crop rotation with legumes has been a regular practice in Indian Agricultural production scenario.

Soil microbes play a major role in recycling the nutrients, enhancing the productivity of soil and the crop plants. Plant roots surface are nutrient rich as the roots secrete sugars, amino acids and readily available nutrients and several microbial communities thrive here. The area surrounding the roots is known as rhizosphere, extends a few millimetres from the root surface and occupies a substantial area. Rhizosphere microbes play a vital role in nutrient mobilization and plant growth and serve as first line of defense against soil borne pathogens. They also live as endophytes protecting plants against harmful organisms by triggering internal defenses of plants. Microbial population in the rhizosphere is dynamic and population fluctuation depends on the soil amendments. The host root place a key role to select the microbes through the root exudate. Plants recruit the microbial communities as per their need, and there is intense competition in the rhizosphere when compared to bulk soil as it accommodates microflora, microfauna, protozoa, algae, soil arthropods beneficial and pathogenic organisms.

Identification of efficient organisms, development of cost effective formulation, setting standards for mass production and inoculation techniques makes cultivable microbes much more attractive research targets among numerous and diverse soil microbes. Beneficial microbial inoculants in agriculture are mainly plant growth-promoting rhizobacteria (PGPR) and fungi, and they function through different mechanisms to increase the plant fitness under biotic/abiotic stresses. When an organism is introduced in the rhizosphere there is an array of changes and lead to microbial succession.

Once the beneficial microorganisms are identified, it is cultured in the laboratory, mixed with a carrier medium and delivered to the field by making formulations with inert materials. Generally dry formulations

are made using inert materials like talc and lignite. Use of peat, agricultural by products like coffee husk, tea dust, whole grains of cereals and pulses are in vogue. Liquid formulations that can be further diluted and applied are also available. But often the biological control fails as there are several factors that control the success in the introduced environment the key factor being the number of viable propagules in the formulations. Secondly, the competition of the introduced organism with other microbes in soil and the third the rhizosphere competition. The formulations being bulky poses several logistic problems besides the maintenance of shelf life. Novel delivery methods like seed coating and biocapsules have been developed tested and transferred to entrepreneurs at ICAR- Indian Institute of Spices Research, Kozhikode. In seed coating the beneficial microbes are delivered on the seeds and as the seedling emerges it gets the benefit of colonization. This was tested for seed spices and demonstrated in farmers field. Farmers were able to record 20-30% enhanced yield besides managing the soil borne diseases. In the Biocapsule technology, the volume is reduced by one hundred times as in place of 1kg talc ten capsules can be used.

In order to make the biocontrol technology viable and popular it must be easy to adopt and must show visible benefits in the field. This underlines the importance of quality of the products in terms of shelf life and efficiency in the field.

LP (S6) 28

Growth and development of technological aspects of biological control of plant diseases

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Agriculture could not have begun without the benefits of naturally occurring biological control (natural control) but modern biological control achieved with introduced microorganisms. As a result of recent scientific advances, there are now exciting new possibilities and opportunities for the management of plant diseases through biological control methods. The concerns in environment over the proliferation of usage of chemical pesticides have prompted for intensive research for development of practically applicable alternative methods such as biological control using microbial antagonists effective against plant pathogens. Such biocontrol agents also have a merit to be useful in organic farming and they need to be certified organic. Since success of derivation of useful biological control methods is dependent upon screening of the appropriate antagonist under similar conditions in which they are used and by determining the effects of varying environmental parameters and mechanism of action of biocontrol agents, it is appropriate to historically review and highlight as to how these methods and concepts of mechanism of action (mycoparasitism, antibiosis, competition, induced defence response, cross protection etc.) have come to this stage of their technological use in plant health management. The present paper thus deals with reviewing historical development and evaluation of different methods of biological control of plant diseases.

LP (S6) 29

Current status of commercialization of bioagents: Development of formulation and delivery system

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The extensive use of agrochemicals has posed a serious risk on human and environment health and also proved to be disastrous for soil microbiota. Post-green revolution, application of large scale agri-inputs has rendered many areas unfertile thereby causing sincere concern. Since agricultural productivity depends upon the organic and inorganic composition of soil, thus, in the current scenario, it has become crucial to search for possible eco-friendly alternatives, for efficiently managing pests and diseases.

Biopesticides are naturally occurring biologically safe microorganisms, used for the management of pests and diseases. The global market demand for microbial based bioinoculants is increasing annually. The estimated market for biopesticides has grown at a rate of 14 % and is predicted to generate US\$ 1.88 billion worldwide by 2020 and US\$ 1.95 billion by 2022. Currently, it is estimated that nearly 80 % of the global market revenues are recorded in Europe and Latin America. However, the acceptance and diffusion of biopesticides in Indian agricultural market is quite limited as is quite evident with the Indian contribution to the global biopesticide market being 2.89% during 2005, and a meager improvement to 5% by 2016.

Initially, nine microbes namely *Bacillus subtilis*, *Gliocladium* spp., *Trichoderma* spp., *Pseudomonas fluorescens*, *Beauveria bassiana*, *Metarrhizium anisopliae*, *Verticillium lecanii*, Grannulosis viruses and nuclear polyhedral viruses (NPV) have been included in a schedule vide an amendment in insecticides Act, 1968 for the commercial production of biopesticides and published in the Gazette of India dated 26th March 1999 and currently 26 microbes have been included in the schedule to the Insecticide Act 1968 for production of microbial biopesticides. *T. viride*, *T. harzianum*, *Pseudomonas*, *B. bassiana*, *M. anisopliae* and *B. subtilis* have carved a niche for themselves in India as important biocontrol agents for management of various pests and diseases.

The poor penetration and acceptance of biopesticides in India can be attributed to the mushrooming of some fly-by-night spurious companies. This not only sows the seeds of doubt in farmers mind about the profitability of microbial biopesticides but also the ill effects of these biopesticides. The research on biocontrol agents can be fruitful only when we commercialize and register the product based on superior strains with Central Insecticide Board and Registration Committee (CIB&RC). To achieve this, certain norms specified by CIB are to be followed strictly. During the presentation emphasis will be given on development of formulation and delivery system, role of CIB&RC in registration, quality control and commercialization of biopesticides.

LP (S6) 30

Developing a consortium of *Trichoderma harzianum* and *Bacillus firmus* for managing soil borne plant pathogens

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Under warm growing conditions, crops experience long and short duration of moisture stress. Under these conditions, *Macrophomina phaseolina* (Tassi) Goid., causes dry root rot in legumes and oil seed crops. In irrigated pockets of the region cumin (*Cuminum cyminum* L) is cultivated during winter season. However, this crop suffers heavily by wilt caused by *Fusarium oxysporum* f. sp. *cumini*. Concerted efforts were made for the last many decades to develop a host of technologies to reduce the population of soil borne plant pathogens below the economic threshold level. Several management strategies like moisture conservation practices, soil solarization, *Brassica* residues, composts and bio control agents have been advocated for managing soil borne plant pathogens. Among these uses of native bio-control agents hold great promise as an integral component of integrated disease management. In recent years, native strains of bio agents' viz., *Bacillus firmus* and *Trichoderma harzianum* have been isolated from arid soils. *B. firmus* was found specific antagonist to *M. phaseolina* in various laboratory tests. Therefore, efforts were made to develop bio-formulated products of these bio-pesticides. After a series of experimentations, a consortium of bacterial and fungal bio agents was developed in a bio-formulated product, which was able to maintain shelf life for a period of 120 day of both the bio-agents. This consortium has been coined as *Misritmarusena*. The temperature studies have shown that it can be stored at room temperature even at farmer's field. After developing consortium, it was thought worthwhile to test effectiveness as seed treatment at growers' field during rainy seasons in order to disseminate this cheap and easy management strategy in the region. Studies revealed that seed treatment with bio-agents significantly reduced incidence of dry root rot on legumes and oilseeds and that of wilt on cumin in all the demonstrations resulting in increased seed yield. Consortium of both the bio-agents also increased seed yield by 22.9% in guar. These demonstrations have resulted in wider acceptance by rain-fed farmers and more by cumin growers. Studies conducted on farmers field in the form of demonstrations have shown that 40-50 kg increase in seed yield is achieved in legumes grown in rainy season and 50 kg seed yield or some time more depending on soil condition of the farmers field will be achieved in cumin by adoption of this consortium. It is expected that in this way *Trichoderma* will also be established in the soil. It is expected that approx. 10-15 % seed yield will be enhanced by use of this easy application technology. The process and development of this consortium has been submitted for patenting and the same has been now published in Indian patent site in 2015. In this presentation and efforts have been made to summarized the salient findings emerged from this study.

LP (S6) 31

Impact of biological control of plant diseases in soil and plant health

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Biological control has emerged as an important alternative in managing seed and soil borne plant diseases in recent years, as it is more eco-friendly. A wide ranging list of *Trichoderma* spp. based biofungicide agents are commercially available (Navi and Bandyopadhyay, 2002) and used against fungal plant diseases. *Trichoderma* spp. is a soil borne organism predominantly inhabited in soil, compete with several microorganisms in the ecosystem by way of competition, mycoparasitism, antagonism, growth promotion, modification of physiological changes and induction of systemic resistance in the plants thereby benefitting the plant community in their survival on this earth. They may be applied as seed priming, bio inoculants, soil application, foliar application, can be multiplied in FYM and compatible with many agro chemicals. The incidence of damping off of seedlings was found to be lowest (4.60%) in seed treatment (4g/kg of seeds) and soil application of nursery beds (0.5g/m²) when treated with *T. viride* (local strain) as against maximum mortality of 21.80 percent in case of farmers practice at KVK, Kanker. Fusarial wilt of brinjal was also found to be less (21.25%) when seed, nursery treatment and application of *T. viride* (4 kg mixed with 40 kg FYM) was done at the time of last ploughing in the field compared to higher incidence (32.15%) in farmers practice. Increase in yield by 7.71% in treated one with B:C ratio of 1:2.31 as against 1:1.97 in farmers practice at KVK, Bijapur was observed. Use of *T. viride* @ 4gm/kg as seed and soil application (@ 400gm/1q of FYM) resulted in almost 50% reduction (12.30%) in incidence of collar rot of chickpea. As a result, there was increase in yield by 11.56 percent in treated compared to farmers practice with B:C ratio of 1:3.02 as against 1: 2.75 in farmers practice at KVK, Kabirdham. Paddy straw when decomposed using *T. viride* took only 33 days for its decomposition as against 70 days in farmers practice. In another study, paddy straw when inoculated with *T. viride* culture took only 58 days for its decomposition as against 165 days in treatment with no culture of *T. viride*.

Oral Papers

OP (S6) 39

Mass multiplication of *Trichoderma harzianum* and standardization of effective dose for management of soil borne diseases

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It is widely known that *Trichoderma harizanium* shows antagonistic behaviour towards soil borne diseases. This biological means to control the disease offers and eco- friendly way to increase the production of agricultural and horticultural crops. Hence, it becomes imperative to characterize the antagonist means of its usage for disease control. A study was taken up to standardize the effective dose for management of soil borne diseases. Initially mother culture of the antagonist fungal biomass broth were mixed with talc powder at 1:2 ratio. The mixture was air dried later mixed with carboxy methylcellulose (CMC) @ 5g/kg of the product. Thus, obtained mixed powder was then packed in polythene bags used in further studies. They were then incubated at 28^oC. Samples of *T. harizanium* talc were drawn at 0, 15, 20, 30, 60, 90, 120, 150 days after inoculation the population was estimated by serial dilution method using TSM. The results of the study to assess longevity indicate that the population of *T. harzianum* decreased significantly (10×10^6 to 2×10^6 cfu/g) over the period of its storage (150 days). In a study to ascertain optimal application levels for disease control, the red gram seeds of variety ASHA (ICPL -87119) were treated with formulations of *T. harzianum* at the rate of 2, 4, 6, 8 and 10 g/kg of seeds. They were then planted in pots containing the inoculum of *Fusarium* sps. @ 10 seeds per pot. *Fusarium* wilt incidence was showed decreasing trend with increased application of talc-based formulation of *T. harzianum*. It reduced from 30.60% (in control) to 4.8% (10g/kg seeds). Application of carbendazim was also found to be compatible with antagonist (*T. harzianum*).

OP (S6) 40

Development of mutant bio-control agents for the control/management of chilli *Fusarium* wilt

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Wilt of chilli (*Capsicum annuum*) caused by *Fusarium solani* is an important and destructive soil borne disease causing yield losses in most popular cultivars of chilli especially in Northern Telangana zone of Telangana state during both *kharif* and *rabi* seasons during recent years. Detailed investigations were carried out on biological control of this disease by developing mutants of *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescens* and studying the role of mutants on the antagonistic activity against the pathogen. Also the mutant biocontrol agents were tested against commonly used agrochemicals to develop an integrated disease management strategy. The wild young culture slants of *T. viride*, *T. harzianum* and *P. fluorescens* were exposed to four doses of gamma irradiation viz., 50, 75, 100 and 125 K rad provided from ⁶⁰Co-source. The exposure of slants at 125 K rad, produced eight and three each of *T. viride* and *T. harzianum* (designated from TvM1 – TvM8 and ThM1 – ThM3), carbendazim tolerant mutants. Wherein, the mutants tolerated higher concentrations of carbendazim as indicated by their growth on fungicide amended medium. While, gamma irradiation did not induce any distinguishable mutants of *P. fluorescens*. The mutants of *T. viride* and *T. harzianum* differed from their wild types in colony morphology, growth habit and rates, sporulation, antagonism and disease control potentials against *F. solani* and tolerance to carbendazim. Among the eight and three mutants of each of *T. viride* and *T. harzianum*, mutants TvM1 and ThM1 recorded a faster growth rate of 82 mm within 72 h and could antagonize the test pathogen *F. solani* by forming zone of inhibition within 96 h, with 90.85 and 90.24 per cent inhibitions respectively compared to the rest of the mutants and wild types. While the bacterial biocontrol agent *P. fluorescens* (commercial isolate) inhibited the growth of *F. solani* to an extent of 65.9 per cent. Further the scanning electron microscopic studies revealed a perfect phenomenon of mycoparasitism involving chemotrophic stimulus, coiling, secretion of extra cellular enzymes and lysis of the pathogen's mycelium / hyphae. Of the different pesticides tested against the mutants, carbendazim, fipronil and fluchloralin were found to be compatible with the mutants of *T. viride*, *T. harzianum* and *P. fluorescens* at their recommended and half the recommended dosages.

OP (S6) 41

Mass production, formulation, quality control and delivery of *Trichoderma* for plant disease management in India

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Trichoderma have carved a niche for themselves in India as important biocontrol agents for management of soil-borne plant pathogenic fungi, suppressive effects on some root nematodes and capable of promoting growth of certain crops. A number of successful products based on different species of *Trichoderma* have been commercialized in India. There are two chief methods of inoculum production of *Trichoderma* spp. viz., solid state fermentation and liquid state fermentation. In solid state fermentation, the *Trichoderma* is grown on different cereal grains, agricultural wastes and by products. The solid state production is very much labour intensive and fit for cottage industry. These products are used mainly for direct soil application to suppress the soil-borne pathogens. In liquid state fermentation, *Trichoderma* is grown in inexpensive media like molasses and yeast medium in deep tanks on a commercial scale. The low viability of the *Trichoderma* during storage and field application along with lack of knowledge regarding the best carrier in conventional formulation are the major drawbacks for the two types of formulation. Commercialization of the *Trichoderma* products is primarily hindered due to the poor shelf life. Quality control is very much obligatory to retain the confidence of the farmers on the efficacy of biocontrol agents. Being the living organisms, their population in a product influences the shelf life. The population load of the antagonists decides the minimum level of requirement for bringing the effective biological control of the plant diseases Hence, research should be concentrated to increase the shelf life of the formulation by developing superior strains that support the increased shelf life, enhancing biocontrol through manipulation of the environment, using mixtures of beneficial organisms, physiological and genetic enhancement of biocontrol mechanisms and manipulation of formulations that support the maximum shelf life with low level of contaminants for making biocontrol as a commercial venture. Lately, microencapsulation has been developed to prolong shelf life and control release from formulations and thus enhancing their application efficiency. Currently *Trichoderma* has entered the genomic era and part of genomic sequences are publically available. We must focus on one of the main factors that are potential to affect economic feasibility of a *Trichoderma* product is formulation technology. Commonly used *Trichoderma* based biopesticide formulation include granules, pellets, dry powder and wet powder. The success of biological control of plant pathogens using *Trichoderma* does not rely solely on effective antagonists but also on the method of delivery or application on the seed, root and soil. In addition, *Trichoderma* rely on their placement on the infection court to effect successful protection and control. Timing of delivery and application is also crucial and *Trichoderma* is generally only effective as a preventative measure but can be integrated with other disease management options particularly when the disease has already established. Delivery systems must ensure that *Trichoderma* will grow well and achieve their purpose. The delivery and application

processes must be developed on a crop by crop and application by application basis. No common solutions exist, and so biocontrol systems ought to be developed for each crop. The present article focuses mass production, formulation, enhancement of biocontrol potential, quality control and application under field conditions to manage important diseases of crops.

OP (S6) 42

Biofilm-based strategy for management of wilt and collar rot disease of chickpea

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Biofilms is well organized, cooperating community of microorganisms. Microbial cells attach to the surfaces and develop a biofilm that represent mixed group of microorganisms in which cells stick to each other within a self-produced matrix of extracellular polymeric substances (EPS); however, their utility as biofertilizers cum biocontrol agent has not been fully explored. The present investigation was geared towards *in vitro* development of biofilms using fungal mycelia (*Trichoderma viride*) as matrices and *Bacillus subtilis* as partners and used them as a biofilm-biofertilizers, BFBFs as well as biocontrol agents for control of soil borne diseases like wilt (*Fusarium oxysporum ciceri*) and collar rot (*Sclerotium rolfsii*) of chickpea. The biofilm developed using Z medium was tested for assessing its efficacy against wilt and collar rot diseases *in vitro*. Biofilm treated seeds showed enhanced germination and vigour index, reduced diseases incidence and had positive effect on biochemical parameters as compared to control. The results indicated that the specific activity of Peroxidase (POD) was significantly up-regulated from zero hours onwards after inoculation and reached at its peak at 24 hr after inoculation (3.8 U/mg protein) when 2.44 fold increase in enzymatic activity was noticed in biofilm treatment. The highest specific activity of Polyphenol oxidase (0.75 U/mg protein) and superoxide dismutase (1.3 U/mg protein) was recorded for Biofilm treatment as compared to respective control (0.27, 0.56 U/mg protein) after 24 hr of inoculation. Plants treated with biofilm, fusarium and sclerotium showed more accumulation of total phenol than the control and for plants pretreated with biofilm alone. The maximum total phenol content (1.48 mg/g) shown for the Biofilm treatment and the minimum (0.59 mg/g) for control. The accumulation of phenol increased from the 12 hr after challenge inoculation with FOC and SR. Biofilm based biofertilizers obviate the need of developing combi-formulation of fungal and bacterial biocontrol agents which is cumbersome.

OP (S6) 43

Mass production protocols of bioagents with special reference to *Trichoderma*

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The ill effects of the pesticides have forced us to the search for alternative forms of pest management. In this respect, biological control has emerged as one of the promising tool for the effective, economic and environment friendly technique to manage the pests and diseases. Biological control has been defined as the reduction of inoculum density or disease producing activities of a pathogen by one or more organisms, accomplished naturally or through manipulation of environment, host or antagonists, or by mass introduction of one or more antagonists. The benefits of biological control are many. *Trichoderma* is an exceptionally good model of biocontrol agent as it is ubiquitous, easy to isolate and culture, multiply rapidly on many substrates, act as mycoparasite, competes well for food, site and oxygen, produce antibiotics like trichodermin, viridian and has the enzymes like chitinases, chitosanases, cellulases, pectinase, cutinase and endoglucanase which are modus operandi to act as mycoparasite Various Protocols currently available for the mass production of *Trichoderma* spp. viz. Talc based formulation: Biocapsules: PESTA granules- Press Mud formulation Oil Based Formulation- Coconut Water. NIPHM (National Institute of Plant Health Management) media Mass production of *Trichoderma harzainum* in wheat bran saw dust medium.

OP (S6) 44

Seed biopriming: Bioprospection in agriculture

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Seed is the most vital and crucial input in crop production as ninety percent of the food crops are grown from seed itself. So, seed health is very important to ensure good crop health and to achieve desire quantity and quality of crop produce. But this crop health is mostly threatened by many seed borne and soil borne pathogens. Devastating consequences of these pathogens results in poor crop stand. To combat such problems, protecting seeds and germinating seedlings will be our prime target. This can be possible through seed treatment with various means. Among them, seed treatment with chemical pesticides is an age old practice, but mostly has limited use due to ecological, economical, and environmental safety concern. Moreover, non-target effects on beneficial microbial population, chances to resistance development within pathogen population, contamination to food chain, toxicity to plants, as well as human, animal health raise

the demand for sound and environment friendly safer alternatives that can easily be fitted with intensified crop production to ensure global food security and improved agricultural productivity. Seed treatments with bioinoculants provide very good protection to germinating seedlings against seed borne as well as soil borne pathogens. Thus, it is considered as an excellent alternative to harmful chemicals.

Biopriming is a technique involving bioprotection with matricconditioning. That's why it is also known as biomatricconditioning. It integrates biological seed treatment (inoculation of seed with beneficial organism to protect seed) with physiological aspects (seed hydration) of disease management. Seed biopriming involves hydration of seeds in a microbial suspension for a pre-determined time period, followed by incubation for proper microbial colonization. Whereas, seed treatment is simple coating of bioinoculant to the seed shell/coat. In biological seed treatment, microbial antagonists are only stick to the outer surface of the seed, which is available to protect the seed externally in soil, while in case of seed biopriming microbial antagonists penetrate within the seed before germination and behave like endophytes to protect seed as well as seedlings which lead to improve seed viability and vigor, plant growth and yield of various crops. Primary aim of seed biopriming is proper germination of seeds, colonization of root rhizosphere to manage seed and soil borne pathogens. Various agriculturally beneficial microorganisms including biological control agents, plant growth promoting rhizobacteria (PGPR), and endophytes can be used for seed biopriming. Various commercially exploited biological control agents, viz., *Trichoderma spp.*, *Pseudomonas fluorescens*, *P. aureofaciens*, *Bacillus subtilis*, *Rhizobium sp.*, *Serratia marcescens*, etc. are used in biopriming mediated suppression of various seed and soil borne diseases. In spite of their direct application, it helps in provide growth promotion, crop protection and mitigation of biotic and abiotic stresses. Efficacy of seed biopriming have been reviewed by various researchers and now it could be suggested that combined application of hydro-priming and seed coating with biocontrol agents would be a sound alternative to the chemical seed treatment. Additionally, it has explored a new dimension of biocontrol and could be exploited by agricultural industries and organic farmers in sustainable agriculture.

OP (S6) 45

Strain improvement of *Bacillus amyloliquefaciens* (DSBA-11) for biocontrol of bacterial wilt disease of tomato caused by *Ralstonia solanacearum*

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Bacterial wilt caused by *Ralstonia solanacearum* (Smith) is a serious disease of tomato (*Solanum lycopersicum* L.) in the world including India. For management of this disease through biocontrol using microbes is an eco-friendly approach. The antagonistic property of *B. amyloliquefaciens* DSBA-11 was enhanced by random mutagenesis by ultra-violet (UV) irradiation, Nitrous acid (HNO₂), and N-Methyl-

N-nitro-N-nitroso guanidine (NTG) treatment. Mutants were screened for antagonistic activity against *R. solanacearum* *in vitro*. MUV-19 obtained after 30 minutes of UV exposure time showed 3.42 cm² of inhibition zone, mutant MNTG-19 treated with NTG, and MHNO2-20 treated with HNO₂ at the concentration of 500 µg/ml showed 3.56 cm² and 4.6 cm² of inhibition zone respectively, wild strain *B. amyloliquefaciens* DSBA-11 showed 3.35 cm₂ of inhibition zone. By GC-MS analysis total sixteen major compounds were detected. The maximum production of 3- Isobutyl hexahydropyrrolo (1, 2,α) pyrazine-1, 4-dione compound was detected in the mutant MHNO2-20 with higher retention time (RT) 43.19. These mutants and wild strain DSBA-11 were further taken for bacterial wilt disease control of tomato at Phytotron under controlled conditions. 21 days old seedling of tomato cultivar Pusa Ruby (susceptible) was taken for this experiment. The minimum disease intensity (9.28 %) was recorded in mutant MHNO2-20 treated plants followed by 20.28 % by MUV-19, 20.28 % by MNTG-21 and 15.40 % by wild strain DSBA-11 after 30 days of inoculation. Maximum wilt disease 82.57 % was observed in *R. solanacearum* treated plants. The improved strain of *B. amyloliquefaciens* DSBA-11 possesses both traits that is, PGP attributes and wilt disease control.

OP (S6) 46

Management of seed associated *Fusarium oxysporum* and *Macrophomina phaseolina* in chickpea through seed biopriming and soil application of *Trichoderma harzianum* strains

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A field experiment was conducted to determine efficacy of biological agents and influence of priming on the seeds associated *Fusarium oxysporum* and *Macrophomina phaseolina*. All the treatments differed significantly from each other for vigour index, percent root rot incidence and yield. Maximum vigour index (VI) *i.e.* 7624 with minimum disease incidence (DI) of 14.7% having highest yield of 1360 kg/ha was observed in treatment no. 6 *i.e.* seed biopriming for 10 hrs. with suspension of solid talc based formulation (2x10⁸cfu/g) of *Trichoderma harzianum* AAU isolates @ 50 g in 250 ml of water/kg of seed + soil application of *T. harzianum* enriched FYM (10 g *T. harzianum* / kg FYM) @ 100 g /m² of soil/furrow, which was followed by treatment no. 2 *i.e.* seed biopriming for 10 hrs. with suspension of solid talc based formulation (2x10⁸ cfu/g) of *Trichoderma harzianum* PAU isolates @ 50 g in 250 ml of water/kg of seed, which recorded 7247 VI, 14.67% DI and grain yield of 1291 kg/ha as compared to treated check (4221 VI, 41% DI and grain yield of 701 kg/ha) and untreated check (4799 VI, 18.67% DI and grain yield of 994 kg/ha).

OP (S6) 47

Induction of Fusarium wilt resistance in tomato by a biocontrol consortium

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Vascular wilt of tomato incited by *Fusarium oxysporum* f. sp. *lycopersici* (FOL), a serious soil-borne fungal disease, needs to be contained through efficient sustainable management practice to promote yield and quality of marketable produce. To enhance the consistency and degree of bio-control of the disease in employing a single antagonist, effective strain mixtures consisting of *Pseudomonas putida* (TEPF), *Bacillus subtilis* (S2BC-1), *Trichoderma harzianum* (S17TH) and *Chaetomium* spp (CG-A) were designed based on growth, antagonism and antifungal gene expression assays. In dual cultures, the microbial strains in general exhibited positive interactions for the tested parameters with no adverse effects on their cell density or mycelial growth, antagonism and chitinase gene expressions. In pot experiments, seed treatment and soil applications of 7 different strain mixtures were assessed for their efficacies in vascular wilt control over individual strains upon challenge inoculation with FOL under polyhouse (18–20°C and 75% relative humidity) conditions. Among the treatments, application of a strain mixture comprising of TEPF+CGA+S17TH under challenge inoculated conditions resulted in significantly lower incidence (63.5% reduction) of Fusarium wilt relative to the pathogen control without biocontrol agents. This lower incidence was associated with an increase in root and shoot length of 145.2%, relative to the pathogen control. In further studies on elucidating the role of defence genes of tomato plants in vascular wilt suppression by the biocontrol consortia, though strong expressions of PR3b and PR5, the marker genes for salicylic acid modulated pathway and glu B, the marker gene for ethylene modulated pathway were observed over the pathogenic and untreated controls, in the treatments, S2BC-1+CGA+S17TH and S2BC-1+TEPF+S17TH, a reduction in expression was observed for the best performing strain mixture, TEPF+CGA+S17TH. The reduced expression indicated that novel genes other than the candidate ones might be playing a role in the induction of resistant response. The possibility of direct antimicrobial activity by the strain mixture appears to be remote as the strain constituting the consortia have earlier been demonstrated to induce resistance in plants. Increase in plant growth by the best performing strain mixture could be attributed to the suppression of the pathogenic fungi besides other mechanisms that need to be studied in detail. The identified consortium has the potential as a preferred input in integrated disease management systems once formulated and tested under field conditions.

OP (S6) 48

Development of native PGPR consortium formulation fortified with phyto-extracts for biocontrol of *Meloidogyne incognita* (root knot nematode)

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Present research was carried out to develop native fortified PGPR formulation comprising of bacterial strains *Providencia vermicola* AAU PR1 (NCBI ACCN: KJ161325), *Pseudomonas putida* AAU PR2 (NCBI ACCN: KJ161326) and *P. fluorescens* AAU PR3 (NCBI ACCN: KJ161327) with phyto-extracts viz *Azadirachta indica* (neem), *Ipomoea carnea* (Besharam) and *Brassica juncea* (mustard) cake having biocontrol activity. Individual bacterial strains were found compatible with each other as well as phyto-extracts @ 10% aqueous concentration; rather there was an increase in population of bacterial count in presence of phyto-extracts. Native fortified PGPR consortium was prepared having initial bacterial count 1.3×10^9 and its longevity studied for 12 months maintaining count up to 7.3×10^7 cfu/ml. Native fortified PGPR consortium showed reduction in egg hatching of *Meloidogyne incognita* under laboratory conditions. Inoculation of cucumber with Native fortified PGPR consortium showed reduction of root knot nematode infestation in pot and field trial as well as farmers' field demonstration.

Policy issues in Registration and commercialization of bioagents

Lead Papers

LP (S7) 32

Development, registration and commercialization of smart AgBiologicals for sustainable crop protection: Recent trends and opportunities

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AgBiologicals are gaining increasing acceptance from farmers worldwide as a fundamental part of crop protection: biological crop protection agents offer highly targeted ways of controlling pests and diseases, and provide new resistance management strategies. More and more Novel Smart biological products are reaching the marketplace with greater investment in proof of efficacy, broader testing across geographies and with advanced technology for fine tuning to meet the growers' pest control needs.

Successful commercialization of AgBiologicals hinges on the outcome of the development process and is often limited by a lack of knowledge and experience with biological control agent production and formulation techniques. Critical steps in development, Registration and commercialization of successful Smart Microbial Bio-control Agents (Bio-pesticides), recent Trends, Opportunities, Constraints and future strategies would be deliberated at length.

LP (S7) 33

Emerging Paradigms in research and commercial production of bioagents: current status and prospects

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Biocontrol agents *viz.*, *Trichoderma* spp., *Pseudomonas* spp., *Bacillus* spp., etc. have been proved beneficial for the management of biotic stresses i.e. seed and soil borne diseases of the crops particularly wilt (*Fusarium* spp.), root rot (*M. phaseolina*, *R. solani*), collar rot (*A. niger*), stem rot (*S. rolfsii*), etc. which cause huge qualitative and quantitative crop yield losses. The importance of these bioagents in plant disease management has been realized to overcome the resistance against pesticides in the pathogens, production of agricultural commodities free of the pesticides in the wake of globalization of trade, checking of the pollution of hazardous chemical in the precious natural resources, etc. A galaxy of Bioagents has tremendous potential for production of cell wall degrading enzymes and useful secondary metabolites triggering systemic resistance in plants against plant diseases, enhancing crop growth mobilizing nutrients, control of nematodes, etc. However, there are some key issues related to the success of bioagents in crop disease management under changing climate and increasing demand of organic crop cultivation globally.

Looking to the multifaceted advantages of bioagents in plant health management, there arises a great demand of efficient bioagents by farmers. There are many issues and challenges for identifying and developing efficient quality species/ strains of Bioagents for commercial production to meet out the demand of users. There is agencies *viz.*, RKVY, DBT, DST, NFSM, State Govt. etc., which are funding for commercial production of quality bioagents and ICAR has sanctioned experiential learning units in SAU's to train their graduate to become entrepreneurs. To produce efficient and good quality Bioagents, there is need to address the issues and challenges as below:

a) Isolation and collection of native rhizosphere competent bioagents from different agroecological regions; b) Molecular identification of Bioagents by gene specific molecular markers c) Gene mining for important genes from efficient strains of bioagents for production of enzymes and antibiotics; d) Evaluation of biocontrol and crop growth promoting efficiency of bioagents in managing biotic i.e. pathogenic and abiotic i.e., drought, salinity, allelopathic, agrochemical residues stresses *in vitro* and *in vivo*; e) Compatibility with agrochemicals *viz.*, fungicides, insecticides, herbicides, etc; and other bioagents; f) Development of pesticides tolerance in efficient strains used simultaneously for management of seed and soil borne diseases; g) Testing efficacy and toxicity and registration with CIB & RC and Distribution of CIB registered strains for commercial production; h) Developing mass production technology under liquid fermentation for their longevity and quality formulations; i) Developing liquid and talc based formulations with enhance shelf life; j) Delivery system i.e. bio-priming of seeds and enrichment of FYM and organic cakes for synergistic action with different formulations; k) Organizing Workshops, Winter/Summer schools

for training the scientific community; l) Training for manpower to become entrepreneurs for various intricacies of developing and multiplying efficient bioagents; m) Creation of bio-resource complex for production, training and guidance of needy human resource; n) Establishment of collaborative research groups nationwide as well as globally for innovative outcomes.

LP (S7) 34

Indigenous technology knowledge as one of components of biological control under organic agriculture in India: An overview

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Crop diseases take heavy toll in agriculture. The estimated loss due to diseases alone ranges between 15 to 20% annually. The loss is more in perishable and storage lossess. The contamination by several storage fungi has often lead to aflatoxin production and food spoilage. This has lead to increased concern over food safety and security in India. The recent thrust on organic agriculture will answer all these questions. Diseases management strategies in organic agriculture aim at long term sustainable management strategies in holistic approach. Under organic agriculture traditional methods form the basis of management of plant diseases in low in put situations. The ancient Indian literature documents use of plant products, animal products and wastes for curing diseases of human beings and plants. The research efforts made on managing the diseases of banana, black pepper, tobacco and soybean are discussed in this paper.

LP (S7) 35

Nematode biocontrol need for popularization and commercialization

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Biological control is one of the best natural methods of keeping the population of a pathogen under control. This method may prove further effective against phytonematodes, because of the general soil borne nature and slow multiplication rate of soil nematodes. Among nematode biocontrol agents, parasitic and predacious fungi constitute the largest groups, numbering hundreds of species, importantly *Purpureocillium lilacinum*, *Pochonia chlamydosporia*, *Fusarium solani*, *Trichoderma* spp., *Nematoctona*, *Nematophthora* spp., *Arthrobotrys* spp., *Dactylaria* spp., *Monacrosporium* spp. etc. Plant growth promoting microorganisms

such as *Pseudomonas fluorescens*, *Aspergillus niger*, *Penicillium avamorii* and *Bacillus subtilis* may also contribute in the nematode management. Some fungi and a few bacteria have shown substantial potential in suppressing phytonematodes that warrants their exploitation for commercial use in agricultural fields. Commercial formulations of *A. niger*, *A. robusta*, *D. candida*, *P. lilacinum*, *P. chlamydosporia*, *P. fluorescens*, *T. harzianum* and *T. virens* have been developed and are being currently market largely for the management of plant diseases caused by fungi. Among bacteria, *Pasteuria penetrans* is an excellent biocontrol agent. It has a very wide host range and has been found to infect numerous nematode species, but strict obligate nature has limited its wide use in nematode management. Despite of availability of commercial formulations of atleast some of the above biocontrol agents in every district, the pesticide dealer as well as farmers are unaware of usefulness of these biopesticides in increasing the crop productivity that may occur through nematode suppression. Hence, oriented extension efforts through demonstration and visit programmes are required to make aware the farmers regarding the seriousness of phytonematodes and usefulness as well as need of biopesticides in the nematode management. Further, pesticide manufacturing companies should be encouraged to commercialize the formulation of nematode biocontrol agents, and to popularize the products at block/ village level.

Oral Papers

OP (S7) 49

Commercialization of *Trichoderma* based biopesticides in India

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Indian government is promoting research, production, registration, and adoption of biopesticides with open hand, through various rules, regulations, policies, and schemes. The National Farmer Policy (2007) has strongly recommended the promotion of biopesticides for increasing agricultural production, sustaining the health of farmers and environment. It also includes the clause that biopesticides would be treated at par with chemical pesticides in terms of support and promotion. Popularization of biopesticides is very slow as compared to chemicals and only 4% biopesticides are available in India. Till date, 34 microorganisms have been permitted by Central Insecticide Board (CIB) for commercialization in agriculture application in Gazette of India. Among the different biopesticides, *Trichoderma* is most exploited and have many success stories. Of late, many small and large entrepreneurs have entered into the commercial production of bio control agents resulting into the entry of various bio-control products into the national market. Some of the commercial formulations of *Trichoderma* Biosecure F, Bioguard, Funginil, Tricco, Echoderma, Trisul, Trichodermin-6. Commercialization of *Trichoderma* based biopesticide is a multi-step process involving a wide range of activities viz., Isolation and screening of antagonist, mass production and formulation

development, toxicology, industrial linkages, pot test and field efficacy, quality control and registration of bioagents. The success of *Trichoderma* based biopesticides to suppress diseases depends on the availability of *Trichoderma* as a product or formulation, which facilitate the technology to transfer from lab to land. The constraints to *Trichoderma* based biopesticides development and utilization mirror some of those factors that limit the development in India. The constraints includes lack of right screening protocol for the selection of promising candidate of *Trichoderma*, paucity of sufficient knowledge on the microbial ecology of *Trichoderma* and plant pathogens, optimization of fermentation technology and mass production of *Trichoderma*, inconsistent performance and poor shelf life, Lack of patent protection, prohibitive registration cost, awareness, training and education shortfalls, lack of multi-disciplinary approach and technology constraint. Despite the constraints, the Indian market for *Trichoderma* based biopesticides has been on a growth course as a result of considerable research infrastructure and constructive public support and policies. In total, at least 410 biopesticide production units have been established in India, 130 in the private sector. Demand of *Trichoderma* based biopesticide in India is anticipate to exhibit high growth in terms of both volume and value. The future research should focus on the aspects suitability of *Trichoderma* for control of the foliar/aerial pathogens, development of liquid/oil formulations suitable for foliar applications, formulations with prolonged shelf life, field persistence and suitable for dry weather conditions, scaling up of solid state production systems with Industry collaboration, large scale demonstration of biocontrol technologies in farmers' fields and fast track registration for better utilization of *Trichoderma* as a biocontrol agent for plant disease management. A determined effort of research institutions, universities, NGOs and government organizations is required to lift up the stature of *Trichoderma* based biopesticides. The present paper will discuss with about commercialization and registration issues of *Trichoderma* based biopesticides in India.

OP (S7) 50

Biological control in 21st century: Opportunities and challenges in subsistence farming system of India

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Biological control has appeared as the most acceptable alternatives of chemical disease management to combat the adverse effects of environment pollution, damage to below ground biodiversity, evolution of resistant pathogen population and health hazards in particular. Although several antagonists of fungal and bacterial origin have been reported along with their genomic sequence, only a few have been registered for commercialization. In the sphere of biological control *Trichoderma* and fluorescent pseudomonads are the most widely studied BCAs wherein 60% of the registered biofungicides belong to *Trichoderma* origin and in India alone, about 250 products are available for field application. The domain of biological control

encompasses exploitation of fungal and bacterial antagonists with the aim to kill pathogenic microbes (except viruses) and certain nematodes. They are also known to induce resistance against plant pathogens, impart abiotic stress tolerance, improve plant growth and vigor, solubilize plant nutrients, and bioremediate heavy metals and environmental pollutants, produce secondary metabolites of clinical significance and enzymes with widespread industrial application. It has been realized that the mixed inoculation of different strains having different mode of action would give better performance than the individuals, compatibility with other PGPMs are also required for absolute selection of bioinoculant consortia. It is also to be determined the growth induction, increase in systemic resistance in plants, microbial dynamics in different crop ecosystems in addition to physical and nutritional status of soil upon application of bioinoculants. The suitable mass production technology of the bioinoculants needs to be formulated keeping the view of their long term survival, ease in application and inclusion in value added chain. Establishment of district level evaluation and production centres under the supervision of Indian Council of Agriculture Research / State Agriculture Universities with the aid of financial subsidy required for registration may only fulfil the demand to some extent.

Session 8

**Farmers, producers, entrepreneurs, scientists and policy makers'
interaction to prepare road map on bioagents**

Oral Papers

OP (S8) 51

Experiential learning programme for preparing youth for entrepreneurship

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Successful commercialization of Ag-Biologicals hinges on the outcome of the development process and is often limited by a lack of knowledge and experience with Experiential Learning (EL) is a useful programme for the students for developing competence, capability, capacity building, acquiring skills, expertise and confidence to start their own business and turn into "Job Creators rather than Job Seekers". This is one of the steps towards "Earn while Learn". The Rural Awareness Works Experience (RAWE) motivates student in understanding the rural situations, technologies adopted by farmers, resolve farmer's problem and try to improve the work skill of the farmers. There is requirement for enriching the practical knowledge of the agriculture graduates. Hands-on training help students to turn laboratory experience in to reality. Student project work provides several opportunities to understand several aspects that cannot be taught in a class room or laboratory. For this purpose student READY programme has been launched by ICAR in order to provide such opportunities to the graduates of agricultural science. There are several scope for agriculture students to develop entrepreneurship for their bright future. Agricultural entrepreneurship contributes towards country's economic development by creating employment for the unemployed in direct and indirect ways, improving nutrition, and contributing to food security. Earning money from an agricultural entrepreneurship requires knowledge of farming practices should have some experience in developing a business. Some of the most profitable agriculture business ideas which can help entrepreneurs in deciding their preferred business to start with are bio-control agent production and botanical pesticide production. The increase trend of organic farming and requirement of residue free agricultural products will lead to increase in demand of biopesticides by the farmers. In India, biopesticides viz. *Trichoderma*, *Bacillus*, *Pseudomonas* etc. represent only 4.2% of the overall pesticide market and is expected to exhibit an impressive annual growth rate of about 10% in the coming years. Hence, there is huge opportunities for students to develop their own business after gaining knowledge and training through experiential learning programme.

OP (S8) 52

Knowledge management for stimulating new research on microbial antagonists

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The increased demand for healthy and safe food by consumers have led to constant efforts over the years for improving crop health through new innovative technologies and pest management strategies. Due to this "Biopesticides" mainly microbial antagonists have become an important component of IPM strategies for plant protection due to their capability of maintaining the food safety. All our research, commercial production, field applications and publications, etc. are heavily focused on only a few of these organisms for over 7 to 8 decades. About 90% of the microbial biopesticides are derived from just one entomopathogenic bacterium, *Bacillus thuringiensis*. More than 200 products are being sold in the US market, compared to only 60 comparable products in the EU. More than 225 microbial biopesticides are manufactured in 30 OECD countries. The NAFTA countries (USA, Canada, and Mexico) use about 45% of the biopesticides sold, while Asia lacks behind with the use of only 5% of biopesticides sold worldwide. Therefore, there are lot of opportunities for their expansion and exploration of new technologies and products. In order to achieve this, an understanding of the challenges which are limiting their expansion is required vis-à-vis interventions are also required to stimulate the research work for development and testing of new products. The experiences shared by different actors in this field shows that there had been challenges to develop a formulation and application method that can not only be implemented commercially but at the same time must be effective, reliable, consistent, economically feasible and with a wider spectrum. There has been a limited use of microbial antagonists for pathogens. Continual laboratory works followed by field experiments are needed to establish effective biocontrol agents particularly against plant fungal pathogens. The absence of experience and knowledge sharing with regard to production technology and different ongoing initiatives has also been the major factor contributing to duplicacy of efforts whereas also been time consuming. Even the duplication of research is happening within the same country and across the countries. The knowledge management platform/s are lacking and their development can be instrumental to share the country specific experiences and lessons learnt to strengthen partnerships link which is critical for broadening initiatives. Sharing of good practice examples through these platforms would also be key in guiding new interventions hence promoting partnerships. It would further enhance the development pipeline and shorten the timelines, thus allowing introduction of advanced biopesticides in the local as well as international markets.

OP (S8) 53

Entrepreneurial efforts for popularization of bioagents

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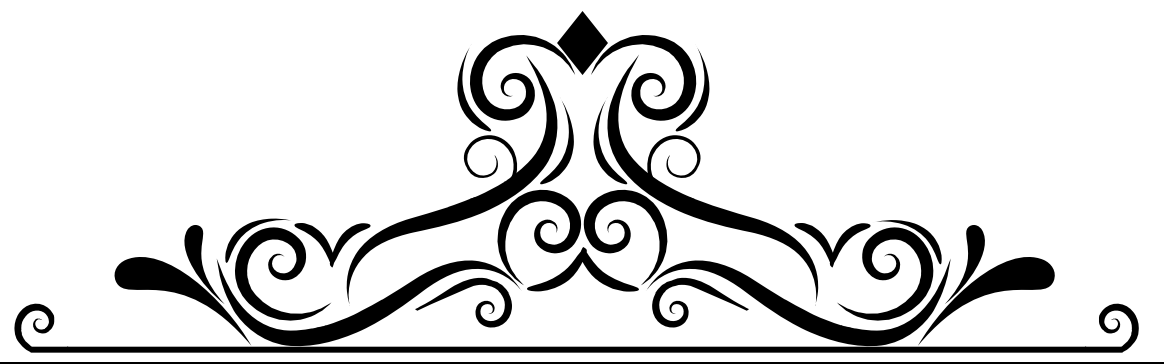
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Biocontrols are increasing popularity because of demand for low-residues and organic foods, heightened focus on environmental protection, desire to preserve biodiversity, a revival of academia's interest in biocontrol and interest from the investment community and traditional plant protection companies. The commercial success of the bioagents formulations depends up on the bioefficacy of bioagents, shelf life and quick knock down effects and cheapest multiplication on the suitable readily available and economical substrate. Screening of efficient strains isolated from natural sources. Bioagents further development w.r.t. high temperature, saline, alkaline tolerance, development of fungicide-insecticide-biofertilizer compatible strains as well as cost effective at mass production. Development of innovative thermostable, long shelf life, sustained release polymer matrix based bioformulations for better efficacies and wide adaptability on different agro climatic conditions. Development of consortia formulation of *Trichoderma*, *Paecilomyces*, *Bacillus* and *Pseudomonas* to achieve broad-spectrum activities of biofungicides against wide range of diseases in single application to save time and cost to the farmers. Development suitable formulation for drip compliant system and user friendly formulation for large scale application in short time to save labour cost to farmers.

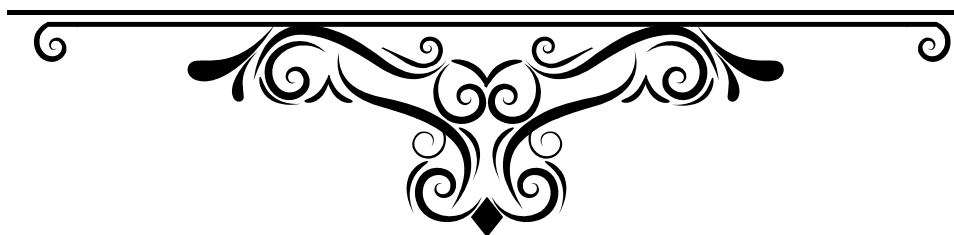
Registration guideline of formulations with Central Insecticide Board authorities need to be defined clearly for hassle-free, easy to adopt and time consuming registration process. Queries, if any, related to product registration from CIB, it should be informed to respective companies immediately with necessary guideline. In addition, representatives from industry needs to be included in the registration committee to appraise difficulties in implementing various existing protocol from CIB guideline. Unless regulators (Central Insecticide Board) more quickly to amend the registration process for biocontrols in India, farmers will not have the tools they need to control pest-disease pressures. Development of stringent quality-control and quality-assessment protocols at per CIB rules and regulations to prevent non-registered manufacturers. Single window and centralized sale permission of biopesticides to avoid permission from state wise authorities to ease time consuming distribution process all over India. Audit of biocontrol and PGPR agent must be done by state or central level committee to avoid unauthorised manufacturer. Uniform bio-efficacy charges across universities under ICAR incorporating product testing as a part of routine departmental activities instead of separate project.

At the end it can be concluded that there are tremendous scope for basic fundamental research on development of user friendly formulations and extension activities for enhancement of biointensive, environmentally sound, economically viable, user friendly, socially acceptable disease and resistance management systems with the help of biocontrol agents. Guide lines for registration and hassle-free registration as well uniform polices across the organization needs to be in place. Industry-universities/

institution interaction needs to be strengthen to encash the research findings toward making commercially feasible technology for farming communities. Biocontrol could represent a new green revolution without pesticides residue by understanding how biological products interact with plants and pathogens. There is an opportunity for us to change the way of agriculture works around the world. Increasing awareness of use of bioagents program at farmers and easily available quality product of bioagents at village level.



POSTER PAPERS



Session 1

Ecology, biodiversity and taxonomy of antagonists/ bio-agents

PP (S1) 01

Biodiversity and biocontrol potential of cold adapted yeasts: A conceptual note with special reference to biological control of post-harvest diseases

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Post harvest diseases cause considerable economic losses to harvested fruits and vegetables in the supply chain. The use of synthetic chemicals/fungicides is the primary approach to combat post harvest decay. However, in the recent trend towards safer and eco-friendly alternatives for the management of post harvest diseases the microbial antagonists are gaining momentum. Among different microbial antagonists reported against post harvest diseases the cold adapted yeasts attract considerable attention as they offer distinct advantage over other antagonists and prevent post harvest decay during storage in cold storage units. Numerous cold adapted yeasts have been isolated from cold environments/cold stored commodities and their biocontrol efficacy against decay pathogens under cold storage conditions is well established. *Aureobasidium pullulans*, *Candida sake*, *Cryptococcus albidus*, *Dabaryomyces hansenii*, *Pichia guilliermondii* and *Rhodotorula glutinis* are the important cold adapted antagonistic yeasts isolated from natural cold habitats. Nutritional competition, rapid colonization in the cold, synthesis of cold active lytic enzymes and induction of resistance in harvested commodity are the important strategies of antagonistic yeasts to suppress post harvest diseases. At international level different yeast, based bioproducts *viz.*, Aspire, Yield Plus, Shemer, Candifruit, Nexy and Boni-Protect have been developed and registered for use. But, this field of study is still in its infancy in India and it is necessary to explore the potential use of antagonistic yeasts in order to increase the potential of biocontrol as a viable alternative to synthetic chemicals.

PP (S1) 02

Arbuscular mycorrhizal fungi used to induce plant disease resistance: A review

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The majority of plants live in close association with a diversity of soil organisms among which arbuscular mycorrhizal fungi (AMF) play an essential role. In present years, the induced disease resistance of plant by Arbuscular Mycorrhizal Fungi (AMF) has become an important in chemo-ecological study and in bio control of plant disease and much information indicating that AMF had antagonistic function to soil borne disease pathogen, or could repress the growth of pathogen, and increase the resistance or tolerance of mycorrhizal plants to soil borne disease. In mycorrhizosphere, there are interactions among microbial community, in which, AMF could suppress the growth of pathogen and promote the growth of beneficial microbe. AMF may use as biocontrol fungi with other antagonism microbes. In future the use AMF as a new biocontrol method to control soil borne disease in ecofriendly-agriculture and also to promote agricultural productivity.

PP (S1) 03

Occurrence, distribution and characterization of native *Trichoderma* spp. from Latur district

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Trichoderma spp. are promising antagonistic organisms and are one of the best alternatives to manage soil borne pathogens. In present study, 18 rhizosphere soil samples were collected from Latur district and subjected to isolation on PDA, by serial dilution and plating technique. Result revealed that of the 18 soil samples, 10 plates exhibited typical cultural growth of *T. harzianum* and rest 8 plates exhibited typical growth of *T. hamatum*, and their percentages were 55.56 and 44.44, respectively. Based on cultural, morphological and microscopic features, the most predominant species identified were *Trichoderma harzianum* and *Trichoderma hamatum*. Among *T. harzianum* isolates, pale green colony colour was exhibited by six isolates (Thr-1, 3, 4, 6, 8 and 9) and rest four isolates (Thr-2, 5, 7 and 10) had yellowish

green to dark green colony colour. Colony colour of *T. hamatum* four isolates was dark green (Thm-1, 2, 4 and 7), two isolates (Thm-3 and 6) had yellow to olive green colony colour and rest two isolates (Thm-5, 8) exhibited yellow to pea green colony colour. Phialides shape and their branching patterns of the test isolates of *T. harzianum* were globose, ampuliform and irregularly branched, narrow; whereas, of the isolates of *T. hamatum*, phialides shape was ellipsoidal to ovoidal and their branching pattern was undulate, regularly branched.

Molecular applications in characterization of bio-agents

PP (S2) 04

DNA fingerprints of *Trichoderma* isolates and their biocontrol potentials against *Aspergillus niger* van Tieghem

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The most common biological control agents of the genus *Trichoderma* have been reported to be strains of *Trichoderma virens*, *T. harzianum* and *T. viride*. Since *Trichoderma* uses different mechanisms of bio-control and varies with different strains or isolates, it is very important to identify proper *Trichoderma* isolates against specific plant disease for their practical use in agriculture. Random Amplified Polymorphic DNA (RAPD) was used to examine the genetic identity among twelve isolates of *Trichoderma* representing three strains (*T. virens*, *T. harzianum* and *T. viride*), and their ability to antagonize *Aspergillus niger* van Tieghem causing collar rot in groundnut. One hundred and three of the 108 bands, using random decamer fungal primers, were polymorphic with an average frequency of 11.4 bands. The primer index showed that Rfu C-5 gave best results of polymorphism among the primer used in the experiment. The RAPD analysis showed 10 marker loci for diagnosis of *T. viride* 60 and/or *T. harzianum* 2J, first two highest inhibitory acting antagonists. A UPGMA dendrogram constructed on the basis of Jaccard's similarity coefficient using NTSYS 2.2 program which illustrated two distinct clusters of 12 isolates of *Trichoderma* and *A. niger* pathogen, and shared only 19% similarity. However, the *in vitro* highest *A. niger* growth inhibitory *Trichoderma* isolates – *T. viride* 60 (86.2%) and *T. harzianum* 2J (80.4%) were in same out group and shared 63% similarity.

PP (S2) 05

Application of molecular tools for enhancement of biocontrol agents

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During a green revolution era, chemical pesticides played a major role in increasing production and productivity through reducing losses due to pests and diseases. However, the continuous use of pesticides raised several harmful concerns, including residual pesticides, their phytotoxicity and environmental pollution due to them and human health hazards that followed them. Biocontrol agents are the good and eco-friendly mandatory tool for disease control. Till up to date, only few biocontrol agents have been efficiently applied because of largely unstable under diverse environmental conditions. This emphasized the need for evolving a superior biocontrol agent that could withstand many adverse conditions and still perform well to reduce pathogen loads. Furthermore, the desirable properties of a given biocontrol agents are not determined by a single character; on the contrary desirable biocontrol behavior of the agent is decided by its competitive ability, nature of antibiosis involved, lysis of the target pathogen and induced systemic resistance of the host plant. Yet a comprehensive knowledge of the nature of genes involved in biocontrol mechanism and molecular mechanism of biocontrol properties of the agent is still elusive. Detail information of genes involved in biocontrol properties of a biocontrol agent with respect to individual mechanism of action is very essential for their improvement. The advancements in biotechnology have opened up several new avenues in biocontrol research and development. Recently, a considerable degree of attention has been devoted towards the identification of useful genes that control biocontrol activity which in turn helps in evolving a superior biocontrol agent. Several approaches have been made towards achieving the following goals: i) Identification and characterization of genes encoding the specific biocontrol property against a given pathogen and ii) Designer biocontrol strains with potential genes responsible for superior biocontrol properties. Characterization of the genes involved in biocontrol property has been predicted using targeted gene sequences analysis. In this technique the sequence specific primer are used in PCR for characterization. Expressed sequence tags are another tool for characterization and study of the efficiency of particular strain of bioagent. The whole genome sequence of different bioagents provides the molecular mechanism of biocontrol properties. Protoplast fusion and genetic engineering are the new approaches for improvement of biocontrol agents. Therefore, biotechnological tools are good for characterization of bioagents as well as for their improvements.

PP (S2) 06

Molecular characterization of microflora isolated from fenugreek rhizosphere and their antagonistic effect against *Fusarium oxysporum* Schlecht *in vitro*

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Fenugreek (*Trigonella foenum-graecum* L.) is an important spices crop, which was found to be suffering from wilt disease in Gujarat state, (India). Now a days in Saurashtra region of Gujarat state fenugreek wilt caused by *Fusarium oxysporum* Schlecht. is a major problem. The microflora was isolated from the rhizosphere of fenugreek wilted plants from different locations by serial dilution method. Isolated microflora was identified by proper molecular tools. The antagonistic potentiality of selected microflora were tested against pathogen *Fusarium oxysporum* *in vitro* condition. Among fungal isolates, *Trichoderma viride* was found most effective and gave 75.89 per cent growth inhibition of pathogen. Among bacterial isolates, the maximum growth inhibition (36.60 per cent) of pathogen was found in *Providencia rettgeri*.

PP (S2) 07

Significance of molecular techniques in identification and characterization of *Trichoderma* species: An antagonist against soil borne pathogens

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Trichoderma species is one of the primary potential biocontrol agents against soil borne plant pathogens, which work by means of mechanism such as mycoparasitism, antibiosis, enzyme secretion and competition. Several potentially useful strains of *Trichoderma* found in the rhizosphere soil are difficult to distinguish from other strains which make a constraint in its exploitation as wide application. An important component for the development of a fungal antagonist is selection of the most effective isolate for disease management. Identification of isolates to species level is difficult and confusing due to the complexity and closely related characters of the species. Precise and accurate identification will encourage its commercial application against soil borne plant pathogens. Identification based on morphological means is not sufficient and not authenticate to distinguish *Trichoderma* species. Thus, there is a need to find ways to examine those useful unidentified strains for its application to natural pathosystems. Identification of *Trichoderma* species requires a complex molecular biology based identification involving amplification and sequencing of multiple genes. The internal transcribed spacer (ITS) and *tef-1* regions of ribosomal DNA are the most reliable method for identification of *Trichoderma* species. Besides, among the molecular

techniques, Random amplified polymorphic DNA (RAPD) markers, sequence characterised amplified region (SCAR), Inter simple sequence repeats (ISSR) markers may be successfully used for identification purposes, estimation of genetic variation among different strains of *Trichoderma species* and it can clearly distinguish strain from other closely related *Trichoderma* strains. Strains belonging to *Trichoderma* species which are pathogenic to cultivated mushrooms can be eliminated by these methods. Moreover, molecular markers offer a means of creating quality control tests which are essential throughout the developmental processes of these useful biocontrol agents. It provide a quick method for the identification of most useful superior species of biocontrol agents which will make sure in its commercial application for managing several soil borne plant pathogens.

**Secondary metabolism of the bioagents in crop growth
promotion through host interactions**

PP (S3) 08

**Managing oxidative stress in chickpea (*Cicer arietinum* L.) through
Trichoderma enriched FYM**

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Trichoderma spp. Are endophytic plant symbionts that are widely used as seed treatments to control diseases and to enhance plant growth and yield. Although their abilities to alleviate abiotic stresses, specific knowledge of mechanisms, abilities to control multiple plant stress factors, their effects on seed and seedlings is studied moreover. The effect of *Trichoderma viride* singly and *Trichoderma* enriched FYM were observed and recorded in increasing seedling vigor and ameliorates stress by inducing physiological protection in plants against oxidative damage. The highest seed germination of 72.78 per cent, seedling shoot length of 10.23 cm, root length of 8.06 cm, vigour index of 1331.14, and growth parameters i.e. the root length of 14.33 cm, shoot length of 26.03 cm, no. of branches 4.86, pods per plant 24.90, seed yield 891.44 kg/ha and dry matter weight per plant of 8.95 gm were recorded with seed treatment of *T. viride* +*Trichoderma* enriched FYM as compared to *T. viride* singly, oxidative stress control and untreated control. The induced systemic resistance (ISR) activity was tested by estimating peroxidase (POD) and polyphenol oxidase (PPO) activity in chickpea plant roots at 7, 14, 21, 28 and 35 DAS where maximum POD activity of 3.100 Δ OD/min/ g tissue at 7 DAS, 3.507 Δ at 14 DAS, 4.077 Δ at 21 DAS, 3.460 Δ at 28 DAS and 2.973 Δ at 35 DAS were recorded in seed treatment of *T. viride* +*Trichoderma* enriched FYM as compared to *T. viride* singly, oxidative stress control and untreated control. Similarly, maximum PPO activity i.e. 0.836 Δ OD/min/ g tissue at 7 DAS, 0.921 Δ at 14 DAS, 1.123 Δ at 21 DAS, 0.957 Δ at 28 DAS and 0.803 Δ at 35 DAS were recorded in seed treatment of *T. viride* +*Trichoderma* enriched FYM and other treatments including both the controls.

PP (S3) 09

Induction of growth and defence in *Glycine max* by *Serratia marcescens*

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Plant Growth Promoting Rhizobacteria (PGPR) have important role in sustainable crop production. PGPR not only have a capacity to improve plant's health, but also help to maintain soil fertility. Usage of chemically synthesized fertilizers may cause some deleterious effects on health of plants as well as humans. Our study is focused on isolating plant growth promoting organism from the rhizosphere of soybean (*Glycine max* L.). It is a number one oilseed crop produced across the world but it gets infected by many phytopathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Pythium* spp., *Phomopsis*, etc. In our studies, total 45 bacteria were isolated and their morphological and biochemical characteristics were studied. Among them five isolates showed most promising PGP characteristics. MN12, ML2, ML4, MP3, MR1 are isolates which produced IAA, ammonia, solubilises nutrients in soil such as phosphate, potassium and zinc and produces hydrolytic enzymes such as protease and cellulase. Seed germination and pot assay studies were carried out to evaluate In vitro and in vivo growth promoting characteristics of these isolates in presence or absence of its phytopathogens *Fusarium oxysporum*. Among these five isolates MP3 showed promising plant growth promotion in pot assay. MP3 was identified using BLAST analysis of its 16s rRNA gene sequence as *Serratia marcescens*. *S. marcescens* was also found to induce PAL, PPO, PO, catalase, lipoxygenase, defence related enzymes in *F. oxysporum* infected Soybean plants.

PP (S3) 10

Signalling molecules of microbes for plant growth and development

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Plants are sessile, multicellular organisms, generally rely on developmental and metabolic changes for growth by tight coordination of the spatial and temporal organization of cell division, cell expansion and cell differentiation. The anatomical configuration of the root, stem and shoot systems to build a plant is known as plant architecture and it is crucial factor in the agronomic success of crops also vital consideration for plant breeders. Orchestration of these events requires the exchange of signalling molecules between the root and shoot, which can be affected by both biotic and abiotic factors. Fungal and bacterial species are able to detect the plant host and initiate their colonization strategies in the rhizosphere by producing

canonical plant growth-regulating substances such as auxins or cytokinins. While other side plants are able to recognize microbe-derived compounds and adjust their defense and growth responses according to the type of microorganism encountered and estimated that between 20 to 40% of all photosynthetically fixed carbon is eventually transferred to the rhizosphere. The interactions that occur between plants and their associated microorganisms could lead to the development of novel agricultural applications, like root-secreted malic acid recruits the beneficial soil bacteria *Bacillus subtilis* to the root and this interaction plays a role in plant protection against the foliar pathogen *Pseudomonas syringae*. Elicitors are molecules involved in plant defense responses, many of them are directly derived from beneficial or pathogenic microbes. Exogenous application of defense signaling molecules, such as salicylic acid, methyl jasmonate and nitric oxide induces the accumulation of a wide range of secondary metabolites including indole glucosinolates, phytoalexins and alkaloids, which may play a role in communication with microbial populations. Plants produce a wide range of organic compounds including sugars, organic acids and vitamins, which can be used as nutrients or signals by microbial populations while other side microorganisms release phytohormones, small molecules or volatile compounds, which may act directly or indirectly to activate plant immunity or regulate plant growth and morphogenesis. In the identification of signals from free-living bacteria and fungi that interact with plants in a beneficial way. Classic plant signals such as auxins and cytokinins can be produced by microorganisms to efficiently colonize the root and modulate root system architecture. Other classes of signals, including N-acyl-L-homoserine lactones, which are used by bacteria for cell-to-cell communication, can be perceived by plants to modulate gene expression, metabolism and growth. The role played by volatile organic compounds released by certain plant growth-promoting rhizobacteria in plant immunity and developmental processes. The picture that emerges is one in which plants and microbes communicate themselves through transkingdom signaling systems involving classic and novel signals.

PP (S3) 11

Bioagents and non-conventional mediated induced resistance in chickpea cultivars against *Rhizoctonia bataticola* causing root rot disease

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A pot culture study was undertaken on chickpea resistant (SAKI-9516) and susceptible (Chaffa) cultivars to ascertain the induction of root rot disease resistance through biochemical analysis of total protein, accumulation of phenols and activities of peroxidase, polyphenol oxidase and chitinase at 14, 28 and 42 days after sowing. For which, the respective chickpea cultivars were treated with *Pseudomonas fluorescens* pf1 strain @ 10 g/kg seed, *Trichoderma viride* @ 4 g/kg seed and Thirum + Carbendazim @ 2:1 g kg⁻¹ seed. The seeds of different cultivars were also soak in non-conventional chemical (10⁻³ and 10⁻⁶ M) viz.; salicylic acid, cycloheximide, cycocel and IAA. According to the results, per cent root rot in resistant cultivar (SAKI-9516) is comparatively less than susceptible cultivar (Chaffa) during different days interval.

Seed treatment of *T. viride* @ 4 g/kg seed recorded minimum root rot incidence i.e. 8.88 per cent in resistant cultivar and 21.1 per cent in susceptible cultivar. Levels of total soluble protein in all induction treatments of non-conventional chemicals and bioagents, increased with increasing time of *R. bataticola* infection in both susceptible and resistant cultivars.

PP (S3) 12

***Trichoderma* volatiles in plant growth enhancement**

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Trichoderma species are the widely exploited bioagents against soil borne pathogens. They are known for their plant growth promotion via induced systemic resistance, antibiosis, enhanced nutrient efficiency and myco-parasitism. Many metabolites of *Trichoderma* have antifungal, antibacterial, and anti cancerous properties, hence of medical and agricultural importance. Induced resistance to plant pathogens has been reported with volatile metabolites of *Trichoderma* leading to improved plant health. Volatile metabolites, also known as volatile organic compounds (VOCs), are low molecular mass compounds with high vapor pressure (>0.01 kPa), low boiling point, and low polarity. These metabolites are diverse chemically and can be hydrocarbons, aromatics, amines, thiols, and terpenes. Few secondary metabolites such as peptaibols and polyketides are known for inducing resistance to plant pathogens; or as toxins. The coconut odor volatile, 6-pentyl-2Hpyran-2-one (6PP), an earliest characterize volatile compound from *Trichoderma* was reported to have effects on plant growth. Direct application of 6PP solution to plant leaves or addition of 6PP (0.166–1 mg/l) to plant growth media induced growth promotion and reduced disease symptoms. Exposure of plants to *Trichoderma* strains resulted in much larger size and more green plants. Such improved growth characteristics were observed with the exposure of model plant, *Arabidopsis thaliana* to *T. viride* -derived VOCs too. Increased plant size, fresh weight, root growth, chlorophyll, and number of flowers were noticed even in the absence of pathogen threat. The volatile-mediated plant growth promotion was dependent on *Trichoderma* species, culture, developmental stage of the plants, and duration of the exposure. Characterization of potential volatile and non-volatile metabolites from antagonistic *Trichoderma* spp. can be done with gas chromatography/mass spectrometry (GC/MS) metabolite profiling and foot printing.

Session 4

**Bioagents/ antagonists in integrated management of
biotic and abiotic stresses of crops**

PP (S4) 13

Biopriming of tomato seed for the management of damping-off disease

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Biopriming is a new technique of seed treatment that integrates biological and physiological aspects (seed hydration) of disease control. It is recently used as an alternative method for controlling many seed and soil borne pathogens. It is an ecological approach using selected fungal antagonists against the soil and seed borne pathogens. Biopriming has great promise for enhancing the efficacy, shelf life, and consistent performance of biological control agents. During 2014 *in vitro* as well as field trial was conducted for the plant growth promoting activity of tomato seed and management of damping-off of tomato disease respectively. Plant growth promoting activity was found highest in biopriming of tomato seed with *Trichoderma harzianum*@10gm/lit. water containing 10⁸ cfu/ml, seed germination (95.33 %), shoot length (6.33 cm) and root length (10.64 cm) and root colonization (6.33 x 10⁴ cfu/ml). In biopriming of tomato seed with different bioagents, seed biopriming with *T. harzianum* @10gm/kg seed containing 10⁸ cfu/gm (13.72 %) was found significantly superior over the rest and reduced damping-off tomato.

PP (S4) 14

Biological management of gray leaf blight disease on mango under green house

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Mango (*Mangifera indica* L.), an important fruit crop grown in grown tropical and subtropical regions of south-east Asia. In India, it is cultivated in the states of Uttar Pradesh, Andhra Pradesh, Karnataka, Bihar, Gujarat, Tamil Nadu, Orrisa, West Bengal, Jharkhand, Kerala and Maharashtra. In Gujarat it is

cultivated over an area of about 130.1 thousand hectares with annual production of 911.3 thousand tones with productivity of 7.01 tones/ha. Considering the seriousness of the problem of gray leaf blight on mango caused by *Pastelotia anacardii* the biological control is an effective, ecofriendly and alternative approach for any disease management practice. Antagonists are well known for their potentiality against various diseases causing pathogen the present investigation was carried out to find out suitable biological agents for the management of disease. Among them minimum per cent disease intensity was observed in *Trichoderma viride* (11.24 %) followed by *T. harzianum* (14.21%) and *Pseudomonas. fluorescens* (21.12%) and maximum per cent disease control (74.07%) was also observed in *Trichoderma viride*, which was found superior over rest of the treatments and were found promising as potential biological control agents against grey leaf blight of mango.

PP (S4) 15

Efficacy of tebuconazole alone and in combination with bio control agents in management of groundnut disease

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A field experiment was conducted to know the comparative efficacy of tebuconazole alone and in combination with biological control agent for controlling stem rot, collar rot, leaf spot and rust diseases of groundnut at Main Oilseeds Research Station, Junagadh Agricultural University, Junagadh during *kharif* 2013-14 to 2015-16. The collar rot (1.84%) and stem rot (1.27%) incidence were recorded significantly lowest in seed treatment with tebuconazole 1.5g/kg seed + two spray of Tebuconazole @ 1ml/L. Whereas the lowest late leaf spot (23.82%) and rust (21.57%) disease was recorded in seed treatment with *T. viride* @ 10g/kg seed + furrow application of *T. viride* at the time of sowing @ 4 kg enriched in 50 kg FYM/ha + Broadcasting *T. viride* @ 4 kg enriched in 50 kg FYM/ha at 40 DAS + two spray of tebuconazole @ 1ml/L, starting from the initiation of foliar diseases and 2nd spray at 15 days interval. The maximum pod (2230 kg/ha) and haulm yield (5256 kg/ha) were recorded in treatment with tebuconazole and combinations with biocontrol agent treatments.

PP (S4) 16

Efficacy of biocontrol agents against *Colletotrichum coffeanum* causing die back of okra

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Okra is an important vegetable crop of the tropical countries and most popular in India. Okra (*Abelmoschus esculentus* L.) also known as 'Lady's finger' in English and "Bhendi" in Hindi belongs to family Malvaceae. It is generally grown in summer and rainy season. Its wide climate and soil adaptability makes it popular among vegetable growers. Okra suffering from many diseases, among them die back of okra is an important disease. This disease is caused by *Colletotrichum coffeanum*. Evaluation of different biocontrol agents against *Colletotrichum coffeanum* under *in vitro* condition, for find out effective biocontrol agents. Testing of six bioagents, four species of *Trichoderma*, *Aspergillus niger* and *Pseudomonas fluorescens*. *Trichoderma viride* was reported maximum growth inhibition of *Colletotrichum coffeanum* (66.62%) followed by *Trichoderma harzianum*(65.89%)and *Pseudomonas fluorescens* (64.52%).

PP (S4) 17

Efficacy of bioagents against foliar fungal pathogens of green gram

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Green gram suffers from many diseases caused by fungi, bacteria and viruses. Among the fungal diseases, Alternaria leaf spot (*Alternaria alternata*), Phoma leaf spot (*Phoma medicaginis*) and Macrophomina leaf blight (*Macrophomina phaseolina*) are important fungal diseases. Investigation on management of foliar fungal pathogens of green gram with fungal and bacterial bioagents *in vitro* was carried out during 2016-2017. Maximum mycelial growth inhibition (85.33%) of *Macrophomina phaseolina* (*Rhizoctonia bataticola*), *Alternaria alternata* (78.33%) and *Phoma medicaginis* (71.94%) were observed with *Trichoderma viride* followed by *Pseudomonas fluorescens* and *Bacillus subtilis*.

PP (S4) 18

Role of *Aspergillus* isolates as a biocontrol agent

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In the present study, soil samples collected from different locations exhibited 9 isolates of two genera capable of degrading chitin on colloidal chitin agar media. Based on morphological and cultural characteristics isolates were categorised as four of *Aspergillus niger* (AN1 to AN4) and five of *Aspergillus flavus* (AF1 to AF5). All the isolates were found effective against *Fusarium udum*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* when tested by dual culture technique. The isolates AN3 of *Aspergillus niger* from Chandur and AF4 of *Aspergillus flavus* from Borgaon exhibited maximum antagonistic activity among their genera against *F. udum* inhibiting the pathogen by 84.28 and 87.63, per cent respectively. *R. bataticola* was inhibited by 69.62 and 82.07 per cent respectively by AN3 and AF5. In case of *S. rolfsii* AN3 and AF5 exhibited maximum percent growth inhibition i.e. 69.61 and 81.05 percent respectively. All isolates of *A. niger* could grow by more than 75% of control due to insecticides and weedicides. Fungicide Carboxin + Thiram and Thiram alone inhibited the growth of *A. niger* by approximately 90% in all isolates. *A. flavus* isolates were inhibited by 100% due to Carboxin + Thiram and Carbendazim. Overall insecticides and weedicides were less toxic to the all the isolates compared to fungicides.

PP (S4) 19

Management of collar rot of groundnut caused by *Aspergillus niger* van Teighem in *in vitro* and *in vivo* conditions

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Groundnut (*Arachis hypogaea* L.) is a very important legume and oilseed crop grown in India. Collar rot is one of the most important disease of groundnut in Karnataka caused by *Aspergillus niger* van Teighem. Pathogen is a well-known polyphagous, ubiquitous, non-target and the most destructive soil and seed inhabiting fungus reported so far. To manage these disease twenty different fungicides, thirteen bioagents and ten botanicals were evaluated in lab condition and different fungicide treated seeds were evaluated in field conditions. Systemic fungicides viz., propiconazole, tebuconazole, fluzilazole and combi-product fungicides viz., fluzilazole 12.5% + carbendazim 25% SE recorded 100 per cent mycelial growth inhibition at all concentrations. Among non-systemic fungicides chlorothalonil showed maximum per cent

inhibition of 64.63 at 3000 ppm concentration. *Bacillus subtilis* P-21, showed maximum mycelial inhibition of 84.81 per cent, among fungal bioagents evaluated maximum inhibition per cent of 66.48 was recorded by *Trichoderma harzianum*– 16. Neem and garlic recorded maximum mycelial inhibition per cent of 45.18, 51.33, 63.66 and 51.66, 55.33, 60.0 at concentration of 5, 10, 15 per cent, respectively. Tebuconazole seed treatment recorded lowest disease incidence of 7 per cent with highest pod yield of 12.8 q/ha and haulm yield of 31.6 q/ha.

PP (S4) 20

Antagonistic action of fungal biocontrol agents against *Rhizoctonia solani* Kühn under *in vitro* condition

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Coriander (*Coriandrum sativum* L.) is a major *Rabi* spice crop of South Saurashtra region known to affect by root rot disease caused by *Rhizoctonia solani* particularly in Junagadh district of Gujarat state, India. To study the antagonistic action of different fungal biocontrol agents viz, *Trichoderma harzianum*, *T. virens*, *T. koningii*, *T. viride* and *T. hamatum* against *R. solani*, an experiment was conducted at the Department of Plant Pathology, Junagadh Agricultural University, Junagadh during *Rabi* 2016-17 by adopting dual culture technique. The results indicated that different fungal biocontrol agents proved effective against mycelial growth inhibition of *R. solani* under laboratory conditions. Among five fungal biocontrol agents tested, *Trichoderma harzianum* showed significantly the maximum mycelial growth inhibition of 66.10% and was remained statistically at par with *T. viride* (64.36%) and *T. koningii* (63.03%). While, *T. hamatum* (53.91%) and *T. virens* (50.36%) found comparatively poor in inhibiting the mycelial growth of *R. solani*.

PP (S4) 21

Management of groundnut major disease by tebuconazole alone and in combination with bio-control agent and their impact on yield

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A field experiment with nine treatment combinations of chemical and bio control agent was carried out at Main Oilseeds Research Station, Junagadh Agricultural University, Junagadh during three year *kharif*: 2013-14 to 2015-16. Result revealed that the collar rot (2.64%) and stem rot (1.27%) incidence was minimum in Tebuconazole 1.5 g/kg seed treatment and two spray of same fungicide at 15 days interval. Late leaf spot (23.82 %) and rust (21.57%) severity were minimum in seed treatment with *T. viride* @ 10g/kg seed, furrow application of *T. viride* at the time of sowing @ 4 kg enriched in 50 kg FYM/ha, Broadcasting *T. viride* @ 4 kg enriched in 50 kg FYM/ha at 40 DAS, accompanied by 2 spray of Tebuconazole @ 1ml/lit at 15 days intervals. Whereas maximum pod (2230 kg/ha) and haulm (5256 kg/ha) yield recorded in Tebuconazole 1.5g/kg seed, furrow application of *T. viride* at time of sowing @ 4 kg enriched in 50 kg FYM/ha same was enriched and applied at 40 DAS and two spray of Tebuconazole @ 1ml/lit at fortnightly intervals.

PP (S4) 22

Biological control of castor root rot incited by *Macrophomina phaseolina* (Tassi) Goid

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Castor (*Ricinus communis* L.) is an important non-edible annual oilseed crop of India. The root rot disease of castor incited by *Macrophomina phaseolina* (Tassi) Goid cause considerable crop loss and become major hurdle in production. As it is soil born pathogen, it is very difficult to control with only chemical. Seven different fungal and bacterial isolates were evaluated for their biocontrol potential against *M. phaseolina* under *in vitro* study (dual culture method). Among them *Trichoderma viride* (74.40%) showed maximum growth inhibition and followed by *T. harzianum* (72.41%). Sclerotial formation was also absent in *T. viride* and *T. harzianum*. While bacterial bioagent *Pseudomonas fluorescens* showed 67.27 %

growth inhibition. *T. virens* and *T. hamatum* were moderately effective to inhibit fungal growth. Similarly, moderate sclerotial formation and least growth inhibition were observed in *T. koningii* and *B. subtilis*. Among all the antagonists *T. viride* and *T. harzianum* consistently showed strong antagonistic property against *M. phaseolina* compared to the other antagonists tested hence considered as potential antagonists.

PP (S4) 23

Effect of bio-agents against post-harvest mango disease causing pathogens *in vitro*

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Mango (*Mangifera indica* L.) is an important fruit crop of India and other sub-tropical and tropical countries of the world. Mango fruit is one of the most popular, nutritionally rich fruits with unique flavour, fragrance, taste, and health promoting qualities, making it number one among new functional foods, often labelled as "super fruits". In post-harvest condition, several diseases are found on the mango fruit, which reduces marketability and shelf life after harvest of the fruits. Widely used fungicides have led to increased activity in the development of biological control against plant pathogens. Hence, the present investigation on the biological control of mango diseases was carried out in *in vitro* condition, the efficacy of bio-control agent on the post-harvest disease of anthracnose, stem end rot of mango by dual culture technique. All the tested bio-control agents were found significantly superior in inhibiting the mycelial growth of the pathogen over control. Significantly the least radial growth of *C. gloeosporioides* (5.25 mm) was observed with *T. harzianum* whereas *T. viride* was gave least radial growth of *B. theobromae* (9.50 mm) over the control.

PP (S4) 24

Effect of seed mycoflora on seedling growth, seed quality parameters and their biological management in pigeonpea [*Cajanus cajan* (L.) Millsp.]

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Seed mycoflora is one of the major constrains for pigeon pea (*Cajanus cajan* L.) growing farmer community. Seeds are regarded as mean of transporting plant pathogen. The fungi associated with the seed at the stage of harvest, transport, processing and under storage bring about several undesirable changes, making them unfit for consumption and sowing. Mycoflora associated with seeds at contaminant may cause seed abortion, seed rot, seed necrosis, and reduction of germination capacity as well as seed quality, *i.e.*, protein, lipids, soluble sugar, starch, crude fibre, vitamins and minerals. Seed samples were examined under agar plate and blotter method showed association of nine different fungi belonging to six genera *i.e.*, *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *F. moniliforme*, *F. udum*, *Curvularia lunata*, *Drechslera* sp., *Rhizoctonia* sp. and *Alternaria alternata*. Among them, the most dominant fungi were *A. niger*, *A. flavus*, *F. oxysporum*, *F. moniliforme*, *F. udum* and *A. alternata*. Culture filtrate of seed mycoflora caused considered reduction in germination percentage and seedling growth as compared to untreated check. Significantly lowest seed germination (43.00%) recorded in *A. niger* culture filtrate. Bio-chemical analysis of artificially inoculated pigeonpea seeds with nine different fungi decreased the percentage of protein, TSS and lipid content when compared with healthy seeds. Significantly, lowest protein and TSS was observed in discoloured seeds *i.e.* 8.70 and 1.20 per cent, respectively while lowest lipid (1.40 %) recorded in shrivelled seeds. Seed treatment for controlling plant diseases has been termed as the "pain less method" for farmers; it is known to improve seed germination and vigour index. Seed treatment with bio-agents is eco-friendly, cheaper as compare to chemical, safer to environment and human. While, chemicals are costly and hazardous to environment as well as human health. Seed bio-priming with *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were found to enhance seedling growth and seed quality parameters. Seed bio priming with different bio-agents among them, *Trichoderma viride* @ 0.4 % was found superior in seed germination (88.00%), shoot length (9.03 cm), root length (11.17 cm) and vigour index (1777.46) followed by *T. harzianum* as compared to untreated check.

PP (S4) 25

***In vitro* and *in vivo* evaluation of antagonistic potential of bioagents against *Macrophomina phaseolina* causing dry root rot in mungbean**

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Mungbean [*Vigna radiata* (L.) Wilczek] is one of the most important and extensively cultivated pulse crop belonging to the family *Fabaceae*. It is an excellent source of high quality protein. The crop is affected by a number of pests and disease of which dry root rot incited by *Macrophomina phaseolina* is an economically important disease. Antagonistic potentiality of five fungal and two bacterial antagonists was evaluated against *Macrophomina phaseolina* by dual culture technique under laboratory condition showed the strong antagonistic effect of *Trichoderma viride* against the pathogen with highest growth inhibition. Field study on effect of seed treatment (ST) and soil application (SA) of bioagents on *Macrophomina* dry root rot of mungbean revealed that application of commercial formulation of *T. viride* (2×10^8 CFU/ml) through ST @ 10 ml/kg seed + SA of *T. viride* enriched FYM (10 g *T. viride*/ kg FYM) @ 100 g/m² of soil/furrow showed least disease incidence with highest seed germination (96.98%), vigour index (1421) and highest grain yield (818 kg/ha). It was at par with seed treatment with commercial formulation of *T. viride* and *P. fluorescens* (2×10^8 CFU/ml) @ 10 ml/kg seeds which recorded lower disease incidence (20.28%), higher germination (95.76%), vigour index (1312) and grain yield (792 kg/ha).

PP (S4) 26

Effect of bio-agents against damping-off of tomato (*Pythium aphanidermatum*) *in vitro*

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Six antagonists viz., *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma virens*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Pseudomonas putida* were studied for their antagonism against *P. aphanidermatum* by dual culture method. Significantly reduced the mycelia growth of the pathogen over control. Significantly maximum inhibition of mycelial growth of the pathogen after 72 hrs. of incubation was obtained with *Bacillus subtilis* (70.44%). The next best antagonist in order of merit was *Trichoderma harzianum* (62.55%). It was followed by *Trichoderma viride* (54.22%) and *Pseudomonas peutila*

(53.33%) which were at par with each other. The next best treatment in order of merit was *Pseudomonas fluorescens* (50.77%). Minimum per cent growth inhibition (42.11%) was recorded in *Trichoderma virens*. In present findings, bacterial bioagent viz. *Bacillus subtilis* was found most effective to inhibit the *Pythium aphanidermatum*.

PP (S4) 27

Efficacy of various bio agents against *Fusarium solani* causing wilt of chilli (*Capsicum annum* L.)

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Chilli (*Capsicum annum* L.) is an important spice and vegetable crop of India. The wilt disease of chilli incited by *Fusarium solani* cause considerable crop loss in Saurashtra specially near Gondal region of Gujarat state. Antagonistic activity of different bio agents *F. solani* was studied for its effective management. By means of dual culture method, six bio agents were examined for their antagonistic effect on *F. solani*. Based on observations of radial growth of antagonist and test fungus, per cent inhibition was calculated. All the bioagents treatments significantly inhibited the mycelial growth of *F. solani* as compared to control and gave more than 72 % growth inhibition. Maximum reduction of pathogen (87.50 %) was observed in the presences of *T. harzianum*- II followed by *T. harzianum*- I (85.40 %). The *Bacillus subtilis* and *T. viride* was also found better with 84.70 and 81.35, respectively per cent reduction in growth of pathogen. *T. hamatum* and *Pseudomonas fluorescens* gave minimum growth inhibition with 78.35% and 72.30%, respectively.

PP (S4) 28

Field evaluation of different biological control agents against chilli anthracnose disease

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In chilli production, anthracnose/ fruit rot disease caused by *Colletotrichum* spp. is one of the major constraints leading to huge economic losses worldwide. Although different chemical fungicides

are being recommended and used to combat the disease, there is a pressing need to explore biocontrol based strategy for the management of crop diseases in the present arena of organic agriculture. Under All India Coordinated Research Project on Biological Control of Crop Pests (AICRP-BC) field experiments were conducted in the year 2015-16 and 2016-17 to evaluate the efficacy of different biocontrol agents against chilli anthracnose disease. Biocontrol yeast isolates *Pichia guillermondii* (Y-12), *Hansanospora uvarum* (Y-73) and *Trichoderma harzianum* (Th-3), *Pseudomonas fluorescens* (Pf-1) were applied through seed treatment, seedling dip and foliar spray @ concentration of 2×10^8 cfu ml⁻¹/g⁻¹. The recommended fungicide carbendazim (0.1%) was used as a chemical check. Among different biocontrol agents tested, the lowest mean disease intensity (MDI), highest disease control (DC) over untreated control were recorded in the treatment *P. guillermondii* (Y-12) (5.50 % MDI, 64.72 % DC (2015-16) & 6.23% MDI and 63.69% DC (2016-17)) and this was found at par with the treatment *P. fluorescens* (Pf-1) (6.30 % MDI, 59.58 % DC (2015-16) & 6.58 % MDI and 61.66 % DC (2016-17)). However, *T. harzianum* (Th-3), reported to be satisfactory in disease control. Hence, the biocontrol yeast isolates could be a promising component in integrated disease management of chilli anthracnose. To note that, the present study has proved the biocontrol efficacy of yeast isolate against chilli anthracnose disease and in addition serves as the base for further exploration and exploitation of yeast isolates for biological control of crop diseases.

PP (S4) 29

Isolation of microbial antagonists from phyllosphere of turmeric and their effect on control of turmeric anthracnose [*Colletotrichum capsici* (Syd.) Butler and Bisby]

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Turmeric (*Curcuma longa* L.) is one of the most important spice crop cultivated in India. India is considered as the largest producer, consumer and exporter of turmeric in the globe. The crop yield is affected by several biotic and abiotic factors, among them, anthracnose of turmeric caused by *C. capsici* was found increasing and occurring regularly every year. It has become as major constraint in successful cultivation of turmeric in Gujarat. The disease appears usually during August and September. The information available on this disease as well as its biological control by using microbial antagonists appears to be very scanty. Biocontrol method for the management of disease has great practical significance. Hence, in this study, antagonistic fungi and bacterium strains were isolated from the surface of turmeric leaves and identified as *Trichoderma* isolate- 1, *Trichoderma* isolate- 2, *Pseudomonas fluorescens* and *Bacillus subtilis* based on morphological characteristics. Among phyllosphere antagonists, *Trichoderma* isolate-1 was found most effective in growth inhibition (53.00%) of pathogen. The literature in this review helps in future research programmes that aim to promote *Trichoderma* spp. as a potential bio-pesticide for augmentative biological control of anthracnose disease of turmeric.

PP (S4) 30

In vitro* evaluation of different bio-agent against *Fusarium oxysporum* f. sp. *ciceri

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Chickpea (*Cicer arietinum* L.) is world's third most important pulse crop grown in tropical, subtropical and temperate regions, in over 40 countries after common bean and pea. India is the largest producer, contributing 67% to the world production. Chickpea occupies first position among the pulses grown in India. Disease is a major constrain in economic crop production as they inflict many losses. More than 78 pathogens are known to attack chickpea. Among them wilt is economic important diseases which causes loss up to 10 to 15 % and sometime more than 80%. The residues of synthetic chemical pesticides create food safety problems and these chemicals are also creating hazards to the environment. Hence, biological control is better for an environmental and economic sense because it offers durable, safe and cost effective alternative of chemicals. In laboratory screening three fungal bio-agents and five bacterial antagonists were evaluated against *F. oxysporum* f.sp. *ciceri* by dual culture technique. The results revealed that all the antagonists screened against *F. oxysporum* f.sp. *ciceri* were significantly superior over the control. Out of these, minimum growth of the pathogen was recorded in *Pseudomonas fluorescens* (6.00 mm) as compared to the rest. Next best in order of merit was *Trichoderma harzianum* (15.15 mm), followed by *Bacillus subtilis* (16.27 mm), *Pseudomonas aeruginosa* (16.75 mm), *Bacillus aerius* (17.25 mm), *T. viride* (17.67mm), *T. faciculatum* (20.00 mm) and *Rhizobium mediterraneum* (33.93 mm).

PP (S4) 31

Use of non-chemical methods for management of black rot of citrus

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Among all bioagents, *Trichoderma harzianum* found most potent antagonist in inhibiting the mycelial growth (49.60%) of *A. niger* as well as it was found most effective in reducing the Aspergillus fruit rot severity both in pre- (16.00%) and post-inoculation (15.50%) treatments. Significantly, lowest Aspergillus fruit rot severity was recorded in fruits treated with mustard oil (5%) in both pre- (20.75%) and post- inoculation (18.50%) treatments. Severity of Aspergillus rot was significantly lowest in fruits exposed to aerated steam treatment at 50°C for 5 min. (8.00%) and hot water treatment at 50°C for 10 min. (14.75%) without hampering the quality of fruits.

PP (S4) 32

***In vitro* evaluation of bio-agents against wilt of okra (*Fusarium oxysporum* Schlecht)**

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Okra (*Abelmoschus esculentus* L.) is an important vegetable crop, belongs to Malvaceae family and grown in India. However, many fungal disease were reported in okra, among them wilt disease of okra caused by *Fusarium oxysporum* occurs severely in okra growing areas of South Gujarat. The present investigation was carried out to evaluate the potential bio control agents for management of the wilt of okra. Different bio control agents viz., *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma fasciculatum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were tested by dual culture technique. The results revealed that, inhibition of *Fusarium oxysporum* was found to be highest in *Trichoderma harzianum* (77.78%), followed by *Trichoderma viride* (72.22%).

PP (S4) 33

Management of powdery mildew of cluster bean

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Cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) is an important drought resistant crop of India. The crop has excellent industrial importance due to its content of galactomannan gum in the seed. Cluster bean is attacked by a number of fungal, bacterial and viral diseases, which cause economical loss on seed yield. Among them, powdery mildew caused by *Leveillula taurica* (Lev.) Arn. Is an important disease particularly in the Saurashtra region of Gujarat. Hence, present investigation was carried out to develop economical & effective management strategies of, powdery mildew cluster bean. In the laboratory screening of systemic fungicides, propiconazole at 250 ppm and hexaconazole at 250 ppm and non-systemic fungicides, wettable sulphur at 500 ppm and dinocap at 500 ppm were proved effective in the inhibition of spore germination of *Leveillula taurica* (Lev.) Arn.. Effect of insecticides also testing in vitro, reveal that, dicofol at 500 ppm, fenazaquine at 1000 ppm and ethion at 500 ppm were found effective for the inhibition of spore germination. While, extracts of neem, tulsi and jatropa were proved to be the best sources of plant origin for the inhibition of spore germination. With the use of different fungal and bacterial cultural filtrates, the inhibition of spore germination was maximum reported in the *T. viride*. In field evaluation, three spray

of different fungicides at 15 days interval during kharif 2013-14, the minimum per cent disease intensity of 16.42 per cent was recorded in the treatment of propiconazole (0.025 %) with 74.88 per cent disease control. In summer (2014-15), powdery mildew was managed satisfactorily by three foliar application of propiconazole (0.025 %) with 16.27 per cent disease intensity and 76.54 and per cent disease control with maximum yield of 1446 kg/ha.

PP (S4) 34

Effect of *Trichoderma* spp. and its culture filtrate antagonists on growth and management of *Rhizopus* rot of tomato fruit *in vitro* and *in vivo*

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For eco-friendly and effective management of *Rhizopus* rot of tomato fruit various antagonists and its culture filtrate viz., *Trichoderma viride*, *T. harzianum*, *T. virens*, *T. atroviride*, *T. asperellum*, *T. fasciculatum* were carried out to know their efficacy in managing the *Rhizopus* rot of tomato *in vitro* and *in vivo*. *In vitro*, all the six antagonists significantly helped in inhibiting the mycelial growth of *R. oryzae* over control. Lowest mycelium growth (22.75 mm) with highest mycelial growth inhibition was recorded in *T. asperellum* (74.72 %), while *T. virens* gave minimum mycelial growth inhibition (62.50 %) after 7th days of incubation. In case of *in vivo* study, *T. asperellum* found significantly superior both in pre- (0.45%) and post-inoculation (0.45%) treatments over control (99.27%) after 5th day of inoculation. The culture filtrate of all the antagonists found effective in inhibiting the mycelial growth of *R. oryzae* over control. Complete mycelial growth inhibition was recorded in *T. asperellum* and *T. viride* on 5th day after incubation in *in vitro*. While in *in vivo* study, the culture filtrate of *T. asperellum* and *T. viride* found most effective both in pre- (0.45 & 0.45%) and post-inoculation (0.45 & 0.45 %) treatments in managing *Rhizopus* rot severity on 5th day after inoculation.

PP (S4) 35

Bioefficacy of antagonists against black mold rot [*Aspergillus niger* van Tieghem] of garlic (*Allium sativum* L.).

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For eco-friendly and effective management of black mold rot of garlic, eight antagonist viz., *Trichoderma viride*, *T. harzianum*, *T. virens*, *T. atroviride*, *T. asperellum*, *T. fasciculatum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were selected and screened by dual culture technique, *Trichoderma asperellum* showed lowest mycelial growth (24.33 mm) with highest per cent mycelial growth inhibition (72.96%) after seven days of incubation. Similarly, Pre and Post- inoculation treatment with *Trichoderma asperellum* on Garlic bulb found significantly superior in reducing rot severity after 7 and 14 day of inoculation, respectively.

PP (S4) 36

Microbial antagonist for plant fungal disease

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Fungal disease is the most deleterious factor in agriculture that causes severe production loss. Biological control of fungal disease has been accepted as a viable alternative method to chemical control. In plant pathology, biocontrol is applied to the use of microbial antagonists to suppress disease. The fungicide is the main source to protect the crop from the disease pron. But, because of the environmental concerns, health conscious attitude of human beings and other hazards associated with the use of chemicals, use of bio agents to suppress the disease causing activity of plant pathogens is gaining importance. During life cycle, the plants and pathogens interact to the various microbes which affect plant health in different ways. Different modes of action of the biocontrol active microorganism are hyperparasitism, predation, antibiosis, cross protection, competition for site and nutrients and induced resistance. Generally three methods are used to apply biocontrol agents to infected plants: spread at the infected part at high population, apply at one place in lower population to allow it to multiply and spread to whole infected part for protection against disease, and one time or occasional application that maintain the pathogen population at below the threshold level. Biological control involves the use of beneficial organisms, their genes, and/or products,

such as metabolites, that reduce the negative effects of plant pathogens and promote positive responses by the plant. Commercial use of the biocontrol agent has slow response because of environmental effects. To overcome this problem, it is accessory to develop the new formulation of the biocontrol agents with high degree of stability and survival. Majority of the biocontrol products are applied against seed borne and soil born fungal pathogens like seed rot and root rot. In some cases, biocontrol microorganisms are applied as a spray on foliar diseases including powdery mildew, downy mildew, blight and leaf spots. Post-harvest fungal disease can also be control by the use of antagonist fungus and bacteria. Biocontrol microorganisms are also applied in the form of compost in some plants. Having more effective biological control strategies in the future, it needs the more research on development of novel formulations; study the impact of environmental factors on biocontrol agents, mass production of biocontrol microorganisms. Very recent technologies like biotechnology and Nano-technology will be used for improvements of biocontrol mechanisms and strategies. The future scenario of the biocontrol of plant disease is very bright and promising because of the demand of the biocontrol agents by growers. The biocontrol practice is an effective strategy to manage plant diseases, increase yield, and protect environment and biological recourses for sustainable agricultural system.

PP (S4) 36

**Potential of *Trichoderma viride* against banded leaf and sheath blight of maize
(*Rhizoctonia solani* f. sp. *Sasakii*)**

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Rhizoctonia solani f. sp. *Sasakii* causing banded leaf and sheath blight diseases is one of the important fungi of maize world wide and destructive diseases of maize due to its wide host range and adaptability in worldwide. Banded leaf and sheath blight of maize continuously appears in moderate to severe form in maize growing areas of Gujarat. The disease manifests on leaves, leaf sheaths, stalk lesions or rind spotting resulting in stem breakage, clumping and cracking of styles and ear rot *etc.*, resulting in cent per cent yield loss and lodging in severe conditions. The fungus is commonly controlled by using fungicide because no resistant variety available. To manage this disease *Trichoderma viride* were evaluated as seed treatment, soil application and also as spray application alone and in combinations infield for two consecutive rainy (kharif) seasons 2015 and 2016. Treatments with combination of seed treatment, soil application and spray application were more effective than seed treatment, soil application and spray application alone and in combination between two applications. The study was conducted at the Main Maize Research Station, Anand Agricultural University, Anand in kharif using GM-6 variety as test plant. The experiments were designed in a randomized block design (RBD) with three replications. The observation on seed germination, plant height, no. of cobs/plot, diseases severity and grain and fodder yield were recorded and found significant

results as compare to control. The study revealed that all the treatments were found significantly superior over control. Among the treatments, seed treatment + soil application + spray application with *Trichoderma viride* was found significantly control to banded leaf and sheath blight (BLSB) disease severity (27.2%) resulted in highest grain yield (3549 kg/ha) and dry fodder (5116 kg/ha) yield. In addition, among the bioagent treatments, seed treatment + soil application + spray application with *Trichoderma viride* was found significantly control to maydis leaf blight, turcicum leaf blight and Curvularia leaf spot with 18.1, 17.5 and 19.1% disease severity, respectively. The studies showed the potential of *Trichoderma viride* for commercialization as a seed treatment, soil application and spray application for the control of banded leaf and sheath blight disease (*R. solani*) in maize.

PP (S4) 38

Management of stem rot of cluster bean caused by *Sclerotium rolfsii* Sacc. through ecofriendly approach

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Cluster bean or guar (*Cyamopsis tetragonoloba* L.) Taub. Synonym *Cyamopsis psoralioides* DC is cultivated in many parts of India and has been known for green fodder, cattle feed, vegetable and green manuring purposes. Stem rot of cluster bean caused by *Sclerotium rolfsii* Sacc. Has become a serious problem in recent years and caused heavy economic loss by reducing yield in Gujarat state. Survey of stem rot disease in Banaskantha district revealed that disease incidence of stem rot was recorded in different tehsils ranging from 4.8 to 9.8 per cent, where maximum being recorded in Dantiwada tehsil (9.8 %) followed by Palanpur (7.6 %), Dhanera (6.2 %), Tharad (5.8 %), and Vav (4.8%). Eight different isolates of *Trichoderma* spp. And one bacterial species (*Pseudomonas fluorescens*) were evaluated in vitro for their antagonistic efficacy against stem rot pathogen of cluster bean by dual culture method. In this method, *T. harzianum* (Sardarkrushinagar), *T. harzianum* (Junagadh) isolates and *T. harzianum* (Navsari) isolate appeared as potential antagonists against cluster bean stem rot pathogen. Six different organic amendments (castor cake, cotton cake, FYM, mustard cake, neem cake and vermin compost) were evaluated against *S. rolfsii* in vitro. The results revealed that all the amendments significantly inhibited the growth of *S. rolfsii* in vitro except FYM. Among all the organic amendments, castor cake recorded significantly highest per cent inhibition of growth and sclerotial production of *S. rolfsii* in all the three the concentrations (10, 20 and 30 %) tested. The superiority of organic amendments for inhibiting the growth and sclerotial production of the pathogen might be due to release of some fungitoxic substances in the medium which suppressed the growth and sclerotial production of *S. rolfsii*. Pot culture experiments on integrated management of stem rot of clusterbean showed that, when tebuconazole applied as soil drenching @ 4 ml/lit of water along with seed treatment of *T. harzianum* @ 10 g/kg of seed, effect might be synergistically enhanced and recorded

disease incidence only 23.33 per cent. When this treatment was supplemented with castor cake as soil organic amendment, significantly the least disease incidence (15.00) was recorded among all the treatments.

PP (S4) 39

Evaluation of different bio control agents against charcoal rot of maize caused by *Macrophomina phaseolina* (Tassi) Goid *in vitro*

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Maize (*Zea mays* L.) is an important staple food in many parts of the world. Various factor are responsible for low productivity of maize. Among them diseases are major concern. The Charcoal rot disease of maize incited by *Macrophomina phaseolina* (Tassi) Goid. Cause crop grayish streak and shredding of bark and stem which wilted crop cause considerable crop loss and become major hurdle in production. In present widely used fungicides have led to increase the residual problem of soil and reduce fertility of soil, activity in the development of biological control against plant pathogens. Hence, the present investigation on the biological control of soil borne pathogens by four different fungal and bacterial isolates were evaluated for their bio control potential against *M. phaseolina* under in vitro (dual culture method). Among them *Trichoderma viride* (92.36 %) showed maximum growth inhibition and followed by *T. asperellum* (90.28%), *T. harzianum* (88.33 %) at par. While least growth inhibition was observed in *P. fluorescens* (73.89 %) against *M. phaseolina*.

PP (S4) 40

***In vitro* evaluation of bioagents against *Alternaria longipes* causing leaf blight in little gourd**

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Leaf blight of little gourd *Coccinia grandis* (L) Voigt, disease caused by *Alternaria longipes* is most serious and destructive disease in South Gujarat Region. Present research on leaf blight of little gourd was carried out to assess suitable management strategies. Looking towards the importance of biological control as a component of Integrated Disease Management of plant pathogens, five bioagents were tested for their efficacy in the laboratory. Interaction studies on the effect of known antagonists carried out by dual culture

method revealed that *Trichoderma viride* showed strong antagonistic effect (90.08 %) against *A. longipes* under dual culture method. Next best in reducing average diameter of *A. longipes* was *Bacillus subtilis* with (86.36 %) growth inhibition. *A. niger* and *P. fluorescens*, were also found effective in inhibiting the growth of the pathogen. The bioagents could be used in organic farming.

PP (S4) 41

Management of seed mycoflora of cowpea

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In vitro evaluation of three fungicides, two phytoextracts, *Trichoderma viride* and cow urine as seed dresser revealed that thiram, carbendazim and carboxin were superior. Thiram proved to be most effective with least number of fungal species followed by carbendazim. *Trichoderma viride* and phytoextracts proved its potential as promising approach against seed mycoflora. Cow urine appeared ineffective.

PP (S4) 42

***Pseudomonas fluorescens*: A powerful antagonist for plant disease management**

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Fluorescent Pseudomonads important group of bacteria that play a major role in the plant growth promotion induced systemic resistance, biological control of pathogens etc. *Pseudomonas fluorescens* belong to plant Growth Promoting Rhizobacteria (PGPR), non-pathogenic saprophytes that colonize soil, water and plant surface environments and common gram negative, rod-shaped bacterium. It secretes a soluble greenish fluorescent pigment called fluorescein, particularly when low iron availability. Because they are well adapted in soil, *P. fluorescens* strains are being investigated extensively for use in applications that require the release and survival of bacteria in the soil in which are biocontrol of pathogens in agriculture and bioremediation of various organic compounds. Many strains of *P. fluorescens* are known to enhance plant growth promotion and reduce severity of various diseases due to result of the production of a number of secondary metabolites including antibiotics, siderophores and hydrogen cyanide and competitive exclusion of pathogens as the result of rapid colonization of the rhizosphere by *P. fluorescens*. The anti-fungal metabolite 2,4-diacetyl phloroglucinol play a major role in the biocontrol capabilities of *P. fluorescens*. The

efficacy of bacterial antagonists in controlling fungal diseases was often better as alone, and sometimes in combination with fungicides. *P. fluorescens* producing an antibiotic compound, pyrrolnitrin, which inhibited growth of *M. phaseolina* by producing an inhibition zone of 12 mm. *P. fluorescens* 2-79 produce the antibiotic phenazine-1-carboxylic acid and suppress take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici*. Foliar spray of *P. fluorescens*, in addition to LFA and 45 kg potash/ha significantly reduced the rice blast incidence and increased crop yield. The future research programmes that aim to promote *P. fluorescens* as a potential bio-pesticide for augmentative biological control of many diseases of agriculture and horticultural importance while environmental and consumer concerns have focused interest on the development of biological control agents as an alternative, eco-friendly strategy for the protection of agricultural and horticultural crops against phytopathogens. However, a better understanding of the factors involved the signalling interaction among antagonist, pathogen, soil and plants, are yet to be revealed to promote the biocontrol agents as wide applicable bio-pesticides in future.

PP (S4) 43

Plant Growth Promoting Rhizobacteria (PGPR): Their ability as biocontrol agents

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The term 'plant growth promoting rhizobacteria' refers to bacteria that colonize plant roots and enhance plant growth. PGPR enhance plant growth by direct & indirect means. PGPR directly promote plant growth by producing phytohormones or facilitating the uptake of certain nutrient from the environment, while indirect mechanism involves the ability of PGPR to reduce the harmful effect of plant pathogen on crop yield. Thus, PGPR also act as a biocontrol agent. They suppress plant disease through induced systemic resistance (ISR) and through production of antimicrobial compound that includes antibiotics and siderophores. Rhizobacteria belonging to genera *Pseudomonas* and *Bacillus* are well known for their antagonistic effects and their ability to triggers ISR. In pigeonpea, strain RRLJ 04 of *Pseudomonas aeruginosa* and strain BS 03 of *Bacillus cereus* has been reported induce resistance against Fusarium wilt, Collar rot, dry root rot and Pigeonpea Sterility Mosaic Virus. Some PGPR produces siderophores in iron deficient soil. Siderophores are low molecular weight iron binding proteins that chelate the iron and make it available to plant and unavailable to many soil pathogens (*Pythium*, *Rhizoctonia*, *Fusarium* etc.) that require iron for their growth. Among most of the siderophores studied, those produced by *Pseudomonas* are known for their higher affinity to ferric ion. A pseudobactin siderophores produced by *P. putida* B10 strain is able to suppress *Fusarium udum* in iron deficient soil. Biological control has also proved useful and economical in the management of plant disease because of the harmful effect of fungicides to human and environment. Therefore, PGPR can be used as a potential biocontrol agent for the management of plant diseases to protect the consumers from ill effects of chemical pesticides.

PP (S4) 44

Effect of bio-control agents against seed mycoflora of sorghum

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Sorghum [*Sorghum bicolor* (L.) Moench] is a vital life sustaining food crop for human being as well as for livestock in many parts of world. Seed borne mycoflora are major pathogens that cause damage in sorghum seeds. The commonly occurring seed mycoflora are *Colletotrichum* spp., *Fusarium moniliforme* and *Alternaria alternata*. These pathogens are effectively controlled by bio-control agents. Hence, the present investigation on biological control of seed borne mycoflora of sorghum is carried out in in-vitro condition. From which seed treated with different bio-control agents from which *Trichoderma viride* recorded highest seed germination (83.00%) and shoot length (7.10 cm) and the maximum root length recorded in *Trichoderma harzianum* (6.90 cm) while maximum seedling vigour index recorded in *Pseudomonas fluorescens* (1137.60%).

PP (S4) 45

Efficacy of different bio control agents against *Meloidogyne incognita* and *Fusarium oxysporum* on tomato

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Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetable crops grown in the world, next to potato. It is used as a fresh vegetable and also can be processed and canned as a paste, juice, sauce, powder or as a whole (Barone and Frusciante, 2007). The experiment was conducted in ten cm earthen pots containing 500 cm sterilized soil employing six bio-control agents viz, *Trichoderma viride*, *Trichoderma harzianum*, *Paecilomyces lilacinus*, *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Pochonia chlamydosporia* were used in present studies. The fungus *P. lilacinus* and *Trichoderma viride* were isolated from the soils of J.N.K.V.V Jabalpur farm by sprinkling it on Petri plates containing sterilized Potato Dextrose Agar (PDA) medium. Biological control is considered as new efficient method that becomes widely used for controlling plant parasitic nematodes, as aim to decrease the extent of environment degradation and the effect of the excessive toxic nematicides. So, this study was done to investigate the role of some bacterial

genera as biocontrol agent against *Meloidogyne incognita*. The results of in pot house experiments indicated that, all tested bacteria have a greatly significant effectiveness for suppressing *M. incognita*. Pot house results showed that significantly increased (80.32 cm) plant height was recorded with *Pacilomyces lilacinus* followed by *Bacillus subtilis* (72.23 cm), *T. harzianum* (68.77 cm) and *P. chlamydosporia* (64.52 cm) over control. The effects of *T. viride*, *T. harzianum* and *Pacilomyces lilacinus* tills were though non-significant but superior over control (52.54 cm). Maximum root length (20.67 cm) was recorded with *Pacilomyces lilacinus* followed by *Bacillus subtilis* (17.63 cm) and *T. harzianum* (14.67 cm).

PP (S4) 46

Impact and effect of different antagonists on the development of *Alternaria* fruit rot on lime fruit caused by *Alternaria citri*

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Citrus is the world's premiere fruit crop, grown in over 100 countries on six continents. The serious neglect in postharvest management of citrus results in very high postharvest losses of this valued fruit. The use of chemicals in postharvest treatment of lime fruits has possibility of direct exposure to the consumers. Bio-control agents have been proved promising in reducing postharvest diseases of fruits and vegetables. These bioagents establish and colonize more efficiently on fruit surface to inhibit the growth of the pathogen when applied as postharvest treatment under controlled conditions. Hence, the use of bioagents appear more feasible in postharvest treatments of fruits without loosing their efficacy in long-term storage as compared to chemicals. *Trichoderma harzianum*, *T. viride*, *B. subtilis* and *P. fluorescens* antagonists were tested against fruit rot of lime. The ripe citrus lime fruits of equal size were first inoculated with different antagonists separately by stem-end method and after 24 h of incubation, the fruits were inoculated on the same site with the pathogen. In lime fruits, all the antagonists were found promising, which significantly reduced the fruit rot of lime. Here, *T. harzianum* and *T. viride* significantly reduced the fruit rot with the lesion diameter of 05.49 and 05.94 mm respectively. However, *B. subtilis* and *P. fluorescens* were also effective with the lesion diameter of 07.58 and 07.72 mm growth of the pathogen. In control, the lesion diameter was 16.87 mm after ten days of incubation. On cut, fungal pathogen was only found in the control fruits.

PP (S4) 47

Management of root-knot nematode *Meloidogyne incognita* using different bio-agents in pot condition

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An experiment was conducted to study the effects of different bio-agents viz., *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, *Pseudomonas fluorescens*, *Trichoderma viride* and *Trichoderma harzianum* each bio-agent @ 0.1% (w/w) enriched with FYM @ 10% (w/w) against *Meloidogyne incognita* on mungbean. All bio-agents application improved plant growth characters and reduced root-knot index and root and soil nematode population. Maximum plant height was recorded in *Paecilomyces lilacinus* followed by *Pseudomonas fluorescens* and *Trichoderma viride*. Similarly, maximum fresh shoot and root weight was obtained in *Paecilomyces lilacinus* treatment followed by *Pseudomonas fluorescens* and *Trichoderma viride*. Root-knot index was lowest in *Paecilomyces lilacinus* (1.20). Maximum reduction in root and soil nematode population was also recorded in *Paecilomyces lilacinus*.

PP (S4) 48

Management of wilt of pomegranate (*Punica granatum* L.) caused by *Fusarium solani* (Mart.) Sacc.

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Pomegranate is regarded as the "Fruit of Paradise". It is one of the most adaptable semi arid fruit crop and its cultivation is increasing very rapidly due to its medicinal properties as well as its nutritional values with excellent keeping quality and remunerative prices in domestic as well as export market. It is affected by number of diseases caused by fungi and bacteria at all stages of its growth from the plants in the nursery to the fruits in transit and storage. Among them, wilt caused by *Ceratocystis fimbriata* is a major threat. Despite that many factors conducive for the high severity of disease, seedlings selection for planting, soil borne nature of pathogen and also its association with shot hole borer and plant parasitic nematodes. This might be the reason for the current rampant spread of the disease in Indian states. Therefore, for the management of wilt pathogen, biological control is analysed as a suitable ecofriendly strategy. Different antagonists of *Trichoderma* spp. and two bacterial species were evaluated *in vitro* for their antagonistic efficacy against *Fusarium solani* causing wilt of pomogranate by dual culture method.

All the *Trichoderma* spp. Exhibited high antagonism against *F. solani*. Among them, *Trichoderma harzianum* (Navsari) and *Trichoderma viride* (Sardarkrushinagar) appeared as potential antagonists to wilt pathogen of pomegranate which could be useful very well in managing the disease. Bacterial bio-agents *Bacillus subtilis* and *Pseudomonas fluorescens* were also shown potential the good potential. The maximum fungal growth inhibition (62.30%) of the pathogen was recorded in *Trichoderma harzianum* (Navsari) which was statistically at par with *Bacillus subtilis* (Navsari) (61.96%) followed by *T. viride* (Sardarkrushinagar) (60.53%) and other treatments with control. Hence, wilt has been observed as limiting factor in the pomegranate growing areas, although biocontrol agents are giving good response in the suppression of the pathogen but still there is need to exploit this strategy as a novel approach of integrated disease management.

PP (S4) 49

Management of leaf blight disease of groundnut caused by *Alternaria alternata* (Fr.) Keissler

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Groundnut (*Arachis hypogaea* L.) is an important legume crop in many tropical and subtropical countries of the world. It is known as the 'King of oilseed' crops. It is one of the important food, edible oil and cash crops of our country. Groundnut occupies first place in oil seed crop growing in India. Gujarat is the leading state in groundnut production. Groundnut is prone to attack by number of diseases including fungi, bacteria, viruses, mycoplasma, and nematodes, of which fungal diseases cause the majority of yield loss. The foliar fungal diseases, early leaf spot (*Cercospora arachidicola* Hori.), late leaf spot [*Phaeoisariopsis personata* (Berk. And M.A. Curtis) van Arx] and rust (*Puccinia arachidis* Speg.) are wide spread and destructive. The leaf blight disease caused by different species of *Alternaria* has been until now a minor disease. The extent of yield loss depended on the time of appearance and severity of the disease. There was a reduction in pod bearing of the diseased plants and the kernels from such plants were by and large shriveled. All the antagonists significantly inhibited the growth of *A. alternata* over control. Out of four antagonists *Trichoderma harzianum*, *T. viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* tested, *T. harzianum* showed significantly maximum growth inhibition (76.87 %) and grew over the *A. alternata* colonies followed by *T. viride* (67.25 %). *P. fluorescens* is next best antagonist in inhibiting fungal growth. Minimum growth inhibition was recorded in *B. subtilis* as 59.25 per cent.

PP (S4) 50

Studies on Fusarium wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Synder and Hans

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Wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Synder and Hans has been found to affect severally and causes accountable qualitative as well as quantitative losses, more than 50% in chickpea. Present investigation on *Fusarium oxysporum* f. sp. *ciceri* were carried out during Rabi 2010 with the objectives namely isolation, pathogenicity, *in vitro* evolution of fungicides, plant extracts and bioagents (Plate and Pot experiment) at the Department of Plant Pathology, College of Agriculture, Latur and *in vivo* evaluation of fungicides, plant extract and bioagents, effect of sowing date on wilt incidence and varietal/germplasm screening at A.R.S. Badnapur. The test pathogen was successfully isolated on Potato dextrose agar and proved its pathogenicity on chickpea Cv. JG-62 under screen house condition by different inoculation method and soil inoculation was found most effective method to create disease. Among all the fungicides used *in vitro*, Carbendazim at 1000 and 2000 ppm inhibited growth of *F. oxysporum* f. sp. *ciceri* 79.63 and 100 per cent, respectively. Bioagents, *T. viride* (58.82) recorded highest mycelial inhibition *i.e.* 55.50 and 60.03 per cent. The seed treatment with Carbendazim + Thiram (0.2% each) recorded highest disease control in both pot and field experiment *i.e.* 79.60 and 81.42 per cent, respectively. Early sowing of chickpea during first fortnight of October is advisable, so as to minimize the wilt incidence. Chickpea germplasm screening against *Fusarium oxysporum* f. sp. *ciceri* under field conditions revealed that, out of 72 genotypes 5 immune/ disease free, 52 resistant, 9 moderately resistant and 8 susceptible lines were observed.

PP (S4) 51

***In vitro* evaluation of bioagents against *Macrophomina phaseolina* (Tassi) Goid. in mungbean**

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Mungbean is the third important pulse crop globally grown in over 40 countries after chickpea and pigeon pea. The crop suffers from many diseases caused by biotic stresses *viz.*, fungi, bacteria, viruses, nematodes and abiotic stresses. The major fungal diseases which causes heavy yield losses in mungbean

are dry root rot, web blight, powdery mildew, Cercospora leaf spot, anthracnose etc. Among them, dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid. Is one of the economically important disease and has been rated as most virulent and destructive pathogen. The pathogen attacks on all parts of plant i.e. root, stem, branches, petioles, leaves, pods and seeds. Moreover, seed infection of *M. phaseolina* ranges from 2.2-15.7 per cent which causes 10.8 per cent reduction in grain yield and 12.3 per cent protein content of seed in mungbean. The pathogen *M. phaseolina* were isolated from mungbean. The bioagents viz., *Trichoderma asperellum*, *T. viride*, *T. harzianum*, *T. virens* and *Pseudomonas fluorescens* were tested for their antagonistic property against *M. phaseolina* by dual culture method. Among all the biological agents *T. asperellum* (79.85%) was significantly superior with highest percent growth inhibition of *M. phaseolina* followed by *T. viride* (76.25%), *T. harzianum* (67.41%), *T. virens* (63.47%) and *P. fluorescens* (57.67%) over control.

PP (S4) 52

Bioefficacy of various antagonists against two major seed-borne pathogenic fungi of sunflower

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Sunflower (*Helianthus annuus* L.), like other crops suffers from many fungal, bacterial and viral diseases, among which some are seed-borne in nature. These seed-borne mycoflora hamper seed germination, cause mortality, reduce optimum plant stand per unit area and thereby reduce significantly the potential yield. Therefore, in present in vitro study, the bioefficacy of various bioagents against two major seed-borne pathogenic fungi viz., *Alternaria alternata* and *Fusarium oxysporum*, isolated from stored seeds of sunflower Cv. Morden and Hyb. LSFH-171 was evaluated by Dual culture technique, planned in CRD and all the treatments replicated thrice. The results revealed that seven fungal and two bacterial bioagents tested were found efficient in inhibiting mycelial growth of *A. alternata* and *F. oxysporum*. The mycelial growth inhibition in *A. alternata* was ranged from 38.40 to 79.80 per cent and in that of *F. oxysporum*, it ranged from 74.70 to 76.20 per cent. In respect of *A. alternata*, maximum mycelial growth inhibition was resulted with *A. niger* (79.80%), followed by *T. virens* (79.40%), *T. viride* (78.10%), *T. koningii* (77.50%), *T. harzianum* and *T. lignorum* (each 77.20%) and *T. hamatum* (74.60%), except *T. hamatum* all were on par to each other. In case of *F. oxysporum*, significantly highest mycelial growth inhibition was recorded with *T. koningii* (76.20%), followed by *T. lignorum* (75.50), *A. niger* (75.30%) and *T. harzianum* (73.10%), all of which were on par to each other *P. fluorescens* followed by *B. subtilis* were found comparatively less effective against both of the test fungi.

PP (S4) 53

Microbiome cocktail therapy to managerial control of plant disease: A new approach and application

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New era of sequencing and computational data analysis technique is revealing that a community of microbes and their genes, called "the microbiome", which are located in the gut and rhizosphere, is responsible for maintaining the normal health of humans and plants respectively. Recent studies have shown that transfer of cultural mixture of core gut microbiome or fecal microbiome of a healthy person when transferred to the diseased person results in re-establishment of the normal microbiome and later on it helps in alleviation from the diseased condition. Similarly, establishment of rumen microbiome in diseased animal from healthy animal has been successfully implicated. Not exactly in agriculture it could be applied but recent studies has been shown that transfer of microbiome via soil solutions has shown ability to alleviate plant disease. The exact practice of transferring artificially cultivated beneficiary microbiome till now not attempted for plant disease managerial control. As the gut and rhizosphere microbiome are known to share many common traits, and their presence suggested good scope of similar studies in agriculture too. Based on information and recent studies drawn from all recent works in microbiome studies of gut and rhizosphere, we propose that therapy of microbiome cocktail containing of diseased suppressive soil/ healthy soil, filtered solution of rhizosphere microbiome of a healthy plant and biological agent can be a successful managerial control for plant disease and it can be a great success in agriculture.

PP (S4) 54

***In vitro* efficacy of *Trichoderma* spp against *Fusarium oxysporum* f.sp *lycopersici* causing wilt in tomato**

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The prospects of most promising biocontrol agents of the genus *Trichoderma* has been evidenced since long back, for managing soil borne plant pathogens, especially *Fusarium* spp. Therefore, present study was conducted to assess bioefficacy of native / local strains of *Trichoderma* spp against *Fusarium oxysporum* f.sp *lycopersici*, causing wilt in tomato. An *in vitro* experiment was planned in CRD and a total of

nine local strains isolated of *Trichoderma* spp were evaluated by applying Dual culture technique on Potato dextrose agar Medium. The results revealed that all of the nine strains of *Trichoderma* spp as antagonistic against the test pathogen. The result obtained on mycelia growth and inhibition of *Fusarium oxysporum* f.sp *lycopersici* with isolates of *Trichoderma* spp antagonist indicates that, isolates obtained from Aurangabad district T1 (Thr-Ab) recorded least mycelia growth 38.22 mm with highest zone of inhibition 57.03 per cent followed by T3 (Thm-L) and T2 (Tv-H) recorded mycelia growth 38.50 mm 38.79 mm with zone of inhibition 56.81 and 56.33 per cent, respectively.

PP (S4) 55

***In vitro* evaluation of rhizosphere microflora against *Ceratocystis fimbriata* causing wilt of pomegranate**

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Wilt of pomegranate caused by *Ceratocystis fimbriata* (Ell and Hast) is a major threat for Pomegranate cultivation. PGPR acts as a biocontrol agents by production of antibiotics, triggering induced local or systemic resistance or preventing the deleterious effects of xenobiotic by degradation which acts as rhizoremedicators. Considering the beneficial effect of PGPR present studies were undertaken to evaluate different rhizosphere microflora against *Ceratocystis fimbriata* *in vitro* by dual culture technique. The data revealed that all the rhizosphere microflora exhibited fungistatic/antifungal activity against *C. fimbriata* and significantly inhibited its growth over untreated control. Amongst the different antagonists tested, *Trichoderma harzianum* was found most effective and recorded least linear mycelial growth (19.00 mm) with highest mycelial inhibition (77.88%) of the test pathogen. The second and third best antagonists found were *Bacillus subtilis* and *Pseudomonas fluorescens* which recorded mycelial growth of 23.67 mm and 25.33 mm, respectively and inhibition of 73.70 and 71.85 per cent, respectively. This was followed by *Aspergillus niger* (col. Dia.: 27.00 mm and inhibition: 70.00%), and *T. viride* (col. Dia.: 29.33 mm and inhibition: 67.41%), *T. koningii* (col. Dia.: 34.33 mm and inhibition: 61.85%) and *Micrococcus flavus* (col. Dia.: 35.67 mm and inhibition: 60.37%). Amongst all the antagonists, *Flectobacillus roseus* was found less effective with 57.67 mm linear mycelial growth and 35.92 per cent mycelial inhibition.

PP (S4) 56

In vitro* bioefficacy of bioagents against *Fusarium oxysporum* f. sp. *ciceri

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Chickpea wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri* (Padw.) Syd. And Hans. Is one of the most destructive and wide spread disease of chickpea causing cent per cent losses under favourable conditions. Present *in vitro* study was attempted to assess *in vitro* bioefficacy of six bioagents against *Fusarium oxysporum* f. sp. *ciceri* in Completely Randomised Design and all the treatments replicated thrice. The results revealed that, out of the five fungal and one bacterial antagonists tested, *Trichoderma viride* was found to be most effective and recorded least mycelial growth (22.99 mm) with highest mycelial inhibition (74.45 %) of *F. oxysporum* f. sp. *ciceri*. The second and third best antagonists found were *T. harzianum* (24.44 mm and 72.84 %) and *T. koningii* (27.00 mm and 70.00 %). The least effective fungal bioagents were *T. virens* (34.88 mm and 61.31 %) and *T. hamatum* (32.43 mm and 63.96 %). The bacterial antagonist *Pseudomonas fluorescens* was comparatively least effective than the fungal bioagents and recorded 44.66 mm growth of *F. oxysporum* f. sp. *ciceri* as compared to 90.00 mm growth of test pathogen in untreated control.

PP (S4) 57

Compatibility of native *Trichoderma* spp. with insecticides

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Trichoderma a genus of asexually reproducing saprophytic fungi, ubiquitously present in nearly all temperate and tropical soils, decaying plant tissues and root ecosystems. Combined application of biocontrol agents with fungicides and insecticides may result either in synergistic/ antagonistic effects. In this context, present study was attempted to assess *in vitro* compatibility of native *Trichoderma* spp. With 7 insecticides, in CRD and all the treatments replicated thrice. The results revealed that in *T. harzianum*, among the insecticides tested, acephate 75% SP was found highly compatible with least mycelial growth inhibition (20.12%), followed by fenvalerate 0.4% DP (26.60%), imidacloprid 17.8% SL (32.28%), acetamiprid 20% SP (32.49%) and thiamethoxam 25% WG (35.50%). However, rest of two insecticides viz., quinalphos 25% EC and profenophos 50% EC were found incompatible with more than 50% mycelial growth inhibition (70.37 and 73.22 %, respectively). Whereas, in *T. hamatum*, fenvalerate 0.4% DP was found highly compatible with least mycelial growth inhibition (20.31%), followed by acephate 75% SP

(21.36%), imidacloprid 17.8% SL (21.48%), acetamiprid 20% SP (27.34%) and thiamethoxam 25% WG (27.81%), however, rest of two insecticides viz., quinalphos 25% EC and profenophos 50% EC were found incompatible with more than 50% mycelial growth inhibition (69.75 and 72.22%, respectively) of *T. hamatum*.

PP (S4) 58

Efficacy of fungicides and bioagents against *Pythium aphanidermatum* causing rhizome rot of turmeric

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Rhizome rot (*Pythium aphanidermatum*) is one of the most wide spread, destructive disease of turmeric (*Curcuma longa* L.), which accounts for about 30 to 80 per cent yield losses. All the fungicides tested significantly inhibited mycelial growth of *P. aphanidermatum*, over untreated control. Average mycelial growth inhibition recorded with the test systemic fungicides was ranged from 73.32 (Propiconazole) to 100 (Metalaxyl) per cent. However, it was cent per cent with Metalaxyl (100 %), followed by Carbendazim (97.67 %), Azoxystrobin (94.55 %), Thiophanate methyl (94.15 %), Fosetyl-AL (86.64 %), Hexaconazole (85.76 %) and Difenconazole (82.85). Whereas, it was comparatively minimum with Propiconazole (73.32 %) and Penconazole (81.14 %). Average mycelial growth inhibition recorded with the test non systemic and contact fungicides was ranged from 50.94 (Metalaxyl 8 % WP + Mancozeb 64 % WP) to 100 (Carbendazim 12 WP + Mancozeb 63 WP) per cent. However, Carbendazim 12 WP + Mancozeb 63 WP gave cent per cent (100%) mycelial inhibition. The next fungicides with significantly least mycelial growth were Copper oxychloride (97.36 %), followed by Chlorothalonil (76.16 %), Mancozeb (70.62 %). However, Metalaxyl 8 % WP + Mancozeb 64 % WP and Cymoxanil 8 % + Mancozeb 64 % WP were found less effective with minimum mycelial inhibition of 50.94 and 55.23 per cent, respectively.

PP (S4) 59

Integrated management of turmeric rhizome rot caused by *Pythium aphanidermatum*

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Turmeric (*Curcuma longa* L) rhizome rot caused by *Pythium aphanidermatum* was one of the divesting disease and causes accountable losses. All fungicides, bioagents, botanicals and soil amendments (alone and in combination) tested *in vitro* for management of turmeric rhizome rot was found effective against *P. aphanidermatum*. However, significantly highest reduction in average mortality was recorded with Metalaxyl (RT) + its drenching (85.37 %), followed by T4 + T5 (RT) + T1 (SA) (74.54 %), Carbendazim 12 % WP + Mancozeb 63 % WP (RT) + its drenching (73.30 %), T4 + T5 (RT) + T3 (SA) (71.87 %), Bioagent consortia (RT) + Its Drenching 71.20 %, Copper oxychloride (RT) + its drenching (68.79 %), T4 + T5 (RT) + T2 (SA) (68.40 %), T4 + T5 (RT) + Bioagent consortia (SA) (68.07 %), Metalaxyl (RT) (68.80 %), Carbendazim 12 % WP + Mancozeb 63 % WP (RT) (62.60 %), Copper oxychloride (RT) (61.31 %), *Trichoderma viride* (RT) (54.86 %), Neem leaf extract (RT) (51.22 %) and NSKE (SA) (48.09 %).

PP (S4) 60

Effect of different bio control agents against watermelon root rot caused by *Sclerotium rolfsii* Saccardo *in vitro*

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Watermelon (*Citrullus lanatus* L.) is one of the important horticultural crop belonging to cucurbitaceae family. Diseases are one of the factors for reduction in yield. One of the major disease of watermelon is root rot. The root rot disease of watermelon incited by *Sclerotium rolfsii* Sacc infect root and base of the crop plants that cause rotting which progress upwards with white mould or mycelial growth and infected plants wilt following yellowing of leaves. *Sclerotium rolfsii* Sacc is a soil borne fungus. Since it is soil borne pathogen, management is challenging with fungicides alone, hence use of biological agent becomes more important. Hence, the present investigation on the biological control of soil borne pathogens by four different fungal and bacterial isolates were evaluated for their bio control potential against *S. rolfsii* under *in vitro* (dual culture method). Among them *Trichoderma harzianum* (88.03%) showed maximum growth inhibition and followed by *T. asperellum* (85.30%), *T. viride* (77.44%), while least growth inhibition was observed in *P. fluorescens* (68.97%) against *S. rolfsii*.

PP (S4) 61

Field evaluation of biocontrol agents against powdery mildew of grape caused by *Uncinula necator* (Schw.) Burr.

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Powdery mildew is second important disease of grape after downy mildew. Powdery mildew is caused by *Uncinula necator* is most economical and causes losses up to 65% in fruit yield and 40% vine size. Keeping economic importance of crop and disease present field experiment was planned using design RBD, replications three and variety Thompson seedless during, 2014-15. The result on field evaluation of biocontrol agents against powdery mildew of grape revealed that, all the five biocontrol agents tested were found effective and significantly reduce the disease incidence. The average powdery mildew disease incidence in all the treatments after four spraying was ranged from 35.61 to 77.68 per cent. Among all the biocontrol agents, *B. subtilis* was recorded significantly least incidence (35.61%). Second best biocontrol was *Pseudomonas fluorescens* (37.62%) and third was *Trichoderma viride* (43.46%) over unsprayed control. Result on disease severity revealed that, all the biocontrol agents tested were found effective and significantly reduce the disease severity. The average powdery mildew disease severity in all the treatments after spraying was ranged from 28.47 to 72.87%. Among all the biocontrol agents *B. subtilis* was recorded least severity (28.47%) followed by *Pseudomonas fluorescens* (30.48%) and *Trichoderma viride* (36.82%) over unsprayed control.

PP (S4) 62

Evaluation of biocontrol agents with *Colletotrichum dematium* inciting fruit rot of papaya

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Nine antagonists viz., *Trichoderma viride*, *T. harzianum*, *T. virens*, *T. asperellum*, *T. fasciculatum*, *T. atroviride*, *Pseudomonas fluorescens*, *P. putida* and *Bacillus subtilis* were considered for their antagonism against *Colletotrichum dematium* causing fruit rot of papaya by dual culture method. The study also carried out *in vivo* by pre inoculation and post inoculation methods. Significantly highest per cent mycelial growth inhibition was observed in *Bacillus subtilis* after 8 days of incubation. In pre-inoculation method, *B. subtilis* was found significantly superior in reducing the *Colletotrichum* fruit rot severity by 1.53 and 3.60 per cent on 4th and 8th day after inoculation, respectively. The similar trend of results was obtained in post-inoculation method.

PP (S4) 63

Biological control of blast of finger millet (*Magnaporthe grisea* (Cke) Sacc.)

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Biological control is a potential alternative to chemically based disease control. An experiment was conducted in field condition from 2009-10 to 2011-12 at Hill Millet Research Station, Waghai to evaluate the efficacy of native strains of *Pseudomonas* spp. Isolated from the rice, finger millet, castor, banana, farm pond and river soil/ rhizosphere. Isolation and biochemical tests were done in the Biopesticide and Biofertilizer Unit, Department of Plant Pathology, N. M. College of Agriculture, N.A.U., Navsari. Based upon the biochemical tests, four isolates were confirmed as *P. aeruginosa* and three as *P. fluorescens*. Bio-efficacy of the seven native strains of *Pseudomonas* spp. was compared along with local commercial available biopesticides and Hinosan. Three times spraying of *P. aeruginosa* Rambhas strain at 0.6% (2×10^9 cfu/ml) at 15 days interval, starting after 21 days of transplanting was found significantly effective in lower disease intensity and higher grain as well as fodder for economical management of the leaf blast of finger millet.

PP (S4) 64

Efficacy of bioagents against *Colletotrichum lindemuthianum* causing anthracnose of cowpea [*Vigna unguiculate* (L.) Walp]

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The *Colletotrichum lindemuthianum* is a pathogen with an exceptionally broad host range. The *Colletotrichum* on infection produce anthracnose symptom in the form of lesions as small angular brown spots on the leaf petiole, the lower surface of leaves and leaf veins of cowpea grown under different cropping patterns. The experiment was conducted in the laboratory of Department of Plant Pathology, N. M. College of Agriculture, NAU, Navsari, during 2015 – 2016 to efficacy of bioagents against *Colletotrichum lindemuthianum* causing anthracnose of cowpea. Seven antagonists viz. *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens*, *T. fasciculatum*, *T. longibrachyatum*, *T. koningii* and *Bacillus subtilis* were tested *in vitro* for their efficacy to *C. lindemuthianum* by dual culture method. In this method *Trichoderma viride*, *T. harzianum* and *T. fasciculatum* were appeared as strong and potent antagonists of *C. lindemuthianum* followed by *T. longibrachyatum*, *T. koningii*, *Pseudomonas fluorescens* and *Bacillus subtilis*.

PP (S4) 65

Efficacy of different bio-control agents against *Macrophomina phaseolina* causing root rot disease of cluster bean, under *in vitro* conditions

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Cluster bean is one of important leguminous crop, which is infected with root rot. Bioagents can be used in IDM practice for management of this disease. Bio-control agents are in great demand for replacing the chemical pesticides to control fungal plant pathogens because chemical control results in accumulation of harmful chemical residues, which may lead to serious ecological problems. In this study the bio efficacy of six antagonistic micro-organisms *Viz. Trichoderma harzianum, T. viride, T. koningii, T. hamatum, Pseudomonas fluorescens* and *Bacillus subtilis* was tested through dual culture technique against *M. phaseolina* causing root rot of cluster bean under *in vitro* conditions. Antagonism studies revealed that among six bio-control agents *T. harzianum* showed maximum inhibition (92.40%) and superior over the other bio-control agents followed by *T. viride* (85.89%) inhibition over control and both are scanty sclerotial formation. Least inhibition was observed in *Bacillus subtilis* (39.10%) and abundant sclerotial formation. The results indicated that the application of these microorganisms successfully decreases the root rot incidence and also increases the growth of the cluster bean plants.

PP (S4) 66

Use of *Chaetomium globosum* as a biofertilizer for wheat and sorghum plant

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A biofertilizer is a substance which contain living microorganisms which when applied to seeds, plant surface, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. The use of certain fungi like *Trichoderma viride* and *Trichoderma harzianum* has been tried by various scientists. In the present investigation, use of *Chaetomium globosum* was done to observed change in the plant height, plant biomass and chlorophyll content in comparison to control treatment increase growth was observed in treatment with *C. globosum* or cellulose degrading fungus. *C. globosum* is reported as endophytic to many plants. It's asymptomatic colonization supports plant tolerance to metal toxicity. Heavy metals such as copper, suppress plant growth and disrupt metabolic processes, e.g. photosynthesis.

Bioagents in enrichment of organic substrates

PP (S5) 67

Enrichment of farm yard manure by Plant Growth Promoting Rhizobacteria

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In intensive agriculture, uses of high analyze inorganic fertilizers, little or scarce use of FYM and considerable reduction in recycling of crop residues resulted in deficiencies of nutrients in soil. Consequently, the deficiencies of macro and micronutrients in soil have become major constraint for maintaining soil productivity. Use of enriched FYM is one of the methods to decrease the nutrient losses. For better growth crop requires more nutrients to improve the yield components. Enriching FYM with Plant Growth Promoting Rhizobacteria (PGPR) boost crop grain yield, and harvest index significantly through improving the physico-chemical properties of the soil and sustain its productivity over years. In the last few years, the number of PGPR strain has seen a great importance, mainly due to role of the rhizosphere as an ecosystem that gained importance in the functioning of the biosphere. Various species of rhizobacteria like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to enhance the plant growth by providing nutrients and Phytohormones. The use of Plant Growth Promoting Rhizobacteria offers an attractive way to replace chemical fertilizer (as biofertilizer), pesticides (as biocontrol), and supplements; most of the PGPR isolates result in a significant increase in plant height, root length, and dry matter production of shoot and root of plants. They also help in the suppression of disease in plants. PGPR is as a component factor in the integrated management systems in which reduced rates of agrochemicals and cultural control practices are used as biocontrol agents. The known quantity of FYM was filled in pits. After 21 days, a composite microbial culture consisting of PGPR consortium (*Azospirillum brasiliense*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Paenibacillus polymyxa*, *Trichoderma harzianum*) should be added and thoroughly mixed to ensure complete contact with the decomposing materials (FYM). PGPR consortium added after 21 days of initial composting to protect the microbes from direct exposure to excess heat generated from the materials. The moisture percentage of organic manures after mixing with PGPR should be maintained around 60% throughout enrichment process. The cattle dung slurry @ 1% was applied as starter inoculum of microorganism to boost up the microbiological activities for enhancement of natural process of composting. The pits should cover by polythene sheet and allowed to decompose. The mixture will turn over weekly and also maintain moisture. The Plant Growth Promoting Rhizobacteria enriched organic manures being cheaper and eco-friendly and could be used as an alternative of chemical fertilizers for improving crop productivity and sustainability of the system. However, profitability of this technology needs to be tested at different locations and in different seasons in the different part of India.

PP (S5) 68

Microbial antagonist and prevention of plant disease through traditional way of indigenous cow dependent farming

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Indigenous cows are the assets of India. Traditionally, farmers are dependent on cow's milk, dung and urine for the various purposes. Farming was the main India's functional business even when chemical fertilizers were not exists. After tremendous increase in population, demand of food, fertilizer, man power etc. were raised to cop up with demand. But as a side effect, simultaneously there is also increased in various crop diseases, pest and detriment of soil. To counter this, we have to come again on traditional farming which is of cow dependent farming, one of the functional ways of organic farming. Cow's urine and dung have different role with various components. Even milk by products have great values. The main preparation of "panchgavya" is acting as a microbial antagonist. The method of preparation brings various beneficiary bacteria, fungi and actinomycetes even with nitrogen fixation microbes. After preparation, the beneficial microbial load increased per week up to 21 day and can be stored for long-term usage. Use of panchgavya in replace of fertilizer is beneficiary for crops and soil. It will recycle the nutrients of soil and prevent from diseases and pest. The ferment is enriched in different beneficial microbes, natural enzymes, minerals and essential amino acids. Though it is old and traditional method but have greater impact. More studies must require to identify, isolate, and characterization of selected microbes and biological agents. Use of panchgavya must be incorporate in farming for the pesticide free and diseased free agriculture product.

PP (S5) 69

Mass multiplication of *Trichoderma harzianum* (THCs) in different organic matter and agricultural wastes

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In order to develop a simple and effective technology for mass multiplication different organic matter (well decomposed FYM, Neem cake, groundnut cake, castor cake, poultry manure and vermicompost), agricultural wastes (groundnut shell, wheat bran, castor husk, bajra husk, sorghum leaves and dried

mango leaves) and different grain (sorghum, bajra, maize and wheat) were used as substrates for the mass multiplication of *T. harzianum* and population in term of cfu per gram were recorded. Different organic matters tested maximum population was observed in well decomposed farm yard manure (700×10^5 cfu/g) which was statistically at par with vermicompost (633.33×10^5 cfu/g). Different agricultural wastes significantly higher population was found in wheat bran (270×10^5 cfu/g) Next best in order of merit was castor husk (156.67×10^5 cfu/g) followed by groundnut shell (110×10^5 cfu/g), bajra husk (86.67×10^5 cfu/g), dried mango leaves (83.33×10^5 cfu/g) and sorghum leaves (56.67×10^5 cfu/g) were medium effective sources. The result indicated that farmer waste can be utilized as a good source for multiplication of Trichoderma and decomposition of agriculture waste. This can be applied as enriched organic manure. Farmers can be trained for such multiplication. Thus, conversion of agri-waste into a value added organic product can prove a novel field level acceptable technique.

PP (S5) 70

Bioagent enriched organic manures for sustainable crop production

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Sustainable crop production ensures maintenance of biodiversity with the use of bioagents: a cost effective method. Bioagents include biofertilizers, bio-pesticides and bio-control agents. Most of the organic manures are low in nutrient content, which is insufficient to meet the nutritional requirement of the crops. So, fortification of organic manures with permitted additives like beneficial microbial cultures becomes a feasible option for nutrient supplementation in food production. Use of bio-agents benefit the soil by solubilization of essential minerals, offering micronutrient in more utilizable form for plants, suppressing the growth of plant pathogens or by production of hormones such as auxin and taking part in BNF too. Plant growth promoting microorganisms (PGPMs) include *Azospirillum*, *Azotobacter*, *Phosphobacteria*, *Rhizobia* and *Cyanobacteria*, which are capable of putting forth advantageous properties on growth and yield characteristics of several cultivable crops in different parts of the world, whereas disease management can be assured by inoculation with *Trichoderma* sp. Generally, maximum population of inoculated organisms is expected around 12-16 days in almost all organic manures ensuring an increase in the nutrient concentration. For achieving sustained production and providing optimal growth requirements, crops can be supplemented with bioagent enriched organic manures.

Development of formulations and delivery systems for bioagents/antagonists

PP (S6) 71

Biosynthesis of silver nanoparticles by using *Trichoderma harzianum*

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Trichoderma spp. are promising antagonistic organism and are one of the best alternative to manage soil borne pathogens. Myconanotechnology is the study of nanoparticles synthesis using fungi and their applications. The present study carried on mycosynthesis and characterization of silver nanoparticles produced by the fungus *Trichoderma harzianum*, which was collected from tomato rhizosphere. *Trichoderma* culture filtrate was used for the reduction of silver ions (Ag⁺) in silver nitrate (AgNO₃) solution to the silver nanoparticles. The different ages (4 days, 6 day, 8 days, 12 days and 15 days) of culture filtrates were treated with 1 mM silver nitrate (AgNO₃) solution. Silver nitrate solution treated with 4 days and 6 days incubated culture filtrates turned into dark brown colour after 24 hrs incubation. Among all the culture filtrate, 6 days culture filtrate treated with silver nitrate solution which show dark brown colour was used for further study. These synthesized silver nanoparticles were characterized using UV-Vis spectrophotometer and Transmission Electron Microscopy (TEM). Synthesized silver nanoparticles from 6 days *Trichoderma harzianum* culture filtrate showed the UV absorption peak at 420 nm with maximum intensity after 24 hrs incubation. The Transmission Electron Microscopy (TEM) microphotographs showed the spherical shaped silver nanoparticles with an average size of 50 nm.

PP (S6) 72

Development of formulation using different carrier material and testing longevity of *Trichoderma harzianum* (THCa)

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The success of *Trichoderma* as bio-control agent (BCA) is due to their high reproductive capacity, ability to survive under very unfavorable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi and efficiency in promoting plant growth and defense mechanism. These properties have made *Trichoderma* a ubiquitous genus present in any habitat and at high population density (Mishra and Prasad, 2013). Different carrier materials were tested for the preparation of formulation of *Trichoderma harzianum* (THCa), the populations in terms of colony forming unit per gram(cfu/g) were recorded at monthly interval up to 120 days to check out the self life by using dilution plate technique. Talc supported significantly higher population (5.67×10^8 cfu/g) of *T. harzianum* (THCa). Next in order of merit was starch (5.00×10^8 cfu/g) followed by Kaolin (4.33×10^8 cfu/g), charvoal powder (4.00×10^8 cfu/g) and Acacia powder (4.00×10^8 cfu/g). Further, the talc based formulation of *T. harzianum* (THCa) was prepared and the longevity of the formulation was tested by calculating the population in terms of cfu/g at monthly periodical interval for 180 days using dilution plate technique. The cfu of *T. harzianum* (THCa) decreased gradually by prolonging storage time upto 180 days. Initially the population in the formulation was 5.67×10^8 cfu/g which reduced to 3.67×10^8 cfu/g. Though, the trend was decreasing, sufficient cfu were recorded after six months of storage meeting the standard level of the product.

PP (S6) 73

Effect of various methods of application of *Trichoderma hamatum* on *Sclerotium rolfsii* causing stem rot of tomato

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Trichoderma spp. are one of the most promising antagonists and are one of the best alternatives to manage soil borne plant pathogens. Though effective, but their inconsistent field performance has always been influenced by their effective delivery system. Therefore, present Pot culture study was conducted to evaluate various methods of application of *Trichoderma hamatum* to manage *Sclerotium rolfsii*, causing

stem rot disease in tomato. The experiment was planned in CRD using seven application methods and planting tomato Cv.ArkaVikas. The result revealed that Seed treatment +soil application was found most effective, compared to other treatments, which recorded 100% seed germination, least pre-emergence (14.99%)and post-emergence mortality(3.70%) and Total mortality due to pathogen 17-70%, respectively .The second best treatment found was seedling root dip + soil application of the test pathogen.

PP (S6) 74

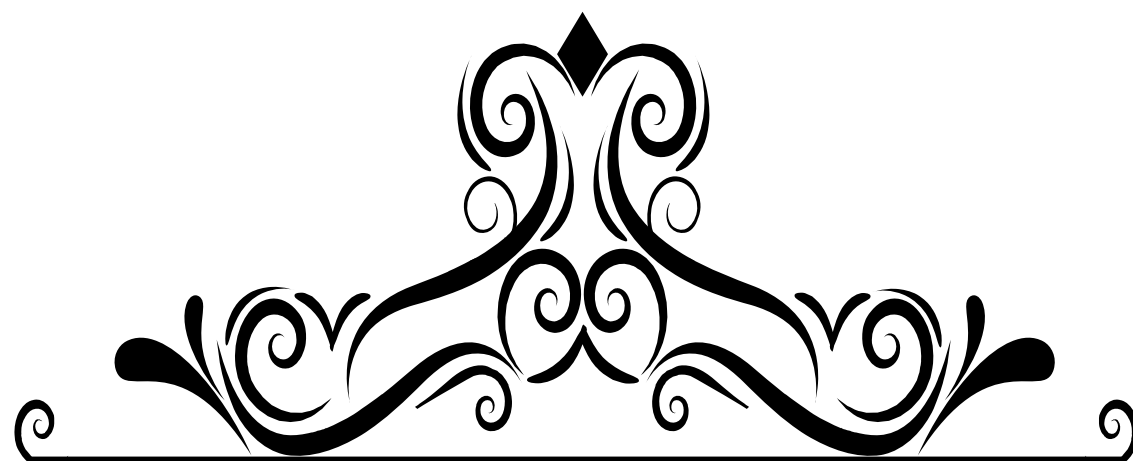
Field evaluation of seed biopriming on seed germination, yield and *Macrophomina* blight in green gram

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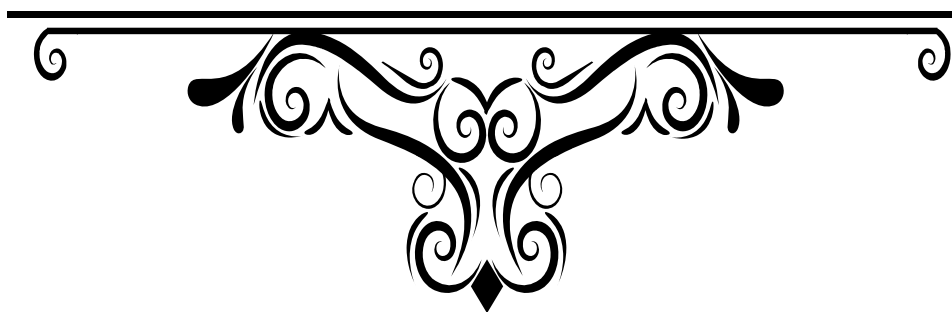
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A two years field experiment was conducted on seed biopriming on seed germination and *Macrophomina* blight in green gram at college farm, NMCA, NAU, Navsari. Seed biopriming with six different bioagents viz., *T. viride*, *T. harzianum*, *T. fascicuatum* *P. fluorescens-I*, *P. fluorescens-II*, *P. aeruginosa* were taken as bioprimed treatments while, hydroprimed seeds and untreated seeds were acted as treated and absolute control respectively. Data on average seed germination, Percent disease control and yield were taken in present experiment. Average seed germination (10.80 to 21.52 per cent), Percent disease control (44.21 to 51.70 per cent) and yield (30.30 to 51.86 per cent) were found to be increased significantly in all the treatments over absolute control. The highest seed germination, Percent Disease Control and yield were obtained in seed biopriming with *T. harzianum* followed by *T. viride*, *P. aeruginosa* and all the other treatments over control. Seed biopriming with *T. harzianum*, *T. viride* or *P. aeruginosa* @ 10 g talc base formulation/kg seeds was recommended not only to get better seed germination and yield but also to manage *Macrophomina* blight significantly.



**SUPPLEMENTARY
POSTER PAPERS**



Session 3

Secondary metabolism of the bioagents in crop growth promotion through host interactions

SPP (S3) 01

Induced systemic resistance with increased antioxidant synthesis in tomato pv. Pusa Ruby with phosphorus solubilizing indigenous *Aspergillus niger* aggregates and management of fungus-nematode wilt disease complex caused by *Fusarium oxysporum* f.sp. *lycopersice* and *Meloidogyne incognita*

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A large number of isolates of *Aspergillus niger* (236 isolates) from 32 different crop fields in 40 districts of the state of Uttar Pradesh, India were isolated. Out of them, 6 isolates SkNAn5, VAn4, AnC2, AnR3, ANAn4 and BuAn3 solubilized more phosphorus, had negative production of ochratoxin-A and were found to possess relatively stronger ability to suppress the *Fusarium oxysporum* f. sp. *lycopersici* through antibiosis (production of volatile compounds) and mycoparasitism (dual culture test). Culture filtrates of these six isolates of *A. niger* inhibited the hatching of eggs and induced mortality to juveniles of *Meloidogyne incognita*. These isolates were also found more compatible with common fungicides viz., carbendazim (Bavistin 50 WP), captan (Captaf 50 WP), mancozeb (Dithane M-45 75 WP), metalaxyl (Apron 35 SD), thiram (TMTD 75 WP), and two nematicides viz., carbofuran (Furadan 3G) and nemacur (Fenamiphos) than rest of the isolates. The isolates were evaluated against wilt disease complex of tomato, *Lycopersicon esculentum* cv. Pusa Ruby in pot culture caused by *F. oxysporum* f. sp. *lycopersici* and *Meloidogyne incognita*. The *A. niger* isolates were applied through nursery treatment in tomato (10 g fungus/100 seedlings) for 10 minutes. Inoculation with *F. oxysporum* f. sp. *lycopersici* caused wilting which appeared on 7-8 weeks old plants and gradually intensified during vegetative growth. Tomato cv. Pusa Ruby was also found susceptible to the infection by *M. incognita* and developed characteristic root galls and egg masses. Concomitant inoculation with both the pathogens resulted to a greater wilting but lesser galling ($P \leq 0.01$). The infected plants exhibited significantly reduced plant growth and yield. Phenol and salicylic acid contents of leaves and roots of tomato were considerably greater in the plants inoculated with wilt fungus and root knot nematode singly or concomitantly. Application of *A. niger* isolates significantly checked the suppressive effect of the pathogens resulting to corresponding increase in the plant growth and yield variables; checked the loss of chlorophyll content of leaves and further increased the phenol content, salicylic acid of leaves and roots and antioxidant (lycopene) content of the tomato fruit. Among the treatments, *A. niger* SkNAn5 showed greatest effectiveness as it decreased the wilt severity by 68% and increased the yield by 88%.

SPP (S4) 02

Evaluation of biopesticides based on *Pochonia chlamydosporia*, *Trichoderma harzianum* and *Bacillus subtilis* for the management of wilt disease complex of chickpea (*Cicer arietinum*)

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Effectiveness of three biopesticides viz., Bionem-X (based on *Pochonia chlamydosporia*), Biowilt-X (based on *Trichoderma harzianum*) and Biocure-X (based on *Bacillus subtilis*) was tested against wilt (*Fusarium oxysporum* f. sp. *ciceri*), root-knot (*Meloidogyne incognita*) and the wilt disease complex (*F. oxysporum* f. sp. *ciceri* + *M. incognita*) of chickpea cv. BG-256. The biopesticides were developed on a powder formulations consisting of flyash, sawdust and molasses, and were applied to seeds (5 g/kg seed) and soil (40 g/microplot) to evaluate their effectiveness against the target diseases under field conditions. The pesticides viz., carbendazim and nemacur were applied @ 1.25 kg a.i./ha and 6.0 kg a.i./ha as soil application, and 2g/kg seed as seed treatment to compare effectiveness of the biopesticides. Chickpea grown in the plots infested with pathogens singly or concomitantly developed characteristic wilt and root-knot symptoms, and exhibited significant yield decline. Application of biopesticides checked the severity of the diseases and reduced the yield loss. The biopesticides Bionem-X and Biocure-X were found effective against root-knot disease and suppressed the galling by 20 and 12% and promoted the yield of infected chickpea plants by 28 and 34% in comparison to the control. Application of Biocure-X was found highly effective against the fungus-nematode wilt disease complex; its seed treatment substantially controlled the wilt and root-knot, and increased the yield of concomitantly infected chickpea by 49%. Biocure-X was found more effective in increasing the nodulation as compared to other treatments. Effect of carbendazim and nemacur was significant ($P < 0.05$) in checking the wilt, root knot and disease complex, but was not as effective as biopesticides. The present study has demonstrated that biological management fungus-nematode wilt disease complex can be successfully achieved with the application of the biopesticides based on *B. subtilis*.

SPP (S4) 03

Relative antagonistic effect of twelve native rhizospheric biocontrol agents against sheath blight disease of rice (*Rhizoctonia solani* AG1-IA)

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The relative antagonistic effect of twelve native rhizospheric isolates of fungal and bacterial biocontrol agents (BCAs) was screened *in vitro* against sheath blight (ShB) pathogen of rice, *Rhizoctonia solani*. All the BCAs significantly inhibited the mycelial growth of *R. solani*, however; substantial variability in virulence among the isolates was observed. Six most efficient isolates were selected to ascertain their effectiveness against *R. solani* under pot condition on rice cv. PS-5. Two sets of pot were maintained, inoculated or uninoculated with *R. solani*. The BCAs were applied as soil application at 2 mL/pot ($2-3 \times 10^6$ CFU/mL) in both sets. Plants grown in *R. solani* inoculated soil developed necrotic lesions and plant suffered 24-30% decrease in growth and yield. Among the BCAs applied, *Pseudomonas putida* AMUPP-2 was found most effective and suppress the disease severity by 69% with correspondingly 21-49% increase in plant growth and yield. *Trichoderma harzianum* was next in effectiveness followed by *P. fluorescens*, *Bacillus subtilis*, *T. viride* and *Aspergillus niger*. The greatest build-up in the rhizospheric population was also recorded with *P. putida* followed by *P. fluorescens* and *T. harzianum*. The study revealed the antagonistic potential of *P. putida* for the first time against *R. solani* and the relative effectiveness of *P. putida* was higher than *P. fluorescens* and *T. harzianum*. Field trials under naturally infested plots also validated the efficacy of *P. putida* and satisfactorily controlled ShB with 61% enhancement in rice yield. The effect of *P. putida* was also at par with the fungicides, propiconazole.

SPP (S4) 04

Biological management of root-rot of mungbean caused by *Macrophomina phaseolina*

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A study was undertaken to evaluate the efficacy of seed treatment with some biocontrol bacteria and fungi viz., *Pseudomonas aeruginosa*, *Trichoderma viride*, *T. hamatum* and *T. virens* against root-rot disease of mungbean (cv. T-44) caused by *Macrophomina phaseolina* under pot condition. Treatment with the biocontrol agents (BCA) resulted in reduced root-rot severity and enhanced plant growth and yield of infected mungbean plants. Plants grown in the fungus infested soil without BCAs exhibited rotting

of roots and significant decline in the plant growth (32-45%) and yield (36%) compared to the control. Greatest suppression in the disease was observed with *T. viride* (47%) which improved the growth and yield parameters by 26-28% and 29% ($P \leq 0.05$). The bacterium, *P. aeruginosa* was next in effectiveness which led to 44% suppression of the disease and 26% enhancement in the yield. *T. virens* was the least effective which resulted in lowest decrease in the disease severity (31%) and minimum increase in plant yield (17%). *T. viride* and *P. aeruginosa* also appeared to be most effective for enhancing the root nodulation and led to 52-57% increase in the functional nodules/root system in the infected plants with 22-23% greater leghaemoglobin content in the nodules. The application of these two BCAs also improved the leaf chlorophylls (30-36%) and carotenoids (31-32%) greater than the other antagonists. Soil population of *M. phaseolina* was 41-67% less in the pots treated with the BCAs than untreated pots. Application of biocontrol agents caused marked decline in the soil population of *M. phaseolina*, with a maximum effect of *T. viride* and *P. aeruginosa*.

SPP (S4) 05

Evaluation of some fungal biocontrol agents against root-knot nematode, *Meloidogyne incognita* on two pigeonpea cultivars under field condition

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Field trial was conducted in microplots to ascertain the performance of seed treatments with five biocontrol fungi viz., *Purpureocillium lilacinum*, *Aspergillus niger*, *Trichoderma harzianum*, *Pochonia chlamydosporia* and *Fusarium solani* against root-knot nematode, *Meloidogyne incognita* on two pigeonpea cultivars CO 6 and UPAS 120. Freshly hatched second stage juveniles (J_2) of *M. incognita* were inoculated @ 2000 /kg soil in 15 cm² top soil. The pigeonpea plants grown in the nematode infested soil developed characteristic galls, numbering 56 and 44/root system in the cv.CO 6 and UPAS 120, respectively. Application of the biocontrol agents suppressed the disease with greatest decrease in the galling recorded with *P. lilacinum* (38% in UPAS 120 and 32% in CO 6) followed by *A. niger* (31% in UPAS 120 and 28% in CO 6), *T. harzianum* (26% in UPAS 120 and 23% in CO 6, $P \leq 0.05$). Egg mass production also decreased greatly with the order of: *P. lilacinus* > *A. niger* > *T. harzianum* > *P. chlamydosporia* > *F. solani*. Pigeonpea plants showed good nodulation when treated with *P. lilacinum* (15.4% in UPAS 120 and 21.1% in CO 6) or *A. niger* (11.5% in UPAS 120 and 15.8% in CO 6). In nematode infected plants, a significant decrease (25%) in nodulation was observed. Pigeonpea cultivars grown in nematode infested soil produced significantly less yield over control ($P \leq 0.05$). Application of the biocontrol agents, however, improved the yield of both the cultivars. Seed treatment with *P. lilacinum* resulted in a significant ($P \leq 0.05$) increase in grain yield of pigeonpea cv. CO 6 (18.3%) and UPAS 120 (15.0%) as compared to inoculated control. Significant increase in grain yield of UPAS 120 (13%) and CO 6 (17%) was also recorded with *A. niger*. A reduction in the

nematode population in BCAs treated plots occurred and the order of decrease was: *P. lilacinum* > *A. niger* > *T. harzianum* > *P. chlamydosporia* > *F. solani*. The soil population of BCAs increased over time in both nematode inoculated and uninoculated plots, being greater in the former.

SPP (S4) 06

Effect of *Meloidogyne incognita* on some leafy vegetables and its bio-management

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Some important leafy vegetables viz., spinach (*Spinacia oleracea*), fenugreek (*Trigonella foenum-graecum*) and dill soa (*Anethum graveolens*) were screened against *Meloidogyne incognita* (2000 J2/kg soil) in order to determine damage potential of the nematode. Further, in view of risk of human toxicity involved in the consumption of pesticides treated foliage effectiveness of some important biocontrol agents viz, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Aspergillus niger* through seed and soil application was evaluated against the nematode. All vegetables were found susceptible to *M. incognita* and the order of susceptibility was spinach > dill soa > fenugreek. Seed or soil application of biocontrol agents suppressed the galling, egg mass production and soil population of *M. incognita* and improved the plant growth of the vegetables to a varying extend. Treatment with *P. fluorescens* provided maximum improvement in the plant growth parameters as well as biomass production. Seed and soil application with this biocontrol agent significantly suppressed the galling by 16-36% and improved the fresh shoot and root weight of dill soa (17 and 11%), spinach (18 and 10%) and fenugreek (20 and 12%) over control, respectively. The BCA treatment also caused decline in the soil population of *M. incognita* by 20-40% at two and four-month stage, respectively. Next in effectiveness was *A. niger* which suppressed the galling by 16-30% and improved the foliage production by 10-14%, respectively. The treatments with *B. subtilis* were also found effective in suppressing the nematode, but considerably less than *A. niger*. Application of biocontrol agents caused substantial decline in the soil population of *M. incognita* (12-40%), which was in the order of *A. niger*, *P. fluorescens* and *B. subtilis*.

SPP (S4) 07

**Effect of *Trichoderma* species on the root-knot of tomato caused by
*Meloidogyne incognita***

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Effects of culture suspension and culture filtrate of soil application of five *Trichoderma* spp. viz., *T. harzianum*, *T. hamatum*, *T. viride*, *T. virens* and *T. koningii* against *Meloidogyne incognita* on causing root-knot in tomato were examine *in vitro* and *in vivo*. *In vitro*, culture suspension and culture filtrate at different concentrations (12.5-50%) inhibited egg hatching of egg and induced mortality to the juvenile of *M. incognita*. These effects were found to be dependent on the concentration and *Trichoderma* spp. Treatments with *T. harzianum* were found to be most effective at causing highest decrease in hatching and 100% mortality of juveniles at 50% concentration, followed by *T. hamatum* *T. virens* and *T. viride* over control. The inoculation with 2000 freshly hatched juveniles of *M. incognita* caused characteristic oval and fleshy galls on the roots of tomato cv. K-21, numbering around 90 galls/root system. Over 70 egg masses/root system were also formed. The nematode infection caused significant decrease in the plant growth and yield variables of tomato. The culture suspension and culture filtrate of *Trichoderma* spp. suppressed the galling, egg mass production, fecundity and soil population of the nematode and improved the plant growth of tomato that varied with treatment. Treatment with culture suspension or culture filtrate of *T. harzianum* provided maximum control of the nematode and increase in the plant growth, biomass production and yield of tomato over control. Next in effectiveness was *T. hamatum* and *T. viren*. The *Trichoderma* spp. established in the soil and their populations increased over time; however, nematode population decreased with the progress of time. Over all, culture suspension performed better against the nematode as compared to the culture filtrate.

SPP (S4) 08

**Effect of *Trichoderma harzianum* isolated from mustard leaves on *Alternaria brassicae*
in vitro and *in vivo***

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The present experiment was conducted to determine the effect of *Trichoderma harzianum* isolated from mustard leaf surface on *Alternaria brassicae* causing leaf spot in mustard. *In vitro* effect of *T. harzianum* was examined on the colonization of *A. brassicae* using dual inoculation test. The test showed that *T.*

harzianum inhibited the radial growth of *A. brassicae* upto 72% over control. Effect of spore suspension and culture filtrate of *T. harzianum* was examined on the disease development and phylloplane population of *A. brassicae* on mustard. The culture filtrate of *T. harzianum* at the highest concentration caused maximum inhibition in the colonization and conidial germination of *A. brassicae*. To examine the effect of culture and cultural filtrate of *T. harzianum* mustard cv. Varuna was grown in a field. Seven-eight week old plants were inoculated by *A. brassicae* by spraying the leaves with conidia suspension of the pathogen (10^5 conidia/ml, 5ml/plants in two sprays on consecutive days). Two days later, the plants were sprayed with the spore suspension of *T. harzianum* (10^7 spores/ml, 5ml/plant in two sprays, with a week interval). *T. harzianum* proved quite effective as its conidial suspension caused upto 17 and 12% reduction in disease intensity on the leaves and pods. Whereas, its culture filtrate caused 24 and 19% reduction in the disease severity. The treatment also caused some significant decline in the phylloplane population of *A. brassicae*. The *T. harzianum* was re-isolated from the mustard leaves, although its population was much low. These results have revealed some prospects of using *T. harzianum* to control foliar disease such as Alternaria blight of mustard.

SPP (S4) 09

Management of root-knot disease of cucumber by antagonistic biocontrol fungi and bacteria under poly house cultivation

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An experiment was conducted to evaluate the effectiveness of five biocontrol fungi and bacteria viz, *Aspergillus niger*, *Trichoderma harzianum*, *T. viride*, *T. hamatum* and *Pseudomonas fluorescens* in the management of root knot nematode (*Meloidogyne incognita*) on cucumber in pots under polyhouse condition. The BCAs were applied through seed treatment (2g/kg seed) and soil application (5g/kg soil) separately. Cucumber seedlings were inoculated with second stage juveniles of *M. incognita* @ $1500J_2$ / kg soil. The inoculated plant showed numerous characteristic galls small as well as large on the root system of cucumber. The nematode infection resulted in 20-34% reduction in the plant growth and biomass production. However, application BCAs combat with negative effect of the nematode. Maximum reduction of root-knot disease was observed with seed treatment of *T. harzianum* (30%), followed by *T. viride* (23%), *A. niger* (19%), *T. hamatum* (15%) and *P. fluorescens* (11%). Overall, seed treatment was found to be more effective as compared to soil application. Besides direct suppression, the BCAs also enhanced the plant growth and biomass production of cucumber. Maximum enhancement in the plant growth parameters of cucumber was recorded with seed treatment of *P. fluorescens* (15-20%) as compared to uninoculated plants.

SPP (S4) 10

Comparison of three different isolates of *Trichoderma* spp. against tomato wilt

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Tomato is the most important vegetable crop but its productivity is very low due to several fungal diseases. One of them, wilt of tomato is a major problem in U.P. *Fusarium oxysporum* f. sp. *lycopersici* is responsible for wilt disease of tomato which results in extensive to the crop. In this experiment, the three isolates *Trichoderma* spp. from different districts of U.P. were compared to *Fusarium oxysporum* f. sp. *lycopersici* isolate. Antagonistic behavior of bioagent was evaluated against *Fusarium oxysporum* f. sp. *lycopersici* by dual culture techniques. All three spp of *Trichoderma* were also evaluated under field condition and data of disease incidence were recorded at 30, 60 and 90 DAT (Days After Transplanting). Among all treatments the *Trichoderma* spp. of Allahabad was found very effective under in vitro and field both condition. In the present study, the local strain of *Trichoderma* spp. from same field gave very good results against *Fusarium oxysporum* f. sp. *lycopersici*.

Development of formulations and delivery systems for bioagents/antagonists

SPP (S6) 11

Effectiveness of different modules of application of biocontrol agents rice root-knot nematode, *Meloidogyne graminicola*

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Investigations were carried out to evaluate the effectiveness of different combination of root dip (RD) and soil application (SA, 2 ml/kg soil) at different days after planting (DAP) of *Aspergillus niger*, *Pochonia chlamydosporia*, *Bacillus subtilis* and *Pseudomonas fluorescens* against root-knot nematode, *Meloidogyne graminicola* on rice cv. PS-5. The biocontrol agents ($2-3 \times 10^6$ CFU/ml) were applied in the nematode infested (1089 ± 114) second-stage juveniles/kg soil) and non-infested soil through seven treatments viz., RD, SA 15 DAP, SA 30 DAP, SA 15 + 30 DAP, RD + SA 15 DAP, RD + SA 30 DAP and RD + SA 15 + 30 DAP. Rice plants grown in non-infested soil and applied with *P. fluorescens* through RD + SA showed significant improvement in the length and dry weight of root and shoot ($P \leq 0.05$). In the nematode infested soil, terminal and spiral galls developed on the roots, and plants suffered 19-31% decrease in the plant growth compare to non-infested control. The RD + SA 15 DAP with *P. chlamydosporia* or *A. niger* was found highly effective against the nematode. The treatments suppressed the gall formation (22-25%), egg mass production (21-29%) and soil population (16-60%) of *M. graminicola*, and subsequently improved the plant growth variables by 11-21%. Relatively greater nematode control was recorded with RD + SA 15 + 30 DAP, but statistically the effect was at par with RD + SA 15 DAP.

SPP (S6) 12

Biomangement of white mould of pea caused by *Sclerotinia sclerotiorum* through seed treatment and soil application of *Trichoderma* species

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An investigation was undertaken to evaluate relative effectiveness of seed treatment and soil application of *Trichoderma harzianum*, *T. virens*, *T. viride*, *T. hamatum*, *T. koningii*, *T. atroviride*, *T. auroviride* and *T. longibrachiatum* against white mould fungus, *Sclerotinia sclerotiorum* @ 2g/kg soil on

pea, *Pisum sativum* cv. Vasundhra grown in earthen pots. *Trichoderma* spp. were applied @ 4g/kg seed (seed treatment) or 4g/kg soil (soil application). In case of soil application *Trichoderma* spp. were applied 3 days before the pathogen inoculation. The pea cv. Vasundhara was found highly susceptible to the white mould fungus and developed characteristic symptoms. Irregular water soaked spots appeared on the stem, which spread to branches and leaves. The lesions later enlarged followed by soft watery rot of the affected parts. The treatments with *Trichoderma* spp. suppressed the disease severity and improved the plant growth and yield of pea. Application of *T. harzianum* resulted in the maximum disease control and subsequent increase in the plant growth and yield over control with relatively greater effects of seed treatment. Next in effectiveness was *T. virens* which increased the plant growth and yield. Seed treatment with *Trichoderma* spp. synergized the root nodule forming bacteria, and substantially enhanced the root nodulation, especially the treatments with *T. harzianum* or *T. virens*. The overall, the order of effectiveness of *Trichoderma* spp. in controlling the disease and enhancing the pea yield was: *T. harzianum* > *T. virens* > *T. viride* > *T. hamatum* > *T. koningii* > *T. longibrachiatum* > *T. atroviride* > *T. auroviride*. The study has demonstrated that seed treatment with *T. harzianum* or *T. virens* may effectively control the white mould and significantly improve pea yield.

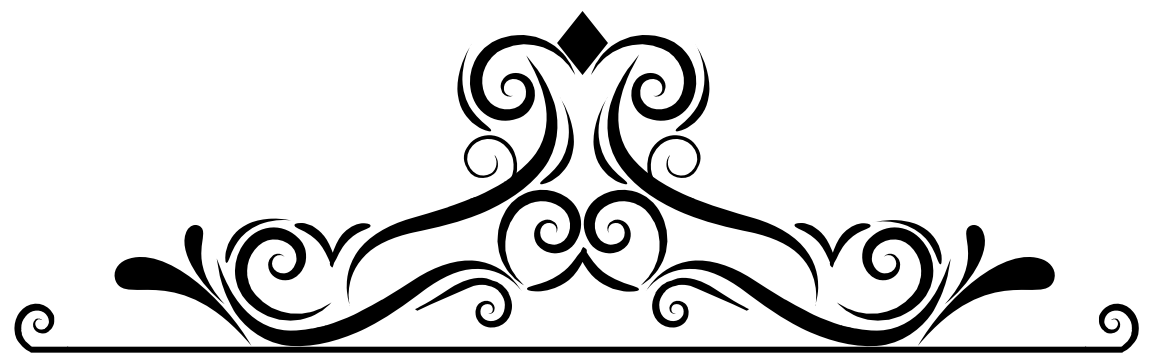
SPP (S6) 13

Evaluation of seed treatment of *Trichoderma* spp. against Fusarium wilt disease of chickpea and yield enhancement in pot conditions

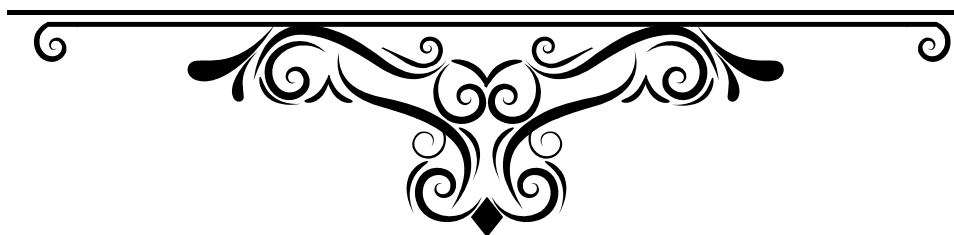
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An experiment was conducted to evaluate relative effectiveness of seed treatment with *Trichoderma harzianum*, *T. viride*, *T. virens* and *T. hamatum* against the wilt of chickpea. The chickpea wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri* is one of the major yield limiting factors in chickpea. Earthen pots filled with 2 kg autoclaved soil were inoculated the wilt fungus colonized on sorghum seeds (2 g/kg soil), whereas *Trichoderma* spp. were applied @ 2 g/Kg seed. The seed treatment with *Trichoderma* spp. significantly increased the seed germination of chickpea as compared to non-treated seeds. Chickpea cv. C104 was found highly susceptible to Fusarium wilt and showed characteristic symptoms of wilting of leaves. The *Trichoderma* treatments reduced the severity of wilt and provided considerable protection to plants, and resulted in significant increase in the quality and quantity of chickpea yield. The highest disease control and yield enhancement were recorded with the seed treatment of *T. harzianum*, followed by *T. viride*, *T. virens* and *T. hamatum*. The *Trichoderma* spp. established in the soil and expressed highest competitive saprophytic and colonizing ability in the chickpea rhizosphere. The study has demonstrated that seed treatment with *T. harzianum* or *T. viride* can serve as a handy tool, thus can be integrated with normal agronomic practice of applying rhizobium to achieve biomanagement of wilt disease in chickpeas.



WEST ZONE SESSIONS



Lead papers

LP 1

Research development and future priorities of Brassica host-pathosystem

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Brassica encompasses large number of edible oil yielding crops (rapeseed-mustard), cruciferous vegetable crops, forage crops, and numerous weeds. These plants like all other living organisms do not live in isolation but are under the influence of interaction among themselves as well as environmental factors. Under natural conditions plants are always challenged simultaneously by biotic and abiotic stresses. The production and productivity of these crops is limited by a number of biotic and abiotic stresses. Cruciferous are known to be inflected by more than forty diseases all over the world. However, diseases like *Alternaria* blight (*Alternaria brassicae* (Berk.) Sacc.), white rust (*Albugo candida* (Pers. ex. Lev.) Kuntze], downy mildew (*Hyaloperonospora parasitica* (Gaum.) Goker.), powdery mildew (*Erysiphe cruciferarum* Opiz. ex. Junell), and *Sclerotinia* rot (*Sclerotinia sclerotiorum* Lib. de Bary) are of major consequences because of their widespread distribution, and devastating nature causing heavy yield losses. In future, the disease scenario in these groups of crops is bound change from major to minor and vice-versa because of intensive cropping, large, and continuous area under same *Brassica* crop, and cultivar, heavy, and rapid irrigation, higher fertilization (Nitrogen), closer spacing, and rapid change in climatic conditions. Although lot of information have been generated on major diseases with respect to geographical distribution, losses incurred, symptomatology, disease cycle, epidemiology, host-parasite interaction, nature, and mechanisms of resistance, physiologic specialization, and integrated diseases management but still much more is required to overcome the annual losses caused through bridging the gaps in research, and comprehension with a holistic view. In the past research efforts have mostly been limited to individual stresses, but it is now quite apparent that a true picture would only emerge if these biotic, and abiotic stresses are studied holistically. Applications of modern science and techniques including omics data analysis, and functional characterization of individual's genes have revealed a convergence of signalling pathways for biotic and abiotic stress adaption. Multiple stress responses are coordinated by complex signalling network involving reactive oxygen species, and phyto-hormones (H₂O₂, and abscisic acid). Future research priority areas of Brassica host-pathosystem should be standardization of host differentials, and nomenclature of pathogenic races, identification of broad spectrum sources of resistance, resistant loci, and resistant genes in each locus, identification of slow blighting, slow mildewing, slow rusting, tolerant, partial resistant, strong, and weak genes, with suitable combinations, genetics of virulence, and virulence spectrum, mapping, cloning, characterization, and identification of genes for resistance, and virulence, biochemical basis, and genetics of *Albugo-Hyaloperonospora* association, comparative study of *Alternaria* spp. in relation to pathogenesis, transfer of detoxifying enzymes/genes from *B. alba* to *B. juncea*, induced resistance, role of phytotoxins in pathogenesis, and host resistance, identification, and use of hypo-virulent isolates of *Sclerotinia*, as well as identification of convergent points involved in the multiple stress signalling mechanism. There is need to percolate the technologies to the end user for the best use of IPM, and IDM technology for integrated *Brassica* crops management.

Oral Papers

OP 1

***Invitro* antifungal efficacy of silver nanoparticles against *Fusarium oxysporum* f. sp. *lycopersici* in tomato**

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Silver nanoparticles, which have high antimicrobial effects as compared to the bulk silver. The prospectus of most promising biocontrol agents of the genus *Trichoderma* has evidenced since long back for soil borne plant pathogens especially *Fusarium* spp. Therefore, present investigation on mycosynthesis of silver nanoparticles and its antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici* in tomato was carried out. Characterization of silver nanoparticles were carried out by UV-Vis spectroscopy and Transmission Electron Microscopy (TEM) which revealed that synthesized nanoparticles were having the UV absorption peak at 420 nm and nanoparticle size was 50 nm. Silver nanoparticles demonstrated significant antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici* in tomato by using Agar well diffusion method and Poisoned food technique. In Agar well diffusion method, the highest zone of inhibition 18.66 mm was recorded at 100 ppm concentration than other treatments. In poisoned food technique, the suspension of silver nanoparticles at 100 ppm concentration recorded highest (75.19%) inhibition. This was followed by 50 ppm, 30 ppm, 10 ppm conc. and *Trichoderma* culture filtrate which recorded 66.67%, 58.89%, 54.45% and 51.45% inhibition, respectively.

OP 2

Management of major diseases of pigeon pea: An integrated approach

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Pigeon pea (*Cajanus cajan* L. Millisp) is a most important legume crop worldwide. India is the largest producer and consumer of pigeonpea in the world. It is a protein rich staple food, contains about 22 percent protein, which is almost three times that of cereals. Pigeonpea supplies a major share of protein requirement of vegetarian population of the country. Its split grain is mainly consumed in the form of Dal, which is an essential supplement of cereal based diet. The crop is affected by many biotic and abiotic stresses at various stages of its growth. Among the biotic stresses, Wilt and Phytophthora stem blight (PSB) are the

most important yield limiting factors. Every year, these diseases cause enormous economic loss and exports are restricted owing to poor quality produce. For the management of these diseases, chemical pesticides are generally used. It is well established that agricultural chemicals cause not only environmental pollution but also pose serious health hazards as their injudicious use often results in toxicity to man, plants, domestic animals and wild life and therefore are regarded as ecologically unacceptable. The management of wilt and PSB by chemicals has not yielded the anticipated results. Therefore, a renewed knowledge is needed to assess the current severity of these problems and to develop or refine integrated disease management (IDM) modules to protect crops in a cost-effective and eco-friendly manner. IDM is a multidisciplinary approach that manages diseases effectively by integration of cultural, physical, biological, safer chemical and molecular approaches. These approaches can play a major role in reducing the losses due to the disease under subsistence farming conditions. Since IDM comprises of many approaches, it will definitely prove to be an effective strategy for enhancing pigeon pea production under the changing climate scenario.

OP 3

Influence of weather parameters on bacterial blight of *hirsutum* varieties of cotton

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Cotton has preeminent status among all cash crops in the country, among various diseases occurring on cotton, bacterial leaf blight caused by *Xanthomonas axonopodis* pv. *malvacearum* causing serious loss to cotton. Since many years, it has been occurring in an epiphytotic form on commercially grown cotton varieties, leading to severe defoliation and substantial yield losses. A field trial was conducted during *Kharif*, 2016 on the experimental field of Plant Pathology Section, College of Agriculture, Nagpur. Studies were conducted to assess progress of bacterial blight disease on 11 varieties of *G. hirsutum* groups, in relation to the environmental factors. Bacterial blight disease was recorded with its first appearance and subsequently at weekly interval till it prevailed on *G. hirsutum* cotton varieties and its incidence varied from 0.37 to 21.11 per cent. Disease was first appeared in 33th Met. Week (2nd week of August) on the test cotton varieties, of which intensity ranged from 0.37 to 1.85 per cent and further prevailed up to 1st Met. week (1st week of January). BLB initiation and development were influenced by weather parameters. Highest disease intensity (21.11%) was recorded during 43rd Met. week on AKH-10-10. The disease started declining after 48th Met. week i.e. during last week December, 2016. The correlation studies revealed that during the year 2016-17 maximum temperature and bright sunshine hours of preceding week had positive and significant correlation with bacterial leaf blight disease intensity. While, total rainfall, total rainy days, relative humidity (evening and morning) mean relative humidity of preceding week showed negative and non-significant correlation with bacterial leaf blight disease intensity.

OP 4

Detection and Molecular Characterization of *Bean Common Mosaic Virus* in mungbean
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Bean common mosaic virus (BCMV) is an important seed borne pathogen. The virus caused downward leaf rolling, thickening of leaves, mosaic, leaf deformation, necrosis of tissues of abaxial side of leaves, necrosis of apical stem portion. *Vigna mungo*, *Panicum granatum*, *Vigna unguiculata* and *Ricinus communis* gave positive reaction to BCMV antisera and potyvirus antisera. The infected leaves of IR-16, Meha and K-851 gave strong positive reaction with BCMV specific antisera with O.D. value of 3.99, 3.91 and 3.37, respectively as compared to positive control with OD value of 2.20. Transmission electron microscope studies revealed the presence of BCMV as flexuous particles of 823 nm long. The RT-PCR amplified product when run on 1 per cent agarose revealed the presence of predicted ~1300 bp product.

Evaluation of 65 Mungbean Genotypes/Varieties to Bean Common Mosaic Virus screened under field conditions in middle Gujarat. The incidence of the virus was recorded from 2-52% on different varieties of mungbean. The maximum disease incidence was recorded in Meha variety of mungbean. Among Sixty-five genotypes/varieties of mungbean, only one genotypes ie LGG-460 was found highly resistance. Whereas, seventeen were resistant. Twenty-two were moderately resistance and fourteen were found moderately susceptible, while, five genotypes were found susceptible and six varieties viz., IPM 02-3 RED, PDM-139, IPM 02-14, K-851, MEHA, IR-16 were highly susceptible against the disease.

Poster Papers

PP 1

Mushroom flora of western ghat region of Maharashtra with special reference to *Pleurotus* spp.

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The present investigation entitled "Mushroom flora of western ghat of Maharashtra with special reference to *Pleurotus* spp." was carried out at Plant Pathology Section, College of Agriculture, Kolhapur. The survey was carried out with object to study biodiversity of mushroom flora in western Maharashtra and to identify mushroom species on the basis of morphological characteristics. Collection of mushroom specimen was conducted during monsoon season of 2015. During the present investigation, seven species of the genus *Pleurotus* viz., *P. ostreatus* (2), *P. pulmonarius*, *P. flabellatus*, *P. porrigens*, *P. lignatilis* and *P. djamor* were observed in this investigation, the extensive survey of forests and existing places near Kolhapur, Satara, Sindudurg and Pune of Maharashtra was carried out. Identification of *Pleurotus* mushroom specimen collected was done on the basis of their macroscopic (morphological) and microscopic characters.

Pleurotus show variation among their pileus shape i.e. *P. ostreatus*, *P. lignatilis*, species had oyster or kidney shape, *P. pulmonarius*, *P. flabellatus* species showed fan or shell shape, *P. porrigens* species of oyster mushroom showed tongue shape structure. Other characteristics include form of mushroom i.e. single, group or connate (united). Out of those oyster species *P. ostreatus*, *P. pulmonarius*, *P. flabellatus*, *P. djamor*, are edible but not consumed by people. *P. porrigens* is Inedible, poisonous so not consumed locally. *Pleurotus* spp. of mushroom, *P. ostreatus* had Lilac spore print, *P. porrigens* had creamish spore print, remaining all 4 species i.e. *P. pulmonarius*, *P. flabellatus*, *P. lignatilis*, *P. djamor*, showed white spore print.

The study revealed that macro fungal diversity in the western ghat region of Maharashtra is needs to be conserve properly especially for edible and medicinal purpose.

PP 2

Screening of sugarcane genotypes against red rot pathogen

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Thirty six promising genotype of sugarcane (*Saccharum Spp.* complex) were screening artificially for their reaction to red rot disease at Main sugarcane research station, Navsari Agriculture University,

Gujarat, India using plug and cotton swab method of inoculation for red rot during 2014-2015. Among IVT early and midlate entries none was found resistant reaction while ninety i.e. Co 11001, Co 11004, Co 11006, Co 11018, CoM 11084, CoN 11071, CoT 11366, Co 11005, Co 11007, Co 11012, Co 11019, Co 11021, Co 11022, Co 11023, Co 11024, CoM 11086, CoM 11087, CoN 11073 and CoN 11074 were showed moderately resistant reaction against in plug method. In cotton swab method all variety exhibited resistant reaction. Among AVT early and mid-late entries none of the entries showed resistant reaction whereas for three entries i.e. Co 09004, CoN 09072, Co 09009 found moderately resistant. By cotton swab method all AVT entries showed resistant. In plug method check varieties Co 94008 and Co 99004 exhibited moderately resistant reaction and in cotton swab method check variety CoC 671 exhibited susceptible reaction. Rest of entries include with check displayed moderately susceptible reaction to red rot by plug and cotton swab method.

PP 3

Effect of crop rotations/sequences in bidi tobacco field on population dynamics of nematodes

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Tobacco, an economically important non-food narcotic cash crop, is attacked by root-knot, reniform and stunt nematodes in Gujarat. No information is available about the impact of crop rotations/sequences on population dynamics of nematodes; therefore, the present investigation was carried out using six treatments like 1. Tobacco MRGTH 1 (*Kharif-Rabi*) alone – Summer fallow (T-T-F), 2. Groundnut GG 2 (*Kharif*) – Potato Kufri Badshah (*Rabi*) – Summer fallow (G-P-F), 3. Maize HQPM 1 (*Kharif*) – Potato (*Rabi*) – Summer fallow (M-P-F), 4. Pigeon pea GT 1+ Pearl millet GHB 558 (*Kharif-Rabi*) – Cluster bean Guj Guar 1 (Summer) (PP+PM-CB), 5. Sesamum Guj Till 1 (*Kharif*) – Potato (*Rabi*) – Summer fallow (S-P-F) and 6. Tobacco (*Kharif-Rabi*) – Pearl millet (Summer) (T-T-PM) in RBD. Each treatment was replicated four times keeping the plot size of 5.4 X 7.5 m. All agronomic practices in vogue were followed. For estimation soil population of nematodes, soil sample (1000 cc) was collected at sowing/planting and thereafter at monthly interval throughout the year. The nematodes were extracted and counted by processing well mixed 200 cc soil using Petri dish Assembly Method.

The results revealed that *kharif* season (up to 120 DAS) favoured the population of stunt and reniform nematodes for multiplication than summer season (270 to 330 DAS). The population of lesion nematodes survived and multiplied on favourable host during all the three seasons. Overall *kharif* season favoured and supported the multiplication of plant parasitic nematodes while *kharif* and *rabi* season (150 to 240 DAS) favoured non-plant parasitic nematodes. Stunt nematodes was favoured by the crop sequences of M-P-F, PP+PM-CB, T-T-F and T-T-PM, reniform nematode was favoured by the crop sequences of S-P-F, PP+PM-CB and T-T-F, while lesion nematodes was favoured by the treatments of M-P-F, PP+PM-CB and T-T-PM.

PP 4

Bioefficacy of plant botanicals and virus inhibitory chemicals against mung bean yellow mosaic India virus in moth bean under *in vitro* condition

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Yellow mosaic disease of moth bean *Vigna conitifolia* (Jacq.) Marechal caused by mung bean yellow mosaic India virus. MYMIV is member of the whitefly (*Bemisia tabaci* Genn.) transmitted bipartite gemini virus group in the family Geminiviridae. Efficacy of some plant botanicals and virus inhibitory chemicals were tested at Department of Plant Pathology, B. A. College of Agriculture, Anand during 2016-17. The results indicated that pre-inoculated sprays of plant botanicals and virus inhibitory chemicals reduced the yellow mosaic virus infection in moth bean plants compared to control. The spray with (T7) salicylic acid @ 0.05 per cent and (T5) NSKE @ 5.0 per cent significantly reduced MYMIV infection by 77.50 and 74.32 per cent, respectively they were at par and followed by the treatment of (T8) benzoic acid @ 0.05 per cent, (T4) neem oil @ 3.0 per cent, (T9) barium chloride @ 0.05 per cent and (T3) *Azadirachta indica* @ 10 per cent significantly reduced disease infection viz., 60.91, 57.51, 57.65 and 54.20 per cent, respectively.

PP 5

Antagonism of phylloplane fungal antagonists against mango anthracnose caused by *Colletotrichum gloeosporioides*

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Colletotrichum gloeosporioides causing anthracnose of mango was isolated from tender mango leaves on potato dextrose agar medium. Isolation of the phylloplane fungal antagonists was done by leaf impression method on semi solid potato dextrose agar medium. The pure cultures of three fungal isolates viz. *Nigrospora sphaerica*, *Gliocladium roseum* and *Aspergillus* spp. were obtained from mango leaves in repeated isolations. These three fungi were tested against *Colletotrichum gloeosporioides*, *in vitro* by dual culture technique. The results of the experiment revealed that all the three fungal antagonists were effective against the pathogen but maximum per cent inhibition (72%) was recorded by *Aspergillus* spp. Experiment on the evaluation of isolated phylloplane fungal antagonists against *C. gloeosporioides* under field conditions revealed that, *Nigrospora sphaerica* was the best phylloplane antagonist as it recorded minimum anthracnose severity (20.08%) as compared to control (42.43 %).

The compatibility of isolated fungal antagonists with three effective and recommended fungicides for disease management in mango was assessed by poisoned food technique. The antagonist *Nigrospora sphaerica* recorded mean colony diameter of 8.60 cm and *Aspergillus* spp. recorded mean colony diameter of 8.20 cm when cultured in sulphur (WP- 0.2%) amended PDA whereas *Gliocladium roseum*, was compatible with Hexaconazole (0.05%).

PP 6

Effects of methods of application of fungicides in management of damping-off in bidi tobacco nursery

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Damping-off, *Pythium aphanidermatum* is a serious threat in biditobacco nursery. Metalaxyl MZ is recommended to manage the disease since 1987 as drenching using the fungicidal solution 2 l/m² requiring 20,000 l solution/ha. Present investigation was carried out to reduce the volume of solution using the fungicides as spray drench (5,000 l/ha). Twenty eight beds, each of 1.2 x 1.2 m, were prepared applying manures and fertilizer as per the recommendation. The beds were inoculated with the pathogen a week prior to seeding of susceptible bidi tobacco variety Anand 119 @ 5 kg seeds/ha. The seven treatments i.e. 1. metalaxyl MZ @ 0.144 % (met. MZ 68 WP @ 0.2 % i.e. 2 g/l of water) (MSD 2), 2. metalaxyl MZ @ 0.0432 % (met. MZ 68 WP @ 0.064 % i.e. 0.64 g/l of water) (MSD 3), 3. metalaxyl MZ @ 2.16 kg a.i./ha (met. MZ 68 WP @ 3.176 kg/ha) (MD 3), 4. fenamidon + mancozeb @ 0.12 % (fenamidon 10 % + mancozeb 50 % i.e. 60 WG @ 0.2 % i.e. 2 g/l of water) (SSD 2), 5. fenamidon + mancozeb @ 0.054 % (fenamidon 10% + mancozeb 50 % i.e. 60 WG @ 0.09 % i.e. 0.9 g/l of water) (SSD 4), 6. fenamidon + mancozeb @ 2.7 kg a.i./ha (fenamidon 10 % + mancozeb 50 % i.e. 60 WG @ 4.5 kg/ha) (SSD 4) and 7. Control (CON) were tried. The treatment No. 1, 2, 4 & 5 were applied using 500 ml solution/sq. m through knapsack sprayer as spray drench; while, 2 l solution/m² through rose-can as drench in treatment no. 3 & 6 at initiation of the disease. Each treatment was replicated four times in RBD. All agronomic practices in vogue were followed. Observations on damped-off seedlings and other parameters were recorded at appropriate time. The data were statistically analyzed. The results revealed that all the treatment significantly reduced damped-off seedlings and increased number of transplants compared to control. Comparison of drench treatment of the fungicides with its higher dose of spray drench significantly reduced damped-off seedlings but could not help to increase transplants compared to its lower dose and drench treatments. Thus, it concluded that looking to the nursery situation i.e. dry or wet, application of metalaxyl MZ @ 2.16 kg/ha as spray drench (5,000 l water/ha, ICBR 1:8.77) or drench (20,000 l water/ha, ICBR 1:6.05) in dry or wet nursery situation, respectively can be recommended for effective and economical management of damping-off and obtaining healthy transplants of bidi tobacco. Fenamidon 10 % + Mancozeb 50 % were next in order.

PP 7

Exploration of genetic resources and other strategies for management of downy mildew disease of isabgol (*Plantago ovata* Forsk.)

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Isabgol (*Plantago ovata* Forsk.) is one of the commercially and exported oriented important medicinal crop grown in India. Several biotic and abiotic factors affect the crop production. Among the biotic factors downy mildew caused by *Peronospora plantaginis* is the major factor drastically affect the crop yield and limiting the cultivation of the crop. The disease development and incidence is also highly influenced by the abiotic factors viz., includes temperature and relative humidity. Symptoms of the disease observed before and at the anthesis stage when the temperature reached at 12-18 °C and coincide with due precipitation favoured the disease development. Early infection is usually symptomless until the congenial environmental conditions coincide. Dew precipitation is required for sporangiospores germination, take place early in the morning, positively correlated with the incidence and severity of the disease. Rainfall at the flowering stage causes severe losses in crop yield. Early maturing varieties completed the reproduction cycle and escaped the vulnerable stages to the infection of downy mildew. Early sowing and balanced nitrogenous fertilizer application minimizes the disease incidence and yield loss. Three consecutive sprays of Ridomil MZ, (0.2% solution) reduces about 70% disease incidence and found most effective for management of the disease. The repeatedly use of the same fungicides can also lead the fungicide resistance as observed in several studies. Host resistance is the best strategy of the diseases management, therefore in isabgol resistance or moderately resistance sources for downy mildew disease were identified by the field screening from available isabgol genetic resources. The total screening material comprised of 225 breeding lines, 160 recombinant inbred lines and 75 breeding germplasm accessions among them DPO-185, DPO-188, RIL-26, RIL-30, RIL-72, RIL-111 were observed and recorded resistance or moderately resistance lines for downy mildew disease in isabgol.

PP 8

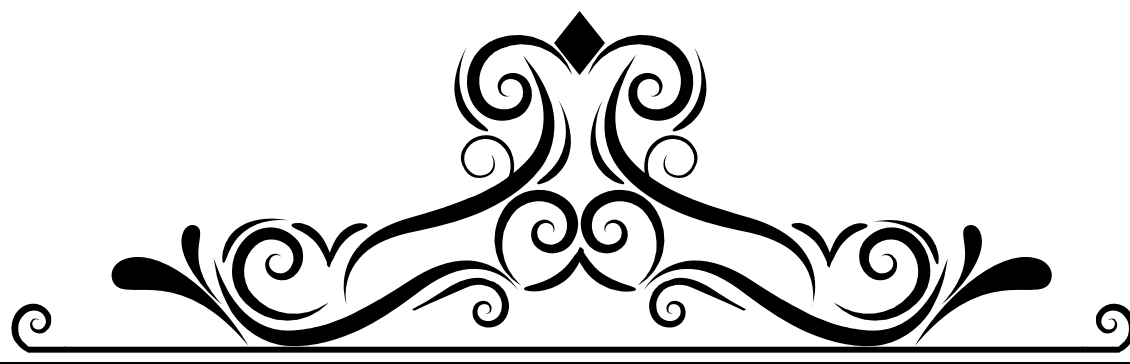
Management of pathogens infecting grain spawn using phytoextract

Savaliya Renishkumar Rameshbhai, Anjali Suansia and Priya John

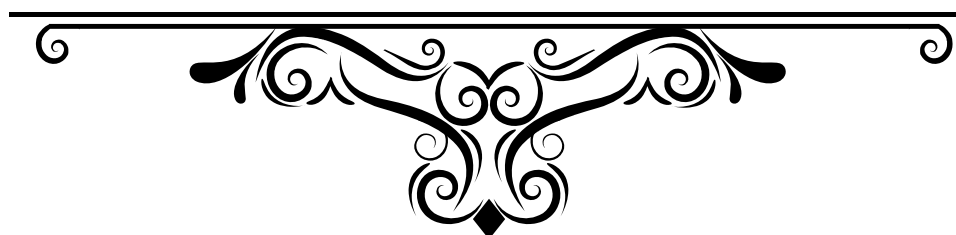
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Infection by different pathogen is one of the major constraints in spawn production of mushroom. Biological control is very important to minimize the cost of cultivation, to avoid health hazards and it does

not hamper nutritional value. Six grain spawns viz., wheat, sorghum, bajra, nagli, maize and rye were used for spawn production of oyster mushroom and evaluated for percent disease incidence by pathogens. *Trichoderma harzianum*, *Aspergillus niger*, *Aspergillus flavus* were found to be common contaminants in six grain spawns. Lowest disease incidence was observed in sorghum grain spawn which showed 6.67%, 0 % (no infection of *Aspergillus niger*) and 6.67% respectively. The next best was maize grain spawn viz., 10%, 6.67%, 13.33% respectively. Whereas the highest incidence was recorded on nagli grain spawn with 36.67%, 33.33% and 36.67% respectively. Spawn infecting pathogens can be managed either by biologically or chemically. Botanical extracts viz., neem (*Azadirachta indica*), garlic (*Allium sativum*), datura (*Datura stramonium*), ginger (*Zingiber officinalis*), turmeric (*Curcuma longa*) and tulsi (*Ocimum sanctum*) were tested against these pathogens at 5% and 10% concentration. The extracts of neem proved to be excellent in inhibiting mycelial growth of all three pathogens showing 43.33%, 55%, 45.55% inhibition respectively. Whereas lowest inhibition of mycelial growth of all three pathogens were observed with tulsi extract showing 19.26%, 23.33%, 19.45% inhibition respectively. The least inhibition of *Pleurotus sajor-caju* was observed in tulsi (18.15%).



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Oral papers

OP 1

**Pathological investigation and management of *Colletotrichum falcatum* Went
- An incitant of sugarcane red rot**

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Red rot is major concern disease for sugarcane growers in South Gujarat. Hence, present investigation was carried out during 2016-17 at Navsari Agricultural University, Navsari, Gujarat. In vitro and in vivo investigation was carried out at PG research laboratory, Plant Pathology Department, N.M. College of Agriculture and at farm of Main Sugarcane Research Station (MSRS), respectively. Pathological investigation revealed association of *Colletotrichum falcatum* Went and proved as an incitant of sugarcane red rot as the identification was done on the basis of cultural and morphological characters. *In vitro* testing of Trifloxystrobin 25% + Tebuconazole 50% 75 WG was carried out with 250, 500 and 1000 ppm concentrations by using poisoned food technique and it revealed the growth of pathogen suppressed at all concentrations. Antagonism of *Trichoderma viride* and *Aspergillus niger* tested by dual culture technique and revealed strong suppression of pathogen. Sugarcane varieties/genotypes viz., 2011 N 444, 2012 N 1738, CoN 13072, Co 13013 and CoC 671 were screened for resistance to red rot by using plug method and cotton swab method *in vivo*. In plug method, out of five sugarcane varieties/genotypes none of found resistant in reaction. Three entries viz., 2011 N 444, 2012 N 1378 and CoN 13072 found moderately resistant while, none of the varieties were moderately susceptible. Only one variety i.e. Co 13013 found susceptible. In cotton swab method, four entries viz., 2011 N 444, 2012 N 1378, CoN 13072 and Co 13013 found resistant in reaction. CoC 671 found highly susceptible in both methods.

OP 2

Molecular characterization and management of *Fusarium solani* (Mart.) Sacc. causing dry rot of potato tubers

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Fusarium dry rot of potato is devastating postharvest disease worldwide and is caused 25 to 60 per cent yield loss in India. Among the fungicides screened *in vitro*, carbendazim completely inhibited the mycelial growth of *F. solani*. The result of *in vivo* study revealed that the dry rot severity was not observed in tubers treated with carbendazim. Complete mycelial growth inhibition of *F. solani* was recorded in aluminium acetate and aluminium chloride salts. Further no dry rot severity was recorded in tubers treated with aluminium acetate. Red Kaner found significantly superior in inhibiting the mycelial growth (69.17%) of *F. solani*. Significantly lowest dry rot severity was noticed in tubers treated with Red Kaner (0.60%). *Trichoderma asperellum* found most efficient antagonist in inhibiting the mycelial growth of *F. solani* (93.99%) in *in vitro* and *in vivo*. Variation in cultures was observed among 25 isolates in growth, pigmentation and conidial size. The primer OPA-6, OPA-7, OPA-11 and OPA-13 showed the highest percentage (100%) of polymorphism. Highest similarity was observed between FS-10 and FS-13 (0.86). The dendrogram exhibited one big cluster of twenty four isolates, while only one isolate FS-6 was separately situated with similarity matrix of 0.349 with FS-1. The PCR amplification with fuminosin mycotoxin specific primer FUM1 showed that all isolates have a potential to produce the toxin fuminosin except FS-6, FS-13 and FS-14. Potato tubers when inoculated with *F. solani* showed progressive decrease in total soluble sugar (2.90%), total reducing sugar (1.02%), non reducing sugar (1.88%) and starch (3.41%), while the total phenol (3.74%) was increased as the incubation period is increased.

OP 3

Epidemiology and management of pigeon pea stem canker

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Pigeon pea (*Cajanus cajan* (L.) Millsp.) is one of the important pulse crop of India. Occurrence of stem canker disease has become a major constraint in recent years for successful and profitable cultivation of pigeon pea in Gujarat. Considering the serious threat to pigeon pea cultivation, the present investigation

was carried out on various pathological aspects to generate scientific information of pathogen which causes this disease as well as to find out suitable source of control. The studies included, identification of causal organism, symptomatology, pathogenicity, superior media for growth of pathogen, epidemiology, biological and chemical control in vitro and integrated disease management in pot conditions. The tissue isolation from stem of infected plant revealed the association of *Macrophomina* sp. which was identified after purification and cultural and morphological studies revealed as *Macrophomina phaseolina* (Tassi.) Goid. and identification was also confirmed by Indian Type Culture Collection, Division of Plant Pathology, I.A.R.I. New Delhi-110 012 (I.T.C.C. No. 9572.14). The pathogenicity of the fungus was confirmed by three different artificial inoculation methods viz., pin prick method, carborandum powder and tooth brush method. All these methods successfully produced spindle shaped lesions and found to be most efficient in producing the symptoms. Eight different media including synthetic and semi synthetic in solid and liquid state were tested for their suitability to the growth and sclerotial formation of *M. phaseolina*. Among the various solid media tested, the growth of *M. phaseolina* was significantly more on potato dextrose agar as compared to the rest but was followed by potato carrot sucrose agar media and in case of liquid media tested, significantly maximum dry mycelial weight was yielded in Richards' medium as compared to the rest but was at par with Czapek's Dox medium. The epidemiological studies showed that relative humidity and sunshine hours were significantly and positively correlated while maximum temperature, minimum temperature, rainfall and wind speed were significantly and negatively correlated with stem canker intensity. Among all the weather parameters, relative humidity and sunshine hours was found to have key role on stem canker disease development during kharif -2014. Seven known antagonists were tested in vitro for their antagonism to *M. phaseolina* by dual culture method. In this method, *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescens* appeared as strong and potent antagonists against *M. phaseolina* followed by *T. fasciculatum*, *T. longibrachyatum*, *T. koningii* and *Bacillus subtilis*. The phytoextracts of commonly available seven plant species (sterilized and unsterilized solution) were evaluated in vitro by poisoned food technique against *M. phaseolina*. The extract of turmeric rhizomes (*Curcuma longa* L.) was proved excellent in inhibiting mycelial growth and sclerotial formation followed by leaves extract of marigold (*Tagetes erecta* L). The eight organic manure/cakes extracts were tested against *M. phaseolina* by poisoned food technique in vitro. Significantly least growth of mycelium was recorded in extracts of neem cake followed by coconut cake. Next best in order of merit were FYM and mustard cake. Ten fungicides at three different concentrations were screened in vitro by poisoned food technique for evaluating their efficacy against *M. phaseolina*, in which mancozeb (dithane M-45, 75% WP), carbendanzim (bavistin, 50% WP), carbendanzim + mancozeb (sixer, 75%WP) and metalexyl 18% + mancozeb 64% (ridomil, 75% WP) were proved to be highly toxic to the growth of the *M. phaseolina*. The effective biocontrol agents, plant extracts, organic manures/cakes and chemicals which were found promising under laboratory studies were further evaluated for the management of stem canker (*M. phaseolina*) disease of pigeon pea under pot condition. Considering disease incidence seed treatment of *Trichoderma viride* @ 6g/kg seed + soil application of carbendanzim 50% WP @ 10g/pot has lowest disease incidence was followed by soil application of carbendanzim 50% WP 10g/pot, Seed treatment of *Trichoderma viride* @ 6g/kg seed and Foliar application of Neem cake extract 10% + Turmeric rhizome extract 5%.

OP 4

Management of finger millet (*Eleusine coracana* L. Gaertn.) blast (*Pyricularia grisea* (Cke.) Sacc.) on the basis of screening of germplasms for resistance to blast and seed treatment as well as foliar spray

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Finger millet blast caused by *Pyricularia grisea* (Cke.) Sacc. is serious disease of finger millet under south Gujarat condition and causes severe losses in yield. In order to manage this disease, the experiments were carried out at Hill millet Research Station, Rajendrapur, Navsari Agricultural University, Waghai, Gujarat. Biochemical parameter exhibited antimicrobial properties that inhibit the growth and development of the pathogen in the plant. The mechanism of resistance revealed that the higher amount of total phenol in finger millet resulted lower disease incidence. The finger millet genotypes GN-5, GPU-28, GPU-48, KOPN-235, KMR-204 and MR-6 are having maximum amount of total phenols. Correlation between total phenol with disease severity at different growth stages shows that there was a negative correlation in all the finger millet genotypes. Among all the tested fungicides and bioagents, seed treatment with carbendazim, 2g/kg seed + 2 sprays of tricyclazole, seed treatment with carbendazim, 2g/kg seed + 2 sprays of tebuconazole and seed treatment with *Pseudomonas fluorescens*, 10g/kg seed + 2 sprays of *P. fluorescens* found superior for the control of blast disease of finger millet as well as for getting higher grain and fodder yield.