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Real-time PCR- based detection of citrus greening disease

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Citrus greening disease aka Huanglongbing (HLB, Chinese name meaning yellow dragon disease) is one of the most serious diseases prevalent in many parts of the citrus producing regions of India. The causal agent, "*Candidatus* Liberibacter asiaticus" is a Gram-negative Alpha-proteobacterium residing in the phloem tissues (sieve tube cells) of the host plant. This fastidious prokaryote, that has not yet been cultured *in vitro*, is naturally transmitted through the sap-sucking psyllid vector *Diaphorina citri* and by means of graft transmission during propagation of contaminated nursery planting materials. Typical disease symptoms include blotchy chlorosis and/ or mottling of leaves; yellowish shoot; vein corking; stunted growth; poor root growth. Infected fruits are small, misshapen and have a bitter taste, do not colour properly, remaining green on the stylar end (bottom half) of the fruit (Hence the name 'greening') (Fig. 1). Some of these symptoms are pretty similar to the symptoms caused by nutritional deficiencies, but many infections may prevail non-symptomatic which later advance into disease.

Fig. 1.Greening infected Nagpur mandarin tree showing mottled leaves and stylar end greening fruits

Next-Generation bioformulations as a Strategy for Sustainable Agriculture

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The future scenario in the agricultural sector, challenged by regulatory pressure, public concern and environmental issues, continues to motivate the development of alternative methods to chemicals for applications as fertilizers and pesticides. Among these, the use of microbial biocontrol agents represents the most promising soil management strategy that may contribute both directly and indirectly to crop production and plant health. The successful production of microbial bioformulation and delivery of the quality product in the market is a challenging task. Since bio formulated products employ living microbial species, it needs extreme care to maintain the microbial load and vigor without having any product contamination. Additionally, during manufacturing processes production technologies requires advanced level of sophisticated instruments to maintain high-quality products. The quality of microbial inoculants in bio formulated products plays a crucial role in supplying nutrients, managing plant pathogens, therefore, contamination should be avoided for achieving maximum benefits. Apart from this, the major constraints that could be associated with effective bioformulation development may include high production cost, shelf life, and inconsistent performance. Furthermore, "living microbial cells" employed in bioformulations have high sensitivity to temperature and other external conditions and requires enormous caution at the stage of manufacture/culture, transportation/distribution, and application. The rapid decline in number of active cells from microbial population affects their potential in a unique environment. The soil environment is a heterogeneous system with mixed biota under fluctuating local conditions, temporal, and spatial aspects, pertaining to the introduction should be critically evaluated for each

The presence of greening disease in India was first suspected in 1960s. Thereafter it was reported from different citrus growing regions of India and was considered to be principal cause of citrus dieback and decline. Once a citrus plant is infected in field, the pathogen remains within it so long the plant is alive. There is no permanent cure or remedy available at present for this serious disease. Prevention and early diagnosis of the bacterium can help in plant health management and delimit the spread and devastation of the disease.

A real time qPCR (quantitative polymerase chain reaction) method has been standardized and developed for rapid and sensitive detection of greening bacterium with primers and probe (Taqman) specific for '*Ca.* Liberibacter asiaticus' at ICAR- Central Citrus Research Institute, Nagpur. The real-time PCR system (Fig. 2) is a more efficient tool in the early diagnosis of the disease, and it is capable of detecting the casual bacterium in asymptomatic young tissues of infected plants before detection of the pathogens is possible by the conventional PCR systems. Additionally, this high-throughput technique has improved the current diagnostic systems in use, achieving high speed, specificity, sensitivity and reliability, as well as reducing the risks of cross-contamination compared with conventional PCR method. Therefore real-time PCR technique promises to be a valuable component in large-scale diagnosis and management practices pertaining to citrus greening disease.

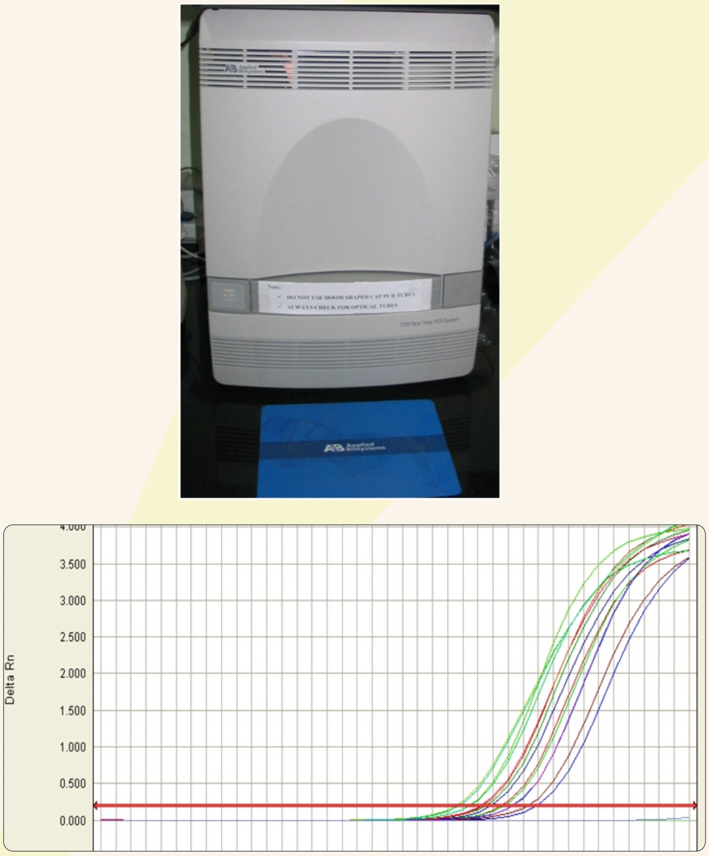

Fig. 2.Detection of citrus greening bacterium using Real-time PCR method.

release. Soil microbiotas play a crucial role in growth/survival-inhibitory effect of the inoculant. The availability of nutrient resources and the hostility of the soil environment to incoming microbes under the presence of various abiotic and biotic factors influence the growth and survivorship of the inoculant and their potential for disease management, crop growth and productivity.

Formulation is often called an enabling technology; in that it enables one or more active ingredients to work most effectively in a particular application. Due to limitations in bioformulations application purposes now the bio formulated derivatives are developed along with microbial metabolites and other additives, which ensure the prolonged shelf life, quick delivery, and stability of the system. Exopolysaccharides (EPS) include the group of important metabolites having a crucial role in root nodulation, root colonization, biofilm formation, carbon utilization, and toxin neutralization etc. EPS supplementation in bioformulated products protects microbial cell from extreme pH, harmful radiations, osmotic shock, dessication, and protection from predators. The supplementation of bioformulated products with the precursor of plant hormones such as L-tryptophan [precursor for indole 3-acetic acid (IAA) production] results into the enhancement of root hair formation, grain yield, and even capacity to fight pathogens. Likewise, L-methionine (precursor of ethylene) which, when amended with PGP microbes resulted in enhanced growth. The use of phytohormones including gibberellic acid and cytokinins as additives in bioformulations produced excellent performance under field condition. The Myc factors secreted by mycorrhizae activate signal transduction pathway or common symbiosis pathway in the host plants can be used in bioformulated derivatives for promoting the microbial association with plants.The multiinoculants Rhizo Myco contains formultions of eighteen endo and ecto mycorrhizae and growth-promoting substances and can be used for nutrient acquisition, root hair formation and plant growth promotion.

In last few years, the development of microbial biostimulants or biopesticides for enhancing plant growth and disease eradication has emerged as an alternative of chemicals. The market for biocontrol agents is a fast growing one with increasing expectations. Formulation will be key to meeting these expectations and will follow mainly the same path as that for conventional plant protection products with the exception that the changes are likely to be much quicker. Market expectations for the quality and performance of biological products will probably increase, and this is also likely to drive investment in formulation technology across the industry.

Point-of-care (POC) diagnostics for Disease Diagnosis and Management

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Early detection of pests and pathogens is of paramount importance in reducing agricultural losses. One approach to early detection is point-of-care (POC) diagnostics, which can provide early warning and therefore allow fast deployment of preventive measures to slow down the establishment of crop diseases. Point-of-care (POC) diagnostics tools are required for their ability to perform analysis and provide prompt responses outside the laboratory, in order to achieve early diagnosis, match surveillance purposes, and prevent large production losses. This is a well-known concept in the human health context with specific criteria described by the World Health Organization under the acronym "ASSURED": Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, and Deliverable. Early detection assumes significance as in human health diagnostics account for less than 5% of hospital costs and ~1.6% of all Medicare costs but they influence up to 60–70% of healthcare decision making. Point-of-care tests (POCTs) are defined as, "a fully or partially automated table-top, portable or disposable device able to be operated in a non-laboratory environment by non-technical staff to deliver a same-day, on-site, clinically relevant, diagnostic test result". Many different POCT platforms and formats exist, ranging from paper-based lateral flow assays (LFAs) and dipsticks to portable nucleic acid detection systems, to handheld nanopore sequencing devices, wearable electronic sensors, "smart" textiles and more. Many POCTs utilize smartphones, Wi-Fi and/or Bluetooth connections to allow data transmission between remote field sites and central databases. Among the available diagnostic technologies, nucleic acid amplification–based diagnostics provide the highest sensitivity and specificity, and those technologies that forego the requirement for thermocycling show the most potential for use at POC.

Several portable and hand-held devices have been developed to translate these laboratory-based methods to point-of-care (POC) settings. POC diagnostic devices could potentially play an important role in environmental monitoring, health, and food safety. Use of a smartphone for nucleic acid testing has shown promising progress in endpoint as well as real-time analysis of various disease conditions. The emergence of smartphone-based POC devices together with paper-based sensors, microfluidic chips and digital droplet assays are used currently in many situations to provide quantitative detection of nucleic acid targets. Smartphones can record sensing patterns of optical, fluorescence, colorimetric, and electrochemical/ electrical techniques to detect bio-analytes using their features like high-resolution cameras, facile video documenting systems, and integrated applications with portable electrochemical transducers.

POC test requires less amount of sample for detection that can be collected by the farmer itself and the results will be displayed within a few minutes after introducing the samples to the test kit. Various POC detection technologies such as nucleic acid lateral flow assays (LFA), nanomaterial-based sensors, and electrochemical sensors have been used for more rapid detection of a broad range of plant diseases. Interestingly, molecular assays based on the amplification of nucleic acid are widely preferred among these techniques. Advances in biomarker and biosensor technologies enabled the innovation of reliable and affordable POC devices. Various prospective biosensors such as label-free electrochemical sensors, nanoparticle-based surface plasmon resonance (SPR) based sensors, and later flow assays biosensors have been developed to provide an improved diagnosis in POC devices.

Lateral flow assays (LFAs): Rapid POC tests such as LFA are new-generation diagnostics. This technology offers several advantages as it is robust, less time-consuming, and cost-effective. The strip consists of a membrane and functional pads that contain a sample pad, a conjugate pad, and an absorbent pad. Test and control line capture samples (either antibody or nuclear acids) are immobilized on the membranes of nitrocellulose. A small volume of samples is applied to the sample pad when testing is performed. The pad is pre-treated with a buffer. The gold nanoparticles are conjugated to the antibody or peptide or nucleotide probe and immobilized on the conjugated pad. Target molecules from the sample pad migrate to the conjugate pad and bind to the gold-conjugated antibody and migrate to the test line, where the target analyte is captured leading to a positive result.

Nanomaterial-based sensors: The synthesis of spherical nanoparticles is relatively simple, so it is the more widely used shape in nanoparticles. A functional nanosphere is prepared by incorporating biomarkers with spherical nanoparticles which enhances the specificity and ensures the high signal in the presence of analyte or pathogen hence serves as an excellent biological label. Biomarker analytes include nucleic acids, proteins, peptides, lipids, metabolites, and other small molecules. This nanomaterial-based method stands out due to its exceptional bio-affinity, strong color change, and catalytic properties. Hence, it is highly preferred in optical and electrochemical POC devices.

Electrochemical sensors: Electrochemical biosensors are simple and less expensive, and their sensitivity can be enhanced via signal amplification. Ultra-highly sensitive electrochemical biosensor for plant virus detection was developed using differential pulse voltammetry and electrochemical impedance spectroscopy. The genetic material in the virus is either

RNA or DNA. In a DNA biosensor, single strand of the oligonucleotide is immobilized on the transducer or electrode surface, which detects the complementary DNA sequence resulting in surface hybridization. Thus, a formed hybrid sends an analytical signal via a transducer. The quantitative measurement is obtained with the help of an electrochemical reaction which is often coupled with a DNA hybridization event. The modulation in the probe-target redox reactions at the electrode surface is translated to analytical signal. These sensors are very sensitive that they can even detect attomole (10^{-18} of a mole) concentration of plant RNA.

A very simple method proposed for DNA or RNA extraction is the so-called "toothpick method," in which a sterile toothpick is used to pierce the sample tissue several times. The tip of the toothpick is then dipped directly in the amplification reaction mix, releasing target genetic material. Despite the extreme simplicity, this method needs to be tested and optimized for each specific need. A modern version of the "toothpick method" was recently proposed using disposable polymeric microneedle patches (MP) to pierce plant leaves. These patches are made of polyvinyl alcohol (PVA), a water-absorbing polymer that showed a good combination of mechanical strength, chemical resistance, and biocompatibility. The use of MP to detect *Phytophthora infestans* from tomato leaves showed a slightly lower sensitivity in comparison to the standard CTAB extraction method, especially during the very early stages of infection. On the other hand, the extraction time was reduced to 1 min, instead of 3–4 h of the CTAB method, suggesting that MP could be easily used for in-field applications.

POC testing will make the difference in term of management decision, clarifying when to start treatment or to require a confirmation test. POC can support farm animal monitoring, health management, and plant pathogen identification, in taking rapid disease control measures. Digital farming is the current trend in agriculture which might improvise the POC devices in future by coupling them with mobile phone apps to visualize and store the data in the phone. With digital farming, the future of POC devices is very bright. The potential advantages of POC analysis are numerous, going from the speed of the analysis to the lower risk of sample contamination and pathogen dispersion. It would allow extensive and fast screening of plants in order to prevent disease spreading. Moreover, imported plants and products could be analyzed directly on-site before they can even enter a country.

Phytoplasma and Phytoplasma Diseases: An ominous Threat to Temperate Horticulture

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Phytoplasma are among the most recently discovered plant pathogens associated with diseases affecting several hundred plant species. They are wallless prokaryotic plant pathogens restricted to phloem tissues belonging to the class Mollicutes of the order Acholeplasmatales and family Acholeplasmataceae. Phytoplasma were discovered in 1967 and were named mycoplasma-like organisms (MLOs), due to their morphological and ultrastructural similarity to mycoplasmas, already known as etiologic agents in animal and human diseases. Later, with the advancement in detection techniques using molecular biology tools MLOs were renamed *Candidatus Phytoplasma*. Phytoplasma are similar to bacterial bodies of small dimensions, varying from 200 nm to 800 nm in diameter, delimited by a plasma membrane, but devoid of the cell wall. The absence of a rigid cell wall allows them to be highly pleomorphic and to change shape by adapting to the environment. This feature is probably associated with the fact that, as the other Mollicutes, phytoplasma are obligate parasites. Phytoplasma lives inside the cells of plants and insect vectors and with a unique life cycle, they replicate intracellularly in both. Phytoplasma enters the vector through insect nutrition activity on infected plants. Phytoplasma are transmitted by phloem-feeding insect species within the Order Hemiptera, such as Cicadellidae (leafhoppers), Fulgoridea (cicada) and Psyllidae (psyllids). The insects must feed for an extended period of time to acquire a sufficient titer of phytoplasma to establish infection. During a latent period in the vector, phytoplasma pass from the alimentary canal through the midgut into the hemolymph, they invade salivary gland cells, multiply and are incorporated into saliva. Then they are transmitted to a new host plant by injection into phloem tissue during insect feeding, during the so-called inoculation access period. Inside the sieve elements, the phytoplasma move systemically through the plant.

The phytoplasma infection causes an impressive impact on many vegetable and fruit crops, ornamental and timber plants, although not all infections are necessarily deleterious, the great majority of phytoplasma diseases cause stunting of overall plant growth, general decline, loss of productivity, and, in some cases, plant death. To date, no effective curative strategy has been developed thus the detection of these micro-organisms is a prerequisite for the management of phytoplasma-associated diseases and, for this reason, the development of sensitive detection and quantification protocols has been a continuous effort in the last decades. Over recent years, there has been a drive toward simpler and quicker detection methods that can be performed for border inspection or detection of phytoplasma infection in field. The availability of rapid and sensitive techniques for phytoplasma detection as well as the possibility to study their relationship with the host plants is a prerequisite for the management of phytoplasma-associated diseases.

Phytoplasma infection induces Ca2+ influx into the sieve elements, leading to sieve-tube blockage. In fact, transmission electron microscopy (TEM) images revealed sieve-element filament formation, agglutination, and callose deposition at the sieve plate level. In addition, Phytoplasma infection can lead to the involvement of other important signal and defence molecules such as hydrogen peroxide (H_2O_2) and different phytohormones besides also downregulation of photosynthetic proteins in phytoplasma-infected plants accompanied by a reduction of total chlorophyll content.

Yellow leaf roll phytoplasma of peach

A new disease in peach was observed in 1995 in the Rajgarh valley of district Sirmour and resulted in quick decline and rapid death of peach plants in the valley. The disease subsequently spread to other peach-growing areas of the state probably with the movement of infected planting material. The disease was characterized by the inward rolling of leaves, thickening of mid veins, prominent yellowing of leaves turning red in colour. Plants in infected orchards could easily be identified from a distance due to the promiment red-coloured plants. Infected plants exhibited typical partial infection symptoms and the presence of mummified fruits on the infected trees. Research findings on this phytoplasma disease of peach resulted in checking the spread of the disease as well as reducing yield losses. Recommendations on the use of phytoplasma –free bud wood and nursery plants along with pruning of the diseased twigs and use of antibiotic treatment of ailing peach trees is practically followed by the peach growers to manage the diseases. The Department of Horticulture, Govt. of H.P. has also followed the recommendation on the restriction of movement of budwood from infected s to the disease-free areas. Research conducted on the molecular detection and characterization of the phytoplasma associated with yellow leaf roll disease of peach has clearly indicated that phytoplasma responsible for this disease belongs to the Elm Yellows group of phytoplasma on the basis of amplification of 16 S ribosomal DNA.

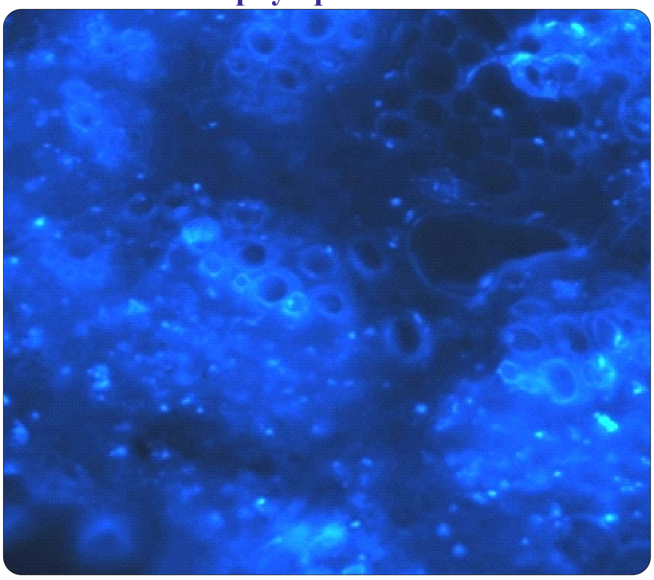
Yellow leaf roll phytoplasma of peach

Phytoplasma diseases of cherry

A new disease was observed in cherry orchards in Baghi area of district Shimla in September, 2022 causing rapid drying up of the infected plants. Visual symptoms in the infected trees indicated a possible association of phytoplasma with the new disease. In order to ascertain the cause of the disease, diagnostic

Partial infection and reddening of leaves in phytoplasma infected cherry tree

Leaf hopper vector of cherry phytoplasma



Phytoplasma bodies illuminating in fluorescent microscope

visit was conducted by the team of scientists of the Department of Plant Pathology, YSPUHF, Nauni and the team recorded the symptoms and interacted with growers of the valley on this issue. The team collected samples from symptomatic trees, weeds, and suspected vectors. The samples were brought to the Plant Virology laboratory and after processing sections of the leaves and leaf hoppers, the samples were analyzedby fluorescent microscope using DAPI stain. The results revealed the phytoplasma bodies in sieve tube elements of the infected plants. Besides, Phytoplasma bodies were also detected in the leaf hopper vector, thereby confirming the association of Phytoplasma with this malady. Subsequently, a Farmer Awareness Camp was organized by the Department of Plant Pathology, YSPUHF, Nauni at Baghi in the month of October, 2022 and detailed information with regard to the identification of symptoms, leafhopper vectors, management strategies and application of trunk injection of Oxytetracycline hydrochloride (OTC-HCL) antibiotic was demonstrated.

Adhoc strategies for the management of Phytoplasma disease of Sweet Cherry

1. Use healthy planting material for raising disease-free nursery as the infected plants serve as foci for spread of the phytoplasma.
2. Remove and destroy diseased twigs / branches during pruning
3. Restriction on movement of planting material and avoid the use of bud sticks from symptomatic trees
4. Oxytetracycline hydrochloride (OTC-HCL) antibiotic @ 2 g + citric acid @ 10 mg dissolved in 10 ml of water should be poured drop by drop through the holes made into the trunk of trees with the help of a pipette. Plug the holes with clay soil and FYM paste (1:1 w/w) after the absorption of solution.
5. For the control of leaf hopper vector, Spray Imidacloprid 17.85 SL @ 50 ml/200 L of water or Oxy-demeton Methyl 25 % EC @ 200 ml/200 L of water.
6. Clean orchard floor management practices should be adopted and weeds growing in the vicinity of orchards should be destroyed as weeds such as ferns serve as carrier of phytoplasma.

Kiwifruit Root Rot in Himachal Pradesh

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Kiwifruit (*Actinidia deliciosa*) is a dioecious, perennial and deciduous warm temperate fruit that belongs to the family *Actinidiaceae*. It is subject to a number of diseases at all stages of its development i.e., from nursery to the consumption of fruits. Among all diseases infecting kiwifruit, the root rot is a new emerging disease posing a threat to kiwifruit cultivation which is in its nascent stage in Himachal Pradesh. During the survey conducted in different kiwifruit orchards, the disease incidence ranged in between 5.45 to 44.5 per cent in orchards and 0.15 to 50 per cent in nurseries of Solan and Sirmour district of Himachal Pradesh. The symptoms at ground level showed black sunken, necrotic lesion on woody tissue, and symptoms above ground level showed wilting of foliage and browning of leaves. As the disease progressed, bronze and necrotic spots emerged on the leaves. Active lesions frequently progressed aboveground on one or more sides of the lower trunk that leads to sunken area. Dieback symptoms included decline, leaf scorching, and defoliation in infected kiwifruit trees. White coloured mycelium was also observed on roots of some diseased kiwifruit trees. Infected vines roots and root crowns had a red brown rot that could be seen by cutting through the cortical tissues. Feeder roots were absent in infected trees.The pathogens associated with root rot of kiwifruit were morphologically and molecularly

Wilting of foliage and browning of leaves

Rotting and browning of internal tissues of roots

confirmed as *Phytophthora* sp. and *Fusarium oxysporum*. Root rot was favoured by a variety of environmental factors, including abundant soil moisture, optimal temperature for pathogen growth, overwatering, poor drainage, continuous or frequent cropping, and other plant stress factors.

STUDENTS CORNER

Seed biopriming- A green approach towards crop protection

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Seed is a vital source for crop sustainability in agriculture and healthy seeds are a key factor in ensuring food security. Diseases majorly which are seed and soil borne in nature are an important factor responsible for low crop productivity. Out of 16%, annual crop losses due to plant diseases, at least 10% loss is incurred due to seed-borne diseases. Seed priming refers to the use of natural or synthetic compounds to induce a particular physiological state in seedlings before germination. Many strategies other than biopesticides have been used for seed priming, including hydropriming, osmopriming, chemical priming, hormonal priming, biological priming, redox priming, and solid matrix priming. Seed priming is a feasible, pre-sowing enhancement technique and has been widely employed in the commercial production of crop seeds. Seed priming initiates multiple pre-germinative metabolisms, including enzyme activation, energy production, metabolites biosynthesis, and DNA repair. Seed priming could secure the enhanced and uniformed seed germination and seedling establishment under field conditions, and greatly contributes to the improvement of crop growth and production. Increasing evidence revealed that seed priming could induce plant immune memory that is either stably maintained throughout developmental stages or transmitted over generations. Different types of seed priming approaches such as biological priming, chemical priming, and nanomaterials priming have been successfully established to protect crop plants against pathogen infections.

Biopriming involves the use of beneficial microorganisms for conditioning of seeds. Term seed biopriming was firstly coined by Callan et al (1990). Seed biopriming involves soaking of seeds in liquid suspensions of bioagents for a particular period of time, which initiates the physiological and developmental processes like DNA synthesis, protein accumulation, DNA repair etc within the seeds and thereby preventing radicle emergence before seed is sown. Beneficial effects of seed biopriming involves the greater membrane integrity, counter action of lipid peroxidation, antipathogenic effects, cellular enzymatic repair system and metabolic removal of toxic substances from the primed seed. The application of bio-agents further promotes the cellular mechanisms of a plant including reprogramming of its metabolism, leading to improved performance under diverse agroecology. Due to these physiological processes in the seed, many fold proliferation of the inoculated bioagent occurs within seed that allows seed to withstand adverse conditions and also protects seeds from pathogens attack.

Beneficial microbes such as plant-growth-promoting fungi (PGPFs) *Trichoderma* spp., plant-growth-promoting rhizobacteria (PGPRs), *Pseudomonas* spp., *Paenibacillus* spp., and *Bacillus* spp. have been employed in seed priming on crops. These biopriming agents are effective plant conditioners in improving plant health by preventing the germinating seedlings from seed and soil borne pathogens. Application of bioinoculants as priming agent facilitate biochemical nutrient cycling, promote enzymatic activity thereby improving crop stand and crop health under changing environment conditions. Seed priming of crop plants with elicitors derived from beneficial microbes also could trigger immune memory, as well as induced resistance, throughout their developmental stages. Phytohormones SA and JA and plant natural product BABA could effectively induce crop disease resistance when applied exogenously in seed priming. Synthetic chemical inducers chitosan, INA, and cholic acid-glycine conjugates (CAGCs) have been successfully applied in the seed priming of crop plants for disease resistance improvement. Recently, advanced chemical inducers-synthesis strategies such as computer-aided inducer design have been developed, which would certainly contribute to the advance of seed priming and its application in crop protection. Nanomaterials could effectively trigger crop immune memory and induced disease resistance when applied exogenously in defense priming. Seed priming of tomato with mycogenic selenium nanoparticles (SeNPs) induced accumulation of lignin and hydrogen peroxide, as well as elevated expression levels of *LOX*, *PAL*, *GLU*, and *SOD* genes. These SeNP-primed tomato plants displayed enhanced resistance against the late blight caused by *Phytophthora infestans* throughout their developmental stages, indicating that nanoparticles could be applied in the seed priming for crop protection. Different processes that lead to increased plant growth and resistance to stress involves hydrolytic enzymatic activity, activation of ROS (Reactive Oxygen Species), change in hormonal levels and differential gene expression.

Biological seed treatments for the control of seed and soil borne diseases offer the farmers an alternative, eco-friendly practice in spite of chemicals. Among different methods employed for biological seed treatment like seed biopriming, seed coating, seedling dip and dry treatment, seed biopriming is the most efficient and productive as it utilizes the bio resources judiciously for enhancing crop productivity and protection. Seed biopriming is a promising seed treatment method that enhances stress tolerance to seeds before germination. Seed biopriming was found effective in the development of ISR (Induced Systemic Resistance) in plants. Biopriming mediated suppression of soil and seed borne diseases have been reported in various crops like carrot, chickpea, pea, chilli, tomato, brinjal, bean, maize, rice by various workers. The protective biochemicals, especially antioxidant enzymes act as salutary effects or of seed bio-priming under any form of stress. The performance of cumin seeds improved under drought stress due to seed priming with *Pseudomonas fluorescence* and *Trichoderma harzianum*. An increase in antioxidant enzyme activities such as ascorbate peroxidase (APX) and catalase (CAT) was reported in cumin seeds. The resistance of tomato plants against Fusarium Wilt increased when the seeds were primed with *Trichoderma asperellum* (fungus) or *Ochrobactrum* sp. (bacterium). Integration of different bioresources like plant extracts, microbial products and other biotic agents for managing plant diseases has been considered as a novel approach as it is devoid of chemicals and pollution hazards, reduces cost of control and maintains biological equilibrium. Seed biopriming has provided a new tool of biocontrol to the farmers for sustainability in agriculture. Seed priming is a simple but an effective and a low-cost technology to prepare the germinating seeds for incoming challenges.

Biochar: An alternative approach to control plant diseases

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Plant diseases have been a persistently great challenge to global food security due to the enormity of the losses. Crops constituting our food basket are under continuous threat of diseases and pests and the average yield losses associated with pathogens and pests globally was estimated at 21.5%, 30.0%, 22.5%, 17.2%, and 21.4% for wheat, rice, maize, potato, and soybean, respectively (Savary et al., 2019). Chemical control with fungicides has always been the major and effective method for plant disease control. However, the excessive application of such agrochemicals is detrimental to the ecosystems and even toxic to human beings (Yang et al., 2022). Increasing studies have shown that plant health and productivity are reliant on the ecosystem services provided by indigenous soil and plant-associated microbiomes (Huang et al., 2020). Therefore, healthy soil management is considered as an effective method to plant disease control (Nwokolo et al., 2021). Hence, there is need for alternatives and innovative approaches which provide sustainable solution for the management of diseases in plants. Biochar formed by pyrolysis is a carbon sequestrating soil amendment reported to improve plant performance and reduce the severity of both foliar and soilborne plant diseases. Pyrolysis is a process involving heating of organic materials in an oxygen deficient environment. The physico-chemical properties of biochars are dynamic in nature, dependent upon source of raw organic material as well as processing conditions especially the temperature of pyrolysis. Numerous studies have indicated that pyrolysis temperature is the most important factor that determined biochar physicochemical properties. The application of biochar produced between 350 and 600 °C dramatically reduced plant disease severity. However, no significant differences were observed for biochars produced ≤ 350 °C and ≥ 600 °C. Apart from the surface area, pH, and cation exchange capacity, which help to mediate soil physiochemical properties, the formation of organic compounds, especially those dissolved compounds, would also affect biochar disease suppression efficiency as well as plant growth. The formation of volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dioxins and furans (PCDD/DFs) with the carbonization of organic compounds makes biochar toxic to both soil microorganisms and plants. Furthermore, studies found organic compounds in biochars like benzoic acid, glycol, and phenols, function as plant immunity inducers to enable plants to perform immune response by stimulating systemic resistance.

Biochar has sparked widespread interest as a soil amendment to boost agricultural productivity, owing to its ability to neutralise soil acidity and increase cation exchange capacity (CEC), both of which are desirable properties for soil chemical improvement. The pH of soil has frequently been found to rise when biochar, which is typically alkaline, is added. Moreover, both the solid and water-soluble components of biochar are redox active and can modify the redox potential (Eh) of soil. Because many soil pathogens flourish in narrow Eh-pH ranges, biochar-induced pH changes or coupled Eh-pH changes in the rhizosphere could have a significant impact on pathogen viability. Furthermore, biochar is a material with a high pore volume, which makes it effective at increasing water holding and improving soil aggregation. Besides that, biochar may adsorb toxins and extracellular enzymes produced by soil borne pathogens, immobilisation of pectinolytic, cellulolytic and other cell wall degrading enzymes may reduce their contact with root cell walls, protecting the plant to some level.The optimal application rate of biochar is not yet established, as it may depend upon the type of biochar, soil and target species. However, some studies concerning plant pathogens have revealed that although biochar concentrations lesser than 1 per cent suppress several diseases, concentrations higher than 3 per cent are mostly ineffective.

Biochar has been reported to be effective in suppressing diseases caused by both soil-borne and air-borne plant pathogens such as *Fusarium oxysporum* f. sp. *lycopersici* on tomato, *Rhizoctonia solani* on cucumber, while *Podosphaera aphanis* on strawberry, *Botrytis cinerea*, *Leveillula taurica* on tomato and pepper. The addition of biochar to soil influences various properties of the soil microbiome which may result in the alteration of plant disease intensity. Moreover, it has also been proposed that the combination of biochar and compost induce modifications in physical and chemical properties of soil, leading to better plant growth and production. There was synergistic impact of co-application of biochar and compost for the management of soil-borne diseases and enhancing the activity of beneficial microbial populations of the soil including arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria (PGPR) and other bio-control agents. Biochar suppresses disease through a variety of mechanisms, including increase in the density and activity of beneficial microorganisms such as PGPR (Plant growth promoting rhizobacteria), Nitrogen-fixing bacteria, VAM (Vesicular arbuscular mycorrhiza) fungi and mycoparasites e.g. *Trichoderma* spp. All of the changes brought about by biochar in the soil can alter the composition of the soil community, the microbial biomass and abundance of soil pathogens. In general, the susceptibility of rhizosphere towards the invasion by soil pathogens is inversely related to the heterogeneity of the rhizosphere microbiome, with increased heterogeneity resulting in decreased pathogen virulence. Several studies have revealed an increase in the *Flavobacterium* genus in biochar-amended soil. Indeed, the majority of rhizosphere bacteria and fungi produce metabolites that restrict the growth or activity of competing pathogens. Biochar resulted in induction of systemic resistance in tomato against a soilborne pathogen and this elucidates some of the molecular pathways responsible for improved plant growth and enhanced plant defenses using whole transcriptomic analysis. The presence of biochar primed the plant for potentiated systemic responses to soilborne pathogen infection. In general, genes and pathways associated with plant defense and plant growth such as jasmonic acid, phenylpropanoids, flavonoid, peroxidases, sterol, brassinosteroids, auxin, cellulose, xylan, and lignin were upregulated. Understanding the mechanisms of biochar-mediated systemic resistance against pathogens and improved plant performance are important steps for the adoption of biochar as a beneficial soil amendment. Therefore, there is a need for more research to extend our knowledge about various chemical and physical characteristics of biochar and its ability to suppress plant diseases in different pathosystems.

Endophytes: the hidden microbial treasure, combating biotic and abiotic stresses in plants

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Plants generally come across various biotic and abiotic stresses which hamper their growth and yields.

The biotic stresses are harmful microorganisms due to establishment of relationships with the plant whereas abiotic stresses indirectly affect physiological and biochemical functions of the plant. The search for exploration of internal microbial flora of plants and their interaction has recently attracted the attention of researchers due to the growing concern of sustainable eco-friendly alternatives for biotic and abiotic stress management.

The term "endophyte" refers to a significant group of plant symbionts that exist asymptomatically within plant tissues in almost every plant species without posing a threat to host plants. Fungal and bacterial endophytes have a significant impact on host plants and create a favorable symbiotic relationship with them. Plant growth, immunity, and general development are all enhanced when the endophytic communities coexist within the host plants. The production of secondary metabolites and other bioactive compounds by the endophytes has been reported to increase plant productivity and disease resistance against deleterious phytopathogens. The endophytic plant microbial associations directly accelerate the mechanisms of nitrogen fixation, phosphate solubilization, siderophores, phytohormones and anti-microbial metabolites which indirectly influence the mechanisms of abiotic and biotic resistance, bioremediation, and phytoremediation.

Endophytes managing biotic stresses

It is an indirect mechanism through which endophytes inhibit pathogens. When pathogens attack, endophytes have the ability to reduce disease susceptibility by inducing resistance in their host plant. Resistance patterns notably ISR mediated by phytohormones like ethylene or jasmonic acid and systemic acquired resistance (SAR) connected with salicylic acid regulation is the known signalling mechanisms. Root colonization by endophytes and expression of pathogenesis-related genes is often correlated with the induced systemic resistance. For instance,fungal endophyte *Fusarium solani,* which is isolated from tomato root tissues and induces systemic resistance against the tomato foliar disease *Septoria lycopersici* by causing the expression of the PR genes PR5 and PR7 in the roots. Additionally, compared to uncolonized plants, plants that have been colonised by endophytes are able to show more defence respectively. When cucumber (*Cucumis sativus*) and watermelon (*Citrullus lanatus*) exposed to a non-pathogenic mutant of *Colletotrichum magna* exhibited higher levels of peroxidase activity, lignin deposition, and phenylalanine ammonia lyase activity that resulted in the protection against disease caused by *Colletotrichum orbiculare* and *Fusarium oxysporum*. Also, the endophyte *Neotyphodium lolii* increased the host's superoxide dismutase (SOD) and peroxidase (POD) activity, reducing the lesions on the infected leaves.

Endophytes managing abiotic stresses

Endophytes help plants withstand abiotic stress by either activating the host stress response system or by biosynthesizing anti-stress metabolites such plant growth regulators,1-aminocyclopropane-1-carboxylate (ACC) deaminase, volatiles, bioactive substances, etc. Depending on the forms of abiotic stress, various endophytes adopt various signalling strategies, which may include the synthesis and accumulation of suitable solutes, modification of ion transport, expression of stress-responsive genes, production of ROS scavenger molecules, etc. Endophytes increases plant vigour, which confers drought tolerance and reduced transplanting shock in plants. For instance, Grapevine plants exhibited increased cold stress tolerance due to bacterial endophyte *Burkholderia phytofirmans*. *Paraphaeosphaeri aquadriseptata*, endophyte enhance heat resistance in *Arabidopsis* by producing heat shock protein (HSP90). Fungus endophyte *Piriformospora indica*, found in the outer layer of the host's roots, promotes plant growth, increase chlorophyll content, and yield under drought stress conditions in wheat at various temperature regimes. The endophytic fungus *Exopiala sp*. LHL08 isolated from cucumber root give resistance against salinity and drought stress in rice.

The use of endophytes may play a beneficial role in sustainable agriculture if the endophytes inoculants are potential, the high production costs associated with the use of chemical fertilizers can be reduced. Furthermore, a thorough understanding of the molecular mechanisms of host- endophytes' interactions can provide a broad understanding for biocontrol screening and its successful application.

Table 1: The endophytes responsible for managing biotic and abiotic stress in plant –microbe interactions

Crop	Endophyte (s)	Biotic and abiotic stress management
Tomato	*Beauveria bassiana*	Whitefly/*Bemisia tabaci*
Cucumber	*Penicillium* sp. and *Hypocrea* sp.	Wilt/*Fusarium oxysporum* f. sp. *cucumerinum*
Maize	*Cladosporium oxysporum* and *Rigidoporus vinctus*	Maize stem borer/Chilo partellus
Wheat	*Sarocladium strictum, Anthracocystis floculossa, Anthracocystis floculossa,* and *Penicillium olsonii*	Fusarium head blight /*F. graminearum*
Soybean	*Trichoderma longibrachiatum, T. asperellum*, and *T. atroviride*	Root rot/*Rhizoctonia solani*
Musk melon	*Trichoderma phayaoense*	Gummy stem blight, Wilt/*Stagonosporopsis cucurbitacearum* and *Fusarium equiseti*
Melons	*Trichoderma harzianum, Trichoderma lentiforme, Epicoccum purpurascens*	Rots, collapse, and Wilt/*Macrophomina phaseolina, Monosporascus cannonballus, Fusarium* sp.
Mustard	*Piriformospora indica*	Drought tolerance
Grapevine plants	*Burkholderia phytofirmans*	Cold tolerance

NEWS DESK

Brainstorming Workshop on use of Smart ICT Devices for Disease Prediction

Brainstorming Session and Training on Expert System for Disease Forewarning was organized under the ages of World Bank funded Himachal Pradesh Horticulture Development Project on July 15, 2022. Fifty scientists, farmers and experts participated in the event. Brainstorming session was chaired by Dr TP Sharma, Associate Professor, Department of Computer Science and Engineering NIT, Hamirpur, who delivered an expert talk on "Principles and Technology in the use of Sensor Based Systems for Forewarning of Diseases and Pests". He also interacted with participants about the use of Sensor Based IoT technology in agriculture with special reference to plant protection. This training assumes significance as the Department of Plant Pathology is Collaborating in DST Funded joint research project with NIT Hamirpur and IIT Roorkee. In this workshop, one session was conducted by the experts from Fasal an agri-start up in expert system for disease forewarning and forecasting. Mr Mukesh Madhukar and Mr. Umang gave hands on training to the participants for using Fasal smart farming expert system and its use in smart agriculture.

ICAR-Sponsored Short Course Organized

Department of Plant Pathology was able to get for the first time a 10-day Short Course cum training on 'Recent Development in Organic Production Systems and natural farming which was successfully organized from November 14-23, 2022. The course was funded by the Indian Council of Agriculture Research. Seventeen scientists from different universities of Tamil Nadu, Kerala, Madhya Pradesh, Jammu and Kashmir, Punjab and Himachal took part in the training. Participants were apprised about the different aspects of organic and natural farming starting from basic principles, food production, plant protection, post-harvest processing and the overall economic viability of natural farming. The training covered all the relevant topics like Novel Microbes and their Potential Use in Organic and Natural Farming Systems, Multilayer Farming, Soil Nutrient Dynamics and Analysis, Effective use of Bio-control Agents and New Insights in Diseases Management. Hon'ble Vice-Chancellor of the University, Prof. Rajeshwar Singh Chandel in his thought provoking lecture emphasized the importance of natural farming and said that the country has taken a challenge at the right time to ensure the conservation of soil and water and provide healthy food to society. Prof. Chandel added that natural farming was a farmer-centric approach where the scientific community is learning along with the farmers. He urged the trainees to develop

Inauguration ceremony of the short course

Lecture by the Hon'ble Vice Chancellor in the short course

Hon'ble Vice Chancellor releasing the compendium of lectures of the short course

their own package of practices on natural farming for their areas using the natural items available in that region. Dr HR Gautam- Course Director and Professor and Head of the department of Plant Pathology urged the trainees to act as brand ambassadors and resource persons for natural farming in their institutions and command areas.

Hon'ble Vice Chancellor distributing course completion certificates to the participants

Kisan Mela Organized at Maraug in District Shimla

Department of Plant Pathology organized a Kisan Mela on September 14, 2022 under the World Bank funded Himachal Pradesh Horticulture Development Project at Maraug in Chopal sub-division of district Shimla in which about 150 farmers participated. Professor and Head, Dr HR Gautam, advised the farmers to adopt new technologies like high density apple orchards as in the demonstration orchards University has recorded yield of more than 40 tonnes per hectare. He made the farmers aware about the indiscriminate and overuse of fertilizers and pesticides which has adversely affected our environment and soil health. Soil fertility has also been adversely affected with the overdose of nitrogenous fertilizers. During this Kisan Mela a kit containing PGPR and *Trichoderma* cultures developed by the University was distributed among the farmers. Use of these bio-inputs will drastically reduce the consumption of fertilizers and pesticides. The State Government is giving a lot of emphasis on natural and organic farming, use and availability of such bio-inputs will incline farmers for their use. During this Mela specialized lectures by the scientists of the University on different topics were delivered. Dr Naveen Sharma, of Department of Fruit Science gave emphasis on the plantation of suitable new varieties on specific rootstocks and proper training and pruning of the plants for getting the higher productivity. Dr Rajesh Kaushal from the Department of Soil Science and Water Management highlighted the role of PGPR for good soil health and sustainable productivity from their orchards. Dr Rakesh Kumar from the Department of Entomology gave a detailed account of important pests in apple and their management. As diseases cause huge losses in apple, Dr HR Gautam and Dr Bhupesh Gupta, made people aware about the symptoms and management practices of scab, premature leaf fall, powdery mildew, canker diseases, white root rot and collar rot diseases. Farmers were advised to adhere to the spray schedule of the apple for effective management of the diseases and pests. Dr Ajay Brakta, Plant Virologist made farmers aware about the diseases caused by viruses and viroids and need for virus free and certified apple nursery. Farmer-scientist interaction was also held to answer the important queries of the farmers related to their apple cultivars. We need to gradually move to such farming practices which are based on organic inputs.

Aravinth Kumar A who did his B.Sc. (Agriculture) from Annamalai Universtiy (Tamil Nadu) and M.Sc. from the Department of Plant Pathology, Dr YS Parmar University of Horticulture and Forestry, Nauni Solan HP qualified All India Competitive Examination 2022 and got selected for Doctoral Programme in Indian Agricultural Research Institute (IARI) Pusa New Delhi, India. He also qualified Tamil Nadu Agricultural University Doctoral Programme Entrance Test 2022 and Banaras Hindu University Research Entrance Test 2022